Mail Stop Interference
P.O. Box 1450
Alexandria Va 22313-1450
Tel: 571-272-4683
Fax: 571-273-0042

Filed 2 February 2010

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

PAUL J. **CARTER** AND LEONARD G. PRESTA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR**, DILJEET SINGH ATHWAL,
AND JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

Patent Interference No. 105,744
(Technology Center 1600)

DECLARATION - Bd.R. 203(b)[1]

Part A. Declaration of interference

An interference is declared (35 U.S.C. § 135(a)) between the above-identified parties. Details of the application(s), patent (if any), reissue application (if any), count(s) and claims designated as corresponding or as not corresponding to the count(s) appear in Parts E and F of this DECLARATION.

[1] "Bd.R. x" may be used as shorthand for "37 C.F.R. § 41.x". 69 Fed. Reg. 49960, 49961 (12 Aug. 2004).

Part B. Judge managing the interference

Administrative Patent Judge Sally Gardner Lane has been designated to manage the interference. Bd. R. 104(a).

Part C. Standing order

A Trial Section STANDING ORDER [SO] (Paper 2) accompanies this DECLARATION. The STANDING ORDER applies to this interference.

Part D. Initial conference call

A telephone conference call to discuss the interference is set for **2:00 p.m. on 16 March 2010** (the Board will initiate the call).

No later than **four business days** prior to the conference call, each party shall file and serve (SO ¶¶ 10.1 & 105) a list of the motions (Bd. R. 120; Bd. R. 204; SO ¶¶ 104.2.1, 120 & 204) the party intends to file.

A sample schedule for taking action during the motion phase appears as Form 2 in the STANDING ORDER. Counsel are encouraged to discuss the schedule prior to the conference call and to agree on dates for taking action. A typical motion period lasts approximately eight (8) months. Counsel should be prepared to justify any request for a shorter or longer period.

Part E. Identification and order of the parties

Junior Party

Named inventors: Paul J. Carter
San Francisco, CA

Leonard G. Presta
San Francisco, CA

Involved Patent:	6,407,213, issued 18 June 2002, from application 08/146,206, which was filed 17 November 1993, and was based on international application PCT/US92/05126, filed 15 June 1992.
Title:	METHOD FOR MAKING HUMANIZED ANTIBODIES
Assignee:	Genentech, Inc.

Senior Party

Named Inventors:	John Robert Adair High Wycombe, United Kingdom Diljeet Singh Athwal London, United Kingdom John Spencer Emtage Marlow, United Kingdom
Involved Application:	11/284,261, filed 21 November 2005
Title:	HUMANISED ANTIBODIES
Assignee:	Celltech R & D Limited

The senior party is assigned exhibit numbers 1001-1999. The junior party is assigned exhibit numbers 2001-2999. Bd. R. 154(c)(1); SO ¶ 154.2.1. The senior party is responsible for initiating settlement discussions. SO ¶ 126.1.

Part F. Count and claims of the parties

Count 1

A humanized antibody heavy chain variable domain comprising non-human Complementarity Determining Region (CDR) amino acid residues which bind antigen incorporated into a human antibody variable domain, and further comprising a Framework Region (FR) amino acid substitution at a site selected from the group consisting of: 24H, 71H, 73H, and 78H, utilizing the numbering system set forth in Kabat.

The claims of the parties are:

Carter: 1-82

Adair: 24

The claims of the parties which correspond to Count 1 are:

Carter: 30, 31, 60, 62, 63, 66, 67, 70, 73, 77-81

Adair: 24

The claims of the parties which do not correspond to Count 1, and therefore are not involved in the interference, are:

Carter: 1-29, 32-59, 61, 64, 65, 68, 69, 71, 72, 74-76, 82

Adair: None

The parties are accorded the following benefit for Count 1:

Carter: PCT/US92/05126, filed 15 June 1992; and
07/715,272, filed 14 June 1991, now abandoned.

Adair: 08/846,658, filed 01 May 1997;
08/303,569, filed 07 September 1994, issued as 5,859,205 on 12 January 1999;
07/743,329, filed on 17 September 1991;
PCT/GB90/02017, filed 21 December 1990; and
GB 8928874.0, filed 21 December 1989.

Part G. Heading to be used on papers

The following heading must be used on all papers filed in this interference, see SO & 106.1.1:

PAUL J. **CARTER** AND LEONARD G. PRESTA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR**, DILJEET SINGH ATHWAL,
AND JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

Patent Interference No. 105,744
(Technology Center 1600)

Part H. Order form for requesting file copies

When requesting copies of files, use of SO Form 4 will greatly expedite processing of the request. Please attach a copy of Parts E and F of this DECLARATION with a hand-drawn circle around the patents and applications for which a copy of a file wrapper is requested.

/Sally Gardner Lane/
Administrative Patent Judge

Enc:
Copy of STANDING ORDER
Form PTO-850
Copy U.S. Patent 6,407,213
Copy of claims of 11/284,261

cc (via overnight delivery):

Attorney for Carter:

Sidley Austin, LLP
Attn: DC Patent Docketing
1501 K Street, N.W.
Washington, DC 20005

Attorney for Adair:

Cozen O'Connor, P.C.
1900 Market Street
Philadelphia, PA 19103-3508

Paper ____

UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

ENTERED: 3 January 2006

STANDING ORDER

This Standing Order is entered in, and governs, contested cases assigned to the Trial Division of the Board of Patent Appeals and Interferences. Parties are expected to be familiar with it. The rules reproduced in the Standing Order are current as of the issue date of the Standing Order, but are subject to change. In the event of a change to a rule, the changed rule will control, the Standing Order notwithstanding.

CONTENTS

¶ 1 Applicability of 37 CFR part 1

Part 1 of 37 CFR was drafted principally with patent prosecution in mind, while 37 CFR Part 41 is directed to proceedings before the Board. If Part 41 or the Standing Order expressly requires different procedure than Part 1, then Part 41 or the Standing Order, respectively, will govern. A party that perceives a conflict between Part 1 and either Part 41 or the Standing Order must promptly raise the issue with the Board.

¶ 2 Board organization

¶ 2.1 Trial Division

The Chief Administrative Patent Judge has designated administrative patent judges to constitute the Trial Division, who decide most contested cases, as well as appeals involving patent reexaminations and reissue applications or applications relating to interferences. The Trial Division consists of two sections—

- the Trial Procedures Section and
- the Trial Merits Section.

¶ 2.2 Trial Procedures Section

Within the Trial Division, the Chief Administrative Patent Judge has designated administrative patent judges to constitute the Trial Procedures Section. The principal function of the Trial Procedure Section is to ready interference cases for a merits decision on substantive motions, including interference priority motions. Accordingly,

administrative patent judges will handle all interlocutory matters in contested cases, including but not limited to—

- # declaration of interferences,
- # setting times for taking action, and
- # administering discovery.

¶ 2.3 Trial Merits Section

Within the Trial Division, the Chief Administrative Patent Judge has designated administrative patent judges to constitute the Trial Merits Section. The principal function of the Trial Merits Section is to prepare opinions on the merits of substantive motions, including interference priority motions.

¶ 2.4 Board staff

Board staff other than administrative patent judges may administer some tasks during contested cases. For instance, Trial Division paralegal assistants enter orders authorizing access to files under Bd.R. 109 and authorizing the filing of facsimile transmissions in excess of the five pages permitted under SO ¶ **10.6**. Staff actions are controlling unless countermanded by an administrative patent judge or a Board panel.

¶ 3 Petitions

¶ 3.1 Procedural relief sought through miscellaneous motion

A request for a procedural remedy must be made in the form of a miscellaneous motion. Bd.R. 121(a)(3); SO ¶ **123**. A petition is not appropriate. No fee is required.

¶ 3.2 Petition affecting the merits in a contested case

Any relief affecting the merits of a contested case must be sought in the form of a motion. Bd.R. 121. If the relief would otherwise require the granting of a petition, the petition, including any required fee, must be filed with the motion. Bd.R. 121(c)(2). A party filing a petition in violation of this subparagraph may be subject to sanctions. Bd.R. 128.

¶ 3.3 Petitions under 35 U.S.C. 135(c)

Petitions based on 35 U.S.C. 135(c) are not part of a contested case. Bd.R. 3(b) and Bd.R. 205.

¶ 4 Good cause requirement for untimeliness

¶ 4.1 High standard

The standard for showing good cause under Bd.R. 4(a) is high. Times are set to facilitate the rendering of timely decisions. There are few, if any, circumstances where good cause can be based on the press of other business arising after a time is set by a Board order, particularly where a time period is set or maintained after a conference with counsel.

¶ 4.2 Settlement

An attempt to settle will rarely constitute good cause. Settlement is encouraged, and the administrative patent judge designated to handle a contested case is available to assist in settlement efforts where appropriate, but parties should expect either to settle the contested case or, in the absence of settlement, to meet each deadline.

¶ 5 Pro hac vice

Contested cases can be technically, legally, and procedurally complex. Consequently, a motion to appear pro hac vice will rarely be granted unless the counsel is an experienced litigating attorney and has an established familiarity with the subject matter at issue in the contested case.

The Board may authorize a person other than a registered practitioner to appear as counsel in a specific proceeding.

¶ 6 Publication of opinions

¶ 6.1 Most opinions are publicly available

Most opinions in contested cases will be available for publication during the proceeding. Virtually all become available at the end of the proceeding. The recent experience in contested cases is that redactions are almost never requested.

¶ 6.2 Notice of confidential information

Some opinions are selected for publication to promote public understanding of Trial Division practice or to create uniform practices. If a party believes that its application contains information not otherwise publicly available that should be redacted from any opinion, the party must **within two (2) months** of the initiation of the contested case file as a separate paper a notice specifically identifying such information.

If additional information not otherwise publicly available is introduced into a contested case that a party believes should be redacted from any opinion, the party must promptly file a notice specifically identifying the information.

If, after filing such notice, specifically identified information becomes publicly available (for example, through publication of a collateral application), the party must promptly notify the Board of this change in the status of the information.

¶ 7 Record management

¶ 7.1 Letters between counsel not to be filed

No letter between counsel may be filed unless it is filed as an exhibit cited—

- # in a motion, opposition, or reply, or
- # during cross-examination.

¶ 7.2 No duplicate papers

A party may not file (not even as an appendix or exhibit) a copy of a paper previously filed in the same contested case.

¶ 7.3 Reference to earlier filed paper

A party referring to an earlier filed paper should identify the paper by title and paper number or, if the paper number is not known, by the filing date.

¶ 8 Mandatory notices

¶ 8.1 Real party-in-interest

Within **fourteen (14) days** of the date of the Declaration, each party must file as a separate paper a notice of any and all right, title, or interest in any application or patent involved in the contested case.

¶ 8.2 Related proceedings

Within **fourteen (14) days** of the initiation of a contested case, each party must file and serve as a separate paper a notice identifying the application or patent number of every United States application or patent claiming, or which may claim, the benefit of priority of the filing date of the party's involved patent or application. If there are no such applications or patents the notice must state this fact. If, during the course of the proceeding, a party files an application claiming, or which may claim, the benefit of the filing date of an involved application or patent, a notice of the filing, including the application number, must be promptly served and filed. The Board may order a party to serve on an opponent a copy of any application or patent identified under this paragraph.

¶ 8.3 Notice of judicial review

A party seeking judicial review must notify the Board of the complaint or notice of appeal within 20 days. Bd.R. 8(b). After a contested case ends, administrative tasks remain for the United States Patent and Trademark Office [Office] generally and for the Board particularly. Files need to be distributed, applications need to be allowed or abandoned, and notices of patent claim cancellation need to be published. If the Board

does not receive timely, effective notice of judicial review, it proceeds on the assumption that no review has been sought. The Office may deem an application abandoned or may issue a patent to the opponent. At best, this leaves the litigant with a problem to correct. Failure to provide adequate notice may result in sanctions under Bd.R. 128.

¶ 9 Ownership determined from Office assignment record

In the absence of an assignment of record, the inventor is the presumptive owner of an involved patent or application. It is the responsibility of each party to ensure that its assignments are properly recorded at the Office. Failure to record an assignment can lead to adverse judgment in a contested case.

Cases involving a United States government interest may be referred to the Civil Division of the United States Department of Justice. 28 C.F.R. § 0.45(f).

¶ 10 Communications with the Board

¶ 10.1 Default mode - electronic mail

Electronic mail is the default mode of filing papers in a contested case. See SO ¶ **105**. Papers authorized to be filed electronically must be electronically mailed as an attachment to: **BoxInterferences@USPTO.GOV**. No papers unrelated to the contested case may be filed at this address. Papers relating to the contested case must not be mailed to any other **USPTO.GOV** address.

The subject line may contain only the case number and the assigned administrative patent judge's initials in parentheses, e.g., in Patent Interference 105,000 before Administrative Patent Judge John Doe the subject line would be "105,000 (JD)".

¶ 10.2 Any United States Postal Service, including EXPRESS MAIL

If mailed correspondence is authorized in a contested case, it must be addressed:

Mail Stop INTERFERENCE
Board of Patent Appeals and Interferences
United States Patent and Trademark Office
PO Box 1450
Alexandria Virginia 22313-1450

Caveat: Any delay resulting from an improper address (including any address provided in SO ¶¶ **10.3** & **10.4**) will be attributed to the party.

¶ 10.3 Hand filing

If hand-filed correspondence is authorized in a contested case, the filer must bear in mind that the delivery point lies inside the security zone on the first floor of:

Madison East Building
600 Dulany Street
Alexandria, Virginia

The courier must pass through a magnetometer, and the correspondence must pass through an X-ray sensor. For authorized hand-filed correspondence other than boxes, the correspondence may be deposited in a drop-off box inside the security zone at the first floor lobby of the Madison East Building.

¶ 10.3.1 Boxes

Boxed correspondence cannot be deposited in the drop-off box. Instead, it must be delivered to Madison East, Room 9B55-A. Access to Room 9B55-A is available on business days from 8:30 a.m to 4:45 p.m. (Eastern Time, SO ¶ **100.2**) only.

The courier should approach the security guard station on the first floor of Madison East (near the elevators) and ask the security guard to call the Board at 571-272-9797 to obtain authorization to enter the building for a delivery to Room 9B55-A.

¶ 10.3.2 Correspondence filed after 4:45 p.m.

Authorized hand-filed correspondence filed after 4:45 p.m. (Eastern Time, SO ¶ **100.2**) will receive the next business day's filing date. If the Board receives the paper by 10 a.m. that next business day, it will be deemed to have been timely filed provided the paper was properly served the previous business day.

¶ 10.3.3 Stamped receipt

If a stamped return receipt is required, the courier must personally deliver the correspondence and postcard receipt to Room 9B55-A between 8:30 a.m to 4:45 p.m. (Eastern Time, SO ¶ **100.2**) only, or leave the postcard with the correspondence in the drop-off box. If the postcard is left in the drop-off box, it must include correct postage and the address where the postcard is to be mailed.

¶ 10.4 Overnight delivery services

Papers authorized to be filed using a commercial overnight delivery service (see SO ¶ **10.2** for United States Postal Service) must be addressed:

Board of Patent Appeals and Interferences
Madison Building East, 9th Floor
600 Dulany Street
Alexandria, Virginia 22313

Properly addressed papers filed are deemed filed on the date they are delivered to the overnight delivery service.

¶ 10.5 Telephone calls

Telephone calls to the Board regarding a contested case must be directed to 571-272-9797. A telephone call requesting a conference call must be directed to Trial Division support staff. When arranging a conference call, be prepared to discuss with a Trial Division paralegal why the call is needed and what materials should be before the Board participant during the call.

¶ 10.6 Facsimile

The facsimile number for contested cases is 571-273-0042. Do not send papers exceeding five (5) pages in length without prior permission from Trial Division support staff. Do not file a confirmation copy. Such copies tend to cause duplicate entries (in violation of Bd.R. 7(b)) among other problems.

¶ 11 Ex parte communications

The prohibition on ex parte communications is strictly enforced.

¶ 11.1 Papers

A properly served paper should not result in an ex parte communication.

¶ 11.2 Other forms of communications

Improper communications typically arise in telephone calls, electronic mail messages, or public meetings. Telephone calls should be conference calls with each opposing party included. Electronic mail messages, when authorized, should include the opposing party in the "To" line or the "Cc" line. Discussion of specific pending contested cases should not occur at public meetings.

¶ 11.3 Permissible contacts

The following types of contacts are not ex parte communications—

- # contact of a ministerial nature directed to support staff,
- # contact of a general nature directed to Board management, or
- # contact under a rule requiring reference to a different contested case, provided the contact does not involve the merits of the other contested case.

A call to a Trial Division paralegal to arrange a telephone conference is a typical example of a ministerial contact with support staff. SO ¶ **10.5**. A general comment, made apart from a particular contested case, on a Board rule with examples drawn from actual experiences is an example of the second kind of permissible contact. Identification of a related case pursuant to Bd.R. 8(b) and SO ¶ **8.2** is an example of the third kind of contact.

¶ 11.4 Refusal to participate

The Board may permit a hearing or conference call to take place even if a party refuses to participate. In such instances, the Board may require additional safeguards,

such as the recording of the communication and the entry of the recording into the record.

¶ 12 Citation of authority

¶ 12.1 Copies of authority cited

A party must file and serve a copy of any authority on which it relies. A party must not assume that the opponent or the Board has access to the authority.

An authority reported in (1) United States Reports or West Publishing Company's Supreme Court Reporter, (2) the second or third series of West's Federal Reports, or (3) the Bureau of National Affairs' United States Patents Quarterly is exempt from the requirement to file and serve a copy.

¶ 12.2 Parallel citation

Examples of proper parallel citation as required under Bd.R. 12(a)(2)—

Aelony v. Arni, 547 F.2d 566, 192 USPQ 486 (CCPA 1977).

In re Deckler, 977 F.2d 1449, 24 USPQ2d 1448 (Fed. Cir. 1992).

¶ 12.3 Binding authority

The following types of decisions are binding precedent—

Decisions of the United States Supreme Court.

Decisions of the Federal Circuit, the former Court of Customs and Patent Appeals and the former Court of Claims announced before the close of business on 30 September 1982.

Decisions of the Director of the United States Patent and Trademark Office (formerly the Commissioner of Patents and Trademarks).

- # Decisions of the Board of Patent Appeals and Interferences that have been determined to be binding precedent in accordance with established Board procedures.

- # Trial Division decisions that have been designated as precedential.

Decisions of other federal courts and non-precedential decisions of the Board may be cited, but are not binding precedent.

¶ 12.4 Primary authority

Only primary authority should be cited. Secondary authorities summarizing primary authority are never binding and are inherently less persuasive. Primary authority includes: (1) the United States Code, (2) the Code of Federal Regulations, (3) notices published in the Federal Register or the Official Gazette, and (4) binding precedent.

The Manual of Patent Examining Procedure, a guide for patent examiners prepared by the Office of the Commissioner for Patents, is not itself primary authority except as a statement of patent examining practices.

¶ 20 Fees

No fees are charged for papers filed in the ordinary course of a contested case. Occasionally, a paper filed in a contested case must also comply with another rule. Bd.R. 121(c)(2). Compliance with the other rule may require payment of a fee. If so, then payment must be made at the time the paper is filed.

¶ 100 Days and times

¶ 100.1 Business day

The Board is officially open for business on business days. Occasionally the Board may act or may expressly require a party to act on a day that is not a business day.

¶ 100.2 Time standard

All times stated in the Standing Order and other Board orders (except as expressly provided) are for Eastern Time, the local time for the Board of Patent Appeals and Interferences–either Eastern Standard Time or Eastern Daylight Savings Time, as appropriate.

¶ 101 Notice of proceeding

¶ 101.1 Declaration of interference

The notice initiating an interference is called a Declaration. See 35 U.S.C. 135(a) ("an interference may be declared").

¶ 101.2 Maintaining proper address

While the Board is authorized to provide notice by means other than mailing to the correspondence address of record, it is ultimately the responsibility of the application or patent owner to maintain a proper correspondence address. *Ray v. Lehman*, 55 F.3d 606, 610, 34 USPQ2d 1786, 1788 (Fed. Cir. 1995).

¶ 102 Completion of examination

In most cases the public interest is best served by completion of examination before any contested case is initiated. Exceptions may occur on a case-by-case basis, but the Board may then require parties to address such issues. For example, an applicant might be required to cancel and refile claims that do not correspond to an interference count because such claims are not entitled to a patent term adjustment.

¶ 103 Jurisdiction over related files

¶ 103.1 Access to related files

The Board temporarily may restrict public access to related files, such as paper files for accorded benefit applications, to ensure that the parties can obtain timely copies of such files. See SO ¶ **109**.

¶ 103.2 Action in related files

The Board does not ordinarily take action in a file not involved in the proceeding, but occasionally a decision on a motion will require action in a related file.

¶ 104 Modification of the Standing Order

¶ 104.1 Modification of Standing Order

An administrative patent judge may modify the terms of the Standing Order.

¶ 104.2 Times for motions phases

The Board generally initiates a conference call to set times for the motion periods. For interferences, the date and time for the conference call typically appear in Part D of the Declaration.

¶ 104.2.1 Motions list

A list of proposed motions (see Bd.R. 204(c) and SO ¶ **204**) must typically be filed no later than **four business days** prior to the conference call to set dates. This requirement improves the administration of justice by—

- helping the Board and counsel arrive at an appropriate schedule for taking action,
- permitting the Board to determine whether the listed motions are both necessary and sufficient to resolve the issues raised, and
- revealing the possibility that there may be a dispositive motion, see, e.g., Bd.R. 201 (threshold issue).

With prior Board approval a party may be permitted to file an unlisted motion, but the set times are not likely to be changed to accommodate the unlisted motion.

Sample orders setting times for the preliminary motion (Form **2**) and priority motion (Form **3**) phases of an interference are reproduced in the **Appendix of Forms**. The parties should discuss the appropriate order prior to the conference call and try to agree on dates for taking action. Note that in each order, the parties may usually stipulate changes to the first six times, but not to the last three times.

¶ 104.2.2 Additional discovery

If additional discovery will be needed to support a proposed motion, the movant should include a miscellaneous motion for such discovery in the list of proposed motions. Bd.R. 121(a)(3); SO ¶ **123**.

¶ 105 Electronic filing

¶ 105.1 Filing in paper

Filing in paper is not permitted unless expressly authorized. A party that is unable to comply with the electronic filing requirement must promptly arrange a telephone conference using the procedure for an opposed miscellaneous motion, Bd.R. 123(b)(1)(ii); SO ¶ **123**.

¶ 105.2 Electronic filing procedures

¶ 105.2.1 Time to file

A paper must be filed no later than 5:00 p.m. (Eastern Time, SO ¶ **100.2**) on the day the paper is due.

¶ 105.2.2 Separate files

Each paper (not each page) must be a separate ADOBE® portable document format [pdf] file.

¶ 105.2.3 Papers as an attachment no greater than 1 megabyte

A paper that is not larger than 1 megabyte in size must be electronically mailed as an attachment to: **BoxInterferences@USPTO.GOV**.

No information other than the title of the attachment may appear in the message body, for example, "Jones Motion 1".

The file name of the attachment must concisely identify the document, for example, "Jones Motion 1.pdf".

¶ 105.2.4 Sender

A paper may only be filed from an address at the domain name of the party's counsel of record (or, if no counsel is appointed, the electronic mail address of record for the party).

¶ 105.2.5 Paper larger than 1 megabyte; collections of exhibits

A paper larger than 1 megabyte, or a collection of exhibits, may not be electronically mailed as provided above. Instead, it must be delivered as provided in SO ¶¶ **10.2**-**10.4** on a compact disc or a 3¼ inch diskette that is compatible with MICROSOFT® WINDOWS XP®. If a hearing has been requested, four copies of the disc or diskette must be filed.

¶ 105.3 Service

A paper must be served via electronic mail.

¶ 105.3.1 Simultaneous filing and service

If, when the paper is electronically filed with the Board, the opposing party is included as an addressee, then no additional certificate of service is required. The electronic mail message will function as the certificate of service.

¶ 105.3.2 Delayed service

If the paper is not served via the electronic mail message in which the paper is filed, then the paper must be served in a separate electronic mail message no later than 6:00 p.m. (Eastern Time SO ¶ **100.2**) the day the paper is due. The paper as filed must include a certificate of service stating the time of service in addition to the date and manner of service. Bd.R. 106(f)(3).

¶ 105.4 Format

¶ 105.4.1 Portable document format required

All papers, excluding exhibits, must be filed in ADOBE® portable document format [pdf]. Papers must be filed in **text-searchable** pdf whenever reasonably possible. Each party is responsible for the accuracy of its pdf files.

¶ 105.4.2 Paper size

Each paper created for the contested case should be formatted for printing on 8½ inch × 11 inch paper.

¶ 105.4.3 Waiver of requirements

The following paper formatting requirements in the Board rules are waived for electronically filed papers—

- Bd.R. 106(b)(2) - two-hole punch; and
- Bd.R. 106(c) - working copy.

¶ 105.5 Signature

¶ 105.5.1 Papers other than exhibits

A paper other than an exhibit must be signed using an S-signature (see 37 C.F.R. § 1.4(d)(2)). Board papers will only be signed using an S-signature.

¶ 105.6 Exhibits

An exhibit with a handwritten signature in the exhibit must be scanned to preserve the appearance of the signature even if other portions of the exhibit are converted to a text-searchable format. An affidavit must have an original signature.

¶ 106 Filing and service generally; in paper

A paper or exhibit that is not timely filed and properly served will not ordinarily be considered. One consequence may be that the relief sought is not granted for failure to move or for failure to prove. Cf. SO ¶ **121.3**.

¶ 106.1 General format of papers

¶ 106.1.1 Caption

The heading shown in Part G of the Declaration must be used in all papers other than non-affidavit exhibits. Affidavits other than deposition transcripts must use the heading, but other exhibits do not need to use the heading. Form **1** in the **Appendix of Forms** shows a sample caption for an interference.

¶ 106.1.2 Style

The style of each paper must appear on a single line and must not use the words "et al". Styles for papers other than motions, oppositions, and replies must be simple and descriptive. Examples for papers other than motions, oppositions and replies include—

- # JONES DESIGNATION OF LEAD ATTORNEY
- # SMITH DESIGNATION OF REAL PARTY IN INTEREST
- # JONES REQUEST FOR FILE COPIES
- # SMITH PRIORITY STATEMENT
- # JONES SERVICE OF REFERENCES
- # SMITH CLEAN COPY OF CLAIMS (with drawing numerals)
- # SMITH CLEAN COPY OF CLAIMS (means-plus-function annotation)

¶ 106.1.3 Paper size

The Board's facilities for maintaining paper records are designed to work best with 8½" x 11" paper. Occasionally an exhibit must be reproduced on a larger paper size to preserve detail. In such instances, a larger paper format is permitted, but the paper should be folded to permit entry as a 8½" x 11" paper.

¶ 106.1.4 Line Numbering

For papers other than exhibits, every page (not including the cover sheet, any table of contents, any table of authorities, required appendices, and the certificate of service) must have line numbering to facilitate precise citation between papers. See, e.g., SO ¶ **122.3**.

¶ 106.1.5 Footnotes

The use of footnotes is discouraged. Footnotes must be double-spaced.

¶ 106.2 Combined papers and incorporation not permitted

An opposition must respond to only a single motion and a reply must respond to only a single opposition. Incorporation by reference and combined papers are prohibited to reduce the chance risk of overlooking an argument and to improve the efficiency of decision making. Incorporation of arguments by reference amounts to a self-help increase in the length of the brief and a pointless imposition on the Board's time. Each motion, opposition, and reply must make all arguments accessible to readers, rather than ask them to play archeologist with the record. *DeSilva v. DiLeonardi*, 181 F.3d 865, 866-67 (7th Cir 1999).

The ban on incorporation by reference does not mean that relief in a paper cannot be made expressly contingent on some result arising out of another paper. Indeed, noting such contingencies is strongly encouraged.

¶ 106.3 Transmittal sheets

Do not file a transmittal sheet listing papers being filed *except* an exhibit list may be filed when more than one exhibit is being filed.

¶ 106.4 Service

¶ 106.4.1 Certificate of service

Each paper (other than an exhibit) must have a separate certificate of service, incorporated as the last page of the paper, to permit the Board to verify that each paper has been served.

¶ 106.4.2 Alternatives to EXPRESS MAIL

For service in paper or of electronic media, any mode of service that accomplishes same-day or overnight delivery of the paper (e.g., by hand, facsimile, or a commercial overnight delivery service) may be substituted for EXPRESS MAIL service.

¶ 106.4.3 Papers served but not filed

The following papers must be served on an opponent, but should not be filed with the Board at the time of service—

- An objection to the admissibility of evidence,
- A notice requesting cross-examination, and
- Automatic discovery pursuant to Bd.R. 150(b)(1).

Such papers may be filed later as exhibits if a dispute arises with respect to the paper served. For instance, an objection to the admissibility of evidence may be filed as an exhibit for a motion to exclude. Bd.R. 155(c).

¶ 106.5 Filing in paper

Electronic filing is the default filing mode. SO ¶¶ **10.1** & **105**. The following provisions apply only in the unusual situation that paper filing is authorized—

- The first page of each paper must be pink. Bd.R. 106(b)(1)(ii).
- The paper must have the two holes required in Bd.R. 106(b)(2).
- A working copy must also be filed and must be marked "APJ COPY" at the top first page. Bd.R. 106(c).

¶ 108 Lead and backup counsel

The notice identifying counsel under Bd.R. 108(b) must identify both a lead counsel and a backup lead counsel, and must provide for each the contact information specified in Bd.R. 108(b)(1)-(b)(5).

If lead counsel or backup counsel are not counsel of record (37 CFR §§ 1.32 and 1.34) in the involved application or patent, then a power of attorney must be filed with the Board for entry in the involved patent or application file within the **fourteen (14) day** period of Bd.R. 108(b).

¶ 109 Request for file copies

¶ 109.1 Filing the request

¶ 109.1.1 Time for filing request

A party seeking copies of an involved or benefit file mentioned in the Declaration must, within **fourteen (14) days** of the date of the Declaration, file with the Board (not another part of the Office) a separate paper styled [Name of party] REQUEST FOR FILE COPIES to which is attached a completed FILE COPY REQUEST. See Form **4** in the **Appendix of Forms**. Failure to request copies of files at the beginning of the contested case will rarely constitute a basis for granting an extension of time (Bd.R. 4(a)). Thus, a party should not expect an extension of time based on non-receipt of a requested file if the party did not timely place an order for copies or timely advise the Board of non-receipt of a file.

¶ 109.1.2 Deposit Account

A party may charge the cost of the file request against its deposit account. If so, the party should keep the following points in mind.

¶ 109.1.2.1 Authorization to charge Deposit Account

The individual requesting the file must be authorized to charge a fee to the identified deposit account. "Authorized" means authorized by the records of the Office. "Authorized" to charge a deposit account is *not* the same thing as being an attorney of record in the case—

- # the authorized individual does not have to be a registered practitioner, and
- # a registered practitioner is not automatically authorized to charge a deposit account.

If the individual is not authorized, the Office of Public Records [OPR] will not fill an order even if the Deposit Account has sufficient funds.

¶ 109.1.2.2 Adequate Deposit Account balance

If the balance in a Deposit Account is not sufficient to cover the costs of the requested copies, the order will not be filled. 37 C.F.R. § 1.25.

¶ 109.2 Filling record requests

There are at least three kinds of records—

- # Image File Wrappers [IFW], which are downloaded to a disk and sent to the individual who requested a file copy.
- # Paper files, which are copied and sent in paper form to the individual who requested a file copy.
- # "Artifacts", which includes such things as colored documents, plant patent drawings, or video tapes.

Paper files are copied in one part of OPR and IFW files are downloaded to disks in another part of OPR. An order is considered "filled" when—

An IFW is downloaded, or

A paper file is copied.

Consequently, for the same "file", an IFW portion, an artifact portion, and a paper file portion of the same order may be filled at different times. A party receiving a record from the Office should not be surprised if, for instance, the IFW portion arrives before the paper file portion or the artifact portion.

Patent Cooperation Treaty [PCT] applications

If a benefit or potential benefit application is a PCT application that was filed in the Office, there may be a paper file, but no IFW at this time. If there is a paper file and it is transmitted to OPR, they will copy the PCT paper file and fill an order. The Office does not have copies (IFW or paper) of PCT applications filed elsewhere in the world.

Foreign benefit applications

Foreign benefit applications generally appear as a certified copy in an involved or benefit file. The Office generally does not maintain a separate file for the foreign benefit application. Consequently, before notifying the Board that it has not received a foreign benefit application, a party should first confirm that the application has not been delivered as part of an involved or benefit file.

Copies of a United States patent mentioned in an IFW file

The IFW files generally do not include copies of U.S. Patents cited by the examiner or the applicant. A party receiving a copy of an IFW file should not be surprised that

copies of any U.S. patent cited in the IFW record are not included. Copies of any U.S. patent may be obtained through the PTO's website:

http://www.uspto.gov/patft/index.html

¶ 109.3 Notification on non-receipt

Within **twenty-one (21) days** of the date of the Declaration, the Board forwards all timely requests and necessary files to the Office of Public Records [OPR]. The Trial Division enters an order notifying the parties that their respective orders have been transmitted to OPR. OPR makes the copies, which are shipped via overnight commercial courier within fourteen days of receipt of the order. The shipment may be separated into more than one package. See SO ¶ **109.2**. The transmittal order instructs parties to advise the Board promptly if complete copies are not received consistent with this schedule.

¶ 109.4 Record of contested case

The record for the contested case does not include any involved application or patent or any accorded benefit files, all of which are maintained as separate files. Ordering the record for the contested case will not result in receipt of copies of the involved files. There should rarely be any need for a party to order the record for the contested case during the pendency of the contested case before the Board.

¶ 109.5 Access to paper files at Board

During the pendency of a contested case, involved files (other than files that are maintained as Image File Wrapper records) may be inspected only at the Board. Moreover, paper files are unavailable while OPR is making copies.

¶ 110 Copies of claims

A movant seeking to add a claim must comply with the requirements of Bd.R. 110(c) for the proposed claim.

A movant seeking to have a claim designated as corresponding to a count must provide a clean copy, and where applicable an annotated copy, of the claim as an appendix to the motion unless such copies have already been filed for the claim.

Clean copies of claims and of biotechnology sequences are required because claims and sequences are often spread throughout an application file. In patents, there are often certificates of correction. The filing also provides an early opportunity to identify any divergence between what the party believes its claims to be and what the record actually shows. Annotated copies of the involved claims allow all parties and the Board to understand the precise meaning of limitations in the claims. An example follows:

> An apparatus comprising
> a first valve { **Fig. 2, item 25** },
> means for printing { **page 5, line 8 through page 6, line 1; Fig. 3, items 45 and 46** }, and
> a second valve { **Fig. 3, item 98** } * * *.

¶ 120 Types of notices of basis for requesting relief

The principal types of notices under Bd.R. 120 in interferences are priority statements and motions lists. More detail is provided in Bd.R. 204 and SO ¶ **204**. The Trial Procedures Section will be more flexible in accepting changes in the motions list

than in the priority statement; however, the practice under Bd.R. 120 is intended to be more rigorous than it was prior to the adoption of the current rules on 13 September 2004. An accurate motions list is necessary to provide the Board and the opposing parties adequate notice to plan for the proceeding. Facially inadequate motions lists can no longer be cured by filing whatever motion a party may please whether it was listed or not.

¶ 121 Motions

Relief on the merits of a case must be sought through a substantive or responsive motion. Miscellaneous motions are for seeking procedural relief. All motions require prior Board authorization except unopposed miscellaneous motions, SO ¶ **123**.

¶ 121.1 Title and numbering of motions

Each motion of each party must be numbered consecutively, starting with one, regardless of the type of motion. The title of each motion should state the name of the party, the type of motion, and the motion number. The title of a motion may include a second parenthetical line specifying the nature of the motion. For example—

JONES MISCELLANEOUS MOTION 1
(for additional discovery)

JONES SUBSTANTIVE MOTION 2
(for judgment based on prior art)

JONES RESPONSIVE MOTION 3
(to correct inventorship)

JONES SUBSTANTIVE MOTION 4
(for judgment based on lack of enablement)

¶ 121.2 Page limits in motions

The following page limits (not including a table of contents, a table of authorities, required appendices, and the certificate of service) apply—

- # priority . . . . . 30 pages
- # miscellaneous . . . . . 10 pages
- # other motions . . . . . 20 pages

¶ 121.3 Burden of proof

In addition to complying with any procedural requirements of the rules and the Standing Order, a movant bears a burden to establish its right to any substantive relief requested in the motion. A motion that fails to comply with applicable procedural requirements may be dismissed without reaching the merits, in which case the issue sought to be raised by the motion is deemed not to have been properly presented for decision by the Board. A motion that, while complying with applicable procedural requirements, nevertheless fails to make out a substantive case may be denied on the merits. A motion may be dismissed or denied without considering any opposition, or may be granted without considering a reply. In an interference, the movant should note the presumptions stated in Bd.R. 207.

While the ultimate burden remains with the movant, the burden of production may shift to the opponent after the movant has made out a facially sufficient case. Similarly, the opponent may bare the initial burden with respect to an issue first propounded in the opposition. For example, a movant seeking judgment of anticipation over a reference bears the ultimate burden of establishing anticipation, but the opponent

arguing that the reference is not enabling will have the initial burden of proof on the issue of enablement.

¶ 121.4 Format

Each motion must set out in the following order the precise relief requested and an argument setting out the reasons why relief should be granted. Two examples of a precise statement of relief—

Jones moves to be accorded the benefit for count 1 of the filing date of application X, filed 22 January 1993.

Jones moves for judgment against Smith on the ground that all of Smith's involved claims are unpatentable under 35 U.S.C. 103 over the combined disclosures of U.S. Patent No. Y and French Patent Z.

¶ 121.5 Appendices

¶ 121.5.1 Appendix 1: List of exhibits

Each motion must include a list of the exhibits cited in the motion. The list must appear as "Appendix 1" to the motion. The list must be ordered by exhibit number. Each exhibit must be listed on a separate line. The listing of each exhibit number should state the exhibit number followed by a concise description of the exhibit. For example:

1048 - Second declaration of Prof. John Doe.

In deciding a motion, the Board ordinarily will not consider an exhibit not listed as provided in this paragraph.

¶ 121.5.2 Appendix 2: Statement of material facts

The statement of material facts for each motion, opposition, or reply must be set forth as "Appendix 2" to the motion, opposition, or reply, respectively. Bd.R. 121(d)(2).

Each fact must be set out as a single, short, numbered declaratory sentence that is capable of being admitted or denied. Citation to the evidence must be specific, for example, by—

- column and line of a patent,
- page, column, and paragraph of a journal article,
- page and line of a deposition transcript,
- page and paragraph of an affidavit (other than a deposition transcript), and
- title and chapter number of a DVD.

A motion may be denied if the facts alleged in Appendix 2 are insufficient to state a claim for which relief may be granted. Facts set out only in the argument portion of a motion may be overlooked and may result in a motion being denied. Citations to an entire document or numerous pages of a cross-examination deposition transcript do not comply with the requirement for a specific citation to the record. The Board will not take on the role of advocate for a party, trying to make out a case the party has not adequately stated.

¶ 121.6 Claim chart alternative

As an alternative to a claim chart, a party may reproduce the complete claim in an appendix. Following each limitation in the claim, and within braces { }, insert in bold a specific citation to the information to be compared to the limitation (such as where a prior art reference describes the limitation). Braces { } must be used instead of brackets [ ] because brackets are used to indicate amended portions of claims in reissue applications.

¶ 122 Oppositions and replies

¶ 122.1 Numbering oppositions and replies

Each opposition must use the same number as the motion it opposes (skipping the number of any motion not opposed), but does not need a descriptive second line. For example (with reference to the examples in SO ¶ **121.1**):

SMITH OPPOSITION 1.

Each reply must use the same number as the opposition to which it replies (again, skipping the number of any opposition for which no reply is filed). For example (with reference to the examples in SO ¶ **121.1**):

JONES REPLY 4.

¶ 122.2 Page limits

As with motions, the page limits for oppositions and replies do not include a table of contents, a table of authorities, required appendices, or a certificate of service.

¶ 122.2.1 Opposition

The page limits for oppositions are the same as those for corresponding motions—

- # priority . . . . . 30 pages
- # miscellaneous . . . . . 10 pages
- # other motions . . . . . 20 pages

¶ 122.2.2 Reply

For replies, the pages limits are—

- # priority . . . . . 12 pages
- # miscellaneous . . . . . 5 pages
- # other motions . . . . . 10 pages

¶ 122.3 Format

¶ 122.3.1 Opposition

An argument stating the reason why relief is opposed must be made in the following manner:

> On page x, lines y-z of the motion, it is argued (or stated factually) that _. The response is _.

¶ 122.3.2 Reply

The argument responsive to statements in the opposition must be made in the following manner:

> On page x, lines y- z of the opposition, it is argued (or stated factually) that _. The response is _.

¶ 122.4 Appendices

¶ 122.4.1 Appendix 1: List of exhibits

Each opposition and reply must include as "Appendix 1" an exhibit list formatted like the list required for motions, SO ¶ **121.5.1**.

¶ 122.4.2 Appendix 2: Statements of material fact

Each opposition and reply must include as "Appendix 2" a statement of material facts formatted like the statement required for motions, SO ¶ **121.5.2**.

As provided in the following paragraphs, the statement of material fact is a compilation of all the facts stated for the motion. That is—

- # The statement of facts in an opposition will include the statement of facts from the motion, and
- # The statement of facts in a reply will include the statement of facts from the motion and the opposition.

Since papers should ordinarily be submitted in text-readable, electronic format, the burden associated with creating the compilation should be relatively small. The statements appear in appendices and thus do not count toward the page limits. The last statement of material facts submitted should be a complete compilation of all facts (and admissions, denials, etc.) relating to the motion.

¶ 122.4.2.1 Opposition

Appendix 2 for the opposition must include a statement of material facts in which,

- # Each material fact alleged in the motion is repeated with concise statement admitting, denying, or stating that the opponent is unable to admit or deny the fact.
- # Any additional material fact upon which the opposition relies, with a citation to the evidence. Any additional material fact must be

consecutively numbered beginning with the next number after the last numbered material fact.

¶ 122.4.2.2 Reply

Appendix 2 for the reply must include a statement of material facts in which,

All the material facts stated in the motion are repeated with the opponent's concise statement admitting, denying, or otherwise addressing the motion fact.

Each material fact alleged in the opposition is repeated with concise statement admitting, denying, or stating that the movant is unable to admit or deny the fact.

Any additional material fact upon which the movant relies, with a citation to the evidence. Any additional material fact must be consecutively numbered beginning with the next number after the last numbered material fact.

¶ 122.5 New issues in replies

A reply that raises a new issue or belatedly presents evidence will not be considered and may be returned. The Board will not attempt to sort proper from improper portions of the reply. Examples of indications that a new issue has been raised in a reply include—

new evidence that is necessary to make out a *prima facie* case for the relief requested in the motion,

new evidence that could have been included with the motion, and

a reply that is longer than the corresponding motion or opposition.

¶ 123 Miscellaneous motions

¶ 123.1 Mandatory conference

Before filing a miscellaneous motion, a party must confer with all opponents and, if agreement cannot be reached, arrange a conference call to the Board official administering the contested case.

¶ 123.2 Timeliness

The movant must explain why the motion is timely.

¶ 123.3 Recording conference calls

The parties, at their expense, may retain the services of a court reporter to record any conference call. A written record is often desirable inasmuch as an oral decision may be made with respect to issues raised during the conference call.

¶ 124 Oral argument

¶ 124.1 Request for oral argument

The time for requesting an oral argument on substantive and responsive motions is normally specifically set in an order. Note that the time set in the order controls over the five-day period set in Bd.R. 124(a). See Bd.R. 104(c).

A precise statement of the issues for which oral argument is requested is helpful in determining whether to authorize a hearing at all and in determining the times to allot to the parties. A request may be granted or denied in whole or in part.

¶ 124.2 Attendance; special needs

A party that does not expect to attend must promptly notify the Board. Such notice must be served on opposing party.

A party should advise the Board as soon as possible before an oral hearing of any special need. Examples of such needs include additional space for a wheel chair or for a stenographer, an easel for posters, or an overhead projector. Parties should not make assumptions about the equipment the Board may have on hand. Such requests should be directed in the first instance to a Trial Division paralegal at 571-272-9797. Ultimately, any special equipment needed for oral argument is the responsibility of the party needing the equipment.

¶ 124.3 Security; access

The parties should consult the Board web page for information regarding parking and security processing:

http://www.uspto.gov/web/offices/dcom/bpai/docs/contacts/visitation_info.htm

Attendees must report to the security station in the lobby of the Madison Building East and present the order setting the hearing along with photo identification to security personnel.

¶ 124.4 Demonstrative exhibits

Four copies (one copy for the record and one working copy for each judge) of each demonstrative exhibit must be filed before, or presented at, oral argument. Demonstrative exhibits must be served at least five business days in advance. Bd.R. 124(d).

Elaborate demonstrative exhibits are more likely to impede than help an oral argument. The most effective demonstrative exhibits tend to be a handout or binder containing the demonstrative exhibits. The pages of each demonstrative exhibit should be numbered to facilitate identification of the exhibits during the hearing, particularly if the hearing is recorded.

¶ 124.5 Transcription of oral argument

Transcription of oral argument is strongly encouraged. The party requesting transcription must arrange for the transcription and pay the costs. Parties are encouraged to share the costs.

When an argument is to be transcribed, the party should notify a Trial Division paralegal assistant (571-272-9797) as soon as possible, but at least one business day prior to oral argument, so that arrangements may be made in the hearing room for the reporter.

The transcriber must use a stenography machine, but may also use a tape recording device as a backup. Microphones at individuals' locations are not authorized.

¶ 125 Rehearing of decisions

¶ 125.1 Time for filing

A request for rehearing must be filed within fourteen (14) days of the decision, Bd.R. 125(c)(1), *unless* a judgment accompanies the decision in which case the request must be filed within thirty (30) days of the judgment, Bd.R. 127(d).

¶ 125.2 Format for request for rehearing

A request for rehearing is, in form, a miscellaneous motion, but no prior conference call is required. The argument responsive to the decision must be made with particularity in the following manner:

> On page _, lines _-_, the opinion states _. The opinion is believed to have overlooked [or misapprehended] _. This point was set forth in _ Motion [or Opposition or Reply] _ at page _, lines _-_.

The request must include as an appendix an evidence list setting forth a list (in numerical order by exhibit number) of each exhibit that the party believes was overlooked or misapprehended.

¶ 125.3 Number of requests

A party may file no more than one request for rehearing per motion decision.

¶ 125.4 New evidence on rehearing

Evidence not already of record at the time of the decision will not be admitted absent a showing of excusable neglect for the belated submission. Bd.R. 4(a).

¶ 126 Settlement discussions required

¶ 126.1 Last-named party initiates

The party named last in the caption set in the declaration is responsible for—

- # initiating any settlement discussions,
- # initially drafting any document, and
- # initiating any conference call required by this paragraph.

The parties may agree to permit another party to undertake the obligations placed upon the last-named party.

The Office encourages settlement of contested cases and has designed this process to facilitate settlement. The last named party in the contested case is required to initiate the mandatory settlement discussion to avoid the perception that initiation of settlement talks indicates weakness.

¶ 126.2 Initial conference

Within **three (3) months** of the date of the Declaration, the parties must conduct a settlement conference and must initiate a conference call with the Board official assigned to the case. During the call, the parties should be prepared to report—

- # the outcome of the settlement discussion;
- # whether the parties are actively engaged in settlement negotiations and, if so, what steps have already been taken toward settlement;
- # whether any settlement negotiations are directed toward obviating the need for filing motions;
- # any issues that are not subject to settlement negotiations; and
- # the status of any settlement negotiations, including how much time might be needed to conclude those negotiations.

The Board official assigned to the contested case is available to facilitate settlement discussions.

¶ 126.3 Subsequent conferences

Unless a different time is set in an order, within **two (2) months** after a panel decision on substantive motions, the parties must conduct another settlement

conference and initiate another conference call with the Board on the conference as provided in the preceding paragraph, SO ¶ **126.2**.

¶ 126.4 Filing notice of conferences

Prior to initiating any conference call required by this paragraph, the parties must file (in cases without electronic filing, preferably by facsimile, SO ¶ **10.6**) a joint statement indicating that a good faith effort has been made to settle the contested case.

¶ 127 Estoppel; rehearing of judgment

¶ 127.1 Estoppel

A substantively or procedurally inadequate motion does not avoid estoppel under Bd.R. 127(a)(1).

¶ 127.2 Rehearing

The rehearing practice under SO ¶¶ **125.2**-**125.4** applies to rehearing of judgments as well. The judgment implementing a decision does not provide a second opportunity to request a rehearing of the underlying decision, SO ¶ **125.3**.

Concurrent decision and judgment

Often a judgment implementing a decision is entered with or shortly after entry of the decision itself such that the fourteen (14) day period for requesting rehearing of a decision (Bd.R. 125(c)(1)) runs concurrently with the thirty (30) day period for requesting rehearing of the judgment (Bd.R. 127(d)). In this situation, a single request for rehearing should be filed within the thirty (30) day period set in Bd.R. 127(d). See SO ¶ **125.1**.

¶ 128 Sanctions

As with other procedural remedies, a party seeks a sanction by filing a miscellaneous motion. SO ¶ **123**.

Examples of conduct likely to lead to sanctions—

- filing a petition affecting the merits of a pending contested case with a part of the agency other than the Board (SO ¶ **3.2**), and
- failing to file adequate notice of judicial review with the Board (SO ¶ **8.3**),
- exceeding the Board-authorized scope for compelled testimony or production (SO ¶ **156.3**),
- failing to comply with the **Cross Examination Guidelines** (SO ¶ **157.5**),
- failure to notify the Board promptly of a common interest (SO ¶ **206**), and
- filing a facially insufficient motion alleging inequitable conduct against an opponent (SO ¶ **208.2**).

¶ 150 Discovery

¶ 150.1 Automatic discovery

Automatic discovery (1) places the parties on a level playing field and (2) reduces any difficulty authenticating documents when a party would like to rely on a document cited in an opponent's specification. A party should have access to documents cited in its opponent's specification, but it may be difficult for the party to locate those documents. The parties should be prepared to promptly file copies of the materials served under Bd.R. 150(b).

¶ 150.2 Requesting additional discovery

Discovery before the Board is significantly different than discovery under the Federal Rules of Civil Procedure. A request for additional discovery must be in the form of a miscellaneous motion. Bd.R. 121(a)(3); SO ¶ **123**. The standard for granting such requests is high and requires specific bases for expecting that the discovery will be productive. Bd.R. 150(a) & (c)(1). Additional discovery is rarely authorized because in contested cases, the party usually has equal or better access to relevant information compared to any other source. Additional discovery is most commonly authorized in the context of cross examination. Bd.R. 150(c)(2). Other situations in which additional discovery might be required include proving an on-sale or public-use bar and proving inequitable conduct intent, but these situations require a solid basis for believing the discovery will be productive.

¶ 151 Challenging admissibility

As with other procedural remedies, a party may seek to prevent the entry of evidence (motion in limine) or to exclude entered evidence by filing a miscellaneous motion. See Bd.R. 121(a)(3); SO ¶¶ **123** & **155**.

¶ 152 Rules of evidence

¶ 152.1 Official notice not automatic

The Board may exercise its discretion to take official notice of the records of the United States Patent and Trademark Office. Bd.R. 152; Fed. R. Evid. 201(c). No party should proceed on the assumption that the Board will take notice of Office records sua sponte. As with other procedural remedies, a party may seek official notice by filing a miscellaneous motion. Bd.R. 121(a)(3); SO ¶ **123**.

¶ 152.2 Hearsay

¶ 152.2.1 Specification

A specification of an involved application or patent is admissible as evidence only to prove what the specification or patent describes. If there is data in the specification upon which a party intends to rely to prove the truth of the data, an affidavit by an individual having first-hand knowledge of how the data was generated (i.e., the individual who performed an experiment reported as an example in the specification) must be filed. This individual may be cross examined.

¶ 152.2.2 Laboratory notebooks

Lab notebooks generally do not fall within the business records exception or the catchall exception. *Chen v. Bouchard*, 347 F.3d 1299, 1308 & n.2, 68 USPQ2d 1705, 1711 & n.2 (Fed. Cir. 2003).

¶ 153 Certification of Office records

Records of the United States Patent and Trademark Office are deemed to be self-authenticating within the meaning of Fed. R. Evid. 902(4), provided the record is available to each party in the proceeding. Parties generally have an opportunity to obtain copies of the involved and benefit files for the contested case.

¶ 154 Form of evidence

¶ 154.1 Records of the Office

¶ 154.1.1 Must be submitted as an exhibit

Records of the United States Patent and Trademark Office, including affidavits filed during examination, are not automatically part of the record before the Board. The first party seeking to rely on the record must submit a copy of the record as an exhibit. As with other exhibits, the opponent will have an opportunity to object to the exhibit. In the case of an affidavit filed during examination, the opponent may cross examine the affiant.

¶ 154.1.2 Reliance on a portion of a file

If a motion (or opposition or reply) relies on any document in the file of a patent or application (including a specification), the entire document must be made an exhibit in the contested case. Each document, however, should be a separate exhibit. Do not submit an entire application file as a single exhibit. A document that is not discussed in the motion (or opposition or reply) must not be filed.

¶ 154.2 Exhibit labels

¶ 154.2.1 Unique and consecutive

Each exhibit from a party must be uniquely and consecutively numbered within the range the Board assigns to the party for the proceeding.

Unless otherwise provided in an order, the party named last in the caption set in the declaration is assigned the range 1001-1999, while the first-named party is assigned 2001-2999.

¶ 154.2.2 Material covered on first page

If an exhibit label covers important material on the first page of an exhibit, a copy of the first page of the exhibit must be reproduced and presented as page 1-a of the exhibit.

¶ 154.3 Filing of exhibits

¶ 154.3.1 Electronically

See SO ¶ **105.2**.

¶ 154.3.2 In paper

A set of original exhibits must be filed in a box, an accordion folder, or a comparable folder containing the exhibits in numerical order, separated by a divider that conspicuously identifies each exhibit by number.

If any party requests oral argument, three (3) separate additional sets of exhibits must also be filed; otherwise, one (1) additional set of exhibits must be filed.

¶ 154.3.3 Repeated submission of the same exhibit

Multiple copies of the same exhibit with different exhibit numbers from the same party are prohibited. Bd.R. 7(b).

¶ 154.4 Exhibit list

A current list must be served whenever evidence is served.

The exhibit list must be filed with the exhibits.

¶ 154.5 Evidence on DVD digital disc

Use of a DVD digital disc [DVD] may be appropriate to present some evidence, such as video of a deposition or of an experiment. The Board may also require live testimony before the Board.

¶ 154.5.1 Transcript of deposition

A DVD of a deposition may be filed in addition to, but not instead of, a text transcript of the deposition. Ordinarily the proponent of the direct testimony is responsible for filing the transcript regardless of which party is filing the DVD. Bd.R. 157(f).

¶ 154.5.2 Format

¶ 154.5.2.1 MPEG-2 encoded DVD R media

The Board will not consider a DVD that does not operate on Board equipment. The Board has equipment to operate a DVD properly encoded in MPEG-2 format on DVD R media. The DVD must be indexed (e.g., title and chapter numbers) so that reference to and viewing of a particular portion of the video may be made. Any references to a DVD exhibit must specifically refer to a particular portion in the DVD (e.g., title number and chapter number), analogous to citing a specific particular page and line number in a text

transcript. Four copies of the DVD must be filed. One copy of the DVD must be served on all opponents.

¶ 154.5.2.2 Deposition DVD

A deposition DVD must show only the head and upper torso of the witness. Except for breaks, the DVD must contain the entire deposition of the witness. For example, the DVD should show whether the witness took a long time to answer a particular question or had to review documents unless review of documents takes place off the record during a break.

¶ 154.5.3 Costs

A party recording an experiment or other event, including a deposition, is responsible for all costs of preparing the DVD. The proponent of the direct testimony remains responsible for the other reasonable costs of the deposition, including any court reporter and required transcripts. Bd.R. 157(f). If a party records a deposition, but subsequently decides not to file the DVD prepared, another party may cause the DVD to be prepared and must assume responsibility for the costs of having the DVD prepared. The parties may agree to divide the costs of preparing a DVD in any proportion.

¶ 154.5.4 Notice of intent to video-record cross examination

A party that intends to prepare a DVD of cross-examination must file and serve a notice of intent to prepare a DVD on each opponent before the date of the cross examination. The timing of the notice depends on which party intends to video-record the cross examination—

If the proponent of the direct testimony, notice must be served at least five (5) business days before the cross examination; else

If the opponent, notice must be served at least three (3) business days before the cross examination.

¶ 155 Challenging admissibility

¶ 155.1 Objections

¶ 155.1.1 Objecting to served evidence

An objection to the admissibility of evidence must not be filed except as part of a motion to exclude.

¶ 155.1.2 Waiver of untimely objections

If an objection could have been made before the filing of supplemental evidence and an objection was not made, the objection is waived.

¶ 155.1.3 Responding to an objection

No immediate response to an objection is required other than the filing of supplemental evidence in response to the objection if the proponent of the evidence chooses to do so. Any attack on the correctness of an objection must come in response to a motion to exclude based on the objection. Any other response to an objection is neither necessary nor desirable.

¶ 155.1.4 Serving supplemental evidence

Supplemental evidence responding to an objection to the admissibility of evidence must not be filed until it is used as an exhibit.

¶ 155.2 Motion to exclude evidence; motion in limine

¶ 155.2.1 Form of motion

As with other motions seeking a procedural remedy, motions to exclude and in limine are miscellaneous motions. Bd.R. 121(a)(3); SO ¶ **123**.

¶ 155.2.2 Content

A motion to exclude evidence must—

- # identify where in the record the objection was originally made,
- # identify where in the record the evidence to be excluded was relied upon by an opponent, and
- # address objections to exhibits (in whole or in part) in exhibit numerical order.

When a timely objection has been made (see SO ¶ **155.1**), no conference call is necessary to file a motion to exclude.

¶ 155.3 Time to file objections and motions to exclude

Times for filing objections and for filing and serving motions to exclude are set in the order setting times for motions. Generally, the order will set a later date for filing of objections and motions to exclude than the default time for a miscellaneous motion set in the rule because supplemental evidence may cure any defect in the evidence.

¶ 156 Compelled testimony and production

¶ 156.1 Form of motion

A miscellaneous motion under Bd.R. 156(a) must comply with the requirements for a miscellaneous motion, Bd.R. 121(a)(3), SO ¶ **123**, and must be filed sufficiently in

advance that any authorized testimony can be served with the motion, opposition or reply it is intended to support.

¶ 156.2 Alternative procedures for compelled testimony

If a motion to compel testimony is granted, testimony may be—

ex parte, subject to subsequent cross examination, or

inter partes.

In moving for, or opposing, such testimony, the parties should discuss which procedure is appropriate.

¶ 156.3 Admissibility of compelled testimony and production

Compelled testimony and production will only be admitted to the extent the Board has authorized. Significant deviation from the Board-authorized scope may result in sanctions.

¶ 157 Direct testimony; cross examination

¶ 157.1 Direct testimony as affidavit

Direct testimony, other than compelled testimony, must be in the form of an affidavit. Bd.R.157(a). "Affidavit" is defined to include a declaration or ex parte deposition. Bd.R. 2.

¶ 157.2 Required paragraph for affidavits

Affiants have been known to announce belatedly that appearing for cross examination at a reasonable time and place in the United States might not be possible. Consequently, the following paragraph must be included on the signature page of all

affidavits to prevent surprise and hardship to the party relying on the testimony of the witness:

> In signing this [affidavit], I understand that the [affidavit] will be filed as evidence in a contested case before the Board of Patent Appeals and Interferences of the United States Patent and Trademark Office. I acknowledge that I may be subject to cross examination in the case and that cross examination will take place within the United States. If cross examination is required of me, I will appear for cross examination within the United States during the time allotted for cross examination.

¶ 157.3 Cross examination

The party relying on an affiant must make the affiant available for cross examination; otherwise, the affidavit may be excluded. The parties must confer to reach agreement on reasonable times, dates, and locations for cross examination of witnesses.

¶ 157.3.1 Start date

Unless the parties otherwise agree, cross examination of an affiant may begin no earlier than twenty-one (21) days after service of the affidavit.

¶ 157.3.2 End date

Unless the parties otherwise agree,

- # Cross examination of an affiant relied upon in a motion other than a miscellaneous motion must occur at least ten (10) days before the opposition to the motion is due.

- # Cross examination of an affiant relied upon in an opposition to a motion other than a miscellaneous motion must take place at least ten (10) days before a reply is due.

¶ 157.3.3 Notice of cross examination

Since cross examination builds on testimony that has already been served, the notice requirement is simplified.

The party seeking the cross examination (rather than the proponent of the testimony) prepares the notice requesting cross examination. The notice must be served at least two business days before cross examination, but must not be filed (except as an exhibit to a miscellaneous motion, SO ¶ **123**, in the event of a dispute). The notice must identify the time and place of the deposition, but not the other items required under Bd.R. 157(c)(4).

¶ 157.3.4 Live testimony

Cross-examination might be ordered to take place in the presence of an administrative patent judge. Examples of where such testimony has been ordered include contested cases where inventorship, derivation, or inequitable conduct has been an issue; where testimony has been given through an interpreter; or where close supervision is warranted.

¶ 157.4 Order of cross examination

While a party requesting cross examination may choose the order of the witnesses, Bd.R.157(c)(2), the order must be reasonable.

¶ 157.5 Cross examination guidelines

The **Cross Examination Guidelines** appended to the Standing Order apply to all cross examination in this contested case.

¶ 157.6 Transcript

¶ 157.6.1 Proponent responsible

The proponent of the direct testimony is responsible for securing the services of a court reporter and providing a copy of any transcript to every opponent.

¶ 157.6.2 Filing transcript

An uncertified copy of each deposition transcript must be filed as an exhibit. A certified transcript of testimony need not be filed unless required by the Board.

¶ 157.7 Observations on cross examinations

Cross examination may occur after a party has filed its last substantive paper on an issue (e.g., after the reply). Such cross examination may result in testimony that should be called to the Board's attention but does not merit a motion to exclude. The Board may authorize the filing of observations to identify such testimony and responses to observations. In practice, few parties file observations.

The party taking the cross examination files the observations. The opposing party may file a response to an observation. The opposing party may not file observations without express prior authorization.

An observation must be a concise statement of the relevance of precisely identified testimony to a precisely identified argument or portion of an exhibit (including another part of the same testimony). Any response should be equally concise. An observation (or response) is not an opportunity to raise new issues, to re-argue issues, or to pursue objections. Each observation should be in the following form:

> In exhibit _, on page _, lines_, the witness testified _. This testimony is relevant to the _ on page _ of _. The testimony is relevant because _.

The entire observation should not exceed one short paragraph. The Board might refuse entry of excessively long or argumentative observations (or responses).

¶ 158 Expert testimony; tests and data

¶ 158.1 Expert testimony

¶ 158.1.1 Basis for testimony must be provided

Each affidavit expressing an opinion of an expert must disclose the underlying facts or data upon which the opinion is based. Bd.R. 152; Fed. R. Evid. 705. Opinions expressed without disclosing the underlying facts or data may be given little, or no, weight.

¶ 158.1.2 Expert testimony on patent law

Affidavits of patent law experts on issues of law generally will not be admitted in evidence.

¶ 158.2 Tests and data

¶ 158.2.1 Explanation

Explanation of tests or data must come in the form of an affidavit, preferably accompanied by citation to relevant pages of standard texts (which should be filed as exhibits). In addition to providing the explanation required in Bd.R. 158(b), the proponent of the test evidence should provide any other information it believes would assist the Board in understanding the significance of the test or the data.

¶ 200 Interference procedure

¶ 200.1 Patent claim scope

Every claim before the Board, including a patent claim, is given the broadest reasonable scope consistent with the disclosure with which the claim appears.

¶ 200.2 Pendency

Requests for a stay or an extension are viewed with strong disfavor. Delay in one part of an interference may result in shortening of another part of an interference. An applicant may be required to disclaim patent term for a period related to the delay.

¶ 203 Suggesting another interference; adding an application or a patent

¶ 203.1 Suggesting another interference

Occasionally the declaration of another interference between the same parties or a subset of the parties may be appropriate, for instance, because it would be more faster, less expensive, or more fair. A suggestion to declare another interference must be in the form of a miscellaneous motion. Bd.R. 121(a)(3); SO ¶ **123**. The motion must comply with the requirements of Bd.R. 202(a). The motion must certify that each opponent has been served with a copy of the application file for the applications or patents that would be involved in the new interference.

¶ 203.2 Adding an application or a patent

A suggestion to add an application or patent to an interference must be in the form of a miscellaneous motion. Bd.R. 121(a)(3); SO ¶ **123**. The motion must—

Identify the application or patent to be added;

Certify that a complete copy of the application file for the application or patent has been served on all opponents;

Indicate which claims of the patent or application should be designated as corresponding to the count (and if there is more than one count, which count); and

Explain whether there are alternative remedies; if so, why alternative remedies are not adequate; and what attempts, if any, have been made to have the examiner recommend declaration of another interference involving the application or patent sought to be added to the interference.

¶ 204 Motions list

The motions list is a tool for planning the course of the proceeding, eliminating unnecessary costs and delay, and avoiding abusive practices. All substantive and anticipated responsive motions must be listed on the motions list. No substantive motions or responsive motions may be filed without prior Board authorization. If the need for an unlisted motion arises, the movant should initiate a conference call to obtain such authorization.

¶ 205 Settlement agreements

¶ 205.1 Notice under 35 U.S.C. 135(c)

Notice is hereby given of the requirement of 35 U.S.C. 135(c) for filing in the Office a copy of any agreement "in connection with or in contemplation of the termination of the interference."

¶ 205.2 Petitions under 35 U.S.C. 135(c)

The Chief Administrative Patent Judge decides petitions for acceptance of untimely filed agreements and petitions for access to agreements. A petition under § 135(c) must be formatted as a miscellaneous motion, Bd.R. 121(a)(3), SO ¶ **123**, and served on all parties, Bd.R. 106(e), but no prior Board authorization is required.

¶ 206 Common interests in the invention

The Board generally relies on assignment records of the United States Patent and Trademark Office (SO ¶ **9**) and notices of real parties-in-interest to determine whether common interests in involved cases may require judgment under Bd.R. 206. Failure to notify the Board promptly of a common interest may lead to sanctions, including adverse judgment. Bd.R. 128.

¶ 207 Applicability of prior art

Prior art asserted against an opponent's involved claims is presumed to render the movant's involved claims unpatentable as well. Even if the movant does not adequately contest the presumption, however, the Board may exercise its discretion not to hold the movant's claims unpatentable if in deciding the motion the Board determines the presumption is not appropriate.

¶ 208 Specific substantive motions

The following subparagraphs provide procedural guidance on the necessary content of substantive and responsive motions. Motions must, however, also be substantively sufficient. Thus, even perfect compliance with this guidance does not assure that a movant will satisfy its burden of proof.

¶ 208.1 Obviousness

When obviousness (35 U.S.C. 103) is the basis for a motion for judgment, if a reference does not teach or suggest a limitation, that fact must be explicitly identified as a difference in the statement of material facts. The argument portion of the motion must account for the difference.

An explanation must be made in the body of the motion (not an appendix) why the subject matter of the claim, as a whole, would have been obvious to a person having ordinary skill in the art notwithstanding any difference.

Proving subject matter is not obvious

Proving that subject matter is not obvious may require a negative proof: that no prior art in combination with the subject matter of the count render the claimed subject matter obvious. A party may be able to satisfy its burden of production with testimony from a knowledgeable witness certifying that there is no known prior art that would have overcome the differences between the subject matter of the count and the subject matter of the claim.

¶ 208.2 Adding or substituting a count

A motion to add or substitute a count must—

Propose a new count.

Show why the new count is patentably distinct from every other count the movant believes should remain.

For each of movant's claims, show why the claim does or does not correspond to the proposed new count. Bd.R. 207(b)(1). If none of movant's claims would correspond, then contingently move to add no more than one claim that would correspond (SO ¶ **208.5.1**).

For each opponent's claim that movant believes should correspond to the count, show why the claim corresponds to the proposed new count. If no claim of an opponent would correspond, then contingently move to add no more than one claim to the opponent's involved application or patent.

Accorded benefit

The motion must show why the movant should be accorded any benefit for the proposed count.

The opponent will be presumed to be entitled to its earliest accorded benefit for a proposed count. The movant may overcome this presumption by showing in the motion why the opponent should not be accorded benefit of an earlier application with respect to the proposed count.

¶ 208.3 Claim correspondence

A change in claim correspondence requires comparison of the claim to a count. See Bd.R. 121(e).

¶ 208.3.1 Designating a claim as corresponding

A motion to have a claim designated as corresponding to a count must show why the subject matter of the count, if treated as prior art, would have anticipated or rendered obvious the subject matter of the claim. Bd.R. 207(b)(2).

If no clean or annotated copy of the claim has been filed, then a clean copy, and where applicable an annotated copy, of the claim must be filed as an appendix to the motion. SO ¶ **110**.

A movant seeking to have its own claim designated as corresponding to a count may stipulate that the claim corresponds to the count. The Board is not bound to accept the stipulation. For instance, a stipulation of correspondence that constitutes the sole basis for maintaining an interference-in-fact might be viewed with some skepticism.

¶ 208.3.2 Designating a claim as not corresponding

A motion to have a claim designated as not corresponding to a count must show why the subject matter of the count, if treated as prior art, would not have anticipated or rendered obvious the subject matter of the claim. Bd.R. 207(b)(2).

A movant may not seek to have all of a party's claims designated as not corresponding to the count. Instead, such relief should be sought by way of a motion for judgment of no interference-in-fact.

¶ 208.4 Benefit motions

¶ 208.4.1 For additional accorded benefit

A motion to be accorded the benefit of an application for a particular count must—

- # Certify that a copy of the application has been served on each opponent. See Bd.R. 154(b) (requiring certified translation of documents not in English).

- # If the application was originally filed in the United States Patent and Trademark Office, certify that a complete copy of the application file has been served on each opponent.

Show that the application includes at least one constructive reduction to practice of the count. Bd.R. 201 ("Constructive reduction to practice").

¶ 208.4.2 Attacking accorded benefit

A motion to attack the benefit of an application accorded for a count must explain why the application does not provide a constructive reduction to practice for the count. Bd.R. 201 ("Constructive reduction to practice").

¶ 208.5 Responsive motions

¶ 208.5.1 Adding a claim

The claim may only be added to an involved application or patent. Note the requirement under Bd.R. 110(c) to provide clean and annotated copies of the claim. Adding a patent claim generally requires the filing of a reissue application. If the claim is to be added to the movant's application or patent, an amendment (or if necessary a reissue application, see SO ¶ **208.5.4**) must be filed with the motion. A motion to add a claim must—

Show the written description for the claim in the disclosure of the involved application or patent to which it would be added.

Certify that the movant is not aware of any reason why the claim is not patentable.

A certification that is inconsistent with the prosecution history of an involved or benefit file will be accorded no weight unless the inconsistency is explained. Similarly, if a claim is added to overcome a patentability problem raised in a motion, the motion to add the claim must explain why the proposed claim would overcome the problem.

A motion to add a claim must also comply with the requirements of 37 C.F.R. § 1.75 to the extent they are consistent with the Standing Order. No more than one claim may

be added in response to a motion unless the Board expressly authorizes the addition of more claims.

A claim will not be added if it does not correspond to a count. Correspondence may be stipulated for the movant's claim, but must be shown for an opponent's claim. A showing of claim correspondence requires comparison of the claim to a count. See Bd.R. 121(e).

Opposing a motion to add a claim to an opponent's involved application or patent

If the movant is seeking to add a claim to an opponent's involved application or patent, the opponent may oppose on the basis that the claim is not patentable to the opponent. If the motion is granted, the opponent must add the claim. Failure to add the claim will be treated as a concession that the subject matter of the claim is not patentable to the opponent. Cf. *In re Ogiue*, 517 F.2d 1382, 1390, 186 USPQ 227, 235 (CCPA 1975) (refusal to add claim to permit interference treated as concession).

¶ 208.5.2 Amending a claim

To ensure clarity in the record, amendment of claims is not permitted. Instead, a party may cancel the existing claim and add a new claim as provided above. SO ¶ 208.5.1.

¶ 208.5.3 Substituting an application of the movant

Occasionally it may be necessary to substitute an application for an involved application or patent. For example, the application may be necessary to support an added claim or to provide a better basis for seeking an earlier accorded benefit. A motion to substitute an application must—

Identify the application.

Certify that a complete copy of the application file has been served on each opponent.

¶ 208.5.4 Adding a reissue application

A motion to add the movant's own reissue application must stipulate that every added claim (compared to the original patent) corresponds to a count in the interference. If the reissue application has not been filed in the Office, it must be filed directly with the Board. The Board will see that a filing receipt is promptly issued. Filing directly with the Board avoids processing delays that might prevent consideration of the motion to add the reissue application. The motion must—

Certify that a complete copy of the reissue application file has been served on each opponent.

Make the showings required for adding a claim (SO ¶ **208.5.1**).

Ordinarily, the filing of a reissue application in itself will not precipitate a change in the accorded benefit. If the movant believes that addition of the reissue application would entitle it to different accorded benefit, it should file a contingent motion to change the accorded benefit (SO ¶ **208.4**).

¶ 208.6 Priority

A party seeking judgment on the basis of priority must file a substantive motion seeking such relief. Bd.R. 121(a)(1)(iii).

When diligence is an issue in priority, the priority motion must include as an appendix a diligence chart. The diligence chart must (1) list all days from the beginning of diligence through the end of diligence, (2) state what happened on each day, and

(3) cite the page and line of the motion on which the listed day is discussed. In a case where the invention is "B", an example would be:

Monday, April 5, 2004	Ordered chemical C	p. 17, lines 4-7
Tuesday, April 6, 2004	Waited for chemical C	p. 17, lines 4-7
Wednesday, April 7, 2004	Chemical C arrived	p. 17, lines 4-7
Thursday, April 8, 2004	Used chemical C to make "A"	p. 17, lines 7-8
Friday, April 9, 2004	Sick--not at work	p. 17, lines 18-24
Saturday, April 10, 2004	Weekend	p. 18, lines 2-8
Sunday, April 11, 2004	Weekend	p. 18, lines 2-8
Monday, April 12, 2004	Scheduled vacation	p. 18, lines 2-8
Tuesday April 13, 2004	Scheduled vacation	p. 18, lines 2-8
Wednesday, April 14, 2004	Used "A" to make "B"	p. 17, lines 9-15

Every date gap in the diligence showing must be explained. The fact that there is a gap does not per se establish lack of reasonable diligence. The fact that there is no gap does not per se establish reasonable diligence.

¶ 208.7 Inequitable conduct

A motion alleging inequitable conduct must make out a facially sufficient case of inequitable conduct or fraud. Additional discovery (Bd.R. 150(c)) or a request to take testimony (Bd.R. 156), asserted to be necessary to make out a facially sufficient case, will rarely be authorized. An allegation of inequitable conduct or fraud that fails to make out a facially sufficient case may result in sanctions or a referral to the Office of Enrollment and Discipline.

/Michael R. Fleming/
Chief Administrative Patent Judge

/Marc L. Caroff/
Administrative Patent Judge

/William F. Pate, III/
Administrative Patent Judge

Board of Patent Appeals and Interferences

Trial Division

/John C. Martin/
Administrative Patent Judge

/Fred E. McKelvey/
Senior Administrative Patent Judge

/Richard E. Schafer/
Administrative Patent Judge

/Teddy S. Gron/
Administrative Patent Judge

/Jameson Lee/
Administrative Patent Judge

/Adriene Lepiane Hanlon/
Administrative Patent Judge

/Richard Torczon/
Administrative Patent Judge

/Hubert C. Lorin/
Administrative Patent Judge

/Carol A. Spiegel/
Administrative Patent Judge

/Romulo H. Delmendo/
Administrative Patent Judge

/Sally Gardner Lane/
Administrative Patent Judge

/Sally C. Medley/
Administrative Patent Judge

/Michael P. Tierney/
Administrative Patent Judge

/James T. Moore/
Administrative Patent Judge

/Linda R. Gaudette/
Administrative Patent Judge

/Mark Nagumo/
Administrative Patent Judge

APPENDIX OF FORMS

Form 1. Standard caption for an interference

Filed on behalf of:
[Name of filing party] Paper No. [leave blank]
By: [Name of lead counsel]
[Name of backup counsel]
[Street address]
[City, State, and ZIP Code]
[Telephone number]
[Facsimile number]
[Electronic mail address]

UNITED STATES PATENT AND TRADEMARK OFFICE

_____

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

_____

[Name of junior party]
([Involved application or patent number])
Junior Party,

v.

[Name of Senior party]
([Involved application or patent number])
Senior Party.

_____

Patent Interference [interference number] ([APJ initials])

_____

[TITLE OF PAPER]

Form 2. Typical schedule for motions other than interference priority

Times shown from date of order.

The parties may typically change these six times by stipulation

TIME PERIOD 1 . . . . . Week 6
File substantive motions
File (but serve one week later) priority statements

TIME PERIOD 2 . . . . . Week 9
File responsive motions to motions
filed in TIME PERIOD 1

TIME PERIOD 3 . . . . . Week 15
File oppositions to all motions

TIME PERIOD 4 . . . . . Week 21
File replies

TIME PERIOD 5 . . . . . Week 27
File request for oral argument
File motions to exclude
File observations

TIME PERIOD 6 . . . . . Week 30
File oppositions to motions to exclude
File response to observations

The parties cannot change these three times by stipulation

TIME PERIOD 7 . . . . . Week 32
File replies to oppositions to motions to exclude

TIME PERIOD 8 . . . . . Week 33
File exhibits
File sets of motions

TIME PERIOD 9 . . . . . Week 35
Present oral argument

Form 3. Typical schedule for priority motions in an interference

Times shown from date of order.

The parties may typically change these six times by stipulation

TIME PERIOD 11 . . . . . Week 6
Junior party only file priority brief and serve
(but do not file) priority evidence

TIME PERIOD 12 . . . . . Week 12
Senior party only file priority brief and serve
(but do not file) priority evidence

TIME PERIOD 13 . . . . . Week 18
File opposition to priority briefs
Serve (but do not file) opposition evidence

TIME PERIOD 14 . . . . . Week 24
File reply
Serve (but do not file) reply evidence

TIME PERIOD 15 . . . . . Week 30
Request hearing
File list of issues to be considered
File observations
File motion to exclude

TIME PERIOD 16 . . . . . Week 33
File response to observations
File opposition to motion to exclude

The parties cannot change these three times by stipulation

TIME PERIOD 17 . . . . . Week 35
File reply to opposition to motion to exclude

TIME PERIOD 18 (Last Time) . . . . . Week 36
File and serve the exhibits
File sets of priority motions

TIME PERIOD 19 . . . . . Week 38
Present oral argument

Form 4. File copy request

FILE COPY REQUEST
Contested Case No. [Contested Case number]

Attach a copy of sections E and F of the DECLARATION to this REQUEST. On the copy, circle each patent and application that you are requesting.

Include the following information to facilitate processing of this REQUEST:

1. Charge fees to USPTO Deposit Account No. _____

2. Complete address, including street, city, state, zip code and telephone number (do not list a Post Office box because file copies are sent by commercial overnight courier).

_____

_____

_____

_____

3. Telephone, including area code: _____

APPENDIX: CROSS EXAMINATION GUIDELINES

Introduction

Cross examination can be a useful tool for determining the facts in a case. In contested cases, direct testimony is usually presented by affidavit, Bd.R. 157(a), while cross examination occurs by oral deposition. Bd.R. 157(b).

Cross examination should be a question-and-answer conversation between the examining lawyer and the witness. The defending lawyer must not act as an intermediary, interpreting questions, deciding which questions the witness should answer and helping the witness formulate answers. The witness comes to the cross examination to be questioned. It is the witness, and not the lawyer, who is testifying.

The cross-examination guidelines below are essentially the deposition guidelines set out in *Hall v. Clifton Precision*, 150 F.R.D. 525 (E.D. Pa. 1993) (Gawthrop, J.) The only significant difference, which results from Bd.R. 157(e)(4), is that certain objections must be noted on the record.

Failure to adhere strictly to these guidelines may be a basis for a sanction under Bd.R. 128, which could include a requirement that the witness, on very short notice, may be directed to appear before the Board or elsewhere, as may be appropriate, coupled with any appropriate award of compensatory damages under Bd.R. 128(b)(6). In addition, cross examination undertaken contrary to these guidelines may result in exclusion of an affidavit from evidence or in the assignment of little, if any weight, to the direct testimony of a witness who was cross examined.

Guideline [1]

At the beginning of a cross examination, the party conducting the cross examination must instruct the witness on the record to ask deposing counsel, rather than the witness's own counsel, for clarifications, definitions or explanations of any words, questions or documents presented during the cross examination. The witness must follow these instructions.

Guideline [2]

A party may not direct or request that a witness not answer a question unless:

(a) a party has objected to the question on the ground that the answer would:
    (1) reveal privileged material or
    (2) violate a limitation the Board has imposed and
(b) counsel immediately places a conference call to the Board official assigned to the contested case asking for a ruling on the objection.

Under these circumstances, (i) the cross examination shall be suspended, (ii) the conference call immediately shall be placed to the Board official assigned to the contested case, and (iii) all counsel must be prepared to explain their respective positions during the call. The court reporter for the cross examination shall be available to record the conference call and to read back questions to which an objection has been made.

If the Board cannot be reached, then the party directing a witness not to answer shall within **two (2) business days** file with the Board (and not to the Office Mail Room or any other part of the Office) a miscellaneous motion seeking relief. Bd.R. 121(a)(3), SO ¶ **123**. Any opposition must be filed within **two (2) business days** of service of the motion. While a reply can be filed, the motion will likely be decided before the reply is filed.

Guideline [3]

Counsel must not make objections or statements that even remotely suggest an answer to a witness. Any objection to evidence during cross examination must be stated concisely and in a non-argumentative and non-suggestive manner and must include the legal basis for the objection. Examining counsel must not address the correctness of an objection, but may instead continue with questions to the witness, the objection having been noted on the record as required under Bd.R. 157(e)(4).[1]

Guideline [4]

Counsel and their witness-clients shall not engage in private, off-the-record conferences during cross examinations or during breaks or recesses, except for the purpose of deciding whether to assert a privilege.[2]

---

[1] With respect to this guideline, the following observation by Judge Gawthrop, 150 F.R.D. at 530 n.10, is highly relevant:

> I also note that a favorite objection or interjection of lawyers is, "I don't understand the question; therefore the witness doesn't understand the question." This is not a proper objection. If the witness needs clarification, the witness may ask the deposing lawyer for clarification. A lawyer's purported lack of understanding is not a proper reason to interrupt a deposition. In addition, counsel are not permitted to state on the record their interpretations of questions, since those interpretations are irrelevant and often suggestive of a particularly desired answer.

By way of example, the following comments by defending counsel generally are viewed as suggesting an answer to a witness:

(a) Objection, vague.
(b) Objection to the form of the question.
(c) Take your time in answering the question.
(d) Look at the document before you answer.
(e) Counsel, do you want to show the witness the document?

[2] The term "witness-clients" in the context of this guideline includes all witnesses who are employed by, or otherwise under the control of, the real party-in-interest, including retained expert witnesses, as well as the individual or individuals named in the caption of the contested case. With respect to this guideline, the following observation by Judge Gawthrop, 150 F.R.D. at 528, is highly relevant:

> The fact that there is no judge in the room to prevent private conferences does not mean that such conferences should or may occur. The underlying reason for preventing private conferences is still present: they tend, at the very least, to give the appearance of obstructing the truth.

Guideline [5]

Any conferences that occur pursuant to, or in violation of, guideline [4] are a proper subject for inquiry by deposing counsel to ascertain whether there has been any witness-coaching and, if so, the nature of that coaching.

Guideline [6]

Any conferences that occur pursuant to, or in violation of, guideline [4] shall be noted on the record by the counsel who participated in the conference. The purpose and outcome of the conference shall also be noted on the record.

Guideline [7]

Counsel taking cross-examination shall provide to defending counsel a copy of all documents shown to the witness during the cross examination. The copies shall be provided either before the cross examination begins or contemporaneously with the showing of each document to the witness. The witness and defending counsel do not have a right to discuss documents privately before the witness answers questions about the documents.

APPENDIX: INDEX OF TIMES

Times running from initiation/declaration

Notice of lead and backup counsel (Bd.R. 108(b)) . . . . . 14 days

Clean copy of claims (Bd.R. 110(a)) . . . . . 14 days

Notice of real party-in-interest (SO ¶ **8.1**) . . . . . 14 days

Notice of related proceedings (SO ¶ **8.2**) . . . . . 14 days

Request for file copies (SO ¶ **109.1**) . . . . . 14 days

Annotated copy of claims (Bd.R. 110(b)) . . . . . 28 days

Notice of confidential information (SO ¶ **6.2**) . . . . . 2 months

Initial settlement conference (SO ¶ **126.2**) . . . . . 3 months

Default times before a triggering event

Filing of motions list (SO ¶ **104.2.1**) before conference . . . . . 4 business days

Service of demonstrative exhibit before oral argument (Bd.R. 124(d)) . 5 business days

Notice of transcription before oral argument (SO ¶ **124.5**) . . . . . 1 business day

End of cross examination before opposition or reply (SO ¶ **157.3.2**) . . . . . 10 days

List of documents and things for cross examination
before conference call (Bd.R. 157(c)(3)) . . . . . 3 business days

Notice of deposition before deposition (Bd.R. 157(c)(4)) . . . . . 2 business days

Conference call regarding interpreter before deposition (Bd.R. 157(d)) 5 business days

Default times after a triggering event

Filed on behalf of: **Adair** Paper No:_____
By: Doreen Yatko Trujillo
Michael B. Fein
Cozen O'Connor P.C.
1900 Market St.
Philadelphia, PA 19103
Telephone: (215) 665-5593
Facsimile: (215) 701-2005
dtrujillo@cozen.com

UNITED STATES PATENT AND TRADEMARK OFFICE

_____

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

_____

PAUL J. **CARTER** AND LEONARD G. PRESTA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR,** DILJEET SINGH ATHWAL,
AND JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

Patent Interference No. 105,744
(Technology Center 1600)

ADAIR DESIGNATION OF LEAD COUNSEL

Pursuant to Bd. R. 108(b) and SO ¶ 108, the party Adair designates Doreen Yatko Trujillo, Reg. No. 35,719, as its lead counsel and Michael B. Fein, Reg. No. 25,333, as its backup lead counsel.

Please direct all future correspondence and telephone calls in connection with this interference to the following as lead counsel and backup lead counsel:

Lead Counsel:

Doreen Yatko Trujillo
Cozen O'Connor P.C.
1900 Market St.
Philadelphia, PA 19103
Telephone: (215) 665-5593
Facsimile: (215) 701-2005
dtrujillo@cozen.com

If the above-designated lead counsel should not be available, backup lead counsel, Michael B. Fein, can be contacted at the same address as given above.

Backup Lead Counsel:

Michael B. Fein
Telephone: (215) 665-4622
Facsimile: (215) 701-2246
mfein@cozen.com

Respectfully submitted,

/Doreen Yatko Trujillo/
DOREEN YATKO TRUJILLO
Registration No. 35,719
Lead Counsel for Adair

Date: February 16, 2010

Cozen O'Connor P.C.
1900 Market St.
Philadelphia, PA 19103
Telephone: (215) 665-5593
Facsimile: (215) 701-2005
dtrujillo@cozen.com

Certificate of Service

This will certify that a true copy of this paper was served this date on the party Carter by Federal Express overnight mail directed to:

SIDLEY AUSTIN, LLP
Attn: DC Patent Docketing
1501 K Street, N.W.
Washington, DC 20005

Date: February 16, 2010

/Doreen Yatko Trujillo/
Doreen Yatko Trujillo

Filed on behalf of: **Adair** Paper No:_____
By: Doreen Yatko Trujillo
Michael B. Fein
Cozen O'Connor P.C.
1900 Market St.
Philadelphia, PA 19103
Telephone: (215) 665-5593
Facsimile: (215) 701-2005
dtrujillo@cozen.com

UNITED STATES PATENT AND TRADEMARK OFFICE

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BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

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PAUL J. **CARTER** AND LEONARD G. PRESTA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR,** DILJEET SINGH ATHWAL,
AND JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

Patent Interference No. 105,744
(Technology Center 1600)

ADAIR CLEAN COPY OF CLAIMS

Pursuant to Bd. R. 110(a) and SO ¶ 110, a clean copy of all the claims currently pending in U.S. Application Serial No. 11/284,261 is enclosed herewith.

Respectfully submitted,

/Doreen Yatko Trujillo/
DOREEN YATKO TRUJILLO
Registration No. 35,719
Lead Counsel for Adair

Date: February 16, 2010

Cozen O'Connor P.C.
1900 Market St.
Philadelphia, PA 19103
Telephone: (215) 665-5593
Facsimile: (215) 01-2005
dtrujillo@cozen.com

Certificate of Service

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SIDLEY AUSTIN, LLP
Attn: DC Patent Docketing
1501 K Street, N.W.
Washington, DC 20005

Date: February 16, 2010

/Doreen Yatko Trujillo/
Doreen Yatko Trujillo

Claim 24: A humanised antibody comprising a heavy chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises a non-human amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof, as numbered according to Kabat.

Filed on behalf of: **Adair** Paper No:_____
By: Doreen Yatko Trujillo
Michael B. Fein
Cozen O'Connor P.C.
1900 Market St.
Philadelphia, PA 19103
Telephone: (215) 665-5593
Facsimile: (215) 701-2005
dtrujillo@cozen.com

UNITED STATES PATENT AND TRADEMARK OFFICE

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BEFORE THE BOARD OF PATENT APPEALS
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PAUL J. **CARTER** AND LEONARD G. PRESTA
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(Patent 6,407,213),

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AND JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

Patent Interference No. 105,744
(Technology Center 1600)

ADAIR NOTICE OF RELATED PROCEEDINGS

Pursuant to Standing Order ¶ 8.2, the party Adair hereby states that no application claims the benefit of priority of the filing date of the party's involved application.

Pursuant to Bd. R. 41.8, Adair advises that it is involved in two other interferences involving applications that claim priority to Application Serial No. 08/846,658, to which the present application also claims priority. They are Interference Nos. 105,688 and 105,705, involving U.S. Patent Nos. 5,585,089 and 6,180,379, respectively.

Respectfully submitted,

/Doreen Yatko Trujillo/
DOREEN YATKO TRUJILLO
Registration No. 35,719
Lead Counsel for Adair

Date: February 16, 2010

Cozen O'Connor P.C.
1900 Market St.
Philadelphia, PA 19103
Telephone: (215) 665-5593
Facsimile: (215) 701-2005
dtrujillo@cozen.com

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Attn: DC Patent Docketing
1501 K Street, N.W.
Washington, DC 20005

Date: February 16, 2010

/Doreen Yatko Trujillo/
Doreen Yatko Trujillo

Filed on behalf of: **Adair**
By: Doreen Yatko Trujillo
Michael B. Fein
Cozen O'Connor P.C.
1900 Market St.
Philadelphia, PA 19103
Telephone: (215) 665-5593
Facsimile: (215) 701-2005
dtrujillo@cozen.com

Paper No:_____

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

PAUL J. **CARTER** AND LEONARD G. PRESTA
Junior Party
(Patent 6,407,213),

v.

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AND JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

Patent Interference No. 105,744
(Technology Center 1600)

ADAIR REQUEST FOR FILE COPIES

A completed File Copy Request, and Sections E and F of the Declaration, are attached.

Respectfully submitted,

/Doreen Yatko Trujillo/
DOREEN YATKO TRUJILLO
Registration No. 35,719
Lead Counsel for Adair

Date: February 16, 2010

Cozen O'Connor P.C.
1900 Market St.
Philadelphia, PA 19103
Telephone: (215) 665-5593
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Certificate of Service

This will certify that a true copy of this paper and attachments was served this date on the party Queen by Federal Express overnight mail directed to:

SIDLEY AUSTIN, LLP
Attn: DC Patent Docketing
1501 K Street, N.W.
Washington, DC 20005

Date: February 16, 2010

/Doreen Yatko Trujillo/
Doreen Yatko Trujillo

Involved Patent:	6,407,213, issued 18 June 2002, from application 08/146,206, which was filed 17 November 1993, and was based on international application PCT/US92/05126, filed 15 June 1992.
Title:	METHOD FOR MAKING HUMANIZED ANTIBODIES
Assignee:	Genentech, Inc.

Senior Party

Named Inventors:	John Robert Adair High Wycombe, United Kingdom Diljeet Singh Athwal London, United Kingdom John Spencer Emtage Marlow, United Kingdom
Involved Application:	11/284,261, filed 21 November 2005
Title:	HUMANISED ANTIBODIES
Assignee:	Celltech R & D Limited

The senior party is assigned exhibit numbers 1001-1999. The junior party is assigned exhibit numbers 2001-2999. Bd. R. 154(c)(1); SO ¶ 154.2.1. The senior party is responsible for initiating settlement discussions. SO ¶ 126.1.

Part B. Judge managing the interference

Administrative Patent Judge Sally Gardner Lane has been designated to manage the interference. Bd. R. 104(a).

Part C. Standing order

A Trial Section STANDING ORDER [SO] (Paper 2) accompanies this DECLARATION. The STANDING ORDER applies to this interference.

Part D. Initial conference call

A telephone conference call to discuss the interference is set for **2:00 p.m. on 16 March 2010** (the Board will initiate the call).

No later than **four business days** prior to the conference call, each party shall file and serve (SO ¶¶ 10.1 & 105) a list of the motions (Bd. R. 120; Bd. R. 204; SO ¶¶ 104.2.1, 120 & 204) the party intends to file.

A sample schedule for taking action during the motion phase appears as Form 2 in the STANDING ORDER. Counsel are encouraged to discuss the schedule prior to the conference call and to agree on dates for taking action. A typical motion period lasts approximately eight (8) months. Counsel should be prepared to justify any request for a shorter or longer period.

Part E. Identification and order of the parties

Junior Party

Named inventors: Paul J. Carter
San Francisco, CA

Leonard G. Presta
San Francisco, CA

Part F. Count and claims of the parties

Count 1

A humanized antibody heavy chain variable domain comprising non-human Complementarity Determining Region (CDR) amino acid residues which bind antigen incorporated into a human antibody variable domain, and further comprising a Framework Region (FR) amino acid substitution at a site selected from the group consisting of: 24H, 71H, 73H, and 78H, utilizing the numbering system set forth in Kabat.

The claims of the parties are:

Carter: 1-82

Adair: 24

The claims of the parties which correspond to Count 1 are:

Carter: 30, 31, 60, 62, 63, 66, 67, 70, 73, 77-81

Adair: 24

The claims of the parties which do not correspond to Count 1, and therefore are not involved in the interference, are:

Carter: 1-29, 32-59, 61, 64, 65, 68, 69, 71, 72, 74-76, 82

Adair: None

The parties are accorded the following benefit for Count 1:

Carter: PCT/US92/05126, filed 15 June 1992; and
07/715,272, filed 14 June 1991, now abandoned.

Adair: 08/846,658, filed 01 May 1997;
08/303,569, filed 07 September 1994, issued as 5,859,205 on 12 January 1999;
07/743,329, filed on 17 September 1991;
PCT/GB90/02017, filed 21 December 1990; and
GB 8928874.0, filed 21 December 1989.

Part G. Heading to be used on papers

The following heading must be used on all papers filed in this interference, see SO & 106.1.1:

PAUL J. **CARTER** AND LEONARD G. PRESTA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR**, DILJEET SINGH ATHWAL,
AND JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

Patent Interference No. 105,744
(Technology Center 1600)

Form 4. File copy request

FILE COPY REQUEST
Contested Case No. [Contested Case number]

Attach a copy of sections E and F of the DECLARATION to this REQUEST. On the copy, circle each patent and application that you are requesting.

Include the following information to facilitate processing of this REQUEST:

1. Charge fees to USPTO Deposit Account No. 503111

2. Complete address, including street, city, state, zip code and telephone number (do not list a Post Office box because file copies are sent by commercial overnight courier).

Doreen Yatko Trujillo
Cozen O'Connor P.C.
1900 Market St.
Philadelphia, PA 19103

3. Telephone, including area code: 215-665-5593

Paper No. _____
Filed on behalf of: Party Carter
Filed: February 19, 2010

By: Oliver R. Ashe, Jr., Esq.
ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

Jeffrey P. Kushan, Esq.
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, DC 20005
Tel.: (202) 736-8914
Fax: (202) 736-8711
E-mail: jkushan@sidley.com

UNITED STATES PATENT AND TRADEMARK OFFICE

_____

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

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PAUL J. **CARTER** AND LEONARD G. PRESTA
Junior Party
(Patent 6,407,213),

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AND JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

_____

Patent Interference 105,744 (SGL)
Technology Center 1600

_____

CARTER DESIGNATION OF LEAD AND BACKUP LEAD COUNSEL

CARTER DESIGNATION OF LEAD AND BACKUP LEAD COUNSEL

In accordance with 37 C.F.R. § 41.108(b) and ¶ 108 of the Standing Order (Paper No. 2), Carter hereby identifies the following lead and backup lead counsel in the above-identified interference:

Lead Counsel:	Oliver R. Ashe, Jr. Registration No. 40,491
Backup Lead Counsel:	Jeffrey P. Kushan Registration No. 43,401

The respective mailing address, telephone number, facsimile number, and internet e-mail address for Mr. Ashe and Mr. Kushan are as follows:

By:

Oliver R. Ashe, Jr., Esq.
ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

Jeffrey P. Kushan, Esq.
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, DC 20005
Tel.: (202) 736-8914
Fax: (202) 736-8711
E-mail: jkushan@sidley.com

Carter is concurrently filing a paper titled "Carter Submission of Power of Attorney," which includes an appointment of attorney for Mr. Ashe.

Respectfully submitted,

February 19, 2010

/Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.
Registration No. 40,491
Counsel for Party Carter

ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

CERTIFICATE OF FILING

The undersigned certifies that a copy of the paper entitled **"CARTER DESIGNATION OF LEAD AND BACKUP LEAD COUNSEL"** was filed this 19th day of February, 2010, by e-mail, to:

The Board of Patent Appeals and Interferences
Madison Building East, 9th Floor
600 Dulany Street
Alexandria, VA 22314
Tel.: 571-272-9797
Fax: 571-273-0042
E-mail: BoxInterferences@USPTO.GOV

February 19, 2010 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

CERTIFICATE OF SERVICE

The undersigned hereby certifies that a copy of the paper entitled **"CARTER DESIGNATION OF LEAD AND BACKUP LEAD COUNSEL"** was served this 19th day of February, 2010, by e-mail, on the Attorney of Record for Adair:

Doreen Yatko Trujillo, Esq.
Cozen O'Connor P.C.
1900 Market Street, 7th Floor
Philadelphia, PA 19103
Tel.: 215-665-6593
Fax: 215-701-2005
E-mail: dtrujillo@cozen.com

February 19, 2010 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

Paper No. _____
Filed on behalf of: Party Carter
Filed: February 19, 2010

By: Oliver R. Ashe, Jr., Esq.
ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

Jeffrey P. Kushan, Esq.
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, DC 20005
Tel.: (202) 736-8914
Fax: (202) 736-8711
E-mail: jkushan@sidley.com

UNITED STATES PATENT AND TRADEMARK OFFICE

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BEFORE THE BOARD OF PATENT APPEALS
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PAUL J. **CARTER** AND LEONARD G. PRESTA
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JOHN ROBERT **ADAIR**, DILJEET SINGH ATHWAL,
AND JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

_____

Patent Interference 105,744 (SGL)
Technology Center 1600

_____

CARTER SUBMISSION OF POWER OF ATTORNEY

CARTER SUBMISSION OF POWER OF ATTORNEY

In accordance with 37 C.F.R. § 41.108(a) and ¶ 108 of the Standing Order (Paper No. 2), attached hereto is a Power of Attorney and Statement Under 37 C.F.R. § 3.73(b), recognizing for representation the attorney associated with Customer Number 000066181 on U.S. Patent Application No. 08/146,206, filed November 17, 1993, now U.S. Patent No. 6,407,213, issued June 18, 2002.

Carter is concurrently filing a paper titled "Carter Designation of Lead and Backup Lead Counsel" in which Oliver R. Ashe, Jr. and Jeffrey P. Kushan are identified as Lead Counsel (Mr. Ashe) and Back-up Lead Counsel (Mr. Kushan) for Carter in the above-identified interference.

Respectfully submitted,

February 19, 2010

/Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.
Registration No. 40,491
Counsel for Party Carter

ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants :	Paul J. Carter et al.	Conf. No.:	3992
Appln No. :	08/146,206	Examiner:	Davis, Minh Tam B
Filed:	November 17, 1993	Art Unit:	1642
U.S. Patent No.:	6,407,213	Issued:	June 18, 2002
For :	*METHOD FOR MAKING HUMANIZED ANTIBODIES*		

Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

POWER OF ATTORNEY
AND
STATEMENT UNDER 37 C.F.R. § 3.73(B)

The assignee of the entire right, title, and interest in U.S. Patent No. 6,407,213, Genentech, Inc., hereby appoints the attorney associated with

Customer Number 000066181

as attorney for the above-identified patent to represent the assignee in any and all interference proceedings and to transact all business in the Patent and Trademark Office connected therewith.

Pursuant to 37 C.F.R. § 3.73(b), the undersigned states that Genentech, Inc. is the assignee of the entire right, title and interest in the above-identified patent application by virtue of the assignment from the inventors to Genentech, Inc., as recorded in U.S. Patent No. 6,407,213 on June 28, 1994, at Reel 007035, Frame 0272.

The undersigned, whose title is supplied below, is authorized to act on behalf of the assignee.

Respectfully submitted,
Genentech, Inc.

Feb. 19, 2010
Date

Atulya R. Agarwal
Name:
Title:

Atulya R. Agarwal, Ph.D., J.D.
Associate General Counsel, Director
Genentech, Inc.
Authorized Corporate Signatory

CERTIFICATE OF FILING

The undersigned certifies that a copy of the paper entitled **"CARTER SUBMISSION OF POWER OF ATTORNEY"** attaching a copy of the Power of Attorney and Statement Under 37 C.F.R. § 3.73(b) was filed this 19th day of February, 2010, by e-mail, to:

The Board of Patent Appeals and Interferences
Madison Building East, 9th Floor
600 Dulany Street
Alexandria, VA 22314
Tel.: 571-272-9797
Fax: 571-273-0042
E-mail: BoxInterferences@USPTO.GOV

February 19, 2010 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

CERTIFICATE OF SERVICE

The undersigned hereby certifies that a copy of the paper entitled **"CARTER SUBMISSION OF POWER OF ATTORNEY"** attaching a copy of the Power of Attorney and Statement Under 37 C.F.R. § 3.73(b) was served this 19th day of February, 2010, by e-mail, on the Attorney of Record for Adair:

Doreen Yatko Trujillo, Esq.
Cozen O'Connor P.C.
1900 Market Street, 7th Floor
Philadelphia, PA 19103
Tel.: 215-665-6593
Fax: 215-701-2005
E-mail: dtrujillo@cozen.com

February 19, 2010 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

Paper No. _____
Filed on behalf of: Party Carter
Filed: February 19, 2010

By: Oliver R. Ashe, Jr., Esq.
ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

Jeffrey P. Kushan, Esq.
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, DC 20005
Tel.: (202) 736-8914
Fax: (202) 736-8711
E-mail: jkushan@sidley.com

UNITED STATES PATENT AND TRADEMARK OFFICE

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BEFORE THE BOARD OF PATENT APPEALS
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PAUL J. **CARTER** AND LEONARD G. PRESTA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR**, DILJEET SINGH ATHWAL,
AND JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

_____

Patent Interference 105,744 (SGL)
Technology Center 1600

_____

CARTER NOTICE OF REAL PARTY IN INTEREST

CARTER NOTICE OF REAL PARTY IN INTEREST

In accordance with 37 C.F.R. § 41.8(a)(1) and ¶ 8.1 of the Standing Order (Paper No. 2), Carter hereby notifies the Board that any and all right, title, or interest in the involved U.S. Patent No. 6,407,213 is assigned to Genentech, Inc.

Respectfully submitted,

February 19, 2010

/Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.
Registration No. 40,491
Counsel for Party Carter

ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

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February 19, 2010 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

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E-mail: dtrujillo@cozen.com

February 19, 2010 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

Paper No. _____
Filed on behalf of: Party Carter
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AND JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

_____

Patent Interference 105,744 (SGL)
Technology Center 1600

_____

CARTER NOTICE OF RELATED PROCEEDINGS

CARTER NOTICE OF RELATED PROCEEDINGS

In accordance with ¶ 8.2 of the Standing Order (Paper No. 2), Carter hereby notifies the Board that the following applications claim the benefit of priority of the November 17, 1993, filing date of Carter's involved U.S. Patent No. 6,407,213.

1. U.S. Patent Application No. 08/439,004, filed May 11, 1995, now abandoned;
2. U.S. Patent Application No. 09/115,800, filed July 15, 1998, now abandoned;
3. U.S. Patent Application No. 09/705,392, filed November 2, 2000, now U.S. Patent No. 6,719,971, issued April 13, 2004;
4. U.S. Patent Application No. 09/705,398, filed November 2, 2000, now U.S. Patent No. 6,800,738, issued October 5, 2004;
5. U.S. Patent Application No. 09/705,686, filed November 2, 2000, now U.S. Patent No. 6,639,055, issued October 28, 2003;
6. U.S. Patent Application No. 10/835,641, filed April 30, 2004, now abandoned;
7. U.S. Patent Application No. 11/444,791, filed June 1, 2006, now abandoned; and
8. U.S. Patent Application No. 11/969,430, now pending.

Respectfully submitted,

February 19, 2010

/Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.
Registration No. 40,491
Counsel for Party Carter

ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

CERTIFICATE OF FILING

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The Board of Patent Appeals and Interferences
Madison Building East, 9th Floor
600 Dulany Street
Alexandria, VA 22314
Tel.: 571-272-9797
Fax: 571-273-0042
E-mail: BoxInterferences@USPTO.GOV

February 19, 2010 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

CERTIFICATE OF SERVICE

The undersigned hereby certifies that a copy of the paper entitled **"CARTER NOTICE OF RELATED PROCEEDINGS"** was served this 19th day of February, 2010, by e-mail, on the Attorney of Record for Adair:

Doreen Yatko Trujillo, Esq.
Cozen O'Connor P.C.
1900 Market Street, 7th Floor
Philadelphia, PA 19103
Tel.: 215-665-6593
Fax: 215-701-2005
E-mail: dtrujillo@cozen.com

February 19, 2010 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

Paper No. _____

Filed on behalf of: Party Carter

Filed: February 19, 2010

By: Oliver R. Ashe, Jr., Esq.
ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

Jeffrey P. Kushan, Esq.
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, DC 20005
Tel.: (202) 736-8914
Fax: (202) 736-8711
E-mail: jkushan@sidley.com

UNITED STATES PATENT AND TRADEMARK OFFICE

_____

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

_____

PAUL J. **CARTER** AND LEONARD G. PRESTA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR**, DILJEET SINGH ATHWAL,
AND JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

_____

Patent Interference 105,744 (SGL)
Technology Center 1600

_____

CARTER CLEAN COPY OF CLAIMS

CARTER CLEAN COPY OF CLAIMS

In accordance with 37 C.F.R. § 41.110(a), Carter herewith provides a clean copy of Carter's involved claim, *i.e.*, claims 30, 31, 60, 62, 63, 66, 67, 70, 73, and 77-81 of U.S. Patent No. 6,407,213.

Respectfully submitted,

February 19, 2010

/Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.
Registration No. 40,491
Counsel for Party Carter

ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

Clean Copy of Carter's Involved Claims in U.S. Patent No. 6,407,213

30. An antibody which binds $p185^{HER2}$ and comprises a humanized antibody variable domain, wherein the humanized antibody variable domain comprises non-human Complementarity Determining Region (CDR) amino acid residues which bind $p185^{HER2}$ incorporated into a human antibody variable domain, and further comprises a Framework Region (FR) amino acid substitution at a site selected from the group consisting of: 4L, 38L, 43L, 44L, 46L, 58L, 62L, 65L, 66L, 67L, 68L, 69L, 73L, 85L, 98L, 2H, 4H, 36H, 39H, 43H, 45H, 69H, 70H, 74H, 75H, 76H, 78H and 92H, utilizing the numbering system set forth in Kabat.

31. The antibody of claim 30 wherein the substituted residue is the residue found at the corresponding location of the non-human antibody from which the non-human CDR amino acid residues are obtained.

60. The antibody of claim 30 wherein the residue at site 78H has been substituted.

62. A humanized antibody variable domain comprising non-human Complementarity Determining Region (CDR) amino acid residues which bind an antigen incorporated into a consensus human variable domain, and further comprising an amino acid substitution at a site selected from the group consisting of: 4L, 38L, 43L, 44L, 46L, 58L, 62L, 65L, 66L, 67L, 68L, 69L, 73L, 85L, 98L, 2H, 4H, 36H, 39H, 43H, 45H, 69H, 70H, 74H, 75H, 76H, 78H and 92H, utilizing the numbering system set forth in Kabat.

63. A humanized antibody which lacks immunogenicity compared to a non-human parent antibody upon repeated administration to a human patient in order to treat a

chronic disease in that patient, wherein the humanized antibody comprises non-human Complementarity Determining Region (CDR) amino acid residues which bind an antigen incorporated into a human antibody variable domain, and further comprises an amino acid substitution at a site selected from the group consisting of: 4L, 38L, 43L, 44L, 46L, 58L, 62L, 65L, 66L, 67L, 68L, 69L, 73L, 85L, 98L, 2H, 4H, 36H, 39H, 43H, 45H, 69H, 70H, 74H, 75H, 76H, 78H and 92H, utilizing the numbering system set forth in Kabat.

66. A humanized antibody heavy chain variable domain comprising non-human Complementarity Determining Region (CDR) amino acid residues which bind antigen incorporated into a human antibody variable domain, and further comprising a Framework Region (FR) amino acid substitution at a site selected from the group consisting of: 24H, 73H, 76H, 78H, and 93H, utilizing the numbering system set forth in Kabat.

67. The humanized variable domain of claim 66 wherein the substituted residue is the residue found at the corresponding location of the non-human antibody from which the non-human CDR amino acid residues are obtained.

70. The humanized variable domain of claim 66 wherein the residue at site 24H has been substituted.

73. The humanized variable domain of claim 66 wherein the residue at site 78H has been substituted.

77. The humanized variable domain of claim 66 which further comprises amino acid substitutions at sites 71H, 73H and 78H.

78. An antibody comprising the humanized variable domain of claim 66.

79. A humanized variant of a non-human parent antibody which binds an antigen, wherein the humanized variant comprises Complementarity Determining Region

(CDR) amino acid residues of the non-human parent antibody incorporated into a human antibody variable domain, and further comprises Framework Region (FR) substitutions at heavy chain positions 71H, 73H, 78H and 93H, utilizing the numbering system set forth in Kabat.

80. A humanized antibody variable domain comprising non-human Complementarity Determining Region (CDR) amino acid residues which bind an antigen incorporated into a human antibody variable domain, and further comprising a Framework Region (FR) amino acid substitution where the substituted FR residue:

(a) noncovalently binds antigen directly;

(b) interacts with a CDR; or

(c) participates in the V_L -V_H interface by affecting the proximity or orientation of the V_L and V_H regions with respect to one another, and wherein the substituted FR residue is at a site selected from the group consisting of: 4L, 38L, 43L, 44L, 58L, 62L, 65L, 66L, 67L, 68L, 69L, 73L, 85L, 98L, 2H, 4H, 24H, 36H, 39H, 43H, 45H, 69H, 70H, 73H, 74H, 76H, 78H, 92H and 93H, utilizing the numbering system set forth in Kabat.

81. The humanized variable domain of claim 80 wherein the substituted residue is the residue found at the corresponding location of the non-human antibody from which the non-human CDR amino acid residues are obtained.

CERTIFICATE OF FILING

The undersigned certifies that a copy of the paper entitled **"CARTER CLEAN COPY OF CLAIMS"** attaching a clean set of Carter's involved claims was filed this 19th day of February, 2010, by e-mail, to:

The Board of Patent Appeals and Interferences
Madison Building East, 9th Floor
600 Dulany Street
Alexandria, VA 22314
Tel.: 571-272-9797
Fax: 571-273-0042
E-mail: BoxInterferences@USPTO.GOV

February 19, 2010 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

CERTIFICATE OF SERVICE

The undersigned hereby certifies that a copy of the paper entitled **"CARTER CLEAN COPY OF CLAIMS"** attaching a clean set of Carter's involved claims was served this 19th day of February, 2010, by e-mail, on the Attorney of Record for Adair:

Doreen Yatko Trujillo, Esq.
Cozen O'Connor P.C.
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Tel.: 215-665-6593
Fax: 215-701-2005
E-mail: dtrujillo@cozen.com

February 19, 2010 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

Paper No. _____
Filed on behalf of: Party Carter
Filed: February 19, 2010

By: Oliver R. Ashe, Jr., Esq.
ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

Jeffrey P. Kushan, Esq.
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, DC 20005
Tel.: (202) 736-8914
Fax: (202) 736-8711
E-mail: jkushan@sidley.com

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

PAUL J. **CARTER** AND LEONARD G. PRESTA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR**, DILJEET SINGH ATHWAL,
AND JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

Patent Interference 105,744 (SGL)
Technology Center 1600

CARTER REQUEST FOR FILE COPIES

CARTER REQUEST FOR FILE COPIES

In accordance with 37 C.F.R. § 41.109(a) and ¶ 109.1.1 of the Standing Order (Paper No. 2), Carter hereby requests copies of the following files:

Adair Files:

(i) U.S. Patent Application No. 11/284,261, filed November 21, 2005;

(ii) U.S. Patent Application No. 08/846,658, filed May 1, 1997;

(iii) U.S. Patent Application No. 08/303,569, filed September 7, 1994, now U.S. Patent No. 5,859,205, issued January 12, 1999;

(iv) U.S. Patent Application No. 07/743,329, filed September 17, 1991, now abandoned;

(v) PCT Application No. PCT/GB90/02017, filed December 21, 1990; and

(vi) UK Application No. GB 8928874.0, filed December 21, 1989.

Carter Files:

(i) U.S. Patent Application No. 08/146,206, filed November 17, 1993, now U.S. Patent No. 6,407,213, issued June 18, 2002;

(ii) PCT Application No. PCT/US92/05126, filed June 15, 1992; and

(iii) U.S. Patent Application No. 07/715,272, filed June 14, 1991, now abandoned.

Please ship the files to:

Oliver R. Ashe, Jr., Esq.
ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002

In accordance with Part H of the Notice Declaring Interference (Paper No. 1) and ¶ 109 of the Standing Order (Paper No. 2), a copy of Parts E and F of the Notice Declaring Interference with a hand-drawn circle around the patents and applications for which a copy of a file wrapper is requested and a copy of completed File Copy Request (SO Form 4) are attached hereto.

Respectfully submitted,

February 19, 2010

/Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.
Registration No. 40,491
Counsel for Party Carter

ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

Part B. Judge managing the interference

Administrative Patent Judge Sally Gardner Lane has been designated to manage the interference. Bd. R. 104(a).

Part C. Standing order

A Trial Section STANDING ORDER [SO] (Paper 2) accompanies this DECLARATION. The STANDING ORDER applies to this interference.

Part D. Initial conference call

A telephone conference call to discuss the interference is set for 2:00 p.m. on 16 March 2010 (the Board will initiate the call).

No later than four business days prior to the conference call, each party shall file and serve (SO ¶¶ 10.1 & 105) a list of the motions (Bd. R. 120; Bd. R. 204; SO ¶¶ 104.2.1, 120 & 204) the party intends to file.

A sample schedule for taking action during the motion phase appears as Form 2 in the STANDING ORDER. Counsel are encouraged to discuss the schedule prior to the conference call and to agree on dates for taking action. A typical motion period lasts approximately eight (8) months. Counsel should be prepared to justify any request for a shorter or longer period.

Part E. Identification and order of the parties

Junior Party

Named inventors:	Paul J. Carter San Francisco, CA
	Leonard G. Presta San Francisco, CA

Involved Patent:	6,407,213, issued 18 June 2002, from application 08/146,206, which was filed 17 November 1993, and was based on international application PCT/US92/05126, filed 15 June 1992.
Title:	METHOD FOR MAKING HUMANIZED ANTIBODIES
Assignee:	Genentech, Inc.

Senior Party

Named Inventors:	John Robert Adair High Wycombe, United Kingdom Diljeet Singh Athwal London, United Kingdom John Spencer Emtage Marlow, United Kingdom
Involved Application:	11/284,261, filed 21 November 2005
Title:	HUMANISED ANTIBODIES
Assignee:	Celltech R & D Limited

The senior party is assigned exhibit numbers 1001-1999. The junior party is assigned exhibit numbers 2001-2999. Bd. R. 154(c)(1); SO ¶ 154.2.1. The senior party is responsible for initiating settlement discussions. SO ¶ 126.1.

Part F. Count and claims of the parties

Count 1

A humanized antibody heavy chain variable domain comprising non-human Complementarity Determining Region (CDR) amino acid residues which bind antigen incorporated into a human antibody variable domain, and further comprising a Framework Region (FR) amino acid substitution at a site selected from the group consisting of: 24H, 71H, 73H, and 78H, utilizing the numbering system set forth in Kabat.

The claims of the parties are:

Carter: 1-82

Adair: 24

The claims of the parties which correspond to Count 1 are:

Carter: 30, 31, 60, 62, 63, 66, 67, 70, 73, 77-81

Adair: 24

The claims of the parties which do not correspond to Count 1, and therefore are not involved in the interference, are:

Carter: 1-29, 32-59, 61, 64, 65, 68, 69, 71, 72, 74-76, 82

Adair: None

The parties are accorded the following benefit for Count 1:

Carter:	PCT/US92/05126, filed 15 June 1992; and 07/715,272, filed 14 June 1991, now abandoned.
Adair:	08/846,658, filed 01 May 1997; 08/303,569, filed 07 September 1994, issued as 5,859,205 on 12 January 1999; 07/743,329, filed on 17 September 1991; PCT/GB90/02017, filed 21 December 1990; and GB 8928874.0, filed 21 December 1989.

Part G. Heading to be used on papers

The following heading must be used on all papers filed in this interference, see SO & 106.1.1:

PAUL J. CARTER AND LEONARD G. PRESTA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT ADAIR, DILJEET SINGH ATHWAL,
AND JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

Patent Interference No. 105,744
(Technology Center 1600)

Form 4. File Copy request

FILE COPY REQUEST
Contested Case No.: Interference No. 105,744

Attach a copy of sections E and F of the DECLARATION to this REQUEST. On the copy, circle each patent and application that you are requesting.

Include the following information to facilitate processing of this REQUEST:

1. Charge fees to USPTO Deposit Account No. **50-4028**

2. Complete address, including street, city, state, zip code and telephone number (do not list a Post Office box because file copies are sent by commercial overnight courier).

Oliver R. Ashe, Jr.

ASHE, P.C.

11440 Isaac Newton Sq. North, Suite 210

Reston, VA 20190

3. Telephone, including area code: **(703) 467-9001 ext. 201**

CERTIFICATE OF FILING

The undersigned certifies that a copy of the paper entitled **"CARTER REQUEST FOR FILE COPIES"** attaching Parts E and F of the Notice Declaring Interference and completed SO Form 4 was filed this 19th day of February, 2010, by e-mail, to:

The Board of Patent Appeals and Interferences
Madison Building East, 9th Floor
600 Dulany Street
Alexandria, VA 22314
Tel.: 571-272-9797
Fax: 571-273-0042
E-mail: BoxInterferences@USPTO.GOV

February 19, 2010 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

CERTIFICATE OF SERVICE

The undersigned hereby certifies that a copy of the paper entitled **"CARTER REQUEST FOR FILE COPIES"** attaching Parts E and F of the Notice Declaring Interference and completed SO Form 4 was served this 19th day of February, 2010, by e-mail, on the Attorney of Record for Adair:

Doreen Yatko Trujillo, Esq.
Cozen O'Connor P.C.
1900 Market Street, 7th Floor
Philadelphia, PA 19103
Tel.: 215-665-6593
Fax: 215-701-2005
E-mail: dtrujillo@cozen.com

February 19, 2010 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

Filed by: Trial Section
Mail Stop Interferences
Board of Patent Appeals & Interferences
U.S. Patent & Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
Telephone: 571-272-4683
BoxInterferences@USPTO.gov

Paper 14
Entered: 23 February 2010

UNITED STATES PATENT AND TRADEMARK OFFICE
BOARD OF PATENT APPEALS AND INTERFERENCES

Patent Interference 105,744 (SGL)
Technology Center 1600

PAUL J. CARTER and LEONARD G. PRESTA
Junior Party,
Patent 6,407,213,

v.

JOHN ROBERT ADAIR, DILJEET SINGH ATHWAL
and JOHN SPENCER EMTAGE
Senior Party,
Application 11/284,261.

Order – Bd.R. 109(b) – Authorizing copies of Office records

(Transmitting file copy requests to the Office of Public Records)

The requests of the parties for file copies, along with those requested files at the Board, have been forwarded to the Office of Public Records of the United States Patent and Trademark Office. It is the responsibility of each party to inform the Board if a complete copy is not received **within 21**

days of this order. Bd.R. 109(c). If a party does not promptly notify the Board of missing or incomplete files, the party may not request an extension of time, Bd.R. 4(a), due to non-receipt of files.

Note that files come in many different formats, see SO ¶ 109.2, and thus different files may arrive at different times.

Upon consideration of the receipt of requests for files (see Part H of the DECLARATION), it is---

ORDERED that the notice to the Board that a file copy has not been received or is incomplete must be transmitted via electronic mail addressed to BoxInterferences@uspto.gov, with "Attention Paralegal" in the subject line of the electronic mail message.

/Yolunda R. Townes/
Paralegal Specialist
Trial Division

cc: Office of Public Records, USPTO
Revised: 23 January 2006

cc (via electronic filing):

Attorney for Carter:

Oliver R. Ashe, Jr., Esq.
ASHE, P.C.
11440 Isaac Newton Square North
Suite 210
Reston, VA 20190

Tel: 703-467-9001
Email: oashe@ashepc.com

Jeffrey P. Kushan, Esq.
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, D.C. 20005

Tel: 202-736-8914
Email: jkushan@sidley.com

Attorney for Adair:

Doreen Yatko Trujillo, Esq.
Michael B. Fein, Esq.
COZEN O'CONNOR P.C.
1900 Market Street
Philadelphia, PA 19103

Tel: 215-665-5593
Email: dtrujillo@cozen.com

Mail Stop Interference
P.O. Box 1450
Alexandria Va 22313-1450
Tel: 571-272-9797
Fax: 571-273-0042

Paper 15
Filed: 24 February 2010

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

PAUL J. **CARTER** AND LEONARD G. PRESTIA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR**, DILGEET SINGH ATHWAL, and JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

Patent Interference No. 105,744
(Technology Center 1600)

ORDER – Miscellaneous – 104(a)

A conference call was held on 24 February 2010 at approximately 10:30 am.

Paritcipating in the call were:

(1) Oliver Ashe and Jeffrey P. Kushan for Junior Party Carter,

(2) Doreen Trujillo and Michael B. Fein for Senior Party Adair, and

(3) Sally Gardner Lane, Administrative Patent Judge.

There was a delay in receipt of the declaration of the interference and other relevant papers by Carter. Moreover, the parties expressed that other delays have been caused by the major snowstorm that occurred in the Washington, D.C. area the week of February 8th.

The parties moved for a delay of the initial conference call. The motion is GRANTED.

It is

ORDERED that the conference call is rescheduled for **15 April 2010 at 10:00 am,** and

FURTHER ORDERED that the call will be initiated by the Board.

/Sally Gardner Lane/
Administrative Patent Judge

cc (via overnight delivery):

Attorney for Carter:

Oliver R. Ashe, Jr., Esq.
ASHE, P.C.
11440 Isaac Newton Square North
Suite 210
Reston, VA 20190

Tel: 703-467-9001
Email: oashe@ashepc.com

Jeffrey P. Kushan, Esq.
SIDLEY AUSTIN LLP
1501 K Street, NW
Washington, DC 20005

Tel: 202-736-8914
Email: jkushan@sidley.com

Attorney for Adair:

Doreen Yatko Trujillo, Esq.
Michael B. Fein, Esq
COZEN O'CONNOR P.C.
1900 Market Street
Philadelphia, PA 19103

Tel: 215-665-5593
Email: dtrujillo@cozen.com

Mail Stop Interference
P.O. Box 1450
Alexandria Va 22313-1450
Tel: 571-272-4683
Fax: 571-273-0042

Filed 3 March 2010

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

PAUL J. **CARTER** AND LEONARD G. PRESTIA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR**, DILGEET SINGH ATHWAL, and JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

Patent Interference No. 105,744
(Technology Center 1600)

ORDER – Miscellaneous – 104(a)

A conference call was held on 3 March 2010 at approximately 2:30 pm.

Participating in the call were:

(1) Oliver Ashe for Carter,

(2) Doreen Trujillo for Adair, and

(3) Sally Gardner Lane, Administrative Patent Judge.

The parties asked for the call to determine if they are required to file an annotated copy of the claims, and if so, for additional time to file the annotated copy.

Parties are required to file "[f]or each involved claim having a limitation that is illustrated in a drawing or biotechnology material sequence, file an annotated copy of the claim indicating in bold face between braces ({}) where each limitation is shown in the drawing or sequence." Bd. R. 110(b)(1).

The parties indicated that, and a review of the claims indicates, that the claims do not recite any particular sequence. (Clean claim copies, Adair at Paper 5 and Carter at Paper 12). Under these particular circumstances, the parties need not file annotated copies of the claims.

It is

ORDERED that the parties need not file annotated copies of the claims. Bd. R. 110(b)(1).

/Sally Gardner Lane/
Administrative Patent Judge

cc (electronic filing):

Attorney for Carter:

Oliver R. Ashe, Jr., Esq.
ASHE, P.C.
11440 Isaac Newton Square North
Suite 210
Reston, VA 20190

Tel: 703-467-9001
Email: oashe@ashepc.com

Jeffrey P. Kushan, Esq.
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, D.C. 20005

Tel: 202-736-8914
Email: jkushan@sidley.com

Attorney for Adair:

Doreen Yatko Trujillo, Esq.
Michael B. Fein, Esq.
COZEN O'CONNOR P.C.
1900 Market Street
Philadelphia, PA 19103

Tel: 215-665-5593
Email: dtrujillo@cozen.com
Email: mfein@cozen.com

Paper No. _____
Filed on behalf of: Party Carter
Filed: March 16, 2010

By: Oliver R. Ashe, Jr., Esq.
ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

Jeffrey P. Kushan, Esq.
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, DC 20005
Tel.: (202) 736-8914
Fax: (202) 736-8711
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UNITED STATES PATENT AND TRADEMARK OFFICE

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BEFORE THE BOARD OF PATENT APPEALS
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PAUL J. **CARTER** AND LEONARD G. PRESTA
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AND JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

_____

Patent Interference 105,744 (SGL)
Technology Center 1600

_____

CARTER NOTICE OF INCOMPLETE FILE COPIES

CARTER NOTICE OF INCOMPLETE FILE COPIES

In accordance with the instructions in the Order dated February 23, 2010 (Paper No. 14, Order - Bd.R. 109(b) - Authorizing copies of Office records), Carter hereby notifies the Board that Carter has received a copy of all requested file histories.

The following papers are either missing from the files, incomplete (*e.g.*, page(s) missing), or illegible:

File	Paper requested	Reason for request
GB 8928874.0 to Adair et al.	GB Application No. 8928874.0, filed December 21, 1989.	Illegible (faint text)
USSN 08/303,569 to Adair et al.	Office Action mailed May 16, 1995 (Paper No. 37).	Page 1 (front page) is missing
	Protest Under 37 CFR § 1.291, filed April 1, 1997 (Paper No. 45).	Missing
	Communication mailed June 23, 1997 (Paper No. 46).	Missing

In view of the substantive nature of the above-identified documents, Carter respectfully requests the Board to promptly forward copies of these documents to the undersigned. In the event the Board does not have immediate access to these documents, immediate production should be required from Adair. See Bd.R. 150. The documents should be directed to:

Oliver R. Ashe, Jr.
ASHE, P.C.
11440 Isaac Newton Square North
Suite 210
Reston, VA 20190

Please charge any associated costs to Deposit Account No. 50-4028.

Respectfully submitted,

March 16, 2010

/Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.
Registration No. 40,491
Counsel for Party Carter

CERTIFICATE OF FILING

The undersigned certifies that a copy of the paper entitled **"CARTER NOTICE OF INCOMPLETE FILE COPIES"** was filed this 16th day of March, 2010, by e-mail, to:

The Board of Patent Appeals and Interferences
Madison Building East, 9th Floor
600 Dulany Street
Alexandria, VA 22314
Tel.: 571-272-9797
Fax: 571-273-0042
E-mail: BoxInterferences@USPTO.GOV

March 16, 2010 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

CERTIFICATE OF SERVICE

The undersigned hereby certifies that a copy of the paper entitled **"CARTER NOTICE OF INCOMPLETE FILE COPIES"** was served this 16th day of March, 2010, by e-mail, on the Attorney of Record for Adair:

Doreen Yatko Trujillo, Esq.
Cozen O'Connor P.C.
1900 Market Street, 7th Floor
Philadelphia, PA 19103
Tel.: 215-665-6593
Fax: 215-701-2005
E-mail: dtrujillo@cozen.com

March 16, 2010 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

Filed on behalf of: **Adair**
By: Doreen Yatko Trujillo
Michael B. Fein
Cozen O'Connor P.C.
1900 Market St.
Philadelphia, PA 19103
Telephone: (215) 665-5593
Facsimile: (215) 701-2005
dtrujillo@cozen.com

Paper No:_____
Date Filed: March 16, 2010

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

PAUL J. **CARTER** AND LEONARD G. PRESTA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR,** DILJEET SINGH ATHWAL,
AND JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

Patent Interference No. 105,744
(Technology Center 1600)

ADAIR NOTICE OF INCOMPLETE FILE COPIES

In accordance with the instructions in the Order dated February 23, 2010 (Paper No. 14, Order - Bd.R. 109(b) - Authorizing copies of Office records), Adair hereby notifies the Board that Adair has received a copy of all requested file histories. However, as indicated in the attached documents, Adair has not received most of the references cited by or to the Patent Office in the file history of U.S. Application Serial No. 07/715,272. References Adair received are crossed-out. Also, Adair was only able to open the first 2230 of 6819 pages of the file wrapper for Application Serial No. 08/146,206. For pages beyond 2230, there is a message of an I/O error. Both file histories were supplied on diskette.

Respectfully submitted,

/Doreen Yatko Trujillo/
DOREEN YATKO TRUJILLO
Registration No. 35,719
Lead Counsel for Adair

Date: March 16, 2010

Cozen O'Connor P.C.
1900 Market St.
Philadelphia, PA 19103
Telephone: (215) 665-5593
Facsimile: (215) 701-2005
dtrujillo@cozen.com

Certificate of Service

This will certify that a true copy of this paper and attachments was served this date on the party Carter by electronic mail directed to :

Oliver R. Ashe, Jr.
ASHE, P.C.
11440 Isaac Newton Square North
Suite 210
Reston, VA 20190
Tel.: (703)467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

Date: March 16, 2010

/Doreen Yatko Trujillo/
Doreen Yatko Trujillo

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PATENT DOCKET 708

#19 5-18-92

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

RECEIVED
MAY 0 8 1992
GROUP 180

In re Application of

Paul J. Carter et al.

Serial No. 07/715272

Filed: June 14, 1991

For: Immunoglobulin Variants

Group Art Unit:

Examiner:

460 Point San Bruno Boulevard
South San Francisco, CA 94080
(415) 266-2614

INFORMATION DISCLOSURE STATEMENT

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C., 20231 on April 30, 1992
(Date of Deposit)
LOUISE STRASBAUGH
Name of Depositing Party
Louise Strasbaugh
Signature of Depositing Party
April 30, 1992
Date of Signature

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

The following items are supplied to the United States Patent and Trademark Office to advance the prosecution of the subject application.

Chothia *et al.*, *J. Mol. Biol.* **186**:651-663 (1985)

Novotny and Haber, *Proc. Natl. Acad. Sci. USA* **82**:4592-4596 (1985)

Cabilly *et al.*, U.S. patent No. 4,816,567

~~Morrison, S. L. *et al.*, *Proc. Natl. Acad. Sci. USA* **81**:6851-6855 (1984)~~

Boulianne, G. L. *et al.*, *Nature* **312**:643-646 (1984)

Neuberger, M. S. *et al.*, *Nature* **314**:268-270 (1985)

Brüggemann, M. *et al.*, *J. Exp. Med.* **166**:1351-1361 (1987)

Riechmann, L. *et al.*, *Nature* **332**:323-327 (1988)

Love *et al.*, *Methods in Enzymology* **178**:515-527 (1989)

Bindon *et al.*, *J. Exp. Med.* **168**:127-142 (1988)

Jones, P. T. *et al.*, *Nature* **321**:522-525 (1986)

Verhoeyen, M. *et al.*, *Science* **239**:1534-1536 (1988)

Hale, G. *et al.*, *Lancet* **i**:1394-1399 (1988)

~~Queen, C. *et al.*, *Proc. Natl. Acad. Sci. USA* **86**:10029-10033 (1989)~~

Co *et al.*, *Proc. Natl. Acad. Sci. USA* **88**:2869-2873 (1991)

Gorman *et al.*, *Proc. Natl. Acad. Sci. USA* **88**:4181-4185 (1991)

Daugherty *et al.*, *Nucleic Acids Research* **19(9)**:2471-2476 (1991)

Brown *et al., Proc. Natl. Acad. Sci. USA* **88**:2663-2667 (1991)

Junghans *et al., Cancer Research* **50**:1495-1502 (1990)

Davies, D. R. *et al., Ann. Rev. Biochem.* **59**:439-473 (1990)

Chothia, C. & Lesk, A. M., *J. Mol. Biol.* **196**:901-917 (1987)

Chothia, C. *et al., Nature* **342**:877-883 (1989)

Tramontano, A. *et al., J. Mol. Biol.* **215**:175-182 (1990)

Margolies *et al., Proc. Natl. Acad. Sci. USA* **72**:2180-2184 (1975)

Pluckthun, *Biotechnology* **9**:545-51 (1991)

Spiegelberg *et al., Biochemistry* **9**:4217-4223 (1970)

Wallick *et al., J. Exp. Med.* **168**:1099-1109 (1988)

Sox *et al., Proc. Natl. Acad. Sci. USA* **66**:975-982 (1970)

Margni *et al., Ann. Rev. Immunol.***6**:535-554 (1988)

Fendly, B. M. *et al., Cancer Res.* **50**:1550-1558 (1990)

~~Neuberger *et al., Nature* **312**:604-608 (1984)~~

~~Takeda *et al., Nature* **314**:452-454 (1985)~~

Snow and Amzel, *Protein: Structure, Function, and Genetics* 1:267-279, Alan R. Liss, Inc. pubs. (1986)

Cheetham, J., *Protein Engineering*, **2(3)**: 170-172 (1988)

WO 91/09967, pub. 07/11/91, Adair *et al.*

One copy of each item cited above is supplied, along with a completed Form PTO-1449. The Examiner is requested to make the citations of record.

This submission is understood to complement the results of the Examiner's own independent search. The submission of this Disclosure Statement should not be construed as a representation that a search was made, or that the cited itms are inclusive of all the relevant and amterial citations that may be available publicly.

The citation of any item is not an admission that the item is prior art. The right is reserved to antedate any item in adherence with standard procedures.

Respectfully submitted,
GENENTECH, INC.

Carolyn R. Adler
Reg. No. 32,324

Dated: April 30, 1992

FORM PTO-1449 | U.S. Dept. of Commerce Patent and Trademark Office

Atty Docket No.	Serial No.
709	07/715,272

LIST OF DISCLOSURES CITED BY APPLICANT

(Use several sheets if necessary)

Applicant	
Paul J. Carter et al.	#14
Filing Date	**Group**
June 14, 1991	1806

U.S. PATENT DOCUMENTS

*Examiner Initials		Document Number	Date	Name	Class	Subclass	Filing Date
	AA						
	AB						
	AC						
	AD						
	AE						
	AF						
	AG						
	AH						
	AI						
	AJ						
	AK						

FOREIGN PATENT DOCUMENTS

		Document Number	Date	Country	Class	Subclass	Translation Yes	Translation No
CR	AL	WO 90/07861	7/26/90	PCT				
	AM							
	AN							
	AO							
	AP							

OTHER DISCLOSURES (Including Author, Title, Date, Pertinent Pages, Etc.)

CR	AR	Carter et al., Proc. Natl. Acad. Sci., 89: 4285-4289 (1992)
	AS	
	AT	
	AU	
	AV	
	AW	
	AX	

Examiner [signature] Date Considered 4/29/93

*Examiner: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

TO SEPARATE, D TOP AND BOTTOM EDGES, SNAP-APART AN DISCARD CARBON

FORM PTO-892 (REV. 2-92) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	SERIAL NO.	GROUP ART UNIT	ATTACHMENT TO PAPER NUMBER
NOTICE OF REFERENCES CITED	07/715,272	1806	26
	APPLICANT(S): Carter et al.		

U.S. PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE
	A						
	B						
	C						
	D						
	E						
	F						
	G						
	H						
	I						
	J						
	K						

FOREIGN PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	COUNTRY	NAME	CLASS	SUB-CLASS	PERTINENT SHTS. DWG	PERTINENT PP. SPEC.
	L								
	M								
	N								
	O								
	P								
	Q								

OTHER REFERENCES (Including Author, Title, Date, Pertinent Pages, Etc.)

R ~~Queen et al. PNAS 86 1023-1033 (1989)~~

S ~~Fendly et al. Cancer Research 5,130-138 (1990)~~

T Hudziak et al. Molecular and Cellular Biology 1989 p. 1165-1172

U

EXAMINER [signature] DATE 1/11/94

* A copy of this reference ... th this office action. on 707.05 (a).)

Filed on behalf of: **Adair**
By: Doreen Yatko Trujillo
Michael B. Fein
Cozen O'Connor P.C.
1900 Market St.
Philadelphia, PA 19103
Telephone: (215) 665-5593
Facsimile: (215) 701-2005
dtrujillo@cozen.com

Paper No:_____
March 18, 2010

UNITED STATES PATENT AND TRADEMARK OFFICE

_____

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

_____

PAUL J. **CARTER** AND LEONARD G. PRESTA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR,** DILJEET SINGH ATHWAL,
AND JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

Patent Interference No. 105,744
(Technology Center 1600)

ADAIR NOTICE OF PRODUCTION OF DOCUMENTS

Adair is today forwarding, by overnight mail, copies of the following documents that Carter identified as missing from the file history of U.S. Application Serial No. 08/303,569 in "Carter Notice of Incomplete File Copies" filed March 16, 2010:

1. Office Action mailed May 16, 1995(Paper No. 37), Page 1 (front page) and Office Action;

2. Protest Under 37 CFR § 1.291, filed April 1, 1997 ("Protest", Paper No. 45); and

3. Communication mailed June 23, 1997 (Paper No. 46).

As discussed and agreed to by Mr. Ashe, all attachments to the Protest, with the exception of the two U.S. patents, were also forwarded.

Respectfully submitted,

/Doreen Yatko Trujillo/
DOREEN YATKO TRUJILLO
Registration No. 35,719
Lead Counsel for Adair

Date: February 16, 2010

Cozen O'Connor P.C.
1900 Market St.
Philadelphia, PA 19103
Telephone: (215) 665-5593
Facsimile: (215) 01-2005
dtrujillo@cozen.com

Certificate of Service

This will certify that a true copy of this paper and the documents referenced herein were served this date on the party Carter by Federal Express overnight mail directed to:

Oliver R. Ashe, Jr.
ASHE, P.C.
11440 Isaac Newton Square North
Suite 210
Reston, VA 20190
Tel.: (703)467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

Date: March 18, 2010

/Doreen Yatko Trujillo/
Doreen Yatko Trujillo

Filed on behalf of: **Adair**
By: Doreen Yatko Trujillo
Michael B. Fein
Cozen O'Connor P.C.
1900 Market St.
Philadelphia, PA 19103
Telephone: (215) 665-5593
Facsimile: (215) 701-2005
dtrujillo@cozen.com

Paper No:_____
Filed: April 9, 2010

UNITED STATES PATENT AND TRADEMARK OFFICE

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BEFORE THE BOARD OF PATENT APPEALS
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PAUL J. **CARTER** AND LEONARD G. PRESTA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR,** DILJEET SINGH ATHWAL,
AND JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

Patent Interference No. 105,744
(Technology Center 1600)

ADAIR MOTIONS LIST

Pursuant to the Declaration of Interference, Bd. R. 120, Bd. R. 204(B), and SO ¶¶ 104.2.1., 120, and 204, Adair submits the following list of proposed motions.

1. Motion under Bd. R. 208(a)(2) and SO ¶ 208.2 to add the following count:

> A humanized antibody light chain variable domain comprising non-human Complementarity Determining Region (CDR) amino acid residues which bind antigen incorporated into a human antibody variable domain, and further comprising a Framework Region (FR) amino acid substitution at residue 58L, utilizing the numbering system set forth in Kabat.

2. Contingent upon the granting of Motion 1, a motion under Bd. R. 208(a)(2) and SO ¶ 208.2 to add the following claim to Adair's application:

> Claim 26 (new). A humanised antibody comprising a light chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises a non-human amino acid substitution at residue 58 as numbered according to Kabat.

3. Contingent upon the granting of Motion 1, a motion under Bd. R. 208(a)(2) and SO ¶ 208.2 to designate the following claims of U.S. Patent No. 6,407,213 ("the '213 patent") as corresponding to the new count: 1-3, 9, 38, and 39.

4. Motion under Bd. Rule 121(a)(1)(iii) and SO ¶ 208.6 for judgment on priority.

5. Motion under Bd. Rule 208(a)(2) and SO ¶ 208.3.1 to designate claims 65 (as corrected by the Certificate of Correction to depend from claim 79), 68, 71, 75, 76, and 82 of the '213 patent as corresponding to Count 1.

6. Motion under Bd. Rule 121(a)(1)(iii) for judgment that the claims of the '213 patent designated as corresponding to the count are unpatentable under 35 U.S.C. § 112, first paragraph, for failure to comply with the written description requirement. There is no written descriptive support in the specification of the '213 patent for the subgenus of residues recited in the involved claims. In particular, there is no written descriptive

support for the subgenus of residues recited in claim 30 regarding an antibody which binds $p185^{HER2}$.

7. Motion under Bd. R. 121(a)(1)(iii) for judgment that all involved claims of the '213 patent are unpatentable under 35 U.S.C. §102(b) as anticipated by, or, under 35 U.S.C. §103(a) as obvious over, at least one or more of the following references, or combinations thereof:

- Slide presentation by Dr. Riechmann at the "Advances in the Application of Monoclonal Antibodies in Clinical Oncology" held at the Wolfson Institute in London, England May 6-8, 1987 (as evidenced by Declaration of Dr. Riechmann submitted by Genentech, Inc. in the Opposition of European Patent No. 451,216B1, and present in the file history of the '213 patent);
- Chothia et al., *J. Mol. Biol.*, 186:651-663 (1985);
- Sheriff et al., *Proc. Natl. Acad. Sci., USA*, 84:8075-8079 (1987);
- WO 88/09344 (published December 1, 1988);
- Foote et al., *Nova acta Leopoldina 61*, 269:103-110 (1989);
- Queen et al., *Proc. Natl. Acad. Sci. USA*, 86:10029-10033 (1989);
- WO 90/07861 (published July 26, 1990);
- Co et al., *Proc. Natl. Acad. Sci.,*88:2869-2873, April, 1991;

Adair's position is that the '213 patent is not entitled to priority earlier than its filing date of June 15, 1992. If the '213 patent is entitled to priority, then WO 90/07861 and Co et al. become references under 35 U.S.C. §102(a).

8. Motion under Bd. R. 121(a)(1)(iii) for judgment that claims of the '213 patent are unpatentable under 35 U.S.C. §102(e) as anticipated by, or, under 35 U.S.C. §103(a)

as obvious over, at least one or more of the following references, or combinations thereof with each other or in combination with one or more of the references listed in paragraph 7:

- U.S. Patent No. 5,132,405 (priority to May 21, 1987);
- U.S. Patent No. 6,548,640 (priority to May 3, 1988);
- U.S. Patent No. 6,632,927 (priority to December 21, 1990); and
- U.S. Patent No. 5,859,205 (priority to December 21, 1990).

9. Motion under Bd. R. 208(a)(2) and SO 208.2 to add an additional count, wherein the additional count is directed to the subject matter of claim 1 of U.S. Patent No. 6,639,055 ("the '055 patent") or claim 24 of U.S. Application Serial No. 11/284,260. The '055 patent claims priority to the '213 patent. Adair is currently pursuing an interference with the '055 patent in their Application Serial No. 11/284,260.

10. Contingent upon the granting of Motion 9, a motion under Bd. R. 208(a)(2) and 203(d) and SO ¶¶ 123 and 203.2 to add the '055 patent.

11. Contingent upon the granting of Motion 9, a motion under Bd. R. 208(a)(2) and 203(d) and SO ¶¶ 123 and 203.2 to add Adair Application Serial No. 11/284,260.

Respectfully submitted,

/Doreen Yatko Trujillo/
DOREEN YATKO TRUJILLO
Registration No. 35,719
Lead Counsel for Adair

Date: April 9, 2010

Cozen O'Connor P.C.
1900 Market St.
Philadelphia, PA 19103
Telephone: (215) 665-5593
Facsimile: (215) 701-2005
dtrujillo@cozen.com

Certificate of Service

This will certify that a true copy of this paper was served this date on the party Carter by electronic mail directed to:

Oliver R. Ashe, Jr.
ASHE, P.C.
11440 Isaac Newton Square North
Suite 210
Reston, VA 20190
Tel.: (703)467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

Date: April 9, 2010

/Doreen Yatko Trujillo/
Doreen Yatko Trujillo

Paper No. _____

Filed on behalf of: Party Carter

Filed: April 9, 2010

By: Oliver R. Ashe, Jr., Esq.
ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

Jeffrey P. Kushan, Esq.
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, DC 20005
Tel.: (202) 736-8914
Fax: (202) 736-8711
E-mail: jkushan@sidley.com

UNITED STATES PATENT AND TRADEMARK OFFICE

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BEFORE THE BOARD OF PATENT APPEALS
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PAUL J. **CARTER** AND LEONARD G. PRESTA
Junior Party
(Patent 6,407,213),

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JOHN ROBERT **ADAIR**, DILJEET SINGH ATHWAL,
AND JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

_____

Patent Interference 105,744 (SGL)
Technology Center 1600

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CARTER MOTIONS LIST

CARTER MOTIONS LIST

Pursuant to Part D of the Declaration of Interference (Paper No. 1), 37 C.F.R. §§ 41.120 and 204, and ¶¶ 104.2.1, 120, and 204 of the Standing Order (Paper No. 2), Carter herein provides a list of motions that Carter presently intends to file.

1. A motion under 37 C.F.R. § 41.121(a)(1)(iii) seeking judgment that Adair's claim 24 is not patentable to Adair under 35 U.S.C. § 135(b)(1). The claims presented by Adair prior to the critical date were not patentable to Adair and/or were not the same as or for the same or substantially the same subject matter as any of the involved claims of Carter's U.S. Patent No. 6,407,213 ("the '213 patent"). Adair's involved claim 24 was presented over three years after the issuance of the involved Carter '213 patent and is not entitled to the benefit of any pre-critical date claim. For at least these reasons, Adair claim 24 is barred under 35 U.S.C. § 135(b)(1). This motion should be treated as raising a threshold issue. 37 C.F.R. § 41.201.

2. A motion under 37 C.F.R. § 41.121(a)(1)(iii) seeking judgment that Adair's involved claim 24 is not patentable under 35 U.S.C. § 112, first paragraph, for failure to satisfy the written description requirement. Adair's involved specification does not describe a humanized antibody wherein a "framework region comprises a non-human amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof" as recited in Adair's involved claim 24. This motion should be treated as raising a threshold issue. 37 C.F.R. § 41.201.

3. A motion under 37 C.F.R. § 41.121(a)(1)(iii) seeking judgment that Adair's involved claim 24 is not patentable under 35 U.S.C. § 112, second paragraph, because it is indefinite in its recitation of a humanized antibody wherein a "framework region comprises a

non-human amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof."

4. A motion under 37 C.F.R. § 41.121(a)(1)(iii) seeking judgment that, applying a two-way test, an interference-in-fact does not exist between Adair's involved claim 24 and any of Carter's involved patent claims. This motion should be treated as raising a threshold issue. 37 C.F.R. § 41.201.

5. A motion under 37 C.F.R. §§ 41.121(a)(1)(i) and 41.208(a)(2) to designate Carter's involved '213 patent claims 30, 31, 60, 62, 63, 67, 80 and 81 as not corresponding to Count 1. Count 1, if treated as prior art, would not have anticipated or rendered obvious the subject matter of these claims.

6. A motion under 37 C.F.R. §§ 41.121(a)(1)(ii) and 41.208(a)(3) to deny Adair priority benefit of Adair U.K. Application No. 8928874.0 and/or PCT/GB90/02017 because the applications do not provide a described and/or enabled anticipation under 35 U.S.C. 102(g)(1) of the subject matter of Count 1.

7. A motion under 37 C.F.R. § 41.121(a)(1)(iii) seeking judgment that Adair's involved claim 24 is not patentable under 35 U.S.C. §§ 102 and/or 103. The primary references to be addressed in the motion are Chothia et al., *Nature*, 342:877-883 (1989) and Tramontano et al., *J. Mol. Biol.,* 215:175-182 (1990) and, possibly, other related evidence under 35 U.S.C. 102(g). This motion may, in part, address whether Adair claim 24 is entitled to the benefit of one or more of its earlier-filed applications under 35 U.S.C. §§ 119/120. In the event Carter determines that additional primary references will be addressed in this motion, Carter will promptly give notice to Adair and the Board and serve copies of such references on Adair.

8. A motion under 37 C.F.R. § 41.208(a)(4) seeking judgment based on derivation and/or priority.

9. Pursuant to 37 C.F.R. § 41.127(a)(1), the Board should be aware that Adair has requested an interference between Adair's U.S. Patent Application 11/284,260 ("the '260 application") and Carter's U.S. Patent 6,639,055 ("the '055 patent"). In an Office Action dated December 28, 2009, concerning the Adair '260 application, the Examiner correctly noted that examination of the Adair application has not been completed as required under 37 C.F.R. § 41.102(a) and that consideration of a potential interference is premature, citing MPEP 2303. Moreover, in the event such an interference is declared, Carter would maintain that Adair lacks standing to provoke or maintain an interference with the Carter '055 patent and that Adair's claim is not patentable to Adair.

Respectfully submitted,

April 9, 2010

/Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.
Registration No. 40,491
Counsel for Party Carter

ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

CERTIFICATE OF FILING

The undersigned certifies that a copy of the paper entitled **"CARTER MOTIONS LIST"** was filed this 9th day of April, 2010, by e-mail, to:

The Board of Patent Appeals and Interferences
Madison Building East, 9th Floor
600 Dulany Street
Alexandria, VA 22314
Tel.: 571-272-9797
Fax: 571-273-0042
E-mail: BoxInterferences@USPTO.GOV

April 9, 2010 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

CERTIFICATE OF SERVICE

The undersigned hereby certifies that a copy of the paper entitled **"CARTER MOTIONS LIST"** was served this 9th day of April, 2010, by e-mail, on the Attorney of Record for Adair:

Doreen Yatko Trujillo, Esq.
Cozen O'Connor P.C.
1900 Market Street, 7th Floor
Philadelphia, PA 19103
Tel.: 215-665-6593
Fax: 215-701-2005
E-mail: dtrujillo@cozen.com

April 9, 2010 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

Paper No. _____
Filed on behalf of: Party Carter
Filed: April 26, 2010

By: Oliver R. Ashe, Jr., Esq.
ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

Jeffrey P. Kushan, Esq.
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, DC 20005
Tel.: (202) 736-8914
Fax: (202) 736-8711
E-mail: jkushan@sidley.com

UNITED STATES PATENT AND TRADEMARK OFFICE

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AND JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

_____

Patent Interference 105,744 (SGL)
Technology Center 1600

_____

CARTER'S TEST DOCUMENT PURSUANT TO WEB PORTAL INSTRUCTIONS

Mail Stop Interference
P.O. Box 1450
Alexandria Va 22313-1450
Tel: 571-272-4683
Fax: 571-273-0042

Filed 27 April 2010

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
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PAUL J. **CARTER** AND LEONARD G. PRESTIA
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and JOHN SPENCER EMTAGE
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Patent Interference No. 105,744
(Technology Center 1600)

Order - Motion Times - Bd.R. 104(c)

A. Conference call

A conference call was held on 22 April 2010 at approximately 2:00 p.m.

Participating in the call were:

(1) Oliver Ashe and Jeffrey Kushan for Carter,

(2) Doreen Trujillo for Adair, and

(3) Sally Gardner Lane, Administrative Patent Judge.

B. Relevant discussion during conference call

The principal purpose of the conference call was to set times for taking action on motions (other than miscellaneous motions) in the interference. Each party has filed a list of motions that it wishes to file.

Modified schedule

At the beginning of the call the Ms. Trujillo, counsel for Adair informed the Board that she soon will be undergoing hip replacement surgery and does not expect to return to work until the beginning part of June. Mr. Ashe, counsel for Carter, expressed a willingness to cooperate and adjust the schedule to accommodate Ms. Trujillo's unavailability.

Given the particular circumstances, the schedule has been adjusted so that Carter will file its threshold motions first and, if necessary, remaining motions will be filed at a time to allow Ms. Trujillo adequate time to prepare the authorized motions after returning to work. However, as discussed during the call, if, in light of either of the Carter threshold motions, an Adair opposition or responsive motion is authorized, it would be expected that the opposition or responsive motion would be filed as soon as possible (with a precise time to be set if, and when, necessary).

Adair request for a second interference

Adair has proposed an interference with Carter patent 6,639,055 in its application 11/284,260 ('260). Adair confirmed that *ex parte* prosecution in the '260 application is not complete. Declaring an interference involving the '260 claims would be premature at this time. However, Adair should inform the Board if its claims become allowable and

at that time Adair can seek authorization to file a miscellaneous motion suggesting an interference. (Bd. R. 102 and Standing Order at ¶ 203.1).

Authorized Motions

Only the motions specifically authorized in this Order may be filed. If a party finds that a responsive motion is necessary, then a conference call should be arranged well prior to the time such motion would be due.

Each party is authorized to file a motion for priority.[1] Priority motions are deferred. In addition either party may move to defer its opposition to any motion for unpatentability over prior art based upon the party's priority statement.

Carter

Expedited schedule:

Carter is authorized to file the following two threshold motions on or before **01 June 2010**:

1. A motion seeking judgment that the single Adair claim is barred under 35 U.S.C. § 135(b) (1) (item #1 on the Carter list), and

2. A motion seeking judgment that the single Adair claim is unpatentable under the written description requirement of 35 U.S.C. § 112, ¶1 (item 2 of the Carter list).

No Adair opposition or responsive motion to either of the Carter threshold motions is authorized at this time. If an opposition to either motion is necessary, a conference call will be scheduled to decide upon an appropriate time table. At that time Adair should be prepared to discuss any responsive motion it might wish to file.

1 In addition derivation briefing, if any, will be deferred. If a party wishes to allege derivation it must do so in its priority statement.

Regular schedule:

1. Carter is authorized to file the motions at items 3 (§ 112, ¶ 2), 4 (no interference in fact), 6 (benefit) and 7 (unpatentability over prior art) on its list in accordance with the schedule set out in the appendix.

Adair

Expedited schedule:

Adair has not asked to file any motion as a threshold motion.

Regular schedule:

1. Adair is authorized to file the motion at item 5 (Carter claim designations).

2. Adair is authorized to file a single motion that all the Carter claims are unpatentable over the prior art cited at items 7 and 8 of the Adair list. As discussed during the call, Adair has filed an unacceptably long list of prior art. Adair agreed that, at a reasonable time prior to the time it files its motion, Adair will inform Carter what references it intends to rely upon and, as to the § 103 issue, which will be relied upon as primary and which will be relied upon as secondary references. If the number of references relied upon is unreasonable the motion may be returned to Adair.

Adair requested to file other motions however Adair is not authorized to file these motions for the following reasons.

1. The motions at items 1, 9 and 10 were said to relate to the '260 application. As discussed above, because that *ex parte* prosecution has not been completed a miscellaneous motion suggesting a second interference is not authorized. (Bd. R. 102 and Standing Order at ¶ 203.1).

2. The motion at item 2 seeks to present a claim said to be directed to subject matter at claims 1-3, 9, 38 and 39 of the involved Carter patent. Adair indicated that it could claim this subject matter in a pending application. In such case, the claim(s) could be examined by a patent examiner to determine there is allowable subject matter to Adair. Since the claims can be presented elsewhere Adair is not authorized to file the motion at item 2.

4. Adair conceded that if its motion at item 6 were to be granted, the subject matter of the Count would not change. An interference is principally about the priority contest. If the granting of the motion would not change that contest, then its filing usually will not be authorized. Bd. R. 208.

Notwithstanding the Standing Order (e.g., 10 at ¶ 121.2 and ¶122.2), the page limit on motions and oppositions is 25 pages and the page limit for replies is 10 pages. The page limit does not include a table of contents, a table of authorities, a certificate of service, or required appendices **except for the page limit does include the statement of material facts (SO ¶ 121.5.2).**[2] In other words, the statement of material facts counts toward the 25 page limit.

C. Time periods associated with motions

In accordance with discussion during the telephone conference call, the TIME PERIODS described below are set out in an Appendix to this ORDER. Action specified for each TIME PERIOD must be completed by the date specified for the TIME PERIOD.

2 The page limit does not include a party's response to its opponent's statements of material facts.

The parties are authorized to stipulate different times (earlier or later, but not later than TIME PERIOD 7) for TIME PERIODS 1 through 6.[3] A notice of the stipulation must be promptly filed. The notice must be in the form of a photocopy of the Appendix attached to this ORDER with old dates crossed out and new dates inserted by hand. The parties may not stipulate an extension of TIME PERIODS 7-9.

1. TIME PERIOD 1

a. File and serve all authorized motions and

b. Serve but do not file evidence in support of these motions.

If no party files a motion, the SENIOR PARTY must arrange a conference call with the parties and the Board so that appropriate adjustments to the schedule may be made.

2. TIME PERIOD 2

a. File and serve responsive motions (Bd.R. 121(a)(2)) in response to an opponent's motion filed during TIME PERIOD 1 and

b. Serve but do not file evidence in support of these responsive motions.

3. TIME PERIOD 3

a. File and serve oppositions to all motions, including responsive motions and

[3] In stipulating different times, the parties should consider the effect of the stipulation on times (1) to object to evidence (5 business days, Bd.R. 155(b)(1)), (2) to supplement evidence (10 business days, Bd.R. 155(b)(2)), (3) to begin cross examination (no earlier than 21 days after service, SO & 157.3.1) and (4) to conclude cross examination (at least 10 days before the opposition or reply is due, SO & 157.3.2).

b. Serve but do not file evidence in support of these oppositions.

4. TIME PERIOD 4

a. File and serve replies to all oppositions and

b. Serve but do not file evidence in support of these replies.

5. TIME PERIOD 5

a. File and serve any request for oral argument on motions,

b. File and serve motions to exclude evidence (Bd.R. 155(c); SO & 155.2), and

c. File and serve observations on cross examination (SO & 157.7) of reply testimony.

6. TIME PERIOD 6

a. File and serve oppositions to an opponent's motion to exclude evidence and

b. File and serve any response to observations.

7. TIME PERIOD 7

File and serve replies to oppositions to motions to exclude evidence.

D. Deposition transcripts

Transcripts of cross examinations and depositions taken under 35 U.S.C. 24 must be served, but not filed until the exhibits are filed.

E. Serving exhibits relied upon in motions

An exhibit, including an affidavit, cited in connection with a motion, opposition, reply, or affidavit, must be served, but not filed,[4] with the motion, opposition, reply or affidavit in which the exhibit is first mentioned.

F. TIME PERIOD 8: Filing the record for decision on motions

1. File an original set of your exhibits and one working copy of your exhibits;
2. For each of your motions, file one folder (or three folders if an oral argument is set each) containing:
    a. The motion,
    b. Any corresponding opposition,
    c. Any corresponding reply,
    d. Any corresponding observations, and
    e. Any corresponding response to the observations.
3. File CD-ROM a party elects to file.

G. TIME PERIOD 9: Default oral argument date

If a request for oral argument (Bd.R. 124(a); TIME PERIOD 5) is granted, the default date for such argument is TIME PERIOD 9. No oral argument will occur if either no argument is requested or granted.

H. Priority statements

1. At TIME PERIOD 1:

[4] Except when the Board sets an expedited schedule for a particular motion, in which case, all exhibits mentioned in that motion or the corresponding opposition or reply must be filed with the motion, opposition, reply, or affidavit in which the exhibit is first mentioned.

a. File <u>but do not serve</u> a priority statement (Bd.R. 120; Bd.R. 204(a)).

b. File and serve a notice advising each opponent of the filing of the priority statement.

2. A junior party who does not file a priority statement shall not have access to the priority statement of any other party.

3. **Within one (1) week** after TIME PERIOD 1, serve a copy of the priority statement upon each opponent (except for a junior party barred under ¶ H.2 above).

/Sally Gardner Lane/
Administrative Patent Judge

Revised 3 January 2006

cc (via electronic filing):

Attorney for CARTER:

Oliver R. Ashe, Jr., Esq.
ASHE, P.C.
11440 Isaac Newton Square, North
Suite 210
Reston, VA 20190
Tel: 703-467-9001
Email: oashe@ashepc.com

Jeffrey P. Kushan, Esq.
SIDLEY AUSTIN LLP
1501 K Street,N.W.
Washington, D.C. 20005
Tel: 202-736-8914
Email: jkushan@sidley.com

Attorney for ADAIR:

Doreen Yatko Trujillo, Esq.
Michael B. Fein, Esq.
COZEN O'CONNOR P.C.
1900 Market Street
Philadelphia, PA 19103
Tel: 215-665-5593
Email: dtrujillo@cozen.com

Appendix--ORDER - RULE 123(a)
(Times for substantive motions; priority deferred)

Interference 105,744

CARTER THRESHOLD MOTIONS……**1 June 2010**

ADAIR OPPOSITION, RESPONSIVE MOTIONS……to be set if needed

TIME PERIOD 1 (all other authorized motions).....**9 August 2010**
File motions
File (but serve one week later) priority statements

TIME PERIOD 2.....**20 September 2010**
File responsive motions (none authorized at this time)
filed in TIME PERIOD 1

TIME PERIOD 3.....**2 November 2010**
File oppositions to all motions

TIME PERIOD 4.....**14 December 2010**
File all replies

TIME PERIOD 5.....**4 January 2011**
File request for oral argument
File motions to exclude
File observations

TIME PERIOD 6.....**18 January 2011**
File oppositions to motions to exclude
File response to observations

TIME PERIOD 7.....**1 February 2011**
File replies to oppositions to motions to exclude

TIME PERIOD 8.....**8 February 2011**
File exhibits
File sets of motions
File any CD-ROMs

TIME PERIOD 9.....**to be set**
Default oral argument date (if ordered)

Paper No. _____

Filed on behalf of: Party Carter

Filed: April 29, 2010

By: Oliver R. Ashe, Jr., Esq.
ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

Jeffrey P. Kushan, Esq.
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, DC 20005
Tel.: (202) 736-8914
Fax: (202) 736-8711
E-mail: jkushan@sidley.com

UNITED STATES PATENT AND TRADEMARK OFFICE

_____

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

_____

PAUL J. **CARTER** AND LEONARD G. PRESTA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR**, DILJEET SINGH ATHWAL,
AND JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

_____

Patent Interference 105,744 (SGL)
Technology Center 1600

_____

CARTER'S REQUEST FOR CLARIFICATION OF PAPER NO. 23

CARTER'S REQUEST FOR CLARIFICATION OF PAPER NO. 23

The Order - Motion Times - Bd. R. 104(c) dated April 27, 2010 (Paper No. 23) does not expressly address the proposed motion identified in item 5 of Carter Motions List (to designate Carter's involved claims 30, 31, 60, 62-63, 67, and 80-81 as not corresponding to Count 1). Carter respectfully requests the Board to clarify whether Carter is authorized to file such a motion.

Respectfully submitted,

April 29, 2010

/Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.
Registration No. 40,491
Counsel for Party Carter

ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

CERTIFICATE OF FILING

The undersigned certifies that a copy of the paper entitled **"CARTER'S REQUEST FOR CLARIFICATION OF PAPER NO. 23"** was filed this 29th day of April, 2010, via Interference Web Portal (https://acts.uspto.gov/ifiling/), with:

The Board of Patent Appeals and Interferences
Madison Building East, 9th Floor
600 Dulany Street
Alexandria, VA 22314
Tel: 571-272-4683
Fax: 571-273-0042
E-mail: BoxInterferences@USPTO.GOV

April 29, 2010 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

CERTIFICATE OF SERVICE

The undersigned hereby certifies that a copy of the paper entitled **"CARTER'S REQUEST FOR CLARIFICATION OF PAPER NO. 23"** was served this 29th day of April, 2010, via Interference Web Portal (https://acts.uspto.gov/ifiling/), on the Attorney of Record for Adair:

Doreen Yatko Trujillo, Esq.
Cozen O'Connor P.C.
1900 Market Street, 7th Floor
Philadelphia, PA 19103
Tel.: 215-665-6593
Fax: 215-701-2005
E-mail: dtrujillo@cozen.com

April 29, 2010 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

Mail Stop Interference
P.O. Box 1450
Alexandria Va 22313-1450
Tel: 571-272-4683
Fax: 571-273-0042

Filed 3 May 2010

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

PAUL J. **CARTER** AND LEONARD G. PRESTIA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR**, DILGEET SINGH ATHWAL,
and JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

Patent Interference No. 105,744
(Technology Center 1600)

ORDER – Miscellaneous – 104(a)

Carter requests clarification (Paper 24) of the Order setting motions times. (Paper 23). As noted by Carter, the Order does not discuss whether Carter is authorized to file the proposed motion to have certain Carter claims designated as not corresponding to the Count (item 5 on the Carter list).

It is

ORDERED that Carter is authorized to file a motion to designate Carter claims 30, 31, 60, 62-63, 67, and 80-81 as not corresponding to the Count; and

FURTHER ORDERED that the motion shall be filed on the regular (non-expedited) schedule.

/Sally Gardner Lane/
Administrative Patent Judge

cc (via electronic filing):

Attorney for CARTER:

Oliver R. Ashe, Jr., Esq.
ASHE, P.C.
11440 Isaac Newton Square, North
Suite 210
Reston, VA 20190
Tel: 703-467-9001
Email: oashe@ashepc.com

Jeffrey P. Kushan, Esq.
SIDLEY AUSTIN LLP
1501 K Street,N.W.
Washington, D.C. 20005
Tel: 202-736-8914
Email: jkushan@sidley.com

Attorney for ADAIR:

Doreen Yatko Trujillo, Esq.
Michael B. Fein, Esq.
COZEN O'CONNOR P.C.
1900 Market Street
Philadelphia, PA 19103
Tel: 215-665-5593
Email: dtrujillo@cozen.com

(12) **United States Patent**
Carter et al.

(10) **Patent No.: US 6,407,213 B1**
(45) **Date of Patent: Jun. 18, 2002**

(54) **METHOD FOR MAKING HUMANIZED ANTIBODIES**

(75) Inventors: **Paul J. Carter**; **Leonard G. Presta**, both of San Francisco, CA (US)

(73) Assignee: **Genentech, Inc.**, South San Francisco, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **08/146,206**

(22) PCT Filed: **Jun. 15, 1992**

(86) PCT No.: **PCT/US92/05126**

§ 371 (c)(1),
(2), (4) Date: **Nov. 17, 1993**

Related U.S. Application Data

(63) Continuation-in-part of application No. 07/715,272, filed on Jun. 14, 1991, now abandoned.

(51) **Int. Cl.**[7] **C07K 16/00**
(52) **U.S. Cl.** **530/387.3**; 435/69.6; 435/69.7; 435/70.21; 435/91; 536/23.53; 424/133.1
(58) **Field of Search** 435/69.6, 69.7, 435/70.21, 91, 172.2, 240.1, 240.27, 252.3, 320.1, 328; 536/23.53; 424/133.1; 530/387.3

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,816,567	A	3/1989	Cabilly et al.	
4,845,198	A	7/1989	Urdal et al.	530/388.22
5,132,405	A	7/1992	Huston et al.	530/387.3
5,225,539	A	7/1993	Winter	530/389.3
5,530,101	A	6/1996	Queen et al.	530/387.3
5,558,864	A	9/1996	Bendig et al.	424/133.1
5,585,089	A	12/1996	Queen et al.	424/133.1
5,677,171	A	10/1997	Hudziak et al.	435/7.23
5,693,762	A *	12/1997	Queen et al.	530/387.2
5,714,350	A	2/1998	Co et al.	435/69.6
5,772,997	A	6/1998	Hudziak et al.	424/130.1
5,821,337	A	10/1998	Carter et al.	530/387.3
5,834,598	A	11/1998	Lowman et al.	530/399
5,859,205	A	1/1999	Adair et al.	530/387.3

FOREIGN PATENT DOCUMENTS

AU	85058/91	3/1992	C07K/15/12
EP	120694	10/1984	
EP	125023 A1	11/1984	
EP	0 239 400 *	9/1987	C12N/15/00
EP	323806 A1	7/1989	
EP	328404 A1	8/1989	A61K/39/395
EP	338745 A1	10/1989	
EP	365209 A2	4/1990	
EP	365997 A2	5/1990	
EP	368684	5/1990	
EP	403156 A1	12/1990	
EP	438310 A2	7/1991	
EP	438312 A2	7/1991	
EP	440351 A2	8/1991	
EP	0 460 167 B1	12/1991	
EP	0 519 596 A1	12/1992	
EP	0 592 106 A1	4/1994	
EP	0 620 276	10/1994	
EP	682040 A1	11/1995	
EP	451216 B1	1/1996	C12P/21/08
EP	432249 B1	9/1996	
GB	2 188941	10/1987	
WO	WO 87/02671	5/1987	
WO	WO 88/09344	12/1988	
WO	WO 89/01783	3/1989	
WO	WO 89/06692	7/1989	
WO	WO 89/09622	10/1989	
WO	WO 90/07861	7/1990	
WO	90/07861 *	7/1990	C12P/21/00
WO	WO 91/07492	5/1991	
WO	WO 91/07500	5/1991	
WO	WO 91/09966	7/1991	C12P/21/08
WO	WO 91/09968	7/1991	C12P/21/08
WO	WO 91/09967	11/1991	
WO	WO 92/01047	1/1992	
WO	WO 92/04380	3/1992	
WO	WO 92/04381	3/1992	
WO	WO 92/05274	4/1992	
WO	WO 92/11383	7/1992	
WO	WO 92/11018	9/1992	A61K/35/14
WO	WO 92/15683	9/1992	
WO	WO 92/16562	10/1992	
WO	WO 92/22653	12/1992	
WO	WO 93/02191	2/1993	
WO	94/11509	5/1994	
WO	WO 94/12214	6/1994	

OTHER PUBLICATIONS

Riechmann et al. [Nature 332:323–327 (1988)].*
Queen et al. [Proc. Natl. Acad. Sci. 86:10029–10033 (1989)].*
Roitt [*Immunology*, published 1985, by Gower Medical Publishing Ltd. (London, England) p. 5.5].*
Tramontano et al. [J. Mol. Biol. 215:175–182 (1990)].*
"Biosym Technologies" in New Products, Chemical Design Automation 3 (Dec. 1988).
"Polygen Corporation" in New Products, Chemical Design Automation 3 (Nov. 1988).
Adair et al., "Humanization of the murine anti–human CD3 monoclonal antibody OKT3" *Hum. Antibod. Hybridomas* 5:41–47 (1994).
Chothia et al., "Principles of protein–protein recognition" *Nature* 256:705–708 (1975).
Chothia et al., "Transmission of conformational change in insulin" *Nature* 302:500–505 (1983).
Corti et al., "Idiotope Determining Regions of a Mouse Monoclonal Antibody and Its Humanized Versions" *J. Mol. Biol.* 235:53–60 (1994).

(List continued on next page.)

Primary Examiner—Anthony C. Caputa
Assistant Examiner—Minh-Tam Davis
(74) *Attorney, Agent, or Firm*—Wendy M. Lee

(57) **ABSTRACT**

Variant immunoglobulins, particularly humanized antibody polypeptides are provided, along with methods for their preparation and use. Consensus immunoglobulin sequences and structural models are also provided.

82 Claims, 9 Drawing Sheets

OTHER PUBLICATIONS

Couto et al., "Anti–BA46 Monoclonal Antibody Mc3 Humanization Using a Novel Positional Consensus and in Vivo and in Vitro Characterization" *Cancer Research Supplement* 55:1717–1722 (1995).
Couto et al., "Humanization of KC4G3, an Anti–Human Carcinoma Antibody" *Hybridoma* 13:215–219 (1994).
Ellis et al., "Engineered Anti–CD38 Monoclonal Antibodies for Immunotherapy of Multiple Myeloma" *The Journal of Immunology* pp. 925–937 (1995).
Hieter et al., "Evolution of Human Immunoglobulin K J Region Genes" *The Journal of Biological Chemistry* 257:1516–1522 (1982).
Lesk, Arthur M., "How Different Amino Acid Sequences Determine Similar Protein Structures: The Structure and Evolutionary Dynamics of the Globins" *J. Mol. Biol.* 135:225–270 (1980).
Matsumura et al., "Hydrophobic stabilization in T4 lysozyme determined directly by multiple substitutions of Ile 3" *Nature* 334:406–410 (1988).
Morrison, S. L., "Transfectomas Provide Novel Chimeric Antibodies" *Science* 229:1202–1207 (Sep. 20, 1985).
Nakatani et al., "Humanization of mouse anti–human IL–2 receptor antibody B–B10" *Protein Engineering* 7:435–443 (1994).
Ohtomo et al., "Humanization of Mouse ONS–M21 Antibody with the Aid of Hybrid Variable Regions" *Molecular Immunology* 32:407–416 (1995).
Rodrigues et al., "Engineering a humanized bispecific $F(ab')_2$ fragment for improved binding to T cells" *Int. J. Cancer* (Suppl.) 7:45–50 (1992).
Sha et al., "A Heavy–Chain Grafted Antibody that Recognizes the Tumor–Associated TAG72 Antigen" *Cancer Biotherapy* 9:341–349 (1994).
Tempest et al., "Identification of framework residues required to restore antigen binding during reshaping of a monoclonal antibody against the glycoprotein gB of human cytomegalovirus" *Int. J. Biol. Macromol.* 17:37–42 (1995).
Tramontano, "Structural Determinants of the Conformations of Medium–Sized Loops in Proteins" *Proteins* 6:382–394 (1989).
Uchiyama et al., "A Monoclonal Antibody (Anti–Tac) Reactive with Activated and Functionally Mature Human T Cells" *Journal of Immunology* 126:1393–1397 (1981).
Vincenti et al., "Interleukin–2–Receptor Blockade with Daclizumab to Prevent Acute Rejection in Renal Transplantation" *New Engl. J. Med.* 338:161–165 (1998).
Vitetta et al., "Redesigning Nature's Poisons to Create Anti–Tumor Reagents" *Science* 238:1098–1104 (1987).
Waldmann et al., "Interleukin 2 Receptor (Tac Antigen) Expression in HTLV–1–associated Adult T–Cell Leukemia" *Cancer Research* 45:4559s–4562s (1985).
Waldmann, Thomas A., "The Structure, Function, and Expression of Interleukin–2 Receptors on Normal and Malignant Lymphocytes" *Science* 232:727–732 (1986).
Wu et al., "An Analysis of the Sequences of the Variable Regions of Bence Jones Proteins and Myeloma Light Chains and Their Implications for Antibody Complementarity" *Journal of Experimental Medicine* 132:211–250 (1970).
Rhodes, P., "Recombinant antibodies from CHO cells" *Abstr Pap Am Chem Soc* (Abstract No. 60 from the 199th American Chemical Society National Meeting held in Boston, MA Apr. 22–27, 1990) 199(1–2):BIOT 60 (Apr. 1990).
Amzel and Poljak, "Three–dimensional structure of immunoglobulins" *Ann. Rev. Biochem.* 48:961–967 (1979).
Bindon et al., "Human monoclonal IgG isotypes differ in complement activating function at the level of C4 as well as C1q" *Journal of Experimental Medicine* 168(1):127–142 (Jul. 1988).
Boulianne, G. L. et al., "Production of functional chimaeric mouse/human antibody" *Nature* 312(5995):643–646 (Dec. 1984).
Brown et al., "Anti–Tac–H, a humanized antibody to the interleukin 2 receptor, prolongs primate cardiac allograft survival" *Proc. Natl. Acad. Sci. USA* 88:2663–2667 (1991).
Bruccoleri, "Structure of antibody hypervariable loops reproduced by a conformational search algorithm" *Nature* (erratum to article in Nature 335(6190):564–568 and) 336:266 (1988).
Bruggemann, M. et al., "Comparison of the effector functions of human immunoglobulins using a matched set of chimeric antibodies" *Journal of Experimental Medicine* 166:1351–1361 (1987).
Burgess et al., "Possible Dissociation of the Heparin–binding and Mitogenic Activities of Heparin–binding (Acidic Fibroblast) Growth Factor–1 from Its Receptor–binding Activities by Site–directed Mutagenesis of a Single Lysine Residue" *Journal of Cell Biology* 111:2129–2138 (1990).
Carter et al., "Humanization of an anti–$p185^{HER2}$ antibody for human cancer therapy" *Proc. Natl. Acad. Sci.* 89:4285–4289 (1992).
Cheetham, J., "Reshaping the antibody combining site by CDR replacement–tailoting or tinkering to fit?" *Protein Engineering* 2(3):170–172 (1988).
Chothia and Lesk, "Canonical Structures for the Hypervariable Regions" *J. Mol. Biol.* 196:901–917 (1987).
Chothia et al., "The predicted structure of immunoglobulin D1.3 and its comparison with the crystal structure" *Science* 233:755–758 (Aug. 15, 1986).
Chothia, C. et al., "Conformations of immunoglobulin hypervariable regions" *Nature* 342(6252):877–883 (1989).
Chothia, Cyrus, "Domain association in immunoglobulin molecules: The packing of variable domains" *J. Mol. Biol.* 186:651–663 (1985).
Clark et al., "The improved lytic function and in vivo efficacy of monovalent monoclonal CD3 antibodies" *European Journal of Immunology* 19:381–388 (1989).
Co et al., "Humanized antibodies for antiviral therapy" *Proc. Natl. Acad. Sci. USA* 88:2869–2873 (1991).
Coussens et al., "Tyrosine Kinase Receptor with Extensive Homology to EGF Receptor Shares Chromosomal Location with neu Oncogene" *Science* 230:1132–1139 (1985).
Daugherty, BL et al., "Polymerase chain reaction facilitates the cloning, CDR–grafting, and rapid expression of a murine monoclonal antibody directed against the CD18 component of leukocyte integrins" *Nucleic Acids Research* 19(9):2471–2476 (May 11, 1991).
Davies, D. R. et al., "Antibody–Antigen Complexes" *Ann. Rev. Biochem.* 59:439–473 (1990).
Epp et al., "The molecular structure of a dimer composed of the variable portions of the Bence–Jones protein REI refined at 2.0–A resolution" *Biochemistry* 14(22):4943–4952 (1975).
Fendly et al., "Characterization of murine monoclonal antibodies reactive to either the human epidermal growth factor receptor or HER2/neu gene product" *Cancer Research* 50:1550–1558 (1990).

Furey et al., "Structure of a novel Bence–Jones protein (Rhe) fragment at 1.6 A resolution" *J. Mol. Biol.* 167(3):661–692 (Jul. 5, 1983).

Gorman, SD et al., "Reshaping a therapeutic CD4 antibody" *Proc. Natl. Acad. Sci. USA* 88(10):4181–4185 (May 15, 1991).

Gregory et al., "The solution conformations of the subclasses of human IgG deduced from sedimentation and small angle X–ray scattering studies" *Molecular Immunology* 24(8):821–829 (Aug. 1987).

Hale et al., "Remission induction in non–hodgkin lymphoma with reshaped human monoclonal antibody campath–1H" *Lancet* 1:1394–1399 (1988).

Harris and Emery, "Therapeutic antibodies—the coming of age" *Tibtech* 11:42–44 (Feb. 1993).

Huber et al., "Crystallographic structure studies of an IgG molecule and an Fc fragment" *Nature* 264:415–420 (Dec. 2, 1976).

Hudziak et al., "p185^{HER2} Monoclonal Antibody Has Antiproliferative Effects In Vitro and Sensitizes Human Breast Tumor Cells to Tumor Necrosis Factor" *Molecular & Cellular Biology* 9(3):1165–1172 (1989).

Jaffers, G. J. et al., "Monoclonal antibody therapy. Anti–idiotypic and non–anti–idiotypic antibodies to OKT3 arising despite intense immunosuppression" *Transplantation* 41(5):572–578 (May 1986).

Jones, P. T. et al., "Replacing the complementarity–determining regions in a human antibody with those from a mouse" *Nature* 321(6069):522–525 (1986).

Junghans et al., "Anti–Tac–H, a humanized antibody to the interleukin 2 receptor with new features for immunotherapy in malignant and immune disorders" *Cancer Research* 50(5):1495–1502 (Mar. 1, 1990).

Kabat et al. *Sequences of Proteins of Immunological Interest,* Bethesda, MD:National Institutes of Health pp. iii–xxvii, 41–176 (1987).

King et al., "Amplification of a Novel v–erbB–Related Gene in a Human Mammary Carcinoma" *Science* 229:974–976 (1985).

Lazar et al., "Transforming Growth Factor α: Mutation of Aspartic Acid 47 and Leucine 48 Results in Different Biological Activities" *Molecular & Cellular Biology* 8(3):1247–1252 (1988).

Love et al, "Recombinant antibodies possessing novel effector functions" *Methods in Enzymology* 178:515–527 (1989).

Lupu et al., "Direct interaction of a ligand for the erbB2 oncogene product with the EGF receptor and p185^{erbB2}" *Science* 249:1552–1555 (1990).

Margni RA and Binaghi RA, "Nonprecipitating asymmetric antibodies" *Ann. Rev. Immunol.* 6:535–554 (1988).

Margolies et al., "Diversity of light chain variable region sequences among rabbit antibodies elicited by the same antigens." *Proc. Natl. Acad. Sci. USA* 72:2180–84 (Jun. 1975).

Marquart et al., "Crystallographic refinement and atomic models of the intact immunoglobulin molecule Kol and its antigen–binding fragment at 3.0 A and 1.0 A resolution" *J. Mol. Biol.* 141(4):369–391 (Aug. 25, 1980).

Mian, IS et al., "Structure, function and properties of antibody binding sites" *J. Mol. Biol.* 217(1):133–151 (Jan. 5, 1991).

Miller, R. et al., "Monoclonal antibody therapeutic trials in seven patients with T–cell lymphoma" *Blood* 62:988–995 (1983).

Morrison, S. L. et al., "Chimeric human antibody molecules: mouse antigen–binding domains with human constant region domains" *Proc. Natl. Acad. Sci. USA* 81(21):6851–6855 (Nov. 1984).

Neuberger et al., "Recombinant antibodies possessing novel effector functions" *Nature* 312(5995):604–608 (Dec. 1984).

Neuberger, M. S. et al., "A hapten–specific chimaeric IgE antibody with human physiological effector function" *Nature* 314(6008):268–270 (Mar. 1985).

Novotny and Haber, "Structural invariants of antigen binding: comparison of immunoglobulin V_L–V_H and V_L–V_L domain dimers" *Proc. Natl. Acad. Sci. USA* 82(14):4592–4596 (Jul. 1985).

Pluckthun, Andreas, "Antibody engineering: advances from the use of *Escherichia coli* expression systems" *Biotechnology* 9:545–51 (1991).

Queen, M. et al., "A humanized antibody that binds to the interleukin 2 receptor" *Proc. Natl. Acad. Sci. USA* 86:10029–10033 (1989).

Riechmann, L. et al., "Reshaping human antibodies for therapy" *Nature* 332:323–327 (1988).

Roitt et al. *Immunology* (Gower Medical Publishing Ltd., London, England) pp. 5.5 (1985).

Saul et al., "Preliminary refinement and structural analysis of the Fab fragment from human immunoglobulin new at 2.0 A resolution" *Journal of Biological Chemistry* 253(2):585–597 (Jan. 25, 1978).

Schroff, R. et al., "Human anti–murine immunoglobulin responses in patients receiving monoclonal antibody therapy" *Cancer Research* 45:879–885 (1985).

Segal et al., "The three–dimensional structure of a phosphorylcholine–binding mouse immunoglobulin Fab and the nature of the antigen binding site" *Proc. Natl. Acad. Sci. USA* 71(11):4298–4302 (Nov. 1974).

Shalaby et al., "Development of humanized bispecific antibodies reactive with cytotoxic lymphocytes and tumor cells overexpressing the HER2 protooncogene" *Journal of Experimental Medicine* 175(1):217–225 (Jan. 1, 1992).

Shepard and Lewis, "Resistance of tumor cells to tumor necrosis factor" *J. Clin. Immunol.* 8(5):333–395 (1988).

Sheriff et al., "Three–dimensional structure of an antibody–antigen complex" *Proc. Natl. Acad. Sci. USA* 84(22):8075–8079 (Nov. 1987).

Sherman et al., "Haloperidol binding to monoclonal antibodies" *Journal of Biological Chemistry* 263:4064–4074 (1988).

Silverton et al., "Three–dimensional structure of an intact human immunoglobulin" *Proc. Natl. Acad. Sci. USA* 74:5140–5144 (1977).

Slamon et al., "Human Breast Cancer: Correlation of Relapse and Survival with Amplification of the HER–2/neu Oncogene" *Science* 235:177–182 (1987).

Slamon et al., "Studies of the HER–2/neu proto–oncogene in human breast and ovarian cancer" *Science* 244:707–712 (1989).

Snow and Amzel, "Calculating three–dimensional changes in protein structure due to amino–acid substitutions: the variable region of immunoglobulins" *Protein: Structure, Function, and Genetics,* Alan R. Liss, Inc. vol. 1:267–279 (1986).

Sox et al., "Attachment of carbohydrate to the variable region of myeloma immunoglubulin light chains" *Proc. Natl. Acad. Sci. USA* 66:975–82 (Jul. 1970).

Spiegelberg et al., "Localization of the carbohydrate within the variable region of light and heavy chains of human γG myeloma proteins" *Biochemistry* 9:4217–23 (Oct. 1970).
Takeda et al., "Construction of chimaeric processed immunoglobulin genes containing mouse variable and human constant region sequences" *Nature* 314(6010):452–454 (Apr. 1985).
Tao et al., "Role of Carbohydrate in the Structure and Effector Functions Mediated by the H uman IgG Constant Region" *J. Immunol.* 143(8):2595–2601 (1989).
Tramontano et al., "Framework residue 71 is a major determinant of the position and conformation of the second hypervariable region in the VH domains of immunoglobulins" *J–Mol–Biol* 215(1):175–182 (Sep. 5, 1990).
Verhoeyen, M. et al., "Reshaping human antibodies: grafting an antilysozyme activity" *Science* 239(4847):1534–1536 (Mar. 25, 1988).
Waldmann, T., "Monoclonal antibodies in diagnosis and therapy" *Science* 252:1657–1662 (1991).
Wallick et al., "Glycosylation of a VH residue of a monoclonal antibody against alpha (1——6) dextran increases its affinity for antigen" *Journal of Experimental Medicine* 168(3):1099–1109 (Sep. 1988).
Winter and Milstein, "Man–made antibodies" *Nature* 349(6307):293–299 (Jan. 24, 1991).
Yamamoto et al., "Similarity of protein encoded by the human c–erb–B–2 gene to epidermal growth factor receptor" *Nature* 319:230–34 (1986).
Carter et al., "High level *escherichia coli* expression and production of a bivalent humanized antibody fragment" *Bio/Technology* 10:163–167 (1992).
Foote et al., "Antibody Framework Residues Affecting the Conformation of the Hypervariable Loops" *J. Mol. Biol.* 224:487–499 (1992).
Foote, J., "Humanized Antibodies" *Nova acta Leopoldina* 61(269):103–110 (1989).
Kabat et al., "Sequences of Proteins of Immunological Interest", Bethesda, MD:National Institute of Health pp. 14–32 (1983).
Kettleborough et al., "Humanization of a Mouse Monoclonal Antibody by CDR–grafting: the Importance of Framework Residues on Loop Conformation" *Protein Engineering* 4(7):773–783 (1991).
Maeda et al., "Construction of Reshaped Human Antibodies with HIV–neutralizing Activity" *Hum. Antibod. Hybridomas* 2:124–134 (Jul. 1991).
Riechmann et al, "Expression of an Antibody Fv Fragment in Myeloma Cells" *J. Mol. Biol.* 203:825–828 (1988).
Routledge et al., "A Humanized Monovalent CD3 Antibody which Can Activate Homologous Complement" *European Journal of Immunology* 21:2717–2725 (1991).
Shearman et al., "Construction, Expression and Characterization of Humanized Antibodies Directed Against the Human α/β T Cell Receptor" *J. Immunol.* 147(12):4366–4373 (Dec. 15, 1991).
Tempest et al., "Reshaping a Human Monoclonal Antibody to Inhibit Human Respiratory Syncytial Virus Infection In Vivo" *Bio/Technology* 9:266–271 (Mar. 1991).
Brown, Jr. et al., "Anti–Tac–H, a humanized antibody to the interleukin 2 receptor, prolongs primate cardiac allograft survival" *Proc. Natl. Acad. Sci. USA* 88:2663–2667 (1991).
Casale et al., "Use of an anti–IgE humanized monoclonal antibody in ragweed–induced allergic rhinitis" *J. Allergy Clin. Immunol.* 100:110–121 (1997).
Fahy et al., "The Effect of an Anti–IgE Monoclonal Antibody on the Early– and Late–Phase Responses to Allergen Inhalation in Asthmatic Subjects" *Am J. Respir. Crit. Care Med* 155:1828–1834 (1997).
Mathieson et al., "Monoclonal–Antibody Therapy in Systemic Vasculitis" *New England J. of Medicine* pp. 250–254 (Jul. 1990).
Presta et al., "Humanization of an anti–vascular endothelial growth factor monoclonal antibody for the therapy of solid tumors and other disorders" *Cancer Research* 57(20):4593–4599 (Oct. 15, 1997).
Amit et al., "Three–Dimensional Structure of an Antigen–Antibody Complex at 2.8 A Resolution" *Science* 233:747–753 (Aug. 1986).
Amzel et al., "The Three Dimensional Structure of a Combining Region–Ligand Complex of Immunglobulin New at 3.5–A Resolution" *Proc. Natl. Acad. Sci. USA* 71(4):1427–1430 (Apr. 1974).
Baselga et al., "Phase II Study of Weekly Intravenous Recombinant Humanized Anti–p185/HER2 Monoclonal Antibody in Patients With HER2/neu–Overexpressing Metastatic Breast Cancer" *J. Clin. Oncol.* 14(3):737–744 (1996).
Beverley & Callard, "Distinctive functional charcteristics of human "T" lymphocytes defined by E rosetting or a monoclonal anti–T cell antibody" *European Journal of Immunology* 11:329–334 (1981).
Bird et al., "Single–chain antigen–binding proteins" *Science* 242:423–426 (Oct. 1988).
Brennan et al., "Preparation of bispecific antibodies by chemical recombination of monoclonal immunoglobulin G_1 fragments" *Science* 229:81–83 (Jul. 1985).
Bruccoleri et al., "Structure of antibody hypervariable loops reproduced by a conformational search algorithm" *Nature* 335:564–568 (Oct. 1988).
Caron et al., "Biological and Immunological Features of Humanized M195 (Anti–CD33) Monoclonal Antibodies" *Cancer Research* 52:6761–6767 (Dec. 1992).
Chothia & Lesk, "The relation between the divergence of sequence and structure in proteins" *EMBO Journal* 5(4):823–826 (1986).
Co & Queen, "Humanized antibodies for therapy" *Nature* 351:501–502 (Jun. 1991).
Co et al., "Chimeric and Humanized Antibodies with Specificity for the CD33 Antigen" *J. of Immunology* 148(4):1149–1154 (Feb. 1992).
Co et al., "Humanized Anti–Lewis Y Antibodies: In Vitro Properties and Pharmacokinetics in Rhesus Monkeys" *Cancer Research* 56:1118–1125 (Mar. 1996).
Colman et al., "Crystal and Molecular Structure of the Dimer of Variable Domains of the Bence–Jones Protein ROY" *J. Mol. Biol.* 116:73–79 (1977).
Colman et al., "Three–dimensional structure of a complex of antibody with influenza virus neuraminidase" *Nature* 326:358–363 (Mar. 1987).
Cook et al., "A map of the human immunoglbulin V_H locus completed by analysis of the telometric region of chromosome 14q" *Nature Genetics* 7:162–168 (Jun. 1994).
Darsley & Rees, "Nucleotide sequences of five anti–lysozyme monoclonal antibodies" *EMBO Journal* 4(2):393–398 (1985).
Davies & Metzger, "Structural Basis of Antibody Function" *Ann. Rev. Immunol.* 1:87–117 (1983).

Davies et al., "Antibody–Antigen Complexes" *Journal of Biological Chemistry* 263(22):10541–10544 (Aug. 1988).

Eigenbrot et al., "X–Ray Structures of Fragments From Binding and Nonbinding Versions of a Humanized Anti–CD18 Antibody: Structural Indications of the Key Role of V_H Residues 59 to 65" *Proteins* 18:49–62 (1994).

Eigenbrot et al., "X–ray structures of the antigen–binding domains from three variants of humanized anti–p185HER2 antibody 4D5 and comparison with molecular modeling" *J. Mol. Biol.* 229:969–995 (1993).

Ellison et al., "The nucleotide sequence of a human immunoglobulin $C_{\gamma 1}$ gene" *Nucleic Acids Research* 10(13):4071–4079 (1982).

Emery & Adair, "Humanised monoclonal antibodies for therapeutic applications" *Exp. Opin. Invest. Drugs* 3(3):241–251 (1994).

Epp et al., "Crystal and Molecular Structure of a Dimer Composed of the Variable Portions of the Bence–Jones Protein REI" *European Journal of Biochemistry* 45:513–524 (1974).

Fanger et al., "Bispecific antibodies and targeted cellular cytotoxicity" *Immunology Today* 12(2):51–54 (1991).

Fanger et al., "Cytotoxicity mediated by human Fc receptors for IgG" *Immunology Today* 10(3):92–99 (1989).

Feldmann et al., "A Hypothetical Space–Filling Model of the V–Regions of the Galactan–Binding Myeloma Immunoglobulin J539" *Molecular Immunology* 18(8):683–698 (1981).

Fendley et al., "The Extracellular Domain of HER2/neu Is a Potential Immunogen for Active Specific Immunotherapy of Breast Cancer" *J. Biol. Resp. Mod.* 9:449–455 (1990).

Glennie et al., "Preparation and Performance of Bispecific $F(ab'\gamma)_2$ Antibody Containing Thioether–Linked $Fab'\gamma$ Fragments" *J. Immunol.* 139(7):2367–2375 (Oct. 1, 1987).

Gonzalez et al., "Humanization of Murine 6G425:An Anti–IL8 Monoclonal Antibody Which Blocks Binding of IL8 to Human Neutrophils" *1996 Keystone Symposia on Exploring and Exploiting Antibody and Ig Superfamily Combining Sites* (Poster) pp. 1–21 (Feb. 1996).

Gussow & Seemann, "Humanization of Monoclonal Antibodies" *Meth. Enzymology,* Academic Press, Inc. vol. 203:99–121 (1991).

Hieter et al., "Cloned human and mouse kappa immunoglobulin constant and J region genes conserve homology in functional segments" *Cell* 22 (Part 1):197–207 (1980).

Houghton, A., "Building a better monoclonal antibody" *Immunology Today* 9(9):265–267 (1988).

Huston et al., "Protein engineering of antibody binding sites: Recovery of specific activity in an anti–digoxin single–chain Fv analogue produced in *Escherichia coli" Proc. Natl. Acad. Sci. USA* 85:5879–5883 (Aug. 1988).

Isaacs et al., "Humanised Monoclonal Antibody Therapy for Rheumatoid Arthritis" *Lancet* 340:748–752 (Sep. 26, 1992).

Johnson et al., "Biological and Molecular Modeling Studies Comparing Murine Monoclonal Antibodies with Their Engineered Chimeric and Humanized Counterparts" *J. Cell. Biochem. Suppl 0 (13 Part A)* (18th Ann. UCLA Symp on Mol. & Cell. Biol., Park City, UT Jan. 17–22, 1989) pp. 87 (1989).

Kabat E., "Origins of Antibody Complementarity and Specificity—Hypervariable Regions and the Minigenen Hypothesis" *J. of Immunology* 125(3):961–969 (Sep. 1980).

Kabat et al. *Sequences of Proteins of Immunological Interest,* U.S. Dept. of Health and Human Services, NIH, 5th edition vol. 1:103–108, 324–331 (1991).

Kindt & Capra *The Antibody Enigma,* New York:Plenum Press pp. 79–86 (1984).

Lesk & Chothia, "Evolution of Proteins Formed by β–Sheets" *J. Mol. Biol.* 160:325–342 (1982).

Lesk & Chothia, "The response of protein structures to amino–acid sequence changes" *Phil. Trans. R. Soc. Lond.* A 317:345–356 (1986).

Mariuzza et al., "The Structure Basis of Antigen–Antibody Recognition" *Ann. Rev. Biophys. Biophys. Chem.* 16:139–159 (1987).

Nadler et al., "Immunogenicity of Humanized and Human Monoclonal Antibodies" *Clin. Pharmacology & Therapeutics* pp. 180 (Feb. 1994).

Nelson, H., "Targeted Cellular Immunotherapy with Bifunctional Antibodies" *Cancer Cells* 3:163–172 (1991).

Neuberger et al., "Antibody Engineering" *Proceedings 8th Intl. Biotech. Symp.,* Paris II:792–799 (1988).

Newmark, P., "Making Chimeric Antibodies Even More Human" *Bio/Technology* 6:468 (May 1988).

Nishimura et al., "Human c–erbB–2 Proto–Oncogene Product as a Target for Bispecific–Antibody–Directed Adoptive Tumor Immunotherapy" *Int. J. Cancer* 50:800–804 (1992).

Nitta et al., "Preliminary trial of specific targeting therapy against malignant glioma" *Lancet* 335(8686):368–371 (Feb. 17, 1990).

Nitta, T. et al., "Bispecific $F(ab')_2$ monomer prepared with anti–CD3 and anti–tumor monoclonal antibodies is most potent in induction of cytolysis of human T cells" *European Journal of Immunology* 19:1437–1441 (1989).

Nolan et al., "Bifunctional antibodies: concept, production and applications" *Biochimica et Biophysica Acta* 1040:1–11 (1990).

O'Connor et al., "Calcium Dependence of an Anti–Protein C Humanized Antibody Involves Framework Residues" (manuscript).

Orlandi et al., "Cloning Immunoglobulin Variable Domains for Expression by the Polymerase Chain Reaction" *Proc. Natl. Acad. Sci. USA* 86:3833–3837 (May 1989).

Orlandi et al., "Cloning of cDNA Corresponding to Heavy and Light Chain Immunoglobulin Variable Domains" *Protein and Pharmaceutical Engineering* pp. 90 (1989).

Ostberg & Queen, "Human and humanized monoclonal antibodies: preclinical studies and clinical experience" *Biochem. Soc. Transactions* pp. 1038–1043 (1995).

Pedlan et al., "Model–building Studies of Antigen–binding Sites:The Hapten–binding Site of MOPC–315" *Cold Springs Harbor Symposia On Quantitative Biology* XLI:627–637 (1977).

Padlan, E., "Anatomy of the Antibody Molecule" *Molecular Immunology* 31(3):169–217 (1994).

Padlan, E., "Evaluation of the Structural Variation Among Light Chain Variable Domains" *Molecular Immunology* 16:287–296 (1979).

Palm & Hilschmann, "Primary structure of a crystalline monoclonal immunoglobulin K–type L–chain, subgroup I (Bence–Jones preotin Rei); isolation & characterization of the tryptic peptides: . . . " *Hoppes–Seyler's Z. Physiol. Chem.* 356:167–191 (Feb. 1975).

Palm & Hilschmann, "The primary structure of a crystalline, monoclonal immunoglobulin–L–chain of the x–type, subgroup I (Bence–Jones Protein Rei): a contribution to the elucidation of the three–dimensional structure of the immunoglobulins" *Hoppe–Seyler's Z. Physiol. Chem.* 354:1651–1654 (Dec. 1973).

Panka et al., "Variable region framework differences result in decreased or increased affinity of variant anti–digoxin antibodies" *Proc. Natl. Acad. Sci. USA* 85:3080–3084 (May 1988).

Presta et al., "Humanization of an Antibody Directed Against IgE" *J. Immunol.* 151(5):2623–2632 (Sep. 1, 1993).

Preval & Fougereau, "Specific Interaction between V_H and V_L Regions of Human Monoclonal Immunoglobulins" *J. Mol. Biol.* 102:657–678 (1976).

Queen et al., "Construction of Humanized Antibodies and Testing in Primates" *J. Cell. Biochem. Suppl. 15 (Part E)* (20th Ann. Mtg. Keystone Symp. Denver, CO Mar. 10–16, 1991) pp. 137 (1991).

Queen et al., "Humanised antibodies to the IL–2 receptor" *Protein Eng. Antibody Mol. Prophyl. Ther. Appl. Man,* Clark, M., Nottingham, UK:Academic Titles pp. 159–170 (1993).

Rhodes & Birch, "Large–Scale Production of Proteins from Mammalian Cells" *Bio/Technology* 6:518, 521, 523 (May 1988).

Riechmann, "Humanizing of Recombinant Antibodies" (Intl. Symp. on Clin. Appl. of Monoclonal Antibodies, Guildford, England) pp. 33–34 (Sep. 1987).

Riechmann & Winter, "Recombinant Antibodies" (U. of London Royal Postgraduate Medical School, Wolfson Institute, Abstract) (May 1987).

Riechmann et al. *Alignment of VL Sequences* (1988).

Roberts & Rees, "Generation of an antibody with enhanced affinity and specificity for its antigen by protein engineering" *Nature* 328:731–734 (Aug. 1987).

Rostapshov et al., "Effective method for obtaining long nucleotide chains on partially complementary templates" *FEBS Letters* 249(2):379–382 (Jun. 1989).

Schneider et al., "The Anti–Idiotypic Response by Cynomolgus Mondkeys to Humanized Anti–Tac Is Primarily Directed to Complementarity–Determining Regions H1, H2, and L3" *J. of Immunology* 150:3086–3090 (Apr. 1993).

Sedlacek et al., "Monoclonal Antibodies in Tumor Therapy", Karger pp. 119–126, 133–179 (1988).

Shields et al., "Inhibition of Allergic Reactions with Antibodies to IgE" *International Archives of Allergy and Immunology* 107(1–3):308–312 (May 1995).

Sims et al., "A Humanized CD18 Antibody Can Block Function Without Cell Destruction" *The Journal of Immunology* 151(4):2296–2308 (Aug. 1993).

Smith–Gill et al., "A Three–dimensional Model of an Anti–lysozyme Antibody" *Mol. Biol.* 194:713–724 (1987).

Songsivilai et al., "Bispecific antibody: a tool for diagnosis and treatment of disease" *Clin. Exp. Immunol.* 79:315–321 (1990).

Stanford, "A Predictive Method for Determining Possible Three–dimensional Foldings of Immunoglobulin Backbones Around Antibody Combining Sites" *Theor. Biol.* 88:421–439 (1981).

Stickney et al., "Bifunctional Antibody: ZCE/CHA ^{III}Indium BLEDTA–IV Clinical Imaging in Colorectal Carcinoma" *Antibody, Immuno Radiopharm* 2:1–13 (1989).

Tighe et al., "Delayed Allograft Rejection in Primates Treated with Anti–IL–2 Receptor Monoclonal antibody Campath–6" *Transplantation* 45(1):226–228 (Jan. 1988).

Verhoeyen & Riechmann, "Engineering of Antibodies" *BioEssays* 8(2):74–78 (Feb./Mar. 1988).

Verhoeyen et al., "Grafting Hypervariable Regions in Antibodies" *Protein Structure, Folding, and Design 2* (Proc. DuPont–UCLA Symp. Streamboat Springs, CO, Apr. 4–11, 1987), Dale L. Oxender, New York:Alan R. Liss, Inc. pp. 501–502 (1987).

Verhoeyen et al., "Humanising Mouse Antibodies: A Protein Engineering Approach" *Soc. for Analytical Cytology* (XIIth Intl. Mtg. for the Soc. for Analytical Cytology, Cambridge, UK) pp. 22 and slide presented at mtg.

Verhoeyen et al., "Re–shaped human anti–PLAP antibodies" *Monoclonal Antibodies Applications in clinical oncology,* Epenetos, 1st edition, Chapman & Hall Medical pp. 37–43 (1991).

Ward et al., "Expression and Secretion of Repertoires of VH Domains in *Escherichia coli:* Isolation of Antigen Binding Activites" *Progress in Immunology* (7th Intl. Congress Immunol. Berlin, W. Germany), F. Melchers vol. VII:1144–1151 (1989).

Ward, E.S. et al., "Binding activities of a repertoire of single immunoglobulin variable domains secreted from *Escherichia coli" Nature* 341:544–546 (1989).

Werther et al., "Humanization of an Anti–Lymphocyte Function–Associated Antigen (LFA)–1 Monoclonal Antibody and Reengineering of the Humanized Antibody for Binding to Rhesus LFA–1" *J. of Immunology* pp. 4986–4995 (1996).

Whittle et al., "Construction and Expression of A CDR–Grafted Anti–TNF Antibody" *J. Cell Biochem. Suppl. 0* (Symp. on Protein and Pharm. Eng. Mol. and Cell. Biol. Park City, Utah)13 Part A:96 (1989).

Winter & Neuberger, "Restructuring Enzymes and Antibodies" *Investigation and Exploitation of Antibody Combining Sites,* Eric Reid, Plenum Press pp. 139–140 (1985).

Winter et al., "Protein Engineering by Site Directed Mutagenesis" *Chemical Synthesis in Molecular Biology,* H. Blocker et al., VCH pp. 189–197 (1987).

Winter G., "Antibody Engineering" *Phil. Trans. R. Soc. Lond. B* 324:99–109 (1989).

Woodle et al., "Humanized OKT3 Antibodies: Successful Transfer of Immune Modulating Properties and Idiotype Expression" *J. of Immunology* 148(9):2756–2763 (May 1992).

* cited by examiner

FIG. 1A

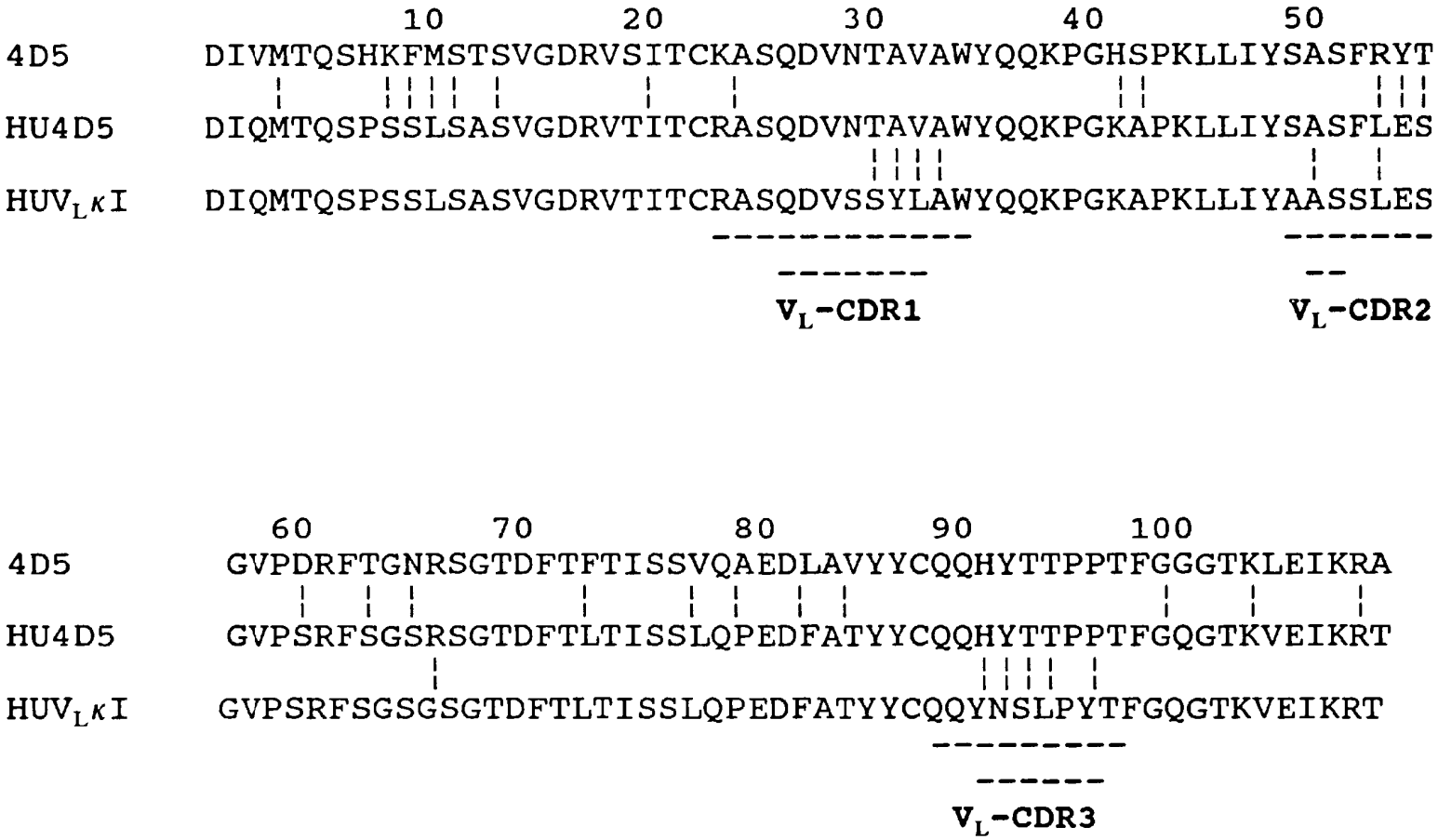
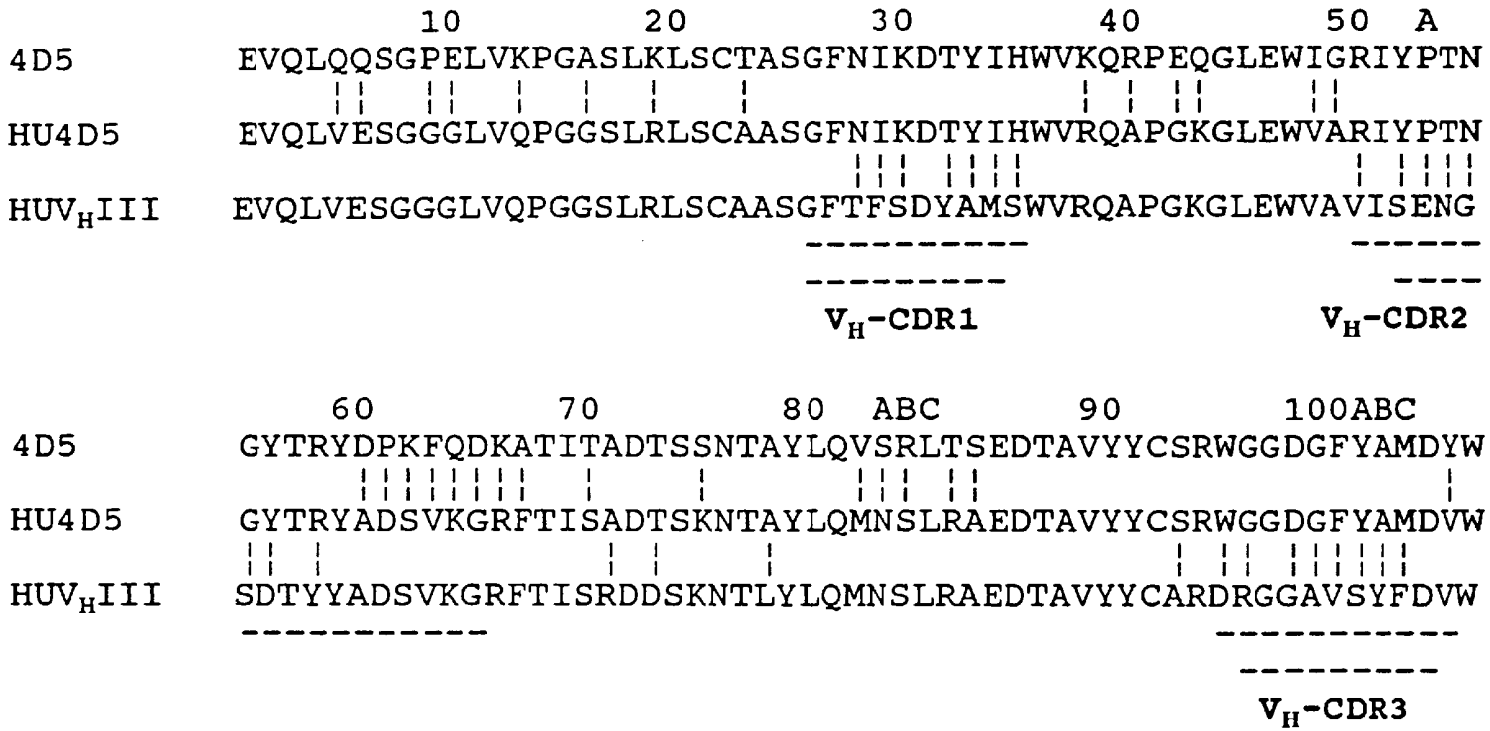


FIG. 1B



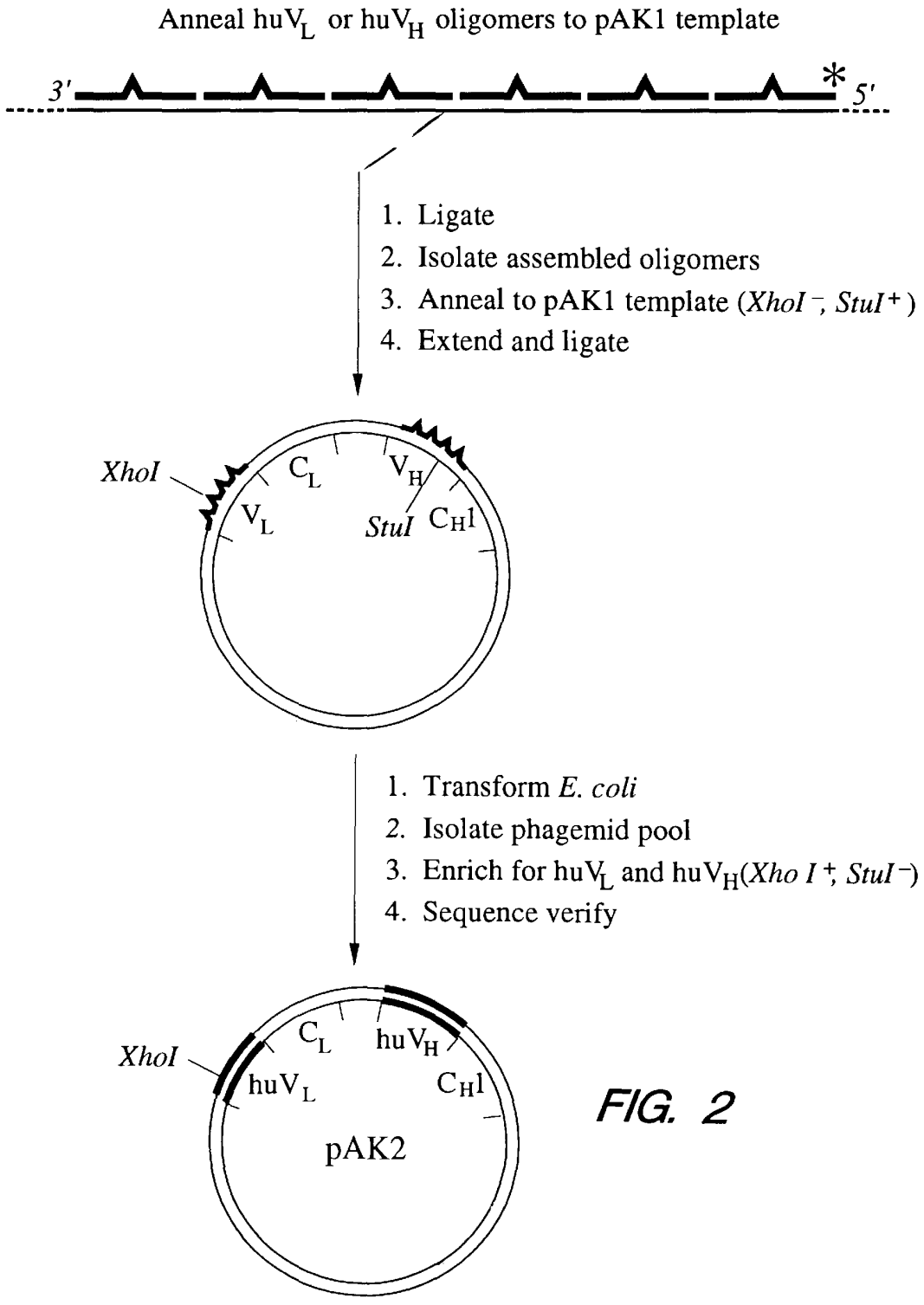

Anneal huV$_L$ or huV$_H$ oligomers to pAK1 template
3'
*
5'
1. Ligate
2. Isolate assembled oligomers
3. Anneal to pAK1 template (*XhoI*$^-$, *StuI*$^+$)
4. Extend and ligate
XhoI
C$_L$
V$_H$
V$_L$
StuI
C$_H$1
1. Transform *E. coli*
2. Isolate phagemid pool
3. Enrich for huV$_L$ and huV$_H$(*Xho I*$^+$, *StuI*$^-$)
4. Sequence verify
XhoI
C$_L$
huV$_H$
huV$_L$
C$_H$1
pAK2

FIG. 2

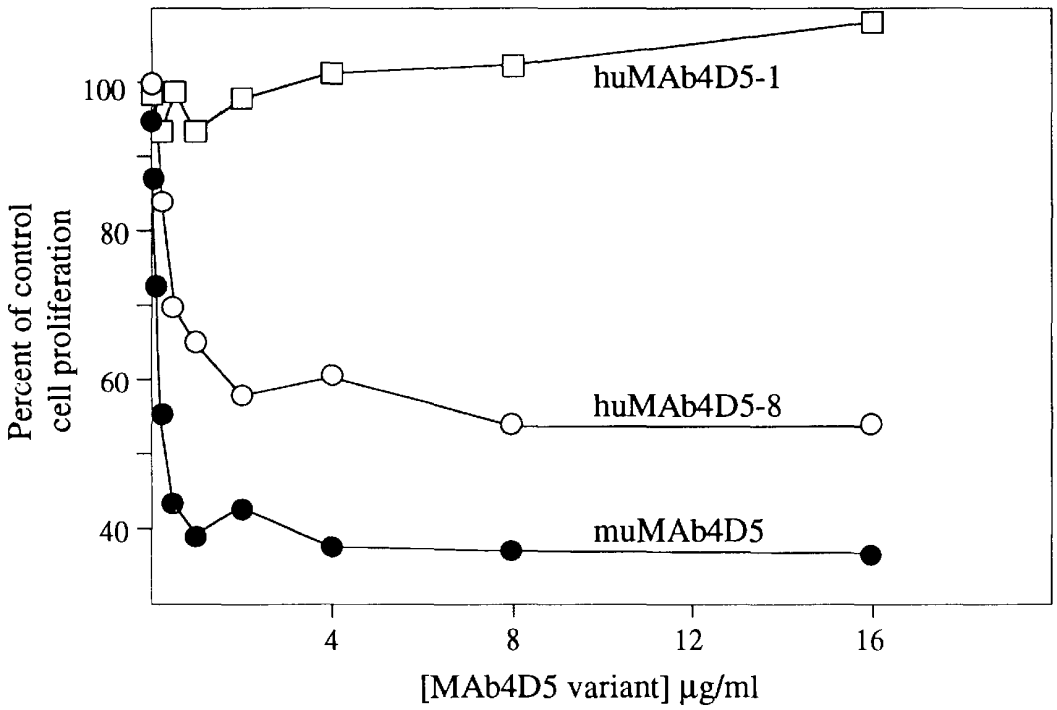

100
80
60
40
Percent of control cell proliferation
huMAb4D5-1
huMAb4D5-8
muMAb4D5
4
8
12
16
[MAb4D5 variant] µg/ml

FIG. 3

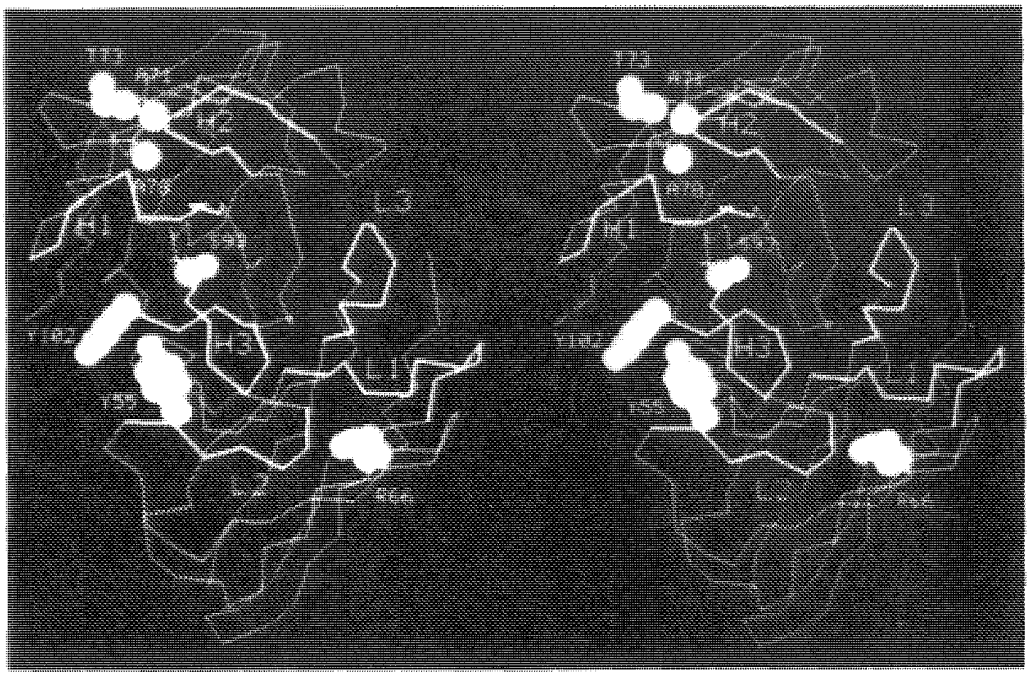


FIG. 4

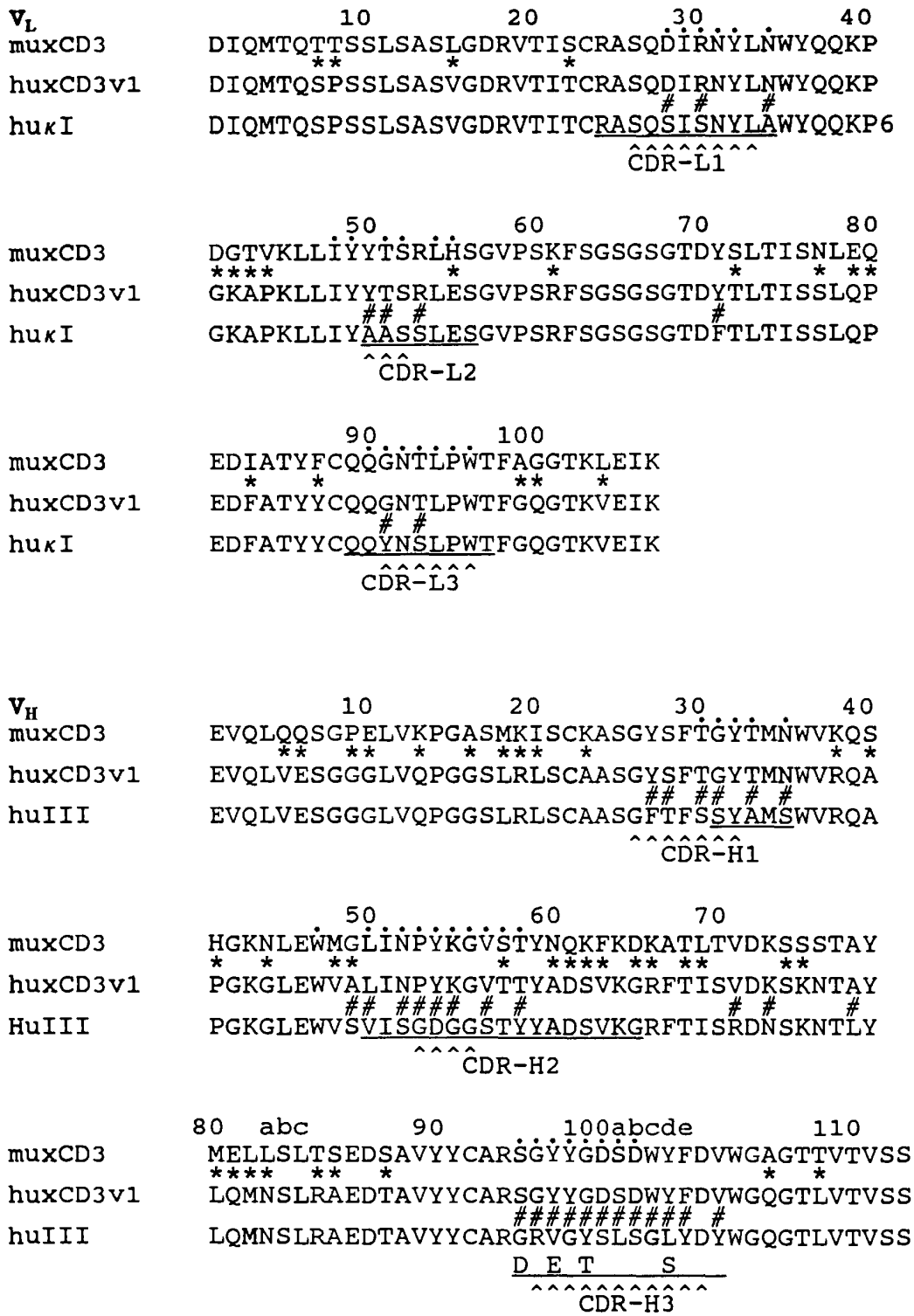

VL
muxCD3
huxCD3v1
huκI
CDR-L1
CDR-L2
CDR-L3
VH
muxCD3
huxCD3v1
huIII
CDR-H1
muxCD3
huxCD3v1
HuIII
CDR-H2
muxCD3
huxCD3v1
huIII
CDR-H3

FIG. 5

FIG. 6A-1

```
                                        10        20        30
H52H4-160                       QVQLQQSGPELVKPGASVKISCKTSGYTFTE
                                .*** .**  **.**.*...** ********
pH52-8.0    MGWSCIILFLVATATGVHSEVQLVESGGGLVQPGGSLRLSCATSGYTFTE
                    10        20        30        40        50

                   40        50        60        70        80
H52H4-160   YTMHWMKQSHGKSLEWIGGFNPKNGGSSHNQRFMDKATLAVDKSTSTAYM
            ******.*. **.***..*.******.********. *..**********
pH52-8.0    YTMHWMRQAPGKGLEWVAGINPKNGGTSHNQRFMDRFTISVDKSTSTAYM
                    60        70        80        90        100

                  90       100       110       120       130
H52H4-160   ELRSLTSEDSGIYYCARWRGLNYGFDVRYFDVWGAGTTVTVSSASTKGPS
            .. ** .**...********************** ** ************
pH52-8.0    QMNSLRAEDTAVYYCARWRGLNYGFDVRYFDVWGQGTLVTVSSASTKGPS
                    110       120       130       140       150

                 140       150       160       170       180
H52H4-160   VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
            ****** *.*** .************************************
pH52-8.0    VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
                    160       170       180       190       200

                 190       200       210       220       230
H52H4-160   QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH
            ************** **..***** ***.********** ** * *
pH52-8.0    QSSGLYSLSSVVTVTSSNFGTQTYTCNVDHKPSNTKVDKTVERKCC---V
                    210       220       230       240

                 240       250       260       270       280
H52H4-160   TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK
             *******  ..************************************.
pH52-8.0    ECPPCPAPP-VAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQ
            250       260       270       280       290
```

FIG. 6A-2

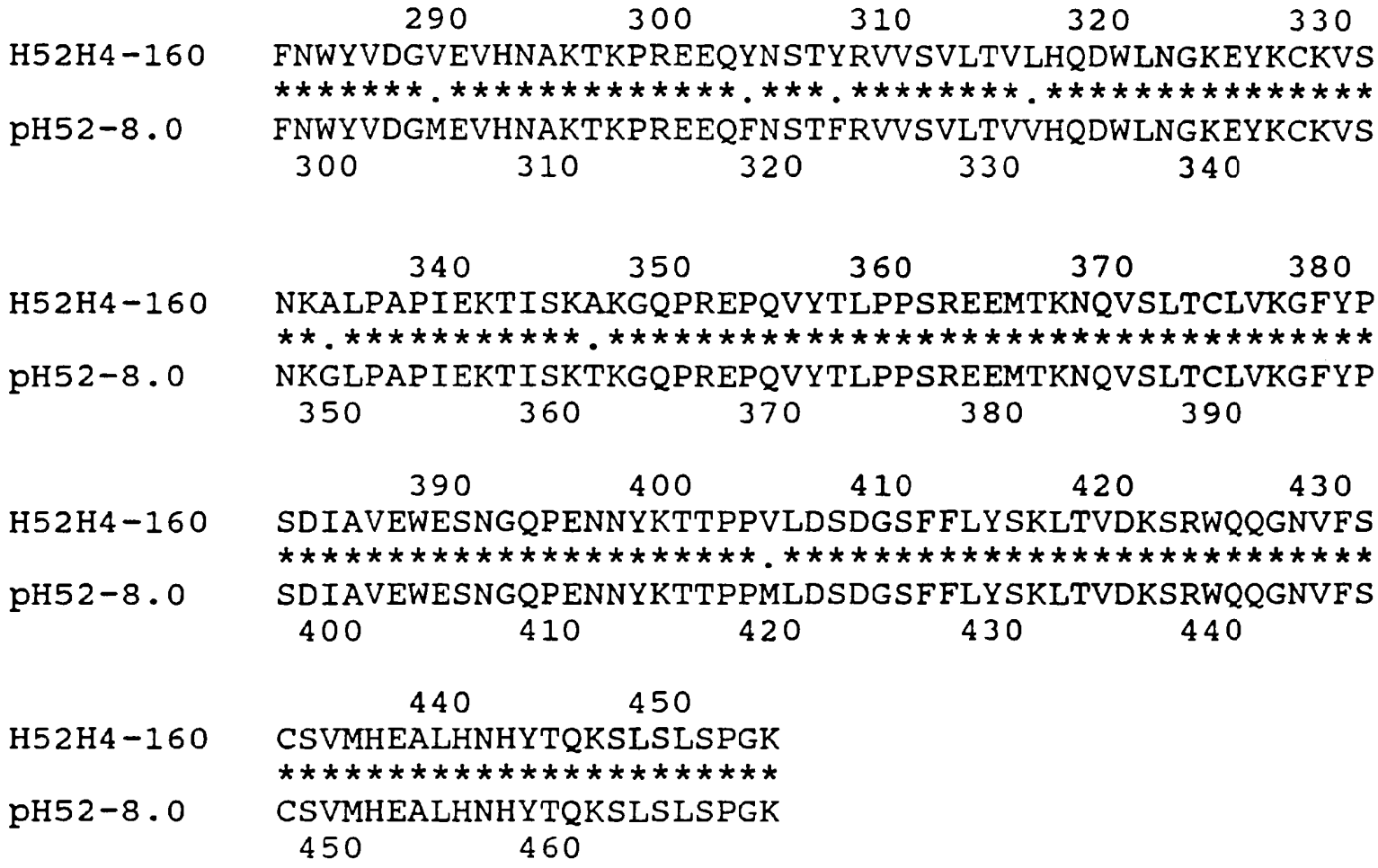


FIG. 6B

```
                                        10        20        30
H52L6-158                          DVQMTQTTSSLSASLGDRVTINCRASQDINN
                                    *.****. ******.****** *********
pH52-9.0    MGWSCIILFLVATATGVHSDIQMTQSPSSLSASVGDRVTITCRASQDINN
                    10        20        30        40        50

                   40        50        60        70        80
H52L6-158   YLNWYQQKPNGTVKLLIYYTSTLHSGVPSRFSGSGSGTDYSLTISNLDQE
            *********  . **************************.****.*. *
pH52-9.0    YLNWYQQKPGKAPKLLIYYTSTLHSGVPSRFSGSGSGTDYTLTISSLQPE
                    60        70        80        90        100

                   90       100       110       120       130
H52L6-158   DIATYFCQQGNTLPPTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS
            *.***.************ ********************************
pH52-9.0    DFATYYCQQGNTLPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS
                    110       120       130       140       150

                  140       150       160       170       180
H52L6-158   VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
            **************************************************
pH52-9.0    VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
                    160       170       180       190       200

                  190       200       210
H52L6-158    SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
             ********************************
pH52-9.0     SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
                    210       220       230
```

METHOD FOR MAKING HUMANIZED ANTIBODIES

CROSS REFERENCES

This application is a continuation-in-part of U.S. application Ser. No. 07/715,272 filed Jun. 14, 1991 (abandoned) which application is incorporated herein by reference and to which application priority is claimed under 35 USC §120.

FIELD OF THE INVENTION

This invention relates to methods for the preparation and use of variant antibodies and finds application particularly in the fields of immunology and cancer diagnosis and therapy.

BACKGROUND OF THE INVENTION

Naturally occurring antibodies (immunoglobulins) comprise two heavy chains linked together by disulfide bonds and two light chains, one light chain being linked to each of the heavy chains by disulfide bonds. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain (V_L) at one end and a constant domain at its other end, the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light and heavy chain variable domains, see e.g. Chothia et al., *J. Mol. Biol.* 186:651–663 (1985); Novotny and Haber, *Proc. Natl. Acad. Sci. USA* 82:4592–4596 (1985).

The constant domains are not involved directly in binding the antibody to an antigen, but are involved in various effector functions, such as participation of the antibody in antibody-dependent cellular cytotoxicity. The variable domains of each pair of light and heavy chains are involved directly in binding the antibody to the antigen. The domains of natural light and heavy chains have the same general structure, and each domain comprises four framework (FR) regions, whose sequences are somewhat conserved, connected by three hyper-variable or complementarity determining regions (CDRs) (see Kabat, E. A. et a., *Sequences of Proteins of Immunological Interest,* National Institutes of Health, Bethesda, Md., (1987)). The four framework regions largely adopt a β-sheet conformation and the CORs form loops connecting, and in some cases forming part of, the β-sheet structure. The CDRs in each chain are held in close proximity by the framework regions and, with the CDRs from the other chain, contribute to the formation of the antigen binding site.

Widespread use has been made of monoclonal antibodies, particularly those derived from rodents including mice, however they are frequently antigenic in human clinical use. For example, a major limitation in the clinical use of rodent monoclonal antibodies is an anti-globulin response during therapy (Miller, R. A. et al., *Blood* 62:988–995 (1983); Schroff, R. W. et al., *Cancer Res.* 45:879–885 (1985)).

The art has attempted to overcome this problem by constructing "chimeric" antibodies in which an animal antigen-binding variable domain is coupled to a human constant domain (Cabilly et al., U.S. Pat. No. 4,816,567; Morrison, S. L. et al., *Proc. Natl. Acad. Sci. USA* 81:6851–6855 (1984); Boulianne, G. L. et al., *Nature* 312:643–646 (1984); Neuberger, M. S. et al., *Nature* 314:268–270 (1985)). The term "chimeric" antibody is used herein to describe a polypeptide comprising at least the antigen binding portion of an antibody molecule linked to at least part of another protein (typically an immunoglobulin constant domain).

The isotype of the human constant domain may be selected to tailor the chimeric antibody for participation in antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (see e.g. Brüggemann, M. et al., *J. Exp. Med.* 166:1351–1361 (1987); Riechmann, L. et al., *Nature* 332:323–327 (1988); Love et al., *Methods in Enzymology* 178:515–527 (1989); Bindon et al., *J. Exp. Med.* 168:127–142 (1988).

In the typical embodiment, such chimeric antibodies contain about one third rodent (or other non-human species) sequence and thus are capable of eliciting a significant anti-globulin response in humans. For example, in the case of the murine anti-CD3 antibody, OKT3, much of the resulting anti-globulin response is directed against the variable region rather than the constant region (Jaffers, G. J. et a., *Transplantation* 41:572–578 (1986)).

In a further effort to resolve the antigen binding functions of antibodies and to minimize the use of heterologous sequences in human antibodies, Winter and colleagues (Jones, P. T. et al., *Nature* 321:522–525 (1986); Riechmann, L. et al., *Nature* 332:323–327 (1988); Verhoeyen, M. et al., *Science* 239:1534–1536 (1988)) have substituted rodent CDRs or CDR sequences for the corresponding segments of a human antibody. As used herein, the term "humanized" antibody is an embodiment of chimeric antibodies wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

The therapeutic promise of this approach is supported by the clinical efficacy of a humanized antibody specific for the CAMPATH-1 antigen with two non-Hodgkin lymphoma patients, one of whom had previously developed an anti-globulin response to the parental rat antibody (Riechmann, L. et al., *Nature* 332:323–327 (1988); Hale, G. et al., *Lancet i:*1394–1399 (1988)). A murine antibody to the interleukin 2 receptor has also recently been humanized (Queen, C. et al., *Proc. Natl. Acad. Sci. USA* 86:10029–10033 (1989)) as a potential immunosuppressive reagent. Additional references related to humanization of antibodies include Co et al., *Proc. Natl. Acad. Sci. USA* 88:2869–2873 (1991); Gorman et al., *Proc. Natl. Acad. Sci. USA* 88:4181–4185 (1991); Daugherty et al., *Nucleic Acids Research* 19(9):2471–2476 (1991); Brown et al., *Proc. Natl. Acad. Sci. USA* 88:2663–2667 (1991); Junghans et al., *Cancer Research* 50:1495–1502 (1990).

In some cases, substituting CDRs from rodent antibodies for the human CDRs in human frameworks is sufficient to transfer high antigen binding affinity (Jones, P. T. et al., *Nature* 321:522–525 (1986); Verhoeyen, M. et al., *Science* 239:1534–1536 (1988)), whereas in other cases it has been necessary to additionally replace one (Riechmann, L. et al., *Nature* 332:323–327 (1988)) or several (Queen, C. et al., *Proc. Natl. Acad. Sci. USA* 86:10029–10033 (1989)) framework region (FR) residues. See also Co et al., supra.

For a given antibody a small number of FR residues are anticipated to be important for antigen binding. Firstly for example, certain antibodies have been shown to contain a few FR residues which directly contact antigen in crystal structures of antibody-antigen complexes (e.g., reviewed in Davies, D. R. et al., *Ann. Rev. Biochem.* 59:439–473 (1990)).

Secondly, a number of FR residues have been proposed by Chothia, Lesk and colleagues (Chothia, C. & Lesk, A. M., *J. Mol. Biol.* 196:901–917 (1987); Chothia, C. et al., *Nature* 342:877–883 (1989); Tramontano, A. et al., *J. Mol. Biol.* 215:175–182 (1990)) as critically affecting the conformation of particular CDRs and thus their contribution to antigen binding. See also Margolies et al., *Proc. Natl. Acad. Sci. USA* 72:2180–2184 (1975).

It is also known that, in a few instances, an antibody variable domain (either V_H or V_L) may contain glycosylation sites, and that this glycosylation may improve or abolish antigen binding, Pluckthun, *Biotechnology* 9:545–51 (1991); Spiegelberg et al., *Biochemistry* 9:4217–4223 (1970); Wallic et al., *J. Exp. Med.* 168:1099–1109 (1988); Sox et al., *Proc. Natl. Acad. Sci. USA* 66:975–982 (1970); Margni et al., *Ann. Rev. Immunol* 6:535–554 (1988). Ordinarily, however, glycosylation has no influence on the antigen-binding properties of an antibody, Pluckthun, supra, (1991).

The three-dimensional structure of immunoglobulin chains has been studied, and crystal structures for intact immunoglobulins, for a variety of immunoglobulin fragments, and for antibody-antigen complexes have been published (see e.g., Saul et al., *Journal of Biological Chemistry* 25:585–97 (1978); Sheriff et al., *Proc. Natl. Acad. Sci. USA* 84:8075–79 (1987); Segal et al., *Proc. Natl. Acad. Sci. USA* 71:4298–4302 (1974); Epp et al., *Biochemistry* 14(22) :4943–4952 (1975); Marquart et al., *J. Mol. Biol.* 141:369–391 (1980); Furey et al., *J. Mol. Biol.* 167:661–692 (1983); Snow and Amzel, *Protein: Structure, Function, and Genetics* 1:267–279, Alan R. Liss, Inc. pubs. (1986); Chothia and Lesk, *J. Mol. Bol.* 196:901–917 (1987); Chothia et al., *Nature* 342:877–883 (1989); Chothia et al., *Science* 233:755–58 (1986); Huber et al., *Nature* 264:415–420 (1976); Bruccoleri et al., *Nature* 335:564–568 (1988) and *Nature* 336:266 (1988); Sherman et al., *Journal of Biological Chemistry* 263:4064–4074 (1988); Amzel and Poljak, *Ann. Rev. Biochem.* 48:961–67 (1979); Silverton et al., *Proc. Natl. Acad. Sci. USA* 74:5140–5144 (1977); and Gregory et al., *Molecular Immunology* 24:821–829 (1987). It is known that the function of an antibody is dependent on its three dimensional structure, and that amino acid substitutions can change the three-dimensional structure of an antibody, Snow and Amzel, supra. It has previously been shown that the antigen binding affinity of a humanized antibody can be increased by mutagenesis based upon molecular modelling (Riechmann, L. et al., *Nature* 332:323–327 (1988); Queen, C. et al., *Proc. Natl. Acad. Sci. USA* 86:10029–10033 (1989)).

Humanizing an antibody with retention of high affinity for antigen and other desired biological activities is at present difficult to achieve using currently available procedures. Methods are needed for rationalizing the selection of sites for substitution in preparing such antibodies and thereby increasing the efficiency of antibody humanization.

The proto-oncogene HER2 (human epidermal growth factor receptor 2) encodes a protein tyrosine kinase ($p185^{HER2}$) that is related to and somewhat homologous to the human epidermal growth factor receptor (see Coussens, L. et al., *Science* 230:1132–1139 (1985); Yamamoto, T. et al., *Nature* 319:230–234 (1986); King, C. R. et al., *Science* 229:974–976 (1985)). HER2 is also known in the field as c-erbB-2, and sometimes by the name of the rat homolog, neu. Amplification and/or overexpression of HER2 is associated with multiple human malignancies and appears to be integrally involved in progression of 25–30% of human breast and ovarian cancers (Slamon, D. J. et al., *Science* 235:177–182 (1987), Slamon, D. J. et al., *Science* 244:707–712 (1989)). Furthermore, the extent of amplification is inversely correlated with the observed median patient survival time (Slamon, supra, Science 1989).

The murine monoclonal antibody known as muMAb4D5 (Fendly, B. M. et al., *Cancer Res.* 50:1550–1558 (1990)), directed against the extracellular domain (ECD) of $p185^{HER2}$, specifically inhibits the growth of tumor cell lines overexpressing $p185^{HER2}$ in monolayer culture or in soft agar (Hudziak, R. M. et al., *Molec. Cell. Biol* 9:1165–1172 (1989); Lupu, R. et al., *Science* 249:1552–1555 (1990)). MuMAb4D5 also has the potential of enhancing tumor cell sensitivity to tumor necrosis factor, an important effector molecule in macrophage-mediated tumor cell cytotoxicity (Hudziak, supra, 1989; Shepard, H. M. and Lewis, G. D. *J. Clinical Immunology* 8:333–395 (1988)). Thus muMAb4D5 has potential for clinical intervention in and imaging of carcinomas in which $p185^{HER2}$ is overexpressed. The muMAb4D5 and its uses are described in PCT application WO 89/06692 published Jul. 27, 1989. This murine antibody was deposited with the ATCC and designated ATCC CRL 10463. However, this antibody may be immunogenic in humans.

It is therefore an object of this invention to provide methods for the preparation of antibodies which are less antigenic in humans than non-human antibodies but have desired antigen binding and other characteristics and activities.

It is a further object of this invention to provide methods for the efficient humanization is of antibodies, i.e. selecting non-human amino acid residues for importation into a human antibody background sequence in such a fashion as to retain or improve the affinity of the non-human donor antibody for a given antigen.

It is another object of this invention to provide humanized antibodies capable of binding $p185^{HER2}$.

Other objects, features, and characteristics of the present invention will become apparent upon consideration of the following description and the appended claims.

SUMMARY OF THE INVENTION

The objects of this invention are accomplished by a method for making a humanized antibody comprising amino acid sequence of an import, non-human antibody and a human antibody, comprising the steps of:

a. obtaining the amino acid sequences of at least a portion of an import antibody variable domain and of a consensus variable domain;

b. identifying Complementarity Determining Region (CDR) amino acid sequences in the import and the human variable domain sequences;

c. substituting an import CDR amino acid sequence for the corresponding human CDR amino acid sequence;

d. aligning the amino acid sequences of a Framework Region (FR) of the import antibody and the corresponding FR of the consensus antibody;

e. identifying import antibody FR residues in the aligned FR sequences that are non-homologous to the corresponding consensus antibody residues;

f. determining if the non-homologous import amino acid residue is reasonably expected to have at least one of the following effects:

1. non-covalently binds antigen directly,

2. interacts with a CDR; or

3. participates in the V_L–V_H interface; and

g. for any non-homologous import antibody amino acid residue which is reasonably expected to have at least one of these effects, substituting that residue for the corresponding amino acid residue in the consensus antibody FR sequence.

Optionally, the method of this invention comprises the additional steps of determining if any non-homologous residues identified in step (e) are exposed on the surface of the domain or buried within it, and if the residue is exposed but has none of the effects identified in step (f), retaining the consensus residue.

Additionally, in certain embodiments the method of this invention comprises the feature wherein the corresponding consensus antibody residues identified in step (e) above are selected from the group consisting of 4L, 35L, 36L, 38L, 43L, 44L, 46L, 58L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71 L, 73L, 85L, 87L, 98L, 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 67H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H (utilizing the numbering system set forth in Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)).

In certain embodiments, the method of this invention comprises the additional steps of searching either or both of the import, non-human and the consensus variable domain sequences for glycosylation sites, determining if the glycosylation is reasonably expected to be important for the desired antigen binding and biological activity of the antibody (i.e., determining if the glycosylation site binds to antigen or changes a side chain of an amino acid residue that binds to antigen, or if the glycosylation enhances or weakens antigen binding, or is important for maintaining antibody affinity). If the import sequence bears the glycosylation site, it is preferred to substitute that site for the corresponding residues in the consensus human if the glycosylation site is reasonably expected to be important. If only the consensus sequence, and not the import, bears the glycosylation site, it is preferred to eliminate that glycosylation site or substitute therefor the corresponding amino acid residues from the import sequence.

Another embodiment of this invention comprises aligning import antibody and the consensus antibody FR sequences, identifying import antibody FR residues which are non-homologous with the aligned consensus FR sequence, and for each such non-homologous import antibody FR residue, determining if the corresponding consensus antibody residue represents a residue which is highly conserved across all species at that site, and if it is so conserved, preparing a humanized antibody which comprises the consensus antibody amino acid residue at that site.

Certain alternate embodiments of the methods of this invention comprise obtaining the amino acid sequence of at least a portion of an import, non-human antibody variable domain having a CDR and a FR, obtaining the amino acid sequence of at least a portion of a consensus antibody variable domain having a CDR and a FR, substituting the non-human CDR for the human CDR in the consensus antibody variable domain, and then substituting an amino acid residue for the consensus amino acid residue at at least one of the following sites:

a. (in the FR of the variable domain of the light chain) 4L, 35L, 36L, 38L, 43L, 44L, 58L, 46L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, or

b. (in the FR of the variable domain of the heavy chain) 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 67H, 68H, 69H, 70H, 73H, 74H, 75H, 78H, 91H, 92H, 93H, and 103H.

In preferred embodiments, the non-CDR residue substituted at the consensus FR site is the residue found at the corresponding location of the non-human antibody.

Optionally, this just-recited embodiment comprises the additional steps of following the method steps appearing at the beginning of this summary and determining whether a particular amino acid residue can reasonably be expected to have undesirable effects.

This invention also relates to a humanized antibody comprising the CDR sequence of an import, non-human antibody and the FR sequence of a human antibody, wherein an amino acid residue within the human FR sequence located at any one of the sites 4L, 35L, 36L, 38L, 43L, 44L, 46L, 58L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 67H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H has been substituted by another residue. In preferred embodiments, the residue substituted at the human FR site is the residue found at the corresponding location of the non-human antibody from which the non-human CDR was obtained. In other embodiments, no human FR residue other than those set forth in this group has been substituted.

This invention also encompasses specific humanized antibody variable domains, and isolated polypeptides having homology with the following sequences.

1. SEQ. ID NO. 1, which is the light chain variable domain of a humanized version of muMAb4D5: DIQMTOSPSSLSASVGDRVTITCRASQD-VNTAVAWYQQKPGKAPKLLIYSASFLES-GVPSRFSGSRSGTDFTLTISSLQPEDFA-TYYCQQHYTTPPTFGQGTKVEIKRT

2. SEQ. ID NO. 2, which is the heavy chain variable domain of a humanized version of muMAb4D5): EVQLVESGGGLVOPGGSLRLSCAASGFNIK DTYIHWVRQAPGKGLEWVARIYPTNGYTRY ADSVKGRFTISADTSKNTAYLQMNSLRAED TAVYYCSRWGGDGFYAMDVWGQGTLVTVSS

In another aspect, this invention provides a consensus antibody variable domain amino acid sequence for use in the preparation of humanized antibodies, methods for obtaining, using, and storing a computer representation of such a consensus sequence, and computers comprising the sequence data of such a sequence. In one embodiment, the following consensus antibody variable domain amino acid sequences are provided:

SEQ. ID NO. 3 (light chain): DDIOMTQSPSSLSAS-VGDRVTITCRASQDVSSYLAWYQQKPGKAPKLL IYAASSLESGVPSRFSGSGSGTDFTLTISSLQP EDFATYYCQQYNSLPYTFGQGTKVEIKRT, and

SEQ. ID NO. 4 (heavy chain): EVQLVESGGGLVQPG GSLRLSCAASGFTFSDYAMSWVRQAPGKGL EWVAVISENGGYTRYADSVKGRFTISADTSKNT AYLQMNSLRAEDTAWYCSRWGGDGFYAMD VWGQGTLVTVSS

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1**A shows the comparison of the V_L domain amino acid residues of muMAb4D5, huMAb4D5, and a consensus sequence (FIG. **1**A, SEQ.ID NO. 5, SEQ. ID NO. 1 and SEQ. ID NO. 3, respectively). FIG. **1**B shows the comparison between the V_H domain amino acid residues of the muMAb4D5, huMAb4D5, and a consensus sequence (FIG. **1**B, SEQ. ID NO. 6, SEQ. ID NO. 2 and SEQ. ID NO. 4, respectively). Both FIGS. **1**A and **1**B use the generally accepted numbering scheme from Kabat, E. A., et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md. (1987)). In both FIG. **1**A

and FIG. 1B, the CDR residues determined according to a standard sequence definition (as in Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)) are indicated by the first underlining beneath the sequences, and the CDR residues determined according to a structural definition (as in Chothia, C. & Lesk, A. M., *J. Mol. Biol.* 196:901–917 (1987)) are indicated by the second, lower underlines. The mismatches between genes are shown by the vertical lines.

FIG. **2** shows a scheme for humanization of muMAb4D5 V_L and V_H by gene conversion mutagenesis.

FIG. **3** shows the inhibition of SK-BR-3 proliferation by MAb4D5 variants. Relative cell proliferation was determined as described (Hudziak, R. M. et al., *Molec. Cell. Biol.* 9:1165–1172 (1989)) and data (average of triplicate determinations) are presented as a percentage of results with untreated cultures for muMAb4D5 (●), huMAb4D5-8 (○) and huMAb4D5-1 (□).

FIG. **4** shows a stereo view of α-carbon tracing for a model of huMAb4D5-8 V_L and V_H. The CDR residues (Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)) are shown in bold and side chains of V_H residues A71, T73, A78, S93, Y102 and V_L residues Y55 plus R66 (see Table 3) are shown.

FIG. **5** shows an amino acid sequence comparison of V_L (top panel) and V_H (lower panel) domains of the murine anti-CD3 monoclonal Ab UCHT1 (muxCD3, Shalaby et al., *J. Exp. Med.* 175, 217–225 (1992) with a humanized variant of this antibody (huxCD3v1). Also shown are consensus sequences (most commonly occurring residue or pair of residues) of the most abundant human subgroups, namely V_L κ 1 and V_H III upon which the humanized sequences are based (Kabat, E. A. et al., *Sequences of Proteins of immunological Interest, 5th* edition, National Institutes of Health, Bethesda, Md., USA (1991)). The light chain sequences—muxCD3, huxCD3v1 and huKI—correspond to SEQ.ID. NOs 16, 17, and 18, respectively. The heavy chain sequences—muxCD3, huxCD3v1 and huxI—correspond to SEQ.ID.NOs 19, 26, and 21, respectively. Residues which differ between muxCD3 and huxCD3v1 are identified by an asterisk (*), whereas those which differ between humanized and consensus sequences are identified by a sharp sign (#). A bullet (•) denotes that a residue at this position has been found to contact antigen in one or more crystallographic structures of antibody/antigen complexes (Kabat et al., 1991; Mian, I. S. et al., *J. Mol. Biol* 217, 133–151 (1991)). The location of CDR residues according to a sequence definition (Kabat et al., 1991) and a structural definition (Chothia and Lesk, supra 1987) are shown by a line and carats (^) beneath the sequences, respectively.

FIG. **6**A compares murine and humanized amino acid sequences for the heavy chain of an anti-CD18 antibody. H52H4-160 (SEQ. ID. NO. 22) is the murine sequence, and pH52–8.0 (SEQ. ID. NO. 23) is the humanized heavy chain sequence. pH52–8.0 residue 143S is the final amino acid in the variable heavy chain domain V_H, and residue 144A is the first amino acid in the constant heavy chain domain C_{H1}.

FIG. **6**B compares murine and humanized amino acid sequences for the light chain of an anti-CD18 antibody. H52L6-158 (SEQ. ID. NO. 24) is the murine sequence, and pH52–9.0 (SEQ. ID. NO. 25) is the humanized light chain sequence. pH52–9.0 residue 128T is the final amino acid in the light chain variable domain V_L, and residue 129V is the first amino acid in the light chain constant domain C_L.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

In general, the following words or phrases have the indicated definitions when used in the description, examples, and claims:

The murine monoclonal antibody known as muMAb4D5 (Fendly, B. M. et al., *Cancer Res.* 50:1550–1558 (1990)) is directed against the extracellular domain (ECD) of $p185^{HER2}$. The muMAb4D5 and its uses are described in PCT application WO 89/06692 published Jul. 27, 1989. This murine antibody was deposited with the ATCC and designated ATCC CRL 10463. In this description and claims, the terms muMAb4D5, chMAb4D5 and huMAb4D5 represent murine, chimerized and humanized versions of the monoclonal antibody 4D5, respectively.

A humanized antibody for the purposes herein is an immunoglobulin amino acid sequence variant or fragment thereof which is capable of binding to a predetermined antigen and which comprises a FR region having substantially the amino acid sequence of a human immunoglobulin and a CDR having substantially the amino acid sequence of a non-human immunoglobulin.

Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are referred to herein as "import" residues, which are typically taken from an "import" antibody domain, particularly a variable domain. An import residue, sequence, or antibody has a desired affinity and/or specificity, or other desirable antibody biological activity as discussed herein.

In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains (Fab, Fab', $F(ab')_2$, Fabc, Fv) in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. Ordinarily, the antibody will contain both the light chain as well as at least the variable domain of a heavy chain. The antibody also may include the CH1, hinge, CH2, CH3, and CH4 regions of the heavy chain.

The humanized antibody will be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including IgG1, IgG2, IgG3 and lgG4. Usually the constant domain is a complement fixing constant domain where it is desired that the humanized antibody exhibit cytotoxic activity, and the class is typically IgG_1. Where such cytotoxic activity is not desirable, the constant domain may be of the IgG_2 class. The humanized antibody may comprise sequences from more than one class or isotype, and selecting particular constant domains to optimize desired effector functions is within the ordinary skill in the art.

The FR and CDR regions of the humanized antibody need not correspond precisely to the parental sequences, e.g., the import CDR or the consensus FR may be mutagenized by substitution, insertion or deletion of at least one residue so that the CDR or FR residue at that site does not correspond to either the consensus or the import antibody. Such mutations, however, will not be extensive. Usually, at least 75% of the humanized antibody residues will correspond to those of the parental FR and CDR sequences, more often 90%, and most preferably greater than 95%.

In general, humanized antibodies prepared by the method of this invention are produced by a process of analysis of the parental sequences and various conceptual humanized products using three dimensional models of the parental and humanized sequences. Three dimensional immunoglobulin models are commonly available and are familiar to those

skilled in the art. Computer programs are available which illustrate and display probable three dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen.

Residues that influence antigen binding are defined to be residues that are substantially responsible for the antigen affinity or antigen specificity of a candidate immunoglobulin, in a positive or a negative sense. The invention is directed to the selection and combination of FR residues from the consensus and import sequence so that the desired immunoglobulin characteristic is achieved. Such desired characteristics include increases in affinity and greater specificity for the target antigen, although it is conceivable that in some circumstances the opposite effects might be desired. In general, the CDR residues are directly and most substantially involved in influencing antigen binding (although not all CDR residues are so involved and therefore need not be substituted into the consensus sequence). However, FR residues also have a significant effect and can exert their influence in at least three ways: They may noncovalently directly bind to antigen, they may interact with CDR residues and they may affect the interface between the heavy and light chains.

A residue that noncovalently directly binds to antigen is one that, by three dimensional analysis, is reasonably expected to noncovalently directly bind to antigen. Typically, it is necessary to impute the position of antigen from the spatial location of neighboring CDRs and the dimensions and structure of the target antigen. In general, only those humanized antibody residues that are capable of forming salt bridges, hydrogen bonds, or hydrophobic interactions are likely to be involved in non-covalent antigen binding, however residues which have atoms which are separated from antigen spatially by 3.2 Angstroms or less may also non-covalently interact with antigen. Such residues typically are the relatively larger amino acids having the side chains with the greatest bulk, such as tyrosine, arginine, and lysine. Antigen-binding FR residues also typically will have side chains that are oriented into an envelope surrounding the solvent oriented face of a CDR which extends about 7 Angstroms into the solvent from the CDR domain and about 7 Angstroms on either side of the CDR domain, again as visualized by three dimensional modeling.

A residue that interacts with a CDR generally is a residue that either affects the conformation of the CDR polypeptide backbone or forms a noncovalent bond with a CDR residue side chain. Conformation-affecting residues ordinarily are those that change the spatial position of any CDR backbone atom (N, Ca, C, O, Cβ) by more than about 0.2 Angstroms. Backbone atoms of CDR sequences are displaced for example by residues that interrupt or modify organized structures such as beta sheets, helices or loops. Residues that can exert a profound affect on the conformation of neighboring sequences include proline and glycine, both of which are capable of introducing bends into the backbone. Other residues that can displace backbone atoms are those that are capable of participating in salt bridges and hydrogen bonds.

A residue that interacts with a CDR side chain is one that is reasonably expected to form a noncovalent bond with a CDR side chain, generally either a salt bridge or hydrogen bond. Such residues are identified by three dimensional positioning of their side chains. A salt or ion bridge could be expected to form between two side chains positioned within about 2.5–3.2 Angstroms of one another that bear opposite charges, for example a lysinyl and a glutamyl pairing. A hydrogen bond could be expected to form between the side chains of residue pairs such as seryl or threonyl with aspartyl or glutamyl (or other hydrogen accepting residues). Such pairings are well known in the protein chemistry art and will be apparent to the artisan upon three dimensional modeling of the candidate immunoglobulin.

Immunoglobulin residues that affect the interface between heavy and light chain variable regions ("the V_L–V_H interface") are those that affect the proximity or orientation of the two chains with respect to one another. Certain residues involved in interchain interactions are already known and include V_L residues 34, 36, 38, 44, 46, 87, 89, 91, 96, and 98 and V_H residues 35, 37, 39, 45, 47, 91, 93, 95, 100, and 103 (utilizing the nomenclature setforth in Kabat et al., *Sequences of Proteins of immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)). Additional residues are newly identified by the inventors herein, and include 43L, 85L, 43H and 60H. While these residues are indicated for IgG only, they are applicable across species. In the practice of this invention, import antibody residues that are reasonably expected to be involved in interchain interactions are selected for substitution into the consensus sequence. It is believed that heretofore no humanized antibody has been prepared with an intrachain-affecting residue selected from an import antibody sequence.

Since it is not entirely possible to predict in advance what the exact impact of a given substitution will be it may be necessary to make the substitution and assay the candidate antibody for the desired characteristic. These steps, however, are per se routine and well within the ordinary skill of the art.

CDR and FR residues are determined according to a standard sequence definition (Kabat et al., *Sequences of Proteins of Immunological Interest,* National Institutes of Health, Bethesda Md. (1987), and a structural definition (as in Chothia and Lesk, *J. Mol. Biol.* 196:901–917 (1987). Where these two methods result in slightly different identifications of a CDR, the structural definition is preferred, but the residues identified by the sequence definition method are considered important FR residues for determination of which framework residues to import into a consensus sequence.

Throughout this description, reference is made to the numbering scheme from Kabat, E. A., et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md. (1987) and (1991). In these compendiums, Kabat lists many amino acid sequences for antibodies for each subclass, and lists the most commonly occurring amino acid for each residue position in that subclass. Kabat uses a method for assigning a residue number to each amino acid in a listed sequence, and this method for assigning residue numbers has become standard in the field. The Kabat numbering scheme is followed in this description.

For purposes of this invention, to assign residue numbers to a candidate antibody amino acid sequence which is not included in the Kabat compendium, one follows the following steps. Generally, the candidate sequence is aligned with any immunoglobulin sequence or any consensus sequence in Kabat. Alignment may be done by hand, or by computer using commonly accepted computer programs; an example of such a program is the Align 2 program discussed in this description. Alignment may be facilitated by using some amino acid residues which are common to most Fab

sequences. For example, the light and heavy chains each typically have two cysteines which have the same residue numbers; in V_L domain the two cysteines are typically at residue numbers 23 and 88, and in the V_H domain the two cysteine residues are typically numbered 22 and 92. Framework residues generally, but not always, have approximately the same number of residues, however the CDRs will vary in size. For example, in the case of a CDR from a candidate sequence which is longer than the CDR in the sequence in Kabat to which it is aligned, typically suffixes are added to the residue number to indicate the insertion of additional residues (see, e.g. residues 100abcde in FIG. **5**). For candidate sequences which, for example, align with a Kabat sequence for residues 34 and 36 but have no residue between them to align with residue 35, the number 35 is simply not assigned to a residue.

Thus, in humanization of an import variable sequence, where one cuts out an entire human or consensus CDR and replaces it with an import CDR sequence, (a) the exact number of residues may be swapped, leaving the numbering the same, (b) fewer import amino acid residues may be introduced than are cut, in which case there will be a gap in the residue numbers, or (c) a larger number of amino acid residues may be introduced then were cut, in which case the numbering will involve the use of suffixes such as 100abcde.

The terms "consensus sequence" and "consensus antibody" as used herein refers to an amino acid sequence which comprises the most frequently occurring amino acid residues at each location in all immunoglobulins of any particular subclass or subunit structure. The consensus sequence may be based on immunoglobulins of a particular species or of many species. A "consensus" sequence, structure, or antibody is understood to encompass a consensus human sequence as described in certain embodiments of this invention, and to refer to an amino acid sequence which comprises the most frequently occurring amino acid residues at each location in all human immunoglobulins of any particular subclass or subunit structure. This invention provides consensus human structures and consensus structures which consider other species in addition to human.

The subunit structures of the live immunoglobulin classes in humans are as follows:

Class	Heavy Chain Subclasses		Light Chain	Molecular Formula
IgG	γ	$\gamma1$, $\gamma2$, $\gamma3$, $\gamma4$	κ or λ	$(\gamma_2\kappa_2)$, $(\gamma_2\lambda_2)$
IgA	α	$\alpha1$, $\alpha2$	κ or λ	$(\alpha_2\kappa_2)_n{}^8$, $(\alpha_2\lambda_2)_n{}^8$
IgM	μ	none	κ or λ	$(\mu_2\kappa_2)_5$, $(\mu_2\lambda_2)_5$
IgD	δ	none	κ or λ	$(\delta_2\kappa_2)$, $(\delta_2\lambda_2)$
IgE	ϵ	none	κ or λ	$(\epsilon_2\kappa_2)$, $(\epsilon_2\lambda_2)$

($^8{}_n$ may equal 1, 2, or 3)

In preferred embodiments of an IgGγl human consensus sequence, the consensus variable domain sequences are derived from the most abundant subclasses in the sequence compilation of Kabat et al., *Sequences of Proteins of Immunological Interest,* National Institutes of Health, Bethesda Md. (1987), namely V_L κ subgroup I and V_H group III. In such preferred embodiments, the V_L consensus domain has the amino acid sequence:

DIQMTQSPSSLSASVGDRVTITCRASQD-VSSYLAWYQQKPGKAPKLLIYAASSLES-GVPSRFSGSGSGTDFTLTISSLQPEDFA-TYYCQQYNSLPYTFGQGTKVEIKRT (SEQ. ID NO. 3);

the V_H consensus domain has the amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYAMSW VRQAPGKGLEWVAVISENGGYTRYADSVKGRFT ISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGD GFYAMDVWGQGTLVTVSS (SEQ. ID NO. 4).

These sequences include consensus CDRs as well as consensus FR residues (see for example in FIG. **1**).

While not wishing to be limited to any particular theories, it may be that these preferred embodiments are less likely to be immunogenic in an individual than less abundant subclasses. However, in other embodiments, the consensus sequence is derived from other subclasses of human immunoglobulin variable domains. In yet other embodiments, the consensus sequence is derived from human constant domains.

Identity or homology with respect to a specified amino acid sequence of this invention is defined herein as the percentage of amino acid residues in a candidate sequence that are identical with the specified residues, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent homology, and not considering any conservative substitutions as part of the sequence identity. None of N-terminal, C-terminal or internal extensions, deletions, or insertions into the specified sequence shall be construed as affecting homology. All sequence alignments called for in this invention are such maximal homology alignments. While such alignments may be done by hand using conventional methods, a suitable computer program is the "Align 2" program for which protection is being sought from the U.S. Register of Copyrights (Align 2, by Genentech, Inc., application filed Dec. 9, 1991).

"Non-homologous" import antibody residues are those residues which are not identical to the amino acid residue at the analogous or corresponding location in a consensus sequence, after the import and consensus sequences are aligned.

The term "computer representation" refers to information which is in a form that can be manipulated by a computer. The act of storing a computer representation refers to the act of placing the information in a form suitable for manipulation by a computer.

This invention is also directed to novel polypeptides, and in certain aspects, isolated novel humanized anti-p185^{HER2} antibodies are provided. These novel anti-p185^{HER2} antibodies are sometimes collectively referred to herein as huMAb4D5, and also sometimes as the light or heavy chain variable domains of huMAb4D5, and are defined herein to be any polypeptide sequence which possesses a biological property of a polypeptide comprising the following polypeptide sequence:

DIQMTQSPSSLSASVGDRVTITCRASODVNTAVAWY QQKPGKAPKLLIYSASFLESGVPSRFSGSRSGT DFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTK VEIKRT (SEQ. ID NO. 1, which is the light chain variable domain of huMAb4D5); or

EVQLVESGGGLVOPGGSLRLSCAASGFNIKDTYIHW VRQAPGKGLEWVARIYPTNGYTRYADSVKGRFT ISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGD GFYAMDVWGQGTLVTVSS (SEQ. ID NO. 2, which is the heavy chain variable domain of huMAb4D5).

"Biological property", as relates for example to anti-p185^{HER2}, for the purposes herein means an in vivo effector or antigen-binding function or activity that is directly or indirectly performed by huMAb4D5 (whether in its native or denatured conformation). Effector functions include p185^{HER2} binding, any hormonal or hormonal antagonist activity, any mitogenic or agonist or antagonist activity, any

cytotoxic activity. An antigenic function means possession of an epitope or antigenic site that is capable of cross-reacting with antibodies raised against the polypeptide sequence of huMAb4D5.

Biologically active huMAb4D5 is defined herein as a polypeptide that shares an effector function of huMAb4D5. A principal known effector function of huMAb4D5 is its ability to bind to $p185^{HER2}$.

Thus, the biologically active and antigenically active huMAb4D5 polypeptides that are the subject of certain embodiments of this invention include the sequence of the entire translated nucleotide sequence of huMAb4D5; mature huMAb4D5; fragments thereof having a consecutive sequence of at least 5, 10, 15, 20, 25, 30 or 40 amino acid residues comprising sequences from muMAb4D5 plus residues from the human FR of huMAb4D5; amino acid sequence variants of huMAb4D5 wherein an amino acid residue has been inserted N- or C-terminal to, or within, huMAb4D5 or its fragment as defined above; amino acid sequence variants of huMAb4D5 or its fragment as defined above wherein an amino acid residue of huMAb4D5 or its fragment as defined above has been substituted by another residue, including predetermined mutations by, e.g., site-directed or PCR mutagenesis; derivatives of huMAb4D5 or its fragments as defined above wherein huMAb4D5 or its fragments have been covalent modified, by substitution, chemical, enzymatic, or other appropriate means, with a moiety other than a naturally occurring amino acid; and glycosylation variants of huMAb4D5 (insertion of a glycosylation site or deletion of any glycosylation site by deletion, insertion or substitution of suitable residues). Such fragments and variants exclude any polypeptide heretofore identified, including muMAb4D5 or any known polypeptide fragment, which are anticipatory order 35 U.S.C. 102 as well as polypeptides obvious thereover under 35 U.S.C. 103.

An "isolated" polypeptide means polypeptide which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, for example, a polypeptide product comprising huMAb4D5 will be purified from a cell culture or other synthetic environment (1) to greater than 95% by weight of protein as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a gas- or liquid-phase sequenator (such as a commercially available Applied Biosystems sequenator Model 470, 477, or 473), or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated huMAb4D5 includes huMAb4D5 in situ within recombinant cells since at least one component of the huMAb4D5 natural environment will not be present. Ordinarily, however, isolated huMAb4D5 will be prepared by at least one purification step.

In accordance with this invention, huMAb4D5 nucleic acid is RNA or DNA containing greater than ten bases that encodes a biologically or antigenically active huMAb4D5, is complementary to nucleic acid sequence encoding such huMAb4D5, or hybridizes to nucleic acid sequence encoding such huMAb4D5 and remains stably bound to it under stringent conditions, and comprises nucleic acid from a muMAb4D5 CDR and a human FR region.

Preferably, the huMAb4D5 nucleic acid encodes a polypeptide sharing at least 75% sequence identity, more preferably at least 80%, still more preferably at least 85%, even more preferably at 90%, and most preferably 95%, with the huMAb4D5 amino acid sequence. Preferably, a nucleic acid molecule that hybridizes to the huMAb4D5 nucleic acid contains at least 20, more preferably 40, and most preferably 90 bases. Such hybridizing or complementary nucleic acid, however, is further defined as being novel under 35 U.S.C. 102 and unobvious under 35 U.S.C. 103 over any prior art nucleic acid.

Stringent conditions are those that (1) employ low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate/0/1% $NaDodSO_4$ at 50° C.; (2) employ during hybridization a denaturing agent such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serumalbumin/0/1% Ficoll/0/1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42° C.; or (3) employ 50% formamide, 5×SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5×Denhardt's solution, sonicated salmon sperm DNA (50 g/ml), 0.1% SDS, and 10% dextran sulfate at 42 C., with washes at 42 C. in 0.2×SSC and 0.1% SDS.

The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, a ribosome binding site, and possibly, other as yet poorly understood sequences. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous and, in the case of a secretory leader, contiguous and in reading phase. However enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, then synthetic oligonucleotide adaptors or linkers are used in accord with conventional practice.

An "exogenous" element is defined herein to mean nucleic acid sequence that is foreign to the cell, or homologous to the cell but in a position within the host cell nucleic acid in which the element is ordinarily not found.

As used herein, the expressions "cell," "cell line," and "cell culture" are used interchangeably and all such designations include progeny. Thus, the words "transformants" and "transformed cells" include the primary subject cell and cultures derived therefrom without regard for the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Mutant progeny that have the same function or biological activity as screened for in the originally transformed cell are included. Where distinct designations are intended, it will be clear from the context.

"Oligonucleotides" are short-length, single- or double-stranded polydeoxynucleotides that are chemically synthesized by known methods (such as phosphotriester, phosphite, or phosphoramidite chemistry, using solid phase techniques such as described in EP 266,032 published May

4, 1988, or via deoxynucleoside H-phosphonate intermediates as described by Froehler et al., *Nucl. Acids Res.,* 14: 5399–5407 [1986]). They are then purified on polyacrylamide gels.

The technique of "polymerase chain reaction," or "PCR," as used herein generally refers to a procedure wherein minute amounts of a specific piece of nucleic acid, RNA and/or DNA, are amplified as described in U.S. Pat. No. 4,683,195 issued Jul. 28, 1987. Generally, sequence information from the ends of the region of interest or beyond needs to be available, such that oligonucleotide primers can be designed; these primers will be identical or similar in sequence to opposite strands of the template to be amplified. The 5' terminal nucleotides of the two primers may coincide with the ends of the amplified material. PCR can be used to amplify specific RNA sequences, specific DNA sequences from total genomic DNA, and cDNA transcribed from total cellular RNA, bacteriophage or plasmid sequences, etc. See generally Mullis et al., *Cold Spring Harbor Symp. Quant. Biol.,* 51: 263 (1987); Erlich, ed., *PCR Technology,* (Stockton Press, N.Y., 1989). As used herein, PCR is considered to be one, but not the only, example of a nucleic acid polymerase reaction method for amplifying a nucleic acid test sample, comprising the use of a known nucleic acid (DNA or RNA) as a primer and utilizes a nucleic acid polymerase to amplify or generate a specific piece of nucleic acid or to amplify or generate a specific piece of nucleic acid which is complementary to a particular nucleic acid.

Suitable Methods for Practicing the Invention

Some aspects of this invention include obtaining an import, non-human antibody variable domain, producing a desired humanized antibody sequence and for humanizing an antibody gene sequence are described below. A particularly preferred method of changing a gene sequence, such as gene conversion from a non-human or consensus sequence into a humanized nucleic acid sequence, is the cassette mutagenesis procedure described in Example 1. Additionally, methods are given for obtaining and producing antibodies generally, which apply equally to native non-human antibodies as well as to humanized antibodies.

Generally, the antibodies and antibody variable domains of this invention are conventionally prepared in recombinant cell culture, as described in more detail below. Recombinant synthesis is preferred for reasons of safety and economy, but it is known to prepare peptides by chemical synthesis and to purify them from natural sources; such preparations are included within the definition of antibodies herein.

Molecular Modeling

An integral step in our approach to antibody humanization is construction of computer graphics models of the import and humanized antibodies. These models are used to determine if the six complementarity-determining regions (CDRs) can be successfully transplanted from the import framework to a human one and to determine which framework residues from the import antibody, if any, need to be incorporated into the humanized antibody in order to maintain CDR conformation. In addition, analysis of the sequences of the import and humanized antibodies and reference to the models can help to discern which framework residues are unusual and thereby might be involved in antigen binding or maintenance of proper antibody structure.

All of the humanized antibody models of this invention are based on a single three-dimensional computer graphics structure hereafter referred to as the consensus structure. This consensus structure is a key distinction from the approach of previous workers in the field, who typically begin by selecting a human antibody structure which has an amino acid sequence which is similar to the sequence of their import antibody.

The consensus structure of one embodiment of this invention was built in five steps as described below.

Step 1

Seven Fab X-ray crystal structures from the Brookhaven Protein Data Bank were used (entries 2FB4, 2RHE, 3FAB, and 1 REI which are human structures, and 2MCP, 1 FBJ, and 2HFL which are murine structures). For each structure, protein mainchain geometry and hydrogen bonding patterns were used to assign each residue to one of three secondary structure types: alpha-helix, beta-strand or other (i.e. non-helix and non-strand). The immunoglobulin residues used in superpositioning and those included in the consensus structure are shown in Table 1.

TABLE I

	Immunoglobulin Residues Used in Superpositioning and Those Included in the Consensus Structure							
Ig[a]	2FB4	2RHE	2MCP	3FAB	1FBJ	2HFL	1REI	Consensus[b]
				$V_L\kappa$ domain				
								2–11
	18–24	18–24	19–25	18–24	19–25	19–25	19–25	16–27
	32–37	34–39	39–44	32–37	32–37	32–37	33–38	33–39
								41–49
	60–66	62–68	67–72	53–66	60–65	60–65	61–66	59–77
	69–74	71–76	76–81	69–74	69–74	69–74	70–75	
	84–88	86–90	91–95	84–88	84–88	84–88	85–89	82–91
								101–105
RMS[c]		0.40	0.60	0.53	0.54	0.48	0.50	
				V_H domain				
								3–8
	18–25		18–25	18–25	18–25	18–25		17–23
	34–39		34–39	34–39	34–39	34–39		33–41
	46–52		46–52	46–52	46–52	46–52		45–51
	57–61		59–63	56–60	57–61	57–61		57–61
	68–71		70–73	67–70	68–71	68–71		66–71
	78–84		80–86	77–83	78–84	78–84		75–82
	92–99		94–101	91–98	92–99	92–99		88–94
								102–108

TABLE I-continued

	Immunoglobulin Residues Used in Superpositioning and Those Included in the Consensus Structure							
Ig[a]	2FB4	2RHE	2MCP	3FAB	1FBJ	2HFL	1REI	Consensus[b]
RMS[c]			0.43	0.85	0.62	0.91		
RMS[d]	0.91		0.73	0.77	0.92			

[a]Four-letter code for Protein Data Bank file.
[b]Residue numbers for the crystal structures are taken from the Protein Data Bank files. Residue numbers for the consensus structure are according to Kabat et al.
[c]Root-mean-square deviation in Å for (N, Cα, C) atoms superimposed on 2FB4.
[d]Root-mean-square deviation in Å for (N, Cα, C) atoms superimposed on 2HFL.

Step 2

Having identified the alpha-helices and beta-strands in each of the seven structures, the structures were superimposed on one another using the INSIGHT computer program (Biosym Technologies, San Diego, Calif.) as follows: The 2FB4 structure was arbitrarily chosen as the template (or reference) structure. The 2FB4 was held fixed in space and the other six structures rotated and translated in space so that their common secondary structural elements (i.e. alpha-helices and beta-strands) were oriented such that these common elements were as close in position to one another as possible. (This superpositioning was performed using accepted mathematical formulae rather than actually physically moving the structures by hand.)

Step 3

With the seven structures thus superimposed, for each residue in the template (2FB4) Fab one calculates the distance from the template alpha-carbon atom (Cα) to the analogous Cα atom in each of the other six superimposed structures. This results in a table of Cα-Cα distances for each residue position in the sequence. Such a table is necessary in order to determine which residue positions will be included in the consensus model. Generally, is if all Cα-Cα distances for a given residue position were ≦1.0 Å, that position was included in the consensus structure. If for a given position only one Fab crystal structure was >1.0 Å, the position was included but the outlying crystal structure was not included in the next step (for this position only). In general, the seven β-strands were included in the consensus structure while some of the loops connecting the β-strands, e.g. complementarity-determining regions (CDRs), were not included in view of Cα divergence.

Step 4

For each residue which was included in the consensus structure after step 3, the average of the coordinates for individual mainchain N, Cα, C, O and Cβ atoms were calculated. Due to the averaging procedure, as well as variation in bond length, bond angle and dihedral angle among the crystal structures, this "average" structure contained some bond lengths and angles which deviated from standard geometry. For purposes of this invention, "standard geometry" is understood to include geometries commonly accepted as typical, such as the compilation of bond lengths and angles from small molecule structures in Weiner, S. J. et. al., *J. Amer. Chem. Soc.,* 106: 765–784 (1984).

Step 5

In order to correct these deviations, the final step was to subject the "average" structure to 50 cycles of energy minimization (DISCOVER program, Biosym Technologies) using the AMBER (Weiner, S. J. et. al., *J. Amer. Chem. Soc.,* 106: 765–784 (1984)) parameter set with only the Ca coordinates fixed (i.e. all other atoms are allowed to move) (energy minimization is described below). This allowed any deviant bond lengths and angles to assume a standard (chemically acceptable) geometry. See Table II.

TABLE II

Average Bond Lengths and Angles for "Average" (Before) and Energy-Minimized Consensus (After 50 Cycles) Structures

	$V_L\kappa$ before (Å)	$V_L\kappa$ after (Å)	V_H before (Å)	V_H after (Å)	Standard Geometry (Å)
N—Cα	1.459(0.012)	1.451(0.004)	1.451(0.023)	1.452(0.004)	1.449
Cα-C	1.515(0.012)	1.523(0.005)	1.507(0.033)	1.542(0.005)	1.522
O═C	1.208(0.062)	1.229(0.003)	1.160(0.177)	1.231(0.003)	1.229
C—N	1.288(0.049)	1.337(0.002)	1.282(0.065)	1.335(0.004)	1.335
Cα-Cβ	1.508(0.026)	1.530(0.002)	1.499(0.039)	1.530(0.002)	1.526
	(*)	(*)	(*)	(*)	(*)
C—N—Cα	123.5(4.2)	123.8(1.1)	125.3(4.6)	124.0(1.1)	121.9
N—Cα-C	110.0(4.0)	109.5(1.9)	110.3(2.8)	109.5(1.6)	110.1
Cα-C—N	116.6(4.0)	116.6(1.2)	117.6(5.2)	116.6(0.8)	116.6
O═C—N	123.1(4.1)	123.4(0.6)	122.2(4.9)	123.3(0.4)	122.9
N—Cα-Cβ	110.3(2.1)	109.8(0.7)	110.6(2.5)	109.8(0.6)	109.5
Cβ-Cα-C	111.4(2.4)	111.1(0.7)	111.2(2.2)	111.1(0.6)	111.1

Values in parentheses are standard deviations. Note that while some bond length and angle averages did not change appreciably after energy-minimization, the corresponding standard deviations are reduced due to deviant geometries assuming standard values after energy-minimization. Standard geometry values are from the AMBER forcefield as implemented in DISCOVER (Biosym Technologies).

The consensus structure might conceivably be dependent upon which crystal structure was chosen as the template on which the others were superimposed. As a test, the entire procedure was repeated using the crystal structure with the worst superposition versus 2FB4, i.e. the 2HFL Fab structure, as the new template (reference). The two consensus structures compare favorably (root-mean-squared deviation of 0.11 Å for all N, Cα and C atoms).

Note that the consensus structure only includes mainchain (N, Cα, C, O, Cβ atoms) coordinates for only those residues which are part of a conformation common to all seven X-ray crystal structures. For the Fab structures, these include the common β-strands (which comprise two β-sheets) and a few non-CDR loops which connect these β-strands. The consensus structure does not include CDRs or sidechains, both of which vary in their conformation among the seven structures. Also, note that the consensus structure includes only the V_L and V_H domains.

This consensus structure is used as the archetype. It is not particular to any species, and has only the basic shape without side chains. Starting with this consensus structure

the model of any import, human, or humanized Fab can be constructed as follows. Using the amino acid sequence of the particular antibody V_L and V_H domains of interest, a computer graphics program (such as INSIGHT, Biosym Technologies) is used to add sidechains and CDRs to the consensus structure. When a sidechain is added, its conformation is chosen on the basis of known Fab crystal structures (see the Background section for publications of such crystal structures) and rotamer libraries (Ponder, J. W. & Richards, F. M., *J. Mol. Biol.* 193: 775–791 (1987)). The model also is constructed so that the atoms of the sidechain are positioned so as to not collide with other atoms in the Fab.

CDRs are added to the model (now having the backbone plus side chains) as follows. The size (i.e. number of amino acids) of each import CDR is compared to canonical CDR structures tabulated by Chothia et al., *Nature,* 342:877–883 (1989)) and which were derived from Fab crystals. Each CDR sequence is also reviewed for the presence or absence of certain specific amino acid residues which are identified by Chothia as structurally important: e.g. light chain residues 29 (CDR1) and 95 (CDR3), and heavy chain residues 26, 27, 29 (CDR1) and 55 (CDR2). For light chain CDR2, and heavy chain CDR3, only the size of the CDR is compared to the Chothia canonical structure. If the size and sequence (i.e. inclusion of the specific, structurally important residues as denoted by Chothia et al.) of the import CDR agrees in size and has the same structurally important residues as those of a canonical CDR, then the mainchain conformation of the import CDR in the model is taken to be the same as that of the canonical CDR. This means that the import sequence is assigned the structural configuration of the canonical CDR, which is then incorporated in the evolving model.

However, if no matching canonical CDR can be assigned for the import CDR, then one of two options can be exercised. First, using a program such as INSIGHT (Biosym Technologies), the Brookhaven Protein Data Bank can be searched for loops with a similar size to that of the import CDR and these loops can be evaluated as possible conformations for the import CDR in the model. Minimally, such loops must exhibit a conformation in which no loop atom overlaps with other protein atoms. Second, one can use available programs which calculate possible loop conformations, assuming a given loop size, using methods such as described by Bruccoleri et al., *Nature* 335: 564–568 (1988).

When all CDRs and sidechains have been added to the consensus structure to give the final model (import, human or humanized), the model is preferably subjected to energy minimization using programs which are available commercially (e.g. DISCOVER, Biosym Technologies). This technique uses complex mathematical formulae to refine the model by performing such tasks as checking that all atoms are within appropriate distances from one another and checking that bond lengths and angles are within chemically acceptable limits.

Models of a humanized, import or human antibody sequence are used in the practice of this invention to understand the impact of selected amino acid residues of the activity of the sequence being modeled. For example, such a model can show residues which may be important in antigen binding, or for maintaining the conformation of the antibody, as discussed in more detail below. Modeling can also be used to explore the potential impact of changing any amino acid residue in the antibody sequence.

Methods for Obtaining a Humanized Antibody Sequence

In the practice of this invention, the first step in humanizing an import antibody is deriving a consensus amino acid sequence into which to incorporate the import sequences. Next a model is generated for these sequences using the methods described above. In certain embodiments of this invention, the consensus human sequences are derived from the most abundant subclasses in the sequence compilation of Kabat et al. (Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)), namely V_L κ subgroup I and V_H group III, and have the sequences indicated in the definitions above.

While these steps may be taken in different order, typically a structure for the candidate humanized antibody is created by transferring the at least one CDR from the non-human, import sequence into the consensus human structure, after the entire corresponding human CDR has been removed. The humanized antibody may contain human replacements of the non-human import residues at positions within CDRs as defined by sequence variability (Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)) or as defined by structural variability (Chothia, C. & Lesk, A. M., *J. Mol. Biol.* 196:901–917 (1987)). For example, huMAb4D5 contains human replacements of the muMAb4D5 residues at three positions within CDRs as defined by sequence variability (Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)) but not as defined by structural variability (Chothia, C. & Lesk, A. M., *J. Mol Bol.* 196:901–917 (1987)): V_L-CDR1 K24R, V_L-CDR2 R54L and V_L-CDR2 T56S.

Differences between the non-human import and the human consensus framework residues are individually investigated to determine their possible influence on CDR conformation and/or binding to antigen. Investigation of such possible influences is desirably performed through modeling, by examination of the characteristics of the amino acids at particular locations, or determined experimentally through evaluating the effects of substitution or mutagenesis of particular amino acids.

In certain preferred embodiments of this invention, a humanized antibody is made comprising amino acid sequence of an import, non-human antibody and a human antibody, utilizing the steps of:

a. obtaining the amino acid sequences of at least a portion of an import antibody variable domain and of a consensus human variable domain;

b. identifying Complementarity Determining Region (CDR) amino acid sequences in the import and the human variable domain sequences;

c. substituting an import CDR amino acid sequence for the corresponding human CDR amino acid sequence;

d. aligning the amino acid sequences of a Framework Region (FR) of the import antibody and the corresponding FR of the consensus antibody;

e. identifying import antibody FR residues in the aligned FR sequences that are non-homologous to the corresponding consensus antibody residues;

f. determining if the non-homologous import amino acid residue is reasonably expected to have at least one of the following effects:

1. non-covalently binds antigen directly,

2. interacts with a CDR; or

3. participates in the V_L–V_H interface; and

g. for any non-homologous import antibody amino acid residue which is reasonably expected to have at least

one of these effects, substituting that residue for the corresponding amino acid residue in the consensus antibody FR sequence.

Optionally, one determines if any non-homologous residues identified in step (e) are exposed on the surface of the domain or buried within it, and if the residue is exposed but has none of the effects identified in step (f), one may retain the consensus residue.

Additionally, in certain embodiments the corresponding consensus antibody residues identified in step (e) above are selected from the group consisting of 4L, 35L, 36L, 38L, 43L, 44L, 46L, 58L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71 L, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 67H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H (utilizing the numbering system set forth in Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)).

In preferred embodiments, the method of this invention comprises the additional steps of searching either or both of the import, non-human and the consensus variable domain sequences for glycosylation sites, determining if the glycosylation is reasonably expected to be important for the desired antigen binding and biological activity of the antibody (i.e., determining if the glycosylation site binds to antigen or changes a side chain of an amino acid residue that binds to antigen, or if the glycosylation enhances or weakens antigen binding, or is important for maintaining antibody affinity). if the import sequence bears the glycosylation site, it is preferred to substitute that site for the corresponding residues in the consensus human sequence if the glycosylation site is reasonably expected to be important. If only the consensus sequence, and not the import, bears the glycosylation site, it is preferred to eliminate that glycosylation site or substitute therefor the corresponding amino acid residues from the import sequence.

Another preferred embodiment of the methods of this invention comprises aligning import antibody and the consensus antibody FR sequences, identifying import antibody FR residues which are non-homologous with the aligned consensus FR sequence, and for each such non-homologous import antibody FR residue, determining if the corresponding consensus antibody residue represents a residue which is highly conserved across all species at that site, and if it is so conserved, preparing a humanized antibody which comprises the consensus antibody amino acid residue at that site.

In certain alternate embodiments, one need not utilize the modeling and evaluation steps described above, and may instead proceed with the steps of obtaining the amino acid sequence of at least a portion of an import, non-human antibody variable domain having a CDR and a FR, obtaining the amino acid sequence of at least a portion of a consensus human antibody variable domain having a CDR and a FR, substituting the non-human CDR for the human CDR in the consensus human antibody variable domain, and then substituting an amino acid residue for the consensus amino acid residue at at least one of the following sites:

a. (in the FR of the variable domain of the light chain) 4L, 35L, 36L, 38L, 43L, 44L, 58L, 46L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, or

b. fin the FR of the variable domain of the heavy chain) 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 67H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H.

Preferably, the non-CDR residue substituted at the consensus FR site is the residue found at the corresponding location of the non-human antibody. If desired, one may utilize the other method steps described above for determining whether a particular amino acid residue can reasonably be expected to have undesirable effects, and remedying those effects.

If after making a humanized antibody according to the steps above and testing its activity one is not satisfied with the humanized antibody, one preferably reexamines the potential effects of the amino acids at the specific locations recited above. Additionally, it is desirable to reinvestigate any buried residues which are reasonably expected to affect the V_L–V_H interface but may not directly affect CDR conformation. It is also desirable to reevaluate the humanized antibody utilizing the steps of the methods claimed herein.

In certain embodiments of this invention, amino acid residues in the consensus human sequence are substituted for by other amino acid residues. In preferred embodiments, residues from a particular non-human import sequence are substituted, however there are circumstances where it is desired to evaluate the effects of other amino acids. For example, if after making a humanized antibody according to the steps above and testing its activity one is not satisfied with the humanized antibody, one may compare the sequences of other classes or subgroups of human antibodies, or classes or subgroups of antibodies from the particular non-human species, and determine which other amino acid side chains and amino acid residues are found at particular locations and substituting such other residues.

Antibodies

Certain aspects of this invention are directed to natural antibodies and to monoclonal antibodies, as illustrated in the Examples below and by antibody hybridomas deposited with the ATCC (as described below). Thus, the references throughout this description to the use of monoclonal antibodies are intended to include the use of natural or native antibodies as well as humanized and chimeric antibodies. As used herein, the term "antibody" includes the antibody variable domain and other separable antibody domains unless specifically excluded.

In accordance with certain aspects of this invention, antibodies to be humanized (import antibodies) are isolated from continuous hybrid cell lines formed by the fusion of antigen-primed immune lymphocytes with myeloma cells. in certain embodiments, the antibodies of this invention are obtained by routine screening. Polyclonal antibodies to an antigen generally are raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the antigen and an adjuvant. It may be useful to conjugate the antigen or a fragment containing the target amino acid sequence to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic is anhydride, $SOCl_2$, or $R^1N{=}C{=}NR$, where R and R^1 are different alkyl groups.

The route and schedule of the host animal or cultured antibody-producing cells therefrom are generally in keeping with established and conventional techniques for antibody stimulation and production. While mice are frequently employed as the test model, it is contemplated that any mammalian subject including human subjects or antibody-producing cells obtained therefrom can be manipulated according to the processes of this invention to serve as the basis for production of mammalian, including human, hybrid cell lines.

Animals are typically immunized against the immunogenic conjugates or derivatives by combining 1 mg or 1 μg of conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with ⅕ to ⅒ the original amount of conjugate in Freund's complete adjuvant (or other suitable adjuvant) by subcutaneous injection at multiple sites. 7 to 14 days later animals are bled and the serum is assayed for antigen titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different protein and/or through a different cross-linking agent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are used to enhance the immune response.

After immunization, monoclonal antibodiesare prepared by recovering immune lymphoid cells—typically spleen cells or lymphocytes from lymph node tissue—from immunized animals and immortalizing the cells in conventional fashion, e.g. by fusion with myeloma cells or by Epstein-Barr (EB)-virus transformation and screening for clones expressing the desired antibody. The hybridoma technique described originally by Kohler and Milstein, *Eur. J. Immunol.* 6:511 (1976) has been widely applied to produce hybrid cell lines that secrete high levels of monoclonal antibodies against many specific antigens.

It is possible to fuse cells of one species with another. However, it is preferable that the source of the immunized antibody producing cells and the myeloma be from the same species.

The hybrid cell lines can be maintained in culture in vitro in cell culture media. The cell lines of this invention can be selected and/or maintained in a composition comprising the continuous cell line in hypoxanthine-aminopterin thymidine (HAT) medium. In fact, once the hybridoma cell line is established, it can be maintained on a variety of nutritionally adequate media. Moreover, the hybrid cell lines can be stored. and preserved in any number of conventional ways, including freezing and storage under liquid nitrogen. Frozen cell lines can be revived and cultured indefinitely with resumed synthesis and secretion of monoclonal antibody. The secreted antibody is recovered from tissue culture supernatant by conventional methods such as precipitation, Ion exchange chromatography, affinity chromatography, or the like. The antibodies described herein are also recovered from hybridoma cell cultures by conventional methods for purification of IgG or IgM as the case may be that heretofore have been used to purify these immunoglobulins from pooled plasma, e.g. ethanol or polyethylene glycol precipitation procedures. The purified antibodies are sterile filtered, and optionally are conjugated to a detectable marker such as an enzyme or spin label for use in diagnostic assays of the antigen in test samples.

While routinely rodent monoclonal antibodies are used as the source of the import antibody, the invention is not limited to any species. Additionally, techniques developed for the production of chimeric antibodies (Morrison et al., *Proc. Natl. Acad. Sci.,* 81:6851 (1984); Neuberger et al., *Nature* 312:604 (1984); Takeda et al., *Nature* 314:452 (1985)) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity (such as ability to activate human complement and mediate ADCC) can be used; such antibodies are within the scope of this invention.

Techniques for creating recombinant DNA versions of the antigen-binding regions of antibody molecules (known as Fab fragments) which bypass the generation of monoclonal antibodies are encompassed within the practice of this invention. One extracts antibody-specific messenger ANA molecules from immune system cells taken from an immunized animal, transcribes these into complementary DNA (cDNA), and clones the CDNA into a bacterial expressions system. One example of such a technique suitable for the practice of this invention was developed by researchers at Scripps/Stratagene, and incorporates a proprietary bacteriophage lambda vector system which contains a leader sequence that causes the expressed Fab protein to migrate to the periplasmic space (between the bacterial cell membrane and the cell wall) or to be secreted. One can rapidly generate and screen great numbers of functional FAb fragments for those which bind the antigen. Such FAb fragments with specificity for the antigen are specifically encompassed within the term "antibody" as it is defined, discussed, and claimed herein.

Amino Acid Sequence Variants

Amino acid sequence variants of the antibodies and polypeptides of this invention (referred to in herein as the target polypeptide) are prepared by introducing appropriate nucleotide changes into the DNA encoding the target polypeptide, or by in vitro synthesis of the desired target polypeptide. Such variants include, for example, humanized variants of non-human antibodies, as well as deletions from, or insertions or substitutions of, residues within particular amino acid sequences. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the target polypeptide, such as changing the number or position of glycosylation sites, altering any membrane anchoring characteristics, and/or altering the intra-cellular location of the target polypeptide by inserting, deleting, or otherwise affecting any leader sequence of the native target polypeptide.

In designing amino acid sequence variants of target polypeptides, the location of the mutation site and the nature of the mutation will depend on the target polypeptide characteristics) to be modified. The sites for mutation can be modified individually or in series, e.g., by (1) substituting first with conservative amino acid choices and then with more radical selections depending upon the results achieved, (2) deleting the target residue, or (3) inserting residues of the same or a different class adjacent to the located site, or combinations of options 1–3. In certain embodiments, these choices are guided by the methods for creating humanized sequences set forth above.

A useful method for identification of certain residues or regions of the target polypeptide that are preferred locations for mutagenesis is called "alanine scanning mutagenesism" as described by Cunningham and Wells (*Science,* 244: 1081–1085 [1989). Here, a residue or group of target residues are identified (e.g., charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with the surrounding aqueous environment in or outside the cell. Those domains demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at or for the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation per se need not be predetermined. For example, to optimize the performance of a mutation at a given site, ala scanning or random mutagenesis may be conducted at the target codon or region and the

expressed target polypeptide variants are screened for the optimal combination of desired activity.

There are two principal variables in the construction of amino acid sequence variants: the location of the mutation site and the nature of the mutation. In general, the location and nature of the mutation chosen will depend upon the target polypeptide characteristic to be modified.

Amino acid sequence deletions of antibodies are generally not preferred, as maintaining the generally configuration of an antibody is believed to be necessary for its activity. Any deletions will be selected so as to preserve the structure of the target antibody.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Intrasequence insertions (i.e., insertions within the target polypeptide sequence) may range generally from about 1 to 10 residues, more preferably 1 to 5, most preferably 1 to 3. Examples of terminal insertions include the target polypeptide with an N-terminal methionyl residue, an artifact of the direct expression of target polypeptide in bacterial recombinant cell culture, and fusion of a heterologous N-terminal signal sequence to the N-terminus of the target polypeptide molecule to facilitate the secretion of the mature target polypeptide from recombinant host cells. Such signal sequences generally will be obtained from, and thus homologous to, the intended host cell species. Suitable sequences include STII or Ipp for *E. coli,* alpha factor for yeast, and viral signals such as herpes gD for mammalian cells.

Other insertional variants of the target polypeptide include the fusion to the N- or C-terminus of the target polypeptide of immunogenic polypeptides, e.g., bacterial polypeptides such as beta-lactamase or an enzyme encoded by the *E. coli* trp locus, or yeast protein, and C-terminal fusions with proteins having a long half-life such as immunoglobulin constant regions (or other immunoglobulin regions), albumin, or ferritin, as described in WO 89/02922 published Apr. 6, 1989.

Another group of variants are amino acid substitution variants. These variants have at least one amino acid residue in the target polypeptide molecule removed and a different residue inserted in its place. The sites of greatest interest for substitutional mutagenesis include sites identified as the active site(s) of the target polypeptide, and sites where the amino acids found in the target polypeptide from various species are substantially different in terms of side-chain bulk, charge, and/or hydrophobicity. Other sites for substitution are described infra, considering the effect of the substitution of the antigen binding, affinity and other characteristics of a particular target antibody.

Other sites of interest are those in which particular residues of the target polypeptides obtained from various species are identical. These positions may be important for the biological activity of the target polypeptide. These sites, especially those falling within a sequence of at least three other identically conserved sites, are substituted in a relatively conservative manner. If such substitutions result in a change in biological activity, then other changes are introduced and the products screened until the desired effect is obtained.

Substantial modifications in function or immunological identity of the target polypeptide are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, lb) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side chain properties:

(1) hydrophobic: norleucine, met, ala, val, leu, ile;

(2) neutral hydrophilic: cys, ser, thr;

(3) acidic: asp, glu;

(4) basic: asn, gin, his, lys, arg;

(5) residues that influence chain orientation: gly, pro; and

(6) aromatic: trp, tyr, phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another. Such substituted residues may be introduced into regions of the target polypeptide that are homologous with other antibodies of the same class or subclass, or, more preferably, into the non-homologous regions of the molecule.

Any cysteine residues not involved in maintaining the proper conformation of target polypeptide also may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking.

DNA encoding amino acid sequence variants of the target polypeptide is prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the target polypeptide. A particularly preferred method of gene conversion mutagenesis is described below in Example 1. These techniques may utilized target polypeptide nucleic acid (DNA or RNA), or nucleic acid complementary to the target polypeptide nucleic acid.

Oligonucleotide-mediated mutagenesis is a preferred method for preparing substitution, deletion, and insertion variants of target polypeptide DNA. This technique is well known in the art as described by Adelman et al., *DNA,* 2: 183 (1983). Briefly, the target polypeptide DNA is altered by hybridizing an oligonucleotide encoding the desired mutation to a DNA template, where the template is the single-stranded form of a plasmid or bacteriophage containing the unaltered or native DNA sequence of the target polypeptide. After hybridization, a DNA polymerase is used to synthesize an entire second complementary strand of the template that will thus incorporate the oligonucleotide primer, and will code for the selected alteration in the target polypeptide DNA.

Generally, oligonucleotides of at least 25 nucleotides in length are used. An optimal oligonucleotide will have 12 to 15 nucleotides that are completely complementary to the template on either side of the nucleotide(s) coding for the mutation. This ensures that the oligonucleotide will hybridize properly to the single-stranded DNA template molecule. The oligonucleotides are readily synthesized using techniques known in the art such as that described by Crea et al. (*Proc. Natl. Acad. Sci. USA,* 75: 5765 [1978]).

Single-stranded DNA template may also be generated by denaturing double-stranded plasmid (or other) DNA using standard techniques.

For alteration of the native DNA sequence (to generate amino acid sequence variants, for example), the oligonucleotide is hybridized to the single-stranded template under suitable hybridization conditions. A DNA polymerizing enzyme, usually the Klenow fragment of DNA polymerase 1, is then added to synthesize the complementary strand of the template using the oligonucleotide as a primer for

synthesis. A heteroduplex molecule is thus formed such that one strand of DNA encodes the mutated form of the target polypeptide, and the other strand (the original template) encodes the native, unaltered sequence of the target polypeptide. This heteroduplex molecule is then transformed into a suitable host cell, usually a prokaryote such as *E. coli* JM101. After the cells are grown, they are plated onto agarose plates and screened using the oligonucleotide primer radiolabeled with 32-phosphate to identify the bacterial colonies that contain the mutated DNA. The mutated region is then removed and placed in an appropriate vector for protein production, generally an expression vector of the type typically employed for transformation of an appropriate host.

The method described immediately above may be modified such that a homoduplex molecule is created wherein both strands of the plasmid contain the mutation(s). The modifications are as follows: The single-stranded oligonucleotide is annealed to the single-stranded template as described above. A mixture of three deoxyribonucleotides, deoxyriboadenosine (dATP), deoxyriboguanosine (dGTP), and deoxyribothymidine (dTTP), is combined with a modified thio-deoxyribocytosine called dCTP-(aS) (which can be obtained from Amersham Corporation). This mixture is added to the template-oligonucleotide complex. Upon addition of DNA polymerase to this mixture, a strand of DNA identical to the template except for the mutated bases is generated. In addition, this new strand of DNA will contain dCTP-(aS) instead of dCTP, which serves to protect it from restriction endonuclease digestion.

After the template strand of the double-stranded heteroduplex is nicked with an appropriate restriction enzyme, the template strand can be digested with ExoIII nuclease or another appropriate nuclease past the region that contains the site(s) to be mutagenized. The reaction is then stopped to leave a molecule that is only partially single-stranded. A complete double-stranded DNA homoduplex is then formed using DNA polymerase in the presence of all four deoxyribonucleotide triphosphates, ATP, and DNA ligase. This homoduplex molecule can then be transformed into a suitable host cell such as *E. coli* JM101, as described above.

DNA encoding target polypeptide variants with more than one amino acid to be substituted may be generated in one of several ways. If the amino acids are located close together in the polypeptide chain, they may be mutated simultaneously using one oligonucleotide that codes for all of the desired amino acid substitutions. If, however, the amino acids are located some distance from each other (separated by more than about ten amino acids), it is more difficult to generate a single oligonucleotide that encodes all of the desired changes. Instead, one of two alternative methods may be employed.

In the first method, a separate oligonucleotide is generated for each amino acid to be substituted. The oligonucleotides are then annealed to the single-stranded template DNA simultaneously, and the second strand of DNA that is synthesized from the template will encode all of the desired amino acid substitutions.

The alternative method involves two or more rounds of mutagenesis to produce the desired mutant. The first round is as described for the single mutants: wild-type DNA is used for the template, an oligonucleotide encoding the first desired amino acid substitution(s) is annealed to this template, and the heteroduplex DNA molecule is then generated. The second round of mutagenesis utilizes the mutated DNA produced in the first round of mutagenesis as the template. Thus, this template already contains one or more mutations. The oligonucleotide encoding the additional desired amino acid substitution(s) is then annealed to this template, and the resulting strand of DNA now encodes mutations from both the first and second rounds of mutagenesis. This resultant DNA can be used as a template in a third round of mutagenesis, and so on.

PCR mutagenesis is also suitable for making amino acid variants of target polypeptide. While the following discussion refers to DNA, it is understood that the technique also finds application with RNA. The PCR technique generally refers to the following procedure (see Erlich, supra, the chapter by R. Higuchi, p. 61–70): When small amounts of template DNA are used as starting material in a PCR, primers that differ slightly in sequence from the corresponding region in a template DNA can be used to generate relatively large quantities of a specific DNA fragment that differs from the template sequence only at the positions where the primers differ from the template. For introduction of a mutation into a plasmid DNA, one of the primers is designed to overlap the position of the mutation and to contain the mutation; the sequence of the other primer must be identical to a stretch of sequence of the opposite strand of the plasmid, but this sequence can be located anywhere along the plasmid DNA. It is preferred, however, that the sequence of the second primer is located within 200 nucleotides from that of the first, such that in the end the entire amplified region of DNA bounded by the primers can be easily sequenced. PCR amplification using a primer pair like the one just described results in a population of DNA fragments that differ at the position of the mutation specified by the primer, and possibly at other positions, as template copying is somewhat error-prone.

If the ratio of template to product material is extremely low, the vast majority of product DNA fragments incorporate the desired mutation(s). This product material is used to replace the corresponding region in the plasmid that served as PCR template using standard DNA technology. Mutations at separate positions can be introduced simultaneously by either using a mutant second primer, or performing a second PCR with different mutant primers and ligating the two resulting PCR fragments simultaneously to the vector fragment in a three (or more)-part ligation.

In a specific example of PCR mutagenesis, template plasmid DNA (1 μg) is linearized by digestion with a restriction endonuclease that has a unique recognition site in the plasmid DNA outside of the region to be amplified. Of this material, 100 ng is added to a PCR mixture containing PCR buffer, which contains the four deoxynucleotide triphosphates and is included in the GeneAmp® kits (obtained from Perkin-Elmer Cetus, Norwalk, Conn. and Emeryville, Calif.), and 25 pmole of each oligonucleotide primer, to a final volume of 50μl. The reaction mixture is overlayed with 35 μl mineral oil. The reaction is denatured for 5 minutes at 100° C., placed briefly on ice, and then 1 μl *Thermus aquaticus* (Taq) DNA polymerase (5 units/μl, purchased from Perkin-Elmer Cetus, Norwalk, Conn. and Emeryville, Calif.) is added below the mineral oil layer. The reaction mixture is then inserted into a DNA Thermal Cycler (purchased from Perkin-Elmer Cetus) programmed as follows: 2 min. at 55° C., then 30 sec. at 72° C., then 19 cycles of the following: 30 sec. at 94° C., 30 sec. at 55° C., and 30 sec. at 72° C.

At the end of the program, the reaction vial is removed from the thermal cycler and the aqueous phase transferred to a new vial, extracted with phenol/chloroform (50:50:vol), and ethanol precipitated, and the DNA is recovered by standard procedures. This material is subsequently subjected to the appropriate treatments for insertion into a vector.

Another method for preparing variants, cassette mutagenesis, is based on the technique described by Wells et al. (*Gene,* 34: 315 [1985]). The starting material is the plasmid (or other vector) comprising the target polypeptide DNA to be mutated. The codon(s) in the target polypeptide DNA to be mutated are identified. There must be a unique restriction endonuclease site on each side of the identified mutation site(s). If no such restriction sites exist, they may be generated using the above-described oligonucleotide-mediated mutagenesis method to introduce them at appropriate locations in the target polypeptide DNA. After the restriction sites have been introduced into the plasmid, the plasmid is cut at these sites to linearize it. A double-stranded oligonucleotide encoding the sequence of the DNA between the restriction sites but containing the desired mutation(s) is synthesized using standard procedures. The two strands are synthesized separately and then hybridized together using standard techniques. This double-stranded oligonucleotide is referred to as the cassette. This cassette is designed to have 3' and 5' ends that are compatible with the ends of the linearized plasmid, such that it can be directly ligated to the plasmid. This plasmid now contains the mutated target polypeptide DNA sequence.

Insertion of DNA into a Cloning Vehicle

The cDNA or genomic DNA encoding the target polypeptide is inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. Many vectors are available, and selection of the appropriate vector will depend on 1) whether it is to be used for DNA amplification or for DNA expression, 2) the size of the DNA to be inserted into the vector, and 3) the host cell to be transformed with the vector. Each vector contains various components depending on its function (amplification of DNA or expression of DNA) and the host cell for which it is compatible. The vector components generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence.

(a) Signal Sequence Component

In general, the signal sequence may be a component of the vector, or it may be a part of the target polypeptide DNA that is inserted into the vector.

The target polypeptides of this invention may be expressed not only directly, but also as a fusion with a heterologous polypeptide, preferably a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the vector, or it may be a part of the target polypeptide DNA that is inserted into the vector. Included within the scope of this invention are target polypeptides with any native signal sequence deleted and replaced with a heterologous signal sequence. The heterologous signal sequence selected should be one that is recognized and processed (i.e. cleaved by a signal peptidase) by the host cell. For prokaryotic host cells that do not recognize and process the native target polypeptide signal sequence, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin 11 leaders. For yeast secretion the native target polypeptide signal sequence may be substituted by the yeast invertase, alpha factor, or acid phosphatase leaders. In mammalian cell expression the native signal sequence is satisfactory, although other mammalian signal sequences may be suitable.

(b) Origin of Replication Component

Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Generally, in cloning vectors this sequence is one that enables the vector to replicate independently of the host chromosomal DNA, and includes origins of replication or autonomously replicating sequences. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2μ plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter).

Most expression vectors are "shuttle" vectors, i.e. they are capable of replication in at least one class of organisms but can be transfected into another organism for expression. For example, a vector is cloned in *E. coli* and then the same vector is transfected into yeast or mammalian cells for expression even though it is not capable of replicating independently of the host cell chromosome.

DNA may also be amplified by insertion into the host genome. This is readily accomplished using Bacillus species as hosts, for example, by including in the vector a DNA sequence that is complementary to a sequence found in Bacillus genomic DNA. Transfection of Bacillus with this vector results in homologous recombination with the genome and insertion of the target polypeptide DNA. However, the recovery of genomic DNA encoding the target polypeptide is more complex than that of an exogenously replicated vector because restriction enzyme digestion is required to excise the target polypeptide DNA.

(c) Selection Gene Component

Expression and cloning vectors should contain a selection gene, also termed a selectable marker. This gene encodes a protein necessary for the survival or growth of transformed host cells grown in a selective culture medium. Host cells not transformed with the vector containing the selection gene will not survive in the culture medium. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g. ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, e.g. the gene encoding D-alanine racemase for Bacilli.

One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene express a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin (Southern et al., *J. Molec. Appl. Genet.,* 1: 327 [1982]), mycophenolic acid (Mulligan et al., *Science=:* 1422 [1980]) or hygromycin (Sugden et al., *Mol. Cell. Biol.,* 5: 410–413 [1985]). The three examples given above employ bacterial genes under eukaryotic control to convey resistance to the appropriate drug G418 or neomycin (geneticin), xgpt (mycophenolic acid), or hygromycin, respectively.

Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the target polypeptide nucleic acid, such as dihydrofolate reductase (DHFR) or thymidine kinase. The mammalian cell transformants are placed under selection pressure which only the transformants are uniquely adapted to survive by virtue of having taken up the marker. Selection pressure is imposed by culturing the transformants

under conditions in which the concentration of selection agent in the medium is successively changed, thereby leading to amplification of both the selection gene and the DNA that encodes the target polypeptide. Amplification is the process by which genes in greater demand for the production of a protein critical for growth are reiterated in tandem within the chromosomes of successive generations of recombinant cells. Increased quantities of the target polypeptide are synthesized from the amplified DNA.

For example, cells transformed with the DHFR selection gene are first identified by culturing all of the transformants in a culture medium that contains methotrexate (Mtx), a competitive antagonist of DHFR. An appropriate host cell when wild-type DHFR is employed is the Chinese hamster ovary (CHO) cell line deficient in DHFR activity, prepared and propagated as described by Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA,* 77: 4216 [1980]. The transformed cells are then exposed to increased levels of methotrexate. This leads to the synthesis of multiple copies of the DHFR gene, and, concomitantly, multiple copies of other DNA comprising the expression vectors, such as the DNA encoding the target polypeptide. This amplification technique can be used with any otherwise suitable host, e.g., ATCC No. CCL61 CHO-K1, notwithstanding the presence of endogenous DHFR if, for example, a mutant DHFR gene that is highly resistant to Mtx is employed (EP 117,060). Alternatively, host cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding the target polypeptide, wild-type DHFR protein, and another selectable marker such as aminoglycoside 3' phosphotransferase (APH) can be selected by cell growth in medium containing a selection agent for the selectable marker such as an aminoglycosidic antibiotic, e.g., kanamycin, neomycin, or G418. See U.S. Pat. No. 4,965,199.

A suitable selection gene for use in yeast is the trp1 gene present in the yeast plasmid YRp7 (Stinchcomb et al., *Nature,* 22: 39 [1979]; Kingsman et al., *Gene,* 7: 141 [1979]; or Tschemper et al., *Gene,* 10: 157 [1980]). The trp1 gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1 (Jones, *Genetics,* 5: 12 [1977]). The presence of the trp1 lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan. Similarly, Leu2-deficient yeast strains (ATCC 20,622 or 38,626) are complemented by known plasmids bearing the Leu2 gene.

(d) Promoter Component

Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to the target polypeptide nucleic acid. Promoters are untranslated sequences located upstream (5') to the start codon of a structural gene (generally within about 100 to 1000 bp) that control the transcription and translation of a particular nucleic acid sequence, such as that encoding the target polypeptide, to which they are operably linked. Such promoters typically fall into two classes, inducible and constitutive. Inducible promoters are promoters that initiate increased levels of transcription from DNA under their control in response to some change in culture conditions, e.g. the presence or absence of a nutrient or a change in temperature. At this time a large number of promoters recognized by a variety of potential host cells are well known. These promoters are operably linked to DNA encoding the target polypeptide by removing the promoter from the source DNA by restriction enzyme digestion and inserting the isolated promoter sequence into the vector. Both the native target polypeptide promoter sequence and many heterologous promoters may be used to direct amplification and/or expression of the target polypeptide DNA. However, heterologous promoters are preferred, as they generally permit greater transcription and higher yields of expressed target polypeptide as compared to the native target polypeptide promoter.

Promoters suitable for use with prokaryotic hosts include the β-lactamase and lactose promoter systems (Chang et al., *Nature,* 275: 615 [1978]; and Goeddel et al., *Nature,* 281: 544 [1979]), alkaline phosphatase, a tryptophan (trp) promoter system (Goeddel, *Nucleic Acids Res.,* 8: 4057 [1980] and EP 36,776) and hybrid promoters such as the tao promoter (deBoer et al., *Proc. Natl. Acad. Sci, USA,* 80: 21–25 [1983]). However, other known bacterial promoters are suitable. Their nucleotide sequences have been published, thereby enabling a skilled worker operably to ligate them to DNA encoding the target polypeptide (Siebenlist et al., *Cell,* 20: 269 [1980]) using linkers or adaptors to supply any required restriction sites. Promoters for use in bacterial systems also generally will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding the target polypeptide.

Suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase (Hitzeman et al., *J. Biol. Chem.,* 255: 2073 [1980]) or other glycolytic enzymes (Hess et al., *J. Adv. Enzyme Reg.,* 2: 149 [1968]; and Holland, *Biochemistry,* 17: 4900 [1978]), such asenolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in Hitzeman et al., EP 73,657A. Yeast enhancers also ate advantageously used with yeast promoters.

Promoter sequences are known for eukaryotes. Virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CXCAAT region where X may be any nucleotide. At the 3' end of most eukaryotic genes is an AATAAA sequence that may be the signal for addition of the poly A tail to the 3' end of the coding sequence. All of these sequences are suitably inserted into mammalian expression vectors.

Target polypeptide transcription from vectors in mammalian host cells is controlled by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published Jul. 5, 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g. the actin promoter or an immunoglobulin promoter, from heat-shock promoters, and from the promoter normally associated with the target polypeptide sequence, provided such promoters are compatible with the host cell systems.

The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that

also contains the SV40 viral origin of replication. Fiers et al., *Nature,* 273:113 (1978); Mulligan and Berg, *Science,* 209: 1422–1427 (1980); Pavlakis et al., *Proc. Natl. Acad. Sci. USA,* 78: 7398–7402 (1981). The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment. Greenaway et al., *Gene,* 18: 355–360 (1982). A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. Pat. No. 4,419,446. A modification of this system is described in U.S. Pat. No. 4,601,978. See also Gray et al., *Nature,* 29: 503–508 (1982) on expressing cDNA encoding immune interferon in monkey cells; , Reyes et al., *Nature,* 297: 598–601 (1982) on expression of human β-interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus, Canaani and Berg, *Proc. Natl. Acad. Sci. USA,* 79: 5166–5170 (1982) on expression of the human interferon β1 gene in cultured mouse and rabbit cells, and Gorman et al., *Proc. Natl. Aced. Sci. USA,* 79: 6777–6781 (1982) on expression of bacterial CAT sequences in CV-1 monkey kidney cells, chicken embryo fibroblasts, Chinese hamster ovary cells, HeLa cells, and mouse NIH-3T3 cells using the Rous sarcoma virus long terminal repeat as a promoter.

(e) Enhancer Element Component

Transcription of DNA encoding the target polypeptide of this invention by higher eukaryotes is often increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10–300 bp, that act on a promoter to increase its transcription. Enhancers are relatively orientation and position independent having been found 5' (Laimins et al., *Proc. Natl. Acad. Sci. USA,* 78: 993 [1981]) and 3' (Lusky et al., *Mol. Cell Bio.* 3: 1108 [1983]) to the transcription unit, within an intron (Banerji et al., *Cell,* 33: 729 [1983]) as well as within the coding sequence itself (Osborne et al., *Mol. Cell Bio.,* 4: 1293 [1984]). Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100–270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, *Nature,* 297: 17–18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the target polypeptide DNA, but is preferably located at a site 5' from the promoter.

(f) Transcription Termination Component

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3' untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding the target polypeptide. The 3' untranslated regions also include transcription termination sites.

Construction of suitable vectors containing one or more of the above listed components the desired coding and control sequences employs standard ligation techniques. Isolated plasmids or DNA fragments are cleaved, tailored, and religated in the form desired to generate the plasmids required.

For analysis to confirm correct sequences in plasmids constructed, the ligation mixtures are used to transform *E. coli* K12 strain 294 (ATCC 31,446) and successful transformants selected by ampicillin or tetracycline resistance where appropriate. Plasmids from the transformants are prepared, analyzed by restriction endonuclease digestion, and/or sequenced is by the method of Messing et al., *Nucleic Acids Res.,* 9: 309 (1981) or by the method of Maxam et al., *Methods in Enzymology* 65: 499 (1980).

Particularly useful in the practice of this invention are expression vectors that provide for the transient expression in mammalian cells of DNA encoding the target polypeptide. In general, transient expression involves the use of an expression vector that is able to replicate efficiently in a host cell, such that the host cell accumulates many copies of the expression vector and, in turn, synthesizes high levels of a desired polypeptide encoded by the expression vector. Transient expression systems, comprising a suitable expression vector and a host cell, allow for the convenient positive identification of polypeptides encoded by cloned DNAs, as well as for the rapid screening of such polypeptides for desired biological or physiological properties. Thus, transient expression systems are particularly useful in the invention for purposes of identifying analogs and variants of the target polypeptide that have target polypeptide-like activity.

Other methods, vectors, and host cells suitable for adaptation to the synthesis of the target polypeptide in recombinant vertebrate cell culture are described in Gething et al., *Nature,* 293: 620–625 [1981]; Mantei et al., *Nature,* 281: 40–46 [1979]; Levinson et al.,; EP 117,060; and EP 117,058. A particularly useful plasmid for mammalian cell culture expression of the target polypeptide is pRK5 (EP pub. no. 307,247) or pSVI6B.

Selection and Transformation of Host Cells

Suitable host cells for cloning or expressing the vectors herein are the prokaryote, yeast, or higher eukaryote cells described above. Suitable prokaryotes include eubacteria, such as Gram-negative or Gram-positive organisms, for example, *E. coli* , Bacilli such as *B. subtilis*, Pseudomonas species such as *P. aeruginosa, Salmonella typhimurium,* or *Serratia marcescans*. One preferred *E. coli* cloning host is *E. coli* 294 (ATCC 31,446), although other strains such as *E. coli* B, *E. coli* X1776 (ATCC 31,537), and *E. coli* W3110 (ATCC 27,325) are suitable. These examples are illustrative rather than limiting. Preferably the host cell should secrete minimal amounts of proteolytic enzymes. Alternatively, in vitro methods of cloning, e.g. PCR or other nucleic acid polymerase reactions, are suitable.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable hosts for target polypeptide-encoding vectors. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *Schizosaccharomyces pombe* [Beach and Nurse, *Nature,* 290: 140 (1981); EP 139,383 published May 2, 1985], Kluyveromyces hosts (U.S. Pat. No. 4,943,529) such as, e.g., *K. lactis* [Louvencourt et al., *J. Bacteriol.,* 737 (1983)], *K. fragilis, K. bulgaricus, K. thermotolerans,* and *K. marxianus,* yarrowia [EP 402,226], *Pichia pastoris* [EP 183,070; Sreekrishna et al., *J. Basic Microbiol.,* 28: 265–278 (1988)], Candida, *Trichoderma reesia* [EP 244,2341], *Neurospora crassa* [Case et al., *Proc. Natl. Acad. Sci. USA,* 76: 5259–5263 (1979)], and filamentous fungi such as, e.g, Neurospora, Penicillium, Tolypocladium [WO 91/00357 published Jan. 10, 1991], and Aspergillus hosts such as *A. nidulans* [Ballance et al., *Biochem. Biophys. Res. Commun.* 112: 284–289 (1983); Tilburn et al., *Gene,* 26: 205–221 (1983); Yelton et al., *Proc. Natl. Acad. Sci. USA,* 81: 1470–1474 (1984)] and *A. niger* [Kelly and Hynes, *EMBO J.,* 4: 475–479 (1985)].

Suitable host cells for the expression of glycosylated target polypeptide are derived from multicellular organisms. Such host cells are capable of complex processing and glycosylation activities. In principle, any higher eukaryotic cell culture is workable, whether from vertebrate or invertebrate culture. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda* (caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosophila melanogaster* (fruitfly), and *Bombyx mori* host cells have been identified. See, e.g., Luckow et al., *Bio/Technologvy* 6: 47–55 (1988); Miller et al., in *Genetic Engineering* Setlow, J. K. et a., eds., Vol. 8 (Plenum Publishing, 1986), pp. 277–279; and Maeda et al., *Nature,* 315: 592–594 (1985). A variety of such viral strains are publicly available, e.g., the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells. Plant cell cutures of cotton, corn, potato, soybean, petunia, tomato, and tobacco can be utilized as hosts. Typically, plant cells are transfected by incubation with certain strains of the bacterium *Agrobacterium tumefaciens,* which has been previously manipulated to contain the target polypeptide DNA. During incubation of the plant cell culture with *A. tumefaciens,* the DNA encoding target polypeptide is transferred to the plant cell host such that it is transfected, and will, under appropriate conditions, express the target polypeptide DNA. In addition, regulatory and signal sequences compatible with plant cells are available, such as the nopaline synthase promoter and polyadenylation signal sequences. Depicker et al., *J. Mol. Appl. Gen.,* 1: 561 (1982). In addition, DNA segments isolated from the upstream region of the T-DNA 780 gene are capable of activating or increasing transcription levels of plant-expressible genes in recombinant DNA-containing plant tissue. See EP 321,196 published Jun. 21, 1989.

However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure in recent years [*Tissue Culture*, Academic Press, Kruse and Patterson, editors (1973)]. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., *J. Gen Virol.,* 36: 59 [1977]); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA,* 77: 4216 [1980]); mouse sertoli cells (TM4, Mather, *Biol. Reprod.,* 23: 243–251 [1980]); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HS 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather et al., *Annals N.Y. Acad. Sci.,* 383: 44–68 [1982]); MRC 5 cells; FS4 cells; and a human hepatoma cell line (Hep G2). Preferred host cells are human embryonic kidney 293 and Chinese hamster ovary cells.

Host cells are transfected and preferably transformed with the above-described expression or cloning vectors of this invention and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Transfection refers to the taking up of an expression vector by a host cell whether or not any coding sequences are in fact expressed. Numerous methods of transfection are known to the ordinarily skilled artisan, for example, $CaPO_4$ and electroporation. Successful transfection is generally recognized when any indication of the operation of this vector occurs within the host cell.

Transformation means introducing DNA into an organism so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integrant. Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in section 1.82 of Sambrook et al., supra, is generally used for prokaryotes or other cells that contain substantial cell-wall barriers. Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw et al., *Gene,* 23: 315 (1983) and WO 89/05859 published Jun. 29, 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method described in sections 16.30–16.37 of Sambrook et al., supra, is preferred. General aspects of mammalian cell host system transformations have been described by Axel in U.S. Pat. No. 4,399,216 issued Aug. 16, 1983. Transformations into yeast are typically carried out according to the method of Van Solingen et al., *J. Bact.,* 130: 946 (1977) and Hsiao et al., *Proc. Natl. Acad. Sci. (USA),* 76: 3829 (1979). However, other methods for introducing DNA into cells such as by nuclear injection, electroporation, or protoplast fusion may also be used.

Culturina the Host Cells

Prokaryotic cells used to produce the target polypeptide of this invention are cultured in suitable media as described generally in Sambrook et al., supra.

The mammalian host cells used to produce the target polypeptide of this invention may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ([MEM], Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ([DMEM], Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham and Wallace, *Meth. Enz.,* 58: 44 (1979), Barnes and Sato, *Anal. Biochem.* 102: 255 (1980), U.S. Pat. Nos. 4,767,704; 4,657,866; 4,927,762; or 4,560,655; WO 90/03430; WO 87/00195; U.S. Pat. No. Re. 30,985, may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleosides (such as adenosine and thymidine), antibiotics (such as Gentamycin™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

The host cells referred to in this disclosure encompass cells in in vitro culture as well as cells that are within a host animal.

It is further envisioned that the target polypeptides of this invention may be produced by homologous recombination, or with recombinant production methods utilizing control elements introduced into cells already containing DNA encoding the target polypeptide currently in use in the field.

For example, a powerful promoter/enhancer element, a suppressor, or an exogenous transcription modulatory element is inserted in the genome of the intended host cell in proximity and orientation sufficient to influence the transcription of DNA encoding the desired target polypeptide. The control element does not encode the target polypeptide of this invention, but the DNA is present in the host cell genome. One next screens for cells making the target polypeptide of this invention, or increased or decreased levels of expression, as desired.

Detecting Gene Amplification/Expression

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, northern blotting to quantitate the transcription of mRNA (Thomas, *Proc. Natl. Acad. Sci. USA,* 77: 5201–5205 [1980]), dot blotting (DNA analysis), or in situ hybridization, using an appropriately labeled probe, based on the sequences provided herein. Various labels may be employed, most commonly radioisotopes, particularly ^{32}P. However, other techniques may also be employed, such as using biotin-modified nucleotides for introduction into a polynucleotide. The biotin then serves as the site for binding to avidin or antibodies, which may be labeled with a wide variety of labels, such as radionuclides, fluorescers, enzymes, or the like. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. With immunohistochemical staining techniques, a cell sample is prepared, typically by dehydration and fixation, followed by reaction with labeled antibodies specific for the gene product coupled, where the labels are usually visually detectable, such as enzymatic labels, fluorescent labels, luminescent labels, and the like. A particularly sensitive staining technique suitable for use in the present invention is described by Hsu et al., *Am. J. Clin. Path.,* 75: 734–738 (1980).

Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared in any mammal. Conveniently, the antibodies may be prepared against a native target polypeptide or against a synthetic peptide based on the DNA sequences provided herein as described further in Section 4 below.

Purification of the Target Polypeptide

The target polypeptide preferably is recovered from the culture medium as a secreted polypeptide, although it also may be recovered from host cell lysates when directly expressed without a secretory signal.

When the target polypeptide is expressed in a recombinant cell other than one of human origin, the target polypeptide is completely free of proteins or polypeptides of human origin. However, it is necessary to purify the target polypeptide from recombinant cell proteins or polypeptides to obtain preparations that are substantially homogeneous as to the target polypeptide. As a first step, the culture medium or lysate is centrifuged to remove particulate cell debris. The membrane and soluble protein fractions are then separated. The target polypeptide may then be purified from the soluble protein fraction and from the membrane fraction of the culture lysate, depending on whether the target polypeptide is membrane bound. The following procedures are exemplary of suitable purification procedures: fractionation on immunoaffinity or ion-exchange columns; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-75; and protein A Sepharose columns to remove contaminants such as IgG.

Target polypeptide variants in which residues have been deleted, inserted or substituted are recovered in the same fashion, taking account of any substantial changes in properties occasioned by the variation. For example, preparation of a target polypeptide fusion with another protein or polypeptide, e.g. a bacterial or viral antigen, facilitates purification; an immunoaffinity column containing antibody to the antigen (or containing antigen, where the target polypeptide is an antibody) can be used to adsorb the fusion. Immunoaffinity columns such as a rabbit polyclonal anti-target polypeptide column can be employed to absorb the target polypeptide variant by binding it to at least one remaining immune epitope. A protease inhibitor such as phenyl methyl sulfonyl fluoride (PMSF) also may be useful to inhibit proteolytic degradation during purification, and antibiotics may be included to prevent the growth of adventitious contaminants. One skilled in the art will appreciate that purification methods suitable for native target polypeptide may require modification to account for changes in the character of the target polypeptide or its variants upon expression in recombinant cell culture.

Covalent Modifications of Target Polypeptides

Covalent modifications of target polypeptides are included within the scope of this invention. One type of covalent modification included within the scope of this invention is a target polypeptide fragment. Target polypeptide fragments having up to about 40 amino acid residues may be conveniently prepared by chemical synthesis, or by enzymatic or chemical cleavage of the full-length target polypeptide or variant target polypeptide. Other types of covalent modifications of the target polypeptide or fragments thereof are introduced into the molecule by reacting specific amino acid residues of the target polypeptide or fragments thereof with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues.

Cysteinyl residues most commonly are reacted with α-haloacetates (and corresponding amines), such as chloroacetic acid or chloroacetamide, to give carboxymethyl or carboxyamidomethyl derivatives. Cysteinyl residues also are derivatized by reaction with bromotrifluoroacetone, α-bromo-β-(5-imidozoyl)propionic acid, chloroacetyl phosphate, N-alkylmaleimides,3-nitro-2-pyridyl disulfide, methyl2-pyridyldisulfide, p-chloromercuribenzoate, 2-chloromercuri-4-nitrophenol, or chloro-7-nitrobenzo-2-oxa-1,3-diazole.

Histidyl residues are derivatized by reaction with diethylpyrocarbonate at pH 5.5–7.0 because this agent is relatively specific for the histidyl side chain. Para-bromophenacyl bromide also is useful; the reaction is preferably performed in 0.1M sodium cacodylate at pH 6.0.

Lysinyl and amino terminal residues are reacted with succinic or other carboxylic acid anhydrides. Derivatization with these agents has the effect of reversing the charge of the lysinyl residues. Other suitable reagents for derivatizing α-amino-containing residues include imidoesters such as methyl picolinimidate; pyridoxal phosphate; pyridoxal;

chloroborohydride; trinitrobenzenesulfonic acid; O-methylisourea; 2,4-pentanedione; and transaminase-catalyzed reaction with glyoxylate.

Arginyl residues are modified by reaction with one or several conventional reagents, among them phenylglyoxal, 2,3-butanedione, 1,2-cyclohexanedione, and ninhydrin. Derivatization of arginine residues requires that the reaction be performed in alkaline conditions because of the high pK_a of the guanidine functional group. Furthermore, these reagents may react with the groups of lysine as well as the arginine epsilon-amino group.

The specific modification of tyrosyl residues may be made, with particular interest in so introducing spectral labels into tyrosyl residues by reaction with aromatic diazonium compounds or tetranitromethane. Most commonly, N-acetylimidizole and tetranitromethane are used to form O-acetyl tyrosyl species and 3-nitro derivatives, respectively. Tyrosyl residues are iodinated using ^{125}I or ^{131}I to prepare labeled proteins for use in radioimmunoassay, the chloramine T method described above being suitable.

Carboxyl side groups (aspartyl or glutamyl) are selectively modified by reaction with carbodiimides (R'—N═C═N—R'), where R and R' are different alkyl groups, such as 1-cyclohexyl-3-(2-morpholinyl-4-ethyl) carbodiimide or 1-ethyl-3-(4-azonia-4,4-dimethylpentyl) carbodiimide. Furthermore, aspartyl and glutamyl residues are converted to asparaginyl and glutaminyl residues by reaction with ammonium ions.

Derivatization with bifunctional agents is useful for crosslinking target polypeptide to a water-insoluble support matrix or surface for use in the method for purifying anti-target polypeptide antibodies, and vice versa. Commonly used crosslinking agents include, e.g., 1,1-bis (diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis (succinimidylpropionate), and bifunctional maleimides such as bis-N-maleimido-1,8-octane. Derivatizing agents such as methyl-3-[(p-azidophenyl)dithio]propioimidate yield photoactivatable intermediates that are capable of forming crosslinks in the presence of light. Alternatively, reactive water-insoluble matrices such as cyanogen bromide-activated carbohydrates and the reactive substrates described in U.S. Pat. Nos. 3,969,287; 3,691,016; 4,195,128; 4,247,642; 4,229,537; and 4,330,440 are employed for protein immobilization.

Glutaminyl and asparaginyl residues are frequently deamidated to the corresponding glutamyl and aspartyl residues, respectively. Alternatively, these residues are deamidated under mildly acidic conditions. Either form of these residues falls within the scope of this invention.

Other modification include hydroxylation of proline and lysine, phophorylation of hydroxyl groups of seryl or threonyl resides, methylation of the α-amino groups of lysine, arginine, and histidine side chains, (T. E. Creighton, *Protein: Structure and Molecular Properties*, W. H. Freeman & Co., San Francisco, pp. 79–86 [1983]), acetylation of the N-terminal amine, and amidaatioon of any C-terminal carboxyl group.

Another type of covalent modification of the target polypeptide included within the scope of this invention comprises altering the native glucosylatuion pattern of the polypeptide. By altering is meant deleting one or more carbohydrate moieties found in the native target polypeptide, and/or adding one or more glycosylation sites that are not present in the native target polypeptide.

Gylcosylation of polypeptides is typically either N-linked or O-linked refers to the attachment of the carbonhydrate moiety to the side chain of an asparagine reisdue. The tri-peptide sequences asparagine-X-resine and asparagine-X-threonine, where X is any aminoe acid except proline, are the recoginition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tri-peptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgactosamine, galactose, or xylose, to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

Addition of glycosylation sites to the target polypeptide is conveniently accomplished by altering the amino acid sequence such that it contains one or more of hte above-described tri-peptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or theonine resides to the native target polypeptide sequence (for O-linked glycosylation sites). For ease, the target polypeptide amino acid sequences is preferably altered through changes at the DNA level, particularly by mutating the DNA encoding the target polypeptide at preselected bases such that condons are generated that will translate into the desired amino acids. The DNA mutation(s) may be made using methods described above under the heading of "Amino Acid Sequence Variants of Target Polypeptide".

Another means of increasing the number of carbohydrate moieties on the target polypeptide is by chemical or enzymatic coupling glycosides to the polypeptides. These procedures are advantageous in that they do not require production of the polypeptide in a host cell that has glycosylation capabilities for N- or O-linked glycosylation. Depending on the couple mode used, the sugar(s) may be attached to (a) arginine and histidine, (b) free carboxyl groups, (c) free sulfhydryl groups such as those of cysteine, (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline, (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan, or (f) the amide group of glutamine. These methods are described in WO 87/05330 published Sep. 11, 1987, and in Aplin and Wriston (*CRC Crit. Rev. Biochem., pp.* 259–306 [1981]).

Removal of carbohydrate moieties present on the native target polypeptide may be accomplished chemically or enzymatically. Chemical deglycosylation requires exposure of the polypeptide to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the polypeptide intact. Chemical deglycosylation is described by Hakimuddin et al. (*Arch. Biochem. Biophys.,* 259:52 [1987]) and by Edge et al. (*Anal. Biochem.,* 118:131 [1981]). Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura et al. (*Meth. Enzymol.* 138:350 [1987]).

Glycosylation at potential glycosylation sites may be prevented by the use of the compound tunicamycin as described by Duskin et al. (*J. Biol. Chem.,* 257:3105 [1982]). Tunicamycin blocks the formation of protein-N-glycoside linkages.

Another type of covalent modification of the target polypeptide comprises linking the target polypeptide to various nonproteinaceous polymers, e.g. polyethylene glycol, polypropylene glycol or polyoxyalkylenes, in the

manner set forth in U.S. Pat. Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337.

The target polypeptide also may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-microcapsules and poly-[methylmethacylate]microcapsules, respectively), in colloidal drug deliverysystems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), or in macroemulsions. Such techniques are disclosed in *Reminaton's Pharmaceutical Sciences,* 16th edition, Osol, A., Ed., (1980).

Target polypeptide preparations are also useful in generating antibodies, for screening for binding partners, as standards in assays for the target polypeptide (e.g. by labeling the target polypeptide for use as a standard in a radioimmunoassay, enzyme-linked immunoassay, or radioreceptor assay), in affinity purification techniques, and in competitive-type receptor binding assays when labeled with radioiodine, enzymes, fluorophores, spin labels, and the like.

Since it is often difficult to predict in advance the characteristics of a variant target polypeptide, it will be appreciated that some screening of the recovered variant will be needed to select the optimal variant. For example, a change in the immunological character of the target polypeptide molecule, such as affinity for a given antigen or antibody, is measured by a competitive-type immunoassay. The variant is assayed for changes in the suppression or enhancement of its activity by comparison to the activity observed for the target polypeptide in the same assay. Other potential modifications of protein or polypeptide properties such as redox or thermal stability, hydrophobicity, susceptibility to proteolytic degradation, stability in recombinant cell culture or in plasma, or the tendency to aggregate with carriers or into multimers are assayed by methods well known in the art.

Diagnostic and Related Uses of the Antibodies

The antibodies of this invention are useful in diagnostic assays for antigen expression in specific cells or tissues. The antibodies are detectably labeled and/or are immobilized on an insoluble matrix.

The antibodies of this invention find further use for the affinity purification of the antigen from recombinant cell culture or natural sources. Suitable diagnostic assays for the antigen and its antibodies depend on the particular antigen or antibody. Generally, such assays include competitive and sandwich assays, and steric inhibition assays. Competitive and sandwich methods employ a phase-separation step as an integral part of the method while steric inhibition assays are conducted in a single reaction mixture. Fundamentally, the same procedures are used for the assay of the antigen and for substances that bind the antigen, although certain methods will be favored depending upon the molecular weight of the substance being assayed. Therefore, the substance to be tested is referred to herein as an analyte, irrespective of its status otherwise as an antigen or antibody, and proteins that bind to the analyte are denominated binding partners, whether they be antibodies, cell surface receptors, or antigens.

Analytical methods for the antigen or its antibodies all use one or more of the following reagents: labeled analyte analogue, immobilized analyte analogue, labeled binding partner, immobilized binding partner and steric conjugates. The labeled reagents also are known as "tracers."

The label used (and this is also useful to label antigen nucleic acid for use as a probe) is any detectable functionality that does not interfere with the binding of analyte and its binding partner. Numerous labels are known for use in immunoassay, examples including moieties that may be detected directly, such as fluorochrome, chemiluminescent, and radioactive labels, as well as moieties, such as enzymes, that must be reacted or derivatized to be detected. Examples of such labels include the radioisotopes ^{32}P, ^{14}C, ^{125}I, ^{3}H, and ^{131}I, fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luceriferases, e.g., firefly luciferase and bacterial luciferase (U.S. Pat. No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, β-galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotintavidin, spin labels, bacteriophage labels, stable free radicals, and the like.

Conventional methods are available to bind these labels covalenily to proteins or polypeptides. For instance, coupling agents such as dialdehydes, carbodiimides, dimaleimides, bis-imidates, bis-diazotized benzidine, and the like may be used to tag the antibodies with the above-described fluorescent, chemiluminescent, and enzyme labels. See, for example, U.S. Pat. No. 3,940,475 (fluorimetry) and U.S. Pat. No. 3,645,090 (enzymes); Hunter et al., *Nature,* 144: 945 (1962); David et al., *Biochemistry,* 13: 1014–1021 (1974); Pain et al., *J. Immunol. Methods,* 40: 219–230 (1981); and Nygren, *J. Histochem. and Cytochem.,* 30: 407–412 (1982). Preferred labels herein are enzymes such as horseradish peroxidase and alkaline phosphatase.

The conjugation of such label, including the enzymes, to the antibody is a standard manipulative procedure for one of ordinary skill in immunoassay techniques. See, for example, O'Sullivan et al., "Methods for the Preparation of Enzyme-antibody Conjugates for Use in Enzyme Immunoassay," in *Methods in in Enzymology*, ed. J. J. Langone and H. Van Vunakis, Vol. 73 (Academic Press, New York, N.Y., 1981), pp. 147–166. Such bonding methods are suitable for use with the antibodies and polypeptides of this invention.

Immobilization of reagents is required for certain assay methods. Immobilization entails separating the binding partner from any analyte that remains free in solution. This conventionally is accomplished by either insolubilizing the binding partner or analyte analogue before the assay procedure, as by adsorption to a water-insoluble matrix or surface (Bennich et al., U.S. Pat. No. 3,720,760), by covalent coupling (for example, using glutaraldehyde cross-linking), or by insolubilizing the partner or analogue afterward, e.g., by immunoprecipitation.

Other assay methods, known as competitive or sandwich assays, are well established and widely used in the commercial diagnostics industry.

Competitive assays rely on the ability of a tracer analogue to compete with the test sample analyte for a limited number of binding sites on a common binding partner. The binding partner generally is insolubilized before or after the competition and then the tracer and analyte bound to the binding partner are separated from the unbound tracer and analyte. This separation is accomplished by decanting (where the binding partner was preinsolubilized) or by centrifuging (where the binding partner was precipitated after the competitive reaction). The amount of test sample analyte is inversely proportional to the amount of bound tracer as measured by the amount of marker substance. Dose-

response curves with known amounts of analyte are prepared and compared with the test results to quantitatively determine the amount of analyte present in the test sample. These assays are called ELISA systems when enzymes are used as the detectable markers.

Another species of competitive assay, called a "homogeneous" assay, does not require a phase separation. Here, a conjugate of an enzyme with the analyte is prepared and used such that when anti-analyte binds to the analyte the presence of the anti-analyte modifies the enzyme activity. In this case, the antigen or its immunologically active fragments are conjugated with a bifunctional organic bridge to an enzyme such as peroxidase. Conjugates are selected for use with antibody so that binding of the antibody inhibits or potentiates the enzyme activity of the label. This method per se is widely practiced under the name of EMIT.

Steric conjugates are used in steric hindrance methods for homogeneous assay. These conjugates are synthesized by covalently linking a low-molecular-weight hapten to a small analyte so that antibody to hapten substantially is unable to bind the conjugate at the same time as anti-analyte. Under this assay procedure the analyte present in the test sample will bind anti-analyte, thereby allowing anti-hapten to bind the conjugate, resulting in a change in the character of the conjugate hapten, e.g., a change in fluorescence when the hapten is a fluorophore.

Sandwich assays particularly are useful for the determination of antigen or antibodies. In sequential sandwich assays an immobilized binding partner is used to adsorb test sample analyte, the test sample is removed as by washing, the bound analyte is used to adsorb labeled binding partner, and bound material is then separated from residual tracer. The amount of bound tracer is directly proportional to test sample analyte. In "simultaneous" sandwich assays the test sample is not separated before adding the labeled binding partner. A sequential sandwich assay using an anti-antigen monoclonal antibody as one antibody and a polyclonal anti-antigen antibody as the other is useful in testing samples for particular antigen activity.

The foregoing are merely exemplary diagnostic assays for the import and humanized antibodies of this invention. Other methods now or hereafter developed for the determination of these analytes are included within the scope hereof, including the bioassays described above.

Immunotoxins

This invention is also directed to immunochemical derivatives of the antibodies of this invention such as immunotoxins (conjugates of the antibody and a cytotoxic moiety). Antibodies which carry the appropriate effector functions, such as with their constant domains, are also used to induce lysis through the natural complement process, and to interact with antibody dependent cytotoxic cells normally present.

For example, purified, sterile filtered antibodies are optionally conjugated to a cytotoxin such as ricin for use in AIDS therapy. U.S. patent application Ser. No. 07/350,895 illustrates methods for making and using immunotoxins for the treatment of HIV infection. The methods of this invention, for example, are suitable for obtaining humanized antibodies for use as immunotoxins for use in AIDS therapy.

The cytotoxic moiety of the immunotoxin may be a cytotoxic drug or an enzymatically active toxin of bacterial, fungal, plant or animal origin, or an enzymatically active fragment of such a toxin. Enzymatically active toxins and fragments thereof used are diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. In another embodiment, the antibodies are conjugated to small molecule anticancer drugs such as cis-platin or 5FU. Conjugates of the monoclonal antibody and such cytotoxic moieties are made using a variety of bifunctional protein coupling agents. Examples of such reagents are SPDP, IT, bifunctional derivatives of imidoesters such as dimethyl adipimidate HCl, active esters such as disuccinimidyl suberate, aldehydes such as glutaraldehyde, bis-azido compounds such as bis (p-azidobenzoyl) hexanediamine, bis-diazonium derivatives such as bis-(p-diazoniumbenzoyl)-ethylenediamine, diisocyanates such as tolylene 2,6-diisocyanate and bis-active fluorine compounds such as 1,5-difluoro-2,4-dinitrobenzene. The lysing portion of a toxin may be joined to the Fab fragment of the antibodies.

Immunotoxins can be made in a variety of ways, as discussed herein. Commonly known crosslinking reagents can be used to yield stable conjugates.

Advantageously, monoclonal antibodies specifically binding the domain of the antigen which is exposed on the infected cell surface, are conjugated to ricin A chain. Most advantageously the ricin A chain is deglycosylated and produced through recombinant means. An advantageous method of making the ricin immunotoxin is described in Vitetta et al., *Science* 238:1098 (1987).

When used to kill infected human cells in vitro for diagnostic purposes, the conjugates will typically be added to the cell culture medium at a concentration of at least about 10 nM. The formulation and mode of administration for in vitro use are not critical. Aqueous formulations that are compatible with the culture or perfusion medium will normally be used. Cytotoxicity may be read by conventional techniques.

Cytotoxic radiopharmaceuticals for treating infected cells may be made by conjugating radioactive isotopes (e.g. I, Y, Pr) to the antibodies. Advantageously alpha particle-emitting isotopes are used. The term "cytotoxic moiety" as used herein is intended to include such isotopes.

In a preferred embodiment, ricin A chain is deglycosylated or produced without oligosaccharides, to decrease its clearance by irrelevant clearance mechanisms (e.g., the liver). In another embodiment, whole ricin (A chain plus B chain) is conjugated to antibody if the galactose binding property of B-chain can be blocked ("blocked ricin").

In a further embodiment toxin-conjugates are made with Fab or $F(ab')_2$ fragments. Because of their relatively small size these fragments can better penetrate tissue to reach infected cells.

In another embodiment, fusogenic liposomes are filled with a cytotoxic drug and the liposomes are coated with antibodies specifically binding the particular antigen.

Antibody Dependent Cellular Cytotoxicity

Certain aspects of this invention involve antibodies which are (a) directed against a particular antigen and (b) belong to a subclass or isotype that is capable of mediating the lysis of cells to which the antibody molecule binds. More specifically, these antibodies should belong to a subclass or isotype that, upon complexing with cell surface proteins, activates serum complement and/or mediates antibody dependent cellular cytotoxicity (ADCC) by activating effector cells such as natural killer cells or macrophages.

Biological activity of antibodies is known to be determined, to a large extent, by the constant domains or Fc region of the antibody molecule (Uananue and Benacerraf,

Textbook of Immunology, 2nd Edition, Williams & Wilkins, p. 218 (1984)). This includes their ability to activate complement and to mediate antibody-dependent cellular cytotoxicity (ADCC) as effected by leukocytes. Antibodies of different classes and subclasses differ in this respect, as do antibodies from the same subclass but different species; according to the present invention, antibodies of those classes having the desired biological activity are prepared. Preparation of these antibodies involves the selection of antibody constant domains are their incorporation in the humanized antibody by known technique. For example, mouse immunoglobulins of the IgG3 and lgG2a class are capable of activating serum complement upon binding to the target cells which express the cognate antigen, and therefore humanized antibodies which incorporate IgG3 and lgG2a effector functions are desirable for certain therapeutic applications.

In general, mouse antibodies of the IgG2a and IgG3 subclass and occasionally IgG1 can mediate ADCC, and antibodies of the IgG3, IgG2a, and IgM subclasses bind and activate serum complement. Complement activation generally requires the binding of at least two IgG molecules in close proximity on the target cell. However, the binding of only one IgM molecule activates serum complement.

The ability of any particular antibody to mediate lysis of the target cell by complement activation and/or AOCC can be assayed. The cells of interest are grown and labeled in vitro; the antibody is added to the cell culture in combination with either serum complement or immune cells which may be activated by the antigen antibody complexes. Cytolysis of the target cells is detected by the release of label from the lysed cells. In fact, antibodies can be screened using the patient's own serum as a source of complement and/or immune cells. The antibody that is capable of activating complement or mediating ADCC in the in vitro test can then be used therapeutically in that particular patient.

This invention specifically encompasses consensus Fc antibody domains prepared and used according to the teachings of this invention.

Therapeutic and Other Uses of the Antibodies

When used in vivo for therapy, the antibodies of the subject invention are administered to the patient in therapeutically effective amounts (i.e. amounts that have desired therapeutic effect). They will normally be administered parenterally. The dose and dosage regimen will depend upon the degree of the infection, the characteristics of the particular antibody or immunotoxin used, e.g., its therapeutic index, the patient, and the patient's history. Advantageously the antibody or immunotoxin is administered continuously over a period of 1–2 weeks, intravenously to treat cells in the vasculature and subcutaneously and intraperitoneally to treat regional lymph nodes. Optionally, the administration is made during the course of adjunct therapy such as combined cycles of radiation, chemotherapeutic treatment, or administration of tumor necrosis factor, interferon or other cytoprotective or immunomodulatory agent.

For parenteral administration the antibodies will be formulated in a unit dosage injectable form (solution, suspension, emulsion) in association with a pharmaceutically acceptable parenteral vehicle. Such vehicles are inherently nontoxic, and non-therapeutic. Examples of such vehicles are water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Nonaqueous vehicles such as fixed oils and ethyl oleate can also be used. Liposomes may be used as carriers. The vehicle may contain minor amounts of additives such as substances that enhance isotonicity and chemical stability, e.g., buffers and preservatives. The antibodies will typically be formulated in such vehicles at concentrations of about 1 mg/ml to 10 mg/ml.

Use of IgM antibodies may be preferred for certain applications, however IgG molecules by being smaller may be more able than IgM molecules to localize to certain types of infected cells.

There is evidence that complement activation in vivo-leads to a variety of biological effects, including the induction of an inflammatory response and the activation of macrophages (Uananue and Benecerraf, *Textbook of Immunology,* 2nd Edition, Williams & Wilkins, p. 218 (1984)). The increased vasodilation accompanying inflammation may increase the ability of various agents to localize in infected cells. Therefore, antigen-antibody combinations of the type specified by this invention can be used therapeutically in many ways. Additionally, purified antigens (Hakomori, *Ann. Rev. Immunol.* 2:103 (1984)) or anti-idiotypic antibodies (Nepom et al., *Proc. Natl. Acad. Sci.* 81:2864 (1985); Koprowski et al., *Proc. Natl. Acad. Sci.* 81:216 (1984)) relating to such antigens could be used to induce an active immune response in human patients. Such a response includes the formation of antibodies capable of activating human complement and mediating ADCC and by such mechanisms cause infected cell destruction.

Optionally, the antibodies of this invention are useful in passively immunizing patients, as exemplified by the administration of humanized anti-HIV antibodies.

The antibody compositions used in therapy are formulated and dosages established in a fashion consistent with good medical practice taking into account the disorder to be treated, the condition of the individual patient, the site of delivery of the composition, the method of administration and other factors known to practitioners. The antibody compositions are prepared for administration according to the description of preparation of polypeptides for administration, infra.

Deposit of Materials

As described above, cultures of the muMAb4D5 have been deposited with the American Type Culture Collection, 10801 University Blvd., Mauassas, Va., USA (ATCC).

This deposit was made under the provisions of the Budapest Treaty on the international Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of viable cultures for 30 years from the date of the deposit. The organisms will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Genentech, Inc. and ATCC, which assures permanent and unrestricted availability of the progeny of the cultures to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures' availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 USC §122 and the Commissioner's rules pursuant thereto (including 37 CFR §1.12 with particular reference to 886 OG 638).

In respect of those designations in which a European patent is sought, a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample. (Rule 28(4) EPC)

The assignee of the present application has agreed that if the cultures on deposit should die or be lost or destroyed

when cultivated under suitable conditions, they will be promptly replaced on notification with a viable specimen of the same culture. Availability of the deposited strain is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by the constructs deposited, since the deposited embodiments are intended to illustrate only certain aspects of the invention and any constructs that are functionally equivalent are within the scope of this invention. The deposit of material herein does not constitute an admission that the written description herein contained is inadequate to enable the practice of any aspect of the invention, including the best mode thereof, nor is it to be construed as limiting the scope of the claims to the specific illustrations that they represent. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

It is understood that the application of the teachings of the present invention to a specific problem or situation will be within the capabilities of one having ordinary skill in the art in light of the teachings contained herein. Examples of the products of the present invention and representative processes for their isolation, use, and manufacture appear below, but should not be construed to limit the invention.

EXAMPLES

Example 1

Humanization of muMAb4D5

Here we report the chimerization of muMAb4D5 (chMAb4D5) and the rapid and simultaneous humanization of heavy (V_H) and light (V_L) chain variable region genes using a novel "gene conversion mutagenesis" strategy. Eight humanized variants (huMAb4D5) were constructed to probe the importance of several FR residues identified by our molecular modeling or previously proposed to be critical to the conformation of particular CDRs (see Chothia, C. & Lesk, A. M., *J. Mol. Biol.* 196:901–917 (1987); Chothia, C. et al., *Nature* 342:877–883 (1989); Tramontano, A. et al., *J. Mol. Biol.* 215:175–182 (1990)). Efficient transient expression of humanized variants in non-myeloma cells allowed us to rapidly investigate the relationship between binding affinity for $p185^{HER2}$ ECD and anti-proliferative activity against $p185^{HER2}$ overexpressing carcinoma cells.

Materials and Methods

Cloning of Variable Region Genes. The muMAb4D5 V_H and V_L genes were isolated by polymerase chain reaction (PCR) amplification of mRNA from the corresponding hybridoma (Fendly, B. M. et al., *Cancer Res.* 50:1550–1558 (1990)) as described by Orlandi et al. (Orlandi, R. et al., *Proc. Natl. Acad. Sci. USA* 86:3833–3837 (1989)). Amino terminal sequencing of muMAb4D5 V_L and V_H was used to design the sense strand PCR primers, whereas the anti-sense PCR primers were based upon consensus sequences of murine framework residues (Orlandi, R. et al., *Proc. Natl. Acad. Sci. USA* 86:3833–3837 (1989); Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)) incorporating restriction sites for directional cloning shown by underlining and listed after the sequences: V_L sense, 5'-TCC GATATCCAGCTGACCCAGTCTCCA-3' (SEQ. ID NO. 7), EcoRV; V_L anti-sense, 5'-GTTTGATCTCCAGCTT GGTACCHSCDCCGAA-3' (SEQ. ID NO. 8), Asp718; V_H sense, 5'-AGGTSMARCTGCAGSAGTCWGG-3' (SEQ. ID NO. 9), PstI and V_H anti-sense, 5'-TGAGGAGAC GGTGACCGTGGTCCCTTGGCCCCAG-3' (SEQ. ID. NO. 10), BstEII; where H=A or C or T, S=C or G, D=A or G or T, M=A or C, R=A or G and W=A or T. The PCR products were cloned into pUC119 (Vieira, J. & Messing, J., *Methods Enzymol.* 153:3–11 (1987)) and five clones for each variable domain sequenced by the dideoxy method (Sanger, F. et al., *Proc. Natl. Acad. Sci. USA* 74:5463–5467 (1977)).

Molecular Modelling. Models for muMAb4D5 V_H and V_L domains were constructed separately from consensus coordinates based upon seven Fab structures from the Brookhaven protein data bank (entries 1FB4, 2RHE, 2MCP, 3FAB, 1FBJ, 2HFL and 1REI). The Fab fragment KOL (Marquart, M. et al., *J. Mol. Biol.* 141:369–391 (1980)) was first chosen as a template for V_L and V_H domains and additional structures were then superimposed upon this structure using their main chain atom coordinates (INSIGHT program, Siosym Technologies). The distance from the template Cα to the analogous Cα in each of the superimposed structures was calculated for each residue position. If all (or nearly all) Cα—Cα distances for a given residue were ≦1 Å, then that position was included in the consensus structure. In most cases the β-sheet framework residues satisfied these criteria whereas the CDR loops did not. For each of these selected residues the average coordinates for individual N, Cα, C, O and Cβ atoms were calculated and then corrected for resultant deviations from non-standard bond geometry by 50 cycles of energy minimization using the DISCOVER program (Biosym Technologies) with the AMBER forcefield (Weiner, S. J. et al., *J. Amer. Chem. Soc.* 106:765–784 (1984)) and Cα coordinates fixed. The side chains of highly conserved residues, such as the disulfide-bridged cysteine residues, were then incorporated into the resultant consensus structure. Next the sequences of muMAb4D5 V_L and V_H were incorporated starting with the CDR residues and using the tabulations of CDR conformations from Chothia et al. (Chothia, C. et al., *Nature* 342:877–883 (1989)) as a guide. Side-chain conformations were chosen on the basis of Fab crystal structures, rotamer libraries (Ponder, J. W. & Richards, F. M., *J. Mol. Biol.* 193:775–791 (1987)) and packing considerations. Since V_H-COR3 could not be assigned a definite backbone conformation from these criteria, two models were created from a search of similar sized loops using the INSIGHT program. A third model was derived using packing and solvent exposure considerations. Each model was then subjected to 5000 cycles of energy minimization.

In humanizing muMAb4D5, consensus human sequences were first derived from the most abundant subclasses in the sequence compilation of Kabat et al. (Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)), namely V_L κ subgroup I and V_H group III, and a molecular model generated for these sequences using the methods described above. A structure for huMAb4D5 was created by transferring the CDRs from the muMAb4D5 model into the consensus human structure. All huMAb4D5 variants contain human replacements of muMAb4D5 residues at three positions within CDRs as defined by sequence variability (Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)) but notas defined by structural variability (Chothia, C. & Lesk, A. M., *J. Mol. Biol.* 196:901–917 (1987)):

V_L-CDR1 K24R, V_L-CDR2 R54L and V_L-CDR2 T56S. Differences between muMAb4D5 and the human consensus framework residues (FIG. 1) were individually modeled to investigate their possible influence on CDR conformation and/or binding to the $p185^{HER2}$ ECD.

Construction of Chimeric Genes. Genes encoding chMAb4D5 light and heavy chains were separately assembled in previously described phagemid vectors containing ihe human cytomegalovirus enhancer and promoter, a 5' intron and SV40 polyadenylation signal (Gorman, C. M. et al., *DNA & Prot. Engin. Tech.* 2:3–10 (1990)). Briefly, gene segments encoding muMAb4D5 V_L (FIG. 1A) and REI human κ_1 light chain C_L (Palm, W. & Hilschmann, N., *Z. Physiol. Chem.* 356:167–191 (1975)) were precisely joined as were genes for muMAb4D5 V_H (FIG. 1B) and human γ1 constant region (Capon, D. J. et al., *Nature* 337:525–531 (1989)) by simple subcloning (Boyle, A., in *Current Protocols in Molecular Biology,* Chapter 3 (F. A. Ausubel et al., eds., Greene Publishing & Wiley-Interscience, New York, 1990)) and site-directed mutagenesis (Carter, P., in *Mutagenesis: A Practical Approach*, Chapter 1 (IRL Press, Oxford, UK 1991)). The γ1 isotype was chosen as it has been found to be the preferred human isotype for supporting ADCC and complement dependent cytotoxicity using matched sets of chimeric (Brüggemann, M. et al., *J. Exp. Med.* 166:1351–1361 (1987)) or humanized antibodies (Riechmann, L. et al., *Nature* 332:323–327 (1988)). The PCR-generated V_L and V_H fragments (FIG. 1) were subsequently mutagenized so that they faithfully represent the sequence of muMAb4D5 determined at the protein level: V_H Q1E, V_L V_{104}L and T109A (variants are denoted by the amino acid residue and number followed by the replacement amino acid). The human γ1 constant regions are identical to those reported by Ellison et al. (Ellison, J. W. et al., *Nucleic Acids Res.* 13:4071–4079 (1982)) except for the mutations E359D and M361L (Eu numbering, as in Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)) which we installed to convert the antibody from the naturally rare A allotype to the much more common non-A allotype (Tramontano, A. et al., *J. Mol. Biol.* 215:175–182 (1990)). This was an attempt to reduce the risk of anti-allotype antibodies interfering with therapy.

Construction of Humanized Genes. Genes encoding chMAb4D5 light chain and heavy chain Fd fragment (V_H and C_H1 domains) were subcloned together into pUC119 (Vieira, J. & Messing, J., *Methods Enzymol.* 153:3–11 (1987)) to create pAK1 and simultaneously humanized in a single step (FIG. 2). Briefly, sets of 6 contiguous oligonucleotides were designed to humanize V_H and V_L (FIG. 1). These oligonucleotides are 28 to 83 nucleotides in length, contain zero to 19 mismatches to the murine antibody template and are constrained to have 8 or 9 perfectly matched residues at each end to promote efficient annealing and ligation of adjacent oligonucleotides. The sets of V_H and V_L humanization oligonucleotides (5 pmol each) were phosphorylated with either ATP or γ-^{32}P-ATP (Carter, P. *Methods Enzymol.* 154: 382–403 (1987)) and separately annealed with 3.7 pmol of pAK1 template in 40 μl 10 mM Tris-HCl (pH 8.0) and 10 mM $MgCl_2$ by cooling from 100° C. to room temperature over ~30 min. The annealed oligonucleotides were joined by incubation with T4 DNA ligase (12 units; New England Biolabs) in the presence of 2 μl 5 mM ATP and 2 μl 0.1 M DTT for 10 min at 14° C. After electrophoresis on a 6% acrylamide sequencing gel the assembled oligonucleotides were located by autoradiography and recovered by electroelution. The assembled oligonucleotides (~0.3 pmol each) were simultaneously annealed to 0.15 pmol single-stranded deoxyuridine-containing pAK1 prepared according to Kunkel et al. (Kunkel, T. A. et al., *Methods Enzymol.* 154:367–382 (1987)) in 10 μl 40 mM Tris-HCl (pH 7.5) and 16 mM $MgCl_2$ as above. Heteroduplex DNA was constructed by extending the primers with T7 DNA polymerase and transformed into *E. coli* BMH 71–18 mutL as previously described (Carter, P., in *Mutagenesis: A Practical Approach,* Chapter 1 (IRL Press, Oxford, UK 1991)). The resultant phagemid DNA pool was enriched first for huV_L by restriction purification using XhoI and then for huV_H by restriction selection using StuI as described in Carter, P., in *Mutagenesis: A Practical Approach,* Chapter 1 (IRL Press, Oxford, UK 1991); and in Wells, J. A. et al., *Phil. Trans. R. Soc. Lond., A* 317:415–423 (1986). Resultant clones containing both huV_L and huV_H genes were identified by nucleotide sequencing (Sanger, F. et al., *Proc. Natl. Acad. Sci. USA* 74:5463–5467 (1977)) and designated pAK2. Additional humanized variants were generated by site-directed mutagenesis (Carter, P., in *Mutagenesis: A Practical Approach,* Chapter 1 (IRL Press, Oxford, UK 1991)). The muMAb4D5 V_L and V_H gene segments in the transient expression vectors described above were then precisely replaced with their humanized versions.

Expression and Purification of MAb4D5 Variants. Appropriate MAb4D5 light and heavy chain cDNA expression vectors were co-transfected into an adenovirus transformed human embryonic kidney cell line, 293 (Graham, F. L. et al., *J. Gen. Virol.* 36:59–72 (1977)) using a high efficiency procedure (Gorman, C. M. et al., *DNA & Prot. Engin. Tech.* 2:3–10 (1990); Gorman, C., in *DNA Cloning,* vol II, pp 143–190 (D. M. Glover, ed., IRL Press, Oxford, UK 1985)). Media were harvested daily for up to 5 days and the cells re-fed with serum free media. Antibodies were recovered from the media and affinity purified on protein A sepharose CL-4B (Pharmacia) as described by the manufacturer. The eluted antibody was buffer-exchanged into phosphate-buffered saline by G25 gel filtration, concentrated by ultrafiltration (Centriprep-30 or Centricon-100, Amicon), sterile-filtered (Millex-GV, Millipore) and stored at 4° C. The concentration of antibody was determined by using both total immunoglobulin and antigen binding ELISAs. The standard used was huMAb4D5-5, whose concentration had been determined by amino acid composition analysis.

Cell Proliferation Assay. The effect of MAb4D5 variants upon proliferation of the human mammary adenocarcinoma cell line, SK-BR-3, was investigated as previously described (Fendly, B. M. et al., *Cancer Res.* 50:1550–1558 (1990)) using saturating MAb4D5 concentrations.

Affinity Measurements. The antigen binding affinity of MAb4D5 variants was determined using a secreted form of the $p185^{HER2}$ ECD prepared as described in Fendly, B. M. et al., *J. Biol. Resp. Mod.* 9:449–455 (1990). Briefly, antibody and $p185^{HER2}$ ECD were incubated in solution until equilibrium was found to be reached. The concentration of free antibody was then determined by ELISA using immobilized $p185^{HER2}$ ECD and used to calculate affinity (K_d) according to Friguet et al. (Friguet, B. et al., *J. Immunol. Methods* 77:305–319 (1985)).

Results

Humanization of muMAb4D5. The muMAb4D5 V_L and V_H gene segments were first cloned by PCR and sequenced (FIG. 1). The variable genes were then simultaneously humanized by gene conversion mutagenesis using preassembled oligonucleotides (FIG. 2). A 311-mer oligonucle-

otide containing 39 mismatches to the template directed 24 simultaneous amino acid changes required to humanize muMAb4D5 V_L. Humanization of muMAb4D5 V_H required 32 amino acid changes which were installed with a 361-mer containing 59 mismatches to the muMAb4D5 template. Two out of 8 clones sequenced precisely encode huMAb4D5-5, although one of these clones contained a single nucleotide imperfection. The 6 other clones were essentially humanized but contained a small number of errors: <3 nucleotide changes and <1 single nucleotide deletion per kilobase. Additional humanized variants (Table 3) were constructed by site-directed mutagenesis of huMAb4D5-5.

Expression levels of huMAb4D5 variants were in the range of 7 to 15 μg/ml as judged by ELISA using immobilized $p185^{HER2}$ ECD. Successive harvests of five 10 cm plates allowed 200 μg to 500 mg of each variant to be produced in a week. Antibodies affinity purified on protein A gave a single band on a Coomassie blue stained SDS polyacrylamide gel of mobility consistent with the expected M_r of ~150 kDa. Electrophoresis under reducing conditions gave 2 bands consistent with the expected M_r of free heavy (48 kDa) and light (23 kDa) chains (not shown). Amino terminal sequence analysis (10-cycles) gave the mixed sequence expected (see FIG. **1**) from an equimolar combination of light and heavy chains (not shown).

huMAb4D5 Variants. In general, the FR residues were chosen from consensus human sequences (Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)) and CDR residues from muMAb4D5. Additional variants were constructed by replacing selected human residues in huMAb4D5-1 with their muMAb4D5 counterparts. These are V_H residues 71, 73, 78, 93 plus 102 and V_L residues 55 plus 66 identified by our molecular modeling. V_H residue 71 has previously been proposed by others (Tramontano, A. et al., *J. Mol. Biol.* 215:175–182 (1990)) to be critical to the conformation of V_H-CDR2. Amino acid sequence differences between huMAb4D5 variant molecules are shown in Table 3, together with their $p185^{HER2}$ ECD binding affinity and maximal anti-proliferative activities against SK-BR-3 cells. Very similar K_d values were obtained for binding of MAb4D5 variants to either SK-BR-3 cells or to $p185^{HER2}$ ECD (Table 3). However, K_d estimates derived from binding of MAb4D5 variants to $p185^{HER2}$ ECD were more reproducible with smaller standard errors and consumed much smaller quantities of antibody than binding measurements with whole cells.

The most potent humanized variant designed by molecular modeling, huMAb4D5-8, contains 5 FR residues from muMAb4D5. This antibody binds the $p185^{HER2}$ ECD 3-fold more tightly than does muMAb4D5 itself (Table 3) and has comparable anti-proliferative activity with SK-BR-3 cells (FIG. **3**). In contrast, huMAb4D5-1 is the most humanized but least potent muMAb4D5 variant, created by simply installing the muMAb4D5 CDRs into the consensus human sequences. huMAb4D5-1 binds the $p185^{HER2}$ ECD 80-fold less tightly than does the murine antibody and has no detectable anti-proliferative activity at the highest antibody concentration investigated (16 μg/ml).

The anti-proliferative activity of huMAb4D5 variants against $p185^{HER2}$ overexpressing SK-BR-3 cells is not simply correlated with their binding affinity for the $p185^{HER2}$ ECD. For example, installation of three murine residues into the V_H domain of huMAb4D5-2 (D73T, L78A and A93S) to create huMAb4D5-3 does not change the antigen binding affinity but does confer significant anti-proliferative activity (Table 3).

The importance of V_H residue 71 (Tramontano, A. et al., *J. Mol. Biol.* 215:175–182 (1990)) is supported by the observed 5-fold increase in affinity for $p185^{HER2}$ ECD on replacement of R71 in huMAb4D5-1 with the corresponding murine residue, alanine (huMAb4D5-2). In contrast, replacing V_H L78 in huMAb4D5-4 with the murine residue, alanine (huMAb4D5-5), does not significantly change the affinity for the $p185^{HER2}$ ECD or change anti-proliferative activity, suggesting that residue 78 is not of critical functional significance to huMAb4D5 and its ability to interact properly with the extracellular domain of $p185^{HER2}$.

V_L residue 66 is usually a glycine in human and murine κ chain sequences (Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)) but an arginine occupies this position in the muMAb4D5 κ light chain. The side chain of residue 66 is likely to affect the conformation of V_L-CDR1 and V_L-CDR2 and the hairpin turn at 68–69 (FIG. **4**). Consistent with the importance of this residue, the mutation V_L G66R (huMAb4D5-3→huMAb4D5-5) increases the affinity for the $p185^{HER2}$ ECD by 4-fold with a concomitant increase in anti-proliferative activity.

From molecular modeling it appears that the tyrosyl side chain of muMAb4D5 V_L residue 55 may either stabilize the conformation of V_H-CDR3 or provide an interaction at the V_L–V_H interface. The latter function may be dependent upon the presence of V_HY102. In the context of huMAb4D5-5 the mutations V_L E55Y (huMAb4D5-6) and V_H V102Y (huMAb4D5-7) individually increase the affinity for $p185^{HER2}$ ECD by 5-fold and 2-fold respectively, whereas together (huMAb4D5-8) they increase the affinity by 11-fold. This is consistent with either proposed role of V_L Y55 and V_H Y102.

Secondary Immune Function of huMAb4D5-8. MuMAb4D5 inhibits the growth of human breast tumor cells which overexpress $p185^{HER2}$ (Hudziak, R. M. et al., *Molec. Cell. Biol.* 9:1165–1172 (1989)). The antibody, however, does not offer the possibility of direct tumor cytotoxic effects. This possibility does arise in huMAb4D5-8 as a result of its high affinity (Kd_d=0.1 μM) and its human IgG_1 subtype. Table 4 compares the ADCC mediated by huMAb4D5-8 with muMAb4D5 on a normal lung epithelial cell line, WI-38, which expresses a low level of $p185^{HER2}$ and on SK-BR-3, which expresses a high level of $p185^{HER2}$. The results demonstrate that: (1) huMAb4D5 has a greatly enhanced ability to carry out ADCC as compared with its murine parent; and (2) that this activity may be selective for cell types which overexpress $p185^{HER2}$.

Discussion

MuMAb4D5 is potentially useful for human therapy since it is cytostatic towards human breast and ovarian tumor lines overexpressing the HER2-encoded $p185^{HER2}$ receptor-like tyrosine kinase. Since both breast and ovarian carcinomas are chronic diseases it is anticipated that the optimal MAb4D5 variant molecule for therapy will have low immunogenicity and will be cytotoxic rather than solely cytostatic in effect. Humanization of muMAb4D5 should accomplish these goals. We have identified 5 different huMAb4D5 variants which bind tightly to $p185^{HER2}$ ECD ($K_d \leqq 1$ nM) and which have significant anti-proliferative activity (Table 3). Furthermore huMAb4D5-8 but not muMAb4D5 mediates ADCC against human tumor cell lines overexpressing $p185^{HER2}$ in the presence of human effector cells (Table 4) as anticipated for a human γ1 isotype (Brcüggemann, M. et al., *J. Exp. Med.* 166:1351–1361 (1987); Riechmann, L. et al., *Nature* 332:323–327 (1988)).

Rapid humanization of huMAb4D5 was facilitated by the gene conversion mutagenesis strategy developed here using long preassembled oligonucleotides. This method requires less than half the amount of synthetic DNA as does total gene synthesis and does not require convenient restriction sites in the target DNA. Our method appears to be simpler and more reliable than a variant protocol recently reported (Rostapshov, V. M. et al., *FEBS Lett.* 249: 379–382 (1989)). Transient expression of huMAb4D5 in human embryonic kidney 293 cells permitted the isolation of a few hundred micrograms of huMAb4D5 variants for rapid characterization by growth inhibition and antigen binding affinity assays. Furthermore, different combinations of light and heavy chain were readily tested by co-transfection of corresponding cDNA expression vectors.

The crucial role of molecular modeling in the humanization of muMAb4D5 is illustrated by the designed variant huMAb4D5-8 which binds the $p185^{HER2}$ ECD 250-fold more tightly than the simple CDR loop swap variant, huMAb4D5-1. It has previously been shown that the antigen binding affinity of a humanized antibody can be increased by mutagenesis based upon molecular modelling (Riechmann, L. et al., *Nature* 332:323–327 (1988); Queen, C. et al., *Proc. Natl. Acad. Sci. USA* 86:10029–10033 (1989)). Here we have extended this earlier work by others with a designed humanized antibody which binds its antigen 3-fold more tightly than the parent rodent antibody. While this result is gratifying, assessment of the success of the molecular modeling must await the outcome of X-ray structure determination. From analysis of huMAb4D5 variants (Table 3) it is apparent that their anti-proliferative activity is not a simple function of their binding affinity for $p185^{HER2}$ ECD. For example the huMAb4D5–8 variant binds $p185^{HER2}$ 3-fold more tightly than muMAb4D5 but the humanized variant is slightly less potent in blocking the proliferation of SK-SR-3 cells. Additional huMAb4D5 variants are currently being constructed in an attempt to identify residues triggering the anti-proliferative activity and in an attempt to enhance this activity.

In addition to retaining tight receptor binding and the ability to inhibit cell growth, the huMAb4D5-8 also confers a secondary immune function (ADCC). This allows for direct cytotoxic activity of the humanized molecule in the presence of human effector cells. The apparent selectivity of the cytotoxic activity for cell types which overexpress $p185^{HER2}$ allows for the evolution of a straightforward clinic approach to those human cancers characterized by overexpression of the HER2 protooncogene.

TABLE 3

$p185^{HER2}$ ECD binding affinity and anti-proliferative activities of MAb4D5 variants

	V_H Residue*					V_L Residue*			
MAb4D5 cell Variant proliferation‡	71 FR3	73 FR3	78 FR3	93 FR3	102 CDR3	55 CDR2	56 FR3	K_d† nM	Relative
huMAb4D5-1	R	D	L	A	V	E	G	25	102
huMAb4D5-2	Ala	D	L	A	V	E	G	4.7	101
huMAb4D5-3	Ala	Thr	Ala	Ser	V	E	G	4.4	66
huMAb4D5-4	Ala	Thr	L	Ser	V	E	Arg	0.82	56
huMAb4D5-5	Ala	Thre	Ala	Ser	V	E	Arg	1.1	48
huMAb4D5-6	Ala	Thr	Ala	Ser	V	Tyr	Arg	0.22	51
huMAb4D5-7	Ala	Thr	Ala	Ser	Tyr	E	Arg	0.62	53
huMAb4D5-8	Ala	Thr	Ala	Ser	Tyr	Tyr	Arg	0.10	54
muMAb4D5	Ala	Thr	Ala	Ser	Tyr	Tyr	Arg	0.30	37

*Human and murine residues are shown in one letter and three letter amino acid code respectively.
†K_d values for the $p185^{HER2}$ ECD were determined using the method of Friguet et al. (43) and the standard error of each estimate is ≦ ± 10%.
‡Proliferation of SK-BR-3 cells incubated for 96 hr with MAb4D5 variants shown as a percentage of the untreated control as described (Hudziak, R. M. et al., Molec. Cell. Biol. 9: 1165–1172 (1989)). Data represent the maximal anti-proliferative effect for each variant (see FIG. 3A) calculated as the mean of triplicate determinations at a MAb4D5 concentration of 8 μg/ml. Data are all taken from the same experiment with an estimated standard error of ≦ ± 15%.

TABLE 4

Selectivity of antibody dependent tumor cell cytotoxicity mediated by huMAb4D5-8

Effector:Target ratio†	WI-38* muMAb4D5	WI-38* huMAb4D5-8	SK-BR-3 muMAb4D5	SK-BR-3 huMAb4D5-8
A.‡				
25:1	<1.0	9.3	7.5	40.6
12.5:1	<1.0	11.1	4.7	36.8
6.25:1	<1.0	8.9	0.9	35.2
3.13:1	<1.0	8.5	4.6	19.6
B.				
25:1	<1.0	3.1	6.1	33.4
12.5:1	<1.0	1.7	5.5	26.2
6.25:1	1.3	2.2	2.0	21.0
3.13:1	<1.0	0.8	2.4	13.4

*Sensitivity to ADCC of two human cell lines (WI-38, normal lung epithelium; and SK-BR-3, human breast tumor cell line) are compared. WI-38 expresses a low level of $p185^{HER2}$ (0.6 pg per μg cell protein) and SK-BR-3 expresses a high level of $p185^{HER2}$ (64 pg $p185^{HER2}$ per μg cell protein), as determined by ELISA (Fendly et al., J. Biol. Resp. Mod. 9:449–455 (1990)).
†ADCC assays were carried out as described in Brüggemann et al., J. Exp. Med. 166:1351–1361 (1987). Effector to target ratios were of IL-2 activated human peripheral blood lymphocytes to either WI-38 fibroblasts or SK-BR-3 tumor cells in 96-well microtiter plates for 4 hours at 37° C. Values given represent percent specific cell lysis as determined by ^{51}Cr release. Estimated standard error in these quadruplicate determinations was ≦±10%.
‡Monoclonal antibody concentrations used were 0.1 μg/ml (A) and 0.1 μg/ml (B).

Example 2
Schematic Method for Humanizing an Antibody Sequence

This example illustrates one stepwise elaboration of the methods for creating a humanized sequence described

above. It will be understood that not all of these steps are essential to the claimed invention, and that steps may be taken in different order.

1. ascertain a consensus human variable domain amino acid sequence and prepare from it a consensus structural model.
2. prepare model of import (the non-human domain to be humanized) variable domain sequences and note structural differences with respect to consensus human model.
3. identify CDR sequences in human and in import, both by using Kabat (supra, 1987) and crystal structure criteria. If there is any difference in CDR identity from the different criteria, use of crystal structure definition of the CDR, but retain the Kabat residues as important framework residues to import.
4. substitute import CDR sequences for human CDR sequences to obtain initial "humanized" sequence.
5. compare import non-CDR variable domain sequence to the humanized sequence and note divergences.
6. Proceed through the following analysis for each amino acid residue where the import diverges from the humanized.
   a. If the humanized residue represents a residue which is generally highly conserved across all species, use the residue in the humanized sequence. If the residue is not conserved across all species, proceed with the analysis described in 6b.
   b. If the residue is not generally conserved across all species, ask if the residue is generally conserved in humans.
      i. If the residue is generally conserved in humans but the import residue differs, examine the structural models of the import and human sequences and determine if the import residue would be likely to affect the binding or biological activity of the CDRs by considering 1) could it bind antigen directly and 2) could it affect the conformation of the CDR. If the conclusion is that an affect on the CDRs is likely, substitute the import residue. If the conclusion is that a CDR affect is unlikely, leave the humanized residue unchanged.
      ii. If the residue is also not generally conserved in humans, examine the structural models of the import and human sequences and determine if the import residue would be likely to affect the binding or biological activity of the CDRs be considering 1) could it bind antigen directly and 2) could it affect the conformation of the CDR. If the conclusion is that an affect on the CDRs is likely, substitute the import residue. If the conclusion is that a CDR affect is unlikely, proceed to the next step.
         a) Examine the structural models of the import and human sequences and determine if the residue is exposed on the surface of the domain or is buried within. If the residue is exposed, use the residue in the humanized sequence. If the residue is buried, proceed to the next step.
            (i) Examine the structural models of the import and human sequences and determine if the residue is likely to affect the V_L–V_H interface. Residues involved with the interface include: 34L, 36L, 38L, 43L, 33L, 36L, 85L, 87L, 89L, 91L, 96L, 98L, 35H, 37H, 39H, 43H, 45H, 47H, 60H, 91H, 93H, 95H, 100H, and 103H. If no effect is likely, use the residue in the humanized sequence. If some affect is likely, substitute the import residue.
7. Search the import sequence, the consensus sequence and the humanized sequence for glycosylation sites outside the CDRs, and determine if this glycosylation site is likely to have any affect on antigen binding and/or biological activity. If no effect is likely, use the human sequence at that site; if some affect is likely, eliminate the glycosylation site or use the import sequence at that site.
8. After completing the above analysis, determine the planned humanized sequence and prepare and test a sample. If the sample does not bind well to the target antigen, examine the particular residues listed below, regardless of the question of residue identity between the import and humanized residues.
   a. Examine particular peripheral (non-CDR) variable domain residues that may, due to their position, possibly interact directly with a macromolecular antigen, including the following residues (where the * indicates residues which have been found to interact with antigen based on crystal structures):
      i. Variable light domain: 36, 46, 49*, 63–70
      ii. Variable heavy domain: 2, 47*, 68, 70, 73–76.
   b. Examine particular variable domain residues which could interact with, or otherwise affect, the conformation of variable domain CDRs, including the following (not including CDR residues themselves, since it is assumed that, because the CDRs interact with one another, any residue in one CDR could potentially affect the conformation of another CDR residue) (L=LIGHT, H=HEAVY, residues appearing in bold are indicated to be structurally important according the Chothia et al., Nature 342:877 (1989), and residues appearing in italic were altered during humanization by Queen et al. (PDL), Proc. Natl. Acad. Sci. USA 86:10029 (1989) and Proc. Natl. Acad. Sci. USA 88:2869 (1991).):
      i. Variable light domain:
         a) CDR-1 (residues 24L–34L): 2L, 4L, 66L-69L, 71L
         b) CDR-2 (residues 50L–56L): 35L, 46L, 47L, 48L, 49L, 58L, 62L, 64L–66L, 71L, 73L
         c) CDR-3 (residues 89L–97L): 2L, 4L, 36L, 98L, 37H, 45H, 47H, 58H, 60H
      ii. Variable heavy domain:
         a) CDR-1 (residues 26H–35H): 2H, 4H, 24H, 36H, 71H, 73H, 76H, 78H, 92H, 94H
         b) CDR-2 (residues 50H–55H): 49H, 69H, 69H, 71H, 73H, 78H
         c) CDR-3 (residues 95H–102H): examine all residues as possible interaction partners with this loop, because this loop varies in size and conformation much more than the other CDRs.
9. If after step 8 the humanized variable domain still is lacking in desired binding, repeat step 8. In addition, re-investigate any buried residues which might affect the V_L–V_H interface (but which would not directly affect CDR conformation). Additionally, evaluate the accessibility of non-CDR residues to solvent.

Example 3

Engineering a Humanized Bisnecific F(ab')$_2$ Fragment

This example demonstrates the construction of a humanized bispecific antibody (BsF(ab')$_2$v1 by separate *E. coli* expression of each Fab' arm followed by directed chemical coupling in vitro. BsF(ab')$_2$v1 (anti-CD3/anti-p185^{HER2}) was demonstrated to retarget the cytotoxic activity of human

$CD3^+$CTL in vitro against the human breast tumor cell line, SK-BR-3, which overexpresses the $p185^{HER2}$ product of the protooncogene HER2. This example demonstrates the minimalistic humanization strategy of installing as few murine residues as possible into a human antibody in order to recruit antigen-binding affinity and biological properties comparable to that of the murine parent antibody. This strategy proved very successful for the anti-$p185^{HER2}$arm of BsF $(ab')_2$v1. In contrast $BsF(ab')_2$ v1 binds to T cells via its anti-CD3 arm much less efficiently than does the chimeric $BsF(ab')_2$ which contains the variable domains of the murine parent anti-CD3 antibody. Here we have constructed additional $BsF(ab')_2$ fragments containing variant anti-CD3 arms with selected murine residues restored in an attempt to improve antibody binding to T cells. One such variant, Ss $F(ab')_2$v9, was created by replacing six residues in the second hypervariable loop of the anti-CD3 heavy chain variable domain of $BsF(ab')_2$v1 with their counterparts from the murine parent anti-CD3 antibody. $BsF(ab')_2$v9 binds to T cells (Jurkat) much more efficiently than does $BsF(ab')_2$v1 and almost as efficiently as the chimeric $BsF(ab')_2$. This improvement in the efficiency of T cell binding of the humanized $BsF(ab')_2$ is an important step in its development as a potential therapeutic agent for the treatment of $p185^{HER2}$-overexpressing cancers.

Bispecific antibodies (BsAbs) with specificities for tumor-associated antigens and surface markers on immune effector cells have proved effective for retargeting effector cells to kill tumor targets both in vitro and in vivo (reviewed by Fanger, M. W. et al., *Immunol. Today* 10: 92–99 (1989); Fanger, M. W. et al., *Immunol. Today* 12: 51–54 (1991); and Nelson, H., *Cancer Cells* 3: 163–172 (1991)). $BsF(ab')_2$ fragments have often been used in preference to intact BsAbs in retargeted cellular cytotoxicity to avoid the risk of killing innocent bystander cells binding to the Fc region of the antibody. An additional advantage of $BsF(ab')_2$ over intact BsAbs is that they are generally much simpler to prepare free of contaminating monospecific molecules (reviewed by Songsivilai, S. and Lachmann, P. J., *Clin. Exp. Immunol.* 79: 315–321 (1990) and Nolan, O. and O'Kennedy, R., *Biochim. Biophys. Acta* 1040: 1–11 (1990)).

$BsF(ab')_2$ fragments are traditionally constructed by directed chemical coupling of Fab' fragments obtained by limited proteolysis plus mild reduction of the parent rodent monoclonal Ab (Brennan, M. et al., *Science* 229, 81–83 (1985) and Glennie, M. J. et al., *J. Immunol.* 139: 2367–2375 (1987)). One such $BsF(ab')_2$ fragment (anti-glioma associated antigen/anti-CD3) was found to have clinical efficacy in glioma patients (Nitta, T. et al., *Lancet* 335: 368–371 (1990) and another $BsF(ab')_2$ (anti-indium chelate/anti-carcinoembryonic antigen) allowed clinical imaging of colorectal carcinoma (Stickney, D. R. et al., *Antibody, Immunoconj. Radiopharm.* 2: 1–13 (1989)). Future $SsF(ab')_2$ destined for clinical applications are likely to be constructed from antibodies which are either human or at least "humanized" (Riechmann, L. et al., *Nature* 332: 323–327 (1988) to reduce their immunogenicity (Hale, G. et al., *Lancet i:* 1394–1399 (1988)).

Recently a facile route to a fully humanized $BsF(ab')_2$ fragment designed for tumor immunotherapy has been demonstrated (Shalaby, M. R. et al., *J. Exp. Med.* 175: 217–225 (1992)). This approach involves separate *E. coli* expression of each Fab' arm followed by traditional directed chemical coupling in vitro to form the $BsF(ab')_2$. One arm of the $BsF(ab')_2$ was a humanized version (Carter, P. et al., *Proc. Natl. Aced. Sci. USA* (1992a) and Carter, P., et al., *Bio/Technology* 10: 163–167 (1992b)) of the murine monoclonal Ab 4D5 which is directed against the $p185^{HER2}$ product of the protooncogene HER2 (c-erbB-2) (Fendly, B. M. et al. *Cancer Res.* 50: 1550–1558 (1989)). The humanization of the antibody 4D5 is shown in Example 1 of this application. The second arm was a minimalistically humanized anti-CD3 antibody (Shalaby et al. supra) which was created by installing the CDR loops from the variable domains of the murine parent monoclonal Ab UCHT1 (Beverley, P. C. L. and Callard, R. E., *Eur. J. Immunol.* 11: 329–334 (1981)) into the humanized anti-$p185^{HER2}$ antibody. The $BsF(ab')_2$ fragment containing the most potent humanized anti-CD3 variant (v1) was demonstrated by flow cytometry to bind specifically to a tumor target overexpressing $p185^{HER2}$ and to human peripheral blood mononuclear cells carrying CD3. In addition, $BsF(ab')_2$v1 enhanced the cytotoxic effects of activated human CTL 4-fold against SK-SR-3 tumor cells overexpressing $p185^{HER2}$. The example descries efforts to improve the antigen binding affinity of the humanized anti-CD3 arm by the judicious recruitment of a small number of additional murine residues into the minimalistically humanized anti-CD3 variable domains.

Materials and Methods

Construction of Mutations in the Anti-CD3 Variable Region Genes

The construction of genes encoding humanized anti-CD3 variant 1 (v1) variable light (V_1) and heavy (V_H) chain domains in phagemid pUC119 has been described (Shalaby et al. supra). Additional anti-CD3 variants were generated using an efficient site-directed mutagenesis method (Carter, P., *Mutagenesis: a practical approach,* (M. J. McPherson, Ed.), Chapter 1, IRL Press, Oxford, UK (1991)) using mismatched oligonucleotides which either install or remove unique restriction sites. Oligonucleotides used are listed below using lowercase to indicate the targeted mutations. Corresponding coding changes are denoted by the starting amino acid in one letter code followed by the residue numbered according to Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest,* 5^{th} edition, National Institutes of Health, Bethesda, Md., USA (1991), then the replacement amino acid and finally the identity of the anti-CD3 variant:

HX11, 5' GTAGATAAATCCtctAACACAGC-CTAtCTGCAAATG 3' (SEQ.ID. NO. 11) V_HK75S, v6;

HX12, 5' GTAGATAAATCCAAAtctACAGC-CTAtCTGCAAATG 3' (SEQ.ID. NO. 12) V_H N76S, v7;

HX13, 5' GTAGATAAATCCtcttctACAGC-CTAtCTGCAAATG 3' (SEQ.ID. NO. 13) V_H K75S:N76S, v8;

X14, 5' CTTATAAAGGTGTTtCcACCTATaaCcA-gAaatTCAAGGatCGTTTCACgATAtc-CGTAGATAAATCC 3' (SEO.ID.NO. 14) V_H T57S:A60N:D61Q:S62K:V63F:G65D, v9;

LX6, 5' CTATACCTCCCGTCTgcatTCTGGAGTCCC 3' (SEQ.ID. NO. 15) V_L E55H, v11.

Oliconucleotides HX11, HX12 and HX13 each remove a site for BspMI, whereas LX6 removes a site for XhoI and HX14 installs a site for EcoRV (bold). Anti-CD3 variant v10 was constructed from v9 by site-directed mutagenesis using oligonucleotide HX13. Mutants were verified by dideoxynucleotide sequencing (Sanger, F. et al., *Proc. Natl. Acad, Sci. USA* 74: 5463–5467 (1977)).

E. coli Expression of Fab' Fragments

The expression plasmid, pAK19, for the co-secretion of light chain and heavy chain Fd' fragment of the most preferred humanized anti-$p185^{HER2}$ variant, HuMAb4D5-8, is described in Carter et al., 1992b, supre. Briefly, the Fab' expression unit is bicistronic with both chains under the

transcriptional control of the ohoA promoter. Genes encoding humanized V_L and V_H domains are precisely fused on their 5' side to a gene segment encoding the heat-stable enterotoxin II signal sequence and on their 3' side to human k_1 C_L and IgG1C_H1 constant domain genes, respectively. The C_H1gene is immediately followed by a sequence encoding the hinge sequence CysAlaAla and followed by a bacteriophage λ t_o transcriptional terminator. Fab' expression plasmids for chimeric and humanized anti-CD3 variants (v1 to v4, Shalaby et al.,supra; v6 to v12, this study) were created from pAK19 by precisely replacing anti-p185^{HER2} V_L and V_H gene segments with those encoding murine and corresponding humanized variants of the anti-CD3 antibody, respectively, by sub-cloning and site-directed mutagenesis. The Fab' expression plasmid for the most potent humanized anti-CD3 variant identified in this study (v9) is designated pAK22. The anti-p185^{HER2} Fab' fragment was secreted from *E. coli* K12 strain 25F2 containing plasmid pAK19 grown for 32 to 40 hr at 37° C. in an aerated 10 liter fermentor. The final cell density was 120–150 OD_{550} and the titer of soluble and functional anti-p185^{HER2} Fab' was 1–2 g/liter as judged by antigen binding ELISA (Carter et al., 1992b, suora). Anti-CD3 Fab' variants were secreted from *E. coli* containing corresponding expression plasmids using very similar fermentation protocols. The highest expression titers of chimeric and humanized anti-CD3 variants were 200 mgaliter and 700 mgaliter, respectively, as judged by total immunoglobulin ELISA.

Construction of BsF(ab')$_2$ Fragments

Fab' fragments were directly recovered from *E. coli* fermentation pastes in the free thiol form (Fab'-SH) by affinity purification on Streptococcal protein G at pH 5 in the presence of EDTA (Carter et al., 1992b supra). Thioether linked BsF(ab')$_2$ fragments (anti-p185^{HER2}/anti-CD3) were constructed by the procedure of Glennie et al. supra with the following modifications. Anti-p185^{HER2} Fab'-SH in 100 mM Tris acetate, 5 mM EDTA (pH 5.0) was reacted with 0.1 vol of 40 mM N,N'-1,2-phenylenedimalemide (o-PDM) in dimethyl formamide for ~1.5 hr at 20° C. Excess o-PDM was removed by protein G purification of the Fab' maleimide derivative (Fab'-mal) followed by buffer exchange into 20 mM sodium acetate, 5 mM EDTA (pH 5.3) (coupling buffer) using centriprep-30 concentrators (Amicon). The total concentration of Fab' variants was estimated from the measured absorbance at 280 nm (HuMAb4D5-8 Fab' $e^{0.1}$%=1.56, Carter et al., 1992b, supra). The free thiol content of Fab' preparations was estimated by reaction with 5,5' -dithiobis (2-nitrobenzoic acid) as described by Creighton, T. E., *Protein structure: a practical approach,* (T. E. Creighton, Ed.), Chapter 7, IRL Press, Oxford, UK (1990). Equimolar amounts of anti-p185^{HER2} Fab'-mal (assuming quantitative reaction of Fab'-SH with o-PDM) and each anti-CD3 Fab'-SH variant were coupled together at a combined concentration of 1 to 2.5 mg/ml in the coupling buffer for 14 to 48 hr at 4° C. The coupling reaction was adjusted to 4 mM cysteine at pH 7.0 and incubated for 15 min at 20 ° C. to reduce any unwanted disulfide-linked F(ab')$_2$ formed. These reduction conditions are sufficient to reduce inter-heavy chain disulfide bonds with virtually no reduction of the disulfide between light and heavy chains. Any free thiols generated were then blocked with 50 mM iodoacetamide. BsF(ab')$_2$ was isolated from the coupling reaction by S100-HR (Pharmacia) size exclusion chromatography (2.5 cm×100 cm) in the presence of PBS. The BsF(ab')$_2$ samples were passed through a 0.2 mm filter flash frozen in liquid nitrogen and stored at −70° C.

Flow Cytometric Analysis of F(ab')$_2$Binding to Jurkat Cells

The Jurkat human acute T cell leukemia cell line was purchased from the American Type Culture Collection (Manassas Va.) (ATCC TIB 152) and grown as. recommended by the ATCC. Aliquots of 10^6 Jurkat cells were incubated with appropriate concentrations of BsF(ab')$_2$ (anti-p185^{HER2}/anti-CD3 variant) or control mono-specific anti-p185^{HER2} F(ab')$_2$ in PBS plus 0.1% (w/v) bovine serum albumin and 10 mM sodium azide for 45 min at 4° C. The cells were washed and then incubated with fluorescein-conjugated goat anti-human F(ab')$_2$ (Organon Teknika, West Chester, Pa.) for 45 min at 4° C. Cells were washed and analyzed on a FACScan® (Becton Dickinson and Co., Mountain View, Calif.). Cells (8×10^3) were acquired by list mode and gated by forward light scatter versus side light scatter excluding dead cells and debris.

Results

Design of Humanized anti-CD3 Variants

The most potent humanized anti-CD3 variant previously identified, v1, differs from the murine parent antibody, UCHT1 at 19 out of 107 amino acid residues within V_L and at 37 out of 122 positions within V_H (Shalaby et al.,supra) 1992). Here we recruited back additional murine residues into anti-CD3 v1 in an attempt to improve the binding affinity for CD3. The strategy chosen was a compromise between minimizing both the number of additional murine residues recruited and the number of anti-CD3 variants to be analyzed. We focused our attentions on a few CDR residues which were originally kept as human sequences in our minimalistic humanization regime. Thus human residues in V_H CDR2 of anti-CD3 v1 were replaced en bloc with their murine counterparts to give anti-CD3 v9: T57S:A60N:D61Q:S62K:V63F:G65D (SEQ ID NO:20). Similarly, the human residue E55 in V_L CDR2 of anti-CD3 v_1 was replaced with histidine from the murine anti-CD3 antibody to generate anti-CD3 v11. In addition, V_H framework region (FR) residues 75 and 76 in anti-CD3 v1 were also replaced with their murine counterparts to create anti-CD3 v8: K75S:N76S. V_H residues 75 and 76 are located in a loop close to V_H CDR1 and CDR2 and therefore might influence antigen binding. Additional variants created by combining mutations at these three sites are described below.

Preparation of BsF(ab')$_2$ Fragments

Soluble and functional anti-p185^{HER2} and anti-CD3 Fab' fragments were recovered directly from corresponding *E. coli* fermentation pastes with the single hinge cysteine predominantly in the free thiol form (75–100% Fab'-SH) by affinity purification on Streptococcal protein G at pH 5 in the presence of EDTA (Carter et al., 1992b, supra). Thioether-linked BsF(ab')$_2$ fragments were then constructed by directed coupling using o-PDM as described by Glennie et al., supra. One arm was always the most potent humanized anti-p185^{HER2} variant, HuMAb4D5-8 (Carter et al., 1992a, supra) and the other either a chimeric or humanized variant of the anti-CD3 antibody. Anti-p185^{HER2} Fab'-SH was reacted with o-PDM to form the maleimide derivative (Fab'-mal) and then coupled to the Fab'-SH for each anti-CD3 variant. F(ab')$_2$ was then purified away from unreacted Fab' by size exclusion chromatography as shown for a representative preparation (BsF(ab')$_2$ v8) in data not shown. The F(ab')$_2$ fragment represents ~54% of the total amount of antibody fragments (by mass) as judged by integration of the chromatograph peaks.

SDS-PAGE analysis of this BsF(ab')$_2$v8 preparation under non-reducing conditions gave one major band with the expected mobility (M, ~96 kD) as well as several very minor

bands (data not shown). Amino-terminal sequence analysis of the major band after electroblotting on to polyvinylidene difluoride 76 are located in a loop close to V_H CDR1 and CDR2 and therefore might membrane Matsudaira, P., *J. Biol. Chem.* 262: 10035–10038 (1987) gave the expected mixed sequence from a stoichiometric 1:1 mixture of light and heavy chains (V_L/V_H: D/E, I/V, Q/D, M/L, T/V, D/E, S/S) expected for BsF(ab')$_2$. The amino terminal region of both light chains are identical as are both heavy chains and correspond to consensus human FR sequences. We have previously demonstrated that F(ab')$_2$ constructed by directed chemical coupling carry both anti-p185^{HER2} and anti-CD3 antigen specificities (Shalaby et al., supra). The level of contamination of the BsF(ab')$_2$ with monospecific F(ab')$_2$ iS likely to be very low since mock coupling reactions with either anti-p185^{HER2} w Fab'-mal or anti-CD3 Fab'-SH alone did not yield detectable quantities of F(ab')$_2$. Furthermore the coupling reaction was subjected to a mild reduction step followed by alkylation to remove trace amounts of disulfide-linked F(ab')$_2$ that might be present. SDS-PAGE of the purified F(ab')$_2$ under reducing conditions gave two major bands with electrophoretic mobility and amino terminal sequence anticipated for free light chain and thioether-linked heavy chain dimers.

Scanning LASER densitometry of a o-PDM coupled F(ab')$_2$ preparation suggest that the minor species together represent ~10% of the protein. These minor contaminants were characterized by amino terminal sequence analysis and were tentatively identified on the basis of stoichiometry of light and heavy chain sequences and their electrophoretic mobility (data not shown). These data are consistent with the minor contaminants including imperfect F(ab')$_2$ in which the disulfide bond between light and heavy chains is missing in one or both arms, trace amounts of Fab' and heavy chain thioether-linked to light chain.

Binding of BsF(ab')$_2$ to Jurkat Cells

Binding of BsF(ab')$_2$ containing different anti-CD3 variants to Jurkat cells (human acute T cell leukemia) was investigated by flow cytometry (data not shown). BsF(ab')$_2$v9 binds much more efficiently to Jurkat cells than does our starting molecule, BsF(ab')$_2$vl, and almost as efficiently as the chimeric BsF(ab')$_2$. Installation of additional murine residues into anti-CD3 v9 to create v10 (V_HK75S:N76S) and v12 (V_HK75S:N76S plus V_L E55H) did not further improve binding of corresponding BsF(ab')$_2$to Jurkat cells. Nor did recruitment of these murine residues into anti-CD3 v1 improve Jurkat binding: V_HK75S (v6), V_HN76S (v7), V_HK75S:N76S (V8), V_LE55H (v11) (not shown). BsF(ab')$_2$v9 was chosen for future study since it is amongst the most efficient variants in binding to Jurkat cells and contains fewest murine residues in the humanized anti-CD3 arm. A monospecific anti-p185^{HEE2} F(ab')$_2$ did not show significant binding to Jurkat cells consistent with the interaction being mediated through the anti-CD3 arm.

Discussion

A minimalistic strategy was chosen to humanize the anti-p185^{HER2} (Carter et al., 1992a, supra) and anti-CD3 arms (Shalaby et al., supra) of the BsF(ab')$_2$ in this study in an attempt to minimize the potential immunogenicity of the resulting humanized antibody in the clinic. Thus we tried to install the minimum number of murine CDR and FR residues into the context of consensus human variable domain sequences as required to recruit antigen-binding affinity and biological properties comparable to the murine parent antibody. Molecular modeling was used firstly to predict the murine FR residues which might be important to antigen binding and secondly to predict the murine CDR residues that might not be required. A small number of humanized variants were then constructed to test these predictions.

Our humanization strategy was very successful for the anti-p185^{HER2} antibody where one out of eight humanized variants (HuMAb4D5-8, IgG1) was identified that bound the p185^{HER2} antigen ~3-fold more tightly than the parent murine antibody (Carter et al., 1992a, supra). HuMAb4D5-8 contains a total of five murine FR residues and nine murine CDR residues, including V_H CDR2 residues 60–65, were discarded in favor of human counterparts. In contrast, BsF(ab')$_2$v1 containing the most potent humanized anti-CD3 variant out of four originally constructed (Shalaby et al., supra) binds J6 cells with an affinity (K_d) of 140 nM which is ~70-fold weaker than that of the corresponding chimeric BsF(ab')$_2$.

Here we have restored T cell binding of the humanized anti-CD3 close to that of the chimeric variant by replacing six human residues in V_H CDR2 with their murine counterparts: T57S:A60N:D61Q:S62K:V63F:G65D (anti-CD3 v9, FIG. **5**). It appears more likely that these murine residues enhance antigen binding indirectly by influencing the conformation of residues in the N-terminal part of V_H CDR2 rather than by directly contacting antigen. Firstly, only N-terminal residues in V_H CDR2 (50–58) have been found to contact antigen in one or more of eight crystallographic structures of antibody/antigen complexes (Kabat et al., supra; and Mian, I. S. et al., *J. Mol. Biol* 217: 133–151 (1991), FIG. **5**). Secondly, molecular modeling suggests that residues in the C-terminal part of V_H CDR2 are at least partially buried (FIG. **5**). BsF(ab')$_2$v9 binds to SK-BR-3 breast tumor cells with equal efficiency to BsF(ab')$_2$v1 and chimeric BsF(ab')$_2$ as anticipated since the anti-p185^{HER2} arm is identical in all of these molecules (Shalaby et al., supra, not shown).

Our novel approach to the construction of BsF(ab')$_2$ fragments exploits an *E. coli* expression system which secretes humanized Fab' fragments at gram per liter titers and permits their direct recovery as Fab'-SH (Carter et al., 1992b, supra). Traditional directed chemical coupling of Fab'-SH fragments is then used to form BsF(ab')$_2$ in vitro (Brennan et al.,supra; and Glennie et al., supra). This route to Fab'-SH obviates problems which are inherent in their generation from intact antibodies: differences in susceptibility to proteolysis and nonspecific cleavage resulting in heterogeneity, low yield as well as partial reduction that is not completely selective for the hinge disulfide bonds. The strategy of using *E. coli*-derived Fab'-SH containing a single hinge cysteine abolishes some sources of heterogeneity in BsF(ab')$_2$ preparation such as intra-hinge disulfide formation and contamination with intact parent antibody whilst greatly diminishes others, eg. formation of F(ab')$_3$ fragments.

BsF(ab')2 fragments constructed here were thioether-linked as originally described by Glennie et al., supra with future in vivo testing of these molecules in mind. Thioether bonds, unlike disulfide bonds, are not susceptible to cleavage by trace amounts of thiol, which led to the proposal that thioether-linked F(ab')$_2$ may be more stable than disulfide-linked F(ab')$_2$ in vivo (Glennie et al., supra). This hypothesis is supported by our preliminary pharmacokinetic experiments in normal mice which suggest that thioether-linked BsF(ab')$_2$ v1 has a 3-fold longer plasma residence time than BsF(ab')$_2$ v1 linked by a single disulfide bond. Disulfide and thioether-linked chimeric BsF(ab')$_2$ were found to be indistinguishable in their efficiency of cell binding and in their retargeting of CTL cytotoxicity, which suggests that o-PDM directed coupling does not compromise binding of the

$BsF(ab')_2$ to either antigen (not shown). Nevertheless the nature of the linkage appears not to be critical since a disulfide-linked $BsF(ab')_2$ (murine anti-$p185^{HER2}$/murine anti-CD3) was recently shown by others (Nishimura et al., Int. *J. Cancer* 50: 800–804 (1992) to have potent anti-tumor activity in nude mice. Our previous study (Shalaby et al., supra) together with this one and that of Nishimura, T. et al., supra improve the potential for using $BsF(ab')_2$ in targeted immunotherapy of $p185^{HER2}$-overexpressing cancers in humans.

Example 4

Humanization of an anti-CD18 Antibody

A murine antibody directed against the leukocyte adhesion receptor β-chain (known as the H52 antibody) was humanized following the methods described above. FIGS. **6**A and **6**B provide amino acid sequence comparisons for the murine and humanized antibody light chains and heavy chains.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 26

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
  1               5                  10                  15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn
                 20                  25                  30

Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
                 35                  40                  45

Leu Leu Ile Tyr Ser Ala Ser Phe Leu Glu Ser Gly Val Pro Ser
                 50                  55                  60

Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile
                 65                  70                  75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
                 80                  85                  90

His Tyr Thr Thr Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu
                 95                 1 00                 1 05

Ile Lys Arg Thr
            109
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 120 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
  1               5                  10                  15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys
                 20                  25                  30

Asp Thr Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
                 35                  40                  45

Glu Trp Val Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr
                 50                  55                  60

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser
                 65                  70                  75
```

-continued

```
Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                 80                  85                  90

Thr Ala Val Tyr Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr
                 95                 1 00                 1 05

Ala Met Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
                110                 115                  120
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
  1               5                  10                  15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser
                 20                  25                  30

Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
                 35                  40                  45

Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser
                 50                  55                  60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
                 65                  70                  75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
                 80                  85                  90

Tyr Asn Ser Leu Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu
                 95                 1 00                 1 05

Ile Lys Arg Thr
            109
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 120 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
  1               5                  10                  15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
                 20                  25                  30

Asp Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
                 35                  40                  45

Glu Trp Val Ala Val Ile Ser Glu Asn Gly Ser Asp Thr Tyr Tyr
                 50                  55                  60

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser
                 65                  70                  75

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                 80                  85                  90

Thr Ala Val Tyr Tyr Cys Ala Arg Asp Arg Gly Gly Ala Val Ser
                 95                 1 00                 1 05

Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
                110                 115                  120
```

-continued

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Asp Ile Val Met Thr Gln Ser His Lys Phe Met Ser Thr Ser Val
1 5 10 15

Gly Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asp Val Asn
20 25 30

Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly His Ser Pro Lys
35 40 45

Leu Leu Ile Tyr Ser Ala Ser Phe Arg Tyr Thr Gly Val Pro Asp
50 55 60

Arg Phe Thr Gly Asn Arg Ser Gly Thr Asp Phe Thr Phe Thr Ile
65 70 75

Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln
80 85 90

His Tyr Thr Thr Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu
95 1 00 1 05

Ile Lys Arg Ala
109

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 120 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly
1 5 10 15

Ala Ser Leu Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys
20 25 30

Asp Thr Tyr Ile His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu
35 40 45

Glu Trp Ile Gly Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr
50 55 60

Asp Pro Lys Phe Gln Asp Lys Ala Thr Ile Thr Ala Asp Thr Ser
65 70 75

Ser Asn Thr Ala Tyr Leu Gln Val Ser Arg Leu Thr Ser Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr
95 1 00 1 05

Ala Met Asp Tyr Trp Gly Gln Gly Ala Ser Val Thr Val Ser Ser
110 115 120

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TCCGATATCC AGCTGACCCA GTCTCCA 27

-continued

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GTTTGATCTC CAGCTTGGTA CCHSCDCCGA A 31

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AGGTSMARCT GCAGSAGTCW GG 22

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TGAGGAGACG GTGACCGTGG TCCCTTGGCC CCAG 34

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GTAGATAAAT CCTCTAACAC AGCCTATCTG CAAATG 36

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GTAGATAAAT CCAAATCTAC AGCCTATCTG CAAATG 36

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GTAGATAAAT CCTCTTCTAC AGCCTATCTG CAAATG 36

-continued

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CTTATAAAGG TGTTTCCACC TATAACCAGA AATTCAAGGA TCGTTTCACG 50

ATATCCGTAG ATAAATCC 68

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CTATACCTCC CGTCTGCATT CTGGAGTCCC 30

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu
1 5 10 15

Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile Arg
20 25 30

Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys
35 40 45

Leu Leu Ile Tyr Tyr Thr Ser Arg Leu His Ser Gly Val Pro Ser
50 55 60

Lys Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile
65 70 75

Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln
80 85 90

Gly Asn Thr Leu Pro Trp Thr Phe Ala Gly Gly Thr Lys Leu Glu
95 1 00 1 05

Ile Lys
107

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile Arg
20 25 30

Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys

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```
                35                  40                  45

 Leu Leu Ile Tyr Tyr Thr Ser Arg Leu Glu Ser Gly Val Pro Ser
                50                  55                  60

 Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile
                65                  70                  75

 Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
                80                  85                  90

 Gly Asn Thr Leu Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu
                95                 1 00                 1 05

 Ile Lys
     107


(2) INFORMATION FOR SEQ ID NO:18:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 107 amino acids
          (B) TYPE: Amino Acid
          (D) TOPOLOGY: Linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

 Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
   1               5                  10                  15

 Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser
                20                  25                  30

 Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
                35                  40                  45

 Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser
                50                  55                  60

 Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
                65                  70                  75

 Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
                80                  85                  90

 Tyr Asn Ser Leu Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu
                95                 1 00                 1 05

 Ile Lys
     107


(2) INFORMATION FOR SEQ ID NO:19:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 122 amino acids
          (B) TYPE: Amino Acid
          (D) TOPOLOGY: Linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

 Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly
   1               5                  10                  15

 Ala Ser Met Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr
                20                  25                  30

 Gly Tyr Thr Met Asn Trp Val Lys Gln Ser His Gly Lys Asn Leu
                35                  40                  45

 Glu Trp Met Gly Leu Ile Asn Pro Tyr Lys Gly Val Ser Thr Tyr
                50                  55                  60

 Asn Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Val Asp Lys Ser
                65                  70                  75

 Ser Ser Thr Ala Tyr Met Glu Leu Leu Ser Leu Thr Ser Glu Asp
                80                  85                  90
```

-continued

```
Ser Ala Val Tyr Tyr Cys Ala Arg Ser Gly Tyr Tyr Gly Asp Ser
                 95                 1 00                 1 05

Asp Trp Tyr Phe Asp Val Trp Gly Ala Gly Thr Thr Val Thr Val
                110                 115                  120

Ser Ser
    122


(2) INFORMATION FOR SEQ ID NO:20:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 122 amino acids
          (B) TYPE: Amino Acid
          (D) TOPOLOGY: Linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
  1               5                  10                  15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Ser Phe Thr
                 20                  25                  30

Gly Tyr Thr Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
                 35                  40                  45

Glu Trp Val Ala Leu Ile Asn Pro Tyr Lys Gly Val Ser Thr Tyr
                 50                  55                  60

Asn Gln Lys Phe Lys Asp Arg Phe Thr Ile Ser Val Asp Lys Ser
                 65                  70                  75

Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                 80                  85                  90

Thr Ala Val Tyr Tyr Cys Ala Arg Ser Gly Tyr Tyr Gly Asp Ser
                 95                 1 00                 1 05

Asp Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val
                110                 115                  120

Ser Ser
    122


(2) INFORMATION FOR SEQ ID NO:21:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 122 amino acids
          (B) TYPE: Amino Acid
          (D) TOPOLOGY: Linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
  1               5                  10                  15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
                 20                  25                  30

Ser Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
                 35                  40                  45

Glu Trp Val Ser Val Ile Ser Gly Asp Gly Gly Ser Thr Tyr Tyr
                 50                  55                  60

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser
                 65                  70                  75

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                 80                  85                  90

Thr Ala Val Tyr Tyr Cys Ala Arg Gly Arg Val Gly Tyr Ser Leu
                 95                 1 00                 1 05

Ser Gly Leu Tyr Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val
                110                 115                  120
```

-continued

Ser Ser
122

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 454 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly
1 5 10 15

Ala Ser Val Lys Ile Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr
20 25 30

Glu Tyr Thr Met His Trp Met Lys Gln Ser His Gly Lys Ser Leu
35 40 45

Glu Trp Ile Gly Gly Phe Asn Pro Lys Asn Gly Gly Ser Ser His
50 55 60

Asn Gln Arg Phe Met Asp Lys Ala Thr Leu Ala Val Asp Lys Ser
65 70 75

Thr Ser Thr Ala Tyr Met Glu Leu Arg Ser Leu Thr Ser Glu Asp
80 85 90

Ser Gly Ile Tyr Tyr Cys Ala Arg Trp Arg Gly Leu Asn Tyr Gly
95 1 00 1 05

Phe Asp Val Arg Tyr Phe Asp Val Trp Gly Ala Gly Thr Thr Val
110 115 120

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
125 130 135

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
140 145 150

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
155 160 165

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
170 175 180

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
185 190 195

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
200 205 210

His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
215 220 225

Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
230 235 240

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
245 250 255

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
260 265 270

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
275 280 285

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
290 295 300

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
305 310 315

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
320 325 330

-continued

```
Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
                335                 340                 345

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
                350                 355                 360

Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
                365                 370                 375

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
                380                 385                 390

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
                395                 400                 405

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                410                 415                 420

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
                425                 430                 435

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
                440                 445                 450

Ser Pro Gly Lys
            454
```

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 469 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr
  1                 5                  10                  15

Gly Val His Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu
                 20                  25                  30

Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Thr Ser Gly
                 35                  40                  45

Tyr Thr Phe Thr Glu Tyr Thr Met His Trp Met Arg Gln Ala Pro
                 50                  55                  60

Gly Lys Gly Leu Glu Trp Val Ala Gly Ile Asn Pro Lys Asn Gly
                 65                  70                  75

Gly Thr Ser His Asn Gln Arg Phe Met Asp Arg Phe Thr Ile Ser
                 80                  85                  90

Val Asp Lys Ser Thr Ser Thr Ala Tyr Met Gln Met Asn Ser Leu
                 95                 1 00                 1 05

Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Trp Arg Gly
                110                 115                 120

Leu Asn Tyr Gly Phe Asp Val Arg Tyr Phe Asp Val Trp Gly Gln
                125                 130                 135

Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
                140                 145                 150

Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr
                155                 160                 165

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
                170                 175                 180

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
                185                 190                 195

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
                200                 205                 210
```

-continued

```
Val Val Thr Val Thr Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr
                215                 220                 225

Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr
                230                 235                 240

Val Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro
                245                 250                 255

Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
                260                 265                 270

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
                275                 280                 285

Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr
                290                 295                 300

Val Asp Gly Met Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
                305                 310                 315

Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr Val
                320                 325                 330

Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
                335                 340                 345

Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
                350                 355                 360

Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
                365                 370                 375

Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
                380                 385                 390

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
                395                 400                 405

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu
                410                 415                 420

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                425                 430                 435

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
                440                 445                 450

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
                455                 460                 465

Ser Pro Gly Lys
            469


(2) INFORMATION FOR SEQ ID NO:24:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 214 amino acids
          (B) TYPE: Amino Acid
          (D) TOPOLOGY: Linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Asp Val Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu
  1               5                  10                  15

Gly Asp Arg Val Thr Ile Asn Cys Arg Ala Ser Gln Asp Ile Asn
                 20                  25                  30

Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asn Gly Thr Val Lys
                 35                  40                  45

Leu Leu Ile Tyr Tyr Thr Ser Thr Leu His Ser Gly Val Pro Ser
                 50                  55                  60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile
                 65                  70                  75
```

-continued

```
Ser Asn Leu Asp Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln
                 80                  85                  90

Gly Asn Thr Leu Pro Pro Thr Phe Gly Gly Gly Thr Lys Val Glu
                 95                 1 00                 1 05

Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
                110                 115                  120

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
                125                 130                  135

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val
                140                 145                  150

Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu
                155                 160                  165

Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr
                170                 175                  180

Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu
                185                 190                  195

Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn
                200                 205                  210

Arg Gly Glu Cys
            214
```

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 233 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr
  1                  5                  10                  15

Gly Val His Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu
                 20                  25                  30

Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser
                 35                  40                  45

Gln Asp Ile Asn Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly
                 50                  55                  60

Lys Ala Pro Lys Leu Leu Ile Tyr Tyr Thr Ser Thr Leu His Ser
                 65                  70                  75

Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr
                 80                  85                  90

Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
                 95                 1 00                 1 05

Tyr Cys Gln Gln Gly Asn Thr Leu Pro Pro Thr Phe Gly Gln Gly
                110                 115                  120

Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe
                125                 130                  135

Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser
                140                 145                  150

Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val
                155                 160                  165

Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
                170                 175                  180

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                185                 190                  195
```

-continued

```
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
                200                 205                 210

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr
                215                 220                 225

Lys Ser Phe Asn Arg Gly Glu Cys
                230          233


(2) INFORMATION FOR SEQ ID NO:26:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 122 amino acids
          (B) TYPE: Amino Acid
          (D) TOPOLOGY: Linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
  1               5                  10                  15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Ser Phe Thr
                20                  25                  30

Gly Tyr Thr Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
                35                  40                  45

Glu Trp Val Ala Leu Ile Asn Pro Tyr Lys Gly Val Thr Thr Tyr
                50                  55                  60

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Val Asp Lys Ser
                65                  70                  75

Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                80                  85                  90

Thr Ala Val Tyr Tyr Cys Ala Arg Ser Gly Tyr Tyr Gly Asp Ser
                95                 1 00                1 05

Asp Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val
                110                 115                 120

Ser Ser
    122
```

We claim:

1. A humanized antibody variable domain comprising non-human Complementarity Determining Region (CDR) amino acid residues which bind an antigen incorporated into a human antibody variable domain, and further comprising a Framework Region (FR) amino acid substitution at a site selected from the group consisting of: 4L, 38L, 43L, 44L, 58L, 62L, 65L, 66L, 67L, 68L, 69L, 73L, 85L, 98L, 2H, 4H, 36H, 39H, 43H, 45H, 69H, 70H, 74H, and 92H, utilizing the numbering system set forth in Kabat.

2. The humanized variable domain of claim **1** wherein the substituted residue is the residue found at the corresponding location of the non-human antibody from which the non-human CDR amino acid residues are obtained.

3. The humanized variable domain of claim **1** wherein no human Framework Region (FR) residue other than those set forth in the group has been substituted.

4. The humanized variable domain of claim **1** wherein the human antibody variable domain is a consensus human variable domain.

5. The humanized variable domain of claim **1** wherein the residue at site 4L has been substituted.

6. The humanized variable domain of claim **1** wherein the residue at site 38L has been substituted.

7. The humanized variable domain of claim **1** wherein the residue at site 43L has been substituted.

8. The humanized variable domain of claim **1** wherein the residue at site 44L has been substituted.

9. The humanized variable domain of claim **1** wherein the residue at site 58L has been substituted.

10. The humanized variable domain of claim **1** wherein the residue at site 62L has been substituted.

11. The humanized variable domain of claim **1** wherein the residue at site 65L has been substituted.

12. The humanized variable domain of claim **1** wherein the residue at site 66L has been substituted.

13. The humanized variable domain of claim **1** wherein the residue at site 67L has been substituted.

14. The humanized variable domain of claim **1** wherein the residue at site 68L has been substituted.

15. The humanized variable domain of claim **1** wherein the residue at site 69L has been substituted.

16. The humanized variable domain of claim **1** wherein the residue at site 73L has been substituted.

17. The humanized variable domain of claim **1** wherein the residue at site 85L has been substituted.

18. The humanized variable domain of claim **1** wherein the residue at site 98L has been substituted.

19. The humanized variable domain of claim **1** wherein the residue at site 2H has been substituted.

20. The humanized variable domain of claim **1** wherein the residue at site 4H has been substituted.

21. The humanized variable domain of claim 1 wherein the residue at site 36H has been substituted.

22. The humanized variable domain of claim 1 wherein the residue at site 39H has been substituted.

23. The humanized variable domain of claim 1 wherein the residue at site 43H has been substituted.

24. The humanized variable domain of claim 1 wherein the residue at site 45H has been substituted.

25. The humanized variable domain of claim 1 wherein the residue at site 69H has been substituted.

26. The humanized variable domain of claim 1 wherein the residue at site 70H has been substituted.

27. The humanized variable domain of claim 1 wherein the residue at site 74H has been substituted.

28. The humanized variable domain of claim 1 wherein the residue at site 92H has been substituted.

29. An antibody comprising the humanized variable domain of claim 1.

30. An antibody which binds $p185^{HER2}$ and comprises a humanized antibody variable domain, wherein the humanized antibody variable domain comprises non-human Complementarity Determining Region (CDR) amino acid residues which bind $p185^{HER2}$ incorporated into a human antibody variable domain, and further comprises a Framework Region (FR) amino acid substitution at a site selected from the group consisting of: 4L, 38L, 43L, 44L, 46L, 58L, 62L, 65L, 66L, 67L, 68L, 69L, 73L, 85L, 98L, 2H, 4H, 36H, 39H, 43H, 45H, 69H, 70H, 74H, 75H, 76H, 78H and 92H, utilizing the numbering system set forth in Kabat.

31. The antibody of claim 30 wherein the substituted residue is the residue found at the corresponding location of the non-human antibody from which the non-human CDR amino acid residues are obtained.

32. The antibody of claim 30 wherein no human Framework Region (FR) residue other than those set forth in the group has been substituted.

33. The antibody of claim 30 wherein the human antibody variable domain is a consensus human variable domain.

34. The antibody of claim 30 wherein the residue at site 4L has been substituted.

35. The antibody of claim 30 wherein the residue at site 38L has been substituted.

36. The antibody of claim 30 wherein the residue at site 43L has been substituted.

37. The antibody of claim 30 wherein the residue at site 44L has been substituted.

38. The antibody of claim 30 wherein the residue at site 46L has been substituted.

39. The antibody of claim 30 wherein the residue at site 58L has been substituted.

40. The antibody of claim 30 wherein the residue at site 62L has been substituted.

41. The antibody of claim 30 wherein the residue at site 65L has been substituted.

42. The antibody of claim 30 wherein the residue at site 66L has been substituted.

43. The antibody of claim 30 wherein the residue at site 67L has been substituted.

44. The antibody of claim 30 wherein the residue at site 68L has been substituted.

45. The antibody of claim 30 wherein the residue at site 69L has been substituted.

46. The antibody of claim 30 wherein the residue at site 73L has been substituted.

47. The antibody of claim 30 wherein the residue at site 85L has been substituted.

48. The antibody of claim 30 wherein the residue at site 98L has been substituted.

49. The antibody of claim 30 wherein the residue at site 2H has been substituted.

50. The antibody of claim 30 wherein the residue at site 4H has been substituted.

51. The antibody of claim 30 wherein the residue at site 36H has been substituted.

52. The antibody of claim 30 wherein the residue at site 39H has been substituted.

53. The antibody of claim 30 wherein the residue at site 43H has been substituted.

54. The antibody of claim 30 wherein the residue at site 45H has been substituted.

55. The antibody of claim 30 wherein the residue at site 69H has been substituted.

56. The antibody of claim 30 wherein the residue at site 70H has been substituted.

57. The antibody of claim 30 wherein the residue at site 74H has been substituted.

58. The antibody of claim 30 wherein the residue at site 75H has been substituted.

59. The antibody of claim 30 wherein the residue at site 76H has been substituted.

60. The antibody of claim 30 wherein the residue at site 78H has been substituted.

61. The antibody of claim 30 wherein the residue at site 92H has been substituted.

62. A humanized antibody variable domain comprising non-human Complementarity Determining Region (CDR) amino acid residues which bind an antigen incorporated into a consensus human variable domain, and further comprising an amino acid substitution at a site selected from the group consisting of: 4L, 38L, 43L, 44L, 46L, 58L, 62L, 65L, 66L, 67L, 68L, 69L, 73L, 85L, 98L, 2H, 4H, 36H, 39H, 43H, 45H, 69H, 70H, 74H, 75H, 76H, 78H and 92H, utilizing the numbering system set forth in Kabat.

63. A humanized antibody which lacks immunogenicity compared to a non-human parent antibody upon repeated administration to a human patient in order to treat a chronic disease in that patient, wherein the humanized antibody comprises non-human Complementarity Determining Region (CDR) amino acid residues which bind an antigen incorporated into a human antibody variable domain, and further comprises an amino acid substitution at a site selected from the group consisting of: 4L, 38L, 43L, 44L, 46L, 58L, 62L, 65L, 66L, 67L, 68L, 69L, 73L, 85L, 98L, 2H, 4H, 36H, 39H, 43H, 45H, 69H, 70H, 74H, 75H, 76H, 78H and 92H, utilizing the numbering system set forth in Kabat.

64. A humanized variant of a non-human parent antibody which binds an antigen and comprises a human variable domain comprising the most frequently occurring amino acid residues at each location in all human immunoglobulins of a human heavy chain immunoglobulin subgroup wherein amino acid residues forming Complementarity Determining Regions (CDRs) thereof comprise non-human antibody amino acid residues, and further comprises a Framework Region (FR) substitution where the substituted FR residue: (a) noncovalently binds antigen directly; (b) interacts with a CDR; (c) introduces a glycosylation site which affects the antigen binding or affinity of the antibody; or (d) participates in the V_L–V_H interface by affecting the proximity or orientation of the V_L and V_H regions with respect to one another.

65. The humanized variant of claim 63 which binds the antigen up to 3-fold more in the binding affinity than the parent antibody binds antigen.

66. A humanized antibody heavy chain variable domain comprising non-human Complementarity Determining

Region (CDR) amino acid residues which bind antigen incorporated into a human antibody variable domain, and further comprising a Framework Region (FR) amino acid substitution at a site selected from the group consisting of: 24H, 73H, 76H, 78H, and 93H, utilizing the numbering system set forth in Kabat.

67. The humanized variable domain of claim **66** wherein the substituted residue is the residue found at the corresponding location of the non-human antibody from which the non-human CDR amino acid residues are obtained.

68. The humanized variable domain of claim **66** wherein no human Framework Region (FR) residue other than those set forth in the group has been substituted.

69. The humanized variable domain of claim **66** wherein the human antibody variable domain is a consensus human variable domain.

70. The humanized variable domain of claim **66** wherein the residue at site 24H has been substituted.

71. The humanized variable domain of claim **66** wherein the residue at site 73H has been substituted.

72. The humanized variable domain of claim **66** wherein the residue at site 76H has been substituted.

73. The humanized variable domain of claim **66** wherein the residue at site 78H has been substituted.

74. The humanized variable domain of claim **66** wherein the residue at site 93H has been substituted.

75. The humanized variable domain of claim **66** which further comprises an amino acid substitution at site 71H.

76. The humanized variable domain of claim **66** which further comprises amino acid substitutions at sites 71H and 73H.

77. The humanized variable domain of claim **66** which further comprises amino acid substitutions at sites 71H, 73H and 78H.

78. An antibody comprising the humanized variable domain of claim **66**.

79. A humanized variant of a non-human parent antibody which binds an antigen, wherein the humanized variant comprises Complementarity Determining Region (CDR) amino acid residues of the non-human parent antibody incorporated into a human antibody variable domain, and further comprises Framework Region (FR) substitutions at heavy chain positions 71H, 73H, 78H and 93H, utilizing the numbering system set forth in Kabat.

80. A humanized antibody variable domain comprising non-human Complementarity Determining Region (CDR) amino acid residues which bind an antigen incorporated into a human antibody variable domain, and further comprising a Framework Region (FR) amino acid substitution where the substituted FR residue:

(a) noncovalently binds antigen directly;

(b) interacts with a CDR; or

(c) participates in the V_L–V_H interface by affecting the proximity or orientation of the V_L and V_H regions with respect to one another, and wherein the substituted FR residue is at a site selected from the group consisting of: 4L, 38L, 43L, 44L, 58L, 62L, 65L, 66L, 67L, 68L, 69L, 73L, 85L, 98L, 2H, 4H, 24H, 36H, 39H, 43H, 45H, 69H, 70H, 73H, 74H, 76H, 78H, 92H and 93H, utilizing the numbering system set forth in Kabat.

81. The humanized variable domain of claim **80** wherein the substituted residue is the residue found at the corresponding location of the non-human antibody from which the non-human CDR amino acid residues are obtained.

82. The humanized variable domain of claim **80** wherein no human Framework Region (FR) residue other than those set forth in the group has been substituted.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,407,213 B1
DATED : June 18, 2002
INVENTOR(S) : Carter et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 88,
Line 63, please delete "63" and insert therefor -- 79 --.

Signed and Sealed this

Third Day of December, 2002

JAMES E. ROGAN
Director of the United States Patent and Trademark Office

DATE FILED: 05/28/2010
DOCUMENT NO: 27

14042 U.S. PTO
112105

EV146601565US

PTO/SB/05 (05-05)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office. U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 C.F.R. 1.53(b))

Attorney Docket No.	CARP0001-112
First Inventor	John R. Adair et al.
Title	HUMANISED ANTIBODIES
Express Mail Label No.	EV146 601 565US

11/28/2261 U.S. PTO 112105

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

1. ☒ **Fee Transmittal Form (e.g., PTO/SB/17)** *(Submit an original and a duplicate for fee processing)*
2. ☐ **Applicant claims small entity status.** See 37 CFR 1.27.
3. ☒ **Specification** [*Total Pages* 70] Both the claims and abstract must start on a new page *(For information on the preferred arrangement, see MPEP 608.01(a))*
4. ☒ **Drawing(s)** (*35 U.S.C.113*) [*Total Sheets* 18]
5. **Oath or Declaration** [*Total Sheets* 03]
   a. ☐ Newly executed (original or copy)
   b. ☒ **Copy from a prior application (37 CFR 1.63 (d))** *(for a continuation/divisional with Box 18 completed)*
      i. ☐ DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b).
6. ☒ **Application Data Sheet.** See 37 CFR 1.76
7. ☐ **CD-ROM or CD-R** in duplicate, large table or Computer Program *(Appendix)*
   ☐ Landscape Table on CD
8. **Nucleotide and/or Amino Acid Sequence Submission** *(if applicable, items a.-c. are required)*
   a. Computer Readable Form (CRF)
      i. ☐ Computer Readable Form (CRF)
      ii. ☒ **Transfer Request (37 CFR 1.821(e))**
   b. Specification Sequence Listing on:
      i. ☐ CD-ROM or CD-R (2 copies); or
      ii. ☒ **Paper Copy**
   c. ☒ **Statements verifying identity of above copies**

ADDRESS TO: **Commissioner for Patents, P.O. Box 1450, Alexandria VA 22313-1450**

ACCOMPANYING APPLICATIONS PARTS

9. ☐ **Assignment Papers** (cover sheet & document(s)) Name of Assignee
10. ☐ **37 C.F.R. 3.73(b) Statement** *(when there is an assignee)* ☒ **Copy of Power of Attorney**
11. ☐ **English Translation Document** *(if applicable)*
12. ☐ **Information Disclosure Statement** (PTO/SB/08 or PTO-1449) ☐ Copies of foreign patent documents, publications & other information
13. ☒ **Preliminary Amendment and Request for Interference under 37 C.F.R. § 42.202**
14. ☒ **Return Receipt Postcard** (MPEP 503) *(Should be specifically itemized)*
15. ☒ **Certified Copy of Priority Document Was Received In Parent Application Serial No. 07/743,329, Filed September 17, 1991** *(if foreign priority is claimed)*
16. ☐ **Nonpublication Request** under 35 U.S.C. 122(b)(2)(B)(i). Applicant must attach form PTO/SB/35 or equivalent.
17. ☒ Other: Copy of Change of Correspondence Address – Application dated December 23, 2002, from Application Serial No. 08/846,658, Filed May 1, 1997.

18. If a CONTINUING APPLICATION, *check appropriate box, and supply the requisite information below and in the first sentence of the specification following the title, or in an Application Data Sheet under 37 CFR 1.76:*

☒ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior Application No. Serial No. 08/846,658, Filed May 1, 1997, which is a Continuation of Application Serial No. 08/303,569, Filed September 7, 1994, now U.S. Patent No. 5,859,205, Issued January 12, 1999, which is a File Wrapper Continuation of Application Serial No. 07/743,329, Filed September 17, 1991, Abandoned, which is a 35 U.S.C. 371 National Stage Application of International Application No. PCT/GB90/02017, International Filing Date of December 21, 1990, which claims priority to Great Britain Application No. 8928874.0, Filed December 21, 1989, all applications incorporated by reference herein in their entireties.

Prior application information: *Examiner* Minh Tam B. Davis *Art Unit:* 1642

19. CORRESPONDENCE ADDRESS

☒ The address associated with Customer Number 34132 *OR* ☐ Correspondence address below

Name					
Address					
City		*State*		*Zip Code*	
Country		*Telephone*		*Email address*	

Signature	Doreen Yatko Trujillo	Date	November 21, 2005
Name (Print/Type)	Doreen Yatko Trujillo	*Registration No. (Attorney/Agent)*	35,719

PTO/SB/17 (12-04v2)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

Effective on 12/08/2004.
Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).

FEE TRANSMITTAL for FY 2005

☐ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT	($) 1,000.00

Complete if Known	
Application Number	Not Yet Assigned
Filing Date	November 21, 2005
First Named Inventor	John R. Adair et al.
Examiner Name	Not Yet Assigned
Art Unit	Not Yet Assigned
Attorney Docket No.	CARP0001-112

METHOD OF PAYMENT (check all that apply)

☐ Check ☐ Credit Card ☐ Money Order ☐ None ☐ Other (please identify) : _____

☒ Deposit Account Deposit Account Number: **50-1275** Deposit Account Name: **Cozen O'Connor, P.C.**

For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

☒ Charge fee(s) indicated below ☐ Charge fee(s) indicated below, **except for the filing fee**

☒ Charge any additional fee(s) or underpayments of fee(s) Under 37 CFR 1.16 and 1.17 ☒ Credit any overpayments

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FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES

	FILING FEES		SEARCH FEES		EXAMINATION FEES		
		Small Entity		Small Entity		Small Entity	
Application Type	Fee ($)	Fee($)	Fee($)	Fee($)	Fee($)	Fee($)	Fees Paid ($)
Utility	300	150	500	250	200	100	$1,000.00
Design	200	100	100	50	130	65	____
Plant	200	100	300	150	160	80	____
Reissue	300	150	500	250	600	300	____
Provisional	200	100	0	0	0	0	____

2. EXCESS CLAIM FEES

Fee Description	Fee ($)	Small Entity Fee ($)
Each claim over 20 (including Reissues)	50	25
Each independent claim over 3 (including Reissues)	200	100
Multiple dependent claims	360	180

Total Claims	Extra Claims	Fee($)	Fee Paid ($)
02 - 20 or HP=	00	x ____	= ____

HP = highest number of total claims paid for, if greater than 20.

Indep. Claims	Extra Claims	Fee($)	Fee Paid ($)
01 - 03 or HP=	00	x ____	= ____

HP = highest number of independent claims paid for, if greater than 3.

Multiple Dependent Claims	
Fee ($)	Fee Paid ($)
____	____

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

Total Sheets	Extra Sheets	Number of each additional 50 or fraction thereof	Fee ($)	Fee Paid ($)
____	- ____ = ____/50 =	____ (round up to a whole number) x	____ =	____

4. OTHER FEE(S)

	Fees Paid ($)
Non-English Specification, $130 fee (no small entity discount)	____
Other (e.g., late filing surcharge) : ____	____

SUBMITTED BY

Signature	Doreen Yatko Trujillo	Registration No. (Attorney/Agent) 35,719	Telephone (215) 665-5593
Name (Print/Type)	Doreen Yatko Trujillo		Date November 21, 2005

PTO/SB/17 (12-04v2)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

Effective on 12/08/2004.
Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).

FEE TRANSMITTAL for FY 2005

☐ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT	($) 1,000.00

Complete if Known	
Application Number	Not Yet Assigned
Filing Date	November 21, 2005
First Named Inventor	John R. Adair et al.
Examiner Name	Not Yet Assigned
Art Unit	Not Yet Assigned
Attorney Docket No.	CARP0001-112

METHOD OF PAYMENT (check all that apply)

☐ Check ☐ Credit Card ☐ Money Order ☐ None ☐ Other (please identify) : _____

☒ Deposit Account Deposit Account Number: **50-1275** Deposit Account Name: **Cozen O'Connor, P.C.**

For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

☒ Charge fee(s) indicated below ☐ Charge fee(s) indicated below, **except for the filing fee**

☒ Charge any additional fee(s) or underpayments of fee(s) Under 37 CFR 1.16 and 1.17 ☒ Credit any overpayments

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FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES

	FILING FEES		SEARCH FEES		EXAMINATION FEES		
		Small Entity		Small Entity		Small Entity	
Application Type	Fee ($)	Fee($)	Fee($)	Fee($)	Fee($)	Fee($)	Fees Paid ($)
Utility	300	150	500	250	200	100	$1,000.00
Design	200	100	100	50	130	65	____
Plant	200	100	300	150	160	80	____
Reissue	300	150	500	250	600	300	____
Provisional	200	100	0	0	0	0	____

2. EXCESS CLAIM FEES

Fee Description	Fee ($)	Small Entity Fee ($)
Each claim over 20 (including Reissues)	50	25
Each independent claim over 3 (including Reissues)	200	100
Multiple dependent claims	360	180

Total Claims	Extra Claims	Fee($)	Fee Paid ($)
02 - 20 or HP=	00	x ____	= ____

HP = highest number of total claims paid for, if greater than 20.

Indep. Claims	Extra Claims	Fee($)	Fee Paid ($)
01 - 03 or HP=	00	x ____	= ____

HP = highest number of independent claims paid for, if greater than 3.

Multiple Dependent Claims	
Fee ($)	Fee Paid ($)
____	____

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

Total Sheets	Extra Sheets	Number of each additional 50 or fraction thereof	Fee ($)	Fee Paid ($)
____	- ____ = ____ /50 =	____ (round up to a whole number) x	____ =	____

4. OTHER FEE(S)

	Fees Paid ($)
Non-English Specification, $130 fee (no small entity discount)	____
Other (e.g., late filing surcharge) : ____	____

SUBMITTED BY

Signature	Doreen Yatko Trujillo	Registration No. (Attorney/Agent) 35,719	Telephone (215) 665-5593
Name (Print/Type)	Doreen Yatko Trujillo		Date November 21, 2005

HUMANISED ANTIBODIES

Field of the Invention

The present invention relates to humanised antibody molecules, to processes for their production using recombinant DNA technology, and to their therapeutic uses.

The term "humanised antibody molecule" is used to describe a molecule having an antigen binding site derived from an immunoglobulin from a non-human species, and remaining immunoglobulin-derived parts of the molecule being derived from a human immunoglobulin. The antigen binding site typically comprises complementarity determining regions (CDRs) which determine the binding specificity of the antibody molecule and which are carried on appropriate framework regions in the variable domains. There are 3 CDRs (CDR1, CDR2 and CDR3) in each of the heavy and light chain variable domains.

In the description, reference is made to a number of publications by number. The publications are listed in numerical order at the end of the description.

Background of the Invention

Natural immunoglobulins have been known for many years, as have the various fragments thereof, such as the Fab, $(Fab')_2$ and Fc fragments, which can be derived by enzymatic cleavage. Natural immunoglobulins comprise a generally Y-shaped molecule having an antigen-binding site towards the end of each upper arm. The remainder of the structure, and particularly the stem of the Y, mediates the effector functions associated with immunoglobulins.

Natural immunoglobulins have been used in assay, diagnosis and, to a more limited extent, therapy. However, such uses, especially in therapy, were hindered until recently by the polyclonal nature of natural immunoglobulins. A significant step towards the realisation of the potential

of immunoglobulins as therapeutic agents was the discovery of procedures for the production of monoclonal antibodies (MAbs) of defined specificity (1).

However, most MAbs are produced by hybridomas which are fusions of rodent spleen cells with rodent myeloma cells. They are therefore essentially rodent proteins. There are very few reports of the production of human MAbs.

Since most available MAbs are of rodent origin, they are naturally antigenic in humans and thus can give rise to an undesirable immune response termed the HAMA (Human Anti-Mouse Antibody) response. Therefore, the use of rodent MAbs as therapeutic agents in humans is inherently limited by the fact that the human subject will mount an immunological response to the MAb and will either remove it entirely or at least reduce its effectiveness. In practice, MAbs of rodent origin may not be used in patients for more than one or a few treatments as a HAMA response soon develops rendering the MAb ineffective as well as giving rise to undesirable reactions. For instance, OKT3 a mouse IgG2a/k MAb which recognises an antigen in the T-cell receptor-CD3 complex has been approved for use in many countries throughout the world as an immunosuppressant in the treatment of acute allograft rejection [Chatenoud et al (2) and Jeffers et al (3)]. However, in view of the rodent nature of this and other such MAbs, a significant HAMA response which may include a major anti-idiotype component, may build up on use. Clearly, it would be highly desirable to diminish or abolish this undesirable HAMA response and thus enlarge the areas of use of these very useful antibodies.

Proposals have therefore been made to render non-human MAbs less antigenic in humans. Such techniques can be generically termed "humanisation" techniques. These

techniques typically involve the use of recombinant DNA technology to manipulate DNA sequences encoding the polypeptide chains of the antibody molecule.

Early methods for humanising MAbs involved production of chimeric antibodies in which an antigen binding site comprising the complete variable domains of one antibody is linked to constant domains derived from another antibody. Methods for carrying out such chimerisation procedures are described in EP0120694 (Celltech Limited), EP0125023 (Genentech Inc. and City of Hope), EP-A-0 171496 (Res. Dev. Corp. Japan), EP-A-0 173 494 (Stanford University), and WO 86/01533 (Celltech Limited). This latter Celltech application (WO 86/01533) discloses a process for preparing an antibody molecule having the variable domains from a mouse MAb and the constant domains from a human immunoglobulin. Such humanised chimeric antibodies, however, still contain a significant proportion of non-human amino acid sequence, i.e. the complete non-human variable domains, and thus may still elicit some HAMA response, particularly if administered over a prolonged period [Begent et al (ref. 4)].

In an alternative approach, described in EP-A-0239400 (Winter), the complementarity determining regions (CDRs) of a mouse MAb have been grafted onto the framework regions of the variable domains of a human immunoglobulin by site directed mutagenesis using long oligonucleotides. The present invention relates to humanised antibody molecules prepared according to this alternative approach, i.e. CDR-grafted humanised antibody molecules. Such CDR-grafted humanised antibodies are much less likely to give rise to a HAMA response than humanised chimeric antibodies in view of the much lower proportion of non-human amino acid sequence which they contain.

The earliest work on humanising MAbs by CDR-grafting was carried out on MAbs recognising synthetic antigens, such as the NP or NIP antigens. However, examples in which a mouse MAb recognising lysozyme and a rat MAb recognising an antigen on human T-cells were humanised by CDR-grafting have been described by Verhoeyen et al (5) and Riechmann et al (6) respectively. The preparation of CDR-grafted antibody to the antigen on human T cells is also described in WO 89/07452 (Medical Research Council).

In Riechmann et al/Medical Research Council it was found that transfer of the CDR regions alone [as defined by Kabat refs. (7) and (8)] was not sufficient to provide satisfactory antigen binding activity in the CDR-grafted product. Riechmann et al found that it was necessary to convert a serine residue at position 27 of the human sequence to the corresponding rat phenylalanine residue to obtain a CDR-grafted product having improved antigen binding activity. This residue at position 27 of the heavy chain is within the structural loop adjacent to CDR1. A further construct which additionally contained a human serine to rat tyrosine change at position 30 of the heavy chain did not have a significantly altered binding activity over the humanised antibody with the serine to phenylalanine change at position 27 alone. These results indicate that changes to residues of the human sequence outside the CDR regions, in particular in the structural loop adjacent to CDR1, may be necessary to obtain effective antigen binding activity for CDR-grafted antibodies which recognise more complex antigens. Even so the binding affinity of the best CDR-grafted antibodies obtained was still significantly less than the original MAb.

Very recently Queen et al (9) have described the preparation of a humanised antibody that binds to the

interleukin 2 receptor, by combining the CDRs of a murine MAb (anti-Tac) with human immunoglobulin framework and constant regions. The human framework regions were chosen to maximise homology with the anti-Tac MAb sequence. In addition computer modelling was used to identify framework amino acid residues which were likely to interact with the CDRs or antigen, and mouse amino acids were used at these positions in the humanised antibody.

In WO 90/07861 Queen et al propose four criteria for designing humanised immunoglobulins. The first criterion is to use as the human acceptor the framework from a particular human immunoglobulin that is unusually homologous to the non-human donor immunoglobulin to be humanised, or to use a consensus framework from many human antibodies. The second criterion is to use the donor amino acid rather than the acceptor if the human acceptor residue is unusual and the donor residue is typical for human sequences at a specific residue of the framework. The third criterion is to use the donor framework amino acid residue rather than the acceptor at positions immediately adjacent to the CDRs. The fourth criterion is to use the donor amino acid residue at framework positions at which the amino acid is predicted to have a side chain atom within about 3 Å of the CDRs in a three-dimensional immunoglobulin model and to be capable of interacting with the antigen or with the CDRs of the humanised immunoglobulin. It is proposed that criteria two, three or four may be applied in addition or alternatively to criterion one, and may be applied singly or in any combination.

WO 90/07861 describes in detail the preparation of a single CDR-grafted humanised antibody, a humanised antibody having specificity for the p55 Tac protein of the

IL-2 receptor. The combination of all four criteria, as above, were employed in designing this humanised antibody, the variable region frameworks of the human antibody Eu (7) being used as acceptor. In the resultant humanised antibody the donor CDRs were as defined by Kabat et al (7 and 8) and in addition the mouse donor residues were used in place of the human acceptor residues, at positions 27, 30, 48, 66, 67, 89, 91, 94, 103, 104, 105 and 107 in the heavy chain and at positions 48, 60 and 63 in the light chain, of the variable region frameworks. The humanised anti-Tac antibody obtained is reported to have an affinity for p55 of 3×10^9 M^{-1}, about one-third of that of the murine MAb.

We have further investigated the preparation of CDR-grafted humanised antibody molecules and have identified a hierarchy of positions within the framework of the variable regions (i.e. outside both the Kabat CDRs and structural loops of the variable regions) at which the amino acid identities of the residues are important for obtaining CDR-grafted products with satisfactory binding affinity. This has enabled us to establish a protocol for obtaining satisfactory CDR-grafted products which may be applied very widely irrespective of the level of homology between the donor immunoglobulin and acceptor framework. The set of residues which we have identified as being of critical importance does not coincide with the residues identified by Queen et al (9).

Summary of the Invention

Accordingly, in a first aspect the invention provides a CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

In preferred embodiments, the heavy chain framework comprises donor residues at positions 23, 24, 49, 71, 73 and 78 or at positions 23, 24 and 49. The residues at positions 71, 73 and 78 of the heavy chain framework are preferably either all acceptor or all donor residues.

In particularly preferred embodiments the heavy chain framework additionally comprises donor residues at one, some or all of positions 6, 37, 48 and 94. Also it is particularly preferred that residues at positions of the heavy chain framework which are commonly conserved across species, i.e. positions 2, 4, 25, 36, 39, 47, 93, 103, 104, 106 and 107, if not conserved between donor and acceptor, additionally comprise donor residues. Most preferably the heavy chain framework additionally comprises donor residues at positions 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

In addition the heavy chain framework optionally comprises donor residues at one, some or all of positions:
1 and 3,
72 and 76,
69 (if 48 is different between donor and acceptor),
38 and 46 (if 48 is the donor residue),
80 and 20 (if 69 is the donor residue),
67,
82 and 18 (if 67 is the donor residue),
91,
88, and
any one or more of 9, 11, 41, 87, 108, 110 and 112.

In the first and other aspects of the present invention reference is made to CDR-grafted antibody products comprising acceptor framework and donor antigen binding regions. It will be appreciated that the invention is widely applicable to the CDR-grafting of antibodies in

general. Thus, the donor and acceptor antibodies may be derived from animals of the same species and even same antibody class or sub-class. More usually, however, the donor and acceptor antibodies are derived from animals of different species. Typically the donor antibody is a non-human antibody, such as a rodent MAb, and the acceptor antibody is a human antibody.

In the first and other aspects of the present invention, the donor antigen binding region typically comprises at least one CDR from the donor antibody. Usually the donor antigen binding region comprises at least two and preferably all three CDRs of each of the heavy chain and/or light chain variable regions. The CDRs may comprise the Kabat CDRs, the structural loop CDRs or a composite of the Kabat and structural loop CDRs and any combination of any of these. Preferably, the antigen binding regions of the CDR-grafted heavy chain variable domain comprise CDRs corresponding to the Kabat CDRs at CDR2 (residues 50-65) and CDR3 (residues 95-100) and a composite of the Kabat and structural loop CDRs at CDR1 (residues 26-35).

The residue designations given above and elsewhere in the present application are numbered according to the Kabat numbering [refs. (7) and (8)]. Thus the residue designations do not always correspond directly with the linear numbering of the amino acid residues. The actual linear amino acid sequence may contain fewer or additional amino acids than in the strict Kabat numbering corresponding to a shortening of, or insertion into, a structural component, whether framework or CDR, of the basic variable domain structure. For example, the heavy chain variable region of the anti-Tac antibody described by Queen et al (9) contains a single amino acid insert (residue 52a) after residue 52 of CDR2 and a three amino

acid insert (residues 82a, 82b and 82c) after framework residue 82, in the Kabat numbering. The correct Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

The invention also provides in a second aspect a CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 1 and/or 3 and 46 and/or 47. Preferably the CDR grafted light chain of the second aspect comprises donor residues at positions 46 and/or 47.

The invention also provides in a third aspect a CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, 58 and 71.

In a preferred embodiment of the third aspect, the framework comprises donor residues at all of positions 46, 48, 58 and 71.

In particularly preferred embodiments of the second and third aspects, the framework additionally comprises donor residues at positions 36, 44, 47, 85 and 87. Similarly positions of the light chain framework which are commonly conserved across species, i.e. positions 2, 4, 6, 35, 49, 62, 64-69, 98, 99, 101 and 102, if not conserved between donor and acceptor, additionally comprise donor residues. Most preferably the light chain framework additionally comprises donor residues at positions 2, 4, 6, 35, 36, 38, 44, 47, 49, 62, 64-69, 85, 87, 98, 99, 101 and 102.

In addition the framework of the second or third aspects optionally comprises donor residues at one, some or all of positions:
1 and 3,
63,
60 (if 60 and 54 are able to form at potential saltbridge),
70 (if 70 and 24 are able to form a potential saltbridge),
73 and 21 (if 47 is different between donor and acceptor),
37 and 45 (if 47 is different between donor and acceptor),
and
any one or more of 10, 12, 40, 80, 103 and 105.

Preferably, the antigen binding regions of the CDR-grafted light chain variable domain comprise CDRs corresponding to the Kabat CDRs at CDR1 (residue 24-34), CDR2 (residues 50-56) and CDR3 (residues 89-97).

The invention further provides in a fourth aspect a CDR-grafted antibody molecule comprising at least one CDR-grafted heavy chain and at least one CDR-grafted light chain according to the first and second or first and third aspects of the invention.

The humanised antibody molecules and chains of the present invention may comprise: a complete antibody molecule, having full length heavy and light chains; a fragment thereof, such as a Fab, $(Fab')_2$ or FV fragment; a light chain or heavy chain monomer or dimer; or a single chain antibody, e.g. a single chain FV in which heavy and light chain variable regions are joined by a peptide linker; or any other CDR-grafted molecule with the same specificity as the original donor antibody. Similarly the CDR-grafted heavy and light chain variable region may be combined with other antibody domains as appropriate.

Also the heavy or light chains or humanised antibody molecules of the present invention may have attached to them an effector or reporter molecule. For instance, it may have a macrocycle, for chelating a heavy metal atom, or a toxin, such as ricin, attached to it by a covalent bridging structure. Alternatively, the procedures of recombinant DNA technology may be used to produce an immunoglobulin molecule in which the Fc fragment or CH3 domain of a complete immunoglobulin molecule has been replaced by, or has attached thereto by peptide linkage, a functional non-immunoglobulin protein, such as an enzyme or toxin molecule.

Any appropriate acceptor variable region framework sequences may be used having regard to class/type of the donor antibody from which the antigen binding regions are derived. Preferably, the type of acceptor framework used is of the same/similar class/type as the donor antibody. Conveniently, the framework may be chosen to maximise/optimise homology with the donor antibody sequence particularly at positions close or adjacent to the CDRs. However, a high level of homology between donor and acceptor sequences is not important for application of the present invention. The present invention identifies a hierarchy of framework residue positions at which donor residues may be important or desirable for obtaining a CDR-grafted antibody product having satisfactory binding properties. The CDR-grafted products usually have binding affinities of at least 10^5 M^{-1}, preferably at least about 10^8 M^{-1}, or especially in the range 10^8-10^{12} M^{-1}. In principle, the present invention is applicable to any combination of donor and acceptor antibodies irrespective of the level of homology between their sequences. A protocol for applying the invention to any particular donor-acceptor antibody pair is given hereinafter. Examples of human frameworks which may be

used are KOL, NEWM, REI, EU, LAY and POM (refs. 4 and 5) and the like; for instance KOL and NEWM for the heavy chain and REI for the light chain and EU, LAY and POM for both the heavy chain and the light chain.

Also the constant region domains of the products of the invention may be selected having regard to the proposed function of the antibody in particular the effector functions which may be required. For example, the constant region domains may be human IgA, IgE, IgG or IgM domains. In particular, IgG human constant region domains may be used, especially of the IgG1 and IgG3 isotypes, when the humanised antibody molecule is intended for therapeutic uses, and antibody effector functions are required. Alternatively, IgG2 and IgG4 isotypes may be used when the humanised antibody molecule is intended for therapeutic purposes and antibody effector functions are not required, e.g. for simple blocking of lymphokine activity.

However, the remainder of the antibody molecules need not comprise only protein sequences from immunoglobulins. For instance, a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain is fused to a DNA sequence encoding the amino acid sequence of a functional polypeptide such as an effector or reporter molecule.

Preferably the CDR-grafted antibody heavy and light chain and antibody molecule products are produced by recombinant DNA technology.

Thus in further aspects the invention also includes DNA sequences coding for the CDR-grafted heavy and light chains, cloning and expression vectors containing the DNA sequences, host cells transformed with the DNA sequences

and processes for producing the CDR-grafted chains and antibody molecules comprising expressing the DNA sequences in the transformed host cells.

The general methods by which the vectors may be constructed, transfection methods and culture methods are well known per se and form no part of the invention. Such methods are shown, for instance, in references 10 and 11.

The DNA sequences which encode the donor amino acid sequence may be obtained by methods well known in the art. For example the donor coding sequences may be obtained by genomic cloning, or cDNA cloning from suitable hybridoma cell lines. Positive clones may be screened using appropriate probes for the heavy and light chain genes in question. Also PCR cloning may be used.

DNA coding for acceptor, e.g. human acceptor, sequences may be obtained in any appropriate way. For example DNA sequences coding for preferred human acceptor frameworks such as KOL, REI, EU and NEWM, are widely available to workers in the art.

The standard techniques of molecular biology may be used to prepare DNA sequences coding for the CDR-grafted products. Desired DNA sequences may be synthesised completely or in part using oligonucleotide synthesis techniques. Site-directed mutagenesis and polymerase chain reaction (PCR) techniques may be used as appropriate. For example oligonucleotide directed synthesis as described by Jones et al (ref. 20) may be used. Also oligonucleotide directed mutagenesis of a pre-exising variable region as, for example, described by Verhoeyen et al (ref. 5) or Riechmann et al (ref. 6) may be used. Also enzymatic filling in of gapped

oligonucleotides using T_4 DNA polymerase as, for example, described by Queen et al (ref. 9) may be used.

Any suitable host cell/vector system may be used for expression of the DNA sequences coding for the CDR-grafted heavy and light chains. Bacterial e.g. E. coli, and other microbial systems may be used, in particular for expression of antibody fragments such as FAb and $(Fab')_2$ fragments, and especially FV fragments and single chain antibody fragments e.g. single chain FVs. Eucaryotic e.g. mammalian host cell expression systems may be used for production of larger CDR-grafted antibody products, including complete antibody molecules. Suitable mammalian host cells include CHO cells and myeloma or hybridoma cell lines.

Thus, in a further aspect the present invention provides a process for producing a CDR-grafted antibody product comprising:

(a) producing in an expression vector an operon having a DNA sequence which encodes an antibody heavy chain according to the first aspect of the invention;

and/or

(b) producing in an expression vector an operon having a DNA sequence which encodes a complementary antibody light chain according to the second or third aspect of the invention;

(c) transfecting a host cell with the or each vector; and

(d) culturing the transfected cell line to produce the CDR-grafted antibody product.

The CDR-grafted product may comprise only heavy or light chain derived polypeptide, in which case only a heavy chain or light chain polypeptide coding sequence is used to transfect the host cells.
For production of products comprising both heavy and light chains, the cell line may be transfected with two vectors, the first vector may contain an operon encoding a light chain-derived polypeptide and the second vector containing an operon encoding a heavy chain-derived polypeptide. Preferably, the vectors are identical, except in so far as the coding sequences and selectable markers are concerned, so as to ensure as far as possible that each polypeptide chain is equally expressed. Alternatively, a single vector may be used, the vector including the sequences encoding both light chain- and heavy chain-derived polypeptides.

The DNA in the coding sequences for the light and heavy chains may comprise cDNA or genomic DNA or both. However, it is preferred that the DNA sequence encoding the heavy or light chain comprises at least partially, genomic DNA, preferably a fusion of cDNA and genomic DNA.

The present invention is applicable to antibodies of any appropriate specificity. Advantageously, however, the invention may be applied to the humanisation of non-human antibodies which are used for <u>in vivo</u> therapy or diagnosis. Thus the antibodies may be site-specific antibodies such as tumour-specific or cell surface-specific antibodies, suitable for use in <u>in vivo</u> therapy or diagnosis, e.g. tumour imaging. Examples of cell surface-specific antibodies are anti-T cell antibodies, such as anti-CD3, and CD4 and adhesion molecules, such as CR3, ICAM and ELAM. The antibodies may have specificity for interleukins (including lymphokines, growth factors and stimulating factors), hormones and other biologically active compounds, and receptors for any of these. For

example, the antibodies may have specificity for any of the following: Interferons α, β, γ or δ, IL1, IL2, IL3, or IL4, etc., TNF, GCSF, GMCSF, EPO, hGH, or insulin, etc.

The the present invention also includes therapeutic and diagnostic compositions comprising the CDR-grafted products of the invention and uses of such compositions in therapy and diagnosis.

Accordingly in a further aspect the invention provides a therapeutic or diagnostic composition comprising a CDR-grafted antibody heavy or light chain or molecule according to previous aspects of the invention in combination with a pharmaceutically acceptable carrier, diluent or excipient.

Accordingly also the invention provides a method of therapy or diagnosis comprising administering an effective amount of a CDR-grafted antibody heavy or light chain or molecule according to previous aspects of the invention to a human or animal subject.

A preferred protocol for obtaining CDR-grafted antibody heavy and light chains in accordance with the present invention is set out below together with the rationale by which we have derived this protocol. This protocol and rationale are given without prejudice to the generality of the invention as hereinbefore described and defined.

Protocol

It is first of all necessary to sequence the DNA coding for the heavy and light chain variable regions of the donor antibody, to determine their amino acid sequences. It is also necessary to choose appropriate acceptor heavy and light chain variable regions, of known amino acid sequence. The CDR-grafted chain is then designed

starting from the basis of the acceptor sequence. It will be appreciated that in some cases the donor and acceptor amino acid residues may be identical at a particular position and thus no change of acceptor framework residue is required.

1. As a first step donor residues are substituted for acceptor residues in the CDRs. For this purpose the CDRs are preferably defined as follows:

Heavy chain	-	CDR1:	residues 26-35
	-	CDR2:	residues 50-65
	-	CDR3:	residues 95-102
Light chain	-	CDR1:	residues 24-34
	-	CDR2:	residues 50-56
	-	CDR3:	residues 89-97

The positions at which donor residues are to be substituted for acceptor in the framework are then chosen as follows, first of all with respect to the heavy chain and subsequently with respect to the light chain.

2. Heavy Chain

2.1 Choose donor residues at all of positions 23, 24, 49, 71, 73 and 78 of the heavy chain or all of positions 23, 24 and 49 (71, 73 and 78 are always either all donor or all acceptor).

2.2 Check that the following have the same amino acid in donor and acceptor sequences, and if not preferably choose the donor: 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

2.3 To further optimise affinity consider choosing donor residues at one, some or any of:

i. 1, 3
ii. 72, 76
iii. If 48 is different between donor and acceptor sequences, consider 69
iv. If at 48 the donor residue is chosen, consider 38 and 46
v. If at 69 the donor residue is chosen, consider 80 and then 20
vi. 67
vii. If at 67 the donor residue is chosen, consider 82 and then 18
viii. 91
ix. 88
x. 9, 11, 41, 87, 108, 110, 112

3. Light Chain

3.1 Choose donor at 46, 48, 58 and 71

3.2 Check that the following have the same amino acid in donor and acceptor sequences, if not preferably choose donor:

2, 4, 6, 35, 38, 44, 47, 49, 62, 64-69 inclusive, 85, 87, 98, 99, 101 and 102

3.3 To further optimise affinity consider choosing donor residues at one, some or any of:

i. 1, 3
ii. 63

iii. 60, if 60 and 54 are able to form potential saltbridge
iv. 70, if 70 and 24 are able to form potential saltbridge
v. 73, and 21 if 47 is different between donor and acceptor
vi. 37, and 45 if 47 is different between donor and acceptor
vii. 10, 12, 40, 80, 103, 105

Rationale

In order to transfer the binding site of an antibody into a different acceptor framework, a number of factors need to be considered.

1. The extent of the CDRs

The CDRs (Complementary Determining Regions) were defined by Wu and Kabat (refs. 4 and 5) on the basis of an analysis of the variability of different regions of antibody variable regions. Three regions per domain were recognised. In the light chain the sequences are 24-34, 50-56, 89-97 (numbering according to Kabat (ref. 4), Eu Index) inclusive and in the heavy chain the sequences are 31-35, 50-65 and 95-102 inclusive.

When antibody structures became available it became apparent that these CDR regions corresponded in the main to loop regions which extended from the β barrel framework of the light and heavy variable domains. For H1 there was a discrepancy in that the loop was from 26 to 32 inclusive and for H2 the loop was 52 to 56 and for L2 from 50 to 53. However, with the exception of H1 the CDR regions encompassed the loop regions and extended into the β strand

frameworks. In H1 residue 26 tends to be a serine and 27 a phenylalanine or tyrosine, residue 29 is a phenylalanine in most cases. Residues 28 and 30 which are surface residues exposed to solvent might be involved in antigen-binding. A prudent definition of the H1 CDR therefore would include residues 26-35 to include both the loop region and the hypervariable residues 33-35.

It is of interest to note the example of Riechmann et al (ref. 3), who used the residue 31-35 choice for CDR-H1. In order to produce efficient antigen binding, residue 27 also needed to be recruited from the donor (rat) antibody.

2. Non-CDR residues which contribute to antigen binding

By examination of available X-ray structures we have identified a number of residues which may have an effect on net antigen binding and which can be demonstrated by experiment. These residues can be sub-divided into a number of groups.

2.1 Surface residues near CDR [all numbering as in Kabat et al (ref. 7)].

2.1.1. Heavy Chain - Key residues are 23, 71 and 73. Other residues which may contribute to a lesser extent are 1, 3 and 76. Finally 25 is usually conserved but the murine residue should be used if there is a difference.

2.1.2 Light Chain - Many residues close to the CDRs, e.g. 63, 65, 67 and 69 are conserved. If conserved none of the surface residues in the light chain are likely to have a major effect. However, if the murine residue at these positions

is unusual, then it would be of benefit to analyse the likely contribution more closely. Other residues which may also contribute to binding are 1 and 3, and also 60 and 70 if the residues at these positions and at 54 and 24 respectively are potentially able to form a salt bridge i.e. 60 + 54; 70 + 24.

2.2 Packing residues near the CDRs.

2.2.1. Heavy Chain - Key residues are 24, 49 and 78. Other key residues would be 36 if not a tryptophan, 94 if not an arginine, 104 and 106 if not glycines and 107 if not a threonine. Residues which may make a further contribution to stable packing of the heavy chain and hence improved affinity are 2, 4, 6, 38, 46, 67 and 69. 67 packs against the CDR residue 63 and this pair could be either both mouse or both human. Finally, residues which contribute to packing in this region but from a longer range are 18, 20, 80, 82 and 86. 82 packs against 67 and in turn 18 packs against 82. 80 packs against 69 and in turn 20 packs against 80. 86 forms an H bond network with 38 and 46. Many of the mouse-human differences appear minor e.g. Leu-Ile, but could have an minor impact on correct packing which could translate into altered positioning of the CDRs.

2.2.2. Light Chain - Key residues are 48, 58 and 71. Other key residues would be 6 if not glutamine, 35 if not tryptophan, 62 if not phenylalanine or tryosine, 64, 66, 68, 99 and 101 if not glycines and 102 if not a threonine. Residues which make a further contribution are 2, 4, 37, 45 and 47. Finally residues 73 and 21 and 19 may make long distance packing contributions of a minor nature.

2.3. Residues at the variable domain interface between heavy and light chains - In both the light and heavy chains most of the non-CDR interface residues are conserved. If a conserved residue is replaced by a residue of different character, e.g. size or charge, it should be considered for retention as the murine residue.

2.3.1. Heavy Chain - Residues which need to be considered are 37 if the residue is not a valine but is of larger side chain volume or has a charge or polarity. Other residues are 39 if not a glutamine, 45 if not a leucine, 47 if not a tryptophan, 91 if not a phenylalanine or tyrosine, 93 if not an alanine and 103 if not a tryptophan. Residue 89 is also at the interface but is not in a position where the side chain could be of great impact.

2.3.2. Light Chain - Residues which need to be considered are 36, if not a tyrosine, 38 if not a glutamine, 44 if not a proline, 46, 49 if not a tyrosine, residue 85, residue 87 if not a tyrosine and 98 if not a phenylalanine.

2.4. Variable-Constant region interface - The elbow angle between variable and constant regions may be affected by alterations in packing of key residues in the variable region against the constant region which may affect the position of V_L and V_H with respect to one another. Therefore it is worth noting the residues likely to be in contact with the constant region. In the heavy chain the surface residues potentially in contact with the variable region are conserved between mouse and human antibodies therefore the variable region contact residues may influence the V-C interaction. In the light chain the amino acids found at a number of the constant

region contact points vary, and the V & C regions are not in such close proximity as the heavy chain. Therefore the influences of the light chain V-C interface may be minor.

2.4.1. Heavy Chain - Contact residues are 7, 11, 41, 87, 108, 110, 112.

2.4.2. Light Chain - In the light chain potentially contacting residues are 10, 12, 40, 80, 83, 103 and 105.

The above analysis coupled with our considerable practical experimental experience in the CDR-grafting of a number of different antibodies have lead us to the protocol given above.

The present invention is now described, by way of example only, with reference to the accompanying Figures 1 - 13.

Brief Description of the Figures

Figure 1 shows DNA and amino acid sequences of the OKT3 light chain;

Figure 2 shows DNA and amino acid sequences of the OKT3 heavy chain;

Figure 3 shows the alignment of the OKT3 light variable region amino acid sequence with that of the light variable region of the human antibody REI;

Figure 4 shows the alignment of the OKT3 heavy variable region amino acid sequence with that of the heavy variable region of the human antibody KOL;

Figure 5 shows the heavy variable region amino acid sequences of OKT3, KOL and various corresponding CDR grafts;

Figure 6 shows the light variable region amino acid sequences of OKT3, REI and various corresponding CDR grafts;

Figure 7 shows a graph of binding assay results for various grafted OKT3 antibodies'

Figure 8 shows a graph of blocking assay results for various grafted OKT3 antibodies;

Figure 9 shows a similar graph of blocking assay results;

Figure 10 shows similar graphs for both binding assay and blocking assay results;

Figure 11 shows further similar graphs for both binding assay and blocking assay results;

Figure 12 shows a graph of competition assay results for a minimally grafted OKT3 antibody compared with the OKT3 murine reference standard, and

Figure 13 shows a similar graph of competition assay results comparing a fully grafted OKT3 antibody with the murine reference standard.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

EXAMPLE 1

CDR-GRAFTING OF OKT3

MATERIAL AND METHODS

1. INCOMING CELLS

Hybridoma cells producing antibody OKT3 were provided by Ortho (seedlot 4882.1) and were grown up in antibiotic free Dulbecco's Modified Eagles Medium (DMEM) supplemented with glutamine and 5% foetal calf serum, and divided to provide both an overgrown supernatant for evaluation and cells for extraction of RNA. The overgrown supernatant was shown to contain 250 ug/mL murine IgG2a/kappa antibody. The supernatant was negative for murine lambda light chain and IgG1, IgG2b, IgG3, IgA and IgM heavy chain. 20mL of supernatant was assayed to confirm that the antibody present was OKT3.

2. MOLECULAR BIOLOGY PROCEDURES

Basic molecular biology procedures were as described in Maniatis et al (ref. 9) with, in some cases, minor modifications. DNA sequencing was performed as described in Sanger et al (ref. 11) and the Amersham International Plc sequencing handbook. Site directed mutagenesis was as described in Kramer et al (ref. 12) and the Anglian Biotechnology Ltd. handbook. COS cell expression and metabolic labelling studies were as described in Whittle et al (ref. 13)

3. RESEARCH ASSAYS

3.1. ASSEMBLY ASSAYS

Assembly assays were performed on supernatants from transfected COS cells to determine the amount of intact IgG present.

3.1.1. COS CELLS TRANSFECTED WITH MOUSE OKT3 GENES

The assembly assay for intact mouse IgG in COS cell supernatants was an ELISA with the following format:

96 well microtitre plates were coated with F(ab')2 goat anti-mouse IgG Fc. The plates were washed in water and samples added for 1 hour at room temperature. The plates were washed and F(ab')2 goat anti-mouse IgG F(ab')2 (HRPO conjugated) was then added. Substrate was added to reveal the reaction. UPC10, a mouse IgG2a myeloma, was used as a standard.

3.1.2. COS AND CHO CELLS TRANSFECTED WITH CHIMERIC OR CDR-GRAFTED OKT3 GENES

The assembly assay for chimeric or CDR-grafted antibody in COS cell supernatants was an ELISA with the following format:

96 well microtitre plates were coated with F(ab')2 goat anti-human IgG Fc. The plates were washed and samples added and incubated for 1 hour at room temperature. The plates were washed and monoclonal mouse anti-human kappa chain was added for 1 hour at room temperature.

The plates were washed and F(ab')2 goat anti-mouse IgG Fc (HRPO conjugated) was added. Enzyme substrate was added to reveal the reaction. Chimeric B72.3 (IgG4) (ref. 13) was used as a standard. The use of a monoclonal anti-kappa chain in this assay allows grafted antibodies to be read from the chimeric standard.

3.2. ASSAY FOR ANTIGEN BINDING ACTIVITY

Material from COS cell supernatants was assayed for OKT3 antigen binding activity onto CD3 positive cells in a direct assay. The procedure was as follows:

HUT 78 cells (human T cell line, CD3 positive) were maintained in culture. Monolayers of HUT 78 cells were prepared onto 96 well ELISA plates using poly-L-lysine and glutaraldehyde. Samples were added to the monolayers for 1 hour at room temperature.

The plates were washed gently using PBS. F(ab')2 goat anti-human IgG Fc (HRPO conjugated) or F(ab')2 goat anti-mouse IgG Fc (HRPO conjugated) was added as appropriate for humanised or mouse samples. Substrate was added to reveal the reaction.

The negative control for the cell-based assay was chimeric B72.3. The positive control was mouse Orthomune OKT3 or chimeric OKT3, when available. This cell-based assay was difficult to perform, and an alternative assay was developed for CDR-grafted OKT3 which was more sensitive and easier to carry out.

In this system CDR-grafted OKT3 produced by COS cells was tested for its ability to bind to the CD3-positive HPB-ALL (human peripheral blood acute lymphocytic leukemia) cell line. It was also tested for its ability to block the binding of murine OKT3 to these cells. Binding was measured by the following procedure: HPB-ALL cells were harvested from tissue culture. Cells were incubated at 4°C for 1 hour with various dilutions of test antibody, positive control antibody, or negative control antibody. The cells were washed once and incubated at 4°C for 1 hour with an FITC-labelled goat anti-human IgG (Fc-

specific, mouse absorbed). The cells were washed twice and analysed by cytofluorography. Chimeric OKT3 was used as a positive control for direct binding. Cells incubated with mock- transfected COS cell supernatant, followed by the FITC-labelled goat anti-human IgG, provided the negative control. To test the ability of CDR-grafted OKT3 to block murine OKT3 binding, the HPB-ALL cells were incubated at 4°C for 1 hour with various dilutions of test antibody or control antibody. A fixed saturating amount of FITC OKT3 was added. The samples were incubated for 1 hour at 4°C, washed twice and analysed by cytofluorography. FITC-labelled OKT3 was used as a positive control to determine maximum binding. Unlabelled murine OKT3 served as a reference standard for blocking. Negative controls were unstained cells with or without mock-transfected cell supernatant. The ability of the CDR-grafted OKT3 light chain to bind CD3-positive cells and block the binding of murine OKT3 was initially tested in combination with the chimeric OKT3 heavy chain. The chimeric OKT3 heavy chain is composed of the murine OKT3 variable region and the human IgG4 constant region. The chimeric heavy chain gene is expressed in the same expression vector used for the CDR-grafted genes. The CDR-grafted light chain expression vector and the chimeric heavy chain expression vector were co-transfected into COS cells. The fully chimeric OKT3 antibody (chimeric light chain and chimeric heavy chain) was found to be fully capable of binding to CD3 positive cells and blocking the binding of murine OKT3 to these cells.

3.3 DETERMINATION OF RELATIVE BINDING AFFINITY

The relative binding affinities of CDR-grafted

anti-CD3 monoclonal antibodies were determined by competition binding (ref. 6) using the HPB-ALL human T cell line as a source of CD3 antigen, and fluorescein-conjugated murine OKT3 (Fl-OKT3) of known binding affinity as a tracer antibody. The binding affinity of Fl-OKT3 tracer antibody was determined by a direct binding assay in which increasing amounts of Fl-OKT3 were incubated with HPB-ALL ($5x10^5$) in PBS with 5% foetal calf serum for 60 min. at 4°C. Cells were washed, and the fluorescence intensity was determined on a FACScan flow cytometer calibrated with quantitative microbead standards (Flow Cytometry Standards, Research Triangle Park, NC). Fluorescence intensity per antibody molecule (F/P ratio) was determined by using microbeads which have a predetermined number of mouse IgG antibody binding sites (Simply Cellular beads, Flow Cytometry Standards). F/P equals the fluorescence intensity of beads saturated with Fl-OKT3 divided by the number of binding sites per bead. The amount of bound and free Fl-OKT3 was calculated from the mean fluorescence intensity per cell, and the ratio of bound/free was plotted against the number of moles of antibody bound. A linear fit was used to determine the affinity of binding (absolute value of the slope).

For competitive binding, increasing amounts of competitor antibody were added to a sub-saturating dose of Fl-OKT3 and incubated with $5x10^5$ HPB-ALL in 200 Ml of PBS with 5% foetal calf serum, for 60 min at 4°C. The fluorescence intensities of the cells were measured on a FACScan flow cytometer calibrated with quantitative microbead standards. The concentrations of bound and free Fl-OKT3 were calculated. The affinities of competing anti-

bodies were calculated from the equation [X]-[ORT3] = (1/Kx) - (1/Ka), where Ka is the affinity of murine ORT3, Kx is the affinity of competitor X, [ ] is the concentration of competitor antibody at which bound/free binding is R/2, and R is the maximal bound/free binding.

4. cDNA LIBRARY CONSTRUCTION

4.1. mRNA PREPARATION AND cDNA SYNTHESIS

ORT3 producing cells were grown as described above and 1.2 x 10^9 cells harvested and mRNA extracted using the guanidinium/LiCl extraction procedure. cDNA was prepared by priming from Oligo-dT to generate full length cDNA. The cDNA was methylated and EcoR1 linkers added for cloning.

4.2. LIBRARY CONSTRUCTION

The cDNA library was ligated to pSP65 vector DNA which had been EcoR1 cut and the 5′ phosphate groups removed by calf intestinal phosphatase (EcoR1/CIP). The ligation was used to transform high transformation efficiency Escherichia coli (E.coli) HB101. A cDNA library was prepared. 3600 colonies were screened for the light chain and 10000 colonies were screened for the heavy chain.

5. SCREENING

E.coli colonies positive for either heavy or light chain probes were identified by oligonucleotide screening using the oligonucleotides:
5′ TCCAGATGTTAACTGCTCAC for the light chain, which is complementary to a sequence in the mouse kappa constant region, and 5′ CAGGGGCCAGTGGATGGATAGAC for the heavy chain which is complementary to a sequence in the mouse IgG2a constant CH1 domain region. 12 light chain and 9 heavy chain clones

were identified and taken for second round screening. Positive clones from the second round of screening were grown up and DNA prepared. The sizes of the gene inserts were estimated by gel electrophoresis and inserts of a size capable of containing a full length cDNA were subcloned into M13 for DNA sequencing.

6. DNA SEQUENCING

Clones representing four size classes for both heavy and light chains were obtained in M13. DNA sequence for the 5′ untranslated regions, signal sequences, variable regions and 3′ untranslated regions of full length cDNAs [Figures 1(a) and 2(a)] were obtained and the corresponding amino acid sequences predicted [(Figures 1(b) and 2(b)]. In Figure 1(a) the untranslated DNA regions are shown in uppercase, and in both Figures 1 and 2 the signal sequences are underlined.

7. CONSTRUCTION OF cDNA EXPRESSION VECTORS

Celltech expression vectors are based on the plasmid pEE6hCMV (ref. 14). A polylinker for the insertion of genes to be expressed has been introduced after the major immediate early promoter/enhancer of the human Cytomegalovirus (hCMV). Marker genes for selection of the plasmid in transfected eukaryotic cells can be inserted as BamH1 cassettes in the unique BamH1 site of pEE6 hCMV; for instance, the neo marker to provide pEE6 hCMV neo. It is usual practice to insert the neo and gpt markers prior to insertion of the gene of interest, whereas the GS marker is inserted last because of the presence of internal EcoR1 sites in the cassette.

The selectable markers are expressed from the SV40 late promoter which also provides an origin of replication so that the vectors can be used for expression in the COS cell transient expression system.
The mouse sequences were excised from the M13 based vectors described above as EcoR1 fragments and cloned into either pEE6-hCMV-neo for the heavy chain and into EE6-hCMV-gpt for the light chain to yield vectors pJA136 and pJA135 respectively.

8. EXPRESSION OF cDNAS IN COS CELLS

Plasmids pJA135 and pJA136 were co-transfected into COS cells and supernatant from the transient expression experiment was shown to contain assembled antibody which bound to T-cell enriched lymphocytes. Metabolic labelling experiments using ^{35}S methionine showed expression and assembly of heavy and light chains.

9. CONSTRUCTION OF CHIMERIC GENES

Construction of chimeric genes followed a previously described strategy [Whittle et al (ref. 13)]. A restriction site near the 3′ end of the variable domain sequence is identified and used to attach an oligonucleotide adapter coding for the remainder of the mouse variable region and a suitable restriction site for attachment to the constant region of choice.

9.1. LIGHT CHAIN GENE CONSTRUCTION

The mouse light chain cDNA sequence contains an AvaI site near the 3′ end of the variable region [Fig. 1(a)]. The majority of the sequence of the variable region was isolated as a 396 bp. EcoR1-AvaI fragment. An oligonucleotide adapter was designed to replace the remainder of the 3′

region of the variable region from the AvaI site and to include the 5' residues of the human constant region up to and including a unique NarI site which had been previously engineered into the constant region.
A HindIII site was introduced to act as a marker for insertion of the linker.
The linker was ligated to the V_L fragment and the 413 bp EcoRI-NarI adapted fragment was purified from the ligation mixture.
The constant region was isolated as an NarI-BamHI fragment from an M13 clone NW361 and was ligated with the variable region DNA into an EcoRI/BamHI/ClP pSP65 treated vector in a three way reaction to yield plasmid JA143. Clones were isolated after transformation into E.coli and the linker and junction sequences were confirmed by the presence of the HindIII site and by DNA sequencing.

9.2 LIGHT CHAIN GENE CONSTRUCTION - VERSION 2

The construction of the first chimeric light chain gene produces a fusion of mouse and human amino acid sequences at the variable-constant region junction. In the case of the OKT3 light chain the amino acids at the chimera junction are:

.....Leu-Glu-Ile-Asn-Arg/ -/Thr-Val-Ala -Ala
VARIABLE CONSTANT

This arrangement of sequence introduces a potential site for Asparagine (Asn) linked (N-linked) glycosylation at the V-C junction. Therefore, a second version of the chimeric light chain oligonucleotide adapter was designed in which the threonine (Thr), the first amino acid of the human constant region, was replaced with the equivalent amino acid from the mouse constant region, Alanine (Ala).

An internal HindIII site was not included in this adapter, to differentiate the two chimeric light chain genes.
The variable region fragment was isolated as a 376 bp EcoR1-AvaI fragment. The oligonucleotide linker was ligated to NarI cut pNW36I and then the adapted 396bp constant region was isolated after recutting the modified pNW361 with EcoR1. The variable region fragment and the modified constant region fragment were ligated directly into EcoR1/C1P treated pEE6hCMVneo to yield pJA137. Initially all clones examined had the insert in the incorrect orientation. Therefore, the insert was re-isolated and recloned to turn the insert round and yield plasmid pJA141. Several clones with the insert in the correct orientation were obtained and the adapter sequence of one was confirmed by DNA sequencing

9.3. HEAVY CHAIN GENE CONSTRUCTION

9.3.1. CHOICE OF HEAVY CHAIN GENE ISOTYPE

The constant region isotype chosen for the heavy chain was human IgG4.

9.3.2. GENE CONSTRUCTION

The heavy chain cDNA sequence showed a BanI site near the 3′ end of the variable region [Fig. 2(a)]. The majority of the sequence of the variable region was isolated as a 426bp. EcoR1/C1P/BanI fragment. An oligonucleotide adapter was designated to replace the remainder of the 3′ region of the variable region from the BanI site up to and including a unique HindIII site which had been previously engineered into the first two amino acids of the constant region.
The linker was ligated to the V_H fragment and the EcoR1-HindIII adapted fragment was purified from the ligation mixture.

The variable region was ligated to the constant region by cutting pJA91 with EcoR1 and Hind111 removing the intron fragment and replacing it with the V_H to yield pJA142. Clones were isolated after transformation into E.coli JM101 and the linker and junction sequences were confirmed by DNA sequencing. (N.B. The Hind111 site is lost on cloning).

10. CONSTRUCTION OF CHIMERIC EXPRESSION VECTORS

10.1. neo AND gpt VECTORS

The chimeric light chain (version 1) was removed from pJA143 as an EcoR1 fragment and cloned into EcoR1/C1P treated pEE6hCMVneo expression vector to yield pJA145. Clones with the insert in the correct orientation were identified by restriction mapping.

The chimeric light chain (version 2) was constructed as described above.

The chimeric heavy chain gene was isolated from pJA142 as a 2.5Kbp EcoR1/BamH1 fragment and cloned into the EcoR1/Bc11/C1P treated vector fragment of a derivative of pEE6hCMVgpt to yield plasmid pJA144.

10.2. GS SEPARATE VECTORS

GS versions of pJA141 and pJA144 were constructed by replacing the neo and gpt cassettes by a BamH1/Sa11/C1P treatment of the plasmids, isolation of the vector fragment and ligation to a GS-containing fragment from the plasmid pRO49 to yield the light chain vector pJA179 and the heavy chain vector pJA180.

10.3. GS SINGLE VECTOR CONSTRUCTION

Single vector constructions containing the cL (chimeric light), cH (chimeric heavy) and GS genes on one plasmid in the order cL-cH-GS, or cH-cL-GS

and with transcription of the genes being head to tail e.g. cL>cH>GS were constructed. These plasmids were made by treating pJA179 or pJA180 with BamH1/ClP and ligating in a Bgl11/Hind111 hCMV promoter cassette along with either the Hind111/BamH1 fragment from pJA141 into pJA180 to give the cH-cL-GS plasmid pJA182 or the Hind111/BamH1 fragment from pJA144 into pJA179 to give the cL-cH-GS plasmid pJA181.

11. EXPRESSION OF CHIMERIC GENES

11.1. EXPRESSION IN COS CELLS

The chimeric antibody plasmid pJA145 (cL) and pJA144 (cH) were co-transfected into COS cells and supernatant from the transient expression experiment was shown to contain assembled antibody which bound to the HUT 78 human T-cell line. Metabolic labelling experiments using ^{35}S methionine showed expression and assembly of heavy and light chains. However the light chain mobility seen on reduced gels suggested that the potential glycosylation site was being glycosylated. Expression in COS cells in the presence of tunicamycin showed a reduction in size of the light chain to that shown for control chimeric antibodies and the OKT3 mouse light chain. Therefore JA141 was constructed and expressed. In this case the light chain did not show an aberrant mobility or a size shift in the presence or absence of tunicamycin. This second version of the chimeric light chain, when expressed in association with chimeric heavy (cH) chain, produced antibody which showed good binding to HUT 78 cells. In both cases antigen binding was equivalent to that of the mouse antibody.

11.2 EXPRESSION IN CHINESE HAMSTER OVARY (CHO) CELLS

Stable cell lines have been prepared from plasmids PJA141/pJA144 and from pJA179/pJA180, pJA181 and pJA182 by transfection into CHO cells.

12. CDR-GRAFTING

The approach taken was to try to introduce sufficient mouse residues into a human variable region framework to generate antigen binding activity comparable to the mouse and chimeric antibodies.

12.1. VARIABLE REGION ANALYSIS

From an examination of a small database of structures of antibodies and antigen-antibody complexes it is clear that only a small number of antibody residues make direct contact with antigen. Other residues may contribute to antigen binding by positioning the contact residues in favourable configurations and also by inducing a stable packing of the individual variable domains and stable interaction of the light and heavy chain variable domains.

The residues chosen for transfer can be identified in a number of ways:

(a) By examination of antibody X-ray crystal structures the antigen binding surface can be predominantly located on a series of loops, three per domain, which extend from the B-barrel framework.

(b) By analysis of antibody variable domain sequences regions of hypervariability [termed the Complementarity Determining Regions (CDRs) by Wu and Kabat (ref. 5)] can be identified. In the most but not all cases these CDRs correspond to, but extend a short way beyond, the loop regions noted above.

(c) Residues not identified by (a) and (b) may contribute to antigen binding directly or indirectly by affecting antigen binding site topology, or by inducing a stable packing of the individual variable domains and stabilising the inter-variable domain interaction. These residues may be identified either by superimposing the sequences for a given antibody on a known structure and looking at key residues for their contribution, or by sequence alignment analysis and noting "idiosyncratic" residues followed by examination of their structural location and likely effects.

12.1.1. LIGHT CHAIN

Figure 3 shows an alignment of sequences for the human framework region RE1 and the OKT3 light variable region. The structural loops (LOOP) and CDRs (KABAT) believed to correspond to the antigen binding region are marked. Also marked are a number of other residues which may also contribute to antigen binding as described in 13.1(c). Above the sequence in Figure 3 the residue type indicates the spatial location of each residue side chain, derived by examination of resolved structures from X-ray crystallography analysis. The key to this residue type designation is as follows:

N - near to CDR (From X-ray Structures)

P - Packing B - Buried Non-Packing

S - Surface E - Exposed

I - Interface * - Interface

- Packing/Part Exposed

? - Non-CDR Residues which may require to be left as Mouse sequence.

Residues underlined in Figure 3 are amino acids. RE1 was chosen as the human framework because the light chain is a kappa chain and the kappa variable regions show higher homology with the mouse sequences than a lambda light variable region, e.g. KOL (see below). RE1 was chosen in preference to another kappa light chain because the X-ray structure of the light chain has been determined so that a structural examination of individual residues could be made.

12.1.2. HEAVY CHAIN

Similarly Figure 4 shows an alignment of sequences for the human framework region KOL and the OKT3 heavy variable region. The structural loops and CDRs believed to correspond to the antigen binding region are marked. Also marked are a number of other residues which may also contribute to antigen binding as described in 12.1(c). The residue type key and other indicators used in Figure 4 are the same as those used in Figure 3. KOL was chosen as the heavy chain framework because the X-ray structure has been determined to a better resolution than, for example, NEWM and also the sequence alignment of OKT3 heavy variable region showed a slightly better homology to KOL than to NEWM.

12.2. DESIGN OF VARIABLE GENES

The variable region domains were designed with mouse variable region optimal codon usage [Grantham and Perrin (ref. 15)] and used the B72.3 signal sequences [Whittle et al (ref. 13)]. The sequences were designed to be attached to the constant region in the same way as for the chimeric genes described above. Some constructs contained the "Kozak consensus sequence" [Kozak (ref. 16)] directly linked to the 5' of the signal

sequence in the gene. This sequence motif is believed to have a beneficial role in translation initiation in eukaryotes.

12.3. GENE CONSTRUCTION

To build the variable regions, various strategies are available. The sequence may be assembled by using oligonucleotides in a manner similar to Jones et al (ref. 17) or by simultaneously replacing all of the CDRs or loop regions by oligonucleotide directed site specific mutagenesis in a manner similar to Verhoeyen et al (ref. 2). Both strategies were used and a list of constructions is set out in Tables 1 and 2 and Figures 4 and 5. It was noted in several cases that the mutagenesis approach led to deletions and rearrangements in the gene being remodelled, while the success of the assembly approach was very sensitive to the quality of the oligonucleotides.

13. CONSTRUCTION OF EXPRESSION VECTORS

Genes were isolated from M13 or SP65 based intermediate vectors and cloned into pEE6hCMVneo for the light chains and pEE6hCMVgpt for the heavy chains in a manner similar to that for the chimeric genes as described above.

TABLE 1 CDR-GRAFTED GENE CONSTRUCTS

CODE	MOUSE SEQUENCE CONTENT	METHOD OF CONSTRUCTION	KOZAK SEQUENCE -	KOZAK SEQUENCE +
LIGHT CHAIN	ALL HUMAN FRAMEWORK RE1			
121	26-32, 50-56, 91-96 inclusive	SDM and gene assembly	+	n.d.
121A	26-32, 50-56, 91-96 inclusive +1, 3, 46, 47	Partial gene assembly	n.d.	+
121B	26-32, 50-56, 91-96 inclusive + 46, 47	Partial gene assembly	n.d.	+
221	24-24, 50-56, 91-96 inclusive	Partial gene assembly	+	+
221A	24-34, 50-56, 91-96 inclusive +1, 3, 46, 47	Partial gene assembly	+	+
221B	24-34, 50-56, 91-96 inclusive +1, 3	Partial gene assembly	+	+
221C	24-34, 50-56, 91-96 inclusive	Partial gene assembly	+	+
HEAVY CHAIN	ALL HUMAN FRAMEWORK KOL			
121	26-32, 50-56, 95-100B inclusive	Gene assembly	n.d.	+
131	26-32, 50-58, 95-100B inclusive	Gene assembly	n.d.	+
141	26-32, 50-65, 95-100B inclusive	Partial gene assembly	+	n.d.
321	26-35, 50-56, 95-100B inclusive	Partial gene assembly	+	n.d.
331	26-35, 50-58, 95-100B inclusive	Partial gene assembly	+	
		Gene assembly		+
341	26-35, 50-65, 95-100B inclusive	SDM	+	
		Partial gene assembly		+
341A	26-35, 50-65, 95-100B inclusive +6, 23, 24, 48, 49, 71, 73, 76, 78, 88, 91 (+63 = human)	Gene assembly	n.d.	+
341B	26-35, 50-65, 95-100B inclusive + 48, 49, 71, 73, 76, 78, 88, 91 (+63 + human)	Gene assembly	n.d.	+

KEY

n.d.	not done
SDM	Site directed mutagenesis
Gene assembly	Variable region assembled entirely from oligonucleotides
Partial gene assembly	Variable region assembled by combination of restriction fragments either from other genes originally created by SDM and gene assembly or by oligonucleotide assembly of part of the variable region and reconstruction with restriction fragments from other genes originally created by SDM and gene assembly

14. EXPRESSION OF CDR-GRAFTED GENES

14.1. PRODUCTION OF ANTIBODY CONSISTING OF GRAFTED LIGHT (gL) CHAINS WITH MOUSE HEAVY (mH) OR CHIMERIC HEAVY (cH) CHAINS

All gL chains, in association with mH or cH produced reasonable amounts of antibody. Insertion of the Kozak consensus sequence at a position 5′ to the ATG (kgL constructs) however, led to a 2-5 fold improvement in net expression. Over an extended series of experiments expression levels were raised from approximately 200ng/ml to approximately 500 ng/ml for kgL/cH or kgL/mH combinations.

When direct binding to antigen on HUT 78 cells was measured, a construct designed to include mouse sequence based on loop length (gL121) did not lead to active antibody in association with mH or cH. A construct designed to include mouse sequence based on Kabat CDRs (gL221) demonstrated some weak binding in association with mH or cH. However, when framework residues 1, 3, 46, 47 were changed from the human to the murine OKT3 equivalents based on the arguments outlined in Section 12.1 antigen binding was demonstrated when both of the new constructs, which were termed 121A and 221A were co-expressed with cH. When the effects of these residues were examined in more detail, it appears that residues 1 and 3 are not major contributing residues as the product of the gL221B gene shows little detectable binding activity in association with cH. The light chain product of gL221C, in which mouse sequences are present at 46 and 47, shows good binding activity in association with cH.

14.2 PRODUCTION OF ANTIBODY CONSISTING OF GRAFTED HEAVY (gH) CHAINS WITH MOUSE LIGHT (mL) OR CHIMERIC LIGHT (cL) CHAINS

Expression of the gH genes proved to be more difficult to achieve than for gL. First, inclusion of the Kozak sequence appeared to have no marked effect on expression of gH genes. Expression appears to be slightly improved but not to the same degree as seen for the grafted light chain.

Also, it proved difficult to demonstrate production of expected quantities of material when the loop choice (amino acid 26-32) for CDR1 is used, e.g. gH121, 131, 141 and no conclusions can be drawn about these constructs.

Moreover, co-expression of the gH341 gene with cL or mL has been variable and has tended to produce lower amounts of antibody than the cH/cL or mH/mL combinations. The alterations to gH341 to produce gH341A and gH341B lead to improved levels of expression.

This may be due either to a general increase in the fraction of mouse sequence in the variable region, or to the alteration at position 63 where the residue is returned to the human amino acid Valine (Val) from Phenylalanine (Phe) to avoid possible internal packing problems with the rest of the human framework. This arrangement also occurs in gH331 and gH321.

When gH321 or gH331 were expressed in association with cL, antibody was produced but antibody binding activity was not detected.

When the more conservative gH341 gene was used antigen binding could be detected in association with cL or mL, but the activity was only marginally above the background level.

When further mouse residues were substituted based on the arguments in 12.1, antigen binding could be clearly demonstrated for the antibody produced when kgH341A and kgH341B were expressed in association with cL.

14.3 PRODUCTION OF FULLY CDR-GRAFTED ANTIBODY

The kgL221A gene was co-expressed with kgH341, kgH341A or kgH341B. For the combination kgH221A/kgH341 very little material was produced in a normal COS cell expression.

For the combinations kgL221A/kgH341A or kgH221A/kgH341B amounts of antibody similar to gL/cH was produced.

In several experiments no antigen binding activity could be detected with kgH221A/gH341 or kgH221A/kgH341 combinations, although expression levels were very low.

Antigen binding was detected when kgL221A/kgH341A or kgH221A/kgH341B combinations were expressed. In the case of the antibody produced from the kgL221A/kgH341A combination the antigen binding was very similar to that of the chimeric antibody.

An analysis of the above results is given below.

15. DISCUSSION OF CDR-GRAFTING RESULTS

In the design of the fully humanised antibody the aim was to transfer the minimum number of mouse amino acids that would confer antigen binding onto a human antibody framework.

15.1. LIGHT CHAIN

15.1.1. EXTENT OF THE CDRs

For the light chain the regions defining the loops known from structural studies of other antibodies to contain the antigen contacting residues, and

those hypervariable sequences defined by Kabat et al (refs. 4 and 5) as Complementarity Determining Regions (CDRs) are equivalent for CDR2. For CDR1 the hypervariable region extends from residues 24-34 inclusive while the structural loop extends from 26-32 inclusive. In the case of OKT3 there is only one amino acid difference between the two options, at amino acid 24, where the mouse sequence is a serine and the human framework RE1 has glutamine. For CDR3 the loop extends from residues 91-96 inclusive while the Kabat hypervariability extends from residues 89-97 inclusive. For OKT3 amino acids 89, 90 and 97 are the same between OKT3 and RE1 (Fig. 3). When constructs based on the loop choice for CDR1 (gL121) and the Kabat choice (gL221) were made and co-expressed with mH or cH no evidence for antigen binding activity could be found for gL121, but trace activity could be detected for the gL221, suggesting that a single extra mouse residue in the grafted variable region could have some detectable effect. Both gene constructs were reasonably well expressed in the transient expression system.

15.1.2. FRAMEWORK RESIDUES

The remaining framework residues were then further examined, in particular amino acids known from X-ray analysis of other antibodies to be close to the CDRs and also those amino acids which in OKT3 showed differences from the consensus framework for the mouse subgroup (subgroup VI) to which OKT3 shows most homology. Four positions 1, 3, 46 and 47 were identified and their possible contribution was examined by substituting the mouse amino acid for the human amino acid at each position. Therefore gL221A (gL221 + D1Q, Q3V, L46R, L47W,

see Figure 3 and Table 1) was made, cloned in EE6hCMVneo and co-expressed with cH (pJA144). The resultant antibody was well expressed and showed good binding activity. When the related genes gL221B (gL221 + D1Q, Q3V) and gL221C (gL221 + L46R, L47W) were made and similarly tested, while both genes produced antibody when co-expressed with cH, only the gL221C/cH combination showed good antigen binding. When the gL121A (gL121 + D1Q, Q3V, L46R, L47W) gene was made and co-expressed with cH, antibody was produced which also bound to antigen.

15.2. HEAVY CHAIN

15.2.1. EXTENT OF THE CDRs

For the heavy chain the loop and hypervariability analyses agree only in CDR3. For CDR1 the loop region extends from residues 26-32 inclusive whereas the Kabat CDR extends from residues 31-35 inclusive. For CDR2 the loop region is from 50-58 inclusive while the hypervariable region covers amino acids 50-65 inclusive. Therefore humanised heavy chains were constructed using the framework from antibody KOL and with various combinations of these CDR choices, including a shorter choice for CDR2 of 50-56 inclusive as there was some uncertainty as to the definition of the end point for the CDR2 loop around residues 56 to 58. The genes were co-expressed with mL or cL initially. In the case of the gH genes with loop choices for CDR1 e.g. gH121, gH131, gH141 very little antibody was produced in the culture supernatants. As no free light chain was detected it was presumed that the antibody was being made and assembled inside the cell but that the heavy chain was aberrant in some way, possibly incorrectly folded, and therefore the antibody was

being degraded internally. In some experiments trace amounts of antibody could be detected in ^{35}S labelling studies.
As no net antibody was produced, analysis of these constructs was not pursued further.
When, however, a combination of the loop choice and the Kabat choice for CDR1 was tested (mouse amino acids 26-35 inclusive) and in which residues 31 (Ser to Arg), 33 (Ala to Thr), and 35 (Tyr to His) were changed from the human residues to the mouse residue and compared to the first series, antibody was produced for gH321, kgH331 and kgH341 when co-expressed with cL. Expression was generally low and could not be markedly improved by the insertion of the Kozak consensus sequence 5′ to the ATG of the signal sequence of the gene, as distinct from the case of the gL genes where such insertion led to a 2-5 fold increase in net antibody production. However, only in the case of gH341/mL or kgH341/cL could marginal antigen binding activity be demonstrated. When the kgH341 gene was co-expressed with kgL221A, the net yield of antibody was too low to give a signal above the background level in the antigen binding assay.

15.2.2. FRAMEWORK RESIDUES

As in the case of the light chain the heavy chain frameworks were re-examined. Possibly because of the lower initial homology between the mouse and human heavy variable domains compared to the light chains, more amino acid positions proved to be of interest. Two genes kgH341A and kgH341B were constructed, with 11 or 8 human residues respectively substituted by mouse residues compared to gH341, and with the CDR2 residue 63 returned to the human amino acid potentially to

improve domain packing. Both showed antigen binding when combined with cL or kgL221A, the kgH341A gene with all 11 changes appearing to be the superior choice.

15.3 INTERIM CONCLUSIONS

It has been demonstrated, therefore, for OKT3 that to transfer antigen binding ability to the humanised antibody, mouse residues outside the CDR regions defined by the Kabat hypervariability or structural loop choices are required for both the light and heavy chains. Fewer extra residues are needed for the light chain, possibly due to the higher initial homology between the mouse and human kappa variable regions.

Of the changes seven (1 and 3 from the light chain and 6, 23, 71, 73 and 76 from the heavy chain) are predicted from a knowledge of other antibody structures to be either partly exposed or on the antibody surface. It has been shown here that residues 1 and 3 in the light chain are not absolutely required to be the mouse sequence; and for the heavy chain the gH341B heavy chain in combination with the 221A light chain generated only weak binding activity. Therefore the presence of the 6, 23 and 24 changes are important to maintain a binding affinity similar to that of the murine antibody. It was important, therefore, to further study the individual contribution of othe other 8 mouse residues of the kgH341A gene compared to kgH341.

16. FURTHER CDR-GRAFTING EXPERIMENTS

Additional CDR-grafted heavy chain genes were prepared substantially as described above. With reference to Table 2 the further heavy chain genes were based upon the gh341 (plasmid pJA178) and

gH341A (plasmid pJA185) with either mouse OKT3 or human KOL residues at 6, 23, 24, 48, 49, 63, 71, 73, 76, 78, 88 and 91, as indicated. The CDR-grafted light chain genes used in these further experiments were gL221, gL221A, gL221B and gL221C as described above.

TABLE 2

OKT3 HEAVY CHAIN CDR GRAFTS

1. gH341 and derivatives

RES NUM	6	23	24	48	49	63	71	73	76	78	88	91	
OKT3vh	Q	K	A	I	G	F	T	K	S	A	A	Y	
gH341	E	S	S	V	A	F	R	N	N	L	G	F	JA178
gH341A	Q	K	A	I	G	V	T	K	S	A	A	Y	JA185
gH341E	Q	K	A	I	G	V	T	K	S	A	G	G	JA198
gH341*	Q	K	A	I	G	V	T	K	N	A	G	F	JA207
gH341*	Q	K	A	I	G	V	R	N	N	A	G	F	JA209
gH341D	Q	K	A	I	G	V	T	K	N	L	G	F	JA197
gH341*	Q	K	A	I	G	V	R	N	N	L	G	F	JA199
gH341C	Q	K	A	V	A	F	R	N	N	L	G	F	JA184
gH341*	Q	S	A	I	G	V	T	K	S	A	A	Y	JA203
gH341*	E	S	A	I	G	V	T	K	S	A	A	Y	JA205
gH341B	E	S	S	I	G	V	T	K	S	A	A	Y	JA183
gH341*	Q	S	A	I	G	V	T	K	S	A	G	F	JA204
gH341*	E	S	A	I	G	V	T	K	S	A	G	F	JA206
gH341*	Q	S	A	I	G	V	T	K	N	A	G	F	JA208
KOL	E	S	S	V	A		R	N	N	L	G	F	

OKT3 LIGHT CHAIN CDR GRAFTS

2. gL221 and derivatives

RES NUM	1	3	46	47	
OKT3vl	Q	V	R	W	
GL221	D	Q	L	L	DA221
gL221A	Q	V	R	W	DA221A
gL221B	Q	V	L	L	DA221B
GL221C	D	Q	R	W	DA221C
REI	D	Q	L	L	

MURINE RESIDUES ARE UNDERLINED

The CDR-grafted heavy and light chain genes were co-expressed in COS cells either with one another in various combinations but also with the corresponding murine and chimeric heavy and light chain genes substantially as described above. The resultant antibody products were then assayed in binding and blocking assays with HPB-ALL cells as described above.

The results of the assays for various grafted heavy chains co-expressed with the gL221C light chain are given in Figures 7 and 8 (for the JA184, JA185, JA197 and JA198 constructs - see Table 2), in Figure 9 (for the JA183, JA184, JA185 and JA197 constructs) in Figure 10 (for the chimeric, JA185, JA199, JA204, JA205, JA207, JA208 and JA209 constructs) and in Figure 11 (for the JA183, JA184, JA185, JA198, JA203, JA205 and JA206 constructs).

The basic grafted product without any human to murine changes in the variable frameworks, i.e. gL221 co-expressed with gh341 (JA178), and also the "fully grafted" product, having most human to murine changes in the grafted heavy chain framework, i.e. gL221C co-expressed with gh341A (JA185), were assayed for relative binding affinity in a competition assay against murine OKT3 reference standard, using HPB-ALL cells. The assay used was as described above in section 3.3. The results obtained are given in Figure 12 for the basic grafted product and in Figure 13 for the fully grafted product. These results indicate that the basic grafted product has neglibible binding ability as compared with the OKT3 murine reference standard; whereas the "fully grafted" product has a binding ability very similar to that of the OKT3 murine reference standard.

The binding and blocking assay results indicate the following:

The JA198 and JA207 constructs appear to have the best binding characteristics and similar binding abilities, both substantially the same as the chimeric and fully grafted gH341A products. This indicates that positions 88 and 91 and position 76 are not highly critical for maintaining the OKT3 binding ability; whereas at least some of positions 6, 23, 24, 48, 49, 71, 73 and 78 are more important.

This is borne out by the finding that the JA209 and JA199, although of similar binding ability to one another, are of lower binding ability than the JA198 and JA207 constructs. This indicates the importance of having mouse residues at positions 71, 73 and 78, which are either completely or partially human in the JA199 and JA209 constructs respectively.

Moreover, on comparing the results obtained for the JA205 and JA183 constructs it is seen that there is a decrease in binding going from the JA205 to the JA183 constructs. This indicates the importance of retaining a mouse residue at position 23, the only position changed between JA205 and JA183.

These and other results lead us to the conclusion that of the 11 mouse framework residues used in the gH341A (JA185) construct, it is important to retain mouse residues at all of positions 6, 23, 24, 48 and 49, and possibly for maximum binding affinity at 71, 73 and 78.

Similar Experiments were carried out to CDR-graft a number of the rodent antibodies including antibodies having specificity for CD4 (OKT4), ICAM-1 (R6-5), TAG72 (B72.3), and TNFα(61E71, 101.4, hTNF1, hTNF2 and hTNF3).

EXAMPLE 2

CDR-GRAFTING OF A MURINE ANTI-CD4 T CELL RECEPTOR ANTIBODY, OKT4A

Anti OKT4A CDR-grafted heavy and light chain genes were prepared, expressed and tested substantially as described above in Example 1 for CDR-grafted OKT3. The CDR grafting of OKT4A is described in detail in Ortho patent application PCT/GB 90..... of even date herewith entitled "Humanised Antibodies". The disclosure of this Ortho patent application PCT/GB 90 ..... is incorporated herein by reference. A number of CDR-grafted OKT4 antibodies have been prepared. Presently the CDR-grafted OKT4A of choice is the combination of the grafted light chain LCDR2 and the grafted heavy chain HCDR10.

THE LIGHT CHAIN

The human acceptor framework used for the grafted light chains was RE1. The preferred LCDR2 light chain has human to mouse changes at positions 33, 34, 38, 49 and 89 in addition to the structural loop CDRs. Of these changed positions, positions 33, 34 and 89 fall within the preferred extended CDRs of the present invention (positions 33 and 34 in CDR1 and position 89 in CDR3). The human to murine changes at positions 38 and 49 corresponds to positions at which the amino acid residues are preferably donor murine amino acid residues in accordance with the present invention.

A comparison of the amino acid sequences of the donor murine light chain variable domain and the RE1 human acceptor light chain variable further reveals that the murine and human residues are identical at all of positions 46, 48 and 71 and at all of positions 2, 4, 6, 35, 36, 44, 47, 62, 64-69, 85, 87, 98, 99 and 101 and 102. However the amino acid residue at position 58 in LCDR2 is

the human RE1 framework residue not the mouse OKT4 residue as would be preferred in accordance with the present invention.

THE HEAVY CHAIN

The human acceptor framework used for the grafted heavy chains was KOL.

The preferred CDR graft HCDR10 heavy chain has human to mouse changes at positions 24, 35, 57, 58, 60, 88 and 91 in addition to the structural loop CDRs.

Of these positions, positions 35 (CDR1) and positions 57, 58 and 60 (CDR2) fall within the preferred extended CDRs of the present invention. Also the human to mouse change at position 24 corresponds to a position at which the amino acid residue is a donor murine residue in accordance with the present invention. Moreover, the human to mouse changes at positions 88 and 91 correspond to positions at which the amino acid residues are optionally donor murine residues.

Moreover, a comparison of the murine OKT4A and human KOL heavy chain variable amino acid sequences reveals that the murine and human residues are identical at all of positions 23, 49, 71, 73 and 78 and at all of positions 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

Thus the OKT4A CDR-grafted heavy chain HCDR10 corresponds to a particularly preferred embodiment according to the present invention.

EXAMPLE 3

CDR-GRAFTING OF AN ANTI-MUCIN SPECIFIC MURINE ANTIBODY, B72.3

The cloning of the genes coding for the anti-mucin specific murine monoclonal antibody B72.3 and the preparation of B72.3 mouse-human chimeric antibodies has been described previously (ref. 13 and WO 89/01783). CDR-grafted versions of B72.3 were prepared as follows.

(a) B72.3 Light Chain

CDR-grafting of this light chain was accomplished by direct transfer of the murine CDRs into the framework of the human light chain RE1.

The regions transferred were:

CDR Number	Residues
1	24-34
2	50-56
3	90-96

The activity of the resulting grafted light chain was assessed by co-expression in COS cells, of genes for the combinations:

B72.3 cH/B72.3 cL

and B72.3 cH/B72.3 gL

Supernatants were assayed for antibody concentration and for the ability to bind to microtitre plates coated with mucin. The results obtained indicated that, in combination with the B72.3 cH chain, B72.3 cL and B72.3 gL had similar binding properties.

Comparison of the murine B72.3 and REI light chain amino acid sequences reveals that the residues are identical at positions 46, 58 and 71 but are different at position 48.

Thus changing the human residue to the donor mouse residue at position 48 may further improve the binding characteristics of the CDR-grafted light chain, (B72.3 gL) in accordance with the present invention.

(b) B72.3 heavy chain

i. Choice of framework

At the outset it was necessary to make a choice of human framework. Simply put, the question was as follows: Was it necessary to use the framework regions from an antibody whose crystal structure was known or could the choice be made on some other criteria?

For B72.3 heavy chain, it was reasoned that, while knowledge of structure was important, transfer of the CDRs from mouse to human frameworks might be facilitated if the overall homology between the donor and receptor frameworks was maximised.

Comparison of the B72.3 heavy chain sequence with those in Kabat (ref. 4) for human heavy chains showed clearly that B72.3 had poor homology for KOL and NEWM (for which crystal structures are available) but was very homologous to the heavy chain for EU.

On this basis, EU was chosen for the CDR-grafting and the following residues transferred as CDRs.

CDR Number	Residues
1	27-36
2	50-63
3	93-102

Also it was noticed that the FR4 region of EU was unlike that of any other human (or mouse) antibody. Consequently, in the grafted heavy chain genes this was also changed to produce a "consensus" human sequence. (Preliminary experiments showed that grafted heavy chain genes containing the EU FR4 sequence expressed very poorly in transient expression systems.)

ii. Results with grafted heavy chain genes

Expression of grafted heavy chain genes containing all human framework regions with either gL or cL genes produced a grafted antibody with little ability to bind to mucin. The grafted antibody had about 1% the activity of the chimeric antibody.

In these experiments, however, it was noted that the activity of the grafted antibody could be increased to ~ 10% of B72.3 by exposure to pHs of 2-3.5.

This observation provided a clue as to how the activity of the grafted antibody could be improved without acid treatment. It was postulated that acid exposure brought about the protonation of an acidic residue (pKa of aspartic acid = 3.86 and of glutamine acid = 4.25) which in turn caused a change in structure of the CDR loops, or allowed better access of antigen.

From comparison of the sequences of B72.3 (ref. 13) and EU (refs. 4 and 5), it was clear that, in going from the mouse to human frameworks, only two positions had been changed in such a way that acidic residues had been introduced. These

positions are at residues 73 and 81, where K to E and Q to E changes had been made, respectively.
Which of these positions might be important was determined by examining the crystal structure of the KOL antibody. In KOL heavy chain, position 81 is far removed from either of the CDR loops.
Position 73, however, is close to both CDRs 1 and 3 of the heavy chain and, in this position it was possible to envisage that a K to E change in this region could have a detrimental effect on antigen binding.

iii. Framework changes in B72.3 gH gene
On the basis of the above analysis, E73 was mutated to a lysine (K). It was found that this change had a dramatic effect on the ability of the grafted Ab to bind to mucin. Further the ability of the grafted B72.3 produced by the mutated gH/gL combination to bind to mucin was similar to that of the B72.3 chimeric antibody.

iv. Other framework changes
In the course of the above experiments, other changes were made in the heavy chain framework regions. Within the accuracy of the assays used, none of the changes, either alone or together, appeared beneficial.

v. Other
All assays used measured the ability of the grafted Ab to bind to mucin and, as a whole, indicated that the single framework change at position 73 is sufficient to generate an antibody with similar binding properties to B72.3.

Comparison of the B72.3 murine and EU heavy chain sequences reveals that the mouse and human residues are identical at positions 23, 24, 71 and 78.
Thus the mutated CDR-grafted B72.3 heavy chain corresponds to a preferred embodiment of the present invention.

EXAMPLE 4

CDR-GRAFTING OF A MURINE ANTI-ICAM-1 MONOCLONAL ANTIBODY

A murine antibody, R6-5-D6 (EP 0314863) having specificity for Intercellular Adhesion Molecule 1 (ICAM-1) was CDR-grafted substantially as described above in previous examples. This work is described in greater detail in co-pending application, British Patent Application No. 9009549.8, the disclosure of which is incorporated herein by reference.

The human EU framework was used as the acceptor framework for both heavy and light chains. The CDR-grafted antibody currently of choice is provided by co-expression of grafted light chain gL221A and grafted heavy chain gH341D which has a binding affinity for ICAM 1 of about 75% of that of the corresponding mouse-human chimeric antibody.

LIGHT CHAIN

gL221A has murine CDRs at positions 24-34 (CDR1), 50-56 (CDR2) and 89-97 (CDR3). In addition several framework residues are also the murine amino acid. These residues were chosen after consideration of the possible contribution of these residues to domain packing and stability of the conformation of the antigen binding region. The residues which have been retained as mouse are at positions 2, 3, 48 (?), 60, 84, 85 and 87. Comparison of the murine anti-ICAM 1 and human EU light chain amino acid sequences reveals that the murine and human residues are identical at positions 46, 58 and 71.

HEAVY CHAIN

gH341D has murine CDRs at positions 26-35 (CDR1), 50-56 (CDR2) and94-100B (CDR3). In addition murine residues were used in gH341D at positions 24, 48, 69, 71, 73, 80, 88 and 91. Comparison of the murine anti-ICAM 1 and human EU heavy chain amino acid sequences are identical at positions 23, 49 and 78.

EXAMPLE 5

CDR-Grafting of murine anti-TNFα antibodies

A number of murine anti-TNFα monoclonal antibodies were CDR-grafted substantially as described above in previous examples. These antibodies include the murine monoclonal antibodies designated 61 E71, hTNF1, hTNF3 and 101.4 A brief summary of the CDR-grafting of each of these antibodies is given below.

61E71

A similar analysis as described above (Example 1, Section 12.1.) was done for 61E71 and for the heavy chain 10 residues were identified at 23, 24, 48, 49, 68, 69, 71, 73, 75 and 88 as residues to potentially retain as murine. The human frameworks chosen for CDR-grafting of this antibody, and the hTNF3 and 101.4 antibodies were RE1 for the light chain and KOL for the heavy chain.
Three genes were built, the first of which contained 23, 24, 48, 49, 71 and 73 [gH341(6)] as murine residues. The second gene also had 75 and 88 as murine residues [gH341(8)] while the third gene additionally had 68, 69, 75 and 88 as murine residues [gH341(10)]. Each was co-expressed with gL221, the minimum grafted light chain (CDRs only). The gL221/gH341(6) and gL221/gH341(8) antibodies both bound as well to TNF as murine 61E71. The gL221/gH341(10) antibody did not express and this combination was not taken further.
Subsequently the gL221/gH341(6) antibody was assessed in an L929 cell competition assay in which the antibody competes against the TNF receptor on L929 cells for binding to TNF in solution. In this assay the gL221/gH341(6) antibody was approximately 10% as active as murine 61E71.

hTNF1

hTNF1 is a monoclonal antibody which recognises an epitope on human TNF- . The EU human framework was used for CDR-grafting of both the heavy and light variable domains.

Heavy Chain

In the CDR-grafted heavy chain (ghTNF1) mouse CDRs were used at positions 26-35 (CDR1), 50-65 (CDR2) and 95-102 (CDR3). Mouse residues were also used in the frameworks at positions 48, 67, 69, 71, 73, 76, 89, 91, 94 and 108. Comparison of the TNF1 mouse and EU human heavy chain residues reveals that these are identical at positions 23, 24, 29 and 78.

Light Chain

In the CDR-grafted light chain (gLhTNF1) mouse CDRs wre used at positions 24-34 (CDR1), 50-56 (CDR2) and 89-97 (CDR3). In addition mouse residues were used in the frameworks at positions 3, 42, 48, 49, 83, 106 and 108. Comparison of the hTNF1 mouse and EU human light chain residues reveals that these are identical at positions 46, 58 and 71.

The grafted hTNF1 heavy chain was co-expressed with the chimeric light chain and the binding ability of the product compared with that of the chimeric light chain/chimeric heavy chain product in a TNF binding assay. The grafted heavy chain product appeared to have binding ability for TNF slightly better than the fully chimeric product.

Similarly, a grafted heavy chain/grafted light chain product was co-expressed and compared with the fully chimeric product and found to have closely similar binding properties to the latter product.

hTNF3

hTNF3 recognises an epitope on human TNF-α. The sequence of hTNF3 shows only 21 differences compared to 61E71 in the light and heavy chain variable regions, 10 in the light chain (2 in the CDRs at positions 50, 96 and 8 in the framework at 1, 19, 40, 45, 46, 76, 103 and 106) and 11 in the heavy chain (3 in the CDR regions at positions 52, 60 and 95 and 8 in the framework at 1, 10, 38, 40, 67, 73, 87 and 105). The light and heavy chains of the 61E71 and hTNF3 chimeric antibodies can be exchanged without loss of activity in the direct binding assay. However 61E71 is an order of magnitude less able to compete with the TNF receptor on L929 cells for TNF-a compared to hTNF3. Based on the 61E71 CDR grafting data gL221 and gH341(+23, 24, 48, 49 71 and 73 as mouse) genes have been built for hTNF3 and tested and the resultant grafted antibody binds well to TNF-a, but competes very poorly in the L929 assay. It is possible that in this case also the framework residues identified for OKT3 programme may improve the competitive binding ability of this antibody.

101.4

101.4 is a further murine monoclonal antibody able to recognise human TNF-a. The heavy chain of this antibody shows good homology to KOL and so the CDR-grafting has been based on RE1 for the light chain and KOL for the heavy chain. Several grafted heavy chain genes have been constructed with conservative choices for the CDR's (gH341) and which have one or a small number of non-CDR residues at positions 73, 78 or 77-79 inclusive, as the mouse amino acids. These have been co-expressed with cL or gL221. In all cases binding to TNF equivalent to the chimeric antibody is seen and when co-expressed with cL the resultant antibodies are able to compete well in the L929 assay. However, with gL221 the resultant antibodies

are at least an order of magnitude less able to compete for TNF against the TNF receptor on L929 cells.

Mouse residues at other positions in the heavy chain, for example, at 23 and 24 together or at 76 have been demonstrated to provide no improvement to the competitive ability of the grafted antibody in the L929 assay.

A number of other antibodies including antibodies having specificity for interleukins e.g. IL1 and cancer markers such as carcinoembryonic antigen (CEA) e.g. the monoclonal antibody A5B7 (ref. 21), have been successfully CDR-grafted according to the present invention.
It will be appreciated that the foregoing examples are given by way of illustration only and are not intended to limit the scope of the claimed invention. Changes and modifications may be made to the methods described whilst still falling within the spirit and scope of the invention.

References

1. Kohler & Milstein, Nature, 265, 295-497, 1975.

2. Chatenoud et al, (1986), J. Immunol. 137, 830-838.

3. Jeffers et al, (1986), Transplantation, 41, 572-578.

4. Begent et al, Br. J. Cancer 62: 487 (1990).

5. Verhoeyen et al, Science, 239, 1534-1536, 1988.

6. Riechmann et al, Nature, 332, 323-324, 1988.

7. Kabat, E.A., Wu, T.T., Reid-Miller, M., Perry, H.M., Gottesman, K.S., 1987, in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA.

8. Wu, T.T., and Kabat, E.A., 1970, J. Exp. Med. 132 211-250.

9. Queen et al, (1989), Proc. Natl. Acad. Sci. USA, 86, 10029-10033 and WO 90/07861

10. Maniatis et al, Molecular Cloning, Cold Spring Harbor, New York, 1989.

11. Primrose and Old, Principles of Gene Manipulation, Blackwell, Oxford, 1980.

12. Sanger, F., Nicklen, S., Coulson, A.R., 1977, Proc. Natl. Acad. Sci. USA, 74 5463

13. Kramer, W., Drutsa, V., Jansen, H.-W., Kramer, B., Plugfelder, M., Fritz, H.-J., 1984, Nucl. Acids Res. 12, 9441

14. Whittle, N., Adair, J., Lloyd, J.C., Jenkins, E., Devine, J., Schlom, J., Raubitshek, A., Colcher, D., Bodmer, M., 1987, Protein Engineering 1, 499.

15. Sikder, S.S., Akolkar, P.N., Kaledas, P.M., Morrison, S.L., Kabat, E.A., 1985, J. Immunol. 135, 4215.

16. Wallick, S.C., Kabat, E.A., Morrison, S.L., 1988, J. Exp. Med. 168, 1099

17. Bebbington, C.R., Published International Patent Application WO 89/01036.

18. Granthan and Perrin 1986, Immunology Today 7, 160.

19. Kozak, M., 1987, J. Mol. Biol. 196, 947.

20. Jones, T.P., Dear, P.H., Foote, J., Neuberger, M.S., Winter, G., 1986, Nature, 321, 522

21. Harwood et al, Br. J. Cancer, 54, 75-82 (1986).

CLAIMS

1. A CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

2. A CDR-grafted heavy chain according to Claim I comprising donor residues at positions 23, 24, 49, 71, 73 and 78, or at positions 23, 24 and 49.

3. A CDR-grafted heavy chain according to Claim 2 comprising donor residues at positions 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

4. A CDR-grafted heavy chain according to Claim 2 or 3, comprising donor residues at one, some or all of positions:
1 and 3,
69 (if 48 is different between donor and acceptor),
38 and 46 (if 48 is the donor residue),
67,
82 and 18 (if 67 is the donor residue),
91, and
any one or more of 9, 11, 41, 87, 108, 110 and 112.

5. A CDR-grafted heavy chain according to any of the preceding comprising donor CDRs at positions 26-35, 50-65 and 95-100.

6. A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 1 and/or 3 and 46 and/or 47.

7. A CDR-grafted light chain according to Claim 6 comprising donor residues at positions 46 and 47.

8. A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, 58 and 71.

9. A CDR-grafted light chain according to Claim 8 comprising donor residues at positions 46, 48, 58 and 71.

10. A CDR-grafted light chain according to Claim 8 or 9, comprising donor residues at positions 2, 4, 6, 35, 36, 38, 44, 47, 49, 62, 64-69, 85, 87, 98, 99, 101 and 102.

11. A CDR-grafted light chain according to Claim 9 or 10, comprising donor residues at one, some or all of positions:
1 and 3,
63,
60 (if 60 and 54 are able to form a potential saltbridge),
70 (if 70 and 24 are able to form a potential saltbridge),
73 and 21 (if 47 is different between donor and acceptor),
37 and 45 (if 47 if different between donor and acceptor), and
any one or more of 10, 12, 40, 83, 103 and 105.

12. A CDR-grafted light chain according to any one of Claims 6-11, comprising donor CDRs at positions 24-34, 50-56 and 89-97.

13. A CDR-grafted antibody molecule comprising at least one CDR-grafted heavy chain according to any one of Claims 1-5 and at least one CDR-grafted light chain according to any one of Claims 6-12.

14. A CDR-grafted antibody molecule according to Claim 13, which is a site-specific antibody molecule.

15. A CDR-grafted antibody molecule according to Claim 13 which has specificity for an interleukin, hormone or other biologically active compound or a receptor therefor.

16. A CDR-grafted antibody heavy or light chain or molecule according to any one of the preceding claims comprising human acceptor residues and non-human donor residues.

17. A DNA sequence which codes for a CDR-grafted heavy chain according to Claim 1 or a CDR-grafted light chain according to Claim 6 or Claim 8.

18. A cloning or expression vector containing a DNA sequence according to Claim 17.

19. A host cell transformed with a DNA sequence according to Claim 17.

20. A process for the production of a CDR-grafted antibody sequence according to Claim 17 in a transformed host cell.

21. A process for producing a CDR-grafted antibody product comprising:

(a) producing in an expression vector an operon having a DNA sequence which encodes an antibody heavy chain according to Claim 1;

and/or

(b) producing in an expression vector an operon having a DNA sequence which encodes a complementary antibody light chain according to Claim 6 or Claim 8;

(c) transfecting a host cell with the or each vector; and

(d) culturing the transfected cell line to produce the CDR-grafted antibody product.

22. A therapeutic or diagnostic composition comprising a CDR-grafted antibody heavy chain according to Claim 1, or a CDR-grafted light chain according to Claim 6 or Claim 8, or a CDR-grafted antibody molecule according to Claim 13 in combination with a pharmaceutically acceptable carrier, diluent or excipient.

23. A method of therapy or diagnosis comprising administering an effective amount of a CDR-grafted heavy chain according to Claim 1, or a CDR-grafted light chain according to Claim 6 or Claim 8, or a CDR-grafted antibody molecule according to Claim 13 to a human or animal subject.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
C12P 21/08, C12N 15/13
A61K 39/395, C07K 15/06
C12N 5/10, 15/62

A1

(11) International Publication Number: WO 91/09967

(43) International Publication Date: 11 July 1991 (11.07.91)

(21) International Application Number: PCT/GB90/02017

(22) International Filing Date: 21 December 1990 (21.12.90)

(30) Priority data:
8928874.0 21 December 1989 (21.12.89) GB

(71) Applicant *(for all designated States except US)*: CELLTECH LIMITED [GB/GB]; 216 Bath Road, Slough, Berkshire SL1 4EN (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : ADAIR, John, Robert [GB/GB]; 23 George Road, Stokenchurch, High Wycombe, Buckinghamshire HP14 3RN (GB). ATHWAL, Diljeet, Singh [GB/GB]; Flat 35, Knollys House, Tavistock Square, London WC1 (GB). EMTAGE, John, Spencer [GB/GB]; 49 Temple Mill Island, Temple, Marlow, Buckinghamshire SL7 1SQ (GB).

(74) Agent: MERCER, Christopher, Paul; Carpmaels & Ransford, 43 Bloomsbury Square, London WC1A 2RA (GB).

(81) Designated States: AT, AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CF (OAPI patent), CG (OAPI patent), CH, CH (European patent), CM (OAPI patent), DE, DE (European patent), DK, FI, FR (European patent), GA (OAPI patent), GB, GB (European patent), GR, HU, IT (European patent), JP, KP, KR, LK, LU, LU (European patent), MC, MG, ML (OAPI patent), MR (OAPI patent), MW, NL, NL (European patent), NO, RO, SD, SE, SE (European patent), SN (OAPI patent), SU, TD (OAPI patent), TG (OAPI patent), US.

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.
With a request for rectification under Rule 91.1(f).

(54) Title: HUMANISED ANTIBODIES

(57) Abstract

CDR-grafted antibody heavy and light chains comprise acceptor framework and donor antigen binding regions, the heavy chains comprising donor residues at at least one of positions (6, 23) and/or (24, 48) and/or (49, 71) and/or (73, 75) and/or (76) and/or (78) and (88) and/or (91). The CDR-grafted light chains comprise donor residues at at least one of positions (1) and/or (3) and (46) and/or (47) or at at least one of positons (46, 48, 58) and (71). The CDR-grafted antibodies are preferably humanised antibodies, having non human, e.g. rodent, donor and human acceptor frameworks, and may be used for *in vivo* therapy and diagnosis. A generally applicable protocol is disclosed for obtaining CDR-grafted antibodies.

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```
  1  GAATTCCCAA AGACAAAatg gattttcaag tgcagatttt cagcttcctg
 51  ctaatcagtg cctcagtcat aatatccaga ggacaaattg ttctcaccca
101  gtctccagca atcatgtctg catctccagg ggagaaggtc accatgacct
151  gcagtgccag ctcaagtgta agttacatga actggtacca gcagaagtca
201  ggcacctccc ccaaaagatg gatttatgac acatccaaac tggcttctgg
251  agtccctgct cacttcaggg gcagtgggtc tgggacctct tactctctca
301  caatcagcgg catggaggct gaagatgctg ccacttatta ctgccagcag
351  tggagtagta  accattcac gttcggctcg gggacaaagt tggaaataaa
401  ccgggctgat actgcaccaa ctgtatccat cttcccacca tccagtgagc
451  agttaacatc tggaggtgcc tcagtcgtgt gcttcttgaa caacttctac
501  cccaaagaca tcaatgtcaa gtggaagatt gatggcagtg aacgacaaaa
551  tggcgtcctg aacagttgga ctgatcagga cagcaaagac agcacctaca
601  gcatgagcag caccctcacg ttgaccaagg acgagtatga acgacataac
651  agctatacct gtgaggccac tcacaagaca tcaacttcac ccattgtcaa
701  gagcttcaac aggaatgagt gtTAGAGACA AAGGTCCTGA GACGCCACCA
751  CCAGCTCCCA GCTCCATCCT ATCTTCCCTT CTAAGGTCTT GGAGGCTTCC
801  CCACAAGCGC tTACCACTGT TGCGGTGCTC TAAACCTCCT CCCACCTCCT
851  TCTCCTCCTC CTCCCTTTCC TTGGCTTTTA TCATGCTAAT ATTTGCAGAA
901  AATATTCAAT AAAGTGAGTC TTTGCCTTGA AAAAAAAAAA AAA
(SEQ ID NO:4)
```

FIG. 1a

```
  1  MDFQVQIFSF LLISASVIIS RGQIVLTQSP AIMSASPGEK VTMTCSASSS
 51  VSYMNWYQQK SGTSPKRWIY DTSKLASGVP AHFRGSGSGT  SYSLTISGME
101  AEDAATYYCQ QWSSNPFTFG SGTKLEINRA DTAPTVSIFP  PSSEQLTSGG
151  ASVVCFLNNF YPKDINVKWK IDGSERQNGV LNSWTDQDSK  DSTYSMSSTL
201  TLTKDEYERH NSYTCEATHK TSTSPIVKSF NRNEC* (SEQ ID NO:5)
```

FIG. 1b

```
   1  GAATTCCCCT CTCCACAGAC ACTGAAAACT CTGACTCAAC ATGGAAAGGC
  51  ACTGGATCTT TCTACTCCTG TTGTCAGTAA CTGCAGGTGT CCACTCCCAG
 101  GTCCAGCTGC AGCAGTCTGG GGCTGAACTG GCAAGACCTG GGGCCTCAGT
 151  GAAGATGTCC TGCAAGGCTT CTGGCTACAC CTTTACTAGG TACACGATGC
 201  ACTGGGTAAA ACAGAGGCCT GGACAGGGTC TGGAATGGAT TGGATACATT
 251  ATTCCTAGCC GTGGTTATAC TAATTACAAT CAGAAGTTCA AGGACAAGGC
 301  CACATTGACT ACAGACAAAT CCTCCAGCAC AGCCTACATG CAACTGAGCA
 351  GCCTGACATC TGAGGACTCT GCAGTCTATT ACTGTGCAAG ATATTATGAT
 401  GATCATTACT GCCTTGACTA CTGGGGCCAA GGCACCACTC TCACAGTCTC
 451  CTCAGCCAAA ACAACAGCCC CATCGGTCTA TCCACTGGCC CCTGTGTGTG
 501  GAGATACAAC TGGCTCCTCG GTGACTCTAG GATGCCTGGT CAAGGGTTAT
 551  TTCCCTGAGC CAGTGACCTT GACCTGGAAC TCTGGATCCC TGTCCAGTGG
 601  TGTGCACACC TTCCCAGCTG TCCTGCAGTC TGACCTCTAC ACCCTCAGCA
 651  GCTCAGTGAC TGTAACCTCG AGCACCTGGC CCAGCCAGTC CATCACCTGC
 701  AATGTGGCCC ACCCGGCAAG CAGCACCAAG GTGGACAAGA AAATTGAGCC
 801  ACCTCTTGGG TGGACCATCC GTCTTCATCT TCCCTCCAAA GATCAAGGAT
 851  GTACTCATGA TCTCCCTGAG CCCCATAGTC ACATGTGTGG TGGTGGATGT
 901  GAGCGAGGAT GACCCAGATG TCCAGATCAG CTGGTTTGTG AACAACGTGG
 951  AAGTACACAC AGCTCAGACA CAAACCCATA GAGAGGATTA CAACAGTACT
1001  CTCCGGGTGG TCAGTGCCCT CCCCATCCAG CACCAGGACT GGATGAGTGG
1051  CAAGGAGTTC AAATGCAAGG TCAACAACAA AGACCTCCCA GCGCCCATCG
1101  AGAGAACCAT CTCAAAACCC AAAGGGTCAG TAAGAGCTCC ACAGGTATAT
1151  GTCTTGCCTC CACCAGAAGA AGAGATGACT AAGAAACAGG TCACTCTGAC
1201  CTGCATGGTC ACAGACTTCA TGCCTGAAGA CATTTACGTG GAGTGGACCA
1251  ACAACGGGAA AACAGAGCTA AACTACAAGA ACACTGAACC AGTCCTGGAC
1301  TCTGATGGTT CTTACTTCAT GTACAGCAAG CTGAGAGTGG AAAAGAAGAA
1351  CTGGGTGGAA AGAAATAGCT ACTCCTGTTC AGTGGTCCAC GAGGGTCTGC
1401  ACAATCACCA CACGACTAAG AGCTTCTCCC GGACTCCGGG TAAATGAGCT
1451  CAGCACCCAC AAAACTCTCA GGTCCAAAGA GAGACCCACA CTCATCTCCA
1501  TGCTTCCCTT GTATAAATAA AGCACCCAGC AATGCCTGGG ACCATGTAAA
1551  AAAAAAAAAA AAAGGAATTC  (SEQ ID NO:6)
```

FIG. 2a

OKT 3 HEAVY CHAIN PROTEIN SEQUENCE DEDUCED FROM DNA SEQUENCE

```
  1  MERHWIFLLL LSVTAGVHSQ VQLQQSGAEL ARPGASVKMS CKASGYTFTR
 51  YTMHWVKQRP GQGLEWIGYI NPSRGYTNYN QKFKDKATLT TDKSSSTAYM
101  QLSSLTSEDS AVYYCARYYD DHYCLDYWGQ GTTLTVSSAK TTAPSVYPLA
151  PVCGDTTGSS VTLGCLVKGY FPEPVTLTWN SGSLSSGVHT FPAVLQSDLY
201  TLSSSVTVTS STWPSQSITC NVAHPASSTK VDKKIEPRGP TIKPCPPCKC
251  PAPNLLGGPS VFIFPPKIKD VLMISLSPIV TCVVVDVSED DPDVQISWFV
301  NNVEVHTAQT QTHREDYNST LRVVSALPIQ HQDWMSGKEF KCKVNNKDLP
351  APIERTISKP KGSVRAPQVY VLPPPEEEMT KKQVTLTCMV TDFMPEDIYV
401  EWTNNGKTEL NYKNTEPVLD SDGSYFMYSK LRVEKKNWVE RNSYSCSVVH
451  EGLHNHHTTK SFSRTPGK*  (SEQ ID NO:7)
```

FIG. 2b

```
                1                       23                      42
                NN      N                 N         N     N
RES TYPE        SBspSPESssBSbSsSssPSPSPsPSsse*s*p*Pi^ISsSe
Okt3vl          QIVLTQSPAIMSASPGEKVTMTCSASS.SVSYMNWYQQKSGT
REI             DIQMTQSPSSLSASVGDRVTITCQASQDIIKYLNWYQQIPGK
                ? ?
                  CDR1    (LOOP)          *******
                  CDR1    (KABAT)        **********


                          56                                    85
                N  NN
RES TYPE        *IsiPpleesesssSBEsePsPSBSSEsPspsPsseesSPePb
Okt3vl          SPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAAT
REI             APKLLIYEASNLQAGVPSRFSGSGSGTDYTFTISSLQPEDIAT (SEQ
ID NO:8)
                ?  ??                              ?  ?
                      *******  CDR2 (LOOP/KABAT)

                               102   108
RES TYPE        PiPIPies**iPIIsPPSPSPSS
Okt3vl          YYCQQWSSNPFTFGSGTKLEINR (SEQ ID NO:29)
REIvl           YYCQQYQSLPYTFGQGTKLQITR (SEQ ID NO:9)
                              ?    ?
                    ******       CDR3 (LOOP)
                  *********      CRD3(KABAT)
```

FIG. 3

```
          NN N                    23 26    32 35  N39  43
RES TYPE  SESPs^SBssS^sSSsSpSpSPsPSEbSBssBePi^PIpiesss
Okt3h     QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMNHWVKQRPGQ
KOL       QVQLVESGGGVVQPGRSLRLSCSSSGFIFSSYAMYWVRQAPGK
               ?                  ??
                                        ******     CDR1 (LOOP)
                                            *****  CDR1 (KABAT)

                    52a   60     65     N N  N   82abc     89
RES TYPE  IIeIppp^sssssssss^ps^pSSsbSpseSsSseSp^pSpsSBssS^ePb
Okt3vh    GLEWIGYINPSRGYTNTNQKFKRKATLTTDKSSSTAYMQLSSLTSEDSAV
KOL       GLEWVAIIWDDGSDQHYADSVKGRFTISRDNSKNTLFLQMDSLPPEDTGV
             ??                      ? ?  ?  ?  ?            ?
                  ***********         CDR2   (LOOP)
                  ******************  CDR2   (KABAT)

           92 N                   107    113
RES TYPE  PiPIEissssiiisssbibi*EIPIP*spSBSS
Okt3vh    YYCARYYDDHY.......CLDYWGQGTTLTVSS   (SEQ ID NO:30)
KOL       YFCARDGGHGFCSSASCFGPDYWGQGTPVTVSS   (SEQ ID NO:10)
               ***************  CRD4 (KABAT/LOOP)
```

FIG. 4

OKT 3 HEAVY CHAIN CDR GRAFTS

1. gh341 and derivatives

```
          1                                26        35  39  43
Okt3vh    QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQ
gH341     QVQLVESGGGVVQOGRSLRLSCSSSGYTFTRYTMHWVRQAPGK   JA178
gH341A    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA185

gH341E    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA198
gH341*    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA207
gH341*    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA209
gH341D    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA197
gH341*    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA199
gH341C    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA184

gH341*    QVQLVQSGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA203
gH341*    QVQLVESGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA205
gH341B    QVQLVESGGGVVQPGRSLRLSCSSSGYTFTRYTMHWVRQAPGK   JA183
gH341*    QVQLVQSGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA204
gH341*    QVQLVESGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA206
gH341*    QVQLVQSGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA208
KOL       QVQLVESGGGVVQPGRSLRLSCSSSGFIFSSYAMYWVRQAPGK
```

FIG. 5a

```
          44    50              65                  83
Okt3vh    GLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLT
gH341     GLEWVAYINPSRGYTNYNQKFKDRFTISRDNSKNTLFLQMDSLR   JA178
gH341A    GLEWIGYINPSRGYTNYNQKVKDRFTISIDKSKSTAFLQMDSLR   JA185

gH341E    GLEWIGYINPSRGYTNYNQKVKDRFTISIDKSKSTAFLQMDSLR   JA198
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISIDKSKNTAFLQMDSLR   JA207
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTAFLQMDSLR   JA209
gH341D    GLEWIGYINPSRGYTNYNQKVKDRFTISIDKSKNTLFLQMDSLR   JA197
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTLFLQMDSLR   JA199
gH341C    GLEWVAYINPSRGYTNYNQKFKDRFTISRDNSKNTLFLQMDSLR   JA184

gH341*    GLEWIGYINPSRGYTNYNOKVKDRFTISIDKSKSTAFLQMDSLR   JA207
gH341*    GLEWIGYINPSRGYTNYNOKVKDRFTISIDKSKSTAFLQMDSLR   JA205
gH341B    GLEWIGYINPSRGYTNYNOKVKDRFTISIDKSKSTAFLQMDSLR   JA183
gH341*    GLEWIGYINPSRGYTNYNOKVKDRFTISIDKSKSTAFLQMDSLR   JA204
gH341*    GLEWIGYINPSRGYTNYNOKVKDRFTISIDKSKSTAFLQMDSLR   JA206
gH341*    GLEWIGYINPSRGYTNYNOKVKDRFTISIDKSKNTAFLQMDSLR   JA208
KOL       GLEWVAIIWDDGSDQHYADSVKGRFTISRDNSKNTLFLQMDSLR
```

FIG. 5b

	84 95 102 113		SEQ ID NO:
Okt3vh	SEDSAVYYCARYYDDHY.....CLDYWGQGTTLTVSS		30
gH341	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA178	11
gH341A	PEDTAVYYCARYYDDHY.....CLDYWGQGTTLTVSS	JA185	12
gH341E	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA198	13
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA207	14
gH341D	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA197	15
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA209	16
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA199	17
gH341C	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA184	18
gH341*	PEDTAVYYCARYYDDHY.....CLDYWGQGTTLTVSS	JA203	19
gH341*	PEDTAVYYCARYYDDHY.....CLDYWGQGTTLTVSS	JA205	20
gH341B	PEDTAVYYCARYYDDHY.....CLDYWGQGTTLTVSS	JA183	21
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA204	22
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA206	23
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA208	24
KOL	PEDTGVYFCARDGGHGFCSSASCFGPDYWGQGTPVTVSS		10

FIG. 5c

OKT3 LIGHT CHAIN CDR GRAFTING

1. gL221 and derivatives

```
          1                        24        34      42
Okt3vl    QIVLTQSPAOMSASPGEKVTMTCSASS.SVSYMNWYQQKSGT
gL221     DIQMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
gL221A    QIVMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
gL221B    QIVMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
gL221C    DIQMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
REI       DIQMTQSPSSLSASVGDRVTITCQASQDIIKYLNWYQQTPGK

          43     50    56                          85
Okt3vl    SPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAAT
gL221     APKLLIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
gL221A    APKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
gL221B    APKLLIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
gL221C    APKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
REI       APKLLIYEASNLQAGVPSRFSGSGSGTDYTFTISSLQPEDIAT (SEQ ID NO:8)

          86   91   96         108
Okt3vl    YYCQQWSSNPFTFGSGTKLEINR     (SEQ ID NO:29)
gL221     YYCQQWSSNPETFGQGTKLQITR     (SEQ ID NO:25)
gL221A    YYCQQWSSNPETFGQGTKLQITR     (SEQ ID NO:26)
gL221B    YYCQQWSSNPETFGQGTKLQITR     (SEQ ID NO:27)
gL221C    YYCQQWSSNPETFGQGTKLQITR     (SEQ ID NO:28)
REI       YYCQQYQSLPYTFGQGTKLQITR     (SEQ ID NO:9)
```

CDR'S ARE UNDERLINED

FRAMEWORK RESIDUES INCLUDED IN THE GENE ARE DOUBLE UNDERLINED

FIG. 6

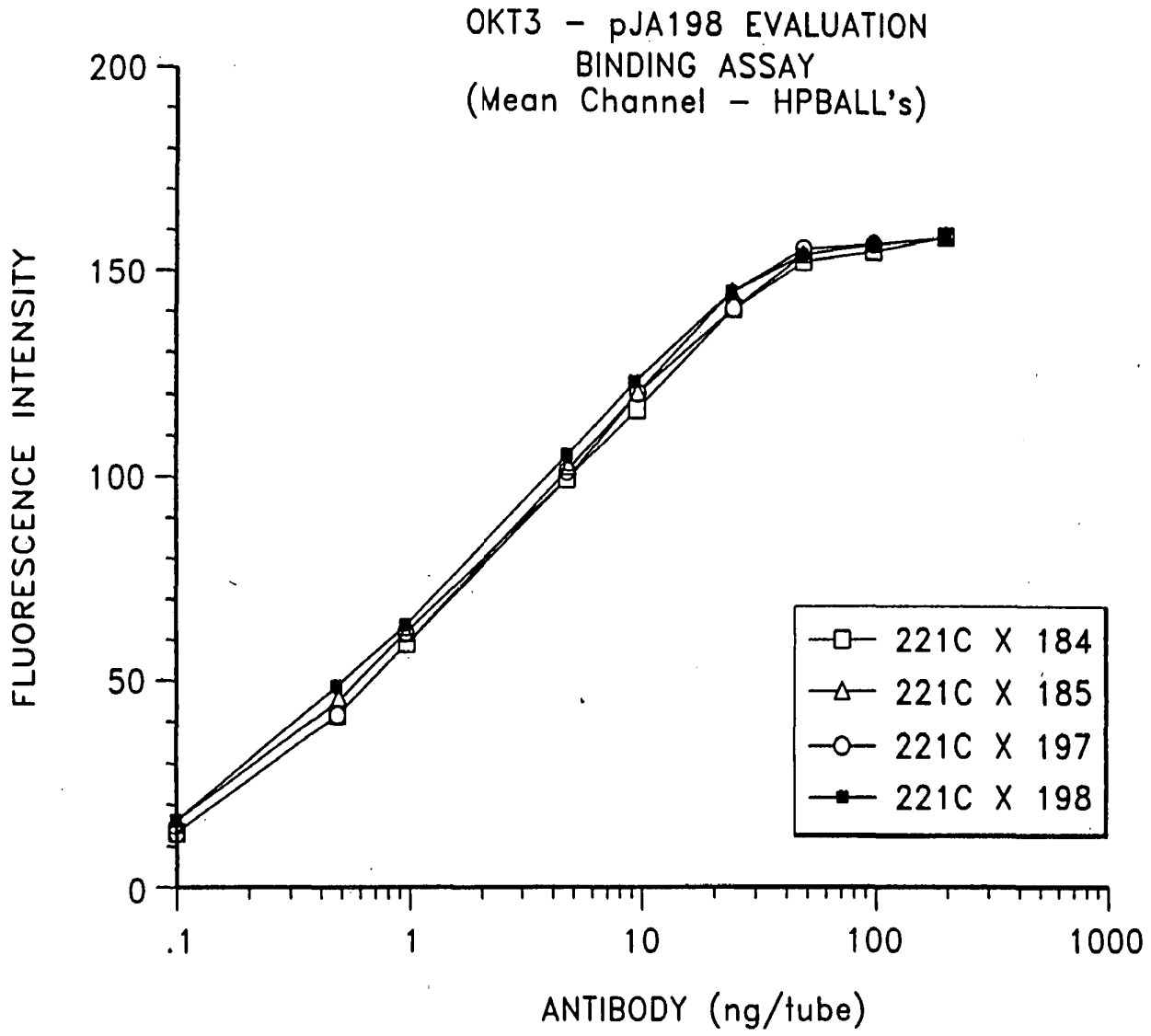

FIG. 7

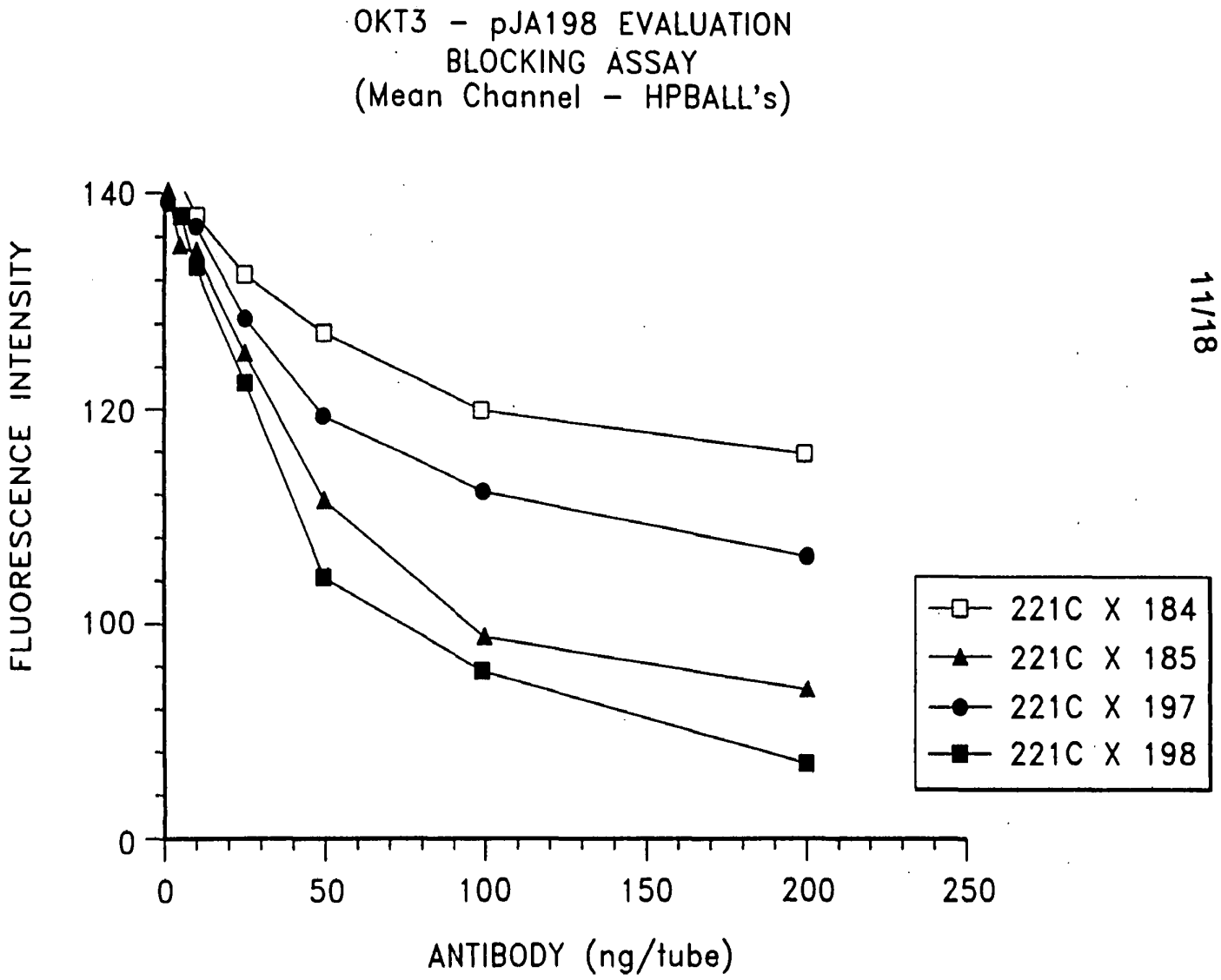

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FIG. 8

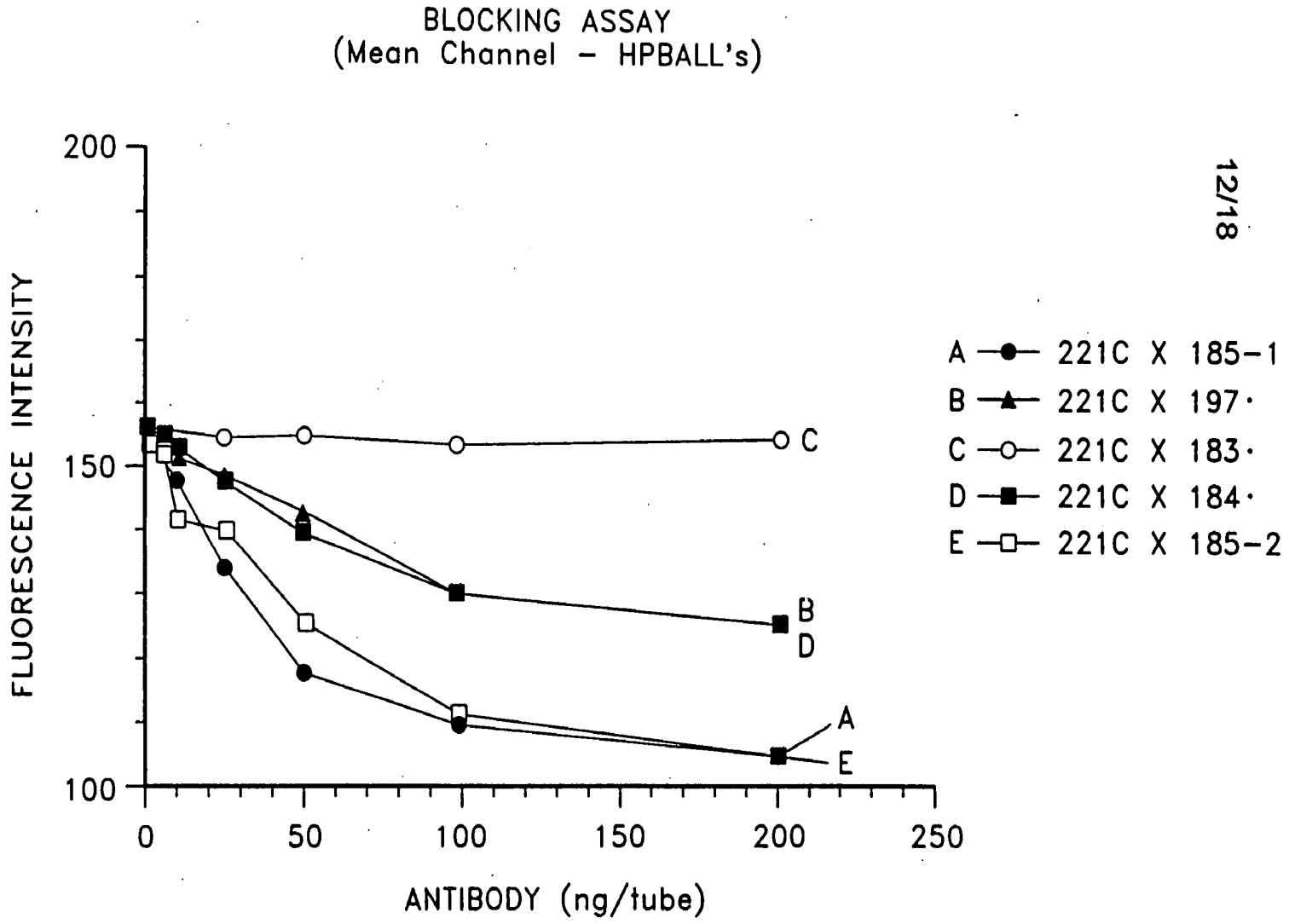

FIG. 9

FIG. 10a

OKT3 – GRAFTED HEAVY CHAINS
BINDING ASSAY
(Mean Channel – HPALL's)
FLUORESCENCE INTENSITY
0
50
100
150
200
ANTIBODY (ng/tube)
.1
1
10
100
1000
A
B
C
D
E
F
G
H
141 X 144
2210 X 185
2210 X 199
2210 X 204
2210 X 205
2210 X 207
2210 X 208
2210 X 209

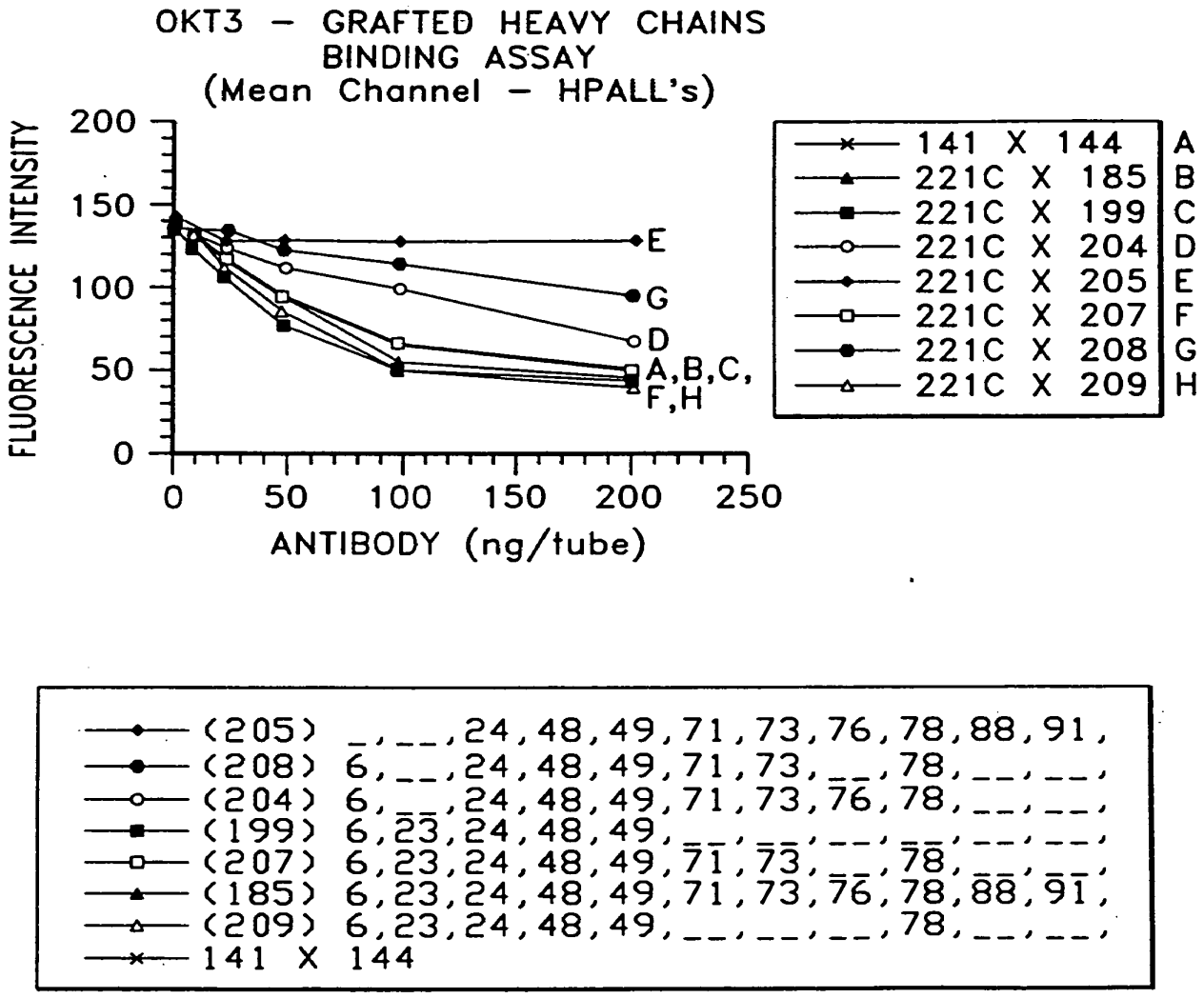
OKT3 – GRAFTED HEAVY CHAINS
BINDING ASSAY
(Mean Channel – HPALL's)
FLUORESCENCE INTENSITY
200
150
100
50
0
0
50
100
150
200
250
ANTIBODY (ng/tube)
E
G
D
A,B,C,
F,H
141 X 144 A
221C X 185 B
221C X 199 C
221C X 204 D
221C X 205 E
221C X 207 F
221C X 208 G
221C X 209 H
(205) _,__,24,48,49,71,73,76,78,88,91,
(208) 6,__,24,48,49,71,73,__,78,__,__,
(204) 6,__,24,48,49,71,73,76,78,__,__,
(199) 6,23,24,48,49,__,__,__,__,__,__,
(207) 6,23,24,48,49,71,73,__,78,__,__,
(185) 6,23,24,48,49,71,73,76,78,88,91,
(209) 6,23,24,48,49,__,__,__,78,__,__,
141 X 144

FIG. 10b

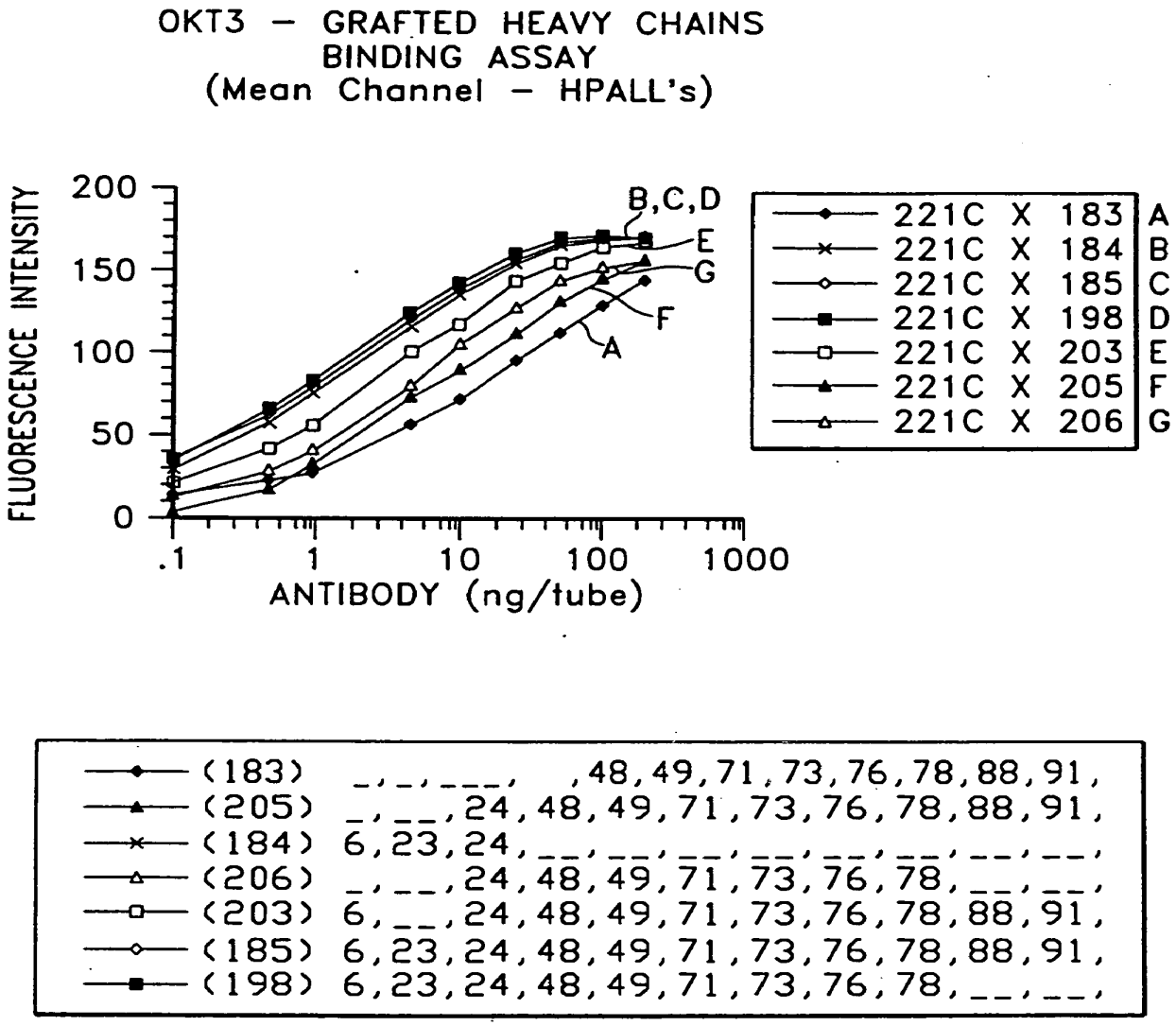
OKT3 – GRAFTED HEAVY CHAINS
BINDING ASSAY
(Mean Channel – HPALL's)
FLUORESCENCE INTENSITY
200
150
100
50
0
.1
1
10
100
1000
ANTIBODY (ng/tube)
B,C,D
E
G
F
A
221C X 183 A
221C X 184 B
221C X 185 C
221C X 198 D
221C X 203 E
221C X 205 F
221C X 206 G
(183) _,_,___, ,48,49,71,73,76,78,88,91,
(205) _,__,24,48,49,71,73,76,78,88,91,
(184) 6,23,24,__,__,__,__,__,__,__,__,
(206) _,__,24,48,49,71,73,76,78,__,__,
(203) 6,__,24,48,49,71,73,76,78,88,91,
(185) 6,23,24,48,49,71,73,76,78,88,91,
(198) 6,23,24,48,49,71,73,76,78,__,__,

FIG. 11a

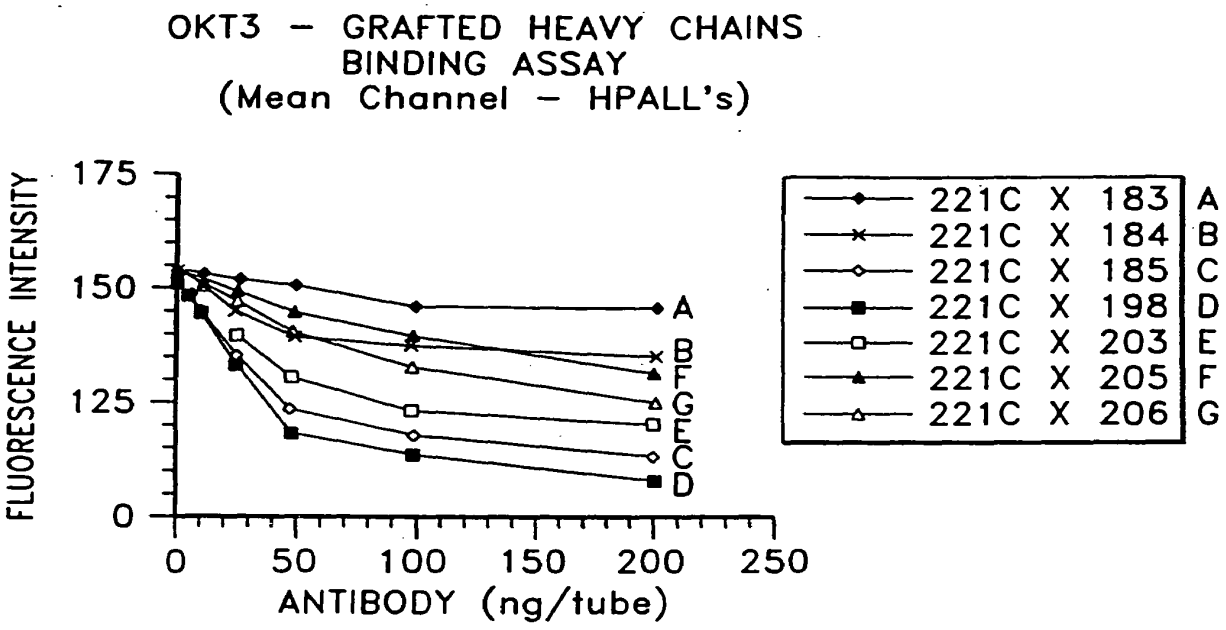
OKT3 – GRAFTED HEAVY CHAINS
BINDING ASSAY
(Mean Channel – HPALL's)
FLUORESCENCE INTENSITY
175
150
125
0
0
50
100
150
200
250
ANTIBODY (ng/tube)
A
B
F
G
E
C
D
221C X 183 A
221C X 184 B
221C X 185 C
221C X 198 D
221C X 203 E
221C X 205 F
221C X 206 G
(183) _,_,___, ,48,49,71,73,76,78,88,91,
(205) _,__,24,48,49,71,73,76,78,88,91,
(184) 6,23,24,__,__,__,__,__,__,__,__,
(206) _,__,24,48,49,71,73,76,78,__,__,
(203) 6,__,24,48,49,71,73,76,78,88,91,
(185) 6,23,24,48,49,71,73,76,78,88,91,
(198) 6,23,24,48,49,71,73,76,78,__,__,

FIG. 11b

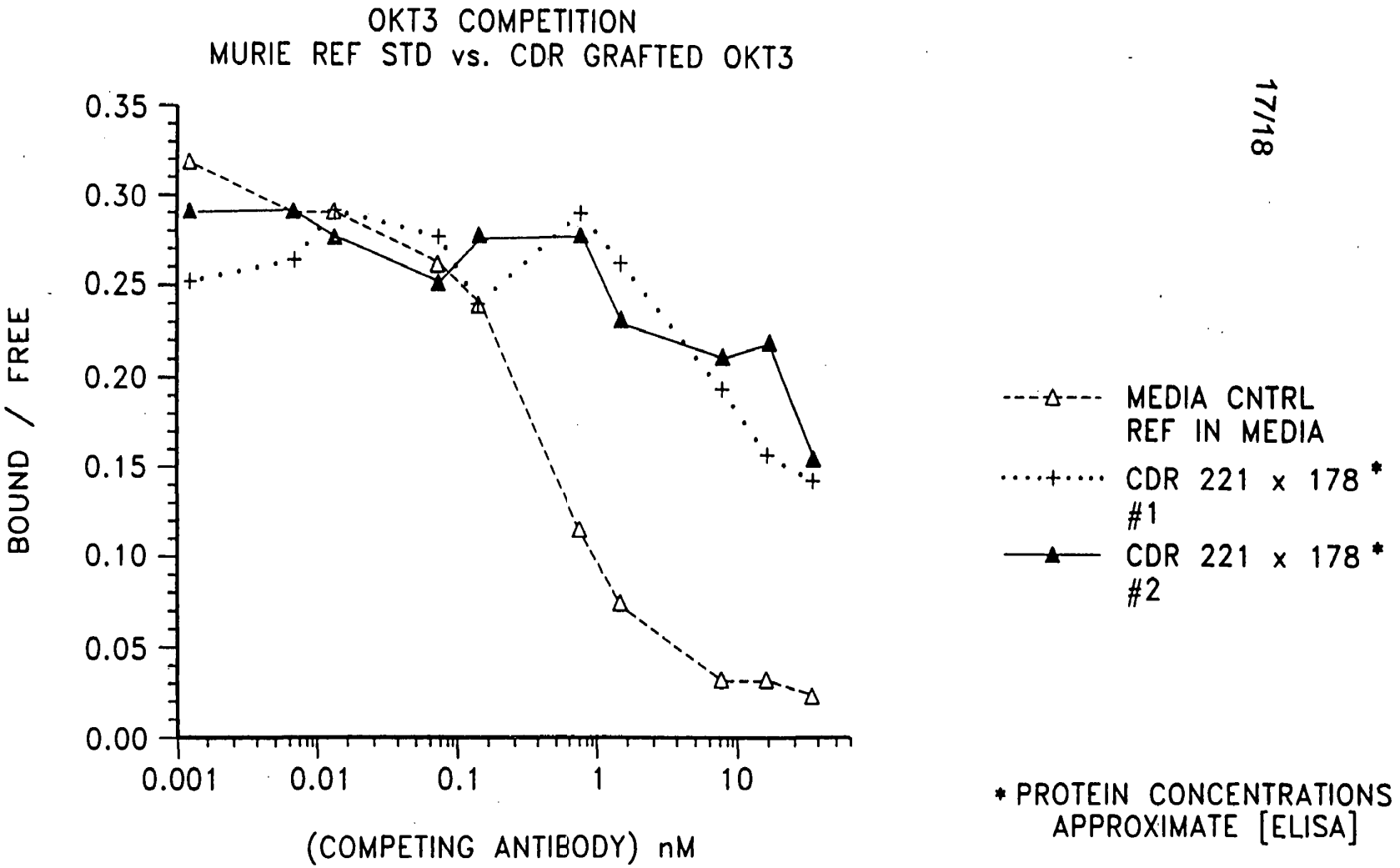

FIG. 12

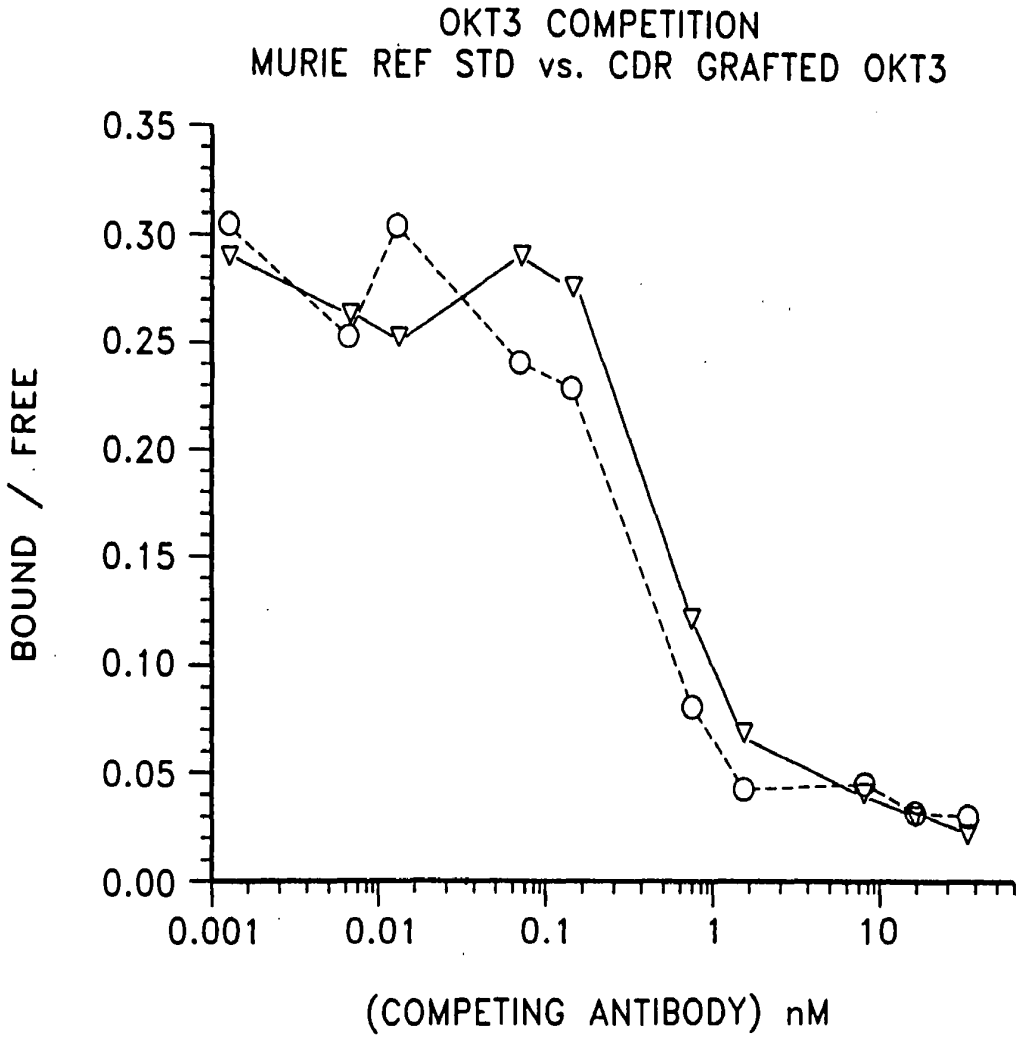

FIG. 13

DOCKET NO.: CARP-0009 PATENT

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name; and

I verily believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: HUMANISED ANTIBODIES the specification of which:

___ is attached hereto.

✓ International was filed on 21 December 1990 as Application Serial No. PCT/GB90/02017 and was amended on _____. (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with 37 CFR § 1.56(a).

I hereby claim foreign priority benefits under 35 U.S.C. § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of any application on which priority is claimed:

Country	Number	Date Filed	Priority Claimed
U.K.	8928874.0	21.12.89	yes
			___
			___

PTO-19

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose material information as defined in 37 CFR § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	Filing Date	Status (patented, pending)

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

Francis A. Paintin

Registration Nos. 19,386

of the firm of **WOODCOCK WASHBURN KURTZ MACKIEWICZ & NORRIS**, One Liberty Place - 46th Floor, Philadelphia, Pennsylvania 19103, and

Address all telephone calls and correspondence to:

Francis A. Paintin

WOODCOCK WASHBURN KURTZ MACKIEWICZ & NORRIS
One Liberty Place - 46th Floor
Philadelphia, PA 19103
Telephone No. **215-568-3100.**

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the

United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1	Full Name JOHN ROBERT ADAIR	Inventor's Signature John Robert Adair	Date 13/8/91
	Residence 23 George Road, Stokenchurch, High Wycombe, Buckinghamshire HP14 3RN, U.K.	Citizenship U.K.	
	Post Office Address 23 George Road, Stokenchurch, High Wycombe, Buckinghamshire HP14 3RN, U.K.		
2	Full Name DILJEET SINGH ATHWAL	Inventor's Signature	Date 13/8/91
	Residence Flat 35, Knollys House, Tavistock Square, London WC1, U.K.	Citizenship U.K.	
	Post Office Address Flat 35, Knollys House, Tavistock Square, London WC1, U.K.		
3	Full Name JOHN SPENCER EMTAGE	Inventor's Signature John Spencer Emtage	Date 13/8/91
	Residence 49 Temple Mill Island, Temple Marlow, Buckinghamshire, SL7 1SQ, U.K.	Citizenship U.K.	
	Post Office Address 49 Temple Mill Island, Temple, Marlow, Buckinghamshire SL7 1SQ, U.K.		
4	Full Name	Inventor's Signature	Date
	Residence	Citizenship	
	Post Office Address		
5	Full Name	Inventor's Signature	Date
	Residence	Citizenship	
	Post Office Address		

DOCKET NO.: CARP0001-112 **PATENT**
PRELIMINARY AMENDMENT AND REQUEST FOR INTERFERENCE UNDER 37 C.F.R. § 42.202 DATED NOVEMBER 21, 2005

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of: John R. Adair, Diljeet S. Athwal and John S. Emtage

Serial No.: Not Yet Assigned **Art Unit: Not Yet Assigned**

Filing Date: November 21, 2005 **Examiner: Not Yet Assigned**

For: HUMANISED ANTIBODIES

Customer No.: 34132

EXPRESS MAIL LABEL NO.: EV146 601 565US
DATE OF DEPOSIT: November 21, 2005

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

PRELIMINARY AMENDMENT
AND
REQUEST FOR INTERFERENCE UNDER 37 CFR § 42.202

Applicant respectfully requests entry of the following amendments prior to the calculation of filing fees:

Amendments To The Specification begin on page 2 of this paper.

Listing of the Claims, reflecting current amendments, begins on page 3 of this paper.

Remarks begin on page 4 of this paper.

The **Conclusion** is found on page 12.

Appendix A is found on page 14.

Appendix B is found on page 17.

Appendix C is found on page 19.

Appendix D is found on page 20.

Appendix E is found on page 21.

Carter Exhibit 2003
Carter v. Adair
Interference No. 105,744

AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph containing the cross-reference to related applications on page 1 of the specification with the following:

This application is a Continuation of U.S. Application Serial No. 08/846,658, Filed May 1, 1997, which is a Continuation of U.S. Application Serial No. 08/303,569, Filed September 7, 1994, now U.S. Patent No. 5,859,205, Issued January 12, 1999, which is a File Wrapper Continuation of U.S. Application Serial No. 07/743,329, Filed September 17, 1991, Abandoned, which is a 35 U.S.C. 371 National Stage Application of International Application No. PCT/GB90/02017, International Filing Date of December 21, 1990, which claims priority to Great Britain Application No. 8928874.0, Filed December 21, 1989, all applications are incorporated by reference herein in their entireties.

LISTING OF CLAIMS

This listing of claims represents the current status of the claims.

Claims 1-23 **(cancelled)**

Claim 24 **(new)** A humanised antibody heavy chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises an amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof, as numbered according to Kabat.

Claim 25 **(new)** A humanised antibody comprising the variable domain of claim 24.

REMARKS

Claims 24 to 25 are pending. Applicants hereby request an interference in accordance with 37 CFR § 42.202. It is noted that U.S. Patent No. 6,407,213, whose claims present the basis for an interference, is classified in Class 530, and was examined by Examiners Minh-Tam Davis and Anthony C. Caputa.

Compliance with 37 CFR § 42.202

Applicants respectfully submit that all requirements of 37 CFR § 42.202 have been met and respectfully request examination of the present application and declaration of an interference.

(a) Identifying The Patent

Applicants request that an interference be declared between Applicants' above-identified application and U.S. Patent No. 6,407,213 B1 (hereinafter the 213 patent), issued to Carter et al. on June 18, 2002, a copy of which is enclosed herewith.

(b) Compliance with 35 USC § 135(b)

Although the present rules do not require a showing of compliance under 35 USC § 135(b), Applicants submit the following to advance the examination of the present application to allowability. The present application is a Continuation of U.S. Application Serial No. 08/846,658, Filed May 1, 1997, which is a Continuation of U.S. Application Serial No. 08/303,569, Filed September 7, 1994, now U.S. Patent No. 5,859,205, Issued January 12, 1999, which is a File Wrapper Continuation of U.S. Application Serial No. 07/743,329, Filed September 17, 1991, Abandoned, which is a 35 U.S.C. 371 National Stage Application of International Application No. PCT/GB90/02017, International Filing Date of December 21, 1990 (hereinafter "the PCT application," a copy of which is enclosed herewith), which claims priority to Great Britain Application No. 8928874.0, Filed December 21, 1989. Claims 1-23 as filed in the PCT application are attached as Appendix A.

Under 35 USC § 135(b)(1), Applicants must show that they had a claim to the same, or substantially the same, subject mater as a claim of the 213 patent within one year of the issuance of the 213 patent, or June 18, 2003. The 213 patent issued on June 18, 2002. The PCT application was filed on December 21, 1990, over 10 years earlier than the 213 patent issued. The time limit of Section 135(b)(1) has been complied with fully. See *Corbett v. Chisholm*, 196 USPQ 337 (CCPA 1977).

To meet the "same or substantially the same invention" requirement of Section 135(b)(1), Applicants must show that their claim contained all material limitations, i.e. limitations necessary to patentability, of the claim of the 213 patent alleged to be to the same, or substantially the same, invention. *Corbett v. Chisholm*, 196 USPQ 337 (C.C.P.A. 1977), *citing Wetmore v. Miller*, 477 F.2d 960, 177 USPQ 699 (C.C.P.A. 19730).

As is evident from Appendix A, Applicants made a claim for the same, or substantially the same, subject matter as a claim of the 213 patent well before the issuance of the 213 patent. Claim 16 of the PCT application, as depending from claim 8, is to substantially the same subject matter as at least claim 1 of the 213 patent. For the Office's convenience, all three claims are duplicated below.

> **Claim 8 of the PCT application**: A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, **58** and 71.
>
> **Claim 16 of the PCT application**: A CDR-grafted antibody heavy or **light chain** or molecule according to any one of the preceding claims comprising human acceptor residues and non-human donor residues.
>
> **Claim 1 of the 213 patent**: A humanized antibody variable domain comprising non-human Complementarity Determining Region (CDR) amino acid residues

which bind an antigen incorporated into a human antibody variable domain, and further comprising a Framework Region (FR) amino acid substitution at a site selected from the group consisting of: 4L, 38L, 43L, 44L, **58L**, 62L, 65L, 66L, 67L, 68L, 69L, 73L, 85L, 98L, 2H, 4H, 36H, 39H, 43H, 45H, 69H, 70H, 74H, and 92H, utilizing the numbering system set forth in Kabat.

Both claim 16 of the PCT application, as it depends from claim 8, and claim 1 of the 213 patent are directed to variable domains comprising non-human Complementarity Determining Regions ("CDRs") that bind antigen, i.e., antigen binding regions, human framework regions, and a non-human framework amino acid at residue 58 of the light chain, using Kabat numbering. Both claims also comprise a human framework region; claim 1 of the 213 patent simply recites it differently -- if one incorporates non-human CDR amino acid residues into a human antibody variable domain, one ends up with a human framework region. Further, the 213 patent defines "humanized antibody" as an antibody having a framework region "having substantially the amino acid sequence of a human immunoglobulin," i.e., a human framework region (see column 8, lines 11-17, of the 213 patent). "Antigen binding regions," as recited in claim 8 of the PCT application, refers to CDRs (see page 8, lines 10-13, of the PCT application). As indicated on page 8, lines 22-24 of the PCT application, all numbering is according to Kabat. Claim 16 implicitly contains the recitation that the amino acid be substituted. As indicated on page 17, lines 1-19 of the PCT application, substitution of the non-human framework residues for the human framework residues is required when the donor and framework residue at any of the recited positions differ. While Applicants' claim recites a "light **chain**" and claim 1 of the 213 patent recites a "variable domain," Applicants submit that this is merely a difference in scope, and not a material difference. *See Corbett.*

(c) Presentation Of A Proposed Count

Applicants present in Appendix B, attached hereto, a proposed count that is presented in the "alternative" format as claim 30 or claim 80 of the 213 patent or Applicants' claim 24. All alternatives are to the same patentable invention.

All alternatives recite that the variable domain comprises non-human complementarity determining region amino acid residues which bind an antigen. All alternatives also comprise a human framework region. As discussed above for compliance with Section 135(b), the 213 patent defines "humanized antibody" as an antibody having a framework region "having substantially the amino acid sequence of a human immunoglobulin," i.e., a human framework region (see column 8, lines 11-17, of the 213 patent). And, if one incorporates non-human CDR amino acid residues into a human antibody variable domain, as recited in claims 30 and 80, one ends up with a human framework region. Further, all alternatives require only that one of the listed framework residues be substituted. Claims 30 and 80 of the 213 patent and Applicants' claim 24 recite substituting, *inter alia*, residue 78 of the heavy chain.

Although claim 30 of the 213 patent and Applicants' claim 24 do not recite the alternative recitations (a) through (c) of claim 80 of the 213 patent, e.g., "noncovalently binds antigen directly," such limitations are clearly implicit for all the framework residue substitutions recited in claim 80.

Although claims 30 and 80 of the 213 patent do not recite that the variable domain is from the light chain, it clearly must be the case when a light chain framework residue, i.e., one designated with an "L," is to be substituted.

Claim 30 of the 213 patent recites an "antibody" while the remaining alternatives recite a "variable domain." The antibody recitation is anticipated by and/or obvious over the recitation of variable domain and *vice versa*. "Humanized antibody" is defined in the 213 patent to comprise, *inter alia*, at least one variable domain (see column 8, lines 26-28, of the 213 patent).

Claim 30 of the 213 patent further recites a specific antibody target. The alternatives of the count that do not recite a particular target are clearly anticipated thereby. Considering the target, i.e., the tyrosine kinase receptor for HER2, $p185^{HER2}$, Applicants submit that claim 30 is clearly obvious over the other alternatives of the count for the reasons that follow.

The import of $p185^{HER2}$ to cancer, particularly breast cancer, had been well documented well before the priority date of the 213 patent. See, for example, Slamon, D.J. et al., *Science*, 235:177-182,1987. The development of antibodies against $p185^{HER2}$ for use in therapy had also

been well documented, as was the use of antibodies other than hybridoma-produced monoclonal antibodies. Applicants respectfully submit that, in view of the knowledge of the art at the time, a humanized antibody against $p185^{HER2}$ would have been obvious over the remaining alternatives of the count.

(d) Identification of claims corresponding to the count

Applicants identify all of the 213 patent claims, claims 1-82, and all of Applicants' pending claims, claims 24-25, as corresponding to the proposed count. All of said claims are either anticipated by, or obvious over, the proposed count as required by 37 CFR § 41.207(b)(2).

More specifically, the alternatives of the proposed count recite framework residue substitutions recited in claims 1, 5-28, 34-63, 66, 70-77, and 79 of the 213 patent.

Claims 2, 31, 67, and 81 of the 213 patent recite that the substituted residue is the residue found at the corresponding location of the non-human antibody from which the non-human CDR amino acids are obtained. Such a claim is anticipated by the proposed count – the goal of substituting framework residues is to improve affinity to approach that of the antibody from which the CDRs are obtained. See, for example, Riechmann, et al., *Nature*, 332:323-327, 1988.

Claims 3, 32, 68, and 82 of the 213 patent recite that no human framework residue other than those set forth in the claim from which they depend has been substituted. Such claims are anticipated by, or clearly obvious over, the proposed count in view of the use of the Markush language "selected from the group consisting of." The transitional phrase "consisting of" is closed, meaning that it excludes any element not listed. See MPEP 2111.03.

Claims 29 and 78 recite an antibody comprising the humanized variable domains of the claims from which they depend. Such claims are clearly anticipated by claim 30 of the proposed count.

While claims 4, 33, 62 and 69 of the 213 patent further recite that the variable domain is a "consensus" human variable domain, use of such framework regions in making humanized antibodies was known by the priority date of the 213 patent. See, for example, column 13, lines

6-9, of U.S. Patent No. 5,530,101, issued to Queen et al., filed on December 19, 1990. Claims 62 and 69 are, thus, obvious in view of the proposed count and the art.

The recitation in claim 63 that the humanized antibody lacks immunogenicity when compared to the non-human parent upon repeated administration is the very goal of humanization and is, thus, implicit. The motivation for preparing humanized antibodies was to reduce immunogenicity. *See* column 1, lines 51-58, of the 213 patent, discussing the "Background of the Invention." References from the early 1980's are cited therein disclosing the anti-globulin response to rodent monoclonal antibodies. Claim 63 is, at a minimum, obvious over the proposed count and the art.

The alternatives recited in claim 64 include the alternatives recited in claim 80 of the count. Claim 64 is, thus, anticipated by the proposed count.

The recitation in claim 65 that the variant of claim 63 binds the antigen "**up to** 3-fold more in the binding affinity than the parent antibody binds antigen" broadly includes variants that have binding affinities equal to **and** less than the parent. As recognized in the "Background of the Invention" section of the 213 patent, at column 3, lines 50-55, humanizing antibody while **retaining** high affinity for antigen was difficult to achieve; achieving lower affinity was not. This recitation, thus, is also either anticipated by, or obvious over, the proposed count in view of the art.

All of Applicants claims, claims 24-25, are anticipated by or obvious over the proposed count. Claim 25 is anticipated by claim 30 of the count.

(e) Interference-In-Fact

"An interference-in-fact exists if the subject matter of a claim of one party would, if prior art, have anticipated or rendered obvious the subject matter of a claim of the opposing party and vice versa." 37 CFR § 41.203(a) (2004). Applicants set forth in attached Appendix C a comparison of claim 66 of the 213 patent with Applicants' claim 24, both of which correspond to the present count.

As is clear from Appendix C, each claim anticipates and/or renders obvious the other. Both claims recite a humanized variable domain. Both claims are directed to a heavy chain

variable domain. Both claims also recite that the variable domain comprises non-human complementarity determining region amino acid residues which bind an antigen. Both claims also comprise a human framework region; claim 66 of the 213 patent simply recites it differently -- if one incorporates non-human CDR amino acid residues into a human antibody variable domain, one ends up with a human framework region. Indeed, as discussed above, the 213 patent defines "humanized antibody" as an antibody having a framework region "having substantially the amino acid sequence of a human immunoglobulin," i.e., a human framework region (see column 8, lines 11-17, of the 213 patent). Both claims recite a single amino acid substitution in the framework region to be selected from a Markush group listed thereafter; both recite that residue 24 of the heavy chain is to be substituted. Finally, both claims recite that numbering is according to Kabat.

(f) Support for Applicants Claims

In attached Appendix D, Applicants illustrate the representative support in their disclosure for the limitations of their claims 24 to 25. There is, of course, additional support in Applicants' application omitted herein for the sake of brevity. In Appendix E, Applicants show support for their claim 24, filed December 21, 1989. Methods for preparing exemplary antibodies having framework substitutions are described, *inter alia*, on pages 18-23, Sections 13.1.1 through 15.3, of the GB priority application.

(g) Applicants Will Prevail on Priority

US Serial No. 08/146,206, which issued as the 213 patent, was filed on June 15, 1992 as a PCT continuation-in-part of U.S. Serial No. 07/715,272, filed June 14, 1991.

The present application is a continuation of Application Serial No. 08/846,658, filed May 1, 1997, which is a continuation of Application Serial No. 08/303,569, filed September 7, 1994, now U.S. Patent No. 5,859,205, which is a continuation of Application Serial No. 07/743,329, filed as PCT/GB90/02017, filed December 21, 1990 and which claims priority benefit of GB 8928874.0, filed December 21, 1989. Applicants' earliest constructive reduction to practice date to which they are entitled is at least as early as December 21, 1989, but not later than December

21, 1990. Regardless, both dates are earlier than the earliest filing date of the 213 patent , or June 14, 1991.

CONCLUSION

The Requested Interference Should Be Declared

Early consideration and indication of allowability of all pending claims is respectfully requested. For an interference to be declared, however, only one claim needs to be allowable. MPEP § 2307.02. Should the present examination involve rejections of applicant's claims that would have been equally applicable against the 213 patent claims, applicants respectfully note MPEP § 2307.02, which requires the approval of the Group Director for such a rejection. Applicants are presumptively the prior inventors of the claimed subject matter and only desire an interference to prove that they are the actual prior inventors. Their opportunity to do so should not be unduly delayed. In view of the foregoing, Applicants respectfully request that an interference be declared between the present application and the 213 patent.

To assist the Examiner, Applicants note the following.

(1) the proposed count for the interference should be the Proposed Count set forth in Appendix B.

(2) the claims of the 213 patent which should be designated as corresponding to the count are claims 1-82, all of the issued claims of the patent.

(3) the claims of Applicants that should be designated as corresponding to the count are claims 24-25, all pending claims of the Applicants' above-identified application.

(4) no claims of either party should be designated as not corresponding to the count since none are directed to a separate patentable invention when considering the proposed count.

The examiner is requested to contact the undersigned attorney if an interview, telephonic or personal, would facilitate allowance of the claims or declaration of an interference.

Respectfully submitted,

Doreen Yatko Trujillo
Registration No. 35,719

Date: November 21, 2005

COZEN O'CONNOR, P.C.
1900 Market Street, 6th Floor
Philadelphia, PA 19103-3508
(215) 665-5593 - Telephone
(215) 701-2005 - Facsimile

Attachments: **Appendices A-E**
Copy of U.S. Patent No. 6,407,213 B1
Copy of PCT Publication WO 91/09967

APPENDIX A

1. A CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

2. A CDR-grafted heavy chain according to claim 1 comprising donor residues at positions 23, 24, 49, 71, 73 and 78, or at positions 23, 24 and 49.

3. A CDR-grafted heavy chain according to claim 2 comprising donor residues at positions 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

4. A CDR-grafted heavy chain according to claim 2 or 3, comprising donor residues at one, some or all of positions: 1 and 3, 69 (if 48 is different between donor and acceptor), 38 and 46 (if 48 is the donor residue), 67, 82 and IS (if 67 is the donor residue), 91, and any one or more of 9, 11, 41, 87, 108, 110 and 112.

5. A CDR-grafted heavy chain according to any of the preceding comprising donor CDRs at positions 26-35, 50-65 and 95-100.

6. A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 1 and/or 3 and 46 and/or 47.

7. A CDR-grafted light chain according to claim 6 comprising donor residues at positions 46 and 47.

8. A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor

residues at at least one of positions 46, 48, 58 and 71.

9. A CDR-grafted light chain according to claim 8 comprising donor residues at positions 46, 48, 58 and 71.

10. A CDR-grafted light chain according to claim 8 or 9, comprising donor residues at positions 2, 4, 6, 35, 36, 38, 44, 47, 49, 62, 64-69, 85, 87, 98, 99, 101 and 102.

11. A CDR-grafted light chain according to claim 9 or 10, comprising donor residues at one, some or all of positions: 1 and 3, 63, 60 (if 60 and 54 are able to form a potential saltbridge), 70 (if 70 and 24 are able to form a potential saltbridge), 73 and 21 (if 47 is different between donor and acceptor), 37 and 45 (if 47 if different between donor and acceptor), and any one or more of 10, 12, 40, 83, 103 and 105.

12. A CDR-grafted light chain according to any one of claims 6-11, comprising donor CDRs at positions 24-34, 50-56 and 89-97.

13. A CDR-grafted antibody molecule comprising at least one CDR-grafted heavy chain according to any one of claims 1-5 and at least one CDR-grafted light chain according to any one of claims 6-12.

14. A CDR-grafted antibody molecule according to claim 13, which is a site-specific antibody molecule.

15. A CDR-grafted antibody molecule according to claim 13 which has specificity for an interleukin, hormone or other biologically active compound or a receptor therefor.

16. A CDR-grafted antibody heavy or light chain or molecule according to any one of the preceding claims comprising human acceptor residues and non-human donor residues.

17. A DNA sequence which codes for a CDR-grafted heavy chain according to claim 1 or a

CDR-grafted light chain according to claim 6 or claim 8.

18. A cloning or expression vector containing a DNA sequence according to claim 17.

19. A host cell transformed with a DNA sequence according to claim 17.

20. A process for the production of a CDR-grafted antibody sequence according to claim 17 in a transformed host cell.

21. A process for producing a CDR-grafted antibody product comprising: (a) producing in an expression vector an operon having a DNA sequence which encodes an antibody heavy chain according to claim 1; and/or (b) producing in an expression vector an operon having a DNA sequence which encodes a complementary antibody light chain according to claim 6 or claim 8; (c) transfecting a host cell with the or each vector; and (d) culturing the transfected cell line to produce the CDR-grafted antibody product.

22. A therapeutic or diagnostic composition comprising a CDR-grafted antibody heavy chain according to claim 1, or a CDR-grafted light chain according to claim 6 or claim 8, or a CDR-grafted antibody molecule according to claim 13 in combination with a pharmaceutically acceptable carrier, diluent or excipient.

23. A method of therapy or diagnosis comprising administering an effective amount of a CDR-grafted heavy chain according to claim 1, or a CDR-grafted light chain according to claim 6 or claim 8, or a CDR-grafted antibody molecule according to claim 13 to a human or animal subject.

APPENDIX B

Proposed Count for interference

Claim 30 of the 213 patent

An antibody which binds $p185^{HER2}$ and comprises a humanized antibody variable domain, wherein the humanized antibody variable domain comprises non-human Complementarity Determining Region (CDR) amino acid residues which bind $p185^{HER2}$ incorporated into a human antibody variable domain, and further comprises a Framework Region (FR) amino acid substitution at a site selected from the group consisting of: 4L, 38L, 43L, 44L, 46L, 58L, 62L, 65L, 66L, 67L, 68L, 69L, 73L, 85L, 98L, 2H, 4H, 36H, 39H, 43H, 45H, 69H, 70H, 74H, 75H, 76H, **78H** and 92H, utilizing the numbering system set forth in Kabat.

OR

Claim 80 of the 213 patent

A humanized antibody variable domain comprising non-human Complementarity Determining Region (CDR) amino acid residues which bind an antigen incorporated into a human antibody variable domain, and further comprising a Framework Region (FR) amino acid substitution where the substituted FR residue:

(a) noncovalently binds antigen directly;

(b) interacts with a CDR; or

(c) participates in the V_L - V_H interface by affecting the proximity or orientation of the V_L and V_H regions with respect to one another, and wherein the substituted FR residue is at a site selected from the group consisting of: 4L, 38L, 43L, 44L, 58L, 62L, 65L, 66L, 67L, 68L, 69L, 73L, 85L, 98L, 2H, 4H, 24H, 36H, 39H, 43H, 45H, 69H, 70H, 73H, 74H, 76H, **78H**, 92H and 93H, utilizing the numbering system set forth in Kabat.

OR

Applicants' claim 24

A humanised antibody **heavy chain** variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises an amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and **78**, and combinations thereof, as numbered according to Kabat.

APPENDIX C

Comparison of Applicants' claim 24 and claim 66 of the 213 patent

Applicants' Claim 24	213 patent Claim 66
Claim 24 – A humanised antibody heavy chain variable domain comprising	A humanized antibody heavy chain variable domain comprising
non-human complementarity determining region amino acid residues which bind an antigen and	non-human Complementarity Determining Region (CDR) amino acid residues which bind an antigen
a human framework region wherein said framework region comprises an amino acid substitution at a residue selected from the group consisting of	incorporated into a human antibody variable domain, and further comprising a Framework Region (FR) amino acid substitution at a site selected from the group consisting of:
23, **24**, 49, 71, 73, and 78, and combinations thereof,	**24H**, 73H, 76H, 78H, and 93H
as numbered according to Kabat.	utilizing the numbering system as set forth in Kabat.

APPENDIX D

Support for Applicants' claims in Applicants' Present Specification

Claim	Present Specification
Claim 24 -- A humanised antibody heavy chain variable domain comprising	page 6, lines 29-31
non-human complementarity determining region amino acid residues which bind an antigen and	page 7, line 29, through page 8, line 7 and page 17, lines 6-7
a human framework region wherein said framework region comprises an amino acid substitution at a residue selected from the group consisting of	page 7, line 29, through page 8, line 7 and page 17, lines 1-19
23, 24, 49, 71, 73, and 78, and combinations thereof,	page 7, lines 1-3
as numbered according to Kabat.	page 8, lines 22-24
Claim 25 -- A humanised antibody comprising the variable domain of claim 24.	see above support for claim 24

APPENDIX E

Support For Applicants' Claims in the GB Application, filed December 21, 1989

Claim	1989 GB Application
Claim 24 – A humanised antibody heavy chain variable domain comprising	page 5, lines 8-10
non-human complementarity determining region amino acid residues which bind an antigen and	page 5, lines 8-21
a human framework region wherein said framework region comprises an amino acid substitution at a residue selected from the group consisting of	page 5, line 10 and page 26, lines 31-33
23, 24, 49, 71, 73, and 78, and combinations thereof,	page 6, lines 8-10 and Table 1
as numbered according to Kabat.	page 6, lines 5-7

DOCKET NO.: CARP0001-112 **PATENT**
APPLICATION SERIAL NO. 11/284,261

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re application of: **John R. Adair et al.** Confirmation No. **5305**

Serial No.: **11/284,261** Art Unit: **1643**

Filed: **November 21, 2005** Examiner: **Anne Gussow**

Title: **HUMANISED ANTIBODIES**

Customer No.: **34132**

Via EFS Web:
DATE FILED: September 9, 2009

MAIL STOP AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR RECONSIDERATION

Dear Sir:

This paper is being filed in response to the Non-Final Office Action dated as mailed March 9, 2009. Applicants hereby petition for a three-month extension of time to respond to the Non-Final Office Action and request the Commissioner to charge Deposit Account 50-3111 the appropriate extension of time fee.

LISTING OF THE CLAIMS

Claims 1-23 (**cancelled**)

Claim 24 (**currently amended**): A humanised antibody comprising a heavy chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises a non-human amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof, as numbered according to Kabat.

Claim 25 (**cancelled**)

REMARKS

Claims 24 and 25 were pending. All pending claims were rejected in the Non-Final Rejection. In view of the foregoing amendments and arguments that follow, Applicants respectfully request withdrawal of all rejections upon reconsideration.

Applicants acknowledge with appreciation the Office's withdrawal of the objections to claim 24 under 35 U.S.C. 112, second paragraph, as being indefinite.

Rejection Under 35 U.S.C. § 112, First Paragraph

Claim 24 was amended with the RCE filing and was again rejected as allegedly being indefinite. The Office alleges that the specification is enabling for a humanized antibody comprising a heavy chain variable domain and a light chain variable domain, with all 6 CDRs, and does not provide enablement for a humanized antibody heavy chain variable domain alone. The Office is clearly disregarding the fact that the CDR-grafted chains can be combined with other chains, as disclosed in the specification, including chimeric and mouse chains. Thus, it is not necessary for the claims to recite both chains. Applicants traverse this rejection but have amended claim 24 to recite a humanized antibody comprising a heavy chain variable domain.

Applicants respectfully submit that this rejection has been overcome.

Rejection Under 35 U.S.C. 102(e)

Claims 24 and 25 were rejected under 35 U.S.C. 102(e) as allegedly being anticipated by Queen, et al US Patent 5,585,089 the "'089 patent". Claim 25 has been cancelled. Applicants traverse this rejection as it applies to claim 24.

With all due respect, the Office has apparently misread the claims. Initially, the office states that the claims recite that the framework region comprises a **non-human** amino acid

substitution at a residue selected from 23, 24, 49, 71, 73, and 78, and combinations thereof. The Office then asserts that the "'089 patent" teaches an antibody comprising a humanized heavy chain variable domain comprising human residues at positions except the CDRs, and the following framework positions—27, 93, 95, 98, 107-109, 11, 30, 67, 48, and 68. The Office concludes, thus, that all the remaining framework positions are the human antibody allegedly taught in the "'089 patent". The Office then states that, since the claims recite substitution to **human** residues in the heavy chain in residues 23, 24, 49, 71, 73, and 78, and the "'089 patent" teaches human residues in all of those positions, the claim limitations are met. As the Office previously acknowledged, however, these residues are **non-human** residues in claim 24. The "'089 patent", thus, does not anticipate the Applicants invention.

Applicants respectfully request that this rejection be withdrawn.

CONCLUSION

Applicants respectfully submit that claim 24 is allowable and early allowance of the same. If a telephonic conversation with Applicants' attorney would help expedite the prosecution of the above-identified application, the Examiner is urged to call the undersigned at 215-665-5593.

Respectfully submitted,

/Doreen Yatko Trujillo/

Doreen Yatko Trujillo
Registration No. 35,719

Date: September 9, 2009

COZEN O'CONNOR, P.C.
1900 Market Street, 4th Floor
Philadelphia, PA 19103-3508
Telephone: (215) 665-5593
Facsimile: (215) 701-2005

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :		(11) International Publication Number: WO 91/09967
C12P 21/08, C12N 15/13 A61K 39/395, C07K 15/06 C12N 5/10, 15/62	A1	(43) International Publication Date: 11 July 1991 (11.07.91)

(21) International Application Number: PCT/GB90/02017

(22) International Filing Date: 21 December 1990 (21.12.90)

(30) Priority data:
8928874.0 21 December 1989 (21.12.89) GB

(71) Applicant *(for all designated States except US)*: CELLTECH LIMITED [GB/GB]; 216 Bath Road, Slough, Berkshire SL1 4EN (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : ADAIR, John, Robert [GB/GB]; 23 George Road, Stokenchurch, High Wycombe, Buckinghamshire HP14 3RN (GB). ATHWAL, Diljeet, Singh [GB/GB]; Flat 35, Knollys House, Tavistock Square, London WC1 (GB). EMTAGE, John, Spencer [GB/GB]; 49 Temple Mill Island, Temple, Marlow, Buckinghamshire SL7 1SQ (GB).

(74) Agent: MERCER, Christopher, Paul; Carpmaels & Ransford, 43 Bloomsbury Square, London WC1A 2RA (GB).

(81) Designated States: AT, AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CF (OAPI patent), CG (OAPI patent), CH, CH (European patent), CM (OAPI patent), DE, DE (European patent), DK, FI, FR (European patent), GA (OAPI patent), GB, GB (European patent), GR, HU, IT (European patent), JP, KP, KR, LK, LU, LU (European patent), MC, MG, ML (OAPI patent), MR (OAPI patent), MW, NL, NL (European patent), NO, RO, SD, SE, SE (European patent), SN (OAPI patent), SU, TD (OAPI patent), TG (OAPI patent), US.

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.
With a request for rectification under Rule 91.1(f).

(54) Title: HUMANISED ANTIBODIES

(57) Abstract

CDR-grafted antibody heavy and light chains comprise acceptor framework and donor antigen binding regions, the heavy chains comprising donor residues at at least one of positions (6, 23) and/or (24, 48) and/or (49, 71) and/or (73, 75) and/or (76) and/or (78) and (88) and/or (91). The CDR-grafted light chains comprise donor residues at at least one of positions (1) and/or (3) and (46) and/or (47) or at at least one of positons (46, 48, 58) and (71). The CDR-grafted antibodies are preferably humanised antibodies, having non human, e.g. rodent, donor and human acceptor frameworks, and may be used for *in vivo* therapy and diagnosis. A generally applicable protocol is disclosed for obtaining CDR-grafted antibodies.

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HUMANISED ANTIBODIES

Field of the Invention

The present invention relates to humanised antibody molecules, to processes for their production using recombinant DNA technology, and to their therapeutic uses.

The term "humanised antibody molecule" is used to describe a molecule having an antigen binding site derived from an immunoglobulin from a non-human species, and remaining immunoglobulin-derived parts of the molecule being derived from a human immunoglobulin. The antigen binding site typically comprises complementarity determining regions (CDRs) which determine the binding specificity of the antibody molecule and which are carried on appropriate framework regions in the variable domains. There are 3 CDRs (CDR1, CDR2 and CDR3) in each of the heavy and light chain variable domains.

In the description, reference is made to a number of publications by number. The publications are listed in numerical order at the end of the description.

Background of the Invention

Natural immunoglobulins have been known for many years, as have the various fragments thereof, such as the Fab, $(Fab')_2$ and Fc fragments, which can be derived by enzymatic cleavage. Natural immunoglobulins comprise a generally Y-shaped molecule having an antigen-binding site towards the end of each upper arm. The remainder of the structure, and particularly the stem of the Y, mediates the effector functions associated with immunoglobulins.

Natural immunoglobulins have been used in assay, diagnosis and, to a more limited extent, therapy. However, such uses, especially in therapy, were hindered until recently by the polyclonal nature of natural immunoglobulins. A significant step towards the realisation of the potential

of immunoglobulins as therapeutic agents was the discovery of procedures for the production of monoclonal antibodies (MAbs) of defined specificity (1).

However, most MAbs are produced by hybridomas which are fusions of rodent spleen cells with rodent myeloma cells. They are therefore essentially rodent proteins. There are very few reports of the production of human MAbs.

Since most available MAbs are of rodent origin, they are naturally antigenic in humans and thus can give rise to an undesirable immune response termed the HAMA (Human Anti-Mouse Antibody) response. Therefore, the use of rodent MAbs as therapeutic agents in humans is inherently limited by the fact that the human subject will mount an immunological response to the MAb and will either remove it entirely or at least reduce its effectiveness. In practice, MAbs of rodent origin may not be used in patients for more than one or a few treatments as a HAMA response soon develops rendering the MAb ineffective as well as giving rise to undesirable reactions. For instance, OKT3 a mouse IgG2a/k MAb which recognises an antigen in the T-cell receptor-CD3 complex has been approved for use in many countries throughout the world as an immunosuppressant in the treatment of acute allograft rejection [Chatenoud et al (2) and Jeffers et al (3)]. However, in view of the rodent nature of this and other such MAbs, a significant HAMA response which may include a major anti-idiotype component, may build up on use. Clearly, it would be highly desirable to diminish or abolish this undesirable HAMA response and thus enlarge the areas of use of these very useful antibodies.

Proposals have therefore been made to render non-human MAbs less antigenic in humans. Such techniques can be generically termed "humanisation" techniques. These

techniques typically involve the use of recombinant DNA technology to manipulate DNA sequences encoding the polypeptide chains of the antibody molecule.

Early methods for humanising MAbs involved production of chimeric antibodies in which an antigen binding site comprising the complete variable domains of one antibody is linked to constant domains derived from another antibody. Methods for carrying out such chimerisation procedures are described in EP0120694 (Celltech Limited), EP0125023 (Genentech Inc. and City of Hope), EP-A-0 171496 (Res. Dev. Corp. Japan), EP-A-0 173 494 (Stanford University), and WO 86/01533 (Celltech Limited). This latter Celltech application (WO 86/01533) discloses a process for preparing an antibody molecule having the variable domains from a mouse MAb and the constant domains from a human immunoglobulin. Such humanised chimeric antibodies, however, still contain a significant proportion of non-human amino acid sequence, i.e. the complete non-human variable domains, and thus may still elicit some HAMA response, particularly if administered over a prolonged period [Begent et al (ref. 4)].

In an alternative approach, described in EP-A-0239400 (Winter), the complementarity determining regions (CDRs) of a mouse MAb have been grafted onto the framework regions of the variable domains of a human immunoglobulin by site directed mutagenesis using long oligonucleotides. The present invention relates to humanised antibody molecules prepared according to this alternative approach, i.e. CDR-grafted humanised antibody molecules. Such CDR-grafted humanised antibodies are much less likely to give rise to a HAMA response than humanised chimeric antibodies in view of the much lower proportion of non-human amino acid sequence which they contain.

The earliest work on humanising MAbs by CDR-grafting was carried out on MAbs recognising synthetic antigens, such as the NP or NIP antigens. However, examples in which a mouse MAb recognising lysozyme and a rat MAb recognising an antigen on human T-cells were humanised by CDR-grafting have been described by Verhoeyen et al (5) and Riechmann et al (6) respectively. The preparation of CDR-grafted antibody to the antigen on human T cells is also described in WO 89/07452 (Medical Research Council).

In Riechmann et al/Medical Research Council it was found that transfer of the CDR regions alone [as defined by Kabat refs. (7) and (8)] was not sufficient to provide satisfactory antigen binding activity in the CDR-grafted product. Riechmann et al found that it was necessary to convert a serine residue at position 27 of the human sequence to the corresponding rat phenylalanine residue to obtain a CDR-grafted product having improved antigen binding activity. This residue at position 27 of the heavy chain is within the structural loop adjacent to CDR1. A further construct which additionally contained a human serine to rat tyrosine change at position 30 of the heavy chain did not have a significantly altered binding activity over the humanised antibody with the serine to phenylalanine change at position 27 alone. These results indicate that changes to residues of the human sequence outside the CDR regions, in particular in the structural loop adjacent to CDR1, may be necessary to obtain effective antigen binding activity for CDR-grafted antibodies which recognise more complex antigens. Even so the binding affinity of the best CDR-grafted antibodies obtained was still significantly less than the original MAb.

Very recently Queen et al (9) have described the preparation of a humanised antibody that binds to the

interleukin 2 receptor, by combining the CDRs of a murine MAb (anti-Tac) with human immunoglobulin framework and constant regions. The human framework regions were chosen to maximise homology with the anti-Tac MAb sequence. In addition computer modelling was used to identify framework amino acid residues which were likely to interact with the CDRs or antigen, and mouse amino acids were used at these positions in the humanised antibody.

In WO 90/07861 Queen et al propose four criteria for designing humanised immunoglobulins. The first criterion is to use as the human acceptor the framework from a particular human immunoglobulin that is unusually homologous to the non-human donor immunoglobulin to be humanised, or to use a consensus framework from many human antibodies. The second criterion is to use the donor amino acid rather than the acceptor if the human acceptor residue is unusual and the donor residue is typical for human sequences at a specific residue of the framework. The third criterion is to use the donor framework amino acid residue rather than the acceptor at positions immediately adjacent to the CDRs. The fourth criterion is to use the donor amino acid residue at framework positions at which the amino acid is predicted to have a side chain atom within about 3 Å of the CDRs in a three-dimensional immunoglobulin model and to be capable of interacting with the antigen or with the CDRs of the humanised immunoglobulin. It is proposed that criteria two, three or four may be applied in addition or alternatively to criterion one, and may be applied singly or in any combination.

WO 90/07861 describes in detail the preparation of a single CDR-grafted humanised antibody, a humanised antibody having specificity for the p55 Tac protein of the

IL-2 receptor. The combination of all four criteria, as above, were employed in designing this humanised antibody, the variable region frameworks of the human antibody Eu (7) being used as acceptor. In the resultant humanised antibody the donor CDRs were as defined by Kabat et al (7 and 8) and in addition the mouse donor residues were used in place of the human acceptor residues, at positions 27, 30, 48, 66, 67, 89, 91, 94, 103, 104, 105 and 107 in the heavy chain and at positions 48, 60 and 63 in the light chain, of the variable region frameworks. The humanised anti-Tac antibody obtained is reported to have an affinity for p55 of 3×10^9 M^{-1}, about one-third of that of the murine MAb.

We have further investigated the preparation of CDR-grafted humanised antibody molecules and have identified a hierarchy of positions within the framework of the variable regions (i.e. outside both the Kabat CDRs and structural loops of the variable regions) at which the amino acid identities of the residues are important for obtaining CDR-grafted products with satisfactory binding affinity. This has enabled us to establish a protocol for obtaining satisfactory CDR-grafted products which may be applied very widely irrespective of the level of homology between the donor immunoglobulin and acceptor framework. The set of residues which we have identified as being of critical importance does not coincide with the residues identified by Queen et al (9).

Summary of the Invention

Accordingly, in a first aspect the invention provides a CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

In preferred embodiments, the heavy chain framework comprises donor residues at positions 23, 24, 49, 71, 73 and 78 or at positions 23, 24 and 49. The residues at positions 71, 73 and 78 of the heavy chain framework are preferably either all acceptor or all donor residues.

In particularly preferred embodiments the heavy chain framework additionally comprises donor residues at one, some or all of positions 6, 37, 48 and 94. Also it is particularly preferred that residues at positions of the heavy chain framework which are commonly conserved across species, i.e. positions 2, 4, 25, 36, 39, 47, 93, 103, 104, 106 and 107, if not conserved between donor and acceptor, additionally comprise donor residues. Most preferably the heavy chain framework additionally comprises donor residues at positions 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

In addition the heavy chain framework optionally comprises donor residues at one, some or all of positions:
1 and 3,
72 and 76,
69 (if 48 is different between donor and acceptor),
38 and 46 (if 48 is the donor residue),
80 and 20 (if 69 is the donor residue),
67,
82 and 18 (if 67 is the donor residue),
91,
88, and
any one or more of 9, 11, 41, 87, 108, 110 and 112.

In the first and other aspects of the present invention reference is made to CDR-grafted antibody products comprising acceptor framework and donor antigen binding regions. It will be appreciated that the invention is widely applicable to the CDR-grafting of antibodies in

general. Thus, the donor and acceptor antibodies may be derived from animals of the same species and even same antibody class or sub-class. More usually, however, the donor and acceptor antibodies are derived from animals of different species. Typically the donor antibody is a non-human antibody, such as a rodent MAb, and the acceptor antibody is a human antibody.

In the first and other aspects of the present invention, the donor antigen binding region typically comprises at least one CDR from the donor antibody. Usually the donor antigen binding region comprises at least two and preferably all three CDRs of each of the heavy chain and/or light chain variable regions. The CDRs may comprise the Kabat CDRs, the structural loop CDRs or a composite of the Kabat and structural loop CDRs and any combination of any of these. Preferably, the antigen binding regions of the CDR-grafted heavy chain variable domain comprise CDRs corresponding to the Kabat CDRs at CDR2 (residues 50-65) and CDR3 (residues 95-100) and a composite of the Kabat and structural loop CDRs at CDR1 (residues 26-35).

The residue designations given above and elsewhere in the present application are numbered according to the Kabat numbering [refs. (7) and (8)]. Thus the residue designations do not always correspond directly with the linear numbering of the amino acid residues. The actual linear amino acid sequence may contain fewer or additional amino acids than in the strict Kabat numbering corresponding to a shortening of, or insertion into, a structural component, whether framework or CDR, of the basic variable domain structure. For example, the heavy chain variable region of the anti-Tac antibody described by Queen et al (9) contains a single amino acid insert (residue 52a) after residue 52 of CDR2 and a three amino

acid insert (residues 82a, 82b and 82c) after framework residue 82, in the Kabat numbering. The correct Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

The invention also provides in a second aspect a CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 1 and/or 3 and 46 and/or 47. Preferably the CDR grafted light chain of the second aspect comprises donor residues at positions 46 and/or 47.

The invention also provides in a third aspect a CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, 58 and 71.

In a preferred embodiment of the third aspect, the framework comprises donor residues at all of positions 46, 48, 58 and 71.

In particularly preferred embodiments of the second and third aspects, the framework additionally comprises donor residues at positions 36, 44, 47, 85 and 87. Similarly positions of the light chain framework which are commonly conserved across species, i.e. positions 2, 4, 6, 35, 49, 62, 64-69, 98, 99, 101 and 102, if not conserved between donor and acceptor, additionally comprise donor residues. Most preferably the light chain framework additionally comprises donor residues at positions 2, 4, 6, 35, 36, 38, 44, 47, 49, 62, 64-69, 85, 87, 98, 99, 101 and 102.

In addition the framework of the second or third aspects optionally comprises donor residues at one, some or all of positions:
1 and 3,
63,
60 (if 60 and 54 are able to form at potential saltbridge),
70 (if 70 and 24 are able to form a potential saltbridge),
73 and 21 (if 47 is different between donor and acceptor),
37 and 45 (if 47 is different between donor and acceptor), and
any one or more of 10, 12, 40, 80, 103 and 105.

Preferably, the antigen binding regions of the CDR-grafted light chain variable domain comprise CDRs corresponding to the Kabat CDRs at CDR1 (residue 24-34), CDR2 (residues 50-56) and CDR3 (residues 89-97).

The invention further provides in a fourth aspect a CDR-grafted antibody molecule comprising at least one CDR-grafted heavy chain and at least one CDR-grafted light chain according to the first and second or first and third aspects of the invention.

The humanised antibody molecules and chains of the present invention may comprise: a complete antibody molecule, having full length heavy and light chains; a fragment thereof, such as a Fab, $(Fab')_2$ or FV fragment; a light chain or heavy chain monomer or dimer; or a single chain antibody, e.g. a single chain FV in which heavy and light chain variable regions are joined by a peptide linker; or any other CDR-grafted molecule with the same specificity as the original donor antibody. Similarly the CDR-grafted heavy and light chain variable region may be combined with other antibody domains as appropriate.

Also the heavy or light chains or humanised antibody molecules of the present invention may have attached to them an effector or reporter molecule. For instance, it may have a macrocycle, for chelating a heavy metal atom, or a toxin, such as ricin, attached to it by a covalent bridging structure. Alternatively, the procedures of recombinant DNA technology may be used to produce an immunoglobulin molecule in which the Fc fragment or CH3 domain of a complete immunoglobulin molecule has been replaced by, or has attached thereto by peptide linkage, a functional non-immunoglobulin protein, such as an enzyme or toxin molecule.

Any appropriate acceptor variable region framework sequences may be used having regard to class/type of the donor antibody from which the antigen binding regions are derived. Preferably, the type of acceptor framework used is of the same/similar class/type as the donor antibody. Conveniently, the framework may be chosen to maximise/optimise homology with the donor antibody sequence particularly at positions close or adjacent to the CDRs. However, a high level of homology between donor and acceptor sequences is not important for application of the present invention. The present invention identifies a hierarchy of framework residue positions at which donor residues may be important or desirable for obtaining a CDR-grafted antibody product having satisfactory binding properties. The CDR-grafted products usually have binding affinities of at least 10^5 M^{-1}, preferably at least about 10^8 M^{-1}, or especially in the range 10^8-10^{12} M^{-1}. In principle, the present invention is applicable to any combination of donor and acceptor antibodies irrespective of the level of homology between their sequences. A protocol for applying the invention to any particular donor-acceptor antibody pair is given hereinafter. Examples of human frameworks which may be

used are KOL, NEWM, REI, EU, LAY and POM (refs. 4 and 5) and the like; for instance KOL and NEWM for the heavy chain and REI for the light chain and EU, LAY and POM for both the heavy chain and the light chain.

Also the constant region domains of the products of the invention may be selected having regard to the proposed function of the antibody in particular the effector functions which may be required. For example, the constant region domains may be human IgA, IgE, IgG or IgM domains. In particular, IgG human constant region domains may be used, especially of the IgG1 and IgG3 isotypes, when the humanised antibody molecule is intended for therapeutic uses, and antibody effector functions are required. Alternatively, IgG2 and IgG4 isotypes may be used when the humanised antibody molecule is intended for therapeutic purposes and antibody effector functions are not required, e.g. for simple blocking of lymphokine activity.

However, the remainder of the antibody molecules need not comprise only protein sequences from immunoglobulins. For instance, a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain is fused to a DNA sequence encoding the amino acid sequence of a functional polypeptide such as an effector or reporter molecule.

Preferably the CDR-grafted antibody heavy and light chain and antibody molecule products are produced by recombinant DNA technology.

Thus in further aspects the invention also includes DNA sequences coding for the CDR-grafted heavy and light chains, cloning and expression vectors containing the DNA sequences, host cells transformed with the DNA sequences

and processes for producing the CDR-grafted chains and antibody molecules comprising expressing the DNA sequences in the transformed host cells.

The general methods by which the vectors may be constructed, transfection methods and culture methods are well known per se and form no part of the invention. Such methods are shown, for instance, in references 10 and 11.

The DNA sequences which encode the donor amino acid sequence may be obtained by methods well known in the art. For example the donor coding sequences may be obtained by genomic cloning, or cDNA cloning from suitable hybridoma cell lines. Positive clones may be screened using appropriate probes for the heavy and light chain genes in question. Also PCR cloning may be used.

DNA coding for acceptor, e.g. human acceptor, sequences may be obtained in any appropriate way. For example DNA sequences coding for preferred human acceptor frameworks such as KOL, REI, EU and NEWM, are widely available to workers in the art.

The standard techniques of molecular biology may be used to prepare DNA sequences coding for the CDR-grafted products. Desired DNA sequences may be synthesised completely or in part using oligonucleotide synthesis techniques. Site-directed mutagenesis and polymerase chain reaction (PCR) techniques may be used as appropriate. For example oligonucleotide directed synthesis as described by Jones et al (ref. 20) may be used. Also oligonucleotide directed mutagenesis of a pre-exising variable region as, for example, described by Verhoeyen et al (ref. 5) or Riechmann et al (ref. 6) may be used. Also enzymatic filling in of gapped

oligonucleotides using T_4 DNA polymerase as, for example, described by Queen et al (ref. 9) may be used.

Any suitable host cell/vector system may be used for expression of the DNA sequences coding for the CDR-grafted heavy and light chains. Bacterial e.g. E. coli, and other microbial systems may be used, in particular for expression of antibody fragments such as FAb and $(Fab')_2$ fragments, and especially FV fragments and single chain antibody fragments e.g. single chain FVs. Eucaryotic e.g. mammalian host cell expression systems may be used for production of larger CDR-grafted antibody products, including complete antibody molecules. Suitable mammalian host cells include CHO cells and myeloma or hybridoma cell lines.

Thus, in a further aspect the present invention provides a process for producing a CDR-grafted antibody product comprising:

(a) producing in an expression vector an operon having a DNA sequence which encodes an antibody heavy chain according to the first aspect of the invention;

and/or

(b) producing in an expression vector an operon having a DNA sequence which encodes a complementary antibody light chain according to the second or third aspect of the invention;

(c) transfecting a host cell with the or each vector; and

(d) culturing the transfected cell line to produce the CDR-grafted antibody product.

The CDR-grafted product may comprise only heavy or light chain derived polypeptide, in which case only a heavy chain or light chain polypeptide coding sequence is used to transfect the host cells.

For production of products comprising both heavy and light chains, the cell line may be transfected with two vectors, the first vector may contain an operon encoding a light chain-derived polypeptide and the second vector containing an operon encoding a heavy chain-derived polypeptide. Preferably, the vectors are identical, except in so far as the coding sequences and selectable markers are concerned, so as to ensure as far as possible that each polypeptide chain is equally expressed. Alternatively, a single vector may be used, the vector including the sequences encoding both light chain- and heavy chain-derived polypeptides.

The DNA in the coding sequences for the light and heavy chains may comprise cDNA or genomic DNA or both. However, it is preferred that the DNA sequence encoding the heavy or light chain comprises at least partially, genomic DNA, preferably a fusion of cDNA and genomic DNA.

The present invention is applicable to antibodies of any appropriate specificity. Advantageously, however, the invention may be applied to the humanisation of non-human antibodies which are used for in vivo therapy or diagnosis. Thus the antibodies may be site-specific antibodies such as tumour-specific or cell surface-specific antibodies, suitable for use in in vivo therapy or diagnosis, e.g. tumour imaging. Examples of cell surface-specific antibodies are anti-T cell antibodies, such as anti-CD3, and CD4 and adhesion molecules, such as CR3, ICAM and ELAM. The antibodies may have specificity for interleukins (including lymphokines, growth factors and stimulating factors), hormones and other biologically active compounds, and receptors for any of these. For

example, the antibodies may have specificity for any of the following: Interferons α, β, γ or δ, IL1, IL2, IL3, or IL4, etc., TNF, GCSF, GMCSF, EPO, hGH, or insulin, etc.

The the present invention also includes therapeutic and diagnostic compositions comprising the CDR-grafted products of the invention and uses of such compositions in therapy and diagnosis.

Accordingly in a further aspect the invention provides a therapeutic or diagnostic composition comprising a CDR-grafted antibody heavy or light chain or molecule according to previous aspects of the invention in combination with a pharmaceutically acceptable carrier, diluent or excipient.

Accordingly also the invention provides a method of therapy or diagnosis comprising administering an effective amount of a CDR-grafted antibody heavy or light chain or molecule according to previous aspects of the invention to a human or animal subject.

A preferred protocol for obtaining CDR-grafted antibody heavy and light chains in accordance with the present invention is set out below together with the rationale by which we have derived this protocol. This protocol and rationale are given without prejudice to the generality of the invention as hereinbefore described and defined.

Protocol

It is first of all necessary to sequence the DNA coding for the heavy and light chain variable regions of the donor antibody, to determine their amino acid sequences. It is also necessary to choose appropriate acceptor heavy and light chain variable regions, of known amino acid sequence. The CDR-grafted chain is then designed

starting from the basis of the acceptor sequence. It will be appreciated that in some cases the donor and acceptor amino acid residues may be identical at a particular position and thus no change of acceptor framework residue is required.

1. As a first step donor residues are substituted for acceptor residues in the CDRs. For this purpose the CDRs are preferably defined as follows:

Heavy chain	-	CDR1:	residues 26-35
	-	CDR2:	residues 50-65
	-	CDR3:	residues 95-102
Light chain	-	CDR1:	residues 24-34
	-	CDR2:	residues 50-56
	-	CDR3:	residues 89-97

The positions at which donor residues are to be substituted for acceptor in the framework are then chosen as follows, first of all with respect to the heavy chain and subsequently with respect to the light chain.

2. Heavy Chain

2.1 Choose donor residues at all of positions 23, 24, 49, 71, 73 and 78 of the heavy chain or all of positions 23, 24 and 49 (71, 73 and 78 are always either all donor or all acceptor).

2.2 Check that the following have the same amino acid in donor and acceptor sequences, and if not preferably choose the donor: 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

2.3 To further optimise affinity consider choosing donor residues at one, some or any of:

i. 1, 3
ii. 72, 76
iii. If 48 is different between donor and acceptor sequences, consider 69
iv. If at 48 the donor residue is chosen, consider 38 and 46
v. If at 69 the donor residue is chosen, consider 80 and then 20
vi. 67
vii. If at 67 the donor residue is chosen, consider 82 and then 18
viii. 91
ix. 88
x. 9, 11, 41, 87, 108, 110, 112

3. Light Chain

3.1 Choose donor at 46, 48, 58 and 71

3.2 Check that the following have the same amino acid in donor and acceptor sequences, if not preferably choose donor:

2, 4, 6, 35, 38, 44, 47, 49, 62, 64-69 inclusive, 85, 87, 98, 99, 101 and 102

3.3 To further optimise affinity consider choosing donor residues at one, some or any of:

i. 1, 3
ii. 63

iii. 60, if 60 and 54 are able to form potential saltbridge

iv. 70, if 70 and 24 are able to form potential saltbridge

v. 73, and 21 if 47 is different between donor and acceptor

vi. 37, and 45 if 47 is different between donor and acceptor

vii. 10, 12, 40, 80, 103, 105

Rationale

In order to transfer the binding site of an antibody into a different acceptor framework, a number of factors need to be considered.

1. The extent of the CDRs

The CDRs (Complementary Determining Regions) were defined by Wu and Kabat (refs. 4 and 5) on the basis of an analysis of the variability of different regions of antibody variable regions. Three regions per domain were recognised. In the light chain the sequences are 24-34, 50-56, 89-97 (numbering according to Kabat (ref. 4), Eu Index) inclusive and in the heavy chain the sequences are 31-35, 50-65 and 95-102 inclusive.

When antibody structures became available it became apparent that these CDR regions corresponded in the main to loop regions which extended from the β barrel framework of the light and heavy variable domains. For H1 there was a discrepancy in that the loop was from 26 to 32 inclusive and for H2 the loop was 52 to 56 and for L2 from 50 to 53. However, with the exception of H1 the CDR regions encompassed the loop regions and extended into the β strand

frameworks. In H1 residue 26 tends to be a serine and 27 a phenylalanine or tyrosine, residue 29 is a phenylalanine in most cases. Residues 28 and 30 which are surface residues exposed to solvent might be involved in antigen-binding. A prudent definition of the H1 CDR therefore would include residues 26-35 to include both the loop region and the hypervariable residues 33-35.

It is of interest to note the example of Riechmann et al (ref. 3), who used the residue 31-35 choice for CDR-H1. In order to produce efficient antigen binding, residue 27 also needed to be recruited from the donor (rat) antibody.

2. Non-CDR residues which contribute to antigen binding

By examination of available X-ray structures we have identified a number of residues which may have an effect on net antigen binding and which can be demonstrated by experiment. These residues can be sub-divided into a number of groups.

2.1 Surface residues near CDR [all numbering as in Kabat et al (ref. 7)].

2.1.1. Heavy Chain - Key residues are 23, 71 and 73. Other residues which may contribute to a lesser extent are 1, 3 and 76. Finally 25 is usually conserved but the murine residue should be used if there is a difference.

2.1.2 Light Chain - Many residues close to the CDRs, e.g. 63, 65, 67 and 69 are conserved. If conserved none of the surface residues in the light chain are likely to have a major effect. However, if the murine residue at these positions

is unusual, then it would be of benefit to analyse the likely contribution more closely. Other residues which may also contribute to binding are 1 and 3, and also 60 and 70 if the residues at these positions and at 54 and 24 respectively are potentially able to form a salt bridge i.e. 60 + 54; 70 + 24.

2.2 Packing residues near the CDRs.

2.2.1. Heavy Chain - Key residues are 24, 49 and 78. Other key residues would be 36 if not a tryptophan, 94 if not an arginine, 104 and 106 if not glycines and 107 if not a threonine. Residues which may make a further contribution to stable packing of the heavy chain and hence improved affinity are 2, 4, 6, 38, 46, 67 and 69. 67 packs against the CDR residue 63 and this pair could be either both mouse or both human. Finally, residues which contribute to packing in this region but from a longer range are 18, 20, 80, 82 and 86. 82 packs against 67 and in turn 18 packs against 82. 80 packs against 69 and in turn 20 packs against 80. 86 forms an H bond network with 38 and 46. Many of the mouse-human differences appear minor e.g. Leu-Ile, but could have an minor impact on correct packing which could translate into altered positioning of the CDRs.

2.2.2. Light Chain - Key residues are 48, 58 and 71. Other key residues would be 6 if not glutamine, 35 if not tryptophan, 62 if not phenylalanine or tryosine, 64, 66, 68, 99 and 101 if not glycines and 102 if not a threonine. Residues which make a further contribution are 2, 4, 37, 45 and 47. Finally residues 73 and 21 and 19 may make long distance packing contributions of a minor nature.

2.3. Residues at the variable domain interface between heavy and light chains - In both the light and heavy chains most of the non-CDR interface residues are conserved. If a conserved residue is replaced by a residue of different character, e.g. size or charge, it should be considered for retention as the murine residue.

2.3.1. Heavy Chain - Residues which need to be considered are 37 if the residue is not a valine but is of larger side chain volume or has a charge or polarity. Other residues are 39 if not a glutamine, 45 if not a leucine, 47 if not a tryptophan, 91 if not a phenylalanine or tyrosine, 93 if not an alanine and 103 if not a tryptophan. Residue 89 is also at the interface but is not in a position where the side chain could be of great impact.

2.3.2. Light Chain - Residues which need to be considered are 36, if not a tyrosine, 38 if not a glutamine, 44 if not a proline, 46, 49 if not a tyrosine, residue 85, residue 87 if not a tyrosine and 98 if not a phenylalanine.

2.4. Variable-Constant region interface - The elbow angle between variable and constant regions may be affected by alterations in packing of key residues in the variable region against the constant region which may affect the position of V_L and V_H with respect to one another. Therefore it is worth noting the residues likely to be in contact with the constant region. In the heavy chain the surface residues potentially in contact with the variable region are conserved between mouse and human antibodies therefore the variable region contact residues may influence the V-C interaction. In the light chain the amino acids found at a number of the constant

region contact points vary, and the V & C regions are not in such close proximity as the heavy chain. Therefore the influences of the light chain V-C interface may be minor.

2.4.1. Heavy Chain - Contact residues are 7, 11, 41, 87, 108, 110, 112.

2.4.2. Light Chain - In the light chain potentially contacting residues are 10, 12, 40, 80, 83, 103 and 105.

The above analysis coupled with our considerable practical experimental experience in the CDR-grafting of a number of different antibodies have lead us to the protocol given above.

The present invention is now described, by way of example only, with reference to the accompanying Figures 1 - 13.

Brief Description of the Figures

Figure 1 shows DNA and amino acid sequences of the OKT3 light chain;

Figure 2 shows DNA and amino acid sequences of the OKT3 heavy chain;

Figure 3 shows the alignment of the OKT3 light variable region amino acid sequence with that of the light variable region of the human antibody REI;

Figure 4 shows the alignment of the OKT3 heavy variable region amino acid sequence with that of the heavy variable region of the human antibody KOL;

Figure 5 shows the heavy variable region amino acid sequences of OKT3, KOL and various corresponding CDR grafts;

Figure 6 shows the light variable region amino acid sequences of OKT3, REI and various corresponding CDR grafts;

Figure 7 shows a graph of binding assay results for various grafted OKT3 antibodies'

Figure 8 shows a graph of blocking assay results for various grafted OKT3 antibodies;

Figure 9 shows a similar graph of blocking assay results;

Figure 10 shows similar graphs for both binding assay and blocking assay results;

Figure 11 shows further similar graphs for both binding assay and blocking assay results;

Figure 12 shows a graph of competition assay results for a minimally grafted OKT3 antibody compared with the OKT3 murine reference standard, and

Figure 13 shows a similar graph of competition assay results comparing a fully grafted OKT3 antibody with the murine reference standard.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

EXAMPLE 1

CDR-GRAFTING OF OKT3

MATERIAL AND METHODS

1. INCOMING CELLS

Hybridoma cells producing antibody OKT3 were provided by Ortho (seedlot 4882.1) and were grown up in antibiotic free Dulbecco's Modified Eagles Medium (DMEM) supplemented with glutamine and 5% foetal calf serum, and divided to provide both an overgrown supernatant for evaluation and cells for extraction of RNA. The overgrown supernatant was shown to contain 250 ug/mL murine IgG2a/kappa antibody. The supernatant was negative for murine lambda light chain and IgG1, IgG2b, IgG3, IgA and IgM heavy chain. 20mL of supernatant was assayed to confirm that the antibody present was OKT3.

2. MOLECULAR BIOLOGY PROCEDURES

Basic molecular biology procedures were as described in Maniatis et al (ref. 9) with, in some cases, minor modifications. DNA sequencing was performed as described in Sanger et al (ref. 11) and the Amersham International Plc sequencing handbook. Site directed mutagenesis was as described in Kramer et al (ref. 12) and the Anglian Biotechnology Ltd. handbook. COS cell expression and metabolic labelling studies were as described in Whittle et al (ref. 13)

3. RESEARCH ASSAYS

3.1. ASSEMBLY ASSAYS

Assembly assays were performed on supernatants from transfected COS cells to determine the amount of intact IgG present.

3.1.1. COS CELLS TRANSFECTED WITH MOUSE OKT3 GENES

The assembly assay for intact mouse IgG in COS cell supernatants was an ELISA with the following format:

96 well microtitre plates were coated with F(ab')2 goat anti-mouse IgG Fc. The plates were washed in water and samples added for 1 hour at room temperature. The plates were washed and F(ab')2 goat anti-mouse IgG F(ab')2 (HRPO conjugated) was then added. Substrate was added to reveal the reaction. UPC10, a mouse IgG2a myeloma, was used as a standard.

3.1.2. COS AND CHO CELLS TRANSFECTED WITH CHIMERIC OR CDR-GRAFTED OKT3 GENES

The assembly assay for chimeric or CDR-grafted antibody in COS cell supernatants was an ELISA with the following format:

96 well microtitre plates were coated with F(ab')2 goat anti-human IgG Fc. The plates were washed and samples added and incubated for 1 hour at room temperature. The plates were washed and monoclonal mouse anti-human kappa chain was added for 1 hour at room temperature.

The plates were washed and F(ab')2 goat anti-mouse IgG Fc (HRPO conjugated) was added. Enzyme substrate was added to reveal the reaction. Chimeric B72.3 (IgG4) (ref. 13) was used as a standard. The use of a monoclonal anti-kappa chain in this assay allows grafted antibodies to be read from the chimeric standard.

3.2. ASSAY FOR ANTIGEN BINDING ACTIVITY

Material from COS cell supernatants was assayed for OKT3 antigen binding activity onto CD3 positive cells in a direct assay. The procedure was as follows:

HUT 78 cells (human T cell line, CD3 positive) were maintained in culture. Monolayers of HUT 78 cells were prepared onto 96 well ELISA plates using poly-L-lysine and glutaraldehyde. Samples were added to the monolayers for 1 hour at room temperature.

The plates were washed gently using PBS. F(ab')2 goat anti-human IgG Fc (HRPO conjugated) or F(ab')2 goat anti-mouse IgG Fc (HRPO conjugated) was added as appropriate for humanised or mouse samples. Substrate was added to reveal the reaction.

The negative control for the cell-based assay was chimeric B72.3. The positive control was mouse Orthomune OKT3 or chimeric OKT3, when available. This cell-based assay was difficult to perform, and an alternative assay was developed for CDR-grafted OKT3 which was more sensitive and easier to carry out.

In this system CDR-grafted OKT3 produced by COS cells was tested for its ability to bind to the CD3-positive HPB-ALL (human peripheral blood acute lymphocytic leukemia) cell line. It was also tested for its ability to block the binding of murine OKT3 to these cells. Binding was measured by the following procedure: HPB-ALL cells were harvested from tissue culture. Cells were incubated at 4°C for 1 hour with various dilutions of test antibody, positive control antibody, or negative control antibody. The cells were washed once and incubated at 4°C for 1 hour with an FITC-labelled goat anti-human IgG (Fc-

specific, mouse absorbed). The cells were washed twice and analysed by cytofluorography. Chimeric OKT3 was used as a positive control for direct binding. Cells incubated with mock- transfected COS cell supernatant, followed by the FITC-labelled goat anti-human IgG, provided the negative control. To test the ability of CDR-grafted OKT3 to block murine OKT3 binding, the HPB-ALL cells were incubated at 4°C for 1 hour with various dilutions of test antibody or control antibody. A fixed saturating amount of FITC OKT3 was added. The samples were incubated for 1 hour at 4°C, washed twice and analysed by cytofluorography. FITC-labelled OKT3 was used as a positive control to determine maximum binding. Unlabelled murine OKT3 served as a reference standard for blocking. Negative controls were unstained cells with or without mock-transfected cell supernatant. The ability of the CDR-grafted OKT3 light chain to bind CD3-positive cells and block the binding of murine OKT3 was initially tested in combination with the chimeric OKT3 heavy chain. The chimeric OKT3 heavy chain is composed of the murine OKT3 variable region and the human IgG4 constant region. The chimeric heavy chain gene is expressed in the same expression vector used for the CDR-grafted genes. The CDR-grafted light chain expression vector and the chimeric heavy chain expression vector were co-transfected into COS cells. The fully chimeric OKT3 antibody (chimeric light chain and chimeric heavy chain) was found to be fully capable of binding to CD3 positive cells and blocking the binding of murine OKT3 to these cells.

3.3 DETERMINATION OF RELATIVE BINDING AFFINITY

The relative binding affinities of CDR-grafted

anti-CD3 monoclonal antibodies were determined by competition binding (ref. 6) using the HPB-ALL human T cell line as a source of CD3 antigen, and fluorescein-conjugated murine OKT3 (Fl-OKT3) of known binding affinity as a tracer antibody. The binding affinity of Fl-OKT3 tracer antibody was determined by a direct binding assay in which increasing amounts of Fl-OKT3 were incubated with HPB-ALL ($5x10^5$) in PBS with 5% foetal calf serum for 60 min. at 4°C. Cells were washed, and the fluorescence intensity was determined on a FACScan flow cytometer calibrated with quantitative microbead standards (Flow Cytometry Standards, Research Triangle Park, NC). Fluorescence intensity per antibody molecule (F/P ratio) was determined by using microbeads which have a predetermined number of mouse IgG antibody binding sites (Simply Cellular beads, Flow Cytometry Standards). F/P equals the fluorescence intensity of beads saturated with Fl-OKT3 divided by the number of binding sites per bead. The amount of bound and free Fl-OKT3 was calculated from the mean fluorescence intensity per cell, and the ratio of bound/free was plotted against the number of moles of antibody bound. A linear fit was used to determine the affinity of binding (absolute value of the slope).

For competitive binding, increasing amounts of competitor antibody were added to a sub-saturating dose of Fl-OKT3 and incubated with $5x10^5$ HPB-ALL in 200 Ml of PBS with 5% foetal calf serum, for 60 min at 4°C. The fluorescence intensities of the cells were measured on a FACScan flow cytometer calibrated with quantitative microbead standards. The concentrations of bound and free Fl-OKT3 were calculated. The affinities of competing anti-

bodies were calculated from the equation [X]-[OKT3] = (1/Kx) - (1/Ka), where Ka is the affinity of murine OKT3, Kx is the affinity of competitor X, [ ] is the concentration of competitor antibody at which bound/free binding is R/2, and R is the maximal bound/free binding.

4. cDNA LIBRARY CONSTRUCTION

4.1. mRNA PREPARATION AND cDNA SYNTHESIS

OKT3 producing cells were grown as described above and 1.2 x 10^9 cells harvested and mRNA extracted using the guanidinium/LiCl extraction procedure. cDNA was prepared by priming from Oligo-dT to generate full length cDNA. The cDNA was methylated and EcoR1 linkers added for cloning.

4.2. LIBRARY CONSTRUCTION

The cDNA library was ligated to pSP65 vector DNA which had been EcoR1 cut and the 5′ phosphate groups removed by calf intestinal phosphatase (EcoR1/CIP). The ligation was used to transform high transformation efficiency Escherichia coli (E.coli) HB101. A cDNA library was prepared. 3600 colonies were screened for the light chain and 10000 colonies were screened for the heavy chain.

5. SCREENING

E.coli colonies positive for either heavy or light chain probes were identified by oligonucleotide screening using the oligonucleotides:
5′ TCCAGATGTTAACTGCTCAC for the light chain, which is complementary to a sequence in the mouse kappa constant region, and 5′ CAGGGGCCAGTGGATGGATAGAC for the heavy chain which is complementary to a sequence in the mouse IgG2a constant CH1 domain region. 12 light chain and 9 heavy chain clones

were identified and taken for second round screening. Positive clones from the second round of screening were grown up and DNA prepared. The sizes of the gene inserts were estimated by gel electrophoresis and inserts of a size capable of containing a full length cDNA were subcloned into M13 for DNA sequencing.

6. DNA SEQUENCING

Clones representing four size classes for both heavy and light chains were obtained in M13. DNA sequence for the 5′ untranslated regions, signal sequences, variable regions and 3′ untranslated regions of full length cDNAs [Figures 1(a) and 2(a)] were obtained and the corresponding amino acid sequences predicted [(Figures 1(b) and 2(b)]. In Figure 1(a) the untranslated DNA regions are shown in uppercase, and in both Figures 1 and 2 the signal sequences are underlined.

7. CONSTRUCTION OF cDNA EXPRESSION VECTORS

Celltech expression vectors are based on the plasmid pEE6hCMV (ref. 14). A polylinker for the insertion of genes to be expressed has been introduced after the major immediate early promoter/enhancer of the human Cytomegalovirus (hCMV). Marker genes for selection of the plasmid in transfected eukaryotic cells can be inserted as BamH1 cassettes in the unique BamH1 site of pEE6 hCMV; for instance, the neo marker to provide pEE6 hCMV neo. It is usual practice to insert the neo and gpt markers prior to insertion of the gene of interest, whereas the GS marker is inserted last because of the presence of internal EcoR1 sites in the cassette.

The selectable markers are expressed from the SV40 late promoter which also provides an origin of replication so that the vectors can be used for expression in the COS cell transient expression system.
The mouse sequences were excised from the M13 based vectors described above as EcoR1 fragments and cloned into either pEE6-hCMV-neo for the heavy chain and into EE6-hCMV-gpt for the light chain to yield vectors pJA136 and pJA135 respectively.

8. EXPRESSION OF cDNAS IN COS CELLS

Plasmids pJA135 and pJA136 were co-transfected into COS cells and supernatant from the transient expression experiment was shown to contain assembled antibody which bound to T-cell enriched lymphocytes. Metabolic labelling experiments using ^{35}S methionine showed expression and assembly of heavy and light chains.

9. CONSTRUCTION OF CHIMERIC GENES

Construction of chimeric genes followed a previously described strategy [Whittle et al (ref. 13)]. A restriction site near the 3′ end of the variable domain sequence is identified and used to attach an oligonucleotide adapter coding for the remainder of the mouse variable region and a suitable restriction site for attachment to the constant region of choice.

9.1. LIGHT CHAIN GENE CONSTRUCTION

The mouse light chain cDNA sequence contains an AvaI site near the 3′ end of the variable region [Fig. 1(a)]. The majority of the sequence of the variable region was isolated as a 396 bp. EcoR1-AvaI fragment. An oligonucleotide adapter was designed to replace the remainder of the 3′

region of the variable region from the AvaI site and to include the 5′ residues of the human constant region up to and including a unique NarI site which had been previously engineered into the constant region.

A HindIII site was introduced to act as a marker for insertion of the linker.

The linker was ligated to the V_L fragment and the 413 bp EcoR1-NarI adapted fragment was purified from the ligation mixture.

The constant region was isolated as an NarI-BamH1 fragment from an M13 clone NW361 and was ligated with the variable region DNA into an EcoR1/BamH1/C1P pSP65 treated vector in a three way reaction to yield plasmid JA143. Clones were isolated after transformation into E.coli and the linker and junction sequences were confirmed by the presence of the HindIII site and by DNA sequencing.

9.2 LIGHT CHAIN GENE CONSTRUCTION - VERSION 2

The construction of the first chimeric light chain gene produces a fusion of mouse and human amino acid sequences at the variable-constant region junction. In the case of the OKT3 light chain the amino acids at the chimera junction are:

.....Leu-Glu-Ile-Asn-Arg/ -/Thr-Val-Ala -Ala

VARIABLE CONSTANT

This arrangement of sequence introduces a potential site for Asparagine (Asn) linked (N-linked) glycosylation at the V-C junction. Therefore, a second version of the chimeric light chain oligonucleotide adapter was designed in which the threonine (Thr), the first amino acid of the human constant region, was replaced with the equivalent amino acid from the mouse constant region, Alanine (Ala).

An internal Hind111 site was not included in this adapter, to differentiate the two chimeric light chain genes.

The variable region fragment was isolated as a 376 bp EcoR1-Aval fragment. The oligonucleotide linker was ligated to Nar1 cut pNW361 and then the adapted 396bp constant region was isolated after recutting the modified pNW361 with EcoR1. The variable region fragment and the modified constant region fragment were ligated directly into EcoR1/C1P treated pEE6hCMVneo to yield pJA137. Initially all clones examined had the insert in the incorrect orientation. Therefore, the insert was re-isolated and recloned to turn the insert round and yield plasmid pJA141. Several clones with the insert in the correct orientation were obtained and the adapter sequence of one was confirmed by DNA sequencing

9.3. HEAVY CHAIN GENE CONSTRUCTION

9.3.1. CHOICE OF HEAVY CHAIN GENE ISOTYPE

The constant region isotype chosen for the heavy chain was human IgG4.

9.3.2. GENE CONSTRUCTION

The heavy chain cDNA sequence showed a Ban1 site near the 3′ end of the variable region [Fig. 2(a)]. The majority of the sequence of the variable region was isolated as a 426bp. EcoR1/C1P/Ban1 fragment. An oligonucleotide adapter was designated to replace the remainder of the 3′ region of the variable region from the Ban1 site up to and including a unique HindIII site which had been previously engineered into the first two amino acids of the constant region.

The linker was ligated to the V_H fragment and the EcoR1-Hind111 adapted fragment was purified from the ligation mixture.

The variable region was ligated to the constant region by cutting pJA91 with EcoR1 and Hind111 removing the intron fragment and replacing it with the V_H to yield pJA142. Clones were isolated after transformation into E.coli JM101 and the linker and junction sequences were confirmed by DNA sequencing. (N.B. The Hind111 site is lost on cloning).

10. CONSTRUCTION OF CHIMERIC EXPRESSION VECTORS

10.1. neo AND gpt VECTORS

The chimeric light chain (version 1) was removed from pJA143 as an EcoR1 fragment and cloned into EcoR1/C1P treated pEE6hCMVneo expression vector to yield pJA145. Clones with the insert in the correct orientation were identified by restriction mapping.

The chimeric light chain (version 2) was constructed as described above.

The chimeric heavy chain gene was isolated from pJA142 as a 2.5Kbp EcoR1/BamH1 fragment and cloned into the EcoR1/Bcl1/C1P treated vector fragment of a derivative of pEE6hCMVgpt to yield plasmid pJA144.

10.2. GS SEPARATE VECTORS

GS versions of pJA141 and pJA144 were constructed by replacing the neo and gpt cassettes by a BamH1/Sal1/C1P treatment of the plasmids, isolation of the vector fragment and ligation to a GS-containing fragment from the plasmid pRO49 to yield the light chain vector pJA179 and the heavy chain vector pJA180.

10.3. GS SINGLE VECTOR CONSTRUCTION

Single vector constructions containing the cL (chimeric light), cH (chimeric heavy) and GS genes on one plasmid in the order cL-cH-GS, or cH-cL-GS

and with transcription of the genes being head to tail e.g. cL>cH>GS were constructed. These plasmids were made by treating pJA179 or pJA180 with BamH1/C1P and ligating in a Bgl11/Hind111 hCMV promoter cassette along with either the Hind111/BamH1 fragment from pJA141 into pJA180 to give the cH-cL-GS plasmid pJA182 or the Hind111/BamH1 fragment from pJA144 into pJA179 to give the cL-cH-GS plasmid pJA181.

11. EXPRESSION OF CHIMERIC GENES

11.1. EXPRESSION IN COS CELLS

The chimeric antibody plasmid pJA145 (cL) and pJA144 (cH) were co-transfected into COS cells and supernatant from the transient expression experiment was shown to contain assembled antibody which bound to the HUT 78 human T-cell line. Metabolic labelling experiments using ^{35}S methionine showed expression and assembly of heavy and light chains. However the light chain mobility seen on reduced gels suggested that the potential glycosylation site was being glycosylated. Expression in COS cells in the presence of tunicamycin showed a reduction in size of the light chain to that shown for control chimeric antibodies and the OKT3 mouse light chain. Therefore JA141 was constructed and expressed. In this case the light chain did not show an aberrant mobility or a size shift in the presence or absence of tunicamycin. This second version of the chimeric light chain, when expressed in association with chimeric heavy (cH) chain, produced antibody which showed good binding to HUT 78 cells. In both cases antigen binding was equivalent to that of the mouse antibody.

11.2 EXPRESSION IN CHINESE HAMSTER OVARY (CHO) CELLS

Stable cell lines have been prepared from plasmids PJA141/pJA144 and from pJA179/pJA180, pJA181 and pJA182 by transfection into CHO cells.

12. CDR-GRAFTING

The approach taken was to try to introduce sufficient mouse residues into a human variable region framework to generate antigen binding activity comparable to the mouse and chimeric antibodies.

12.1. VARIABLE REGION ANALYSIS

From an examination of a small database of structures of antibodies and antigen-antibody complexes it is clear that only a small number of antibody residues make direct contact with antigen. Other residues may contribute to antigen binding by positioning the contact residues in favourable configurations and also by inducing a stable packing of the individual variable domains and stable interaction of the light and heavy chain variable domains.

The residues chosen for transfer can be identified in a number of ways:

(a) By examination of antibody X-ray crystal structures the antigen binding surface can be predominantly located on a series of loops, three per domain, which extend from the B-barrel framework.

(b) By analysis of antibody variable domain sequences regions of hypervariability [termed the Complementarity Determining Regions (CDRs) by Wu and Kabat (ref. 5)] can be identified. In the most but not all cases these CDRs correspond to, but extend a short way beyond, the loop regions noted above.

(c) Residues not identified by (a) and (b) may contribute to antigen binding directly or indirectly by affecting antigen binding site topology, or by inducing a stable packing of the individual variable domains and stabilising the inter-variable domain interaction. These residues may be identified either by superimposing the sequences for a given antibody on a known structure and looking at key residues for their contribution, or by sequence alignment analysis and noting "idiosyncratic" residues followed by examination of their structural location and likely effects.

12.1.1. LIGHT CHAIN

Figure 3 shows an alignment of sequences for the human framework region RE1 and the OKT3 light variable region. The structural loops (LOOP) and CDRs (KABAT) believed to correspond to the antigen binding region are marked. Also marked are a number of other residues which may also contribute to antigen binding as described in 13.1(c). Above the sequence in Figure 3 the residue type indicates the spatial location of each residue side chain, derived by examination of resolved structures from X-ray crystallography analysis. The key to this residue type designation is as follows:

N - near to CDR (From X-ray Structures)

P - Packing B - Buried Non-Packing

S - Surface E - Exposed

I - Interface * - Interface

- Packing/Part Exposed

? - Non-CDR Residues which may require to be left as Mouse sequence.

Residues underlined in Figure 3 are amino acids. RE1 was chosen as the human framework because the light chain is a kappa chain and the kappa variable regions show higher homology with the mouse sequences than a lambda light variable region, e.g. KOL (see below). RE1 was chosen in preference to another kappa light chain because the X-ray structure of the light chain has been determined so that a structural examination of individual residues could be made.

12.1.2. HEAVY CHAIN

Similarly Figure 4 shows an alignment of sequences for the human framework region KOL and the OKT3 heavy variable region. The structural loops and CDRs believed to correspond to the antigen binding region are marked. Also marked are a number of other residues which may also contribute to antigen binding as described in 12.1(c). The residue type key and other indicators used in Figure 4 are the same as those used in Figure 3. KOL was chosen as the heavy chain framework because the X-ray structure has been determined to a better resolution than, for example, NEWM and also the sequence alignment of OKT3 heavy variable region showed a slightly better homology to KOL than to NEWM.

12.2. DESIGN OF VARIABLE GENES

The variable region domains were designed with mouse variable region optimal codon usage [Grantham and Perrin (ref. 15)] and used the B72.3 signal sequences [Whittle et al (ref. 13)]. The sequences were designed to be attached to the constant region in the same way as for the chimeric genes described above. Some constructs contained the "Kozak consensus sequence" [Kozak (ref. 16)] directly linked to the 5′ of the signal

sequence in the gene. This sequence motif is believed to have a beneficial role in translation initiation in eukaryotes.

12.3. GENE CONSTRUCTION

To build the variable regions, various strategies are available. The sequence may be assembled by using oligonucleotides in a manner similar to Jones et al (ref. 17) or by simultaneously replacing all of the CDRs or loop regions by oligonucleotide directed site specific mutagenesis in a manner similar to Verhoeyen et al (ref. 2). Both strategies were used and a list of constructions is set out in Tables 1 and 2 and Figures 4 and 5. It was noted in several cases that the mutagenesis approach led to deletions and rearrangements in the gene being remodelled, while the success of the assembly approach was very sensitive to the quality of the oligonucleotides.

13. CONSTRUCTION OF EXPRESSION VECTORS

Genes were isolated from M13 or SP65 based intermediate vectors and cloned into pEE6hCMVneo for the light chains and pEE6hCMVgpt for the heavy chains in a manner similar to that for the chimeric genes as described above.

TABLE 1 CDR-GRAFTED GENE CONSTRUCTS

CODE	MOUSE SEQUENCE CONTENT	METHOD OF CONSTRUCTION	KOZAK SEQUENCE -	KOZAK SEQUENCE +
LIGHT CHAIN	ALL HUMAN FRAMEWORK RE1			
121	26-32, 50-56, 91-96 inclusive	SDM and gene assembly	+	n.d.
121A	26-32, 50-56, 91-96 inclusive +1, 3, 46, 47	Partial gene assembly	n.d.	+
121B	26-32, 50-56, 91-96 inclusive + 46, 47	Partial gene assembly	n.d.	+
221	24-24, 50-56, 91-96 inclusive	Partial gene assembly	+	+
221A	24-34, 50-56, 91-96 inclusive +1, 3, 46, 47	Partial gene assembly	+	+
221B	24-34, 50-56, 91-96 inclusive +1, 3	Partial gene assembly	+	+
221C	24-34, 50-56, 91-96 inclusive	Partial gene assembly	+	+
HEAVY CHAIN	ALL HUMAN FRAMEWORK KOL			
121	26-32, 50-56, 95-100B inclusive	Gene assembly	n.d.	+
131	26-32, 50-58, 95-100B inclusive	Gene assembly	n.d.	+
141	26-32, 50-65, 95-100B inclusive	Partial gene assembly	+	n.d.
321	26-35, 50-56, 95-100B inclusive	Partial gene assembly	+	n.d.
331	26-35, 50-58, 95-100B inclusive	Partial gene assembly	+	
		Gene assembly		+
341	26-35, 50-65, 95-100B inclusive	SDM	+	
		Partial gene assembly		+
341A	26-35, 50-65, 95-100B inclusive +6, 23, 24, 48, 49, 71, 73, 76, 78, 88, 91 (+63 = human)	Gene assembly	n.d.	+
341B	26-35, 50-65, 95-100B inclusive + 48, 49, 71, 73, 76, 78, 88, 91 (+63 + human)	Gene assembly	n.d.	+

KEY

n.d.	not done
SDM	Site directed mutagenesis
Gene assembly	Variable region assembled entirely from oligonucleotides
Partial gene assembly	Variable region assembled by combination of restriction fragments either from other genes originally created by SDM and gene assembly or by oligonucleotide assembly of part of the variable region and reconstruction with restriction fragments from other genes originally created by SDM and gene assembly

14. EXPRESSION OF CDR-GRAFTED GENES

14.1. PRODUCTION OF ANTIBODY CONSISTING OF GRAFTED LIGHT (gL) CHAINS WITH MOUSE HEAVY (mH) OR CHIMERIC HEAVY (cH) CHAINS

All gL chains, in association with mH or cH produced reasonable amounts of antibody.

Insertion of the Kozak consensus sequence at a position 5′ to the ATG (kgL constructs) however, led to a 2-5 fold improvement in net expression. Over an extended series of experiments expression levels were raised from approximately 200ng/ml to approximately 500 ng/ml for kgL/cH or kgL/mH combinations.

When direct binding to antigen on HUT 78 cells was measured, a construct designed to include mouse sequence based on loop length (gL121) did not lead to active antibody in association with mH or cH. A construct designed to include mouse sequence based on Kabat CDRs (gL221) demonstrated some weak binding in association with mH or cH. However, when framework residues 1, 3, 46, 47 were changed from the human to the murine OKT3 equivalents based on the arguments outlined in Section 12.1 antigen binding was demonstrated when both of the new constructs, which were termed 121A and 221A were co-expressed with cH. When the effects of these residues were examined in more detail, it appears that residues 1 and 3 are not major contributing residues as the product of the gL221B gene shows little detectable binding activity in association with cH. The light chain product of gL221C, in which mouse sequences are present at 46 and 47, shows good binding activity in association with cH.

14.2 PRODUCTION OF ANTIBODY CONSISTING OF GRAFTED HEAVY (gH) CHAINS WITH MOUSE LIGHT (mL) OR CHIMERIC LIGHT (cL) CHAINS

Expression of the gH genes proved to be more difficult to achieve than for gL. First, inclusion of the Kozak sequence appeared to have no marked effect on expression of gH genes. Expression appears to be slightly improved but not to the same degree as seen for the grafted light chain.

Also, it proved difficult to demonstrate production of expected quantities of material when the loop choice (amino acid 26-32) for CDR1 is used, e.g. gH121, 131, 141 and no conclusions can be drawn about these constructs.

Moreover, co-expression of the gH341 gene with cL or mL has been variable and has tended to produce lower amounts of antibody than the cH/cL or mH/mL combinations. The alterations to gH341 to produce gH341A and gH341B lead to improved levels of expression.

This may be due either to a general increase in the fraction of mouse sequence in the variable region, or to the alteration at position 63 where the residue is returned to the human amino acid Valine (Val) from Phenylalanine (Phe) to avoid possible internal packing problems with the rest of the human framework. This arrangement also occurs in gH331 and gH321.

When gH321 or gH331 were expressed in association with cL, antibody was produced but antibody binding activity was not detected.

When the more conservative gH341 gene was used antigen binding could be detected in association with cL or mL, but the activity was only marginally above the background level.

When further mouse residues were substituted based on the arguments in 12.1, antigen binding could be clearly demonstrated for the antibody produced when kgH341A and kgH341B were expressed in association with cL.

14.3 PRODUCTION OF FULLY CDR-GRAFTED ANTIBODY

The kgL221A gene was co-expressed with kgH341, kgH341A or kgH341B. For the combination kgH221A/kgH341 very little material was produced in a normal COS cell expression.

For the combinations kgL221A/kgH341A or kgH221A/kgH341B amounts of antibody similar to gL/cH was produced.

In several experiments no antigen binding activity could be detected with kgH221A/gH341 or kgH221A/kgH341 combinations, although expression levels were very low.

Antigen binding was detected when kgL221A/kgH341A or kgH221A/kgH341B combinations were expressed. In the case of the antibody produced from the kgL221A/kgH341A combination the antigen binding was very similar to that of the chimeric antibody.

An analysis of the above results is given below.

15. DISCUSSION OF CDR-GRAFTING RESULTS

In the design of the fully humanised antibody the aim was to transfer the minimum number of mouse amino acids that would confer antigen binding onto a human antibody framework.

15.1. LIGHT CHAIN

15.1.1. EXTENT OF THE CDRs

For the light chain the regions defining the loops known from structural studies of other antibodies to contain the antigen contacting residues, and

those hypervariable sequences defined by Kabat et al (refs. 4 and 5) as Complementarity Determining Regions (CDRs) are equivalent for CDR2. For CDR1 the hypervariable region extends from residues 24-34 inclusive while the structural loop extends from 26-32 inclusive. In the case of OKT3 there is only one amino acid difference between the two options, at amino acid 24, where the mouse sequence is a serine and the human framework RE1 has glutamine. For CDR3 the loop extends from residues 91-96 inclusive while the Kabat hypervariability extends from residues 89-97 inclusive. For OKT3 amino acids 89, 90 and 97 are the same between OKT3 and RE1 (Fig. 3). When constructs based on the loop choice for CDR1 (gL121) and the Kabat choice (gL221) were made and co-expressed with mH or cH no evidence for antigen binding activity could be found for gL121, but trace activity could be detected for the gL221, suggesting that a single extra mouse residue in the grafted variable region could have some detectable effect. Both gene constructs were reasonably well expressed in the transient expression system.

15.1.2. FRAMEWORK RESIDUES

The remaining framework residues were then further examined, in particular amino acids known from X-ray analysis of other antibodies to be close to the CDRs and also those amino acids which in OKT3 showed differences from the consensus framework for the mouse subgroup (subgroup VI) to which OKT3 shows most homology. Four positions 1, 3, 46 and 47 were identified and their possible contribution was examined by substituting the mouse amino acid for the human amino acid at each position. Therefore gL221A (gL221 + D1Q, Q3V, L46R, L47W,

see Figure 3 and Table 1) was made, cloned in EE6hCMVneo and co-expressed with cH (pJA144). The resultant antibody was well expressed and showed good binding activity. When the related genes gL221B (gL221 + D1Q, Q3V) and gL221C (gL221 + L46R, L47W) were made and similarly tested, while both genes produced antibody when co-expressed with cH, only the gL221C/cH combination showed good antigen binding. When the gL121A (gL121 + D1Q, Q3V, L46R, L47W) gene was made and co-expressed with cH, antibody was produced which also bound to antigen.

15.2. HEAVY CHAIN

15.2.1. EXTENT OF THE CDRs

For the heavy chain the loop and hypervariability analyses agree only in CDR3. For CDR1 the loop region extends from residues 26-32 inclusive whereas the Kabat CDR extends from residues 31-35 inclusive. For CDR2 the loop region is from 50-58 inclusive while the hypervariable region covers amino acids 50-65 inclusive. Therefore humanised heavy chains were constructed using the framework from antibody KOL and with various combinations of these CDR choices, including a shorter choice for CDR2 of 50-56 inclusive as there was some uncertainty as to the definition of the end point for the CDR2 loop around residues 56 to 58. The genes were co-expressed with mL or cL initially. In the case of the gH genes with loop choices for CDR1 e.g. gH121, gH131, gH141 very little antibody was produced in the culture supernatants. As no free light chain was detected it was presumed that the antibody was being made and assembled inside the cell but that the heavy chain was aberrant in some way, possibly incorrectly folded, and therefore the antibody was

being degraded internally. In some experiments trace amounts of antibody could be detected in ^{35}S labelling studies.

As no net antibody was produced, analysis of these constructs was not pursued further.

When, however, a combination of the loop choice and the Kabat choice for CDR1 was tested (mouse amino acids 26-35 inclusive) and in which residues 31 (Ser to Arg), 33 (Ala to Thr), and 35 (Tyr to His) were changed from the human residues to the mouse residue and compared to the first series, antibody was produced for gH321, kgH331 and kgH341 when co-expressed with cL. Expression was generally low and could not be markedly improved by the insertion of the Kozak consensus sequence 5′ to the ATG of the signal sequence of the gene, as distinct from the case of the gL genes where such insertion led to a 2-5 fold increase in net antibody production. However, only in the case of gH341/mL or kgH341/cL could marginal antigen binding activity be demonstrated. When the kgH341 gene was co-expressed with kgL221A, the net yield of antibody was too low to give a signal above the background level in the antigen binding assay.

15.2.2. FRAMEWORK RESIDUES

As in the case of the light chain the heavy chain frameworks were re-examined. Possibly because of the lower initial homology between the mouse and human heavy variable domains compared to the light chains, more amino acid positions proved to be of interest. Two genes kgH341A and kgH341B were constructed, with 11 or 8 human residues respectively substituted by mouse residues compared to gH341, and with the CDR2 residue 63 returned to the human amino acid potentially to

improve domain packing. Both showed antigen binding when combined with cL or kgL221A, the kgH341A gene with all 11 changes appearing to be the superior choice.

15.3 INTERIM CONCLUSIONS

It has been demonstrated, therefore, for OKT3 that to transfer antigen binding ability to the humanised antibody, mouse residues outside the CDR regions defined by the Kabat hypervariability or structural loop choices are required for both the light and heavy chains. Fewer extra residues are needed for the light chain, possibly due to the higher initial homology between the mouse and human kappa variable regions.

Of the changes seven (1 and 3 from the light chain and 6, 23, 71, 73 and 76 from the heavy chain) are predicted from a knowledge of other antibody structures to be either partly exposed or on the antibody surface. It has been shown here that residues 1 and 3 in the light chain are not absolutely required to be the mouse sequence; and for the heavy chain the gH341B heavy chain in combination with the 221A light chain generated only weak binding activity. Therefore the presence of the 6, 23 and 24 changes are important to maintain a binding affinity similar to that of the murine antibody. It was important, therefore, to further study the individual contribution of othe other 8 mouse residues of the kgH341A gene compared to kgH341.

16. FURTHER CDR-GRAFTING EXPERIMENTS

Additional CDR-grafted heavy chain genes were prepared substantially as described above. With reference to Table 2 the further heavy chain genes were based upon the gh341 (plasmid pJA178) and

gH341A (plasmid pJA185) with either mouse OKT3 or human KOL residues at 6, 23, 24, 48, 49, 63, 71, 73, 76, 78, 88 and 91, as indicated. The CDR-grafted light chain genes used in these further experiments were gL221, gL221A, gL221B and gL221C as described above.

TABLE 2

OKT3 HEAVY CHAIN CDR GRAFTS

1. gH341 and derivatives

RES NUM	6	23	24	48	49	63	71	73	76	78	88	91	
OKT3vh	Q	K	A	I	G	F	T	K	S	A	A	Y	
gH341	E	S	S	V	A	F	R	N	N	L	G	F	JA178
gH341A	Q	K	A	I	G	V	T	K	S	A	A	Y	JA185
gH341E	Q	K	A	I	G	V	T	K	S	A	G	G	JA198
gH341*	Q	K	A	I	G	V	T	K	N	A	G	F	JA207
gH341*	Q	K	A	I	G	V	R	N	N	A	G	F	JA209
gH341D	Q	K	A	I	G	V	T	K	N	L	G	F	JA197
gH341*	Q	K	A	I	G	V	R	N	N	L	G	F	JA199
gH341C	Q	K	A	V	A	F	R	N	N	L	G	F	JA184
gH341*	Q	S	A	I	G	V	T	K	S	A	A	Y	JA203
gH341*	E	S	A	I	G	V	T	K	S	A	A	Y	JA205
gH341B	E	S	S	I	G	V	T	K	S	A	A	Y	JA183
gH341*	Q	S	A	I	G	V	T	K	S	A	G	F	JA204
gH341*	E	S	A	I	G	V	T	K	S	A	G	F	JA206
gH341*	Q	S	A	I	G	V	T	K	N	A	G	F	JA208
KOL	E	S	S	V	A		R	N	N	L	G	F	

OKT3 LIGHT CHAIN CDR GRAFTS

2. gL221 and derivatives

RES NUM	1	3	46	47	
OKT3vl	Q	V	R	W	
GL221	D	Q	L	L	DA221
gL221A	Q	V	R	W	DA221A
gL221B	Q	V	L	L	DA221B
GL221C	D	Q	R	W	DA221C
RE1	D	Q	L	L	

MURINE RESIDUES ARE UNDERLINED

The CDR-grafted heavy and light chain genes were co-expressed in COS cells either with one another in various combinations but also with the corresponding murine and chimeric heavy and light chain genes substantially as described above. The resultant antibody products were then assayed in binding and blocking assays with HPB-ALL cells as described above.

The results of the assays for various grafted heavy chains co-expressed with the gL221C light chain are given in Figures 7 and 8 (for the JA184, JA185, JA197 and JA198 constructs - see Table 2), in Figure 9 (for the JA183, JA184, JA185 and JA197 constructs) in Figure 10 (for the chimeric, JA185, JA199, JA204, JA205, JA207, JA208 and JA209 constructs) and in Figure 11 (for the JA183, JA184, JA185, JA198, JA203, JA205 and JA206 constructs).

The basic grafted product without any human to murine changes in the variable frameworks, i.e. gL221 co-expressed with gh341 (JA178), and also the "fully grafted" product, having most human to murine changes in the grafted heavy chain framework, i.e. gL221C co-expressed with gh341A (JA185), were assayed for relative binding affinity in a competition assay against murine OKT3 reference standard, using HPB-ALL cells. The assay used was as described above in section 3.3. The results obtained are given in Figure 12 for the basic grafted product and in Figure 13 for the fully grafted product. These results indicate that the basic grafted product has neglibible binding ability as compared with the OKT3 murine reference standard; whereas the "fully grafted" product has a binding ability very similar to that of the OKT3 murine reference standard.

The binding and blocking assay results indicate the following:

The JA198 and JA207 constructs appear to have the best binding characteristics and similar binding abilities, both substantially the same as the chimeric and fully grafted gH341A products. This indicates that positions 88 and 91 and position 76 are not highly critical for maintaining the OKT3 binding ability; whereas at least some of positions 6, 23, 24, 48, 49, 71, 73 and 78 are more important.

This is borne out by the finding that the JA209 and JA199, although of similar binding ability to one another, are of lower binding ability than the JA198 and JA207 constructs. This indicates the importance of having mouse residues at positions 71, 73 and 78, which are either completely or partially human in the JA199 and JA209 constructs respectively.

Moreover, on comparing the results obtained for the JA205 and JA183 constructs it is seen that there is a decrease in binding going from the JA205 to the JA183 constructs. This indicates the importance of retaining a mouse residue at position 23, the only position changed between JA205 and JA183.

These and other results lead us to the conclusion that of the 11 mouse framework residues used in the gH341A (JA185) construct, it is important to retain mouse residues at all of positions 6, 23, 24, 48 and 49, and possibly for maximum binding affinity at 71, 73 and 78.

Similar Experiments were carried out to CDR-graft a number of the rodent antibodies including antibodies having specificity for CD4 (OKT4), ICAM-1 (R6-5), TAG72 (B72.3), and TNFα(61E71, 101.4, hTNF1, hTNF2 and hTNF3).

EXAMPLE 2

CDR-GRAFTING OF A MURINE ANTI-CD4 T CELL RECEPTOR ANTIBODY, OKT4A

Anti OKT4A CDR-grafted heavy and light chain genes were prepared, expressed and tested substantially as described above in Example 1 for CDR-grafted OKT3. The CDR grafting of OKT4A is described in detail in Ortho patent application PCT/GB 90..... of even date herewith entitled "Humanised Antibodies". The disclosure of this Ortho patent application PCT/GB 90 ..... is incorporated herein by reference. A number of CDR-grafted OKT4 antibodies have been prepared. Presently the CDR-grafted OKT4A of choice is the combination of the grafted light chain LCDR2 and the grafted heavy chain HCDR10.

THE LIGHT CHAIN

The human acceptor framework used for the grafted light chains was RE1. The preferred LCDR2 light chain has human to mouse changes at positions 33, 34, 38, 49 and 89 in addition to the structural loop CDRs. Of these changed positions, positions 33, 34 and 89 fall within the preferred extended CDRs of the present invention (positions 33 and 34 in CDR1 and position 89 in CDR3). The human to murine changes at positions 38 and 49 corresponds to positions at which the amino acid residues are preferably donor murine amino acid residues in accordance with the present invention.

A comparison of the amino acid sequences of the donor murine light chain variable domain and the RE1 human acceptor light chain variable further reveals that the murine and human residues are identical at all of positions 46, 48 and 71 and at all of positions 2, 4, 6, 35, 36, 44, 47, 62, 64-69, 85, 87, 98, 99 and 101 and 102. However the amino acid residue at position 58 in LCDR2 is

the human RE1 framework residue not the mouse OKT4 residue as would be preferred in accordance with the present invention.

THE HEAVY CHAIN

The human acceptor framework used for the grafted heavy chains was KOL.

The preferred CDR graft HCDR10 heavy chain has human to mouse changes at positions 24, 35, 57, 58, 60, 88 and 91 in addition to the structural loop CDRs.

Of these positions, positions 35 (CDR1) and positions 57, 58 and 60 (CDR2) fall within the preferred extended CDRs of the present invention. Also the human to mouse change at position 24 corresponds to a position at which the amino acid residue is a donor murine residue in accordance with the present invention. Moreover, the human to mouse changes at positions 88 and 91 correspond to positions at which the amino acid residues are optionally donor murine residues.

Moreover, a comparison of the murine OKT4A and human KOL heavy chain variable amino acid sequences reveals that the murine and human residues are identical at all of positions 23, 49, 71, 73 and 78 and at all of positions 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

Thus the OKT4A CDR-grafted heavy chain HCDR10 corresponds to a particularly preferred embodiment according to the present invention.

EXAMPLE 3

CDR-GRAFTING OF AN ANTI-MUCIN SPECIFIC MURINE ANTIBODY, B72.3

The cloning of the genes coding for the anti-mucin specific murine monoclonal antibody B72.3 and the preparation of B72.3 mouse-human chimeric antibodies has been described previously (ref. 13 and WO 89/01783). CDR-grafted versions of B72.3 were prepared as follows.

(a) B72.3 Light Chain

CDR-grafting of this light chain was accomplished by direct transfer of the murine CDRs into the framework of the human light chain RE1.
The regions transferred were:

CDR Number	Residues
1	24-34
2	50-56
3	90-96

The activity of the resulting grafted light chain was assessed by co-expression in COS cells, of genes for the combinations:

B72.3 cH/B72.3 cL
and B72.3 cH/B72.3 gL

Supernatants were assayed for antibody concentration and for the ability to bind to microtitre plates coated with mucin. The results obtained indicated that, in combination with the B72.3 cH chain, B72.3 cL and B72.3 gL had similar binding properties.

Comparison of the murine B72.3 and REI light chain amino acid sequences reveals that the residues are identical at positions 46, 58 and 71 but are different at position 48.

Thus changing the human residue to the donor mouse residue at position 48 may further improve the binding characteristics of the CDR-grafted light chain, (B72.3 gL) in accordance with the present invention.

(b) B72.3 heavy chain

i. Choice of framework

At the outset it was necessary to make a choice of human framework. Simply put, the question was as follows: Was it necessary to use the framework regions from an antibody whose crystal structure was known or could the choice be made on some other criteria?

For B72.3 heavy chain, it was reasoned that, while knowledge of structure was important, transfer of the CDRs from mouse to human frameworks might be facilitated if the overall homology between the donor and receptor frameworks was maximised.

Comparison of the B72.3 heavy chain sequence with those in Kabat (ref. 4) for human heavy chains showed clearly that B72.3 had poor homology for KOL and NEWM (for which crystal structures are available) but was very homologous to the heavy chain for EU.

On this basis, EU was chosen for the CDR-grafting and the following residues transferred as CDRs.

CDR Number	Residues
1	27-36
2	50-63
3	93-102

Also it was noticed that the FR4 region of EU was unlike that of any other human (or mouse) antibody. Consequently, in the grafted heavy chain genes this was also changed to produce a "consensus" human sequence. (Preliminary experiments showed that grafted heavy chain genes containing the EU FR4 sequence expressed very poorly in transient expression systems.)

ii. Results with grafted heavy chain genes

Expression of grafted heavy chain genes containing all human framework regions with either gL or cL genes produced a grafted antibody with little ability to bind to mucin. The grafted antibody had about 1% the activity of the chimeric antibody.

In these experiments, however, it was noted that the activity of the grafted antibody could be increased to ~ 10% of B72.3 by exposure to pHs of 2-3.5.

This observation provided a clue as to how the activity of the grafted antibody could be improved without acid treatment. It was postulated that acid exposure brought about the protonation of an acidic residue (pKa of aspartic acid = 3.86 and of glutamine acid = 4.25) which in turn caused a change in structure of the CDR loops, or allowed better access of antigen.

From comparison of the sequences of B72.3 (ref. 13) and EU (refs. 4 and 5), it was clear that, in going from the mouse to human frameworks, only two positions had been changed in such a way that acidic residues had been introduced. These

positions are at residues 73 and 81, where K to E and Q to E changes had been made, respectively.

Which of these positions might be important was determined by examining the crystal structure of the KOL antibody. In KOL heavy chain, position 81 is far removed from either of the CDR loops.

Position 73, however, is close to both CDRs 1 and 3 of the heavy chain and, in this position it was possible to envisage that a K to E change in this region could have a detrimental effect on antigen binding.

iii. Framework changes in B72.3 gH gene

On the basis of the above analysis, E73 was mutated to a lysine (K). It was found that this change had a dramatic effect on the ability of the grafted Ab to bind to mucin. Further the ability of the grafted B72.3 produced by the mutated gH/gL combination to bind to mucin was similar to that of the B72.3 chimeric antibody.

iv. Other framework changes

In the course of the above experiments, other changes were made in the heavy chain framework regions. Within the accuracy of the assays used, none of the changes, either alone or together, appeared beneficial.

v. Other

All assays used measured the ability of the grafted Ab to bind to mucin and, as a whole, indicated that the single framework change at position 73 is sufficient to generate an antibody with similar binding properties to B72.3.

Comparison of the B72.3 murine and EU heavy chain sequences reveals that the mouse and human residues are identical at positions 23, 24, 71 and 78.
Thus the mutated CDR-grafted B72.3 heavy chain corresponds to a preferred embodiment of the present invention.

EXAMPLE 4

CDR-GRAFTING OF A MURINE ANTI-ICAM-1 MONOCLONAL ANTIBODY

A murine antibody, R6-5-D6 (EP 0314863) having specificity for Intercellular Adhesion Molecule 1 (ICAM-1) was CDR-grafted substantially as described above in previous examples. This work is described in greater detail in co-pending application, British Patent Application No. 9009549.8, the disclosure of which is incorporated herein by reference.

The human EU framework was used as the acceptor framework for both heavy and light chains. The CDR-grafted antibody currently of choice is provided by co-expression of grafted light chain gL221A and grafted heavy chain gH341D which has a binding affinity for ICAM 1 of about 75% of that of the corresponding mouse-human chimeric antibody.

LIGHT CHAIN

gL221A has murine CDRs at positions 24-34 (CDR1), 50-56 (CDR2) and 89-97 (CDR3). In addition several framework residues are also the murine amino acid. These residues were chosen after consideration of the possible contribution of these residues to domain packing and stability of the conformation of the antigen binding region. The residues which have been retained as mouse are at positions 2, 3, 48 (?), 60, 84, 85 and 87. Comparison of the murine anti-ICAM 1 and human EU light chain amino acid sequences reveals that the murine and human residues are identical at positions 46, 58 and 71.

HEAVY CHAIN

gH341D has murine CDRs at positions 26-35 (CDR1), 50-56 (CDR2) and94-100B (CDR3). In addition murine residues were used in gH341D at positions 24, 48, 69, 71, 73, 80, 88 and 91. Comparison of the murine anti-ICAM 1 and human EU heavy chain amino acid sequences are identical at positions 23, 49 and 78.

EXAMPLE 5

CDR-Grafting of murine anti-TNFα antibodies

A number of murine anti-TNFα monoclonal antibodies were CDR-grafted substantially as described above in previous examples. These antibodies include the murine monoclonal antibodies designated 61 E71, hTNF1, hTNF3 and 101.4 A brief summary of the CDR-grafting of each of these antibodies is given below.

61E71

A similar analysis as described above (Example 1, Section 12.1.) was done for 61E71 and for the heavy chain 10 residues were identified at 23, 24, 48, 49, 68, 69, 71, 73, 75 and 88 as residues to potentially retain as murine. The human frameworks chosen for CDR-grafting of this antibody, and the hTNF3 and 101.4 antibodies were RE1 for the light chain and KOL for the heavy chain.
Three genes were built, the first of which contained 23, 24, 48, 49, 71 and 73 [gH341(6)] as murine residues. The second gene also had 75 and 88 as murine residues [gH341(8)] while the third gene additionally had 68, 69, 75 and 88 as murine residues [gH341(10)]. Each was co-expressed with gL221, the minimum grafted light chain (CDRs only). The gL221/gH341(6) and gL221/gH341(8) antibodies both bound as well to TNF as murine 61E71. The gL221/gH341(10) antibody did not express and this combination was not taken further.
Subsequently the gL221/gH341(6) antibody was assessed in an L929 cell competition assay in which the antibody competes against the TNF receptor on L929 cells for binding to TNF in solution. In this assay the gL221/gH341(6) antibody was approximately 10% as active as murine 61E71.

hTNF1

hTNF1 is a monoclonal antibody which recognises an epitope on human TNF- . The EU human framework was used for CDR-grafting of both the heavy and light variable domains.

Heavy Chain

In the CDR-grafted heavy chain (ghTNF1) mouse CDRs were used at positions 26-35 (CDR1), 50-65 (CDR2) and 95-102 (CDR3). Mouse residues were also used in the frameworks at positions 48, 67, 69, 71, 73, 76, 89, 91, 94 and 108. Comparison of the TNF1 mouse and EU human heavy chain residues reveals that these are identical at positions 23, 24, 29 and 78.

Light Chain

In the CDR-grafted light chain (gLhTNF1) mouse CDRs wre used at positions 24-34 (CDR1), 50-56 (CDR2) and 89-97 (CDR3). In addition mouse residues were used in the frameworks at positions 3, 42, 48, 49, 83, 106 and 108. Comparison of the hTNF1 mouse and EU human light chain residues reveals that these are identical at positions 46, 58 and 71.

The grafted hTNF1 heavy chain was co-expressed with the chimeric light chain and the binding ability of the product compared with that of the chimeric light chain/chimeric heavy chain product in a TNF binding assay. The grafted heavy chain product appeared to have binding ability for TNF slightly better than the fully chimeric product.

Similarly, a grafted heavy chain/grafted light chain product was co-expressed and compared with the fully chimeric product and found to have closely similar binding properties to the latter product.

hTNF3

hTNF3 recognises an epitope on human TNF-α. The sequence of hTNF3 shows only 21 differences compared to 61E71 in the light and heavy chain variable regions, 10 in the light chain (2 in the CDRs at positions 50, 96 and 8 in the framework at 1, 19, 40, 45, 46, 76, 103 and 106) and 11 in the heavy chain (3 in the CDR regions at positions 52, 60 and 95 and 8 in the framework at 1, 10, 38, 40, 67, 73, 87 and 105). The light and heavy chains of the 61E71 and hTNF3 chimeric antibodies can be exchanged without loss of activity in the direct binding assay. However 61E71 is an order of magnitude less able to compete with the TNF receptor on L929 cells for TNF-a compared to hTNF3. Based on the 61E71 CDR grafting data gL221 and gH341(+23, 24, 48, 49 71 and 73 as mouse) genes have been built for hTNF3 and tested and the resultant grafted antibody binds well to TNF-a, but competes very poorly in the L929 assay. It is possible that in this case also the framework residues identified for OKT3 programme may improve the competitive binding ability of this antibody.

101.4

101.4 is a further murine monoclonal antibody able to recognise human TNF-a. The heavy chain of this antibody shows good homology to KOL and so the CDR-grafting has been based on RE1 for the light chain and KOL for the heavy chain. Several grafted heavy chain genes have been constructed with conservative choices for the CDR's (gH341) and which have one or a small number of non-CDR residues at positions 73, 78 or 77-79 inclusive, as the mouse amino acids. These have been co-expressed with cL or gL221. In all cases binding to TNF equivalent to the chimeric antibody is seen and when co-expressed with cL the resultant antibodies are able to compete well in the L929 assay. However, with gL221 the resultant antibodies

are at least an order of magnitude less able to compete for TNF against the TNF receptor on L929 cells.

Mouse residues at other positions in the heavy chain, for example, at 23 and 24 together or at 76 have been demonstrated to provide no improvement to the competitive ability of the grafted antibody in the L929 assay.

A number of other antibodies including antibodies having specificity for interleukins e.g. IL1 and cancer markers such as carcinoembryonic antigen (CEA) e.g. the monoclonal antibody A5B7 (ref. 21), have been successfully CDR-grafted according to the present invention.
It will be appreciated that the foregoing examples are given by way of illustration only and are not intended to limit the scope of the claimed invention. Changes and modifications may be made to the methods described whilst still falling within the spirit and scope of the invention.

References

1. Kohler & Milstein, Nature, 265, 295-497, 1975.

2. Chatenoud et al, (1986), J. Immunol. 137, 830-838.

3. Jeffers et al, (1986), Transplantation, 41, 572-578.

4. Begent et al, Br. J. Cancer 62: 487 (1990).

5. Verhoeyen et al, Science, 239, 1534-1536, 1988.

6. Riechmann et al, Nature, 332, 323-324, 1988.

7. Kabat, E.A., Wu, T.T., Reid-Miller, M., Perry, H.M., Gottesman, K.S., 1987, in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA.

8. Wu, T.T., and Kabat, E.A., 1970, J. Exp. Med. 132 211-250.

9. Queen et al, (1989), Proc. Natl. Acad. Sci. USA, 86, 10029-10033 and WO 90/07861

10. Maniatis et al, Molecular Cloning, Cold Spring Harbor, New York, 1989.

11. Primrose and Old, Principles of Gene Manipulation, Blackwell, Oxford, 1980.

12. Sanger, F., Nicklen, S., Coulson, A.R., 1977, Proc. Natl. Acad. Sci. USA, 74 5463

13. Kramer, W., Drutsa, V., Jansen, H.-W., Kramer, B., Plugfelder, M., Fritz, H.-J., 1984, Nucl. Acids Res. 12, 9441

14. Whittle, N., Adair, J., Lloyd, J.C., Jenkins, E., Devine, J., Schlom, J., Raubitshek, A., Colcher, D., Bodmer, M., 1987, Protein Engineering 1, 499.

15. Sikder, S.S., Akolkar, P.N., Kaledas, P.M., Morrison, S.L., Kabat, E.A., 1985, J. Immunol. 135, 4215.

16. Wallick, S.C., Kabat, E.A., Morrison, S.L., 1988, J. Exp. Med. 168, 1099

17. Bebbington, C.R., Published International Patent Application WO 89/01036.

18. Granthan and Perrin 1986, Immunology Today 7, 160.

19. Kozak, M., 1987, J. Mol. Biol. 196, 947.

20. Jones, T.P., Dear, P.H., Foote, J., Neuberger, M.S., Winter, G., 1986, Nature, 321, 522

21. Harwood et al, Br. J. Cancer, 54, 75-82 (1986).

CLAIMS

1. A CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

2. A CDR-grafted heavy chain according to Claim 1 comprising donor residues at positions 23, 24, 49, 71, 73 and 78, or at positions 23, 24 and 49.

3. A CDR-grafted heavy chain according to Claim 2 comprising donor residues at positions 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

4. A CDR-grafted heavy chain according to Claim 2 or 3, comprising donor residues at one, some or all of positions:
1 and 3,
69 (if 48 is different between donor and acceptor),
38 and 46 (if 48 is the donor residue),
67,
82 and 18 (if 67 is the donor residue),
91, and
any one or more of 9, 11, 41, 87, 108, 110 and 112.

5. A CDR-grafted heavy chain according to any of the preceding comprising donor CDRs at positions 26-35, 50-65 and 95-100.

6. A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 1 and/or 3 and 46 and/or 47.

7. A CDR-grafted light chain according to Claim 6 comprising donor residues at positions 46 and 47.

8. A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, 58 and 71.

9. A CDR-grafted light chain according to Claim 8 comprising donor residues at positions 46, 48, 58 and 71.

10. A CDR-grafted light chain according to Claim 8 or 9, comprising donor residues at positions 2, 4, 6, 35, 36, 38, 44, 47, 49, 62, 64-69, 85, 87, 98, 99, 101 and 102.

11. A CDR-grafted light chain according to Claim 9 or 10, comprising donor residues at one, some or all of positions:
1 and 3,
63,
60 (if 60 and 54 are able to form a potential saltbridge),
70 (if 70 and 24 are able to form a potential saltbridge),
73 and 21 (if 47 is different between donor and acceptor),
37 and 45 (if 47 if different between donor and acceptor), and
any one or more of 10, 12, 40, 83, 103 and 105.

12. A CDR-grafted light chain according to any one of Claims 6-11, comprising donor CDRs at positions 24-34, 50-56 and 89-97.

13. A CDR-grafted antibody molecule comprising at least one CDR-grafted heavy chain according to any one of Claims 1-5 and at least one CDR-grafted light chain according to any one of Claims 6-12.

14. A CDR-grafted antibody molecule according to Claim 13, which is a site-specific antibody molecule.

15. A CDR-grafted antibody molecule according to Claim 13 which has specificity for an interleukin, hormone or other biologically active compound or a receptor therefor.

16. A CDR-grafted antibody heavy or light chain or molecule according to any one of the preceding claims comprising human acceptor residues and non-human donor residues.

17. A DNA sequence which codes for a CDR-grafted heavy chain according to Claim 1 or a CDR-grafted light chain according to Claim 6 or Claim 8.

18. A cloning or expression vector containing a DNA sequence according to Claim 17.

19. A host cell transformed with a DNA sequence according to Claim 17.

20. A process for the production of a CDR-grafted antibody sequence according to Claim 17 in a transformed host cell.

21. A process for producing a CDR-grafted antibody product comprising:

(a) producing in an expression vector an operon having a DNA sequence which encodes an antibody heavy chain according to Claim 1;

and/or

(b) producing in an expression vector an operon having a DNA sequence which encodes a complementary antibody light chain according to Claim 6 or Claim 8;

(c) transfecting a host cell with the or each vector; and

(d) culturing the transfected cell line to produce the CDR-grafted antibody product.

22. A therapeutic or diagnostic composition comprising a CDR-grafted antibody heavy chain according to Claim 1, or a CDR-grafted light chain according to Claim 6 or Claim 8, or a CDR-grafted antibody molecule according to Claim 13 in combination with a pharmaceutically acceptable carrier, diluent or excipient.

23. A method of therapy or diagnosis comprising administering an effective amount of a CDR-grafted heavy chain according to Claim 1, or a CDR-grafted light chain according to Claim 6 or Claim 8, or a CDR-grafted antibody molecule according to Claim 13 to a human or animal subject.

```
   1  GAATTCCCAA AGACAAAatg gattttcaag tgcagatttt cagcttcctg
  51  ctaatcagtg cctcagtcat aatatccaga ggacaaattg ttctcaccca
 101  gtctccagca atcatgtctg catctccagg ggagaaggtc accatgacct
 151  gcagtgccag ctcaagtgta agttacatga actggtacca gcagaagtca
 201  ggcacctccc ccaaaagatg gatttatgac acatccaaac tggcttctgg
 251  agtccctgct cacttcaggg gcagtgggtc tgggacctct tactctctca
 301  caatcagcgg catggaggct gaagatgctg ccacttatta ctgccagcag
 351  tggagtagta acccattcac gttcggctcg gggacaaagt tggaaataaa
 401  ccgggctgat actgcaccaa ctgtatccat cttcccacca tccagtgagc
 451  agttaacatc tggaggtgcc tcagtcgtgt gcttcttgaa caacttctac
 501  cccaaagaca tcaatgtcaa gtggaagatt gatggcagtg aacgacaaaa
 551  tggcgtcctg aacagttgga ctgatcagga cagcaaagac agcacctaca
 601  gcatgagcag caccctcacg ttgaccaagg acgagtatga acgacataac
 651  agctatacct gtgaggccac tcacaagaca tcaacttcac ccattgtcaa
 701  gagcttcaac aggaatgagt gtTAGAGACA AAGGTCCTGA GACGCCACCA
 751  CCAGCTCCCA GCTCCATCCT ATCTTCCCTT CTAAGGTCTT GGAGGCTTCC
 801  CCACAAGCGC tTACCACTGT TGCGGTGCTC tAAACCTCCT CCCACCTCCT
 851  TCTCCTCCTC CTCCCTTTCC TTGGCTTTTA TCATGCTAAT ATTTGCAGAA
 901  AATATTCAAT AAAGTGAGTC TTTGCCTTGA AAAAAAAAAA AAA
```

Fig. 1(a)

```
   1  MDFOVOIFSF LLISASVIIS RGQIVLTQSP AIMSASPGEK VTMTCSASSS
  51  VSYMNWYQQK SGTSPKRWIY DTSKLASGVP AHFRGSGSGT SYSLTISGME
 101  AEDAATYYCQ QWSSNPFTFG SGTKLEINRA DTAPTVSIFP PSSEQLTSGG
 151  ASVVCFLNNF YPKDINVKWK IDGSERQNGV LNSWTDQDSK DSTYSMSSTL
 201  TLTKDEYERH NSYTCEATHK TSTSPIVKSF NRNEC*
```

Fig. 1(b)

SUBSTITUTE SHEET

```
   1 GAATTCCCCT CTCCACAGAC ACTGAAAACT CTGACTCAAC ATGGAAAGGC
  51 ACTGGATCTT TCTACTCCTG TTGTCAGTAA CTGCAGGTGT CCACTCCCAG
 101 GTCCAGCTGC AGCAGTCTGG GGCTGAACTG GCAAGACCTG GGGCCTCAGT
 151 GAAGATGTCC TGCAAGGCTT CTGGCTACAC CTTTACTAGG TACACGATGC
 201 ACTGGGTAAA ACAGAGGCCT GGACAGGGTC TGGAATGGAT TGGATACATT
 251 AATCCTAGCC GTGGTTATAC TAATTACAAT CAGAAGTTCA AGGACAAGGC
 301 CACATTGACT ACAGACAAAT CCTCCAGCAC AGCCTACATG CAACTGAGCA
 351 GCCTGACATC TGAGGACTCT GCAGTCTATT ACTGTGCAAG ATATTATGAT
 401 GATCATTACT GCCTTGACTA CTGGGGCCAA GGCACCACTC TCACAGTCTC
 451 CTCAGCCAAA ACAACAGCCC CATCGGTCTA TCCACTGGCC CCTGTGTGTG
 501 GAGATACAAC TGGCTCCTCG GTGACTCTAG GATGCCTGGT CAAGGGTTAT
 551 TTCCCTGAGC CAGTGACCTT GACCTGGAAC TCTGGATCCC TGTCCAGTGG
 601 TGTGCACACC TTCCCAGCTG TCCTGCAGTC TGACCTCTAC ACCCTCAGCA
 651 GCTCAGTGAC TGTAACCTCG AGCACCTGGC CCAGCCAGTC CATCACCTGC
 701 AATGTGGCCC ACCCGGCAAG CAGCACCAAG GTGGACAAGA AAATTGAGCC
 751 CAGAGGGCCC ACAATCAAGC CCTGTCCTCC ATGCAAATGC CCAGCACCTA
 801 ACCTCTTGGG TGGACCATCC GTCTTCATCT TCCCTCCAAA GATCAAGGAT
 851 GTACTCATGA TCTCCCTGAG CCCCATAGTC ACATGTGTGG TGGTGGATGT
 901 GAGCGAGGAT GACCCAGATG TCCAGATCAG CTGGTTTGTG AACAACGTGG
 951 AAGTACACAC AGCTCAGACA CAAACCCATA GAGAGGATTA CAACAGTACT
1001 CTCCGGGTGG TCAGTGCCCT CCCCATCCAG CACCAGGACT GGATGAGTGG
1051 CAAGGAGTTC AAATGCAAGG TCAACAACAA AGACCTCCCA GCGCCCATCG
1101 AGAGAACCAT CTCAAAACCC AAAGGGTCAG TAAGAGCTCC ACAGGTATAT
1151 GTCTTGCCTC CACCAGAAGA AGAGATGACT AAGAAACAGG TCACTCTGAC
1201 CTGCATGGTC ACAGACTTCA TGCCTGAAGA CATTTACGTG GAGTGGACCA
1251 ACAACGGGAA AACAGAGCTA AACTACAAGA ACACTGAACC AGTCCTGGAC
1301 TCTGATGGTT CTTACTTCAT GTACAGCAAG CTGAGAGTGG AAAAGAAGAA
1351 CTGGGTGGAA AGAAATAGCT ACTCCTGTTC AGTGGTCCAC GAGGGTCTGC
1401 ACAATCACCA CACGACTAAG AGCTTCTCCC GGACTCCGGG TAAATGAGCT
1451 CAGCACCCAC AAAACTCTCA GGTCCAAAGA GACACCCACA CTCATCTCCA
1501 TGCTTCCCTT GTATAAATAA AGCACCCAGC AATGCCTGGG ACCATGTAAA
1551 AAAAAAAAAA AAAGGAATTC
```

Fig. 2(a)

OKT 3 HEAVY CHAIN PROTEIN SEQUENCE DEDUCED FROM DNA SEQUENCE

```
  1  MERHWIFLLL LSVTAGVHSQ VQLQQSGAEL ARPGASVKMS CKASGYTFTR
 51  YTMHWVKQRP GQGLEWIGYI NPSRGYTNYN QKFKDKATLT TDKSSSTAYM
101  QLSSLTSEDS AVYYCARYYD DHYCLDYWGQ GTTLTVSSAK TTAPSVYPLA
151  PVCGDTTGSS VTLGCLVKGY FPEPVTLTWN SGSLSSGVHT FPAVLQSDLY
201  TLSSSVTVTS STWPSQSITC NVAHPASSTK VDKKIEPRGP TIKPCPPCKC
251  PAPNLLGGPS VFIFPPKIKD VLMISLSPIV TCVVVDVSED DPDVQISWFV
301  NNVEVHTAQT QTHREDYNST LRVVSALPIQ HQDWMSGKEF KCKVNNKDLP
351  APIERTISKP KGSVRAPQVY VLPPPEEEMT KKQVTLTCMV TDFMPEDIYV
401  EWTNNGKTEL NYKNTEPVLD SDGSYFMYSK LRVEKKNWVE RNSYSCSVVH
451  EGLHNHHTTK SFSRTPGK*
```

Fig. 2(b)

```
                1                       23                      42
                NN      N                 N        N    N
RES TYPE        SBspSPESssBSbSsSssPSPSPsPSsse*s*p*Pi^ISsSe
Okt3vl          QIVLTQSPAIMSASPGEKVTMTCSASS.SVSYMNWYQQKSGT
REI             DIQMTQSPSSLSASVGDRVTITCQASQDIIKYLNWYQQTPGK
                ? ?
                  CDR1    (LOOP)          *******
                  CDR1    (KABAT)        ***********


                          56                              85
                N  NN
RES TYPE        *IsiPpIeesesssSBEsePsPSBSSEsPspsPsseesSPePb
Okt3vl          SPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAAT
REI             APKLLIYEASNLQAGVPSRFSGSGSGTDYTFTISSLQPEDIAT
                ?  ??                          ?  ?
                      *******  CDR2 (LOOP/KABAT)


                             102   108
RES TYPE        PiPIPies**iPIIsPPSPSPSS
Okt3vl          YYCQQWSSNPFTFGSGTKLEINR
REIvl           YYCQQYQSLPYTFGQGTKLQITR
                             ?    ?
                   ******        CDR3 (LOOP)
                 *********       CRD3 (KABAT)
```

Fig. 3

```
          NN N                  23 26    32 35 N39  43
RES TYPE  SESPs^SBssS^sSSsSpSpSPsPSEbSBssBePiPIpiesss
Okt3h     QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQ
KOL       QVQLVESGGGVVQPGRSLRLSCSSSGFIFSSYAMYWVRQAPGK
               ?                ??
                                    ******    CDR1 (LOOP)
                                       ***** CDR1 (KABAT)

                  52a      60  65     N N  N    82abc     89
RES TYPE IIeIppp^sssssssss^ps^pSSsbSpseSsSseSp^pSpsSBssS^ePb
Okt3vh   GLEWIGYINPSRGYTNTNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAV
KOL      GLEWVAIIWDDGSDQHYADSVKGRFTISRDNSKNTLFLQMDSLRPEDTGV
           ??                    ? ?  ?  ?            ?
              ************         CDR2  (LOOP)
              ******************** CDR2  (KABAT)

           92 N                  107    113
RES TYPE  PiPIEissssiiisssbibi*EIPIP*spSBSS
Okt3vh    YYCARYYDDHY.......CLDYWGQGTTLTVSS
KOL       YFCARDGGHGFCSSASCFGPDYWGQGTPVTVSS
               ***************** CRD3 (KABAT/LOOP)
```

Fig. 4

OKT 3 HEAVY CHAIN CDR GRAFTS

1. gh341 and derivatives

```
          1                          26        35  39  43
Okt3vh    QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQ
gH341     QVQLVESGGGVVQPGRSLRLSCSSSGYTFTRYTMHWVRQAPGK   JA178
gH341A    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA185

gH341E    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA198
gH341*    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA207
gH341*    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA209
gH341D    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA197
gH341*    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA199
gH341C    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA184

gH341*    QVQLVQSGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA203
gH341*    QVQLVESGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA205
gH341B    QVQLVESGGGVVQPGRSLRLSCSSSGYTFTRYTMHWVRQAPGK   JA183
gH341*    QVQLVQSGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA204
gH341*    QVQLVESGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA206
gH341*    QVQLVQSGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA208
KOL       QVQLVESGGGVVQPGRSLRLSCSSSGFIFSSYAMYWVRQAPGK
```

Fig. 5(i)

```
          44     50                  65                        83
Okt3vh    GLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLT
gH341     GLEWVAYINPSRGYTNYNQKFKDRFTISRDNSKNTLFLQMDSLR JA178
gH341A    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR JA185

gH341E    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR JA198
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKNTAFLQMDSLR JA207
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTAFLQMDSLR JA209
gH341D    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKNTLFLQMDSLR JA197
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTLFLQMDSLR JA199
gH341C    GLEWVAYINPSRGYTNYNQKFKDRFTISRDNSKNTLFLQMDSLR JA184

gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR JA207
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR JA205
gH341B    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR JA183
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR JA204
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR JA206
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKNTAFLQMDSLR JA208
KOL       GLEWVAIIWDDGSDQHYADSVKGRFTISRDNSKNTLFLQMDSLR
```

Fig. 5(ii)

```
          84          95              102         113
Okt3vh    SEDSAVYYCARYYDDHY.......CLDYWGQGTTLTVSS
gH341     PEDTGVYFCARYYDDHY.......CLDYWGQGTTLTVSS   JA178
gH341A    PEDTAVYYCARYYDDHY.......CLDYWGQGTTLTVSS   JA185

gH341E    PEDTGVYFCARYYDDHY.......CLDYWGQGTTLTVSS   JA198
gH341*    PEDTGVYFCARYYDDHY.......CLDYWGQGTTLTVSS   JA207
gH341D    PEDTGVYFCARYYDDHY.......CLDYWGQGTTLTVSS   JA197
gH341*    PEDTGVYFCARYYDDHY.......CLDYWGQGTTLTVSS   JA209
gH341*    PEDTGVYFCARYYDDHY.......CLDYWGQGTTLTVSS   JA199
gH341C    PEDTGVYFCARYYDDHY.......CLDYWGQGTTLTVSS   JA184

gH341*    PEDTAVYYCARYYDDHY.......CLDYWGQGTTLTVSS   JA203
gH341*    PEDTAVYYCARYYDDHY.......CLDYWGQGTTLTVSS   JA205
gH341B    PEDTAVYYCARYYDDHY.......CLDYWGQGTTLTVSS   JA183
gH341*    PEDTGVYFCARYYDDHY.......CLDYWGQGTTLTVSS   JA204
gH341*    PEDTGVYFCARYYDDHY.......CLDYWGQGTTLTVSS   JA206
gH341*    PEDTGVYFCARYYDDHY.......CLDYWGQGTTLTVSS   JA208
KOL       PEDTGVYFCARDGGHGFCSSASCFGPDYWGQGTPVTVSS
```

Fig. 5(iii)

OKT3 LIGHT CHAIN CDR GRAFTING

1. gL221 and derivatives

```
          1                        24        34       42
Okt3vl    QIVLTQSPAIMSASPGEKVTMTCSASS.SVSYMNWYQQKSGT
gL221     DIQMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
gL221A    QIVMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
gL221B    QIVMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
gL221C    DIQMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
REI       DIQMTQSPSSLSASVGDRVTITCQASQDIIKYLNWYQQTPGK

          43     50    56                          85
Okt3vl    SPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAAT
gL221     APKLLIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
gL221A    APKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
gL221B    APKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
gL221C    APKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
REI       APKLLIYEASNLQAGVPSRFSGSGSGTDYTFTISSLQPEDIAT

          86   91   96          108
Okt3vl    YYCQQWSSNPFTFGSGTKLEINR
gL221     YYCQQWSSNPFTFGQGTKLQITR
gL221A    YYCQQWSSNPFTFGQGTKLQITR
gL221B    YYCQQWSSNPFTFGQGTKLQITR
gL221C    YYCQQWSSNPFTFGQGTKLQITR
REI       YYCQQYQSLPYTFGQGTKLQITR
```

CDR'S ARE UNDERLINED

FRAMEWORK RESIDUES INCLUDED IN THE GENE ARE DOUBLE UNDERLINED

Fig. 6

OKT3 - pJA198 EVALUATION
BINDING ASSAY
(Mean Channel - HPBALL's)

Fig. 7

OKT3 - pJA198 EVALUATION
BLOCKING ASSAY
(Mean Channel -HPBALL's)

Fig. 8

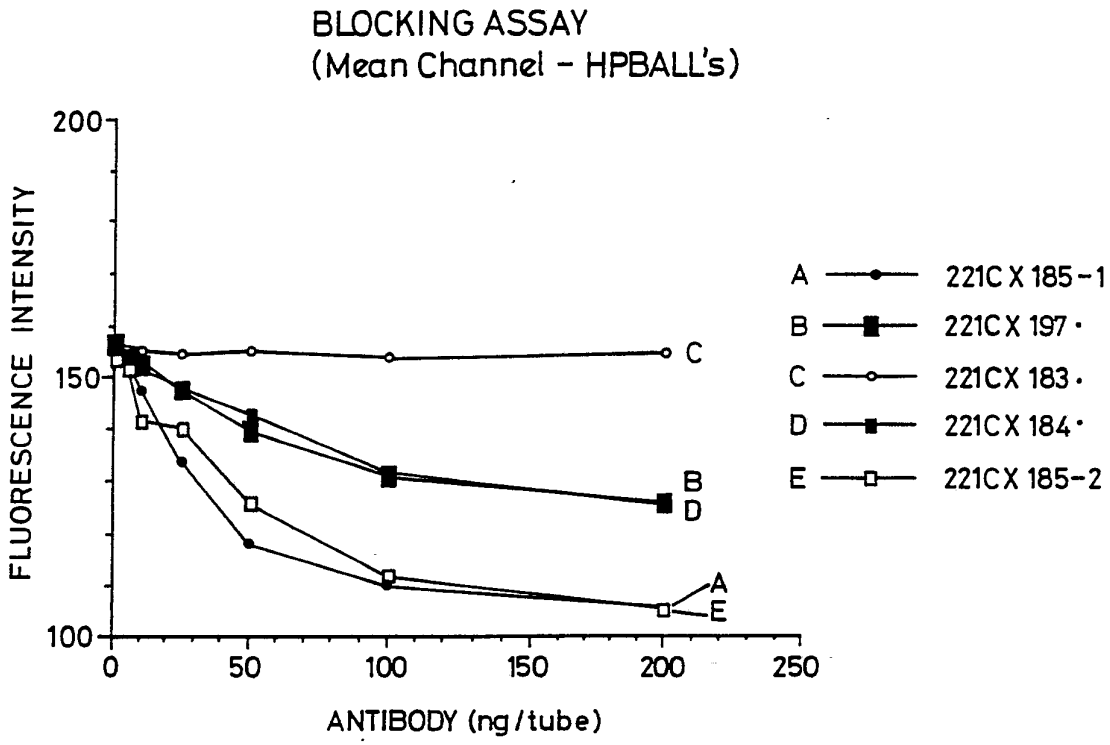
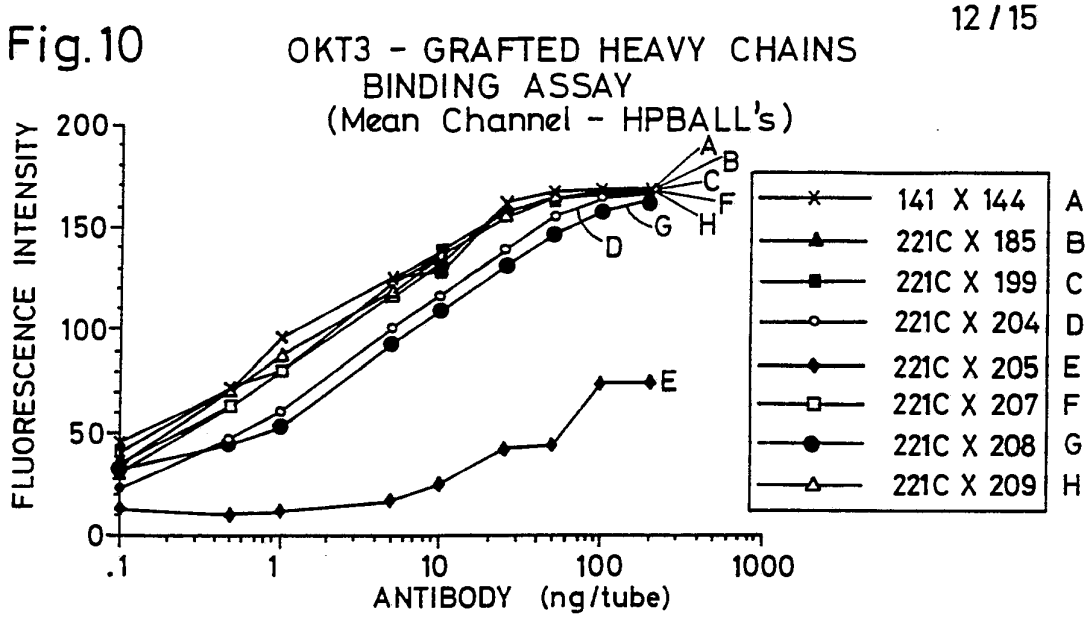
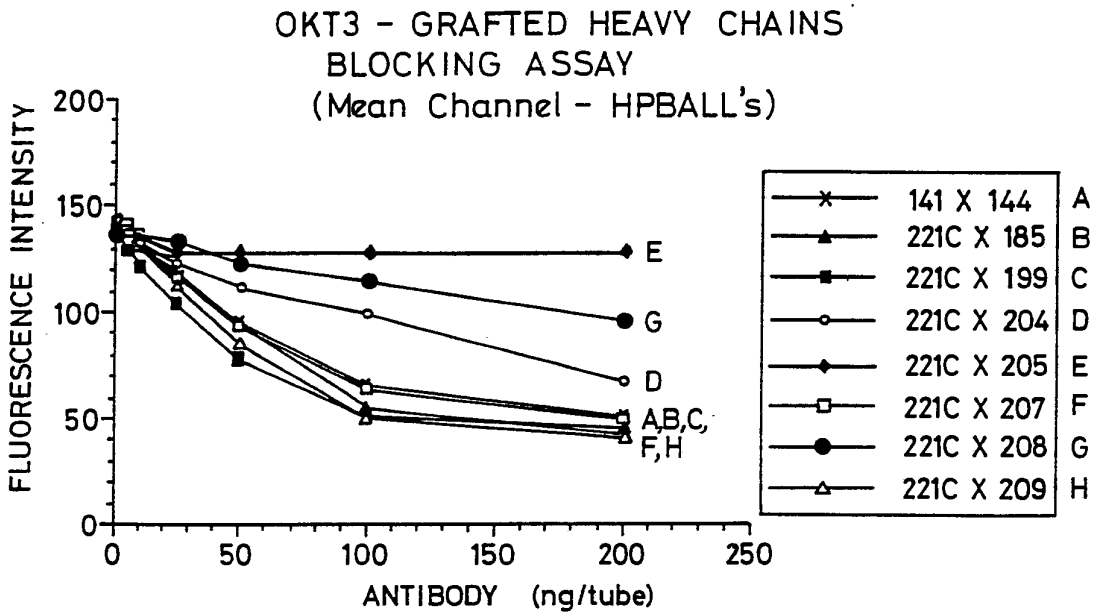

BLOCKING ASSAY
(Mean Channel - HPBALL's)
FLUORESCENCE INTENSITY
200
150
100
0
50
100
150
200
250
ANTIBODY (ng/tube)
A
B
C
D
E
A 221C X 185-1
B 221C X 197
C 221C X 183
D 221C X 184
E 221C X 185-2

Fig. 9

Fig.10

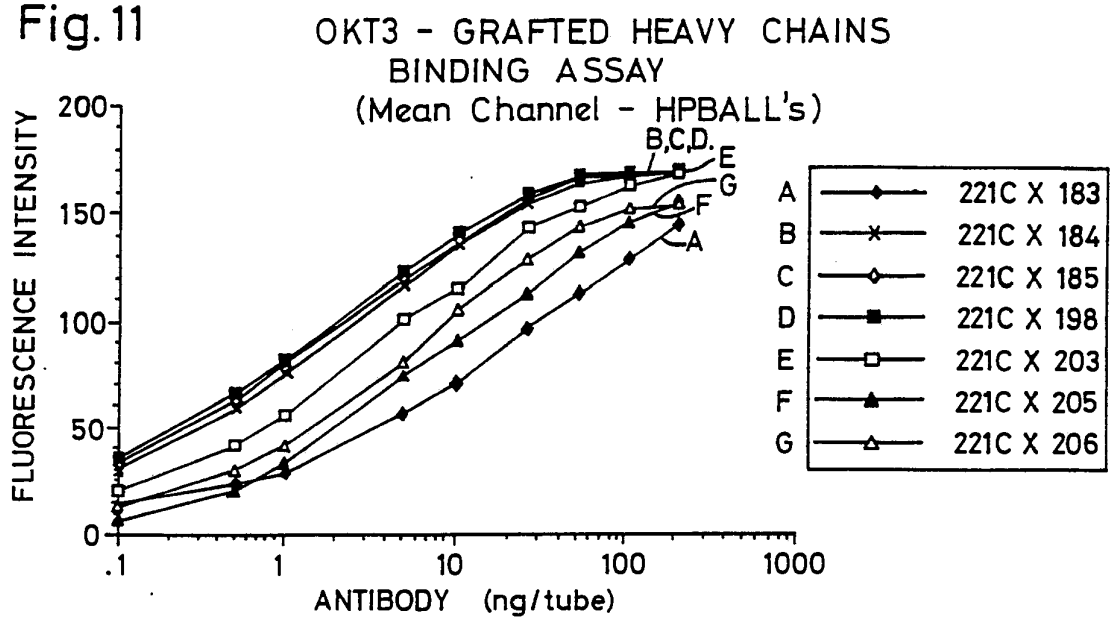


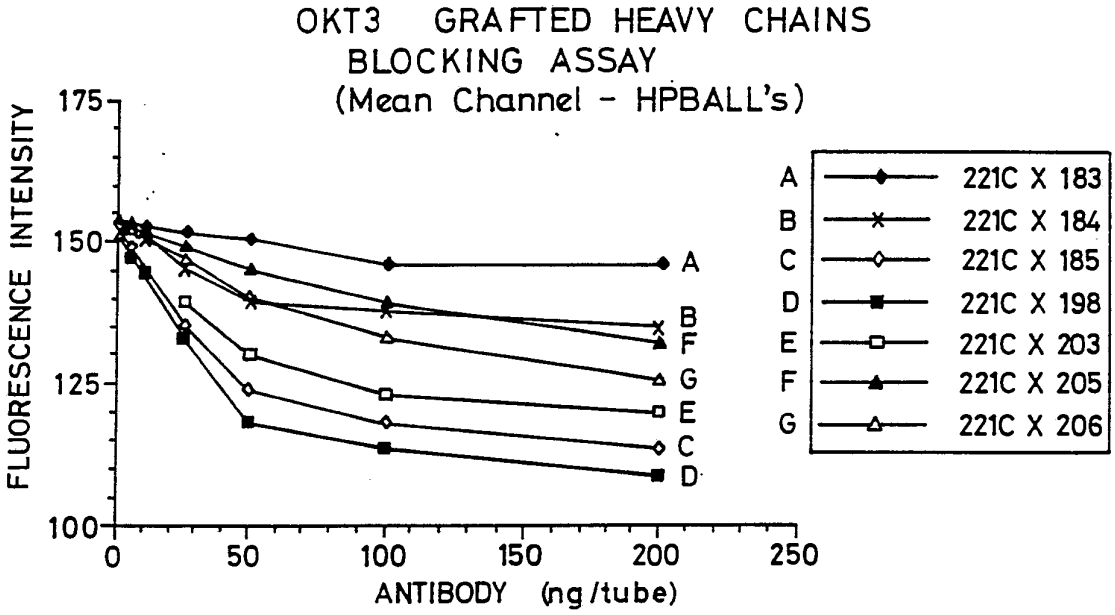


(205) _,__,24,48,49,71,73,76,78,88,91,
(208) 6,__,24,48,49,71,73,__,78,__,__,
(204) 6,__,24,48,49,71,73,76,78,__,__,
(199) 6,23,24,48,49,__,__,__,__,__,__,
(207) 6,23,24,48,49,71,73,__,78,__,__,
(185) 6,23,24,48,49,71,73,76,78,88,91,
(209) 6,23,24,48,49,__,__,__,78,__,__,
141 X 144

SUBSTITUTE SHEET

Fig. 11

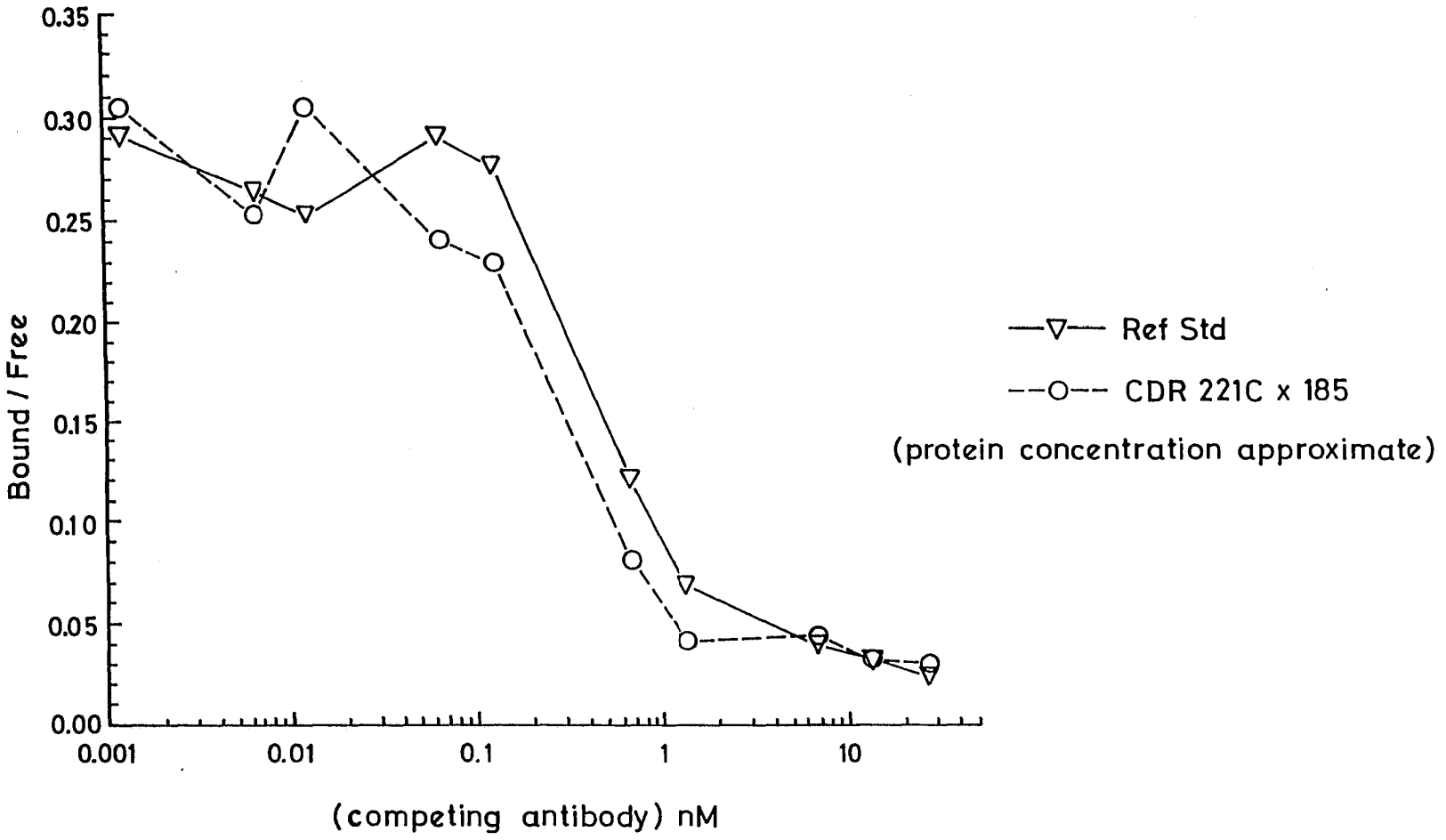




◆	(183)	_,__,__,48,49,71,73,76,78,88,91,
▲	(205)	_,__,24,48,49,71,73,76,78,88,91,
×	(184)	6,23,24,__,__,__,__,__,__,__,__,
△	(206)	_,__,24,48,49,71,73,76,78,__,__,
□	(203)	6,__,24,48,49,71,73,76,78,88,91,
◇	(185)	6,23,24,48,49,71,73,76,78,88,91,
■	(198)	6,23,24,48,49,71,73,76,78,__,__,

Fig. 12

Fig. 13



INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 90/02017

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: C 12 P 21/08, C 12 N 15/13, A 61 K 39/395, C 07 K 15/06
C 12 N 5/10, 15/62

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	C 12 P; C 12 N; A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
P,X	EP, A1, 0403156 (GENZYME CORPORATION ET AL.) 19 December 1990, see examples 8-12 and corresponding tables	1,6,8, 13,14-22
Y	Proc. Natl. Acad. Sci. USA, vol. 86, December 1989, C. Queen et al.: "A humanized antibody that binds to the interleukin 2 receptor ", see page 10029- page 10033 see the whole document and in particular page 10031 right col. - page 10032, left col. and page 10033 left col.	1,6,8, 13,14-22
Y	EP, A1, 0328404 (MEDICAL RESEARCH COUNCIL ET AL.) 16 August 1989, see pages 1-3, page 9, lines 49-54 and the claims	1,6,8, 13,14-22

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
11th April 1991	17.05.91
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	F.W. HECK

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	Nature, vol. 332, March 1988, L. Riechmann et al.: "Reshaping human antibodies for therapy ", see page 323 - page 327 see in particular page 327, right col.	1,6,8, 13,144-22
A	Nature, vol. 321, May 1986, P.T. Jones et al.: "Replacing the complementarity-determining regions in a human antibody with those from a mouse ", see page 522 - page 525 see the whole document	1-22
A	Nature, vol. 328, August 1987, S. Roberts et al.: "Generation of an antibody with enhanced affinity and specificity for its antigen by protein engineering ", see page 731 - page. 734 see the whole document	1,6
A	Science, vol. 239, 1988, M. Verhoeyen et al.: "Reshaping Human Antibodies: Grafting an Antilysozyme Activity ", see page 1534 - page 1536 see the whole document	1,6
A	EP, A2, 0239400 (WINTER, GREGORY PAUL) 30 September 1987, see the whole document	1,6,17-22
A	EP, A1, 0323806 (CIBA-GEIGY AG) 12 July 1989, see pages 2-6	1,6,17-22
A	Nature, vol. 341, October 1989, E.S. Ward et al.: "Binding activities of a repertoire of single immunoglobulin variable domains secreted from Escherichia coli ", see page 544 - page 546	1,6

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. PCT/GB 90/02017

SA 43080

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 28/02/91
The European Patent office is in no way liable for theseparticulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A1- 0403156	19/12/90	NONE		
EP-A1- 0328404	16/08/89	AU-D-	3062689	06/09/89
		GB-A-	2216126	04/10/89
		WO-A-	89/07452	24/08/89
EP-A2- 0239400	30/09/87	GB-A-B-	2188638	07/10/87
		JP-A-	62296890	24/12/87
EP-A1- 0323806	12/07/89	AU-D-	2759588	06/07/89
		JP-A-	2154696	14/06/90

For more details about this annex : see Official Journal of the European patent Office, No. 12/82

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Ind 1

HUMANISED ANTIBODIES

Field of the Invention

The present invention relates to humanised antibody molecules, to processes for their production using recombinant DNA technology, and to their therapeutic uses.

The term "humanised antibody molecule" is used to describe a molecule having an antigen binding site derived from an immunoglobulin from a non-human species, and remaining immunoglobulin-derived parts of the molecule being derived from a human immunoglobulin. The antigen binding site typically comprises complementarity determining regions (CDRs) which determine the binding specificity of the antibody molecule and which are carried on appropriate framework regions in the variable domains. There are 3 CDRs (CDR1, CDR2 and CDR3) in each of the heavy and light chain variable domains.

In the description, reference is made to a number of publications by number. The publications are listed in numerical order at the end of the description.

Background of the Invention

Natural immunoglobulins have been known for many years, as have the various fragments thereof, such as the Fab, $(Fab')_2$ and Fc fragments, which can be derived by enzymatic cleavage. Natural immunoglobulins comprise a generally Y-shaped molecule having an antigen-binding site towards the end of each upper arm. The remainder of the structure, and particularly the stem of the Y, mediates the effector functions associated with immunoglobulins.

Natural immunoglobulins have been used in assay, diagnosis and, to a more limited extent, therapy. However, such uses, especially in therapy, were hindered until recently by the polyclonal nature of natural immunoglobulins. A significant step towards the realisation of the potential

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of immunoglobulins as therapeutic agents was the discovery of procedures for the production of monoclonal antibodies (MAbs) of defined specificity (1).

However, most MAbs are produced by hybridomas which are fusions of rodent spleen cells with rodent myeloma cells. They are therefore essentially rodent proteins. There are very few reports of the production of human MAbs.

Since most available MAbs are of rodent origin, they are naturally antigenic in humans and thus can give rise to an undesirable immune response termed the HAMA (Human Anti-Mouse Antibody) response. Therefore, the use of rodent MAbs as therapeutic agents in humans is inherently limited by the fact that the human subject will mount an immunological response to the MAb and will either remove it entirely or at least reduce its effectiveness. In practice, MAbs of rodent origin may not be used in patients for more than one or a few treatments as a HAMA response soon develops rendering the MAb ineffective as well as giving rise to undesirable reactions. For instance, OKT3 a mouse IgG2a/k MAb which recognises an antigen in the T-cell receptor-CD3 complex has been approved for use in many countries throughout the world as an immunosuppressant in the treatment of acute allograft rejection [Chatenoud et al (2) and Jeffers et al (3)]. However, in view of the rodent nature of this and other such MAbs, a significant HAMA response which may include a major anti-idiotype component, may build up on use. Clearly, it would be highly desirable to diminish or abolish this undesirable HAMA response and thus enlarge the areas of use of these very useful antibodies.

Proposals have therefore been made to render non-human MAbs less antigenic in humans. Such techniques can be generically termed "humanisation" techniques. These

techniques typically involve the use of recombinant DNA technology to manipulate DNA sequences encoding the polypeptide chains of the antibody molecule.

Early methods for humanising MAbs involved production of chimeric antibodies in which an antigen binding site comprising the complete variable domains of one antibody is linked to constant domains derived from another antibody. Methods for carrying out such chimerisation procedures are described in EP0120694 (Celltech Limited), EP0125023 (Genentech Inc. and City of Hope), EP-A-0 171496 (Res. Dev. Corp. Japan), EP-A-0 173 494 (Stanford University), and WO 86/01533 (Celltech Limited). This latter Celltech application (WO 86/01533) discloses a process for preparing an antibody molecule having the variable domains from a mouse MAb and the constant domains from a human immunoglobulin. Such humanised chimeric antibodies, however, still contain a significant proportion of non-human amino acid sequence, i.e. the complete non-human variable domains, and thus may still elicit some HAMA response, particularly if administered over a prolonged period [Begent et al (ref. 4)].

In an alternative approach, described in EP-A-0239400 (Winter), the complementarity determining regions (CDRs) of a mouse MAb have been grafted onto the framework regions of the variable domains of a human immunoglobulin by site directed mutagenesis using long oligonucleotides. The present invention relates to humanised antibody molecules prepared according to this alternative approach, i.e. CDR-grafted humanised antibody molecules. Such CDR-grafted humanised antibodies are much less likely to give rise to a HAMA response than humanised chimeric antibodies in view of the much lower proportion of non-human amino acid sequence which they contain.

The earliest work on humanising MAbs by CDR-grafting was carried out on MAbs recognising synthetic antigens, such as the NP or NIP antigens. However, examples in which a mouse MAb recognising lysozyme and a rat MAb recognising an antigen on human T-cells were humanised by CDR-grafting have been described by Verhoeyen et al (5) and Riechmann et al (6) respectively. The preparation of CDR-grafted antibody to the antigen on human T cells is also described in WO 89/07452 (Medical Research Council).

In Riechmann et al/Medical Research Council it was found that transfer of the CDR regions alone [as defined by Kabat refs. (7) and (8)] was not sufficient to provide satisfactory antigen binding activity in the CDR-grafted product. Riechmann et al found that it was necessary to convert a serine residue at position 27 of the human sequence to the corresponding rat phenylalanine residue to obtain a CDR-grafted product having improved antigen binding activity. This residue at position 27 of the heavy chain is within the structural loop adjacent to CDR1. A further construct which additionally contained a human serine to rat tyrosine change at position 30 of the heavy chain did not have a significantly altered binding activity over the humanised antibody with the serine to phenylalanine change at position 27 alone. These results indicate that changes to residues of the human sequence outside the CDR regions, in particular in the structural loop adjacent to CDR1, may be necessary to obtain effective antigen binding activity for CDR-grafted antibodies which recognise more complex antigens. Even so the binding affinity of the best CDR-grafted antibodies obtained was still significantly less than the original MAb.

Very recently Queen et al (9) have described the preparation of a humanised antibody that binds to the

interleukin 2 receptor, by combining the CDRs of a murine MAb (anti-Tac) with human immunoglobulin framework and constant regions. The human framework regions were chosen to maximise homology with the anti-Tac MAb sequence. In addition computer modelling was used to identify framework amino acid residues which were likely to interact with the CDRs or antigen, and mouse amino acids were used at these positions in the humanised antibody.

In WO 90/07861 Queen et al propose four criteria for designing humanised immunoglobulins. The first criterion is to use as the human acceptor the framework from a particular human immunoglobulin that is unusually homologous to the non-human donor immunoglobulin to be humanised, or to use a consensus framework from many human antibodies. The second criterion is to use the donor amino acid rather than the acceptor if the human acceptor residue is unusual and the donor residue is typical for human sequences at a specific residue of the framework. The third criterion is to use the donor framework amino acid residue rather than the acceptor at positions immediately adjacent to the CDRs. The fourth criterion is to use the donor amino acid residue at framework positions at which the amino acid is predicted to have a side chain atom within about 3 Å of the CDRs in a three-dimensional immunoglobulin model and to be capable of interacting with the antigen or with the CDRs of the humanised immunoglobulin. It is proposed that criteria two, three or four may be applied in addition or alternatively to criterion one, and may be applied singly or in any combination.

WO 90/07861 describes in detail the preparation of a single CDR-grafted humanised antibody, a humanised antibody having specificity for the p55 Tac protein of the

IL-2 receptor. The combination of all four criteria, as above, were employed in designing this humanised antibody, the variable region frameworks of the human antibody Eu (7) being used as acceptor. In the resultant humanised antibody the donor CDRs were as defined by Kabat et al (7 and 8) and in addition the mouse donor residues were used in place of the human acceptor residues, at positions 27, 30, 48, 66, 67, 89, 91, 94, 103, 104, 105 and 107 in the heavy chain (SEQ ID NO:31) and at positions 48, 60 and 63 in the light chain, of the variable region frameworks. The humanised anti-Tac antibody obtained is reported to have an affinity for p55 of 3 x 10^9 M^{-1}, about one-third of that of the murine MAb.

We have further investigated the preparation of CDR-grafted humanised antibody molecules and have identified a hierarchy of positions within the framework of the variable regions (i.e. outside both the Kabat CDRs and structural loops of the variable regions) at which the amino acid identities of the residues are important for obtaining CDR-grafted products with satisfactory binding affinity. This has enabled us to establish a protocol for obtaining satisfactory CDR-grafted products which may be applied very widely irrespective of the level of homology between the donor immunoglobulin and acceptor framework. The set of residues which we have identified as being of critical importance does not coincide with the residues identified by Queen et al (9).

Summary of the Invention

Accordingly, in a first aspect the invention provides a CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

In preferred embodiments, the heavy chain framework comprises donor residues at positions 23, 24, 49, 71, 73 and 78 or at positions 23, 24 and 49. The residues at positions 71, 73 and 78 of the heavy chain framework are preferably either all acceptor or all donor residues.

In particularly preferred embodiments the heavy chain framework additionally comprises donor residues at one, some or all of positions 6, 37, 48 and 94. Also it is particularly preferred that residues at positions of the heavy chain framework which are commonly conserved across species, i.e. positions 2, 4, 25, 36, 39, 47, 93, 103, 104, 106 and 107, if not conserved between donor and acceptor, additionally comprise donor residues. Most preferably the heavy chain framework additionally comprises donor residues at positions 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

In addition the heavy chain framework optionally comprises donor residues at one, some or all of positions:
1 and 3,
72 and 76,
69 (if 48 is different between donor and acceptor),
38 and 46 (if 48 is the donor residue),
80 and 20 (if 69 is the donor residue),
67,
82 and 18 (if 67 is the donor residue),
91,
88, and
any one or more of 9, 11, 41, 87, 108, 110 and 112.

In the first and other aspects of the present invention reference is made to CDR-grafted antibody products comprising acceptor framework and donor antigen binding regions. It will be appreciated that the invention is widely applicable to the CDR-grafting of antibodies in

general. Thus, the donor and acceptor antibodies may be derived from animals of the same species and even same antibody class or sub-class. More usually, however, the donor and acceptor antibodies are derived from animals of different species. Typically the donor antibody is a non-human antibody, such as a rodent MAb, and the acceptor antibody is a human antibody.

In the first and other aspects of the present invention, the donor antigen binding region typically comprises at least one CDR from the donor antibody. Usually the donor antigen binding region comprises at least two and preferably all three CDRs of each of the heavy chain and/or light chain variable regions. The CDRs may comprise the Kabat CDRs, the structural loop CDRs or a composite of the Kabat and structural loop CDRs and any combination of any of these. Preferably, the antigen binding regions of the CDR-grafted heavy chain variable domain comprise CDRs corresponding to the Kabat CDRs at CDR2 (residues 50-65) and CDR3 (residues 95-100) and a composite of the Kabat and structural loop CDRs at CDR1 (residues 26-35).

The residue designations given above and elsewhere in the present application are numbered according to the Kabat numbering [refs. (7) and (8)]. Thus the residue designations do not always correspond directly with the linear numbering of the amino acid residues. The actual linear amino acid sequence may contain fewer or additional amino acids than in the strict Kabat numbering corresponding to a shortening of, or insertion into, a structural component, whether framework or CDR, of the basic variable domain structure. For example, the heavy chain variable region of the anti-Tac antibody described by Queen et al (9) contains a single amino acid insert (residue 52a) after residue 52 of CDR2 and a three amino

acid insert (residues 82a, 82b and 82c) after framework residue 82, in the Kabat numbering. The correct Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

The invention also provides in a second aspect a CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 1 and/or 3 and 46 and/or 47. Preferably the CDR grafted light chain of the second aspect comprises donor residues at positions 46 and/or 47.

The invention also provides in a third aspect a CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, 58 and 71.

In a preferred embodiment of the third aspect, the framework comprises donor residues at all of positions 46, 48, 58 and 71.

In particularly preferred embodiments of the second and third aspects, the framework additionally comprises donor residues at positions 36, 44, 47, 85 and 87. Similarly positions of the light chain framework which are commonly conserved across species, i.e. positions 2, 4, 6, 35, 49, 62, 64-69, 98, 99, 101 and 102, if not conserved between donor and acceptor, additionally comprise donor residues. Most preferably the light chain framework additionally comprises donor residues at positions 2, 4, 6, 35, 36, 38, 44, 47, 49, 62, 64-69, 85, 87, 98, 99, 101 and 102.

In addition the framework of the second or third aspects optionally comprises donor residues at one, some or all of positions:
1 and 3,
63,
60 (if 60 and 54 are able to form at potential saltbridge),
70 (if 70 and 24 are able to form a potential saltbridge),
73 and 21 (if 47 is different between donor and acceptor),
37 and 45 (if 47 is different between donor and acceptor), and
any one or more of 10, 12, 40, 80, 103 and 105.

Preferably, the antigen binding regions of the CDR-grafted light chain variable domain comprise CDRs corresponding to the Kabat CDRs at CDR1 (residue 24-34), CDR2 (residues 50-56) and CDR3 (residues 89-97).

The invention further provides in a fourth aspect a CDR-grafted antibody molecule comprising at least one CDR-grafted heavy chain and at least one CDR-grafted light chain according to the first and second or first and third aspects of the invention.

The humanised antibody molecules and chains of the present invention may comprise: a complete antibody molecule, having full length heavy and light chains; a fragment thereof, such as a Fab, $(Fab')_2$ or FV fragment; a light chain or heavy chain monomer or dimer; or a single chain antibody, e.g. a single chain FV in which heavy and light chain variable regions are joined by a peptide linker; or any other CDR-grafted molecule with the same specificity as the original donor antibody. Similarly the CDR-grafted heavy and light chain variable region may be combined with other antibody domains as appropriate.

Also the heavy or light chains or humanised antibody molecules of the present invention may have attached to them an effector or reporter molecule. For instance, it may have a macrocycle, for chelating a heavy metal atom, or a toxin, such as ricin, attached to it by a covalent bridging structure. Alternatively, the procedures of recombinant DNA technology may be used to produce an immunoglobulin molecule in which the Fc fragment or CH3 domain of a complete immunoglobulin molecule has been replaced by, or has attached thereto by peptide linkage, a functional non-immunoglobulin protein, such as an enzyme or toxin molecule.

Any appropriate acceptor variable region framework sequences may be used having regard to class/type of the donor antibody from which the antigen binding regions are derived. Preferably, the type of acceptor framework used is of the same/similar class/type as the donor antibody. Conveniently, the framework may be chosen to maximise/optimise homology with the donor antibody sequence particularly at positions close or adjacent to the CDRs. However, a high level of homology between donor and acceptor sequences is not important for application of the present invention. The present invention identifies a hierarchy of framework residue positions at which donor residues may be important or desirable for obtaining a CDR-grafted antibody product having satisfactory binding properties. The CDR-grafted products usually have binding affinities of at least 10^5 M^{-1}, preferably at least about 10^8 M^{-1}, or especially in the range 10^8-10^{12} M^{-1}. In principle, the present invention is applicable to any combination of donor and acceptor antibodies irrespective of the level of homology between their sequences. A protocol for applying the invention to any particular donor-acceptor antibody pair is given hereinafter. Examples of human frameworks which may be

used are KOL, NEWM, REI, EU, LAY and POM (refs. 4 and 5) and the like; for instance KOL and NEWM for the heavy chain and REI for the light chain and EU, LAY and POM for both the heavy chain and the light chain.

Also the constant region domains of the products of the invention may be selected having regard to the proposed function of the antibody in particular the effector functions which may be required. For example, the constant region domains may be human IgA, IgE, IgG or IgM domains. In particular, IgG human constant region domains may be used, especially of the IgG1 and IgG3 isotypes, when the humanised antibody molecule is intended for therapeutic uses, and antibody effector functions are required. Alternatively, IgG2 and IgG4 isotypes may be used when the humanised antibody molecule is intended for therapeutic purposes and antibody effector functions are not required, e.g. for simple blocking of lymphokine activity.

However, the remainder of the antibody molecules need not comprise only protein sequences from immunoglobulins. For instance, a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain is fused to a DNA sequence encoding the amino acid sequence of a functional polypeptide such as an effector or reporter molecule.

Preferably the CDR-grafted antibody heavy and light chain and antibody molecule products are produced by recombinant DNA technology.

Thus in further aspects the invention also includes DNA sequences coding for the CDR-grafted heavy and light chains, cloning and expression vectors containing the DNA sequences, host cells transformed with the DNA sequences

and processes for producing the CDR-grafted chains and antibody molecules comprising expressing the DNA sequences in the transformed host cells.

The general methods by which the vectors may be constructed, transfection methods and culture methods are well known per se and form no part of the invention. Such methods are shown, for instance, in references 10 and 11.

The DNA sequences which encode the donor amino acid sequence may be obtained by methods well known in the art. For example the donor coding sequences may be obtained by genomic cloning, or cDNA cloning from suitable hybridoma cell lines. Positive clones may be screened using appropriate probes for the heavy and light chain genes in question. Also PCR cloning may be used.

DNA coding for acceptor, e.g. human acceptor, sequences may be obtained in any appropriate way. For example DNA sequences coding for preferred human acceptor frameworks such as KOL, REI, EU and NEWM, are widely available to workers in the art.

The standard techniques of molecular biology may be used to prepare DNA sequences coding for the CDR-grafted products. Desired DNA sequences may be synthesised completely or in part using oligonucleotide synthesis techniques. Site-directed mutagenesis and polymerase chain reaction (PCR) techniques may be used as appropriate. For example oligonucleotide directed synthesis as described by Jones et al (ref. 20) may be used. Also oligonucleotide directed mutagenesis of a pre-exising variable region as, for example, described by Verhoeyen et al (ref. 5) or Riechmann et al (ref. 6) may be used. Also enzymatic filling in of gapped

oligonucleotides using T_4 DNA polymerase as, for example, described by Queen et al (ref. 9) may be used.

Any suitable host cell/vector system may be used for expression of the DNA sequences coding for the CDR-grafted heavy and light chains. Bacterial e.g. E. coli, and other microbial systems may be used, in particular for expression of antibody fragments such as FAb and $(Fab')_2$ fragments, and especially FV fragments and single chain antibody fragments e.g. single chain FVs. Eucaryotic e.g. mammalian host cell expression systems may be used for production of larger CDR-grafted antibody products, including complete antibody molecules. Suitable mammalian host cells include CHO cells and myeloma or hybridoma cell lines.

Thus, in a further aspect the present invention provides a process for producing a CDR-grafted antibody product comprising:

(a) producing in an expression vector an operon having a DNA sequence which encodes an antibody heavy chain according to the first aspect of the invention;

and/or

(b) producing in an expression vector an operon having a DNA sequence which encodes a complementary antibody light chain according to the second or third aspect of the invention;

(c) transfecting a host cell with the or each vector; and

(d) culturing the transfected cell line to produce the CDR-grafted antibody product.

The CDR-grafted product may comprise only heavy or light chain derived polypeptide, in which case only a heavy chain or light chain polypeptide coding sequence is used to transfect the host cells.
For production of products comprising both heavy and light chains, the cell line may be transfected with two vectors, the first vector may contain an operon encoding a light chain-derived polypeptide and the second vector containing an operon encoding a heavy chain-derived polypeptide. Preferably, the vectors are identical, except in so far as the coding sequences and selectable markers are concerned, so as to ensure as far as possible that each polypeptide chain is equally expressed. Alternatively, a single vector may be used, the vector including the sequences encoding both light chain- and heavy chain-derived polypeptides.

The DNA in the coding sequences for the light and heavy chains may comprise cDNA or genomic DNA or both. However, it is preferred that the DNA sequence encoding the heavy or light chain comprises at least partially, genomic DNA, preferably a fusion of cDNA and genomic DNA.

The present invention is applicable to antibodies of any appropriate specificity. Advantageously, however, the invention may be applied to the humanisation of non-human antibodies which are used for in vivo therapy or diagnosis. Thus the antibodies may be site-specific antibodies such as tumour-specific or cell surface-specific antibodies, suitable for use in in vivo therapy or diagnosis, e.g. tumour imaging. Examples of cell surface-specific antibodies are anti-T cell antibodies, such as anti-CD3, and CD4 and adhesion molecules, such as CR3, ICAM and ELAM. The antibodies may have specificity for interleukins (including lymphokines, growth factors and stimulating factors), hormones and other biologically active compounds, and receptors for any of these. For

example, the antibodies may have specificity for any of the following: Interferons α, β, γ or δ, IL1, IL2, IL3, or IL4, etc., TNF, GCSF, GMCSF, EPO, hGH, or insulin, etc.

The the present invention also includes therapeutic and diagnostic compositions comprising the CDR-grafted products of the invention and uses of such compositions in therapy and diagnosis.

Accordingly in a further aspect the invention provides a therapeutic or diagnostic composition comprising a CDR-grafted antibody heavy or light chain or molecule according to previous aspects of the invention in combination with a pharmaceutically acceptable carrier, diluent or excipient.

Accordingly also the invention provides a method of therapy or diagnosis comprising administering an effective amount of a CDR-grafted antibody heavy or light chain or molecule according to previous aspects of the invention to a human or animal subject.

A preferred protocol for obtaining CDR-grafted antibody heavy and light chains in accordance with the present invention is set out below together with the rationale by which we have derived this protocol. This protocol and rationale are given without prejudice to the generality of the invention as hereinbefore described and defined.

Protocol

It is first of all necessary to sequence the DNA coding for the heavy and light chain variable regions of the donor antibody, to determine their amino acid sequences. It is also necessary to choose appropriate acceptor heavy and light chain variable regions, of known amino acid sequence. The CDR-grafted chain is then designed

starting from the basis of the acceptor sequence. It will be appreciated that in some cases the donor and acceptor amino acid residues may be identical at a particular position and thus no change of acceptor framework residue is required.

1. As a first step donor residues are substituted for acceptor residues in the CDRs. For this purpose the CDRs are preferably defined as follows:

Heavy chain	-	CDR1:	residues 26-35
	-	CDR2:	residues 50-65
	-	CDR3:	residues 95-102
Light chain	-	CDR1:	residues 24-34
	-	CDR2:	residues 50-56
	-	CDR3:	residues 89-97

The positions at which donor residues are to be substituted for acceptor in the framework are then chosen as follows, first of all with respect to the heavy chain and subsequently with respect to the light chain.

2. Heavy Chain

2.1 Choose donor residues at all of positions 23, 24, 49, 71, 73 and 78 of the heavy chain or all of positions 23, 24 and 49 (71, 73 and 78 are always either all donor or all acceptor).

2.2 Check that the following have the same amino acid in donor and acceptor sequences, and if not preferably choose the donor: 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

2.3 To further optimise affinity consider choosing donor residues at one, some or any of:

i. 1, 3
ii. 72, 76
iii. If 48 is different between donor and acceptor sequences, consider 69
iv. If at 48 the donor residue is chosen, consider 38 and 46
v. If at 69 the donor residue is chosen, consider 80 and then 20
vi. 67
vii. If at 67 the donor residue is chosen, consider 82 and then 18
viii. 91
ix. 88
x. 9, 11, 41, 87, 108, 110, 112

3. Light Chain

3.1 Choose donor at 46, 48, 58 and 71

3.2 Check that the following have the same amino acid in donor and acceptor sequences, if not preferably choose donor:

2, 4, 6, 35, 38, 44, 47, 49, 62, 64-69 inclusive, 85, 87, 98, 99, 101 and 102

3.3 To further optimise affinity consider choosing donor residues at one, some or any of:

i. 1, 3
ii. 63

iii. 60, if 60 and 54 are able to form potential saltbridge
iv. 70, if 70 and 24 are able to form potential saltbridge
v. 73, and 21 if 47 is different between donor and acceptor
vi. 37, and 45 if 47 is different between donor and acceptor
vii. 10, 12, 40, 80, 103, 105

Rationale

In order to transfer the binding site of an antibody into a different acceptor framework, a number of factors need to be considered.

1. The extent of the CDRs

The CDRs (Complementary Determining Regions) were defined by Wu and Kabat (refs. 4 and 5) on the basis of an analysis of the variability of different regions of antibody variable regions. Three regions per domain were recognised. In the light chain the sequences are 24-34, 50-56, 89-97 (numbering according to Kabat (ref. 4), Eu Index) inclusive and in the heavy chain the sequences are 31-35, 50-65 and 95-102 inclusive.

When antibody structures became available it became apparent that these CDR regions corresponded in the main to loop regions which extended from the β barrel framework of the light and heavy variable domains. For H1 there was a discrepancy in that the loop was from 26 to 32 inclusive and for H2 the loop was 52 to 56 and for L2 from 50 to 53. However, with the exception of H1 the CDR regions encompassed the loop regions and extended into the β strand

frameworks. In H1 residue 26 tends to be a serine and 27 a phenylalanine or tyrosine, residue 29 is a phenylalanine in most cases. Residues 28 and 30 which are surface residues exposed to solvent might be involved in antigen-binding. A prudent definition of the H1 CDR therefore would include residues 26-35 to include both the loop region and the hypervariable residues 33-35.

It is of interest to note the example of Riechmann et al (ref. 3), who used the residue 31-35 choice for CDR-H1. In order to produce efficient antigen binding, residue 27 also needed to be recruited from the donor (rat) antibody.

2. Non-CDR residues which contribute to antigen binding

By examination of available X-ray structures we have identified a number of residues which may have an effect on net antigen binding and which can be demonstrated by experiment. These residues can be sub-divided into a number of groups.

2.1 Surface residues near CDR [all numbering as in Kabat et al (ref. 7)].

2.1.1. Heavy Chain - Key residues are 23, 71 and 73. Other residues which may contribute to a lesser extent are 1, 3 and 76. Finally 25 is usually conserved but the murine residue should be used if there is a difference.

2.1.2 Light Chain - Many residues close to the CDRs, e.g. 63, 65, 67 and 69 are conserved. If conserved none of the surface residues in the light chain are likely to have a major effect. However, if the murine residue at these positions

is unusual, then it would be of benefit to analyse the likely contribution more closely. Other residues which may also contribute to binding are 1 and 3, and also 60 and 70 if the residues at these positions and at 54 and 24 respectively are potentially able to form a salt bridge i.e. 60 + 54; 70 + 24.

2.2 Packing residues near the CDRs.

2.2.1. Heavy Chain - Key residues are 24, 49 and 78. Other key residues would be 36 if not a tryptophan, 94 if not an arginine, 104 and 106 if not glycines and 107 if not a threonine. Residues which may make a further contribution to stable packing of the heavy chain and hence improved affinity are 2, 4, 6, 38, 46, 67 and 69. 67 packs against the CDR residue 63 and this pair could be either both mouse or both human. Finally, residues which contribute to packing in this region but from a longer range are 18, 20, 80, 82 and 86. 82 packs against 67 and in turn 18 packs against 82. 80 packs against 69 and in turn 20 packs against 80. 86 forms an H bond network with 38 and 46. Many of the mouse-human differences appear minor e.g. Leu-Ile, but could have an minor impact on correct packing which could translate into altered positioning of the CDRs.

2.2.2. Light Chain - Key residues are 48, 58 and 71. Other key residues would be 6 if not glutamine, 35 if not tryptophan, 62 if not phenylalanine or tryosine, 64, 66, 68, 99 and 101 if not glycines and 102 if not a threonine. Residues which make a further contribution are 2, 4, 37, 45 and 47. Finally residues 73 and 21 and 19 may make long distance packing contributions of a minor nature.

2.3. Residues at the variable domain interface between heavy and light chains - In both the light and heavy chains most of the non-CDR interface residues are conserved. If a conserved residue is replaced by a residue of different character, e.g. size or charge, it should be considered for retention as the murine residue.

2.3.1. Heavy Chain - Residues which need to be considered are 37 if the residue is not a valine but is of larger side chain volume or has a charge or polarity. Other residues are 39 if not a glutamine, 45 if not a leucine, 47 if not a tryptophan, 91 if not a phenylalanine or tyrosine, 93 if not an alanine and 103 if not a tryptophan. Residue 89 is also at the interface but is not in a position where the side chain could be of great impact.

2.3.2. Light Chain - Residues which need to be considered are 36, if not a tyrosine, 38 if not a glutamine, 44 if not a proline, 46, 49 if not a tyrosine, residue 85, residue 87 if not a tyrosine and 98 if not a phenylalanine.

2.4. Variable-Constant region interface - The elbow angle between variable and constant regions may be affected by alterations in packing of key residues in the variable region against the constant region which may affect the position of V_L and V_H with respect to one another. Therefore it is worth noting the residues likely to be in contact with the constant region. In the heavy chain the surface residues potentially in contact with the variable region are conserved between mouse and human antibodies therefore the variable region contact residues may influence the V-C interaction. In the light chain the amino acids found at a number of the constant

region contact points vary, and the V & C regions are not in such close proximity as the heavy chain. Therefore the influences of the light chain V-C interface may be minor.

2.4.1. Heavy Chain - Contact residues are 7, 11, 41, 87, 108, 110, 112.

2.4.2. Light Chain - In the light chain potentially contacting residues are 10, 12, 40, 80, 83, 103 and 105.

The above analysis coupled with our considerable practical experimental experience in the CDR-grafting of a number of different antibodies have lead us to the protocol given above.

The present invention is now described, by way of example only, with reference to the accompanying Figures 1 - 13.

Brief Description of the Figures

Figure 1 shows DNA and amino acid sequences of the OKT3 light chain;

Figure 2 shows DNA and amino acid sequences of the OKT3 heavy chain;

Figure 3 shows the alignment of the OKT3 light variable region amino acid sequence with that of the light variable region of the human antibody REI;

Figure 4 shows the alignment of the OKT3 heavy variable region amino acid sequence with that of the heavy variable region of the human antibody KOL;

Figure 5 shows the heavy variable region amino acid sequences of OKT3, KOL and various corresponding CDR grafts;

Figure 6 shows the light variable region amino acid sequences of OKT3, REI and various corresponding CDR grafts;

Figure 7 shows a graph of binding assay results for various grafted OKT3 antibodies'

Figure 8 shows a graph of blocking assay results for various grafted OKT3 antibodies;

Figure 9 shows a similar graph of blocking assay results;

Figure 10a and b shows similar graphs for both binding assay and blocking assay results;

Figure 11a and b shows further similar graphs for both binding assay and blocking assay results;

Figure 12 shows a graph of competition assay results for a minimally grafted OKT3 antibody compared with the OKT3 murine reference standard, and

Figure 13 shows a similar graph of competition assay results comparing a fully grafted OKT3 antibody with the murine reference standard.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

EXAMPLE 1

CDR-GRAFTING OF OKT3

MATERIAL AND METHODS

1. INCOMING CELLS

Hybridoma cells producing antibody OKT3 were provided by Ortho (seedlot 4882.1) and were grown up in antibiotic free Dulbecco's Modified Eagles Medium (DMEM) supplemented with glutamine and 5% foetal calf serum, and divided to provide both an overgrown supernatant for evaluation and cells for extraction of RNA. The overgrown supernatant was shown to contain 250 ug/mL murine IgG2a/kappa antibody. The supernatant was negative for murine lambda light chain and IgG1, IgG2b, IgG3, IgA and IgM heavy chain. 20mL of supernatant was assayed to confirm that the antibody present was OKT3.

2. MOLECULAR BIOLOGY PROCEDURES

Basic molecular biology procedures were as described in Maniatis et al (ref. 9) with, in some cases, minor modifications. DNA sequencing was performed as described in Sanger et al (ref. 11) and the Amersham International Plc sequencing handbook. Site directed mutagenesis was as described in Kramer et al (ref. 12) and the Anglian Biotechnology Ltd. handbook. COS cell expression and metabolic labelling studies were as described in Whittle et al (ref. 13)

3. RESEARCH ASSAYS

3.1. ASSEMBLY ASSAYS

Assembly assays were performed on supernatants from transfected COS cells to determine the amount of intact IgG present.

3.1.1. COS CELLS TRANSFECTED WITH MOUSE OKT3 GENES

The assembly assay for intact mouse IgG in COS cell supernatants was an ELISA with the following format:

96 well microtitre plates were coated with F(ab')2 goat anti-mouse IgG Fc. The plates were washed in water and samples added for 1 hour at room temperature. The plates were washed and F(ab')2 goat anti-mouse IgG F(ab')2 (HRPO conjugated) was then added. Substrate was added to reveal the reaction. UPC10, a mouse IgG2a myeloma, was used as a standard.

3.1.2. COS AND CHO CELLS TRANSFECTED WITH CHIMERIC OR CDR-GRAFTED OKT3 GENES

The assembly assay for chimeric or CDR-grafted antibody in COS cell supernatants was an ELISA with the following format:

96 well microtitre plates were coated with F(ab')2 goat anti-human IgG Fc. The plates were washed and samples added and incubated for 1 hour at room temperature. The plates were washed and monoclonal mouse anti-human kappa chain was added for 1 hour at room temperature.

The plates were washed and F(ab')2 goat anti-mouse IgG Fc (HRPO conjugated) was added. Enzyme substrate was added to reveal the reaction. Chimeric B72.3 (IgG4) (ref. 13) was used as a standard. The use of a monoclonal anti-kappa chain in this assay allows grafted antibodies to be read from the chimeric standard.

3.2. ASSAY FOR ANTIGEN BINDING ACTIVITY

Material from COS cell supernatants was assayed for OKT3 antigen binding activity onto CD3 positive cells in a direct assay. The procedure was as follows:

HUT 78 cells (human T cell line, CD3 positive) were maintained in culture. Monolayers of HUT 78 cells were prepared onto 96 well ELISA plates using poly-L-lysine and glutaraldehyde. Samples were added to the monolayers for 1 hour at room temperature.

The plates were washed gently using PBS. F(ab')2 goat anti-human IgG Fc (HRPO conjugated) or F(ab')2 goat anti-mouse IgG Fc (HRPO conjugated) was added as appropriate for humanised or mouse samples. Substrate was added to reveal the reaction.

The negative control for the cell-based assay was chimeric B72.3. The positive control was mouse Orthomune OKT3 or chimeric OKT3, when available. This cell-based assay was difficult to perform, and an alternative assay was developed for CDR-grafted OKT3 which was more sensitive and easier to carry out.

In this system CDR-grafted OKT3 produced by COS cells was tested for its ability to bind to the CD3-positive HPB-ALL (human peripheral blood acute lymphocytic leukemia) cell line. It was also tested for its ability to block the binding of murine OKT3 to these cells. Binding was measured by the following procedure: HPB-ALL cells were harvested from tissue culture. Cells were incubated at 4°C for 1 hour with various dilutions of test antibody, positive control antibody, or negative control antibody. The cells were washed once and incubated at 4°C for 1 hour with an FITC-labelled goat anti-human IgG (Fc-

specific, mouse absorbed). The cells were washed twice and analysed by cytofluorography. Chimeric OKT3 was used as a positive control for direct binding. Cells incubated with mock- transfected COS cell supernatant, followed by the FITC-labelled goat anti-human IgG, provided the negative control. To test the ability of CDR-grafted OKT3 to block murine OKT3 binding, the HPB-ALL cells were incubated at 4°C for 1 hour with various dilutions of test antibody or control antibody. A fixed saturating amount of FITC OKT3 was added. The samples were incubated for 1 hour at 4°C, washed twice and analysed by cytofluorography. FITC-labelled OKT3 was used as a positive control to determine maximum binding. Unlabelled murine OKT3 served as a reference standard for blocking. Negative controls were unstained cells with or without mock-transfected cell supernatant. The ability of the CDR-grafted OKT3 light chain to bind CD3-positive cells and block the binding of murine OKT3 was initially tested in combination with the chimeric OKT3 heavy chain. The chimeric OKT3 heavy chain is composed of the murine OKT3 variable region and the human IgG4 constant region. The chimeric heavy chain gene is expressed in the same expression vector used for the CDR-grafted genes. The CDR-grafted light chain expression vector and the chimeric heavy chain expression vector were co-transfected into COS cells. The fully chimeric OKT3 antibody (chimeric light chain and chimeric heavy chain) was found to be fully capable of binding to CD3 positive cells and blocking the binding of murine OKT3 to these cells.

3.3 DETERMINATION OF RELATIVE BINDING AFFINITY

The relative binding affinities of CDR-grafted

anti-CD3 monoclonal antibodies were determined by competition binding (ref. 6) using the HPB-ALL human T cell line as a source of CD3 antigen, and fluorescein-conjugated murine OKT3 (Fl-OKT3) of known binding affinity as a tracer antibody. The binding affinity of Fl-OKT3 tracer antibody was determined by a direct binding assay in which increasing amounts of Fl-OKT3 were incubated with HPB-ALL ($5x10^5$) in PBS with 5% foetal calf serum for 60 min. at 4°C. Cells were washed, and the fluorescence intensity was determined on a FACScan flow cytometer calibrated with quantitative microbead standards (Flow Cytometry Standards, Research Triangle Park, NC). Fluorescence intensity per antibody molecule (F/P ratio) was determined by using microbeads which have a predetermined number of mouse IgG antibody binding sites (Simply Cellular beads, Flow Cytometry Standards). F/P equals the fluorescence intensity of beads saturated with Fl-OKT3 divided by the number of binding sites per bead. The amount of bound and free Fl-OKT3 was calculated from the mean fluorescence intensity per cell, and the ratio of bound/free was plotted against the number of moles of antibody bound. A linear fit was used to determine the affinity of binding (absolute value of the slope).

For competitive binding, increasing amounts of competitor antibody were added to a sub-saturating dose of Fl-OKT3 and incubated with $5x10^5$ HPB-ALL in 200 Ml of PBS with 5% foetal calf serum, for 60 min at 4°C. The fluorescence intensities of the cells were measured on a FACScan flow cytometer calibrated with quantitative microbead standards. The concentrations of bound and free Fl-OKT3 were calculated. The affinities of competing anti-

bodies were calculated from the equation [X]-[OKT3] = (1/Kx) - (1/Ka), where Ka is the affinity of murine OKT3, Kx is the affinity of competitor X, [ ] is the concentration of competitor antibody at which bound/free binding is R/2, and R is the maximal bound/free binding.

4. cDNA LIBRARY CONSTRUCTION

4.1. mRNA PREPARATION AND cDNA SYNTHESIS

OKT3 producing cells were grown as described above and 1.2 x 10^9 cells harvested and mRNA extracted using the guanidinium/LiCl extraction procedure. cDNA was prepared by priming from Oligo-dT to generate full length cDNA. The cDNA was methylated and EcoR1 linkers added for cloning.

4.2. LIBRARY CONSTRUCTION

The cDNA library was ligated to pSP65 vector DNA which had been EcoR1 cut and the 5′ phosphate groups removed by calf intestinal phosphatase (EcoR1/CIP). The ligation was used to transform high transformation efficiency Escherichia coli (E.coli) HB101. A cDNA library was prepared. 3600 colonies were screened for the light chain and 10000 colonies were screened for the heavy chain.

5. SCREENING

E.coli colonies positive for either heavy or light chain probes were identified by oligonucleotide screening using the oligonucleotides:

5′ TCCAGATGTTAACTGCTCAC (SEQ ID NO:1) for the light chain, which is complementary to a sequence in the mouse kappa constant region, and 5′ CAGGGGCCAGTGGATGGATAGAC (SEQ ID NO:2) for the heavy chain which is complementary to a sequence in the mouse IgG2a constant CH1 domain region. 12 light chain and 9 heavy chain clones

were identified and taken for second round screening. Positive clones from the second round of screening were grown up and DNA prepared. The sizes of the gene inserts were estimated by gel electrophoresis and inserts of a size capable of containing a full length cDNA were subcloned into M13 for DNA sequencing.

6. DNA SEQUENCING

Clones representing four size classes for both heavy and light chains were obtained in M13. DNA sequence for the 5' untranslated regions, signal sequences, variable regions and 3' untranslated regions of full length cDNAs [Figures 1(a) (SEQ ID NO:4) and 2(a) (SEQ ID NO:6)] were obtained and the corresponding amino acid sequences predicted [(Figures 1(b) (SEQ ID NO:8) and 2(b) (SEQ ID NO:9)]. In Figure 1(a) the untranslated DNA regions are shown in uppercase, and in both Figures 1 and 2 (SEQ ID NO:4+5) the signal sequences are underlined.

7. CONSTRUCTION OF cDNA EXPRESSION VECTORS

Celltech expression vectors are based on the plasmid pEE6hCMV (ref. 14). A polylinker for the insertion of genes to be expressed has been introduced after the major immediate early promoter/enhancer of the human Cytomegalovirus (hCMV). Marker genes for selection of the plasmid in transfected eukaryotic cells can be inserted as BamH1 cassettes in the unique BamH1 site of pEE6 hCMV; for instance, the neo marker to provide pEE6 hCMV neo. It is usual practice to insert the neo and gpt markers prior to insertion of the gene of interest, whereas the GS marker is inserted last because of the presence of internal EcoR1 sites in the cassette.

The selectable markers are expressed from the SV40 late promoter which also provides an origin of replication so that the vectors can be used for expression in the COS cell transient expression system.

The mouse sequences were excised from the M13 based vectors described above as EcoR1 fragments and cloned into either pEE6-hCMV-neo for the heavy chain and into EE6-hCMV-gpt for the light chain to yield vectors pJA136 and pJA135 respectively.

8. EXPRESSION OF cDNAS IN COS CELLS

Plasmids pJA135 and pJA136 were co-transfected into COS cells and supernatant from the transient expression experiment was shown to contain assembled antibody which bound to T-cell enriched lymphocytes. Metabolic labelling experiments using ^{35}S methionine showed expression and assembly of heavy and light chains.

9. CONSTRUCTION OF CHIMERIC GENES

Construction of chimeric genes followed a previously described strategy [Whittle et al (ref. 13)]. A restriction site near the 3′ end of the variable domain sequence is identified and used to attach an oligonucleotide adapter coding for the remainder of the mouse variable region and a suitable restriction site for attachment to the constant region of choice.

9.1. LIGHT CHAIN GENE CONSTRUCTION

The mouse light chain cDNA sequence contains an AvaI site near the 3′ end of the variable region [Fig. 1(a) (SEQ ID NO:4)]. The majority of the sequence of the variable region was isolated as a 396 bp. EcoR1-AvaI fragment. An oligonucleotide adapter was designed to replace the remainder of the 3′

region of the variable region from the AvaI site and to include the 5' residues of the human constant region up to and including a unique NarI site which had been previously engineered into the constant region.
A HindIII site was introduced to act as a marker for insertion of the linker.
The linker was ligated to the V_L fragment and the 413 bp EcoR1-NarI adapted fragment was purified from the ligation mixture.
The constant region was isolated as an NarI-BamHI fragment from an M13 clone NW361 and was ligated with the variable region DNA into an EcoR1/BamH1/C1P pSP65 treated vector in a three way reaction to yield plasmid JA143. Clones were isolated after transformation into E.coli and the linker and junction sequences were confirmed by the presence of the HindIII site and by DNA sequencing.

9.2 LIGHT CHAIN GENE CONSTRUCTION - VERSION 2

The construction of the first chimeric light chain gene produces a fusion of mouse and human amino acid sequences at the variable-constant region junction. In the case of the OKT3 light chain the amino acids at the chimera junction are: (SEQ ID NO:3)

.....Leu-Glu-Ile-Asn-Arg/ -/Thr-Val-Ala -Ala

VARIABLE CONSTANT

This arrangement of sequence introduces a potential site for Asparagine (Asn) linked (N-linked) glycosylation at the V-C junction. Therefore, a second version of the chimeric light chain oligonucleotide adapter was designed in which the threonine (Thr), the first amino acid of the human constant region, was replaced with the equivalent amino acid from the mouse constant region, Alanine (Ala).

An internal Hindlll site was not included in this adapter, to differentiate the two chimeric light chain genes.

The variable region fragment was isolated as a 376 bp EcoR1-Aval fragment. The oligonucleotide linker was ligated to Nar1 cut pNW361 and then the adapted 396bp constant region was isolated after recutting the modified pNW361 with EcoR1. The variable region fragment and the modified constant region fragment were ligated directly into EcoR1/C1P treated pEE6hCMVneo to yield pJA137. Initially all clones examined had the insert in the incorrect orientation. Therefore, the insert was re-isolated and recloned to turn the insert round and yield plasmid pJA141. Several clones with the insert in the correct orientation were obtained and the adapter sequence of one was confirmed by DNA sequencing

9.3. HEAVY CHAIN GENE CONSTRUCTION

9.3.1. CHOICE OF HEAVY CHAIN GENE ISOTYPE

The constant region isotype chosen for the heavy chain was human IgG4.

9.3.2. GENE CONSTRUCTION

The heavy chain cDNA sequence showed a Ban1 site (SEQ ID NO:6) near the 3′ end of the variable region [Fig. 2(a)]. The majority of the sequence of the variable region was isolated as a 426bp. EcoR1/C1P/Ban1 fragment. An oligonucleotide adapter was designated to replace the remainder of the 3′ region of the variable region from the Ban1 site up to and including a unique HindIII site which had been previously engineered into the first two amino acids of the constant region.

The linker was ligated to the V_H fragment and the EcoR1-Hindlll adapted fragment was purified from the ligation mixture.

The variable region was ligated to the constant region by cutting pJA91 with EcoR1 and Hind111 removing the intron fragment and replacing it with the V_H to yield pJA142. Clones were isolated after transformation into E.coli JM101 and the linker and junction sequences were confirmed by DNA sequencing. (N.B. The Hind111 site is lost on cloning).

10. CONSTRUCTION OF CHIMERIC EXPRESSION VECTORS

10.1. neo AND gpt VECTORS

The chimeric light chain (version 1) was removed from pJA143 as an EcoR1 fragment and cloned into EcoR1/C1P treated pEE6hCMVneo expression vector to yield pJA145. Clones with the insert in the correct orientation were identified by restriction mapping.

The chimeric light chain (version 2) was constructed as described above.

The chimeric heavy chain gene was isolated from pJA142 as a 2.5Kbp EcoR1/BamH1 fragment and cloned into the EcoR1/Bc11/C1P treated vector fragment of a derivative of pEE6hCMVgpt to yield plasmid pJA144.

10.2. GS SEPARATE VECTORS

GS versions of pJA141 and pJA144 were constructed by replacing the neo and gpt cassettes by a BamH1/Sa11/C1P treatment of the plasmids, isolation of the vector fragment and ligation to a GS-containing fragment from the plasmid pRO49 to yield the light chain vector pJA179 and the heavy chain vector pJA180.

10.3. GS SINGLE VECTOR CONSTRUCTION

Single vector constructions containing the cL (chimeric light), cH (chimeric heavy) and GS genes on one plasmid in the order cL-cH-GS, or cH-cL-GS

and with transcription of the genes being head to tail e.g. cL>cH>GS were constructed. These plasmids were made by treating pJA179 or pJA180 with BamH1/C1P and ligating in a Bgl11/Hind111 hCMV promoter cassette along with either the Hind111/BamH1 fragment from pJA141 into pJA180 to give the cH-cL-GS plasmid pJA182 or the Hind111/BamH1 fragment from pJA144 into pJA179 to give the cL-cH-GS plasmid pJA181.

11. EXPRESSION OF CHIMERIC GENES

11.1. EXPRESSION IN COS CELLS

The chimeric antibody plasmid pJA145 (cL) and pJA144 (cH) were co-transfected into COS cells and supernatant from the transient expression experiment was shown to contain assembled antibody which bound to the HUT 78 human T-cell line. Metabolic labelling experiments using ^{35}S methionine showed expression and assembly of heavy and light chains. However the light chain mobility seen on reduced gels suggested that the potential glycosylation site was being glycosylated. Expression in COS cells in the presence of tunicamycin showed a reduction in size of the light chain to that shown for control chimeric antibodies and the OKT3 mouse light chain. Therefore JA141 was constructed and expressed. In this case the light chain did not show an aberrant mobility or a size shift in the presence or absence of tunicamycin. This second version of the chimeric light chain, when expressed in association with chimeric heavy (cH) chain, produced antibody which showed good binding to HUT 78 cells. In both cases antigen binding was equivalent to that of the mouse antibody.

11.2 EXPRESSION IN CHINESE HAMSTER OVARY (CHO) CELLS

Stable cell lines have been prepared from plasmids PJA141/pJA144 and from pJA179/pJA180, pJA181 and pJA182 by transfection into CHO cells.

12. CDR-GRAFTING

The approach taken was to try to introduce sufficient mouse residues into a human variable region framework to generate antigen binding activity comparable to the mouse and chimeric antibodies.

12.1. VARIABLE REGION ANALYSIS

From an examination of a small database of structures of antibodies and antigen-antibody complexes it is clear that only a small number of antibody residues make direct contact with antigen. Other residues may contribute to antigen binding by positioning the contact residues in favourable configurations and also by inducing a stable packing of the individual variable domains and stable interaction of the light and heavy chain variable domains.

The residues chosen for transfer can be identified in a number of ways:

(a) By examination of antibody X-ray crystal structures the antigen binding surface can be predominantly located on a series of loops, three per domain, which extend from the B-barrel framework.

(b) By analysis of antibody variable domain sequences regions of hypervariability [termed the Complementarity Determining Regions (CDRs) by Wu and Kabat (ref. 5)] can be identified. In the most but not all cases these CDRs correspond to, but extend a short way beyond, the loop regions noted above.

(c) Residues not identified by (a) and (b) may contribute to antigen binding directly or indirectly by affecting antigen binding site topology, or by inducing a stable packing of the individual variable domains and stabilising the inter-variable domain interaction. These residues may be identified either by superimposing the sequences for a given antibody on a known structure and looking at key residues for their contribution, or by sequence alignment analysis and noting "idiosyncratic" residues followed by examination of their structural location and likely effects.

12.1.1. LIGHT CHAIN

Figure 3 shows an alignment of sequences for the human framework region REI and the OKT3 light variable region. The structural loops (LOOP) and CDRs (KABAT) believed to correspond to the antigen binding region are marked. Also marked are a number of other residues which may also contribute to antigen binding as described in 13.1(c). Above the sequence in Figure 3 the residue type indicates the spatial location of each residue side chain, derived by examination of resolved structures from X-ray crystallography analysis. The key to this residue type designation is as follows:

N - near to CDR (From X-ray Structures)
P - Packing
B - Buried Non-Packing
S - Surface
E - Exposed
I - Interface
* - Interface
- Packing/Part Exposed
? - Non-CDR Residues which may require to be left as Mouse sequence.

Residues underlined in Figure 3 are amino acids. RE1 was chosen as the human framework because the light chain is a kappa chain and the kappa variable regions show higher homology with the mouse sequences than a lambda light variable region, e.g. KOL (see below). RE1 was chosen in preference to another kappa light chain because the X-ray structure of the light chain has been determined so that a structural examination of individual residues could be made.

12.1.2. HEAVY CHAIN

Similarly Figure 4 shows an alignment of sequences for the human framework region KOL and the OKT3 heavy variable region. The structural loops and CDRs believed to correspond to the antigen binding region are marked. Also marked are a number of other residues which may also contribute to antigen binding as described in 12.1(c). The residue type key and other indicators used in Figure 4 are the same as those used in Figure 3. KOL was chosen as the heavy chain framework because the X-ray structure has been determined to a better resolution than, for example, NEWM and also the sequence alignment of OKT3 heavy variable region showed a slightly better homology to KOL than to NEWM.

12.2. DESIGN OF VARIABLE GENES

The variable region domains were designed with mouse variable region optimal codon usage [Grantham and Perrin (ref. 15)] and used the B72.3 signal sequences [Whittle et al (ref. 13)]. The sequences were designed to be attached to the constant region in the same way as for the chimeric genes described above. Some constructs contained the "Kozak consensus sequence" [Kozak (ref. 16)] directly linked to the 5′ of the signal

sequence in the gene. This sequence motif is believed to have a beneficial role in translation initiation in eukaryotes.

12.3. GENE CONSTRUCTION

To build the variable regions, various strategies are available. The sequence may be assembled by using oligonucleotides in a manner similar to Jones et al (ref. 17) or by simultaneously replacing all of the CDRs or loop regions by oligonucleotide directed site specific mutagenesis in a manner similar to Verhoeyen et al (ref. 2). Both strategies were used and a list of constructions is set out in Tables 1 and 2 and Figures 4 and 5a-c. It was noted in several cases that the mutagenesis approach led to deletions and rearrangements in the gene being remodelled, while the success of the assembly approach was very sensitive to the quality of the oligonucleotides.

13. CONSTRUCTION OF EXPRESSION VECTORS

Genes were isolated from M13 or SP65 based intermediate vectors and cloned into pEE6hCMVneo for the light chains and pEE6hCMVgpt for the heavy chains in a manner similar to that for the chimeric genes as described above.

TABLE 1 CDR-GRAFTED GENE CONSTRUCTS

CODE	MOUSE SEQUENCE CONTENT	METHOD OF CONSTRUCTION	KOZAK SEQUENCE -	KOZAK SEQUENCE +
LIGHT CHAIN	ALL HUMAN FRAMEWORK REl			
121	26-32, 50-56, 91-96 inclusive	SDM and gene assembly	+	n.d.
121A	26-32, 50-56, 91-96 inclusive +1, 3, 46, 47	Partial gene assembly	n.d.	+
121B	26-32, 50-56, 91-96 inclusive + 46, 47	Partial gene assembly	n.d.	+
221	24-24, 50-56, 91-96 inclusive	Partial gene assembly	+	+
221A	24-34, 50-56, 91-96 inclusive +1, 3, 46, 47	Partial gene assembly	+	+
221B	24-34, 50-56, 91-96 inclusive +1, 3	Partial gene assembly	+	+
221C	24-34, 50-56, 91-96 inclusive	Partial gene assembly	+	+
HEAVY CHAIN	ALL HUMAN FRAMEWORK KOL			
121	26-32, 50-56, 95-100B inclusive	Gene assembly	n.d.	+
131	26-32, 50-58, 95-100B inclusive	Gene assembly	n.d.	+
141	26-32, 50-65, 95-100B inclusive	Partial gene assembly	+	n.d.
321	26-35, 50-56, 95-100B inclusive	Partial gene assembly	+	n.d.
331	26-35, 50-58, 95-100B inclusive	Partial gene assembly	+	
		Gene assembly		+
341	26-35, 50-65, 95-100B inclusive	SDM	+	
		Partial gene assembly		+
341A	26-35, 50-65, 95-100B inclusive +6, 23, 24, 48, 49, 71, 73, 76, 78, 88, 91 (+63 = human) (SEQ ID NO:8-28)	Gene assembly	n.d.	+
341B	26-35, 50-65, 95-100B inclusive + 48, 49, 71, 73, 76, 78, 88, 91 (+63 + human)	Gene assembly	n.d.	+

KEY

n.d.	not done
SDM	Site directed mutagenesis
Gene assembly	Variable region assembled entirely from oligonucleotides
Partial gene assembly	Variable region assembled by combination of restriction fragments either from other genes originally created by SDM and gene assembly or by oligonucleotide assembly of part of the variable region and reconstruction with restriction fragments from other genes originally created by SDM and gene assembly

14. EXPRESSION OF CDR-GRAFTED GENES

14.1. PRODUCTION OF ANTIBODY CONSISTING OF GRAFTED LIGHT (gL) CHAINS WITH MOUSE HEAVY (mH) OR CHIMERIC HEAVY (cH) CHAINS

All gL chains, in association with mH or cH produced reasonable amounts of antibody. Insertion of the Kozak consensus sequence at a position 5' to the ATG (kgL constructs) however, led to a 2-5 fold improvement in net expression. Over an extended series of experiments expression levels were raised from approximately 200ng/ml to approximately 500 ng/ml for kgL/cH or kgL/mH combinations.

When direct binding to antigen on HUT 78 cells was measured, a construct designed to include mouse sequence based on loop length (gL121) did not lead to active antibody in association with mH or cH. A construct designed to include mouse sequence based on Kabat CDRs (gL221 (SEQ ID NO:28)) demonstrated some weak binding in association with mH or cH. However, when framework residues 1, 3, 46, 47 were changed from the human to the murine OKT3 equivalents based on the arguments outlined in Section 12.1 antigen binding was demonstrated when both of the new constructs, which were termed 121A and 221A were co-expressed with cH. When the effects of these residues were examined in more detail, it appears that residues 1 and 3 are not major contributing residues as the product of the gL221B gene (SEQ ID NO:27) shows little detectable binding activity in association with cH. The light chain product of gL221C (SEQ ID NO:28), in which mouse sequences are present at 46 and 47, shows good binding activity in association with cH.

14.2 PRODUCTION OF ANTIBODY CONSISTING OF GRAFTED HEAVY (gH) CHAINS WITH MOUSE LIGHT (mL) OR CHIMERIC LIGHT (cL) CHAINS

Expression of the gH genes proved to be more difficult to achieve than for gL. First, inclusion of the Kozak sequence appeared to have no marked effect on expression of gH genes. Expression appears to be slightly improved but not to the same degree as seen for the grafted light chain.

Also, it proved difficult to demonstrate production of expected quantities of material when the loop choice (amino acid 26-32) for CDR1 is used, e.g. gH121, 131, 141 and no conclusions can be drawn about these constructs.

Moreover, co-expression of the gH341 gene (SEQ ID NO:11) with cL or mL has been variable and has tended to produce lower amounts of antibody than the cH/cL or mH/mL combinations. The alterations to gH341 (SEQ ID NO:11) to produce gH341A (SEQ ID NO:12) and gH341B (SEQ ID NO:21) lead to improved levels of expression.

This may be due either to a general increase in the fraction of mouse sequence in the variable region, or to the alteration at position 63 where the residue is returned to the human amino acid Valine (Val) from Phenylalanine (Phe) to avoid possible internal packing problems with the rest of the human framework. This arrangement also occurs in gH331 and gH321.

When gH321 or gH331 were expressed in association with cL, antibody was produced but antibody binding activity was not detected.

When the more conservative gH341 gene (SEQ ID NO:11) was used antigen binding could be detected in association with cL or mL, but the activity was only marginally above the background level.

When further mouse residues were substituted based on the arguments in 12.1, antigen binding could be clearly demonstrated for the antibody produced when kgH341A and kgH341B were expressed in association with cL.

14.3 PRODUCTION OF FULLY CDR-GRAFTED ANTIBODY

The kgL221A gene was co-expressed with kgH341, kgH341A or kgH341B. For the combination kgH221A/kgH341 very little material was produced in a normal COS cell expression.

For the combinations kgL221A/kgH341A or kgH221A/kgH341B amounts of antibody similar to gL/cH was produced.

In several experiments no antigen binding activity could be detected with kgH221A/gH341 or kgH221A/kgH341 combinations, although expression levels were very low.

Antigen binding was detected when kgL221A/kgH341A or kgH221A/kgH341B combinations were expressed. In the case of the antibody produced from the kgL221A/kgH341A combination the antigen binding was very similar to that of the chimeric antibody.

An analysis of the above results is given below.

15. DISCUSSION OF CDR-GRAFTING RESULTS

In the design of the fully humanised antibody the aim was to transfer the minimum number of mouse amino acids that would confer antigen binding onto a human antibody framework.

15.1. LIGHT CHAIN

15.1.1. EXTENT OF THE CDRs

For the light chain the regions defining the loops known from structural studies of other antibodies to contain the antigen contacting residues, and

those hypervariable sequences defined by Kabat et al (refs. 4 and 5) as Complementarity Determining Regions (CDRs) are equivalent for CDR2. For CDR1 the hypervariable region extends from residues 24-34 inclusive while the structural loop extends from 26-32 inclusive. In the case of OKT3 (SEQ ID NO:5) there is only one amino acid difference between the two options, at amino acid 24, where the mouse sequence is a serine and the human framework RE1 has glutamine. For CDR3 the loop extends from residues 91-96 inclusive while the Kabat hypervariability extends from residues 89-97 inclusive. For OKT3 amino acids 89, 90 and 97 are the same between OKT3 and RE1 (Fig. 3) (SEQ ID NO. 8 AND 9). When constructs based on the loop choice for CDR1 (gL121) and the Kabat choice (gL221) were made and co-expressed with mH or cH no evidence for antigen binding activity could be found for gL121, but trace activity could be detected for the gL221, suggesting that a single extra mouse residue in the grafted variable region could have some detectable effect. Both gene constructs were reasonably well expressed in the transient expression system.

15.1.2. FRAMEWORK RESIDUES

The remaining framework residues were then further examined, in particular amino acids known from X-ray analysis of other antibodies to be close to the CDRs and also those amino acids which in OKT3 showed differences from the consensus framework for the mouse subgroup (subgroup VI) to which OKT3 shows most homology. Four positions 1, 3, 46 and 47 were identified and their possible contribution was examined by substituting the mouse amino acid for the human amino acid at each position. Therefore gL221A (gL221 + D1Q, Q3V, L46R, L47W,

see Figure 3 and Table 1) was made, cloned in EE6hCMVneo and co-expressed with cH (pJA144). The resultant antibody was well expressed and showed good binding activity. When the related genes gL221B (SEQ ID NO:28) (gL221 + D1Q, Q3V) and gL221C (gL221 + L46R, L47W) were made and similarly tested, while both genes produced antibody when co-expressed with cH, only the gL221C/cH combination showed good antigen binding. When the gL121A (gL121 + D1Q, Q3V, L46R, L47W) gene was made and co-expressed with cH, antibody was produced which also bound to antigen.

15.2. HEAVY CHAIN

15.2.1. EXTENT OF THE CDRs

For the heavy chain the loop and hypervariability analyses agree only in CDR3. For CDR1 the loop region extends from residues 26-32 inclusive whereas the Kabat CDR extends from residues 31-35 inclusive. For CDR2 the loop region is from 50-58 inclusive while the hypervariable region covers amino acids 50-65 inclusive. Therefore humanised heavy chains were constructed using the framework from antibody KOL and with various combinations of these CDR choices, including a shorter choice for CDR2 of 50-56 inclusive as there was some uncertainty as to the definition of the end point for the CDR2 loop around residues 56 to 58. The genes were co-expressed with mL or cL initially. In the case of the gH genes with loop choices for CDR1 e.g. gH121, gH131, gH141 very little antibody was produced in the culture supernatants. As no free light chain was detected it was presumed that the antibody was being made and assembled inside the cell but that the heavy chain was aberrant in some way, possibly incorrectly folded, and therefore the antibody was

being degraded internally. In some experiments trace amounts of antibody could be detected in ^{35}S labelling studies.

As no net antibody was produced, analysis of these constructs was not pursued further.

When, however, a combination of the loop choice and the Kabat choice for CDR1 was tested (mouse amino acids 26-35 inclusive) and in which residues 31 (Ser to Arg), 33 (Ala to Thr), and 35 (Tyr to His) were changed from the human residues to the mouse residue and compared to the first series, antibody was produced for gH321, kgH331 and kgH341 when co-expressed with cL. Expression was generally low and could not be markedly improved by the insertion of the Kozak consensus sequence 5′ to the ATG of the signal sequence of the gene, as distinct from the case of the gL genes where such insertion led to a 2-5 fold increase in net antibody production. However, only in the case of gH341/mL or kgH341/cL could marginal antigen binding activity be demonstrated. When the kgH341 gene was co-expressed with kgL221A (SEQ ID NO: 26), the net yield of antibody was too low to give a signal above the background level in the antigen binding assay.

15.2.2. FRAMEWORK RESIDUES

As in the case of the light chain the heavy chain frameworks were re-examined. Possibly because of the lower initial homology between the mouse and human heavy variable domains compared to the light chains, more amino acid positions proved to be of interest. Two genes kgH341A and kgH341B were constructed, with 11 or 8 human residues respectively substituted by mouse residues compared to gH341, and with the CDR2 residue 63 returned to the human amino acid potentially to

improve domain packing. Both showed antigen binding when combined with cL or kgL221A, the kgH341A gene with all 11 changes appearing to be the superior choice.

15.3 INTERIM CONCLUSIONS

It has been demonstrated, therefore, for OKT3 that to transfer antigen binding ability to the humanised antibody, mouse residues outside the CDR regions defined by the Kabat hypervariability or structural loop choices are required for both the light and heavy chains. Fewer extra residues are needed for the light chain, possibly due to the higher initial homology between the mouse and human kappa variable regions.

Of the changes seven (1 and 3 from the light chain and 6, 23, 71, 73 and 76 from the heavy chain) are predicted from a knowledge of other antibody structures to be either partly exposed or on the antibody surface. It has been shown here that residues 1 and 3 in the light chain are not absolutely required to be the mouse sequence; and for the heavy chain the gH341B heavy chain in combination with the 221A light chain generated only weak binding activity. Therefore the presence of the 6, 23 and 24 changes are important to maintain a binding affinity similar to that of the murine antibody. It was important, therefore, to further study the individual contribution of othe other 8 mouse residues of the kgH341A gene compared to kgH341.

16. FURTHER CDR-GRAFTING EXPERIMENTS

Additional CDR-grafted heavy chain genes were prepared substantially as described above. With reference to Table 2 the further heavy chain genes were based upon the gh341 (plasmid pJA178) and

gH341A (plasmid pJA185) (SEQ ID NO:12) with either mouse OKT3 or human KOL residues at 6, 23, 24, 48, 49, 63, 71, 73, 76, 78, 88 and 91, as indicated. The CDR-grafted light chain genes used in these further experiments were gL221 (SEQ ID NO:15), gL221A (SEQ ID NO:26), gL221B (SEQ ID NO:27) and gL221C (SEQ ID NO:
as described above.

TABLE 2

OKT3 HEAVY CHAIN CDR GRAFTS

1. gH341 and derivatives

RES NUM	6	23	24	48	49	63	71	73	76	78	88	91	
OKT3vh	Q	K	A	I	G	F	T	K	S	A	A	Y	
gH341	E	S	S	V	A	F	R	N	N	L	G	F	JA178
gH341A	Q	K	A	I	G	V	T	K	S	A	A	Y	JA185
gH341E	Q	K	A	I	G	V	T	K	S	A	G	G	JA198
gH341*	Q	K	A	I	G	V	T	K	N	A	G	F	JA207
gH341*	Q	K	A	I	G	V	R	N	N	A	G	F	JA209
gH341D	Q	K	A	I	G	V	T	K	N	L	G	F	JA197
gH341*	Q	K	A	I	G	V	R	N	N	L	G	F	JA199
gH341C	Q	K	A	V	A	F	R	N	N	L	G	F	JA184
gH341*	Q	S	A	I	G	V	T	K	S	A	A	Y	JA203
gH341*	E	S	A	I	G	V	T	K	S	A	A	Y	JA205
gH341B	E	S	S	I	G	V	T	K	S	A	A	Y	JA183
gH341*	Q	S	A	I	G	V	T	K	S	A	G	F	JA204
gH341*	E	S	A	I	G	V	T	K	S	A	G	F	JA206
gH341*	Q	S	A	I	G	V	T	K	N	A	G	F	JA208
KOL	E	S	S	V	A		R	N	N	L	G	F	

A J (SEQ ID NO: 7, 10 and 11-24)

OKT3 LIGHT CHAIN CDR GRAFTS

2. gL221 and derivatives

RES NUM	1	3	46	47	
OKT3v1	Q	V	R	W	
GL221	D	Q	L	L	DA221
gL221A	Q	V	R	W	DA221A
gL221B	Q	V	L	L	DA221B
GL221C	D	Q	R	W	DA221C
RE1	D	Q	L	L	

K J (SEQ ID NO: 8, 8, 9 and 25-28)

MURINE RESIDUES ARE UNDERLINED

The CDR-grafted heavy and light chain genes were co-expressed in COS cells either with one another in various combinations but also with the corresponding murine and chimeric heavy and light chain genes substantially as described above. The resultant antibody products were then assayed in binding and blocking assays with HPB-ALL cells as described above.

The results of the assays for various grafted heavy chains co-expressed with the gL221C light chain (SEQ ID NO:28) are given in Figures 7 and 8 (for the JA184, JA185, JA197 and JA198 constructs - see Table 2), in Figure 9 (for the JA183, JA184, JA185 and JA197 constructs) in Figure 10 a and b (for the chimeric, JA185, JA199, JA204, JA205, JA207, JA208 and JA209 constructs) and in Figure 11 a and b (for the JA183, JA184, JA185, JA198, JA203, JA205 and JA206 constructs).

The basic grafted product without any human to murine changes in the variable frameworks, i.e. gL221 (SEQ ID NO:25) co-expressed with gh341 (JA178) (SEQ ID NO.11), and also the "fully grafted" product, having most human to murine changes in the grafted heavy chain framework, i.e. gL221C (SEQ ID NO:28) co-expressed with gh341A (JA185) (SEQ ID NO:12), were assayed for relative binding affinity in a competition assay against murine OKT3 reference standard, using HPB-ALL cells. The assay used was as described above in section 3.3. The results obtained are given in Figure 12 for the basic grafted product and in Figure 13 for the fully grafted product. These results indicate that the basic grafted product has neglibible binding ability as compared with the OKT3 murine reference standard; whereas the "fully grafted" product has a binding ability very similar to that of the OKT3 murine reference standard.

The binding and blocking assay results indicate the following:

The JA198 and JA207 constructs appear to have the best binding characteristics and similar binding abilities, both substantially the same as the chimeric and fully grafted gH341A products. This indicates that positions 88 and 91 and position 76 are not highly critical for maintaining the OKT3 binding ability; whereas at least some of positions 6, 23, 24, 48, 49, 71, 73 and 78 are more important.

This is borne out by the finding that the JA209 and JA199, although of similar binding ability to one another, are of lower binding ability than the JA198 and JA207 constructs. This indicates the importance of having mouse residues at positions 71, 73 and 78, which are either completely or partially human in the JA199 and JA209 constructs respectively.

Moreover, on comparing the results obtained for the JA205 and JA183 constructs it is seen that there is a decrease in binding going from the JA205 to the JA183 constructs. This indicates the importance of retaining a mouse residue at position 23, the only position changed between JA205 and JA183.

These and other results lead us to the conclusion that of the 11 mouse framework residues used in the gH341A (JA185) construct, it is important to retain mouse residues at all of positions 6, 23, 24, 48 and 49, and possibly for maximum binding affinity at 71, 73 and 78.

Similar Experiments were carried out to CDR-graft a number of the rodent antibodies including antibodies having specificity for CD4 (OKT4), ICAM-1 (R6-5), TAG72 (B72.3), and TNFα(61E71, 101.4, hTNF1, hTNF2 and hTNF3).

EXAMPLE 2

CDR-GRAFTING OF A MURINE ANTI-CD4 T CELL RECEPTOR ANTIBODY, OKT4A

Anti OKT4A CDR-grafted heavy and light chain genes were prepared, expressed and tested substantially as described above in Example 1 for CDR-grafted OKT3. The CDR grafting of OKT4A is described in detail in Ortho patent application PCT/GB 90..... of even date herewith entitled "Humanised Antibodies". The disclosure of this Ortho patent application PCT/GB 90 ..... is incorporated herein by reference. A number of CDR-grafted OKT4 antibodies have been prepared. Presently the CDR-grafted OKT4A of choice is the combination of the grafted light chain LCDR2 and the grafted heavy chain HCDR10.

THE LIGHT CHAIN

The human acceptor framework used for the grafted light chains was RE1. (SEQ ID NO: 8 and 9) The preferred LCDR2 light chain has human to mouse changes at positions 33, 34, 38, 49 and 89 in addition to the structural loop CDRs. Of these changed positions, positions 33, 34 and 89 fall within the preferred extended CDRs of the present invention (positions 33 and 34 in CDR1 and position 89 in CDR3). The human to murine changes at positions 38 and 49 corresponds to positions at which the amino acid residues are preferably donor murine amino acid residues in accordance with the present invention.

A comparison of the amino acid sequences of the donor murine light chain variable domain and the RE1 human acceptor light chain variable further reveals that the murine and human residues are identical at all of positions 46, 48 and 71 and at all of positions 2, 4, 6, 35, 36, 44, 47, 62, 64-69, 85, 87, 98, 99 and 101 and 102. However the amino acid residue at position 58 in LCDR2 is

the human RE1 framework residue not the mouse OKT4 residue as would be preferred in accordance with the present invention.

THE HEAVY CHAIN

The human acceptor framework used for the grafted heavy chains was KOL (SEQ ID NO: 10)

The preferred CDR graft HCDR10 heavy chain has human to mouse changes at positions 24, 35, 57, 58, 60, 88 and 91 in addition to the structural loop CDRs.

Of these positions, positions 35 (CDR1) and positions 57, 58 and 60 (CDR2) fall within the preferred extended CDRs of the present invention. Also the human to mouse change at position 24 corresponds to a position at which the amino acid residue is a donor murine residue in accordance with the present invention. Moreover, the human to mouse changes at positions 88 and 91 correspond to positions at which the amino acid residues are optionally donor murine residues.

Moreover, a comparison of the murine OKT4A and human KOL heavy chain variable amino acid sequences reveals that the murine and human residues are identical at all of positions 23, 49, 71, 73 and 78 and at all of positions 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

Thus the OKT4A CDR-grafted heavy chain HCDR10 corresponds to a particularly preferred embodiment according to the present invention.

EXAMPLE 3

CDR-GRAFTING OF AN ANTI-MUCIN SPECIFIC MURINE ANTIBODY, B72.3

The cloning of the genes coding for the anti-mucin specific murine monoclonal antibody B72.3 and the preparation of B72.3 mouse-human chimeric antibodies has been described previously (ref. 13 and WO 89/01783). CDR-grafted versions of B72.3 were prepared as follows.

(a) B72.3 Light Chain

CDR-grafting of this light chain was accomplished by direct transfer of the murine CDRs into the framework of the human light chain RE1. The regions transferred were:

CDR Number	Residues
1	24-34
2	50-56
3	90-96

The activity of the resulting grafted light chain was assessed by co-expression in COS cells, of genes for the combinations:

B72.3 cH/B72.3 cL

and B72.3 cH/B72.3 gL

Supernatants were assayed for antibody concentration and for the ability to bind to microtitre plates coated with mucin. The results obtained indicated that, in combination with the B72.3 cH chain, B72.3 cL and B72.3 gL had similar binding properties.

Comparison of the murine B72.3 and REI (SEQ ID NO:8 and 9) light chain amino acid sequences reveals that the residues are identical at positions 46, 58 and 71 but are different at position 48.

Thus changing the human residue to the donor mouse residue at position 48 may further improve the binding characteristics of the CDR-grafted light chain, (B72.3 gL) in accordance with the present invention.

(b) B72.3 heavy chain

i. Choice of framework

At the outset it was necessary to make a choice of human framework. Simply put, the question was as follows: Was it necessary to use the framework regions from an antibody whose crystal structure was known or could the choice be made on some other criteria?

For B72.3 heavy chain, it was reasoned that, while knowledge of structure was important, transfer of the CDRs from mouse to human frameworks might be facilitated if the overall homology between the donor and receptor frameworks was maximised.

Comparison of the B72.3 heavy chain sequence with those in Kabat (ref. 4) for human heavy chains showed clearly that B72.3 had poor homology for KOL (SEQ ID NO:10) and NEWM (for which crystal structures are available) but was very homologous to the heavy chain for EU.

On this basis, EU was chosen for the CDR-grafting and the following residues transferred as CDRs.

CDR Number	Residues
1	27-36
2	50-63
3	93-102

Also it was noticed that the FR4 region of EU was unlike that of any other human (or mouse) antibody. Consequently, in the grafted heavy chain genes this was also changed to produce a "consensus" human sequence. (Preliminary experiments showed that grafted heavy chain genes containing the EU FR4 sequence expressed very poorly in transient expression systems.)

ii. Results with grafted heavy chain genes

Expression of grafted heavy chain genes containing all human framework regions with either gL or cL genes produced a grafted antibody with little ability to bind to mucin. The grafted antibody had about 1% the activity of the chimeric antibody.

In these experiments, however, it was noted that the activity of the grafted antibody could be increased to ~ 10% of B72.3 by exposure to pHs of 2-3.5.

This observation provided a clue as to how the activity of the grafted antibody could be improved without acid treatment. It was postulated that acid exposure brought about the protonation of an acidic residue (pKa of aspartic acid = 3.86 and of glutamine acid = 4.25) which in turn caused a change in structure of the CDR loops, or allowed better access of antigen.

From comparison of the sequences of B72.3 (ref. 13) and EU (refs. 4 and 5), it was clear that, in going from the mouse to human frameworks, only two positions had been changed in such a way that acidic residues had been introduced. These

positions are at residues 73 and 81, where K to E and Q to E changes had been made, respectively.

Which of these positions might be important was determined by examining the crystal structure of the KOL antibody. In KOL heavy chain (SEQ ID NO:10), position 81 is far removed from either of the CDR loops.

Position 73, however, is close to both CDRs 1 and 3 of the heavy chain and, in this position it was possible to envisage that a K to E change in this region could have a detrimental effect on antigen binding.

iii. Framework changes in B72.3 gH gene

On the basis of the above analysis, E73 was mutated to a lysine (K). It was found that this change had a dramatic effect on the ability of the grafted Ab to bind to mucin. Further the ability of the grafted B72.3 produced by the mutated gH/gL combination to bind to mucin was similar to that of the B72.3 chimeric antibody.

iv. Other framework changes

In the course of the above experiments, other changes were made in the heavy chain framework regions. Within the accuracy of the assays used, none of the changes, either alone or together, appeared beneficial.

v. Other

All assays used measured the ability of the grafted Ab to bind to mucin and, as a whole, indicated that the single framework change at position 73 is sufficient to generate an antibody with similar binding properties to B72.3.

Comparison of the B72.3 murine and EU heavy chain sequences reveals that the mouse and human residues are identical at positions 23, 24, 71 and 78.
Thus the mutated CDR-grafted B72.3 heavy chain corresponds to a preferred embodiment of the present invention.

EXAMPLE 4

CDR-GRAFTING OF A MURINE ANTI-ICAM-1 MONOCLONAL ANTIBODY

A murine antibody, R6-5-D6 (EP 0314863) having specificity for Intercellular Adhesion Molecule 1 (ICAM-1) was CDR-grafted substantially as described above in previous examples. This work is described in greater detail in co-pending application, British Patent Application No. 9009549.8, the disclosure of which is incorporated herein by reference.

The human EU framework was used as the acceptor framework for both heavy and light chains. The CDR-grafted antibody currently of choice is provided by co-expression of grafted light chain gL221A (SEQ ID NO: 26) and grafted heavy chain gH341D (SEQ ID NO: 16) which has a binding affinity for ICAM 1 of about 75% of that of the corresponding mouse-human chimeric antibody.

LIGHT CHAIN

gL221A has murine CDRs at positions 24-34 (CDR1), 50-56 (CDR2) and 89-97 (CDR3). In addition several framework residues are also the murine amino acid. These residues were chosen after consideration of the possible contribution of these residues to domain packing and stability of the conformation of the antigen binding region. The residues which have been retained as mouse are at positions 2, 3, 48 (?), 60, 84, 85 and 87. Comparison of the murine anti-ICAM 1 and human EU light chain amino acid sequences reveals that the murine and human residues are identical at positions 46, 58 and 71.

HEAVY CHAIN

gH341D has murine CDRs at positions 26-35 (CDR1), 50-56 (CDR2) and94-100B (CDR3). In addition murine residues were used in gH341D at positions 24, 48, 69, 71, 73, 80, 88 and 91. Comparison of the murine anti-ICAM 1 and human EU heavy chain amino acid sequences are identical at positions 23, 49 and 78.

EXAMPLE 5

CDR-Grafting of murine anti-TNFα antibodies

A number of murine anti-TNFα monoclonal antibodies were CDR-grafted substantially as described above in previous examples. These antibodies include the murine monoclonal antibodies designated 61 E71, hTNF1, hTNF3 and 101.4 A brief summary of the CDR-grafting of each of these antibodies is given below.

61E71

A similar analysis as described above (Example 1, Section 12.1.) was done for 61E71 and for the heavy chain 10 residues were identified at 23, 24, 48, 49, 68, 69, 71, 73, 75 and 88 as residues to potentially retain as murine. The human frameworks chosen for CDR-grafting of this antibody, and the hTNF3 and 101.4 antibodies were RE1 for the light chain and KOL for the heavy chain.
Three genes were built, the first of which contained 23, 24, 48, 49, 71 and 73 [gH341(6)] as murine residues. The second gene also had 75 and 88 as murine residues [gH341(8)] while the third gene additionally had 68, 69, 75 and 88 as murine residues [gH341(10)]. Each was co-expressed with gL221, the minimum grafted light chain (CDRs only). The gL221/gH341(6) and gL221/gH341(8) antibodies both bound as well to TNF as murine 61E71. The gL221/gH341(10) antibody did not express and this combination was not taken further.
Subsequently the gL221/gH341(6) antibody was assessed in an L929 cell competition assay in which the antibody competes against the TNF receptor on L929 cells for binding to TNF in solution. In this assay the gL221/gH341(6) antibody was approximately 10% as active as murine 61E71.

hTNF1

hTNF1 is a monoclonal antibody which recognises an epitope on human TNF- . The EU human framework was used for CDR-grafting of both the heavy and light variable domains.

Heavy Chain

In the CDR-grafted heavy chain (ghTNF1) mouse CDRs were used at positions 26-35 (CDR1), 50-65 (CDR2) and 95-102 (CDR3). Mouse residues were also used in the frameworks at positions 48, 67, 69, 71, 73, 76, 89, 91, 94 and 108. Comparison of the TNF1 mouse and EU human heavy chain residues reveals that these are identical at positions 23, 24, 29 and 78.

Light Chain

In the CDR-grafted light chain (gLhTNF1) mouse CDRs wre used at positions 24-34 (CDR1), 50-56 (CDR2) and 89-97 (CDR3). In addition mouse residues were used in the frameworks at positions 3, 42, 48, 49, 83, 106 and 108. Comparison of the hTNF1 mouse and EU human light chain residues reveals that these are identical at positions 46, 58 and 71.

The grafted hTNF1 heavy chain was co-expressed with the chimeric light chain and the binding ability of the product compared with that of the chimeric light chain/chimeric heavy chain product in a TNF binding assay. The grafted heavy chain product appeared to have binding ability for TNF slightly better than the fully chimeric product.

Similarly, a grafted heavy chain/grafted light chain product was co-expressed and compared with the fully chimeric product and found to have closely similar binding properties to the latter product.

hTNF3

hTNF3 recognises an epitope on human TNF-α. The sequence of hTNF3 shows only 21 differences compared to 61E71 in the light and heavy chain variable regions, 10 in the light chain (2 in the CDRs at positions 50, 96 and 8 in the framework at 1, 19, 40, 45, 46, 76, 103 and 106) and 11 in the heavy chain (3 in the CDR regions at positions 52, 60 and 95 and 8 in the framework at 1, 10, 38, 40, 67, 73, 87 and 105). The light and heavy chains of the 61E71 and hTNF3 chimeric antibodies can be exchanged without loss of activity in the direct binding assay. However 61E71 is an order of magnitude less able to compete with the TNF receptor on L929 cells for TNF-a compared to hTNF3. Based on the 61E71 CDR grafting data gL221 and gH341(+23, 24, 48, 49 71 and 73 as mouse) genes have been built for hTNF3 and tested and the resultant grafted antibody binds well to TNF-a, but competes very poorly in the L929 assay. It is possible that in this case also the framework residues identified for OKT3 programme may improve the competitive binding ability of this antibody.

101.4

101.4 is a further murine monoclonal antibody able to recognise human TNF-a. The heavy chain of this antibody shows good homology to KOL and so the CDR-grafting has been based on RE1 for the light chain and KOL for the heavy chain. Several grafted heavy chain genes have been constructed with conservative choices for the CDR's (gH341) (SEQ ID NO:11) and which have one or a small number of non-CDR residues at positions 73, 78 or 77-79 inclusive, as the mouse amino acids. These have been co-expressed with cL or gL221. In all cases binding to TNF equivalent to the chimeric antibody is seen and when co-expressed with cL the resultant antibodies are able to compete well in the L929 assay. However, with gL221 the resultant antibodies

are at least an order of magnitude less able to compete for TNF against the TNF receptor on L929 cells.

Mouse residues at other positions in the heavy chain, for example, at 23 and 24 together or at 76 have been demonstrated to provide no improvement to the competitive ability of the grafted antibody in the L929 assay.

A number of other antibodies including antibodies having specificity for interleukins e.g. IL1 and cancer markers such as carcinoembryonic antigen (CEA) e.g. the monoclonal antibody A5B7 (ref. 21), have been successfully CDR-grafted according to the present invention.
It will be appreciated that the foregoing examples are given by way of illustration only and are not intended to limit the scope of the claimed invention. Changes and modifications may be made to the methods described whilst still falling within the spirit and scope of the invention.

References

1. Kohler & Milstein, Nature, 265, 295-497, 1975.

2. Chatenoud et al, (1986), J. Immunol. 137, 830-838.

3. Jeffers et al, (1986), Transplantation, 41, 572-578.

4. Begent et al, Br. J. Cancer 62: 487 (1990).

5. Verhoeyen et al, Science, 239, 1534-1536, 1988.

6. Riechmann et al, Nature, 332, 323-324, 1988.

7. Kabat, E.A., Wu, T.T., Reid-Miller, M., Perry, H.M., Gottesman, K.S., 1987, in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA.

8. Wu, T.T., and Kabat, E.A., 1970, J. Exp. Med. 132 211-250.

9. Queen et al, (1989), Proc. Natl. Acad. Sci. USA, 86, 10029-10033 and WO 90/07861

10. Maniatis et al, Molecular Cloning, Cold Spring Harbor, New York, 1989.

11. Primrose and Old, Principles of Gene Manipulation, Blackwell, Oxford, 1980.

12. Sanger, F., Nicklen, S., Coulson, A.R., 1977, Proc. Natl. Acad. Sci. USA, 74 5463

13. Kramer, W., Drutsa, V., Jansen, H.-W., Kramer, B., Plugfelder, M., Fritz, H.-J., 1984, Nucl. Acids Res. 12, 9441

14. Whittle, N., Adair, J., Lloyd, J.C., Jenkins, E., Devine, J., Schlom, J., Raubitshek, A., Colcher, D., Bodmer, M., 1987, Protein Engineering 1, 499.

15. Sikder, S.S., Akolkar, P.N., Kaledas, P.M., Morrison, S.L., Kabat, E.A., 1985, J. Immunol. 135, 4215.

16. Wallick, S.C., Kabat, E.A., Morrison, S.L., 1988, J. Exp. Med. 168, 1099

17. Bebbington, C.R., Published International Patent Application WO 89/01036.

18. Granthan and Perrin 1986, Immunology Today 7, 160.

19. Kozak, M., 1987, J. Mol. Biol. 196, 947.

20. Jones, T.P., Dear, P.H., Foote, J., Neuberger, M.S., Winter, G., 1986, Nature, 321, 522

21. Harwood et al, Br. J. Cancer, 54, 75-82 (1986).

CLAIMS

1. A CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

2. A CDR-grafted heavy chain according to Claim 1 comprising donor residues at positions 23, 24, 49, 71, 73 and 78, or at positions 23, 24 and 49.

3. A CDR-grafted heavy chain according to Claim 2 comprising donor residues at positions 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

4. A CDR-grafted heavy chain according to Claim 2 or 3, comprising donor residues at one, some or all of positions:
1 and 3,
69 (if 48 is different between donor and acceptor),
38 and 46 (if 48 is the donor residue),
67,
82 and 18 (if 67 is the donor residue),
91, and
any one or more of 9, 11, 41, 87, 108, 110 and 112.

5. A CDR-grafted heavy chain according to any of the preceding comprising donor CDRs at positions 26-35, 50-65 and 95-100.

6. A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 1 and/or 3 and 46 and/or 47.

7. A CDR-grafted light chain according to Claim 6 comprising donor residues at positions 46 and 47.

8. A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, 58 and 71.

9. A CDR-grafted light chain according to Claim 8 comprising donor residues at positions 46, 48, 58 and 71.

10. A CDR-grafted light chain according to Claim 8 or 9, comprising donor residues at positions 2, 4, 6, 35, 36, 38, 44, 47, 49, 62, 64-69, 85, 87, 98, 99, 101 and 102.

11. A CDR-grafted light chain according to Claim 9 or 10, comprising donor residues at one, some or all of positions:
1 and 3,
63,
60 (if 60 and 54 are able to form a potential saltbridge),
70 (if 70 and 24 are able to form a potential saltbridge),
73 and 21 (if 47 is different between donor and acceptor),
37 and 45 (if 47 if different between donor and acceptor), and
any one or more of 10, 12, 40, 83, 103 and 105.

12. A CDR-grafted light chain according to any one of Claims 6-11, comprising donor CDRs at positions 24-34, 50-56 and 89-97.

13. A CDR-grafted antibody molecule comprising at least one CDR-grafted heavy chain according to any one of Claims 1-5 and at least one CDR-grafted light chain according to any one of Claims 6-12.

14. A CDR-grafted antibody molecule according to Claim 13, which is a site-specific antibody molecule.

15. A CDR-grafted antibody molecule according to Claim 13 which has specificity for an interleukin, hormone or other biologically active compound or a receptor therefor.

16. A CDR-grafted antibody heavy or light chain or molecule according to any one of the preceding claims comprising human acceptor residues and non-human donor residues.

17. A DNA sequence which codes for a CDR-grafted heavy chain according to Claim 1 or a CDR-grafted light chain according to Claim 6 or Claim 8.

18. A cloning or expression vector containing a DNA sequence according to Claim 17.

19. A host cell transformed with a DNA sequence according to Claim 17.

20. A process for the production of a CDR-grafted antibody sequence according to Claim 17 in a transformed host cell.

21. A process for producing a CDR-grafted antibody product comprising:

(a) producing in an expression vector an operon having a DNA sequence which encodes an antibody heavy chain according to Claim 1;

and/or

(b) producing in an expression vector an operon having a DNA sequence which encodes a complementary antibody light chain according to Claim 6 or Claim 8;

(c) transfecting a host cell with the or each vector; and

(d) culturing the transfected cell line to produce the CDR-grafted antibody product.

22. A therapeutic or diagnostic composition comprising a CDR-grafted antibody heavy chain according to Claim 1, or a CDR-grafted light chain according to Claim 6 or Claim 8, or a CDR-grafted antibody molecule according to Claim 13 in combination with a pharmaceutically acceptable carrier, diluent or excipient.

23. A method of therapy or diagnosis comprising administering an effective amount of a CDR-grafted heavy chain according to Claim 1, or a CDR-grafted light chain according to Claim 6 or Claim 8, or a CDR-grafted antibody molecule according to Claim 13 to a human or animal subject.

ABSTRACT

CDR-grafted antibody heavy and light chains comprise acceptor framework and donor antigen binding regions, the heavy chains comprising donor residues at at least one of positions (6, 23) and/or (24, 48) and/or (49, 71) and/or (73, 75) and/or (76) and/or (78) and (88) and/or (91). The CDR-grafted light chains comprise donor residues at at least one of positions (1) and/or (3) and (46) and/or (47) or at at least one of positions (46, 48, 58) and (71). The CDR-grafted antibodies are preferably humanised antibodies, having non human, e.g. rodent, donor and human acceptor frameworks, and may be used for *in vivo* therapy and diagnosis. A generally applicable protocol is disclosed for obtaining CDR-grafted antibodies.

```
   1  GAATTCCCAA AGACAAAatg gattttcaag tgcagatttt cagcttcctg
  51  ctaatcagtg cctcagtcat aatatccaga ggacaaattg ttctcaccca
 101  gtctccagca atcatgtctg catctccagg ggagaaggtc accatgacct
 151  gcagtgccag ctcaagtgta agttacatga actggtacca gcagaagtca
 201  ggcacctccc ccaaaagatg gatttatgac acatccaaac tggcttctgg
 251  agtccctgct cacttcaggg gcagtgggtc tgggacctct tactctctca
 301  caatcagcgg catggaggct gaagatgctg ccacttatta ctgccagcag
 351  tggagtagta acccattcac gttcggctcg gggacaaagt tggaaataaa
 401  ccgggctgat actgcaccaa ctgtatccat cttcccacca tccagtgagc
 451  agttaacatc tggaggtgcc tcagtcgtgt gcttcttgaa caacttctac
 501  cccaaagaca tcaatgtcaa gtggaagatt gatggcagtg aacgacaaaa
 551  tggcgtcctg aacagttgga ctgatcagga cagcaaagac agcacctaca
 601  gcatgagcag caccctcacg ttgaccaagg acgagtatga acgacataac
 651  agctatacct gtgaggccac tcacaagaca tcaacttcac ccattgtcaa
 701  gagcttcaac aggaatgagt gtTAGAGACA AAGGTCCTGA GACGCCACCA
 751  CCAGCTCCCA GCTCCATCCT ATCTTCCCTT CTAAGGTCTT GGAGGCTTCC
 801  CCACAAGCGC tTACCACTGT TGCGGTGCTC tAAACCTCCT CCCACCTCCT
 851  TCTCCTCCTC CTCCCTTTCC TTGGCTTTTA TCATGCTAAT ATTTGCAGAA
 901  AATATTCAAT AAAGTGAGTC TTTGCCTTGA AAAAAAAAAA AAA
```

Fig. 1(a)

```
   1  MDFQVQIFSF LLISASVIIS RGQIVLTQSP AIMSASPGEK VTMTCSASSS
  51  VSYMNWYQQK SGTSPKRWIY DTSKLASGVP AHFRGSGSGT SYSLTISGME
 101  AEDAATYYCQ QWSSNPFTFG SGTKLEINRA DTAPTVSIFP PSSEQLTSGG
 151  ASVVCFLNNF YPKDINVKWK IDGSERQNGV LNSWTDQDSK DSTYSMSSTL
 201  TLTKDEYERH NSYTCEATHK TSTSPIVKSF NRNEC*
```

Fig. 1(b)

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   1 GAATTCCCCT CTCCACAGAC ACTGAAAACT CTGACTCAAC ATGGAAAGGC
  51 ACTGGATCTT TCTACTCCTG TTGTCAGTAA CTGCAGGTGT CCACTCCCAG
 101 GTCCAGCTGC AGCAGTCTGG GGCTGAACTG GCAAGACCTG GGGCCTCAGT
 151 GAAGATGTCC TGCAAGGCTT CTGGCTACAC CTTTACTAGG TACACGATGC
 201 ACTGGGTAAA ACAGAGGCCT GGACAGGGTC TGGAATGGAT TGGATACATT
 251 AATCCTAGCC GTGGTTATAC TAATTACAAT CAGAAGTTCA AGGACAAGGC
 301 CACATTGACT ACAGACAAAT CCTCCAGCAC AGCCTACATG CAACTGAGCA
 351 GCCTGACATC TGAGGACTCT GCAGTCTATT ACTGTGCAAG ATATTATGAT
 401 GATCATTACT GCCTTGACTA CTGGGGCCAA GGCACCACTC TCACAGTCTC
 451 CTCAGCCAAA ACAACAGCCC CATCGGTCTA TCCACTGGCC CCTGTGTGTG
 501 GAGATACAAC TGGCTCCTCG GTGACTCTAG GATGCCTGGT CAAGGGTTAT
 551 TTCCCTGAGC CAGTGACCTT GACCTGGAAC TCTGGATCCC TGTCCAGTGG
 601 TGTGCACACC TTCCCAGCTG TCCTGCAGTC TGACCTCTAC ACCCTCAGCA
 651 GCTCAGTGAC TGTAACCTCG AGCACCTGGC CCAGCCAGTC CATCACCTGC
 701 AATGTGGCCC ACCCGGCAAG CAGCACCAAG GTGGACAAGA AAATTGAGCC
 751 CAGAGGGCCC ACAATCAAGC CCTGTCCTCC ATGCAAATGC CCAGCACCTA
 801 ACCTCTTGGG TGGACCATCC GTCTTCATCT TCCCTCCAAA GATCAAGGAT
 851 GTACTCATGA TCTCCCTGAG CCCCATAGTC ACATGTGTGG TGGTGGATGT
 901 GAGCGAGGAT GACCCAGATG TCCAGATCAG CTGGTTTGTG AACAACGTGG
 951 AAGTACACAC AGCTCAGACA CAAACCCATA GAGAGGATTA CAACAGTACT
1001 CTCCGGGTGG TCAGTGCCCT CCCCATCCAG CACCAGGACT GGATGAGTGG
1051 CAAGGAGTTC AAATGCAAGG TCAACAACAA AGACCTCCCA GCGCCCATCG
1101 AGAGAACCAT CTCAAAACCC AAAGGGTCAG TAAGAGCTCC ACAGGTATAT
1151 GTCTTGCCTC CACCAGAAGA AGAGATGACT AAGAAACAGG TCACTCTGAC
1201 CTGCATGGTC ACAGACTTCA TGCCTGAAGA CATTTACGTG GAGTGGACCA
1251 ACAACGGGAA AACAGAGCTA AACTACAAGA ACACTGAACC AGTCCTGGAC
1301 TCTGATGGTT CTTACTTCAT GTACAGCAAG CTGAGAGTGG AAAAGAAGAA
1351 CTGGGTGGAA AGAAATAGCT ACTCCTGTTC AGTGGTCCAC GAGGGTCTGC
1401 ACAATCACCA CACGACTAAG AGCTTCTCCC GGACTCCGGG TAAATGAGCT
1451 CAGCACCCAC AAAACTCTCA GGTCCAAAGA GACACCCACA CTCATCTCCA
1501 TGCTTCCCTT GTATAAATAA AGCACCCAGC AATGCCTGGG ACCATGTAAA
1551  AAAAAAAAAA AAAGGAATTC
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Fig. 2(a)

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OKT 3 HEAVY CHAIN PROTEIN SEQUENCE DEDUCED FROM DNA SEQUENCE

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  1  MERHWIFLLL LSVTAGVHSQ VQLQQSGAEL ARPGASVKMS CKASGYTFTR
 51  YTMHWVKQRP GQGLEWIGYI NPSRGYTNYN QKFKDKATLT TDKSSSTAYM
101  QLSSLTSEDS AVYYCARYYD DHYCLDYWGQ GTTLTVSSAK TTAPSVYPLA
151  PVCGDTTGSS VTLGCLVKGY FPEPVTLTWN SGSLSSGVHT FPAVLQSDLY
201  TLSSSVTVTS STWPSQSITC NVAHPASSTK VDKKIEPRGP TIKPCPPCKC
251  PAPNLLGGPS VFIFPPKIKD VLMISLSPIV TCVVVDVSED DPDVQISWFV
301  NNVEVHTAQT QTHREDYNST LRVVSALPIQ HQDWMSGKEF KCKVNNKDLP
351  APIERTISKP KGSVRAPQVY VLPPPEEEMT KKQVTLTCMV TDFMPEDIYV
401  EWTNNGKTEL NYKNTEPVLD SDGSYFMYSK LRVEKKNWVE RNSYSCSVVH
451  EGLHNHHTTK SFSRTPGK*
```

Fig. 2(b)

```
                1                       23                  42
                NN      N                 N         N     N
RES TYPE        SBspSPESssBSbSsSssPSPSPsPSsse*s*p*Pi^ISsSe
Okt3v1          QIVLTQSPAIMSASPGEKVTMTCSASS.SVSYMNWYQQKSGT
REI             DIQMTQSPSSLSASVGDRVTITCQASQDIIKYLNWYQQTPGK
                ? ?
                  CDR1    (LOOP)          *******
                  CDR1    (KABAT)        ***********

                          56                              85
                N  NN
RES TYPE        *IsiPpIeesesssSBEsePsPSBSSEsPspsPsseesSPePb
Okt3v1          SPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAAT
REI             APKLLIYEASNLQAGVPSRFSGSGSGTDYTFTISSLQPEDIAT
                ?  ??                        ?  ?
                      *******  CDR2 (LOOP/KABAT)

                               102   108
RES TYPE        PiPIPies**iPIIsPPSPSPSS
Okt3v1          YYCQQWSSNPFTFGSGTKLEINR
REIv1           YYCQQYQSLPYTFGQGTKLQITR
                              ?    ?
                    ******       CDR3 (LOOP)
                   *********     CRD3 (KABAT)
```

Fig. 3

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          NN N                  23 26    32 35 N39  43
RES TYPE  SESPs^SBssS^sSSsSpSpSPsPSEbSBssBePiPIpiesss
Okt3h     QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQ
KOL       QVQLVESGGGVVQPGRSLRLSCSSSGFIFSSYAMYWVRQAPGK
               ?              ??
                                    ******    CDR1 (LOOP)
                                   ***** CDR1 (KABAT)

                  52a      60  65     N N  N    82abc    89
RES TYPE IIeIppp^sssssssss^ps^pSSsbSpseSsSseSp^pSpsSBssS^ePb
Okt3vh   GLEWIGYINPSRGYTNTNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAV
KOL      GLEWVAIIWDDGSDQHYADSVKGRFTISRDNSKNTLFLQMDSLRPEDTGV
           ??                     ? ?  ?  ?            ?
              ************         CDR2  (LOOP)
              ******************** CDR2  (KABAT)

           92 N                  107    113
RES TYPE  PiPIEisssiiisssbibi*EIPIP*spSBSS
Okt3vh    YYCARYYDDHY.......CLDYWGQGTTLTVSS
KOL       YFCARDGGHGFCSSASCFGPDYWGQGTPVTVSS
               ***************** CRD3 (KABAT/LOOP)
```

Fig. 4

SUBSTITUTE SHEET

OKT 3 HEAVY CHAIN CDR GRAFTS

1. gh341 and derivatives

	1 26 35 39 43	
Okt3vh	QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQ	
gH341	QVQLVESGGGVVQPGRSLRLSCSSSGYTFTRYTMHWVRQAPGK	JA178
gH341A	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK	JA185
gH341E	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK	JA198
gH341*	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK	JA207
gH341*	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK	JA209
gH341D	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK	JA197
gH341*	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK	JA199
gH341C	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK	JA184
gH341*	QVQLVQSGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK	JA203
gH341*	QVQLVESGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK	JA205
gH341B	QVQLVESGGGVVQPGRSLRLSCSSSGYTFTRYTMHWVRQAPGK	JA183
gH341*	QVQLVQSGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK	JA204
gH341*	QVQLVESGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK	JA206
gH341*	QVQLVQSGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK	JA208
KOL	QVQLVESGGGVVQPGRSLRLSCSSSGFIFSSYAMYWVRQAPGK	

Fig. 5(i)

SUBSTITUTE SHEET

```
          44    50              65                  83
Okt3vh    GLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLT
gH341     GLEWVAYINPSRGYTNYNQKFKDRFTISRDNSKNTLFLQMDSLR JA178
gH341A    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR JA185

gH341E    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR JA198
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKNTAFLQMDSLR JA207
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTAFLQMDSLR JA209
gH341D    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKNTLFLQMDSLR JA197
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTLFLQMDSLR JA199
gH341C    GLEWVAYINPSRGYTNYNQKFKDRFTISRDNSKNTLFLQMDSLR JA184

gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR JA207
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR JA205
gH341B    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR JA183
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR JA204
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR JA206
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKNTAFLQMDSLR JA208
KOL       GLEWVAIIWDDGSDQHYADSVKGRFTISRDNSKNTLFLQMDSLR
```

Fig. 5(ii)

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	84 95 102 113	
Okt3vh	SEDSAVYYCARYYDDHY.....CLDYWGQGTTLTVSS	
gH341	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA178
gH341A	PEDTAVYYCARYYDDHY.....CLDYWGQGTTLTVSS	JA185
gH341E	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA198
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA207
gH341D	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA197
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA209
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA199
gH341C	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA184
gH341*	PEDTAVYYCARYYDDHY.....CLDYWGQGTTLTVSS	JA203
gH341*	PEDTAVYYCARYYDDHY.....CLDYWGQGTTLTVSS	JA205
gH341B	PEDTAVYYCARYYDDHY.....CLDYWGQGTTLTVSS	JA183
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA204
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA206
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA208
KOL	PEDTGVYFCARDGGHGFCSSASCFGPDYWGQGTPVTVSS	

Fig. 5(iii)

OKT3 LIGHT CHAIN CDR GRAFTING

1. gL221 and derivatives

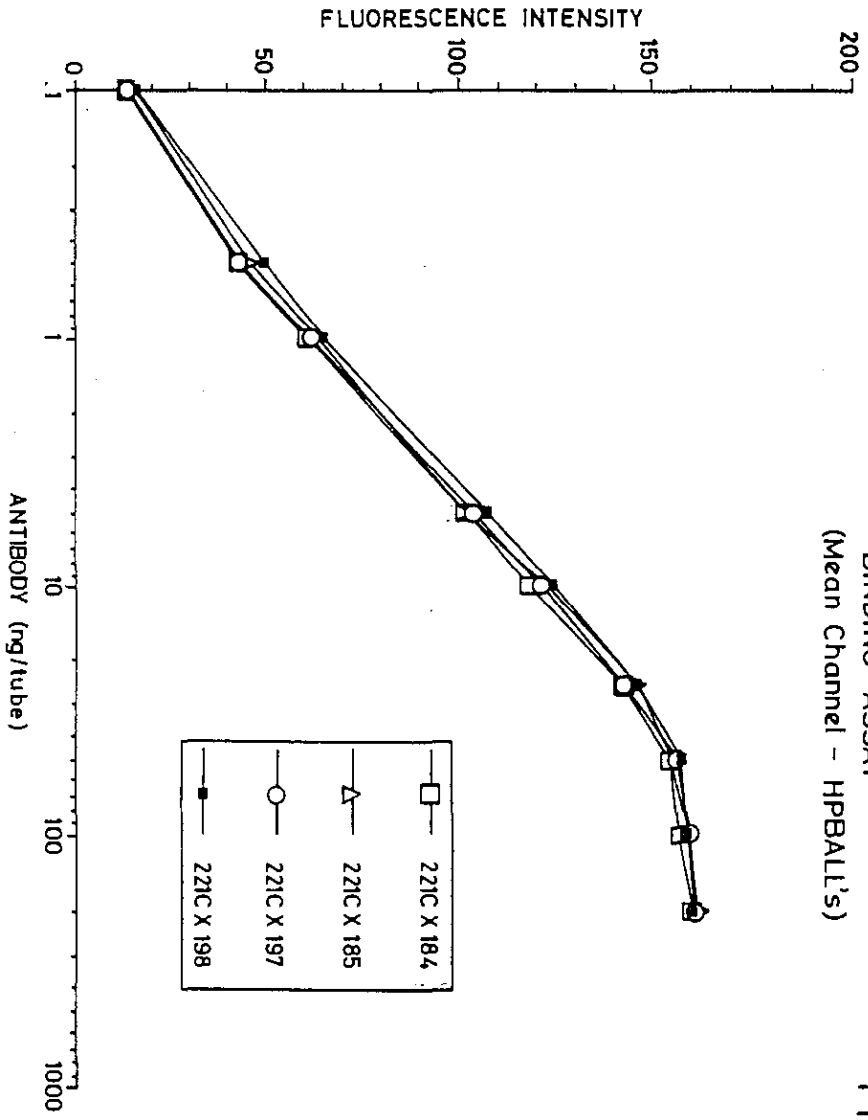
```
          1                        24        34       42
Okt3vl    QIVLTQSPAIMSASPGEKVTMTCSASS.SVSYMNWYQQKSGT
gL221     DIQMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
gL221A    QIVMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
gL221B    QIVMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
gL221C    DIQMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
REI       DIQMTQSPSSLSASVGDRVTITCQASQDIIKYLNWYQQTPGK

          43      50     56                          85
Okt3vl    SPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAAT
gL221     APKLLIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
gL221A    APKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
gL221B    APKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
gL221C    APKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
REI       APKLLIYEASNLQAGVPSRFSGSGSGTDYTFTISSLQPEDIAT

        86   91   96          108
Okt3vl  YYCQQWSSNPFTFGSGTKLEINR
gL221   YYCQQWSSNPFTFGQGTKLQITR
gL221A  YYCQQWSSNPFTFGQGTKLQITR
gL221B  YYCQQWSSNPFTFGQGTKLQITR
gL221C  YYCQQWSSNPFTFGQGTKLQITR
REI     YYCQQYQSLPYTFGQGTKLQITR
```

CDR'S ARE UNDERLINED

FRAMEWORK RESIDUES INCLUDED IN THE GENE ARE DOUBLE UNDERLINED

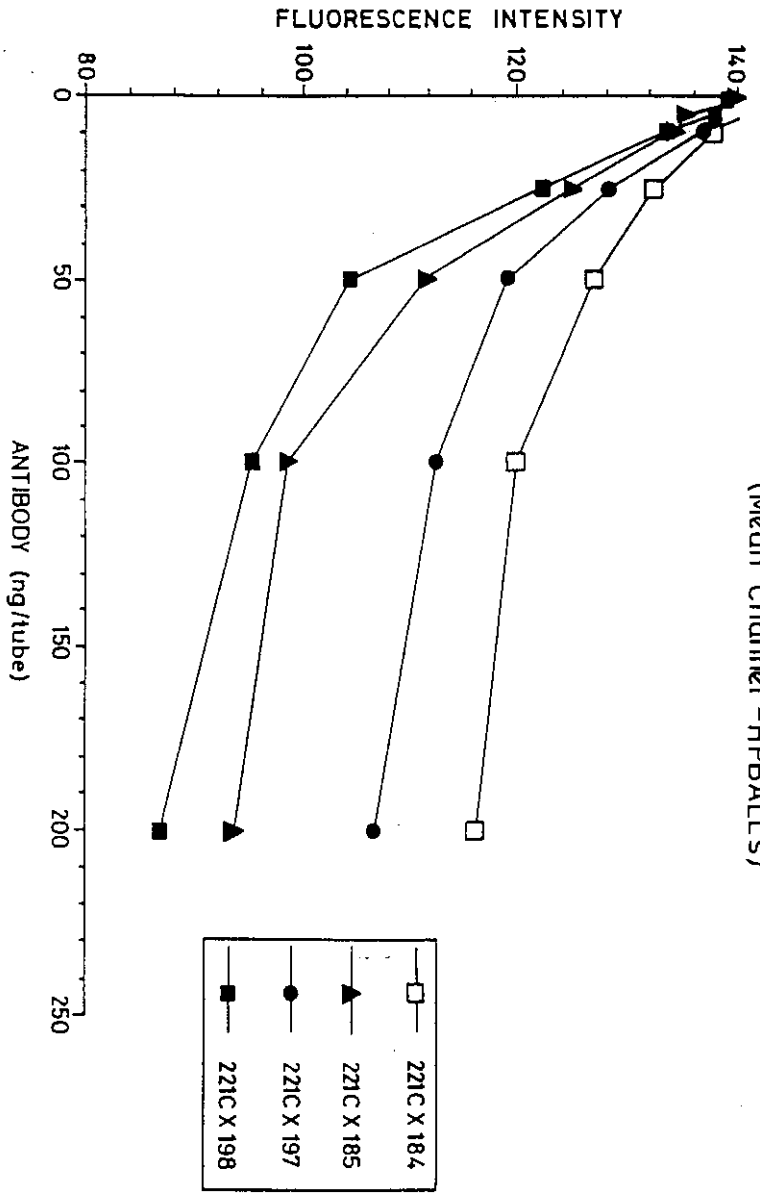
Fig. 6

SUBSTITUTE SHEET



OKT3 - pJA198 EVALUATION
BINDING ASSAY
(Mean Channel - HPBALL's)

Fig. 7



OKT3 - pJA198 EVALUATION
BLOCKING ASSAY
(Mean Channel -HPBALL's)

Fig. 8

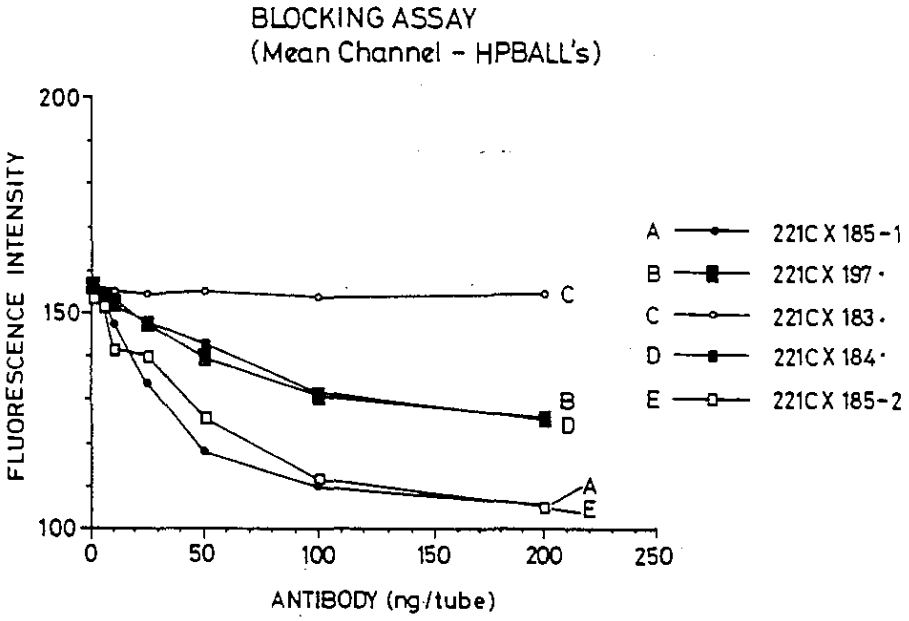
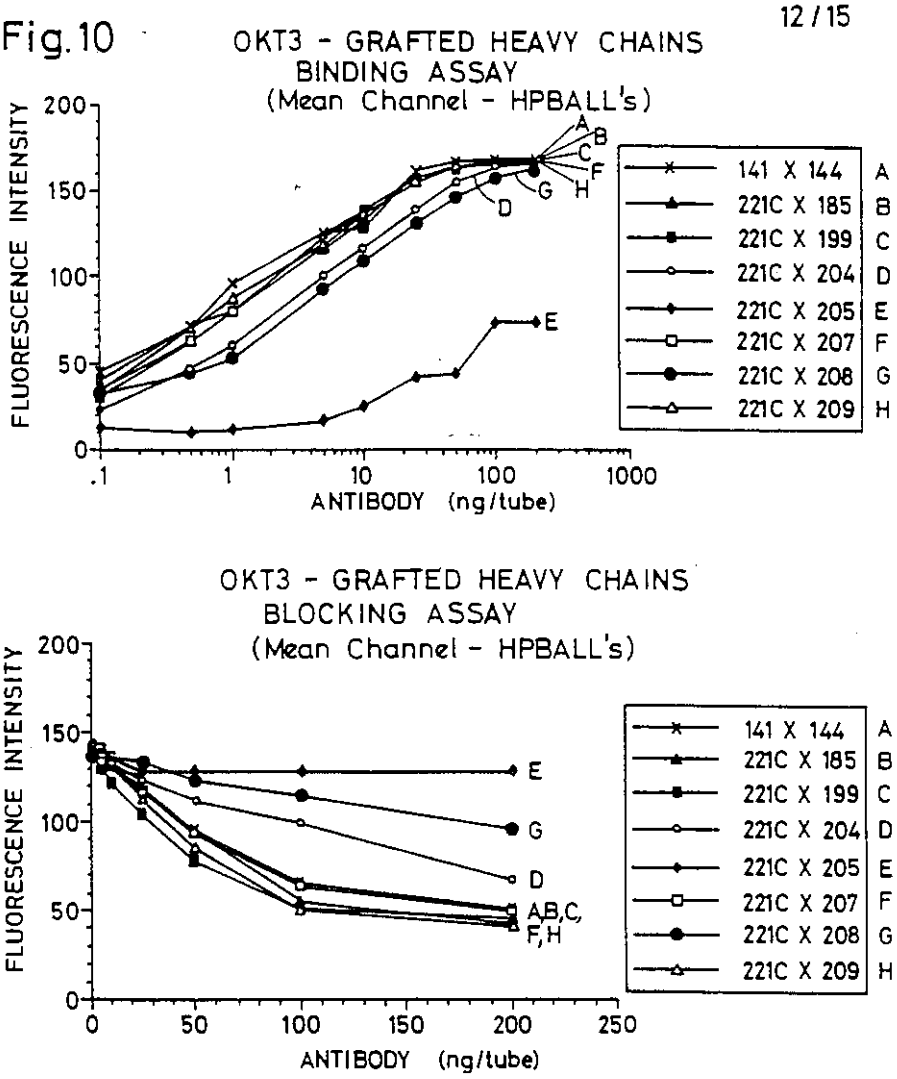
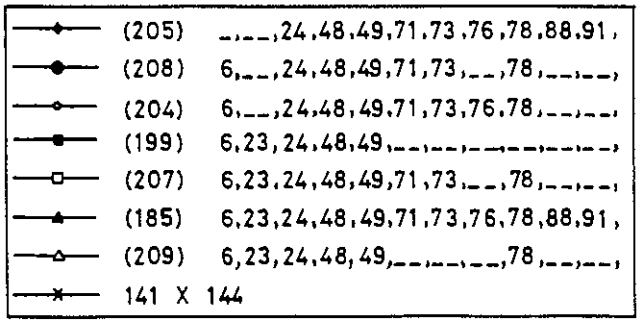


Fig. 9

SUBSTITUTE SHEET

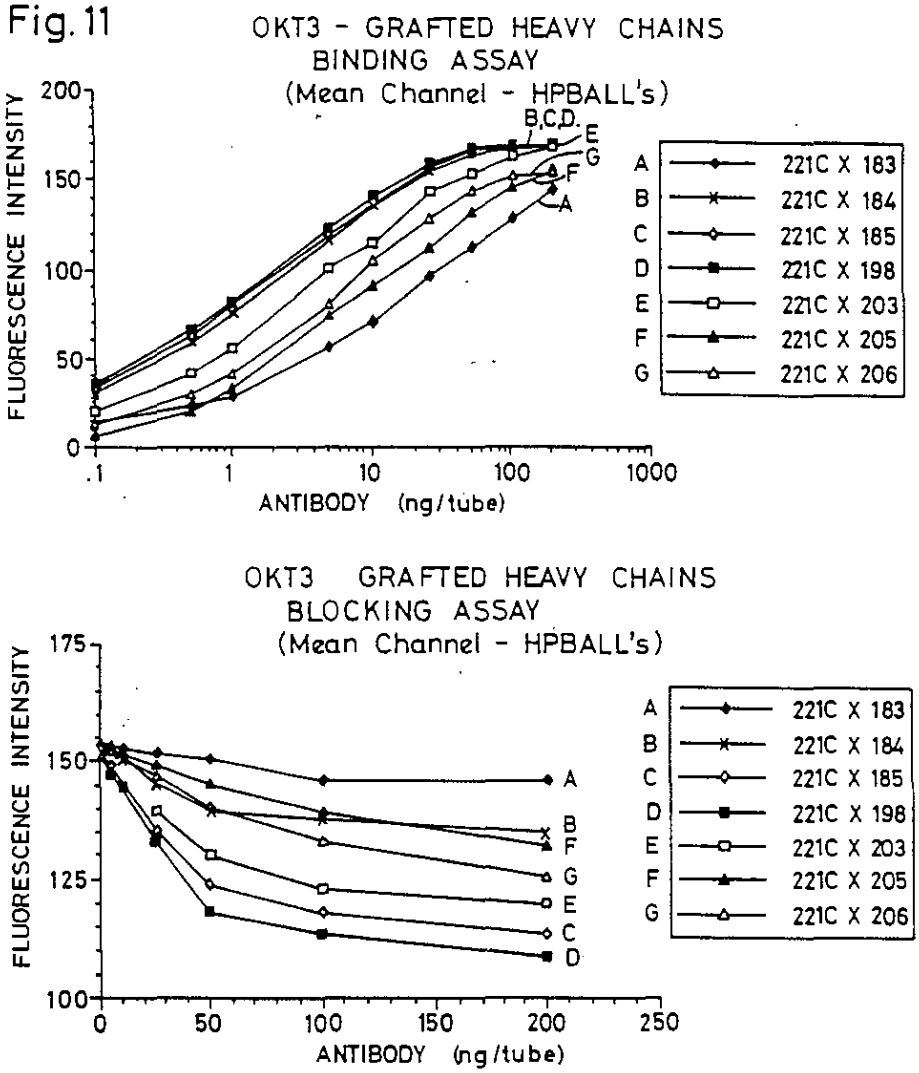
Fig.10





SUBSTITUTE SHEET

Fig. 11



◆	(183)	_,_,_,_,48,49,71,73,76,78,88,91,
▲	(205)	_,_,_,24,48,49,71,73,76,78,88,91,
✕	(184)	6,23,24,_,_,_,_,_,_,_,_,_,_,_,_,
△	(206)	_,_,_,24,48,49,71,73,76,78,_,_,_,
□	(203)	6,_,_,24,48,49,71,73,76,78,88,91,
◇	(185)	6,23,24,48,49,71,73,76,78,88,91,
■	(198)	6,23,24,48,49,71,73,76,78,_,_,_,

SUBSTITUTE SHEET

Fig. 12

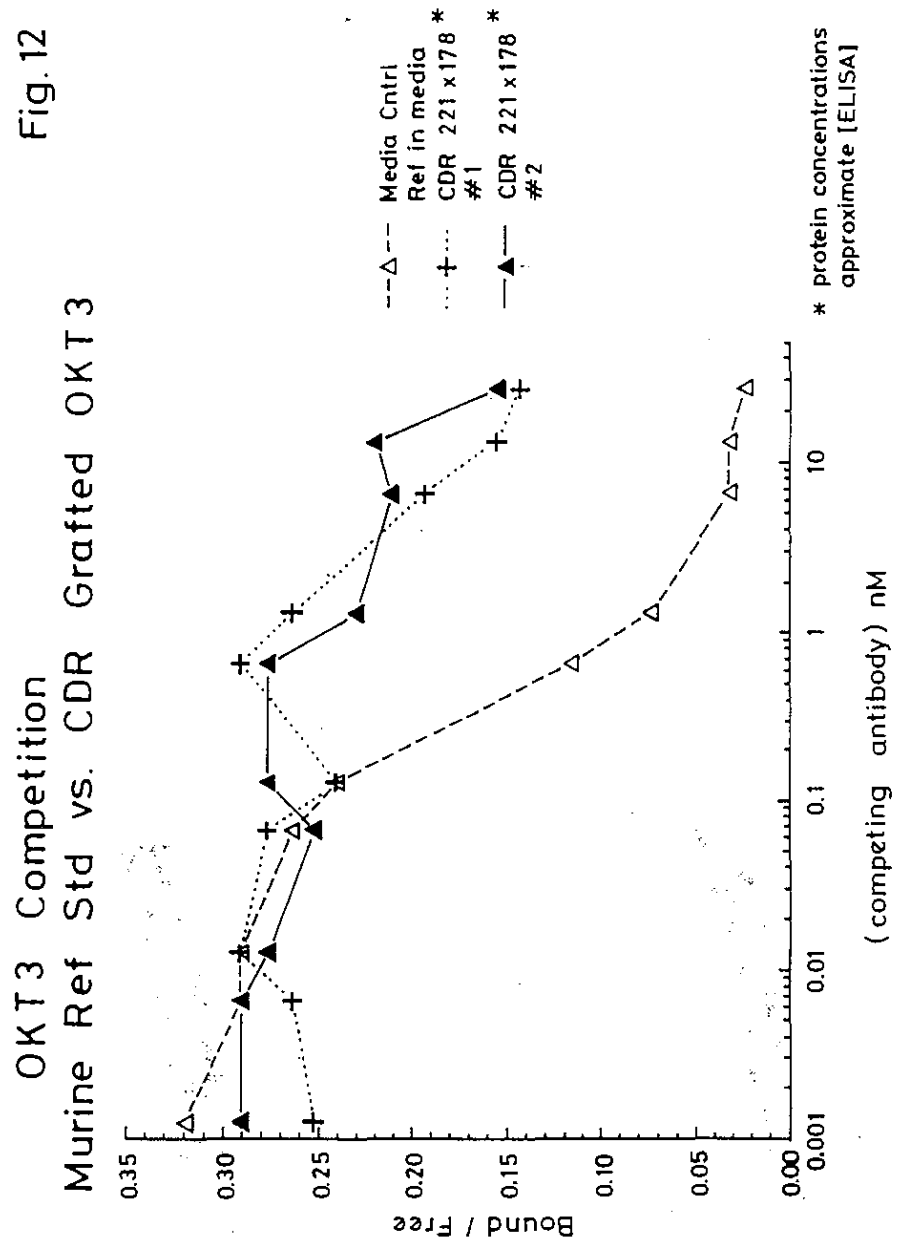
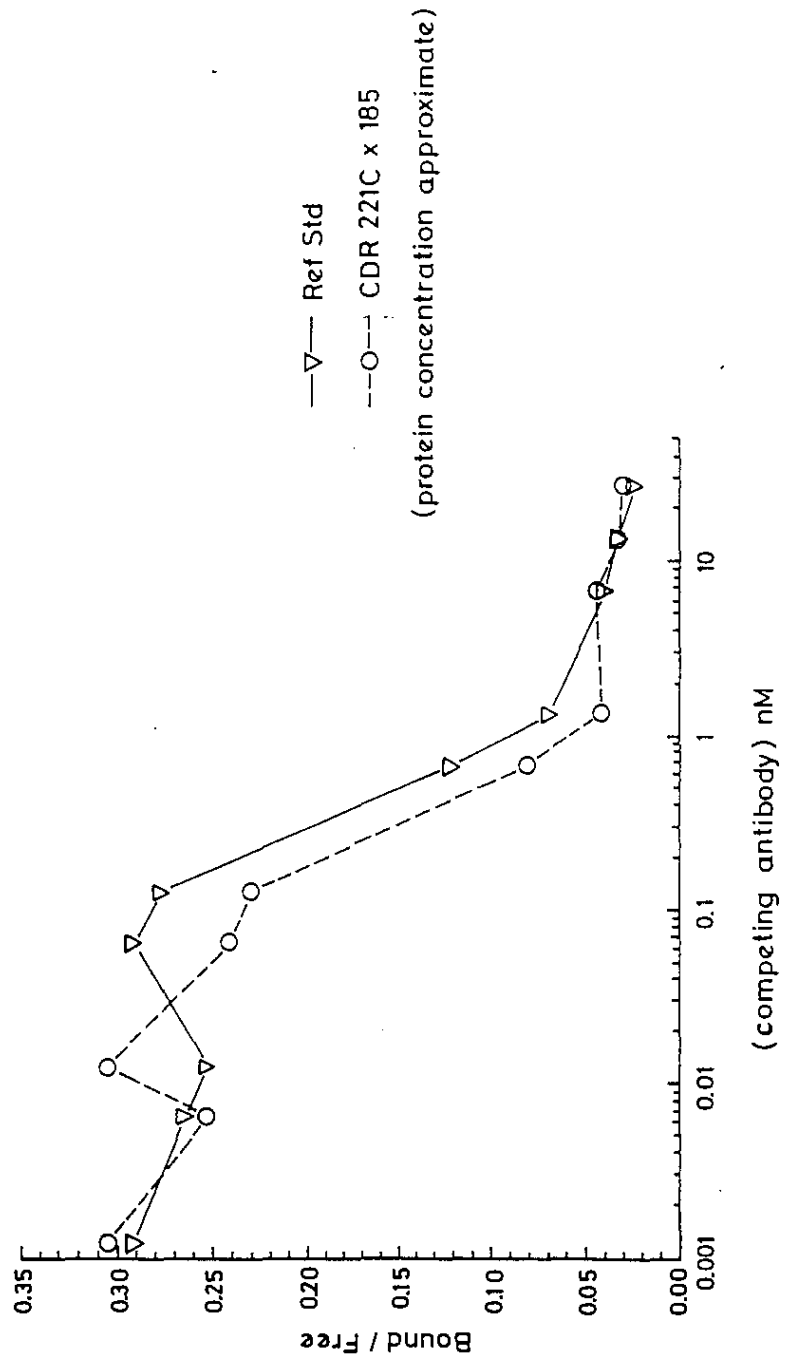

OKT3 Competition
Murine Ref Std vs. CDR Grafted OKT3
Media Cntrl
Ref in media
CDR 221 x178 * #1
CDR 221 x178 * #2
* protein concentrations approximate [ELISA]
Bound / Free
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0.001
0.01
0.1
1
10
(competing antibody) nM

Fig. 13

OKT3 Competition
Murine Ref Std vs. CDR Grafted OKT3



#4

FORM PTO-1390 (REV. 8-87) — U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)

ATTORNEY'S DOCKET NUMBER: CARP-0009

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB90/02017	21 December 1990	21 December 1989

TITLE OF INVENTION: HUMANISED ANTIBODIES

APPLICANT(S) FOR DO/EO/US: ADAIR, John, Robert, ATHWAL, Diljeet, Singh, and EMTAGE, John, Spencer

Applicant herewith submits to the United States Designated/ Elected Office (DO/EO/US) the following items under 35 U.S.C. 371:

1. ☐ This express request to immediately begin national examination procedures (35 U.S.C. 371(f)).
2. ☑ The U.S. National Fee (35 U.S.C. 371(c)(1)) and other fees as follows:

CLAIMS	(1) FOR	(2) NUMBER FILED	(3) NUMBER EXTRA	(4) RATE	(5) CALCULATIONS
	TOTAL CLAIMS	-20-		X$ 20.00	$
	INDEPENDENT CLAIMS	-3-		X$ 60.00	
	MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+$ 200.00	
	BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(4)): ☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) ..... $ 330 ☐ No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445 (a)(2)) ..... $ 370 ☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO..... $ 500 ☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2) to (4)..... $ 50				
	Surcharge of $120 for furnishing the National fee or oath or declaration later than ☑20 ☐30 mos. from the earliest claimed priority date (37 CFR 1.492(e)).				$120.00
			TOTAL OF ABOVE CALCULATIONS		-$120.00
	Reduction by 1/2 for filing by small entity, if applicable. Affidavit musts be filed also. (Note 37 CFR 1.9, 1.27, 1.28.)				$60.00
			SUBTOTAL		+$60.00
	Processing fee of $30 for furnishing the English Translation later than ☐20 ☐30 mos. from the earliest claimed priority date (37 CFR 1.492(f)).				
			TOTAL NATIONAL FEE		$ 60.00
	Fee for recording the enclosed assignment (37 CFR 1.21(h)).				+
			TOTAL FEES ENCLOSED		$ 60.00

a. ☒ A check in the amount of $ 60.00 to cover the above fees is enclosed.
b. ☐ Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.
c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 23-3050. A duplicate copy of this sheet is enclosed.

10/16/91X060 * 00258-00257*

01U3329

25-9 60

"EXPRESS MAIL" Mailing Label No. RB567260177
Date of Deposit September 17, 1991
I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231

Diane M. Kushner
(Typed)
Diane M. Kushner
(Signature)

ATTORNEY'S DOCKET NUMBER
CARP-0009

3. A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
   c. ☐ has been transmitted by the International Bureau.
4. ☐ A translation of the International Application into English (35 U.S.C. 371 (c)(2)).
5. Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
6. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
7. ☒ An oath or declaration of the inventor (35 U.S.C. 371(c)(4)).
8. ☐ A translation of the Annexes to the International Preliminary Examination Report under PCT Article 36(35 U.S.C. 371(c)(5)).

Other document(s) or information included:

9. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
10. ☐ An assignment document for recording.
    Please mail the recorded assignment document to:
    a. ☐ the person whose signature, name & address appears at the bottom of this page.
    b. ☐ the following:

11. The above checked items are being transmitted
    a. ☐ before the 18th month publication.
    b. ☐ after publication and the Article 20 communication but before 20 months from the priority date.
    c. ☒ after 20 months but before 22 months (surcharge and/or processing fee included).
    d. ☐ after 22 months (surcharge and/or processing fee included).
       Note: Petition to revive (37 CFR 1.137(a) or (b)) is necessary if 35 U.S.C. 371 requirements submitted after 22 months *and no proper demand for International Preliminary Examination was made by 19 months from the earliest claimed priority date.*
    e. ☐ by 30 months and a proper demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
    f. ☐ after 30 months but before 32 months and a proper demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date (surcharge and/or processing fee included).
    g. ☐ after 32 months (surcharge and/or processing fee included).
       Note: Petition to revive (37 CFR 1.137(a) or (b)) is necessary if 35 U.S.C. 371 requirements submitted after 32 months and a proper demand for International Preliminary Examination was made by 19 months from the earliest claimed priority date.
12. At the time of transmittal, the time limit for amending claims under Article 19
    a. ☐ has expired and no amendments were made.
    b. ☐ has not yet expired.
13. ☐ Certain requirements under 35 U.S.C. 371 were previously submitted by the applicant on _____ (date), namely:
14. Submission of Verified Statement Claiming Small Entity Status and Request for Refund

Francis A. Paintin
NAME

Woodcock Washburn Kurtz Mackiewicz & Norris
ADDRESS One Liberty Place - 46th Floor

Philadelphia, PA 19103

SIGNATURE 19,386 REGISTRATION NUMBER

Form PTO-1390 (Rev. 8-87) USCOMM-DC-87-3805

#54

DOCKET NO.: CARP-0009 **PATENT**

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name; and

I verily believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: HUMANISED ANTIBODIES
the specification of which:

___ is attached hereto.

✓ International was filed on 21 December 1990 as Application Serial No. PCT/GB90/02017 and was amended on _____. (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with 37 CFR § 1.56(a).

I hereby claim foreign priority benefits under 35 U.S.C. § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of any application on which priority is claimed:

Country	Number	Date Filed	Priority Claimed
U.K.	8928874.0	21.12.89	yes

PTO-19

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose material information as defined in 37 CFR § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	**Filing Date**	**Status (patented, pending)**

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

Francis A. Paintin

Registration Nos. 19,386

of the firm of **WOODCOCK WASHBURN KURTZ MACKIEWICZ & NORRIS**, One Liberty Place - 46th Floor, Philadelphia, Pennsylvania 19103, and

Address all telephone calls and correspondence to:

Francis A. Paintin

WOODCOCK WASHBURN KURTZ MACKIEWICZ & NORRIS
One Liberty Place - 46th Floor
Philadelphia, PA 19103
Telephone No. **215-568-3100.**

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the

United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-00	**Full Name** JOHN ROBERT ADAIR	**Inventor's Signature** John Robert Adair	**Date** 13/8/91
1	**Residence** 23 George Road, Stokenchurch, High Wycombe, Buckinghamshire HP14 3RN, U.K. GBX	**Citizenship** U.K.	
	Post Office Address 23 George Road, Stokenchurch, High Wycombe, Buckinghamshire HP14 3RN, U.K.		
2-00	**Full Name** DILJEET SINGH ATHWAL	**Inventor's Signature**	**Date** 13/8/91
2	**Residence** Flat 35, Knollys House, Tavistock Square, London WC1, U.K. GBX	**Citizenship** U.K.	
	Post Office Address Flat 35, Knollys House, Tavistock Square, London WC1, U.K.		
3-00	**Full Name** JOHN SPENCER EMTAGE	**Inventor's Signature** John Spencer Emtage	**Date** 13/8/91
3	**Residence** 49 Temple Mill Island, Temple Marlow, Buckinghamshire, SL7 1SQ, U.K. GBX	**Citizenship** U.K.	
	Post Office Address 49 Temple Mill Island, Temple, Marlow, Buckinghamshire SL7 1SQ, U.K.		
4	**Full Name**	**Inventor's Signature**	**Date**
	Residence	**Citizenship**	
	Post Office Address		
5	**Full Name**	**Inventor's Signature**	**Date**
	Residence	**Citizenship**	
	Post Office Address		

(12) **United States Patent**
Carter et al.

(10) **Patent No.: US 6,407,213 B1**
(45) **Date of Patent: Jun. 18, 2002**

(54) **METHOD FOR MAKING HUMANIZED ANTIBODIES**

(75) Inventors: **Paul J. Carter**; **Leonard G. Presta**, both of San Francisco, CA (US)

(73) Assignee: **Genentech, Inc.**, South San Francisco, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **08/146,206**

(22) PCT Filed: **Jun. 15, 1992**

(86) PCT No.: **PCT/US92/05126**

§ 371 (c)(1),
(2), (4) Date: **Nov. 17, 1993**

Related U.S. Application Data

(63) Continuation-in-part of application No. 07/715,272, filed on Jun. 14, 1991, now abandoned.

(51) **Int. Cl.**[7] **C07K 16/00**
(52) **U.S. Cl.** **530/387.3**; 435/69.6; 435/69.7; 435/70.21; 435/91; 536/23.53; 424/133.1
(58) **Field of Search** 435/69.6, 69.7, 435/70.21, 91, 172.2, 240.1, 240.27, 252.3, 320.1, 328; 536/23.53; 424/133.1; 530/387.3

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,816,567	A	3/1989	Cabilly et al.	
4,845,198	A	7/1989	Urdal et al.	530/388.22
5,132,405	A	7/1992	Huston et al.	530/387.3
5,225,539	A	7/1993	Winter	530/389.3
5,530,101	A	6/1996	Queen et al.	530/387.3
5,558,864	A	9/1996	Bendig et al.	424/133.1
5,585,089	A	12/1996	Queen et al.	424/133.1
5,677,171	A	10/1997	Hudziak et al.	435/7.23
5,693,762	A	* 12/1997	Queen et al.	530/387.2
5,714,350	A	2/1998	Co et al.	435/69.6
5,772,997	A	6/1998	Hudziak et al.	424/130.1
5,821,337	A	10/1998	Carter et al.	530/387.3
5,834,598	A	11/1998	Lowman et al.	530/399
5,859,205	A	1/1999	Adair et al.	530/387.3

FOREIGN PATENT DOCUMENTS

AU	85058/91		3/1992	C07K/15/12
EP	120694		10/1984	
EP	125023	A1	11/1984	
EP	0 239 400	*	9/1987	C12N/15/00
EP	323806	A1	7/1989	
EP	328404	A1	8/1989	A61K/39/395
EP	338745	A1	10/1989	
EP	365209	A2	4/1990	
EP	365997	A2	5/1990	
EP	368684		5/1990	
EP	403156	A1	12/1990	
EP	438310	A2	7/1991	
EP	438312	A2	7/1991	
EP	440351	A2	8/1991	
EP	0 460 167	B1	12/1991	
EP	0 519 596	A1	12/1992	
EP	0 592 106	A1	4/1994	
EP	0 620 276		10/1994	
EP	682040	A1	11/1995	
EP	451216	B1	1/1996	C12P/21/08
EP	432249	B1	9/1996	
GB	2 188941		10/1987	
WO	WO 87/02671		5/1987	
WO	WO 88/09344		12/1988	
WO	WO 89/01783		3/1989	
WO	WO 89/06692		7/1989	
WO	WO 89/09622		10/1989	
WO	WO 90/07861		7/1990	
WO	90/07861	*	7/1990	C12P/21/00
WO	WO 91/07492		5/1991	
WO	WO 91/07500		5/1991	
WO	WO 91/09966		7/1991	C12P/21/08
WO	WO 91/09968		7/1991	C12P/21/08
WO	WO 91/09967		11/1991	
WO	WO 92/01047		1/1992	
WO	WO 92/04380		3/1992	
WO	WO 92/04381		3/1992	
WO	WO 92/05274		4/1992	
WO	WO 92/11383		7/1992	
WO	WO 92/11018		9/1992	A61K/35/14
WO	WO 92/15683		9/1992	
WO	WO 92/16562		10/1992	
WO	WO 92/22653		12/1992	
WO	WO 93/02191		2/1993	
WO	94/11509		5/1994	
WO	WO 94/12214		6/1994	

OTHER PUBLICATIONS

Riechmann et al. [Nature 332:323–327 (1988)].*
Queen et al. [Proc. Natl. Acad. Sci. 86:10029–10033 (1989)].*
Roitt [*Immunology*, published 1985, by Gower Medical Publishing Ltd. (London, England) p. 5.5].*
Tramontano et al. [J. Mol. Biol. 215:175–182 (1990)].*
"Biosym Technologies" in New Products, Chemical Design Automation 3 (Dec. 1988).
"Polygen Corporation" in New Products, Chemical Design Automation 3 (Nov. 1988).
Adair et al., "Humanization of the murine anti–human CD3 monoclonal antibody OKT3" *Hum. Antibod. Hybridomas* 5:41–47 (1994).
Chothia et al., "Principles of protein–protein recognition" *Nature* 256:705–708 (1975).
Chothia et al., "Transmission of conformational change in insulin" *Nature* 302:500–505 (1983).
Corti et al., "Idiotope Determining Regions of a Mouse Monoclonal Antibody and Its Humanized Versions" *J. Mol. Biol.* 235:53–60 (1994).

(List continued on next page.)

Primary Examiner—Anthony C. Caputa
Assistant Examiner—Minh-Tam Davis
(74) *Attorney, Agent, or Firm*—Wendy M. Lee

(57) **ABSTRACT**

Variant immunoglobulins, particularly humanized antibody polypeptides are provided, along with methods for their preparation and use. Consensus immunoglobulin sequences and structural models are also provided.

82 Claims, 9 Drawing Sheets

OTHER PUBLICATIONS

Couto et al., "Anti–BA46 Monoclonal Antibody Mc3 Humanization Using a Novel Positional Consensus and in Vivo and in Vitro Characterization" *Cancer Research Supplement* 55:1717–1722 (1995).

Couto et al., "Humanization of KC4G3, an Anti–Human Carcinoma Antibody" *Hybridoma* 13:215–219 (1994).

Ellis et al., "Engineered Anti–CD38 Monoclonal Antibodies for Immunotherapy of Multiple Myeloma" *The Journal of Immunology* pp. 925–937 (1995).

Hieter et al., "Evolution of Human Immunoglobulin K J Region Genes" *The Journal of Biological Chemistry* 257:1516–1522 (1982).

Lesk, Arthur M., "How Different Amino Acid Sequences Determine Similar Protein Structures: The Structure and Evolutionary Dynamics of the Globins" *J. Mol. Biol.* 135:225–270 (1980).

Matsumura et al., "Hydrophobic stabilization in T4 lysozyme determined directly by multiple substitutions of Ile 3" *Nature* 334:406–410 (1988).

Morrison, S. L., "Transfectomas Provide Novel Chimeric Antibodies" *Science* 229:1202–1207 (Sep. 20, 1985).

Nakatani et al., "Humanization of mouse anti–human IL–2 receptor antibody B–B10" *Protein Engineering* 7:435–443 (1994).

Ohtomo et al., "Humanization of Mouse ONS–M21 Antibody with the Aid of Hybrid Variable Regions" *Molecular Immunology* 32:407–416 (1995).

Rodrigues et al., "Engineering a humanized bispecific $F(ab')_2$ fragment for improved binding to T cells" *Int. J. Cancer* (Suppl.) 7:45–50 (1992).

Sha et al., "A Heavy–Chain Grafted Antibody that Recognizes the Tumor–Associated TAG72 Antigen" *Cancer Biotherapy* 9:341–349 (1994).

Tempest et al., "Identification of framework residues required to restore antigen binding during reshaping of a monoclonal antibody against the glycoprotein gB of human cytomegalovirus" *Int. J. Biol. Macromol.* 17:37–42 (1995).

Tramontano, "Structural Determinants of the Conformations of Medium–Sized Loops in Proteins" *Proteins* 6:382–394 (1989).

Uchiyama et al., "A Monoclonal Antibody (Anti–Tac) Reactive with Activated and Functionally Mature Human T Cells" *Journal of Immunology* 126:1393–1397 (1981).

Vincenti et al., "Interleukin–2–Receptor Blockade with Daclizumab to Prevent Acute Rejection in Renal Transplantation" *New Engl. J. Med.* 338:161–165 (1998).

Vitetta et al., "Redesigning Nature's Poisons to Create Anti–Tumor Reagents" *Science* 238:1098–1104 (1987).

Waldmann et al., "Interleukin 2 Receptor (Tac Antigen) Expression in HTLV–1–associated Adult T–Cell Leukemia" *Cancer Research* 45:4559s–4562s (1985).

Waldmann, Thomas A., "The Structure, Function, and Expression of Interleukin–2 Receptors on Normal and Malignant Lymphocytes" *Science* 232:727–732 (1986).

Wu et al., "An Analysis of the Sequences of the Variable Regions of Bence Jones Proteins and Myeloma Light Chains and Their Implications for Antibody Complementarity" *Journal of Experimental Medicine* 132:211–250 (1970).

Rhodes, P., "Recombinant antibodies from CHO cells" *Abstr Pap Am Chem Soc* (Abstract No. 60 from the 199th American Chemical Society National Meeting held in Boston, MA Apr. 22–27, 1990) 199(1–2):BIOT 60 (Apr. 1990).

Amzel and Poljak, "Three–dimensional structure of immunoglobulins" *Ann. Rev. Biochem.* 48:961–967 (1979).

Bindon et al., "Human monoclonal IgG isotypes differ in complement activating function at the level of C4 as well as C1q" *Journal of Experimental Medicine* 168(1):127–142 (Jul. 1988).

Boulianne, G. L. et al., "Production of functional chimaeric mouse/human antibody" *Nature* 312(5995):643–646 (Dec. 1984).

Brown et al., "Anti–Tac–H, a humanized antibody to the interleukin 2 receptor, prolongs primate cardiac allograft survival" *Proc. Natl. Acad. Sci. USA* 88:2663–2667 (1991).

Bruccoleri, "Structure of antibody hypervariable loops reproduced by a conformational search algorithm" *Nature* (erratum to article in Nature 335(6190):564–568 and) 336:266 (1988).

Bruggemann, M. et al., "Comparison of the effector functions of human immunoglobulins using a matched set of chimeric antibodies" *Journal of Experimental Medicine* 166:1351–1361 (1987).

Burgess et al., "Possible Dissociation of the Heparin–binding and Mitogenic Activities of Heparin–binding (Acidic Fibroblast) Growth Factor–1 from Its Receptor–binding Activities by Site–directed Mutagenesis of a Single Lysine Residue" *Journal of Cell Biology* 111:2129–2138 (1990).

Carter et al., "Humanization of an anti–$p185^{HER2}$ antibody for human cancer therapy" *Proc. Natl. Acad. Sci.* 89:4285–4289 (1992).

Cheetham, J., "Reshaping the antibody combining site by CDR replacement–tailoting or tinkering to fit?" *Protein Engineering* 2(3):170–172 (1988).

Chothia and Lesk, "Canonical Structures for the Hypervariable Regions" *J. Mol. Biol.* 196:901–917 (1987).

Chothia et al., "The predicted structure of immunoglobulin D1.3 and its comparison with the crystal structure" *Science* 233:755–758 (Aug. 15, 1986).

Chothia, C. et al., "Conformations of immunoglobulin hypervariable regions" *Nature* 342(6252):877–883 (1989).

Chothia, Cyrus, "Domain association in immunoglobulin molecules: The packing of variable domains" *J. Mol. Biol.* 186:651–663 (1985).

Clark et al., "The improved lytic function and in vivo efficacy of monovalent monoclonal CD3 antibodies" *European Journal of Immunology* 19:381–388 (1989).

Co et al., "Humanized antibodies for antiviral therapy" *Proc. Natl. Acad. Sci. USA* 88:2869–2873 (1991).

Coussens et al., "Tyrosine Kinase Receptor with Extensive Homology to EGF Receptor Shares Chromosomal Location with neu Oncogene" *Science* 230:1132–1139 (1985).

Daugherty, BL et al., "Polymerase chain reaction facilitates the cloning, CDR–grafting, and rapid expression of a murine monoclonal antibody directed against the CD18 component of leukocyte integrins" *Nucleic Acids Research* 19(9):2471–2476 (May 11, 1991).

Davies, D. R. et al., "Antibody–Antigen Complexes" *Ann. Rev. Biochem.* 59:439–473 (1990).

Epp et al., "The molecular structure of a dimer composed of the variable portions of the Bence–Jones protein REI refined at 2.0–A resolution" *Biochemistry* 14(22):4943–4952 (1975).

Fendly et al., "Characterization of murine monoclonal antibodies reactive to either the human epidermal growth factor receptor or HER2/neu gene product" *Cancer Research* 50:1550–1558 (1990).

Furey et al., "Structure of a novel Bence–Jones protein (Rhe) fragment at 1.6 A resolution" *J. Mol. Biol.* 167(3):661–692 (Jul. 5, 1983).

Gorman, SD et al., "Reshaping a therapeutic CD4 antibody" *Proc. Natl. Acad. Sci. USA* 88(10):4181–4185 (May 15, 1991).

Gregory et al., "The solution conformations of the subclasses of human IgG deduced from sedimentation and small angle X–ray scattering studies" *Molecular Immunology* 24(8):821–829 (Aug. 1987).

Hale et al., "Remission induction in non–hodgkin lymphoma with reshaped human monoclonal antibody campath–1H" *Lancet* 1:1394–1399 (1988).

Harris and Emery, "Therapeutic antibodies—the coming of age" *Tibtech* 11:42–44 (Feb. 1993).

Huber et al., "Crystallographic structure studies of an IgG molecule and an Fc fragment" *Nature* 264:415–420 (Dec. 2, 1976).

Hudziak et al., "p185^{HER2} Monoclonal Antibody Has Antiproliferative Effects In Vitro and Sensitizes Human Breast Tumor Cells to Tumor Necrosis Factor" *Molecular & Cellular Biology* 9(3):1165–1172 (1989).

Jaffers, G. J. et al., "Monoclonal antibody therapy. Anti–idiotypic and non–anti–idiotypic antibodies to OKT3 arising despite intense immunosuppression" *Transplantation* 41(5):572–578 (May 1986).

Jones, P. T. et al., "Replacing the complementarity–determining regions in a human antibody with those from a mouse" *Nature* 321(6069):522–525 (1986).

Junghans et al., "Anti–Tac–H, a humanized antibody to the interleukin 2 receptor with new features for immunotherapy in malignant and immune disorders" *Cancer Research* 50(5):1495–1502 (Mar. 1, 1990).

Kabat et al. *Sequences of Proteins of Immunological Interest,* Bethesda, MD:National Institutes of Health pp. iii–xxvii, 41–176 (1987).

King et al., "Amplification of a Novel v–erbB–Related Gene in a Human Mammary Carcinoma" *Science* 229:974–976 (1985).

Lazar et al., "Transforming Growth Factor α: Mutation of Aspartic Acid 47 and Leucine 48 Results in Different Biological Activities" *Molecular & Cellular Biology* 8(3):1247–1252 (1988).

Love et al, "Recombinant antibodies possessing novel effector functions" *Methods in Enzymology* 178:515–527 (1989).

Lupu et al., "Direct interaction of a ligand for the erbB2 oncogene product with the EGF receptor and p185^{erbB2}" *Science* 249:1552–1555 (1990).

Margni RA and Binaghi RA, "Nonprecipitating asymmetric antibodies" *Ann. Rev. Immunol.* 6:535–554 (1988).

Margolies et al., "Diversity of light chain variable region sequences among rabbit antibodies elicited by the same antigens." *Proc. Natl. Acad. Sci. USA* 72:2180–84 (Jun. 1975).

Marquart et al., "Crystallographic refinement and atomic models of the intact immunoglobulin molecule Kol and its antigen–binding fragment at 3.0 A and 1.0 A resolution" *J. Mol. Biol.* 141(4):369–391 (Aug. 25, 1980).

Mian, IS et al., "Structure, function and properties of antibody binding sites" *J. Mol. Biol.* 217(1):133–151 (Jan. 5, 1991).

Miller, R. et al., "Monoclonal antibody therapeutic trials in seven patients with T–cell lymphoma" *Blood* 62:988–995 (1983).

Morrison, S. L. et al., "Chimeric human antibody molecules: mouse antigen–binding domains with human constant region domains" *Proc. Natl. Acad. Sci. USA* 81(21):6851–6855 (Nov. 1984).

Neuberger et al., "Recombinant antibodies possessing novel effector functions" *Nature* 312(5995):604–608 (Dec. 1984).

Neuberger, M. S. et al., "A hapten–specific chimaeric IgE antibody with human physiological effector function" *Nature* 314(6008):268–270 (Mar. 1985).

Novotny and Haber, "Structural invariants of antigen binding: comparison of immunoglobulin V_L–V_H and V_L–V_L domain dimers" *Proc. Natl. Acad. Sci. USA* 82(14):4592–4596 (Jul. 1985).

Pluckthun, Andreas, "Antibody engineering: advances from the use of *Escherichia coli* expression systems" *Biotechnology* 9:545–51 (1991).

Queen, M. et al., "A humanized antibody that binds to the interleukin 2 receptor" *Proc. Natl. Acad. Sci. USA* 86:10029–10033 (1989).

Riechmann, L. et al., "Reshaping human antibodies for therapy" *Nature* 332:323–327 (1988).

Roitt et al. *Immunology* (Gower Medical Publishing Ltd., London, England) pp. 5.5 (1985).

Saul et al., "Preliminary refinement and structural analysis of the Fab fragment from human immunoglobulin new at 2.0 A resolution" *Journal of Biological Chemistry* 253(2):585–597 (Jan. 25, 1978).

Schroff, R. et al., "Human anti–murine immunoglobulin responses in patients receiving monoclonal antibody therapy" *Cancer Research* 45:879–885 (1985).

Segal et al., "The three–dimensional structure of a phosphorylcholine–binding mouse immunoglobulin Fab and the nature of the antigen binding site" *Proc. Natl. Acad. Sci. USA* 71(11):4298–4302 (Nov. 1974).

Shalaby et al., "Development of humanized bispecific antibodies reactive with cytotoxic lymphocytes and tumor cells overexpressing the HER2 protooncogene" *Journal of Experimental Medicine* 175(1):217–225 (Jan. 1, 1992).

Shepard and Lewis, "Resistance of tumor cells to tumor necrosis factor" *J. Clin. Immunol.* 8(5):333–395 (1988).

Sheriff et al., "Three–dimensional structure of an antibody–antigen complex" *Proc. Natl. Acad. Sci. USA* 84(22):8075–8079 (Nov. 1987).

Sherman et al., "Haloperidol binding to monoclonal antibodies" *Journal of Biological Chemistry* 263:4064–4074 (1988).

Silverton et al., "Three–dimensional structure of an intact human immunoglobulin" *Proc. Natl. Acad. Sci. USA* 74:5140–5144 (1977).

Slamon et al., "Human Breast Cancer: Correlation of Relapse and Survival with Amplification of the HER–2/neu Oncogene" *Science* 235:177–182 (1987).

Slamon et al., "Studies of the HER–2/neu proto–oncogene in human breast and ovarian cancer" *Science* 244:707–712 (1989).

Snow and Amzel, "Calculating three–dimensional changes in protein structure due to amino–acid substitutions: the variable region of immunoglobulins" *Protein: Structure, Function, and Genetics,* Alan R. Liss, Inc. vol. 1:267–279 (1986).

Sox et al., "Attachment of carbohydrate to the variable region of myeloma immunoglubulin light chains" *Proc. Natl. Acad. Sci. USA* 66:975–82 (Jul. 1970).

Spiegelberg et al., "Localization of the carbohydrate within the variable region of light and heavy chains of human γG myeloma proteins" *Biochemistry* 9:4217–23 (Oct. 1970).
Takeda et al., "Construction of chimaeric processed immunoglobulin genes containing mouse variable and human constant region sequences" *Nature* 314(6010):452–454 (Apr. 1985).
Tao et al., "Role of Carbohydrate in the Structure and Effector Functions Mediated by the H uman IgG Constant Region" *J. Immunol.* 143(8):2595–2601 (1989).
Tramontano et al., "Framework residue 71 is a major determinant of the position and conformation of the second hypervariable region in the VH domains of immunoglobulins" *J–Mol–Biol* 215(1):175–182 (Sep. 5, 1990).
Verhoeyen, M. et al., "Reshaping human antibodies: grafting an antilysozyme activity" *Science* 239(4847):1534–1536 (Mar. 25, 1988).
Waldmann, T., "Monoclonal antibodies in diagnosis and therapy" *Science* 252:1657–1662 (1991).
Wallick et al., "Glycosylation of a VH residue of a monoclonal antibody against alpha (1——6) dextran increases its affinity for antigen" *Journal of Experimental Medicine* 168(3):1099–1109 (Sep. 1988).
Winter and Milstein, "Man–made antibodies" *Nature* 349(6307):293–299 (Jan. 24, 1991).
Yamamoto et al., "Similarity of protein encoded by the human c–erb–B–2 gene to epidermal growth factor receptor" *Nature* 319:230–34 (1986).
Carter et al., "High level *escherichia coli* expression and production of a bivalent humanized antibody fragment" *Bio/Technology* 10:163–167 (1992).
Foote et al., "Antibody Framework Residues Affecting the Conformation of the Hypervariable Loops" *J. Mol. Biol.* 224:487–499 (1992).
Foote, J., "Humanized Antibodies" *Nova acta Leopoldina* 61(269):103–110 (1989).
Kabat et al., "Sequences of Proteins of Immunological Interest", Bethesda, MD:National Institute of Health pp. 14–32 (1983).
Kettleborough et al., "Humanization of a Mouse Monoclonal Antibody by CDR–grafting: the Importance of Framework Residues on Loop Conformation" *Protein Engineering* 4(7):773–783 (1991).
Maeda et al., "Construction of Reshaped Human Antibodies with HIV–neutralizing Activity" *Hum. Antibod. Hybridomas* 2:124–134 (Jul. 1991).
Riechmann et al, "Expression of an Antibody Fv Fragment in Myeloma Cells" *J. Mol. Biol.* 203:825–828 (1988).
Routledge et al., "A Humanized Monovalent CD3 Antibody which Can Activate Homologous Complement" *European Journal of Immunology* 21:2717–2725 (1991).
Shearman et al., "Construction, Expression and Characterization of Humanized Antibodies Directed Against the Human α/β T Cell Receptor" *J. Immunol.* 147(12):4366–4373 (Dec. 15, 1991).
Tempest et al., "Reshaping a Human Monoclonal Antibody to Inhibit Human Respiratory Syncytial Virus Infection In Vivo" *Bio/Technology* 9:266–271 (Mar. 1991).
Brown, Jr. et al., "Anti–Tac–H, a humanized antibody to the interleukin 2 receptor, prolongs primate cardiac allograft survival" *Proc. Natl. Acad. Sci. USA* 88:2663–2667 (1991).
Casale et al., "Use of an anti–IgE humanized monoclonal antibody in ragweed–induced allergic rhinitis" *J. Allergy Clin. Immunol.* 100:110–121 (1997).
Fahy et al., "The Effect of an Anti–IgE Monoclonal Antibody on the Early– and Late–Phase Responses to Allergen Inhalation in Asthmatic Subjects" *Am J. Respir. Crit. Care Med* 155:1828–1834 (1997).
Mathieson et al., "Monoclonal–Antibody Therapy in Systemic Vasculitis" *New England J. of Medicine* pp. 250–254 (Jul. 1990).
Presta et al., "Humanization of an anti–vascular endothelial growth factor monoclonal antibody for the therapy of solid tumors and other disorders" *Cancer Research* 57(20):4593–4599 (Oct. 15, 1997).
Amit et al., "Three–Dimensional Structure of an Antigen–Antibody Complex at 2.8 A Resolution" *Science* 233:747–753 (Aug. 1986).
Amzel et al., "The Three Dimensional Structure of a Combining Region–Ligand Complex of Immunglobulin New at 3.5–A Resolution" *Proc. Natl. Acad. Sci. USA* 71(4):1427–1430 (Apr. 1974).
Baselga et al., "Phase II Study of Weekly Intravenous Recombinant Humanized Anti–p185/HER2 Monoclonal Antibody in Patients With HER2/neu–Overexpressing Metastatic Breast Cancer" *J. Clin. Oncol.* 14(3):737–744 (1996).
Beverley & Callard, "Distinctive functional charcteristics of human "T" lymphocytes defined by E rosetting or a monoclonal anti–T cell antibody" *European Journal of Immunology* 11:329–334 (1981).
Bird et al., "Single–chain antigen–binding proteins" *Science* 242:423–426 (Oct. 1988).
Brennan et al., "Preparation of bispecific antibodies by chemical recombination of monoclonal immunoglobulin G_1 fragments" *Science* 229:81–83 (Jul. 1985).
Bruccoleri et al., "Structure of antibody hypervariable loops reproduced by a conformational search algorithm" *Nature* 335:564–568 (Oct. 1988).
Caron et al., "Biological and Immunological Features of Humanized M195 (Anti–CD33) Monoclonal Antibodies" *Cancer Research* 52:6761–6767 (Dec. 1992).
Chothia & Lesk, "The relation between the divergence of sequence and structure in proteins" *EMBO Journal* 5(4):823–826 (1986).
Co & Queen, "Humanized antibodies for therapy" *Nature* 351:501–502 (Jun. 1991).
Co et al., "Chimeric and Humanized Antibodies with Specificity for the CD33 Antigen" *J. of Immunology* 148(4):1149–1154 (Feb. 1992).
Co et al., "Humanized Anti–Lewis Y Antibodies: In Vitro Properties and Pharmacokinetics in Rhesus Monkeys" *Cancer Research* 56:1118–1125 (Mar. 1996).
Colman et al., "Crystal and Molecular Structure of the Dimer of Variable Domains of the Bence–Jones Protein ROY" *J. Mol. Biol.* 116:73–79 (1977).
Colman et al., "Three–dimensional structure of a complex of antibody with influenza virus neuraminidase" *Nature* 326:358–363 (Mar. 1987).
Cook et al., "A map of the human immunoglbulin V_H locus completed by analysis of the telometric region of chromosome 14q" *Nature Genetics* 7:162–168 (Jun. 1994).
Darsley & Rees, "Nucleotide sequences of five anti–lysozyme monoclonal antibodies" *EMBO Journal* 4(2):393–398 (1985).
Davies & Metzger, "Structural Basis of Antibody Function" *Ann. Rev. Immunol.* 1:87–117 (1983).

Davies et al., "Antibody–Antigen Complexes" *Journal of Biological Chemistry* 263(22):10541–10544 (Aug. 1988).

Eigenbrot et al., "X–Ray Structures of Fragments From Binding and Nonbinding Versions of a Humanized Anti–CD18 Antibody: Structural Indications of the Key Role of V_H Residues 59 to 65" *Proteins* 18:49–62 (1994).

Eigenbrot et al., "X–ray structures of the antigen–binding domains from three variants of humanized anti–p185HER2 antibody 4D5 and comparison with molecular modeling" *J. Mol. Biol.* 229:969–995 (1993).

Ellison et al., "The nucleotide sequence of a human immunoglobulin $C_{\gamma 1}$ gene" *Nucleic Acids Research* 10(13):4071–4079 (1982).

Emery & Adair, "Humanised monoclonal antibodies for therapeutic applications" *Exp. Opin. Invest. Drugs* 3(3):241–251 (1994).

Epp et al., "Crystal and Molecular Structure of a Dimer Composed of the Variable Portions of the Bence–Jones Protein REI" *European Journal of Biochemistry* 45:513–524 (1974).

Fanger et al., "Bispecific antibodies and targeted cellular cytotoxicity" *Immunology Today* 12(2):51–54 (1991).

Fanger et al., "Cytotoxicity mediated by human Fc receptors for IgG" *Immunology Today* 10(3):92–99 (1989).

Feldmann et al., "A Hypothetical Space–Filling Model of the V–Regions of the Galactan–Binding Myeloma Immunoglobulin J539" *Molecular Immunology* 18(8):683–698 (1981).

Fendley et al., "The Extracellular Domain of HER2/neu Is a Potential Immunogen for Active Specific Immunotherapy of Breast Cancer" *J. Biol. Resp. Mod.* 9:449–455 (1990).

Glennie et al., "Preparation and Performance of Bispecific $F(ab'\gamma)_2$ Antibody Containing Thioether–Linked $Fab'\gamma$ Fragments" *J. Immunol.* 139(7):2367–2375 (Oct. 1, 1987).

Gonzalez et al., "Humanization of Murine 6G425:An Anti–IL8 Monoclonal Antibody Which Blocks Binding of IL8 to Human Neutrophils" *1996 Keystone Symposia on Exploring and Exploiting Antibody and Ig Superfamily Combining Sites* (Poster) pp. 1–21 (Feb. 1996).

Gussow & Seemann, "Humanization of Monoclonal Antibodies" *Meth. Enzymology,* Academic Press, Inc. vol. 203:99–121 (1991).

Hieter et al., "Cloned human and mouse kappa immunoglobulin constant and J region genes conserve homology in functional segments" *Cell* 22 (Part 1):197–207 (1980).

Houghton, A., "Building a better monoclonal antibody" *Immunology Today* 9(9):265–267 (1988).

Huston et al., "Protein engineering of antibody binding sites: Recovery of specific activity in an anti–digoxin single–chain Fv analogue produced in *Escherichia coli" Proc. Natl. Acad. Sci. USA* 85:5879–5883 (Aug. 1988).

Isaacs et al., "Humanised Monoclonal Antibody Therapy for Rheumatoid Arthritis" *Lancet* 340:748–752 (Sep. 26, 1992).

Johnson et al., "Biological and Molecular Modeling Studies Comparing Murine Monoclonal Antibodies with Their Engineered Chimeric and Humanized Counterparts" *J. Cell. Biochem. Suppl 0 (13 Part A)* (18th Ann. UCLA Symp on Mol. & Cell. Biol., Park City, UT Jan. 17–22, 1989) pp. 87 (1989).

Kabat E., "Origins of Antibody Complementarity and Specificity—Hypervariable Regions and the Minigenen Hypothesis" *J. of Immunology* 125(3):961–969 (Sep. 1980).

Kabat et al. *Sequences of Proteins of Immunological Interest,* U.S. Dept. of Health and Human Services, NIH, 5th edition vol. 1:103–108, 324–331 (1991).

Kindt & Capra *The Antibody Enigma,* New York:Plenum Press pp. 79–86 (1984).

Lesk & Chothia, "Evolution of Proteins Formed by β–Sheets" *J. Mol. Biol.* 160:325–342 (1982).

Lesk & Chothia, "The response of protein structures to amino–acid sequence changes" *Phil. Trans. R. Soc. Lond.* A 317:345–356 (1986).

Mariuzza et al., "The Structure Basis of Antigen–Antibody Recognition" *Ann. Rev. Biophys. Biophys. Chem.* 16:139–159 (1987).

Nadler et al., "Immunogenicity of Humanized and Human Monoclonal Antibodies" *Clin. Pharmacology & Therapeutics* pp. 180 (Feb. 1994).

Nelson, H., "Targeted Cellular Immunotherapy with Bifunctional Antibodies" *Cancer Cells* 3:163–172 (1991).

Neuberger et al., "Antibody Engineering" *Proceedings 8th Intl. Biotech. Symp.,* Paris II:792–799 (1988).

Newmark, P., "Making Chimeric Antibodies Even More Human" *Bio/Technology* 6:468 (May 1988).

Nishimura et al., "Human c–erbB–2 Proto–Oncogene Product as a Target for Bispecific–Antibody–Directed Adoptive Tumor Immunotherapy" *Int. J. Cancer* 50:800–804 (1992).

Nitta et al., "Preliminary trial of specific targeting therapy against malignant glioma" *Lancet* 335(8686):368–371 (Feb. 17, 1990).

Nitta, T. et al., "Bispecific $F(ab')_2$ monomer prepared with anti–CD3 and anti–tumor monoclonal antibodies is most potent in induction of cytolysis of human T cells" *European Journal of Immunology* 19:1437–1441 (1989).

Nolan et al., "Bifunctional antibodies: concept, production and applications" *Biochimica et Biophysica Acta* 1040:1–11 (1990).

O'Connor et al., "Calcium Dependence of an Anti–Protein C Humanized Antibody Involves Framework Residues" (manuscript).

Orlandi et al., "Cloning Immunoglobulin Variable Domains for Expression by the Polymerase Chain Reaction" *Proc. Natl. Acad. Sci. USA* 86:3833–3837 (May 1989).

Orlandi et al., "Cloning of cDNA Corresponding to Heavy and Light Chain Immunoglobulin Variable Domains" *Protein and Pharmaceutical Engineering* pp. 90 (1989).

Ostberg & Queen, "Human and humanized monoclonal antibodies: preclinical studies and clinical experience" *Biochem. Soc. Transactions* pp. 1038–1043 (1995).

Pedlan et al., "Model–building Studies of Antigen–binding Sites:The Hapten–binding Site of MOPC–315" *Cold Springs Harbor Symposia On Quantitative Biology* XLI:627–637 (1977).

Padlan, E., "Anatomy of the Antibody Molecule" *Molecular Immunology* 31(3):169–217 (1994).

Padlan, E., "Evaluation of the Structural Variation Among Light Chain Variable Domains" *Molecular Immunology* 16:287–296 (1979).

Palm & Hilschmann, "Primary structure of a crystalline monoclonal immunoglobulin K–type L–chain, subgroup I (Bence–Jones preotin Rei); isolation & characterization of the tryptic peptides: . . . " *Hoppes–Seyler's Z. Physiol. Chem.* 356:167–191 (Feb. 1975).

Palm & Hilschmann, "The primary structure of a crystalline, monoclonal immunoglobulin–L–chain of the x–type, subgroup I (Bence–Jones Protein Rei): a contribution to the elucidation of the three–dimensional structure of the immunoglobulins" *Hoppe–Seyler's Z. Physiol. Chem.* 354:1651–1654 (Dec. 1973).

Panka et al., "Variable region framework differences result in decreased or increased affinity of variant anti–digoxin antibodies" *Proc. Natl. Acad. Sci. USA* 85:3080–3084 (May 1988).

Presta et al., "Humanization of an Antibody Directed Against IgE" *J. Immunol.* 151(5):2623–2632 (Sep. 1, 1993).

Preval & Fougereau, "Specific Interaction between V_H and V_L Regions of Human Monoclonal Immunoglobulins" *J. Mol. Biol.* 102:657–678 (1976).

Queen et al., "Construction of Humanized Antibodies and Testing in Primates" *J. Cell. Biochem. Suppl. 15 (Part E)* (20th Ann. Mtg. Keystone Symp. Denver, CO Mar. 10–16, 1991) pp. 137 (1991).

Queen et al., "Humanised antibodies to the IL–2 receptor" *Protein Eng. Antibody Mol. Prophyl. Ther. Appl. Man,* Clark, M., Nottingham, UK:Academic Titles pp. 159–170 (1993).

Rhodes & Birch, "Large–Scale Production of Proteins from Mammalian Cells" *Bio/Technology* 6:518, 521, 523 (May 1988).

Riechmann, "Humanizing of Recombinant Antibodies" (Intl. Symp. on Clin. Appl. of Monoclonal Antibodies, Guildford, England) pp. 33–34 (Sep. 1987).

Riechmann & Winter, "Recombinant Antibodies" (U. of London Royal Postgraduate Medical School, Wolfson Institute, Abstract) (May 1987).

Riechmann et al. *Alignment of VL Sequences* (1988).

Roberts & Rees, "Generation of an antibody with enhanced affinity and specificity for its antigen by protein engineering" *Nature* 328:731–734 (Aug. 1987).

Rostapshov et al., "Effective method for obtaining long nucleotide chains on partially complementary templates" *FEBS Letters* 249(2):379–382 (Jun. 1989).

Schneider et al., "The Anti–Idiotypic Response by Cynomolgus Mondkeys to Humanized Anti–Tac Is Primarily Directed to Complementarity–Determining Regions H1, H2, and L3" *J. of Immunology* 150:3086–3090 (Apr. 1993).

Sedlacek et al., "Monoclonal Antibodies in Tumor Therapy", Karger pp. 119–126, 133–179 (1988).

Shields et al., "Inhibition of Allergic Reactions with Antibodies to IgE" *International Archives of Allergy and Immunology* 107(1–3):308–312 (May 1995).

Sims et al., "A Humanized CD18 Antibody Can Block Function Without Cell Destruction" *The Journal of Immunology* 151(4):2296–2308 (Aug. 1993).

Smith–Gill et al., "A Three–dimensional Model of an Anti–lysozyme Antibody" *Mol. Biol.* 194:713–724 (1987).

Songsivilai et al., "Bispecific antibody: a tool for diagnosis and treatment of disease" *Clin. Exp. Immunol.* 79:315–321 (1990).

Stanford, "A Predictive Method for Determining Possible Three–dimensional Foldings of Immunoglobulin Backbones Around Antibody Combining Sites" *Theor. Biol.* 88:421–439 (1981).

Stickney et al., "Bifunctional Antibody: ZCE/CHA ^{III}Indium BLEDTA–IV Clinical Imaging in Colorectal Carcinoma" *Antibody, Immuno Radiopharm* 2:1–13 (1989).

Tighe et al., "Delayed Allograft Rejection in Primates Treated with Anti–IL–2 Receptor Monoclonal antibody Campath–6" *Transplantation* 45(1):226–228 (Jan. 1988).

Verhoeyen & Riechmann, "Engineering of Antibodies" *BioEssays* 8(2):74–78 (Feb./Mar. 1988).

Verhoeyen et al., "Grafting Hypervariable Regions in Antibodies" *Protein Structure, Folding, and Design 2* (Proc. DuPont–UCLA Symp. Streamboat Springs, CO, Apr. 4–11, 1987), Dale L. Oxender, New York:Alan R. Liss, Inc. pp. 501–502 (1987).

Verhoeyen et al., "Humanising Mouse Antibodies: A Protein Engineering Approach" *Soc. for Analytical Cytology* (XIIth Intl. Mtg. for the Soc. for Analytical Cytology, Cambridge, UK) pp. 22 and slide presented at mtg.

Verhoeyen et al., "Re–shaped human anti–PLAP antibodies" *Monoclonal Antibodies Applications in clinical oncology,* Epenetos, 1st edition, Chapman & Hall Medical pp. 37–43 (1991).

Ward et al., "Expression and Secretion of Repertoires of VH Domains in *Escherichia coli:* Isolation of Antigen Binding Activites" *Progress in Immunology* (7th Intl. Congress Immunol. Berlin, W. Germany), F. Melchers vol. VII:1144–1151 (1989).

Ward, E.S. et al., "Binding activities of a repertoire of single immunoglobulin variable domains secreted from *Escherichia coli" Nature* 341:544–546 (1989).

Werther et al., "Humanization of an Anti–Lymphocyte Function–Associated Antigen (LFA)–1 Monoclonal Antibody and Reengineering of the Humanized Antibody for Binding to Rhesus LFA–1" *J. of Immunology* pp. 4986–4995 (1996).

Whittle et al., "Construction and Expression of A CDR–Grafted Anti–TNF Antibody" *J. Cell Biochem. Suppl. 0* (Symp. on Protein and Pharm. Eng. Mol. and Cell. Biol. Park City, Utah)13 Part A:96 (1989).

Winter & Neuberger, "Restructuring Enzymes and Antibodies" *Investigation and Exploitation of Antibody Combining Sites,* Eric Reid, Plenum Press pp. 139–140 (1985).

Winter et al., "Protein Engineering by Site Directed Mutagenesis" *Chemical Synthesis in Molecular Biology,* H. Blocker et al., VCH pp. 189–197 (1987).

Winter G., "Antibody Engineering" *Phil. Trans. R. Soc. Lond. B* 324:99–109 (1989).

Woodle et al., "Humanized OKT3 Antibodies: Successful Transfer of Immune Modulating Properties and Idiotype Expression" *J. of Immunology* 148(9):2756–2763 (May 1992).

* cited by examiner

FIG. 1A

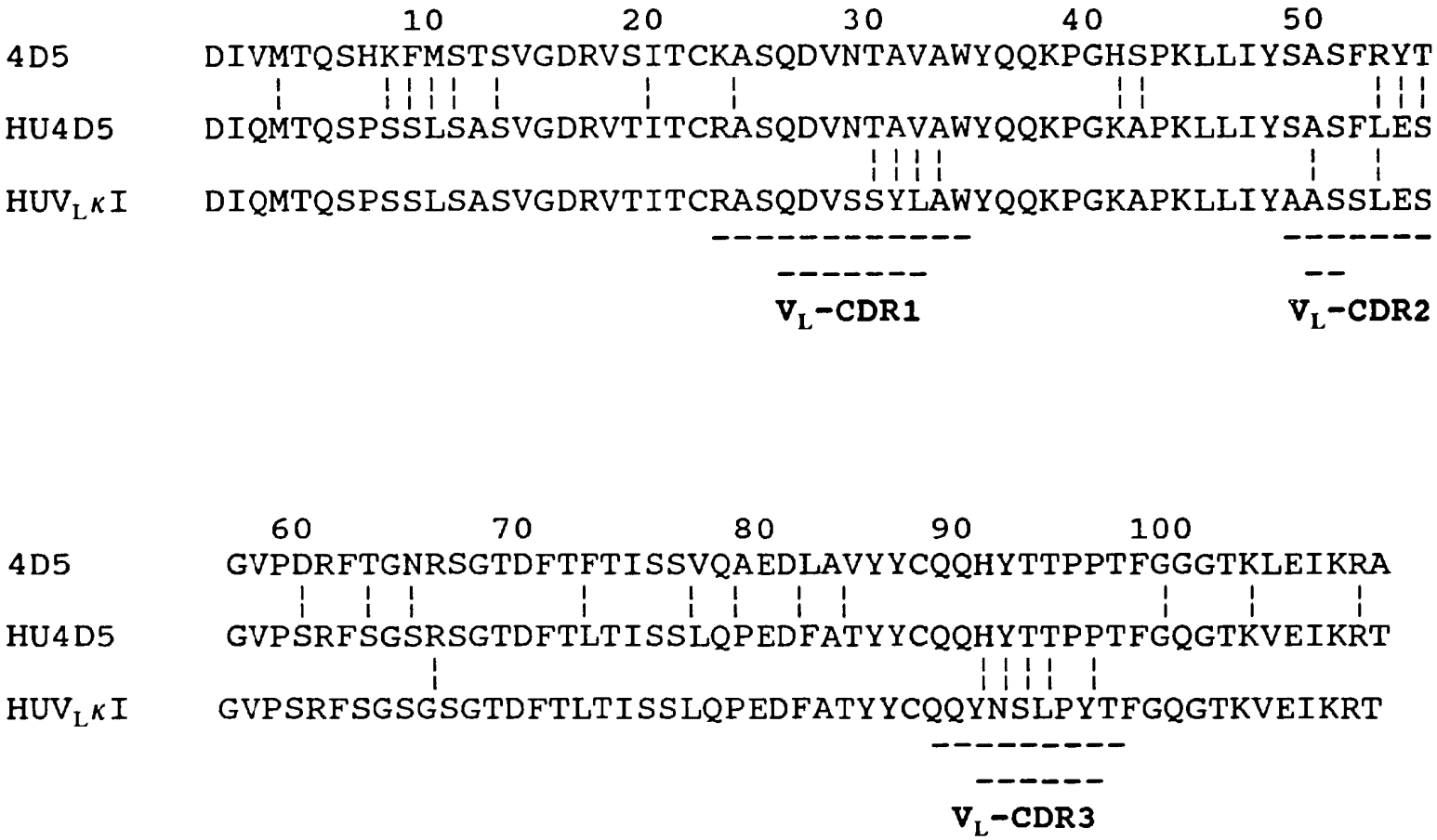
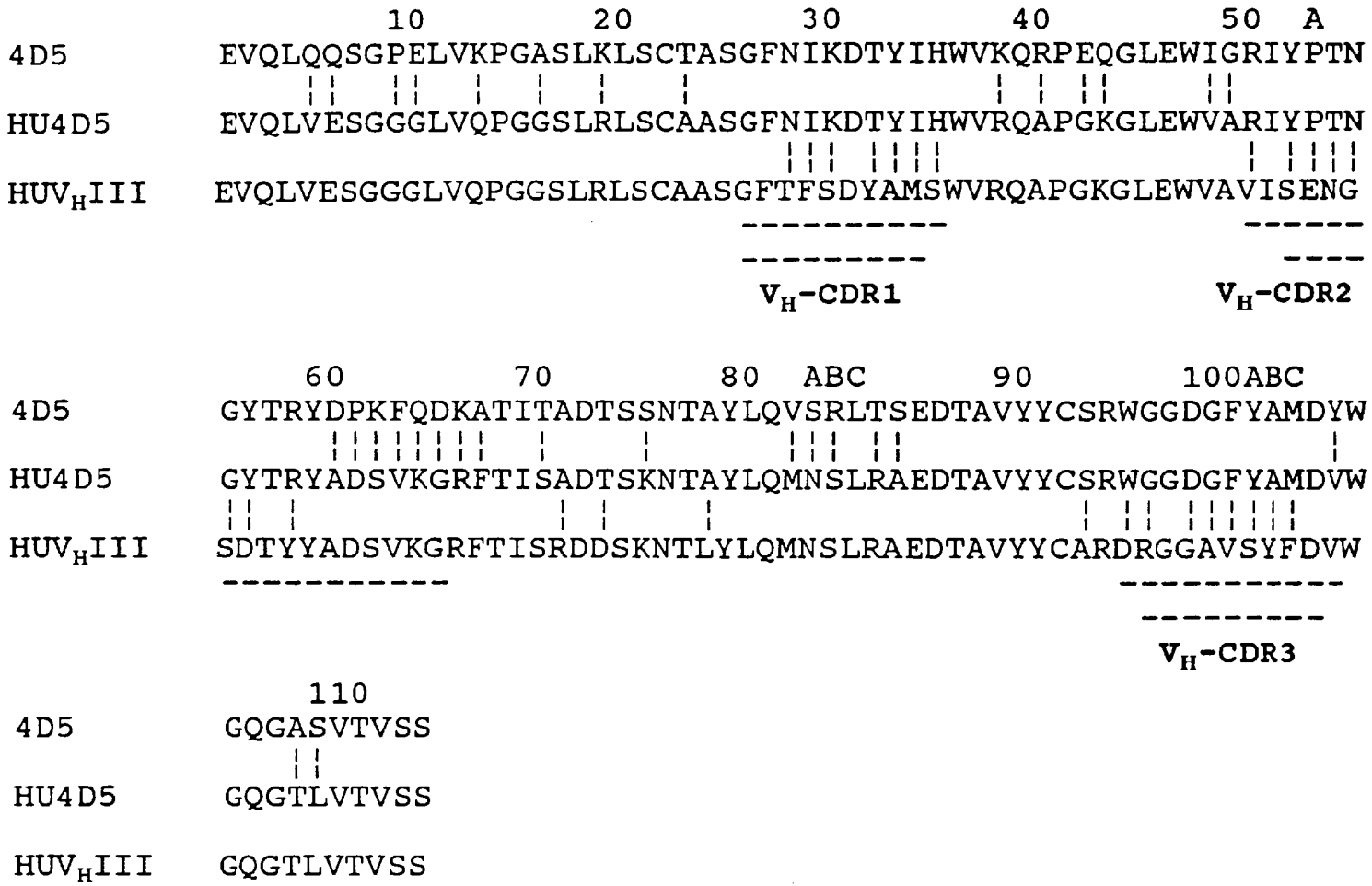


FIG. 1B



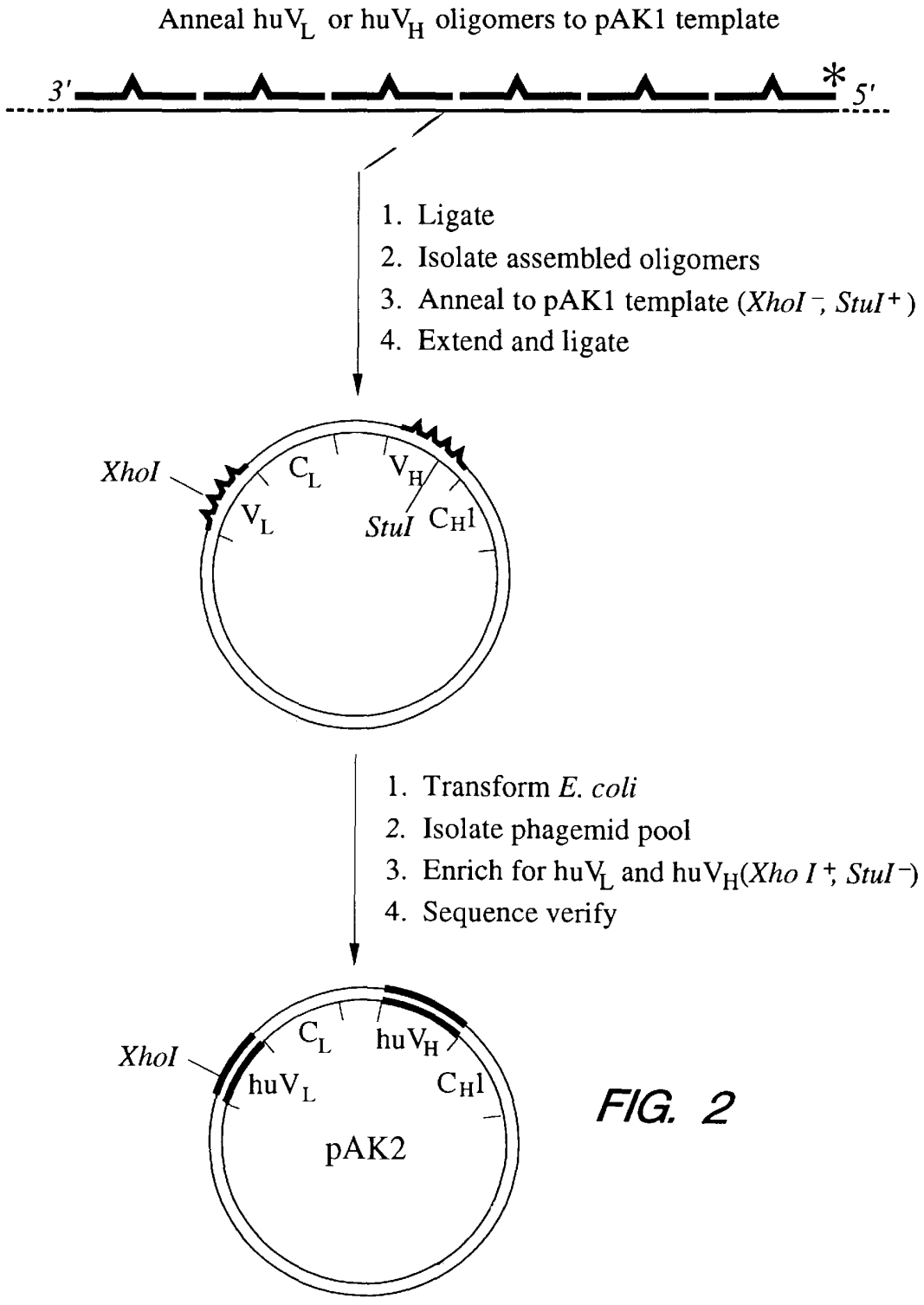

Anneal huV$_L$ or huV$_H$ oligomers to pAK1 template
3'
*
5'
1. Ligate
2. Isolate assembled oligomers
3. Anneal to pAK1 template (*XhoI*$^-$, *StuI*$^+$)
4. Extend and ligate
XhoI
C$_L$
V$_H$
V$_L$
StuI
C$_H$1
1. Transform *E. coli*
2. Isolate phagemid pool
3. Enrich for huV$_L$ and huV$_H$(*Xho I*$^+$, *StuI*$^-$)
4. Sequence verify
XhoI
C$_L$
huV$_H$
huV$_L$
C$_H$1
pAK2

FIG. 2

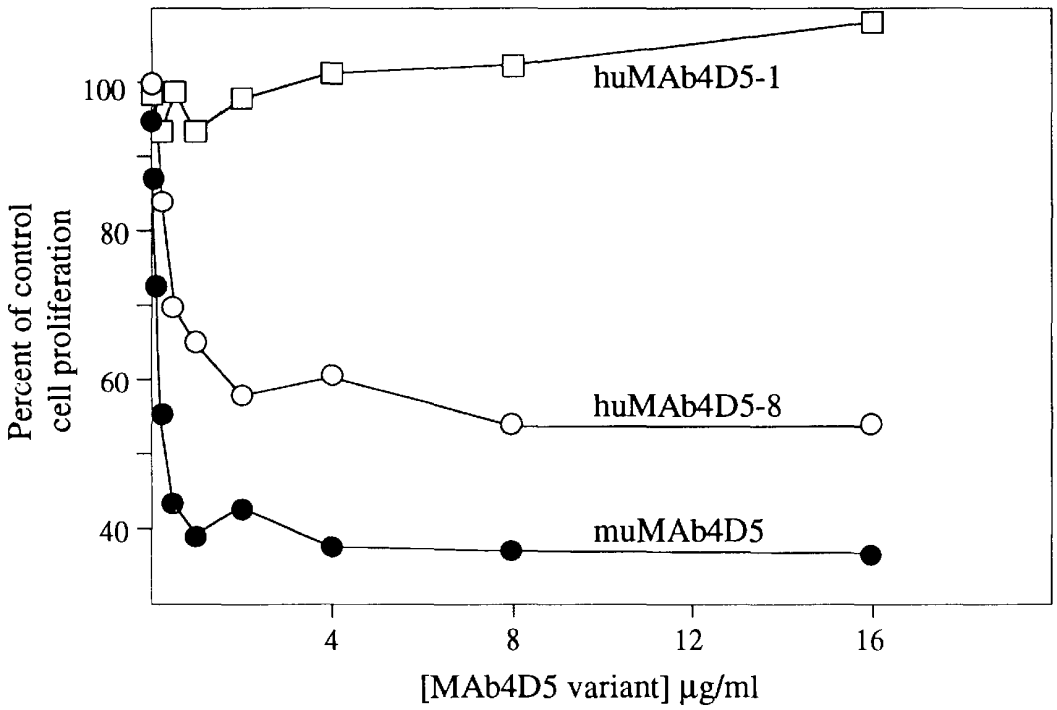

100
80
60
40
Percent of control cell proliferation
huMAb4D5-1
huMAb4D5-8
muMAb4D5
4
8
12
16
[MAb4D5 variant] µg/ml

FIG. 3

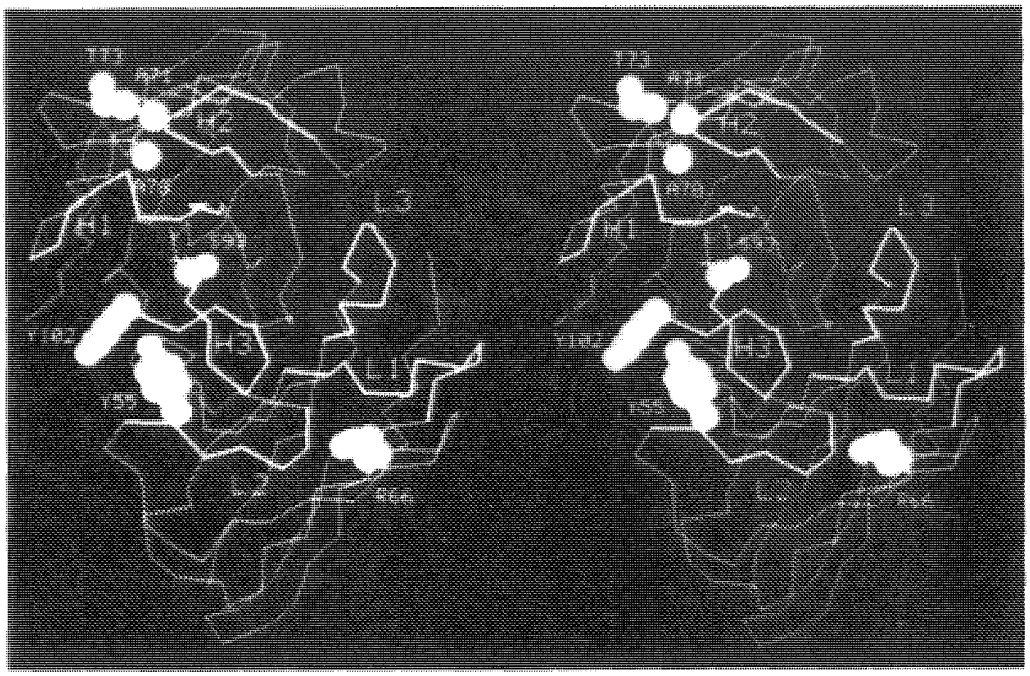


FIG. 4

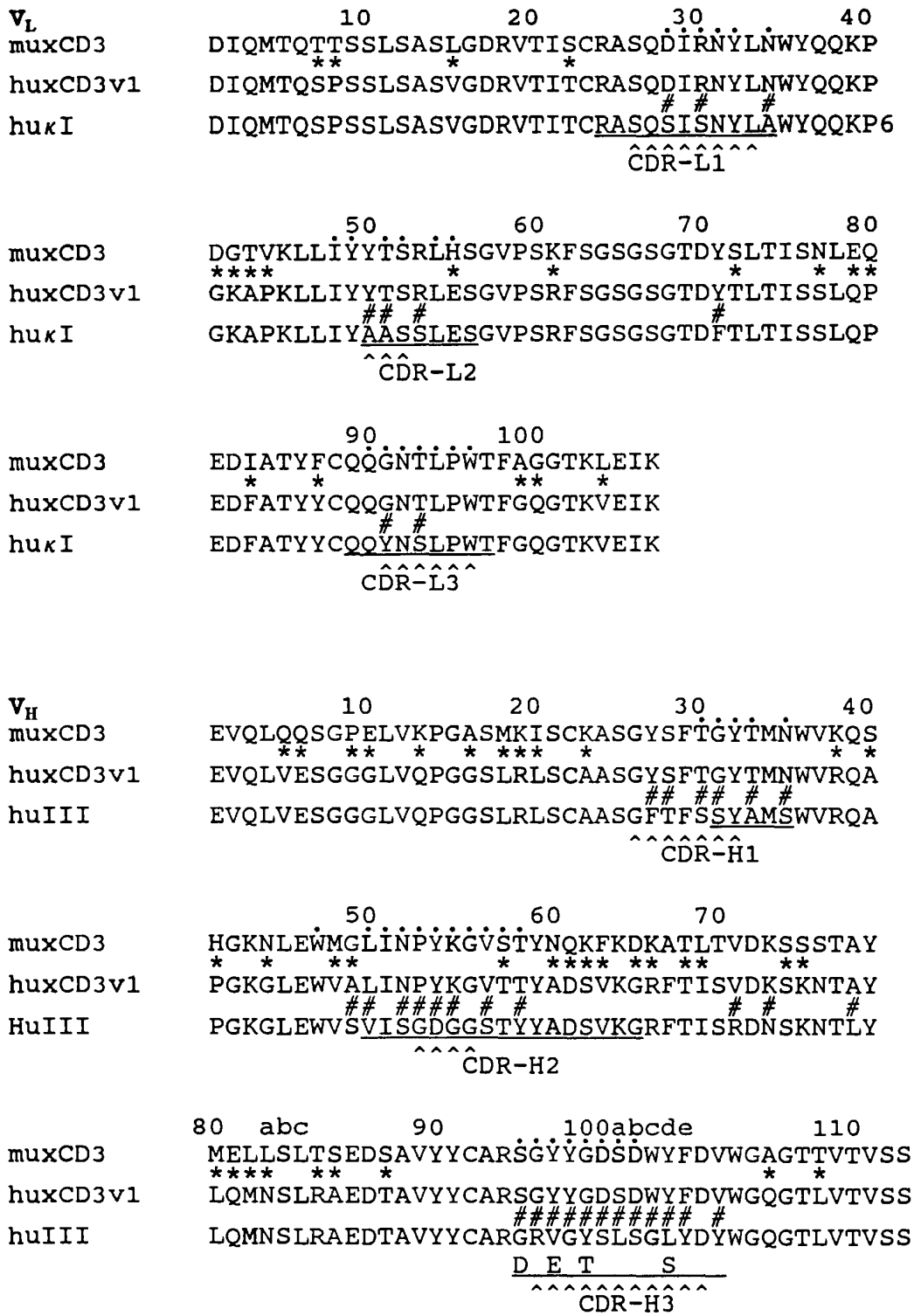

VL
10 20 30 40
muxCD3 DIQMTQTTSSLSASLGDRVTISCRASQDIRNYLNWYQQKP
huxCD3v1 DIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKP
huκI DIQMTQSPSSLSASVGDRVTITCRASQSISNYLAWYQQKP6
CDR-L1
50 60 70 80
muxCD3 DGTVKLLIYYTSRLHSGVPSKFSGSGSGTDYSLTISNLEQ
huxCD3v1 GKAPKLLIYYTSRLESGVPSRFSGSGSGTDYTLTISSLQP
huκI GKAPKLLIYAASSLESGVPSRFSGSGSGTDFTLTISSLQP
CDR-L2
90 100
muxCD3 EDIATYFCQQGNTLPWTFAGGTKLEIK
huxCD3v1 EDFATYYCQQGNTLPWTFGQGTKVEIK
huκI EDFATYYCQQYNSLPWTFGQGTKVEIK
CDR-L3
VH
10 20 30 40
muxCD3 EVQLQQSGPELVKPGASMKISCKASGYSFTGYTMNWVKQS
huxCD3v1 EVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQA
huIII EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQA
CDR-H1
50 60 70
muxCD3 HGKNLEWMGLINPYKGVSTYNQKFKDKATLTVDKSSSTAY
huxCD3v1 PGKGLEWVALINPYKGVTTYADSVKGRFTISVDKSKNTAY
HuIII PGKGLEWVSVISGDGGSTYYADSVKGRFTISRDNSKNTLY
CDR-H2
80 abc 90 100abcde 110
muxCD3 MELLSLTSEDSAVYYCARSGYYGDSDWYFDVWGAGTTVTVSS
huxCD3v1 LQMNSLRAEDTAVYYCARSGYYGDSDWYFDVWGQGTLVTVSS
huIII LQMNSLRAEDTAVYYCARGRVGYSLSGLYDYWGQGTLVTVSS
D E T S
CDR-H3

FIG. 5

FIG. 6A-1

```
                                                                10                  20                  30
H52H4-160                                                       QVQLQQSGPELVKPGASVKISCKTSGYTFTE
                                                                .*** .** **.**.*...** ********
pH52-8.0                MGWSCIILFLVATATGVHSEVQLVESGGGLVQPGGSLRLSCATSGYTFTE
                                10                  20                  30                  40                  50

                                                40                  50                  60                  70                  80
H52H4-160               YTMHWMKQSHGKSLEWIGGFNPKNGGSSHNQRFMDKATLAVDKSTSTAYM
                        *******.*. **.***..*.*******.********** *..**********
pH52-8.0                YTMHWMRQAPGKGLEWVAGINPKNGGTSHNQRFMDRFTISVDKSTSTAYM
                                60                  70                  80                  90                  100

                                                90                  100                 110                 120                 130
H52H4-160               ELRSLTSEDSGIYYCARWRGLNYGFDVRYFDVWGAGTTVTVSSASTKGPS
                        ..  ** .**...********************* ** **********
pH52-8.0                QMNSLRAEDTAVYYCARWRGLNYGFDVRYFDVWGQGTLVTVSSASTKGPS
                                110                 120                 130                 140                 150

                                                140                 150                 160                 170                 180
H52H4-160               VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
                        ****** *.*** .**********************
pH52-8.0                VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
                                160                 170                 180                 190                 200

                                                190                 200                 210                 220                 230
H52H4-160               QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH
                        ********************* **.*****.********** ** * *
pH52-8.0                QSSGLYSLSSVVTVTSSNFGTQTYTCNVDHKPSNTKVDKTVERKCC--V
                                210                 220                 230                 240

                                                240                 250                 260                 270                 280
H52H4-160               TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK
                         *******  .*********************
pH52-8.0                ECPPCPAPP-VAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQ
                        250                 260                 270                 280                 290
```

FIG. 6A-2

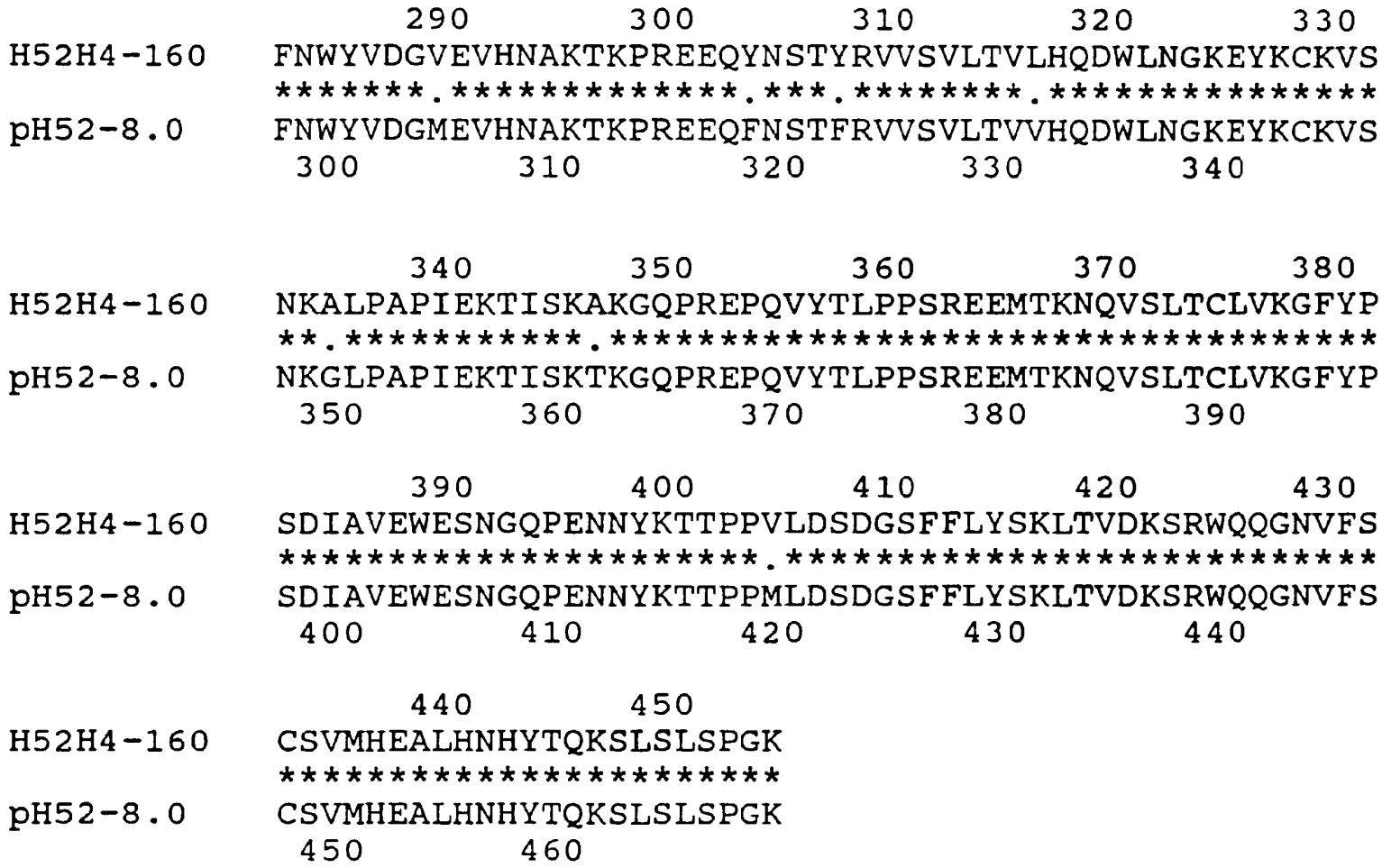


FIG. 6B

```
                                        10        20        30
H52L6-158                          DVQMTQTTSSLSASLGDRVTINCRASQDINN
                                    *.****. ******.****** *********
pH52-9.0    MGWSCIILFLVATATGVHSDIQMTQSPSSLSASVGDRVTITCRASQDINN
                    10        20        30        40        50

                   40        50        60        70        80
H52L6-158   YLNWYQQKPNGTVKLLIYYTSTLHSGVPSRFSGSGSGTDYSLTISNLDQE
            *********  . ****************************.****.*. *
pH52-9.0    YLNWYQQKPGKAPKLLIYYTSTLHSGVPSRFSGSGSGTDYTLTISSLQPE
                    60        70        80        90        100

                   90       100       110       120       130
H52L6-158   DIATYFCQQGNTLPPTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS
            *.***.************ ******************************
pH52-9.0    DFATYYCQQGNTLPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS
                    110       120       130       140       150

                  140       150       160       170       180
H52L6-158   VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
            **************************************************
pH52-9.0    VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
                    160       170       180       190       200

                  190       200       210
H52L6-158    SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
             ********************************
pH52-9.0     SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
                    210       220       230
```

METHOD FOR MAKING HUMANIZED ANTIBODIES

CROSS REFERENCES

This application is a continuation-in-part of U.S. application Ser. No. 07/715,272 filed Jun. 14, 1991 (abandoned) which application is incorporated herein by reference and to which application priority is claimed under 35 USC §120.

FIELD OF THE INVENTION

This invention relates to methods for the preparation and use of variant antibodies and finds application particularly in the fields of immunology and cancer diagnosis and therapy.

BACKGROUND OF THE INVENTION

Naturally occurring antibodies (immunoglobulins) comprise two heavy chains linked together by disulfide bonds and two light chains, one light chain being linked to each of the heavy chains by disulfide bonds. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain (V_L) at one end and a constant domain at its other end, the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light and heavy chain variable domains, see e.g. Chothia et al., *J. Mol. Biol.* 186:651–663 (1985); Novotny and Haber, *Proc. Natl. Acad. Sci. USA* 82:4592–4596 (1985).

The constant domains are not involved directly in binding the antibody to an antigen, but are involved in various effector functions, such as participation of the antibody in antibody-dependent cellular cytotoxicity. The variable domains of each pair of light and heavy chains are involved directly in binding the antibody to the antigen. The domains of natural light and heavy chains have the same general structure, and each domain comprises four framework (FR) regions, whose sequences are somewhat conserved, connected by three hyper-variable or complementarity determining regions (CDRs) (see Kabat, E. A. et a., *Sequences of Proteins of Immunological Interest,* National Institutes of Health, Bethesda, Md., (1987)). The four framework regions largely adopt a β-sheet conformation and the CORs form loops connecting, and in some cases forming part of, the β-sheet structure. The CDRs in each chain are held in close proximity by the framework regions and, with the CDRs from the other chain, contribute to the formation of the antigen binding site.

Widespread use has been made of monoclonal antibodies, particularly those derived from rodents including mice, however they are frequently antigenic in human clinical use. For example, a major limitation in the clinical use of rodent monoclonal antibodies is an anti-globulin response during therapy (Miller, R. A. et al., *Blood* 62:988–995 (1983); Schroff, R. W. et al., *Cancer Res.* 45:879–885 (1985)).

The art has attempted to overcome this problem by constructing "chimeric" antibodies in which an animal antigen-binding variable domain is coupled to a human constant domain (Cabilly et al., U.S. Pat. No. 4,816,567; Morrison, S. L. et al., *Proc. Natl. Acad. Sci. USA* 81:6851–6855 (1984); Boulianne, G. L. et al., *Nature* 312:643–646 (1984); Neuberger, M. S. et al., *Nature* 314:268–270 (1985)). The term "chimeric" antibody is used herein to describe a polypeptide comprising at least the antigen binding portion of an antibody molecule linked to at least part of another protein (typically an immunoglobulin constant domain).

The isotype of the human constant domain may be selected to tailor the chimeric antibody for participation in antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (see e.g. Brüggemann, M. et al., *J. Exp. Med.* 166:1351–1361 (1987); Riechmann, L. et al., *Nature* 332:323–327 (1988); Love et al., *Methods in Enzymology* 178:515–527 (1989); Bindon et al., *J. Exp. Med.* 168:127–142 (1988).

In the typical embodiment, such chimeric antibodies contain about one third rodent (or other non-human species) sequence and thus are capable of eliciting a significant anti-globulin response in humans. For example, in the case of the murine anti-CD3 antibody, OKT3, much of the resulting anti-globulin response is directed against the variable region rather than the constant region (Jaffers, G. J. et a., *Transplantation* 41:572–578 (1986)).

In a further effort to resolve the antigen binding functions of antibodies and to minimize the use of heterologous sequences in human antibodies, Winter and colleagues (Jones, P. T. et al., *Nature* 321:522–525 (1986); Riechmann, L. et al., *Nature* 332:323–327 (1988); Verhoeyen, M. et al., *Science* 239:1534–1536 (1988)) have substituted rodent CDRs or CDR sequences for the corresponding segments of a human antibody. As used herein, the term "humanized" antibody is an embodiment of chimeric antibodies wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

The therapeutic promise of this approach is supported by the clinical efficacy of a humanized antibody specific for the CAMPATH-1 antigen with two non-Hodgkin lymphoma patients, one of whom had previously developed an anti-globulin response to the parental rat antibody (Riechmann, L. et al., *Nature* 332:323–327 (1988); Hale, G. et al., *Lancet i:*1394–1399 (1988)). A murine antibody to the interleukin 2 receptor has also recently been humanized (Queen, C. et al., *Proc. Natl. Acad. Sci. USA* 86:10029–10033 (1989)) as a potential immunosuppressive reagent. Additional references related to humanization of antibodies include Co et al., *Proc. Natl. Acad. Sci. USA* 88:2869–2873 (1991); Gorman et al., *Proc. Natl. Acad. Sci. USA* 88:4181–4185 (1991); Daugherty et al., *Nucleic Acids Research* 19(9):2471–2476 (1991); Brown et al., *Proc. Natl. Acad. Sci. USA* 88:2663–2667 (1991); Junghans et al., *Cancer Research* 50:1495–1502 (1990).

In some cases, substituting CDRs from rodent antibodies for the human CDRs in human frameworks is sufficient to transfer high antigen binding affinity (Jones, P. T. et al., *Nature* 321:522–525 (1986); Verhoeyen, M. et al., *Science* 239:1534–1536 (1988)), whereas in other cases it has been necessary to additionally replace one (Riechmann, L. et al., *Nature* 332:323–327 (1988)) or several (Queen, C. et al., *Proc. Natl. Acad. Sci. USA* 86:10029–10033 (1989)) framework region (FR) residues. See also Co et al., supra.

For a given antibody a small number of FR residues are anticipated to be important for antigen binding. Firstly for example, certain antibodies have been shown to contain a few FR residues which directly contact antigen in crystal structures of antibody-antigen complexes (e.g., reviewed in Davies, D. R. et al., *Ann. Rev. Biochem.* 59:439–473 (1990)).

Secondly, a number of FR residues have been proposed by Chothia, Lesk and colleagues (Chothia, C. & Lesk, A. M., *J. Mol. Biol.* 196:901–917 (1987); Chothia, C. et al., *Nature* 342:877–883 (1989); Tramontano, A. et al., *J. Mol. Biol.* 215:175–182 (1990)) as critically affecting the conformation of particular CDRs and thus their contribution to antigen binding. See also Margolies et al., *Proc. Natl. Acad. Sci. USA* 72:2180–2184 (1975).

It is also known that, in a few instances, an antibody variable domain (either V_H or V_L) may contain glycosylation sites, and that this glycosylation may improve or abolish antigen binding, Pluckthun, *Biotechnology* 9:545–51 (1991); Spiegelberg et al., *Biochemistry* 9:4217–4223 (1970); Wallic et al., *J. Exp. Med.* 168:1099–1109 (1988); Sox et al., *Proc. Natl. Acad. Sci. USA* 66:975–982 (1970); Margni et al., *Ann. Rev. Immunol* 6:535–554 (1988). Ordinarily, however, glycosylation has no influence on the antigen-binding properties of an antibody, Pluckthun, supra, (1991).

The three-dimensional structure of immunoglobulin chains has been studied, and crystal structures for intact immunoglobulins, for a variety of immunoglobulin fragments, and for antibody-antigen complexes have been published (see e.g., Saul et al., *Journal of Biological Chemistry* 25:585–97 (1978); Sheriff et al., *Proc. Natl. Acad. Sci. USA* 84:8075–79 (1987); Segal et al., *Proc. Natl. Acad. Sci. USA* 71:4298–4302 (1974); Epp et al., *Biochemistry* 14(22) :4943–4952 (1975); Marquart et al., *J. Mol. Biol.* 141:369–391 (1980); Furey et al., *J. Mol. Biol.* 167:661–692 (1983); Snow and Amzel, *Protein: Structure, Function, and Genetics* 1:267–279, Alan R. Liss, Inc. pubs. (1986); Chothia and Lesk, *J. Mol. Bol.* 196:901–917 (1987); Chothia et al., *Nature* 342:877–883 (1989); Chothia et al., *Science* 233:755–58 (1986); Huber et al., *Nature* 264:415–420 (1976); Bruccoleri et al., *Nature* 335:564–568 (1988) and *Nature* 336:266 (1988); Sherman et al., *Journal of Biological Chemistry* 263:4064–4074 (1988); Amzel and Poljak, *Ann. Rev. Biochem.* 48:961–67 (1979); Silverton et al., *Proc. Natl. Acad. Sci. USA* 74:5140–5144 (1977); and Gregory et al., *Molecular Immunology* 24:821–829 (1987). It is known that the function of an antibody is dependent on its three dimensional structure, and that amino acid substitutions can change the three-dimensional structure of an antibody, Snow and Amzel, supra. It has previously been shown that the antigen binding affinity of a humanized antibody can be increased by mutagenesis based upon molecular modelling (Riechmann, L. et al., *Nature* 332:323–327 (1988); Queen, C. et al., *Proc. Natl. Acad. Sci. USA* 86:10029–10033 (1989)).

Humanizing an antibody with retention of high affinity for antigen and other desired biological activities is at present difficult to achieve using currently available procedures. Methods are needed for rationalizing the selection of sites for substitution in preparing such antibodies and thereby increasing the efficiency of antibody humanization.

The proto-oncogene HER2 (human epidermal growth factor receptor 2) encodes a protein tyrosine kinase ($p185^{HER2}$) that is related to and somewhat homologous to the human epidermal growth factor receptor (see Coussens, L. et al., *Science* 230:1132–1139 (1985); Yamamoto, T. et al., *Nature* 319:230–234 (1986); King, C. R. et al., *Science* 229:974–976 (1985)). HER2 is also known in the field as c-erbB-2, and sometimes by the name of the rat homolog, neu. Amplification and/or overexpression of HER2 is associated with multiple human malignancies and appears to be integrally involved in progression of 25–30% of human breast and ovarian cancers (Slamon, D. J. et al., *Science* 235:177–182 (1987), Slamon, D. J. et al., *Science* 244:707–712 (1989)). Furthermore, the extent of amplification is inversely correlated with the observed median patient survival time (Slamon, supra, Science 1989).

The murine monoclonal antibody known as muMAb4D5 (Fendly, B. M. et al., *Cancer Res.* 50:1550–1558 (1990)), directed against the extracellular domain (ECD) of $p185^{HER2}$, specifically inhibits the growth of tumor cell lines overexpressing $p185^{HER2}$ in monolayer culture or in soft agar (Hudziak, R. M. et al., *Molec. Cell. Biol* 9:1165–1172 (1989); Lupu, R. et al., *Science* 249:1552–1555 (1990)). MuMAb4D5 also has the potential of enhancing tumor cell sensitivity to tumor necrosis factor, an important effector molecule in macrophage-mediated tumor cell cytotoxicity (Hudziak, supra, 1989; Shepard, H. M. and Lewis, G. D. *J. Clinical Immunology* 8:333–395 (1988)). Thus muMAb4D5 has potential for clinical intervention in and imaging of carcinomas in which $p185^{HER2}$ is overexpressed. The muMAb4D5 and its uses are described in PCT application WO 89/06692 published Jul. 27, 1989. This murine antibody was deposited with the ATCC and designated ATCC CRL 10463. However, this antibody may be immunogenic in humans.

It is therefore an object of this invention to provide methods for the preparation of antibodies which are less antigenic in humans than non-human antibodies but have desired antigen binding and other characteristics and activities.

It is a further object of this invention to provide methods for the efficient humanization is of antibodies, i.e. selecting non-human amino acid residues for importation into a human antibody background sequence in such a fashion as to retain or improve the affinity of the non-human donor antibody for a given antigen.

It is another object of this invention to provide humanized antibodies capable of binding $p185^{HER2}$.

Other objects, features, and characteristics of the present invention will become apparent upon consideration of the following description and the appended claims.

SUMMARY OF THE INVENTION

The objects of this invention are accomplished by a method for making a humanized antibody comprising amino acid sequence of an import, non-human antibody and a human antibody, comprising the steps of:

a. obtaining the amino acid sequences of at least a portion of an import antibody variable domain and of a consensus variable domain;

b. identifying Complementarity Determining Region (CDR) amino acid sequences in the import and the human variable domain sequences;

c. substituting an import CDR amino acid sequence for the corresponding human CDR amino acid sequence;

d. aligning the amino acid sequences of a Framework Region (FR) of the import antibody and the corresponding FR of the consensus antibody;

e. identifying import antibody FR residues in the aligned FR sequences that are non-homologous to the corresponding consensus antibody residues;

f. determining if the non-homologous import amino acid residue is reasonably expected to have at least one of the following effects:

1. non-covalently binds antigen directly,

2. interacts with a CDR; or

3. participates in the V_L–V_H interface; and

g. for any non-homologous import antibody amino acid residue which is reasonably expected to have at least one of these effects, substituting that residue for the corresponding amino acid residue in the consensus antibody FR sequence.

Optionally, the method of this invention comprises the additional steps of determining if any non-homologous residues identified in step (e) are exposed on the surface of the domain or buried within it, and if the residue is exposed but has none of the effects identified in step (f), retaining the consensus residue.

Additionally, in certain embodiments the method of this invention comprises the feature wherein the corresponding consensus antibody residues identified in step (e) above are selected from the group consisting of 4L, 35L, 36L, 38L, 43L, 44L, 46L, 58L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71 L, 73L, 85L, 87L, 98L, 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 67H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H (utilizing the numbering system set forth in Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)).

In certain embodiments, the method of this invention comprises the additional steps of searching either or both of the import, non-human and the consensus variable domain sequences for glycosylation sites, determining if the glycosylation is reasonably expected to be important for the desired antigen binding and biological activity of the antibody (i.e., determining if the glycosylation site binds to antigen or changes a side chain of an amino acid residue that binds to antigen, or if the glycosylation enhances or weakens antigen binding, or is important for maintaining antibody affinity). If the import sequence bears the glycosylation site, it is preferred to substitute that site for the corresponding residues in the consensus human if the glycosylation site is reasonably expected to be important. If only the consensus sequence, and not the import, bears the glycosylation site, it is preferred to eliminate that glycosylation site or substitute therefor the corresponding amino acid residues from the import sequence.

Another embodiment of this invention comprises aligning import antibody and the consensus antibody FR sequences, identifying import antibody FR residues which are non-homologous with the aligned consensus FR sequence, and for each such non-homologous import antibody FR residue, determining if the corresponding consensus antibody residue represents a residue which is highly conserved across all species at that site, and if it is so conserved, preparing a humanized antibody which comprises the consensus antibody amino acid residue at that site.

Certain alternate embodiments of the methods of this invention comprise obtaining the amino acid sequence of at least a portion of an import, non-human antibody variable domain having a CDR and a FR, obtaining the amino acid sequence of at least a portion of a consensus antibody variable domain having a CDR and a FR, substituting the non-human CDR for the human CDR in the consensus antibody variable domain, and then substituting an amino acid residue for the consensus amino acid residue at at least one of the following sites:

a. (in the FR of the variable domain of the light chain) 4L, 35L, 36L, 38L, 43L, 44L, 58L, 46L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, or

b. (in the FR of the variable domain of the heavy chain) 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 67H, 68H, 69H, 70H, 73H, 74H, 75H, 78H, 91H, 92H, 93H, and 103H.

In preferred embodiments, the non-CDR residue substituted at the consensus FR site is the residue found at the corresponding location of the non-human antibody.

Optionally, this just-recited embodiment comprises the additional steps of following the method steps appearing at the beginning of this summary and determining whether a particular amino acid residue can reasonably be expected to have undesirable effects.

This invention also relates to a humanized antibody comprising the CDR sequence of an import, non-human antibody and the FR sequence of a human antibody, wherein an amino acid residue within the human FR sequence located at any one of the sites 4L, 35L, 36L, 38L, 43L, 44L, 46L, 58L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 67H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H has been substituted by another residue. In preferred embodiments, the residue substituted at the human FR site is the residue found at the corresponding location of the non-human antibody from which the non-human CDR was obtained. In other embodiments, no human FR residue other than those set forth in this group has been substituted.

This invention also encompasses specific humanized antibody variable domains, and isolated polypeptides having homology with the following sequences.

1. SEQ. ID NO. 1, which is the light chain variable domain of a humanized version of muMAb4D5: DIQMTOSPSSLSASVGDRVTITCRASQD-VNTAVAWYQQKPGKAPKLLIYSASFLES-GVPSRFSGSRSGTDFTLTISSLQPEDFA-TYYCQQHYTTPPTFGQGTKVEIKRT

2. SEQ. ID NO. 2, which is the heavy chain variable domain of a humanized version of muMAb4D5): EVQLVESGGGLVOPGGSLRLSCAASGFNIK DTYIHWVRQAPGKGLEWVARIYPTNGYTRY ADSVKGRFTISADTSKNTAYLQMNSLRAED TAVYYCSRWGGDGFYAMDVWGQGTLVTVSS

In another aspect, this invention provides a consensus antibody variable domain amino acid sequence for use in the preparation of humanized antibodies, methods for obtaining, using, and storing a computer representation of such a consensus sequence, and computers comprising the sequence data of such a sequence. In one embodiment, the following consensus antibody variable domain amino acid sequences are provided:

SEQ. ID NO. 3 (light chain): DDIOMTQSPSSLSAS-VGDRVTITCRASQDVSSYLAWYQQKPGKAPKLL IYAASSLESGVPSRFSGSGSGTDFTLTISSLQP EDFATYYCQQYNSLPYTFGQGTKVEIKRT, and

SEQ. ID NO. 4 (heavy chain): EVQLVESGGGLVQPG GSLRLSCAASGFTFSDYAMSWVRQAPGKGL EWVAVISENGGYTRYADSVKGRFTISADTSKNT AYLQMNSLRAEDTAWYCSRWGGDGFYAMD VWGQGTLVTVSS

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1**A shows the comparison of the V_L domain amino acid residues of muMAb4D5, huMAb4D5, and a consensus sequence (FIG. **1**A, SEQ.ID NO. 5, SEQ. ID NO. 1 and SEQ. ID NO. 3, respectively). FIG. **1**B shows the comparison between the V_H domain amino acid residues of the muMAb4D5, huMAb4D5, and a consensus sequence (FIG. **1**B, SEQ. ID NO. 6, SEQ. ID NO. 2 and SEQ. ID NO. 4, respectively). Both FIGS. **1**A and **1**B use the generally accepted numbering scheme from Kabat, E. A., et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md. (1987)). In both FIG. **1**A

and FIG. 1B, the CDR residues determined according to a standard sequence definition (as in Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)) are indicated by the first underlining beneath the sequences, and the CDR residues determined according to a structural definition (as in Chothia, C. & Lesk, A. M., *J. Mol. Biol.* 196:901–917 (1987)) are indicated by the second, lower underlines. The mismatches between genes are shown by the vertical lines.

FIG. **2** shows a scheme for humanization of muMAb4D5 V_L and V_H by gene conversion mutagenesis.

FIG. **3** shows the inhibition of SK-BR-3 proliferation by MAb4D5 variants. Relative cell proliferation was determined as described (Hudziak, R. M. et al., *Molec. Cell. Biol.* 9:1165–1172 (1989)) and data (average of triplicate determinations) are presented as a percentage of results with untreated cultures for muMAb4D5 (●), huMAb4D5-8 (○) and huMAb4D5-1 (□).

FIG. **4** shows a stereo view of α-carbon tracing for a model of huMAb4D5-8 V_L and V_H. The CDR residues (Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)) are shown in bold and side chains of V_H residues A71, T73, A78, S93, Y102 and V_L residues Y55 plus R66 (see Table 3) are shown.

FIG. **5** shows an amino acid sequence comparison of V_L (top panel) and V_H (lower panel) domains of the murine anti-CD3 monoclonal Ab UCHT1 (muxCD3, Shalaby et al., *J. Exp. Med.* 175, 217–225 (1992) with a humanized variant of this antibody (huxCD3v1). Also shown are consensus sequences (most commonly occurring residue or pair of residues) of the most abundant human subgroups, namely V_L κ 1 and V_H III upon which the humanized sequences are based (Kabat, E. A. et al., *Sequences of Proteins of immunological Interest, 5th* edition, National Institutes of Health, Bethesda, Md., USA (1991)). The light chain sequences—muxCD3, huxCD3v1 and huKI—correspond to SEQ.ID. NOs 16, 17, and 18, respectively. The heavy chain sequences—muxCD3, huxCD3v1 and huxI—correspond to SEQ.ID.NOs 19, 26, and 21, respectively. Residues which differ between muxCD3 and huxCD3v1 are identified by an asterisk (*), whereas those which differ between humanized and consensus sequences are identified by a sharp sign (#). A bullet (•) denotes that a residue at this position has been found to contact antigen in one or more crystallographic structures of antibody/antigen complexes (Kabat et al., 1991; Mian, I. S. et al., *J. Mol. Biol* 217, 133–151 (1991)). The location of CDR residues according to a sequence definition (Kabat et al., 1991) and a structural definition (Chothia and Lesk, supra 1987) are shown by a line and carats (^) beneath the sequences, respectively.

FIG. **6**A compares murine and humanized amino acid sequences for the heavy chain of an anti-CD18 antibody. H52H4-160 (SEQ. ID. NO. 22) is the murine sequence, and pH52–8.0 (SEQ. ID. NO. 23) is the humanized heavy chain sequence. pH52–8.0 residue 143S is the final amino acid in the variable heavy chain domain V_H, and residue 144A is the first amino acid in the constant heavy chain domain C_{H1}.

FIG. **6**B compares murine and humanized amino acid sequences for the light chain of an anti-CD18 antibody. H52L6-158 (SEQ. ID. NO. 24) is the murine sequence, and pH52–9.0 (SEQ. ID. NO. 25) is the humanized light chain sequence. pH52–9.0 residue 128T is the final amino acid in the light chain variable domain V_L, and residue 129V is the first amino acid in the light chain constant domain C_L.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

In general, the following words or phrases have the indicated definitions when used in the description, examples, and claims:

The murine monoclonal antibody known as muMAb4D5 (Fendly, B. M. et al., *Cancer Res.* 50:1550–1558 (1990)) is directed against the extracellular domain (ECD) of $p185^{HER2}$. The muMAb4D5 and its uses are described in PCT application WO 89/06692 published Jul. 27, 1989. This murine antibody was deposited with the ATCC and designated ATCC CRL 10463. In this description and claims, the terms muMAb4D5, chMAb4D5 and huMAb4D5 represent murine, chimerized and humanized versions of the monoclonal antibody 4D5, respectively.

A humanized antibody for the purposes herein is an immunoglobulin amino acid sequence variant or fragment thereof which is capable of binding to a predetermined antigen and which comprises a FR region having substantially the amino acid sequence of a human immunoglobulin and a CDR having substantially the amino acid sequence of a non-human immunoglobulin.

Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are referred to herein as "import" residues, which are typically taken from an "import" antibody domain, particularly a variable domain. An import residue, sequence, or antibody has a desired affinity and/or specificity, or other desirable antibody biological activity as discussed herein.

In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains (Fab, Fab', $F(ab')_2$, Fabc, Fv) in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. Ordinarily, the antibody will contain both the light chain as well as at least the variable domain of a heavy chain. The antibody also may include the CH1, hinge, CH2, CH3, and CH4 regions of the heavy chain.

The humanized antibody will be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including IgG1, IgG2, IgG3 and lgG4. Usually the constant domain is a complement fixing constant domain where it is desired that the humanized antibody exhibit cytotoxic activity, and the class is typically IgG_1. Where such cytotoxic activity is not desirable, the constant domain may be of the IgG_2 class. The humanized antibody may comprise sequences from more than one class or isotype, and selecting particular constant domains to optimize desired effector functions is within the ordinary skill in the art.

The FR and CDR regions of the humanized antibody need not correspond precisely to the parental sequences, e.g., the import CDR or the consensus FR may be mutagenized by substitution, insertion or deletion of at least one residue so that the CDR or FR residue at that site does not correspond to either the consensus or the import antibody. Such mutations, however, will not be extensive. Usually, at least 75% of the humanized antibody residues will correspond to those of the parental FR and CDR sequences, more often 90%, and most preferably greater than 95%.

In general, humanized antibodies prepared by the method of this invention are produced by a process of analysis of the parental sequences and various conceptual humanized products using three dimensional models of the parental and humanized sequences. Three dimensional immunoglobulin models are commonly available and are familiar to those

skilled in the art. Computer programs are available which illustrate and display probable three dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen.

Residues that influence antigen binding are defined to be residues that are substantially responsible for the antigen affinity or antigen specificity of a candidate immunoglobulin, in a positive or a negative sense. The invention is directed to the selection and combination of FR residues from the consensus and import sequence so that the desired immunoglobulin characteristic is achieved. Such desired characteristics include increases in affinity and greater specificity for the target antigen, although it is conceivable that in some circumstances the opposite effects might be desired. In general, the CDR residues are directly and most substantially involved in influencing antigen binding (although not all CDR residues are so involved and therefore need not be substituted into the consensus sequence). However, FR residues also have a significant effect and can exert their influence in at least three ways: They may noncovalently directly bind to antigen, they may interact with CDR residues and they may affect the interface between the heavy and light chains.

A residue that noncovalently directly binds to antigen is one that, by three dimensional analysis, is reasonably expected to noncovalently directly bind to antigen. Typically, it is necessary to impute the position of antigen from the spatial location of neighboring CDRs and the dimensions and structure of the target antigen. In general, only those humanized antibody residues that are capable of forming salt bridges, hydrogen bonds, or hydrophobic interactions are likely to be involved in non-covalent antigen binding, however residues which have atoms which are separated from antigen spatially by 3.2 Angstroms or less may also non-covalently interact with antigen. Such residues typically are the relatively larger amino acids having the side chains with the greatest bulk, such as tyrosine, arginine, and lysine. Antigen-binding FR residues also typically will have side chains that are oriented into an envelope surrounding the solvent oriented face of a CDR which extends about 7 Angstroms into the solvent from the CDR domain and about 7 Angstroms on either side of the CDR domain, again as visualized by three dimensional modeling.

A residue that interacts with a CDR generally is a residue that either affects the conformation of the CDR polypeptide backbone or forms a noncovalent bond with a CDR residue side chain. Conformation-affecting residues ordinarily are those that change the spatial position of any CDR backbone atom (N, Ca, C, O, Cβ) by more than about 0.2 Angstroms. Backbone atoms of CDR sequences are displaced for example by residues that interrupt or modify organized structures such as beta sheets, helices or loops. Residues that can exert a profound affect on the conformation of neighboring sequences include proline and glycine, both of which are capable of introducing bends into the backbone. Other residues that can displace backbone atoms are those that are capable of participating in salt bridges and hydrogen bonds.

A residue that interacts with a CDR side chain is one that is reasonably expected to form a noncovalent bond with a CDR side chain, generally either a salt bridge or hydrogen bond. Such residues are identified by three dimensional positioning of their side chains. A salt or ion bridge could be expected to form between two side chains positioned within about 2.5–3.2 Angstroms of one another that bear opposite charges, for example a lysinyl and a glutamyl pairing. A hydrogen bond could be expected to form between the side chains of residue pairs such as seryl or threonyl with aspartyl or glutamyl (or other hydrogen accepting residues). Such pairings are well known in the protein chemistry art and will be apparent to the artisan upon three dimensional modeling of the candidate immunoglobulin.

Immunoglobulin residues that affect the interface between heavy and light chain variable regions ("the V_L–V_H interface") are those that affect the proximity or orientation of the two chains with respect to one another. Certain residues involved in interchain interactions are already known and include V_L residues 34, 36, 38, 44, 46, 87, 89, 91, 96, and 98 and V_H residues 35, 37, 39, 45, 47, 91, 93, 95, 100, and 103 (utilizing the nomenclature setforth in Kabat et al., *Sequences of Proteins of immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)). Additional residues are newly identified by the inventors herein, and include 43L, 85L, 43H and 60H. While these residues are indicated for IgG only, they are applicable across species. In the practice of this invention, import antibody residues that are reasonably expected to be involved in interchain interactions are selected for substitution into the consensus sequence. It is believed that heretofore no humanized antibody has been prepared with an intrachain-affecting residue selected from an import antibody sequence.

Since it is not entirely possible to predict in advance what the exact impact of a given substitution will be it may be necessary to make the substitution and assay the candidate antibody for the desired characteristic. These steps, however, are per se routine and well within the ordinary skill of the art.

CDR and FR residues are determined according to a standard sequence definition (Kabat et al., *Sequences of Proteins of Immunological Interest,* National Institutes of Health, Bethesda Md. (1987), and a structural definition (as in Chothia and Lesk, *J. Mol. Biol.* 196:901–917 (1987). Where these two methods result in slightly different identifications of a CDR, the structural definition is preferred, but the residues identified by the sequence definition method are considered important FR residues for determination of which framework residues to import into a consensus sequence.

Throughout this description, reference is made to the numbering scheme from Kabat, E. A., et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md. (1987) and (1991). In these compendiums, Kabat lists many amino acid sequences for antibodies for each subclass, and lists the most commonly occurring amino acid for each residue position in that subclass. Kabat uses a method for assigning a residue number to each amino acid in a listed sequence, and this method for assigning residue numbers has become standard in the field. The Kabat numbering scheme is followed in this description.

For purposes of this invention, to assign residue numbers to a candidate antibody amino acid sequence which is not included in the Kabat compendium, one follows the following steps. Generally, the candidate sequence is aligned with any immunoglobulin sequence or any consensus sequence in Kabat. Alignment may be done by hand, or by computer using commonly accepted computer programs; an example of such a program is the Align 2 program discussed in this description. Alignment may be facilitated by using some amino acid residues which are common to most Fab

sequences. For example, the light and heavy chains each typically have two cysteines which have the same residue numbers; in V_L domain the two cysteines are typically at residue numbers 23 and 88, and in the V_H domain the two cysteine residues are typically numbered 22 and 92. Framework residues generally, but not always, have approximately the same number of residues, however the CDRs will vary in size. For example, in the case of a CDR from a candidate sequence which is longer than the CDR in the sequence in Kabat to which it is aligned, typically suffixes are added to the residue number to indicate the insertion of additional residues (see, e.g. residues 100abcde in FIG. **5**). For candidate sequences which, for example, align with a Kabat sequence for residues 34 and 36 but have no residue between them to align with residue 35, the number 35 is simply not assigned to a residue.

Thus, in humanization of an import variable sequence, where one cuts out an entire human or consensus CDR and replaces it with an import CDR sequence, (a) the exact number of residues may be swapped, leaving the numbering the same, (b) fewer import amino acid residues may be introduced than are cut, in which case there will be a gap in the residue numbers, or (c) a larger number of amino acid residues may be introduced then were cut, in which case the numbering will involve the use of suffixes such as 100abcde.

The terms "consensus sequence" and "consensus antibody" as used herein refers to an amino acid sequence which comprises the most frequently occurring amino acid residues at each location in all immunoglobulins of any particular subclass or subunit structure. The consensus sequence may be based on immunoglobulins of a particular species or of many species. A "consensus" sequence, structure, or antibody is understood to encompass a consensus human sequence as described in certain embodiments of this invention, and to refer to an amino acid sequence which comprises the most frequently occurring amino acid residues at each location in all human immunoglobulins of any particular subclass or subunit structure. This invention provides consensus human structures and consensus structures which consider other species in addition to human.

The subunit structures of the live immunoglobulin classes in humans are as follows:

Class	Heavy Chain Subclasses		Light Chain	Molecular Formula
IgG	γ	$\gamma 1, \gamma 2, \gamma 3, \gamma 4$	κ or λ	$(\gamma_2\kappa_2), (\gamma_2\lambda_2)$
IgA	α	$\alpha 1, \alpha 2$	κ or λ	$(\alpha_2\kappa_2)_n^8, (\alpha_2\lambda_2)_n^8$
IgM	μ	none	κ or λ	$(\mu_2\kappa_2)_5, (\mu_2\lambda_2)_5$
IgD	δ	none	κ or λ	$(\delta_2\kappa_2), (\delta_2\lambda_2)$
IgE	ϵ	none	κ or λ	$(\epsilon_2\kappa_2), (\epsilon_2\lambda_2)$

(^8_n may equal 1, 2, or 3)

In preferred embodiments of an IgGγl human consensus sequence, the consensus variable domain sequences are derived from the most abundant subclasses in the sequence compilation of Kabat et al., *Sequences of Proteins of Immunological Interest,* National Institutes of Health, Bethesda Md. (1987), namely V_L κ subgroup I and V_H group III. In such preferred embodiments, the V_L consensus domain has the amino acid sequence:

DIQMTQSPSSLSASVGDRVTITCRASQD-VSSYLAWYQQKPGKAPKLLIYAASSLES-GVPSRFSGSGSGTDFTLTISSLQPEDFA-TYYCQQYNSLPYTFGQGTKVEIKRT (SEQ. ID NO. 3);

the V_H consensus domain has the amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYAMSW VRQAPGKGLEWVAVISENGGYTRYADSVKGRFT ISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGD GFYAMDVWGQGTLVTVSS (SEQ. ID NO. 4).

These sequences include consensus CDRs as well as consensus FR residues (see for example in FIG. **1**).

While not wishing to be limited to any particular theories, it may be that these preferred embodiments are less likely to be immunogenic in an individual than less abundant subclasses. However, in other embodiments, the consensus sequence is derived from other subclasses of human immunoglobulin variable domains. In yet other embodiments, the consensus sequence is derived from human constant domains.

Identity or homology with respect to a specified amino acid sequence of this invention is defined herein as the percentage of amino acid residues in a candidate sequence that are identical with the specified residues, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent homology, and not considering any conservative substitutions as part of the sequence identity. None of N-terminal, C-terminal or internal extensions, deletions, or insertions into the specified sequence shall be construed as affecting homology. All sequence alignments called for in this invention are such maximal homology alignments. While such alignments may be done by hand using conventional methods, a suitable computer program is the "Align 2" program for which protection is being sought from the U.S. Register of Copyrights (Align 2, by Genentech, Inc., application filed Dec. 9, 1991).

"Non-homologous" import antibody residues are those residues which are not identical to the amino acid residue at the analogous or corresponding location in a consensus sequence, after the import and consensus sequences are aligned.

The term "computer representation" refers to information which is in a form that can be manipulated by a computer. The act of storing a computer representation refers to the act of placing the information in a form suitable for manipulation by a computer.

This invention is also directed to novel polypeptides, and in certain aspects, isolated novel humanized anti-p185^{HER2} antibodies are provided. These novel anti-p185^{HER2} antibodies are sometimes collectively referred to herein as huMAb4D5, and also sometimes as the light or heavy chain variable domains of huMAb4D5, and are defined herein to be any polypeptide sequence which possesses a biological property of a polypeptide comprising the following polypeptide sequence:

DIQMTQSPSSLSASVGDRVTITCRASODVNTAVAWY QQKPGKAPKLLIYSASFLESGVPSRFSGSRSGT DFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTK VEIKRT (SEQ. ID NO. 1, which is the light chain variable domain of huMAb4D5); or

EVQLVESGGGLVOPGGSLRLSCAASGFNIKDTYIHW VRQAPGKGLEWVARIYPTNGYTRYADSVKGRFT ISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGD GFYAMDVWGQGTLVTVSS (SEQ. ID NO. 2, which is the heavy chain variable domain of huMAb4D5).

"Biological property", as relates for example to anti-p185^{HER2}, for the purposes herein means an in vivo effector or antigen-binding function or activity that is directly or indirectly performed by huMAb4D5 (whether in its native or denatured conformation). Effector functions include p185^{HER2} binding, any hormonal or hormonal antagonist activity, any mitogenic or agonist or antagonist activity, any

cytotoxic activity. An antigenic function means possession of an epitope or antigenic site that is capable of cross-reacting with antibodies raised against the polypeptide sequence of huMAb4D5.

Biologically active huMAb4D5 is defined herein as a polypeptide that shares an effector function of huMAb4D5. A principal known effector function of huMAb4D5 is its ability to bind to $p185^{HER2}$.

Thus, the biologically active and antigenically active huMAb4D5 polypeptides that are the subject of certain embodiments of this invention include the sequence of the entire translated nucleotide sequence of huMAb4D5; mature huMAb4D5; fragments thereof having a consecutive sequence of at least 5, 10, 15, 20, 25, 30 or 40 amino acid residues comprising sequences from muMAb4D5 plus residues from the human FR of huMAb4D5; amino acid sequence variants of huMAb4D5 wherein an amino acid residue has been inserted N- or C-terminal to, or within, huMAb4D5 or its fragment as defined above; amino acid sequence variants of huMAb4D5 or its fragment as defined above wherein an amino acid residue of huMAb4D5 or its fragment as defined above has been substituted by another residue, including predetermined mutations by, e.g., site-directed or PCR mutagenesis; derivatives of huMAb4D5 or its fragments as defined above wherein huMAb4D5 or its fragments have been covalent modified, by substitution, chemical, enzymatic, or other appropriate means, with a moiety other than a naturally occurring amino acid; and glycosylation variants of huMAb4D5 (insertion of a glycosylation site or deletion of any glycosylation site by deletion, insertion or substitution of suitable residues). Such fragments and variants exclude any polypeptide heretofore identified, including muMAb4D5 or any known polypeptide fragment, which are anticipatory order 35 U.S.C. 102 as well as polypeptides obvious thereover under 35 U.S.C. 103.

An "isolated" polypeptide means polypeptide which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, for example, a polypeptide product comprising huMAb4D5 will be purified from a cell culture or other synthetic environment (1) to greater than 95% by weight of protein as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a gas- or liquid-phase sequenator (such as a commercially available Applied Biosystems sequenator Model 470, 477, or 473), or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated huMAb4D5 includes huMAb4D5 in situ within recombinant cells since at least one component of the huMAb4D5 natural environment will not be present. Ordinarily, however, isolated huMAb4D5 will be prepared by at least one purification step.

In accordance with this invention, huMAb4D5 nucleic acid is RNA or DNA containing greater than ten bases that encodes a biologically or antigenically active huMAb4D5, is complementary to nucleic acid sequence encoding such huMAb4D5, or hybridizes to nucleic acid sequence encoding such huMAb4D5 and remains stably bound to it under stringent conditions, and comprises nucleic acid from a muMAb4D5 CDR and a human FR region.

Preferably, the huMAb4D5 nucleic acid encodes a polypeptide sharing at least 75% sequence identity, more preferably at least 80%, still more preferably at least 85%, even more preferably at 90%, and most preferably 95%, with the huMAb4D5 amino acid sequence. Preferably, a nucleic acid molecule that hybridizes to the huMAb4D5 nucleic acid contains at least 20, more preferably 40, and most preferably 90 bases. Such hybridizing or complementary nucleic acid, however, is further defined as being novel under 35 U.S.C. 102 and unobvious under 35 U.S.C. 103 over any prior art nucleic acid.

Stringent conditions are those that (1) employ low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate/0/1% $NaDodSO_4$ at 50° C.; (2) employ during hybridization a denaturing agent such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serumalbumin/0/1% Ficoll/0/1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42° C.; or (3) employ 50% formamide, 5×SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5×Denhardt's solution, sonicated salmon sperm DNA (50 g/ml), 0.1% SDS, and 10% dextran sulfate at 42 C., with washes at 42 C. in 0.2×SSC and 0.1% SDS.

The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, a ribosome binding site, and possibly, other as yet poorly understood sequences. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous and, in the case of a secretory leader, contiguous and in reading phase. However enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, then synthetic oligonucleotide adaptors or linkers are used in accord with conventional practice.

An "exogenous" element is defined herein to mean nucleic acid sequence that is foreign to the cell, or homologous to the cell but in a position within the host cell nucleic acid in which the element is ordinarily not found.

As used herein, the expressions "cell," "cell line," and "cell culture" are used interchangeably and all such designations include progeny. Thus, the words "transformants" and "transformed cells" include the primary subject cell and cultures derived therefrom without regard for the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Mutant progeny that have the same function or biological activity as screened for in the originally transformed cell are included. Where distinct designations are intended, it will be clear from the context.

"Oligonucleotides" are short-length, single- or double-stranded polydeoxynucleotides that are chemically synthesized by known methods (such as phosphotriester, phosphite, or phosphoramidite chemistry, using solid phase techniques such as described in EP 266,032 published May

4, 1988, or via deoxynucleoside H-phosphonate intermediates as described by Froehler et al., *Nucl. Acids Res.,* 14: 5399–5407 [1986]). They are then purified on polyacrylamide gels.

The technique of "polymerase chain reaction," or "PCR," as used herein generally refers to a procedure wherein minute amounts of a specific piece of nucleic acid, RNA and/or DNA, are amplified as described in U.S. Pat. No. 4,683,195 issued Jul. 28, 1987. Generally, sequence information from the ends of the region of interest or beyond needs to be available, such that oligonucleotide primers can be designed; these primers will be identical or similar in sequence to opposite strands of the template to be amplified. The 5' terminal nucleotides of the two primers may coincide with the ends of the amplified material. PCR can be used to amplify specific RNA sequences, specific DNA sequences from total genomic DNA, and cDNA transcribed from total cellular RNA, bacteriophage or plasmid sequences, etc. See generally Mullis et al., *Cold Spring Harbor Symp. Quant. Biol.,* 51: 263 (1987); Erlich, ed., *PCR Technology,* (Stockton Press, N.Y., 1989). As used herein, PCR is considered to be one, but not the only, example of a nucleic acid polymerase reaction method for amplifying a nucleic acid test sample, comprising the use of a known nucleic acid (DNA or RNA) as a primer and utilizes a nucleic acid polymerase to amplify or generate a specific piece of nucleic acid or to amplify or generate a specific piece of nucleic acid which is complementary to a particular nucleic acid.

Suitable Methods for Practicing the Invention

Some aspects of this invention include obtaining an import, non-human antibody variable domain, producing a desired humanized antibody sequence and for humanizing an antibody gene sequence are described below. A particularly preferred method of changing a gene sequence, such as gene conversion from a non-human or consensus sequence into a humanized nucleic acid sequence, is the cassette mutagenesis procedure described in Example 1. Additionally, methods are given for obtaining and producing antibodies generally, which apply equally to native non-human antibodies as well as to humanized antibodies.

Generally, the antibodies and antibody variable domains of this invention are conventionally prepared in recombinant cell culture, as described in more detail below. Recombinant synthesis is preferred for reasons of safety and economy, but it is known to prepare peptides by chemical synthesis and to purify them from natural sources; such preparations are included within the definition of antibodies herein.

Molecular Modeling

An integral step in our approach to antibody humanization is construction of computer graphics models of the import and humanized antibodies. These models are used to determine if the six complementarity-determining regions (CDRs) can be successfully transplanted from the import framework to a human one and to determine which framework residues from the import antibody, if any, need to be incorporated into the humanized antibody in order to maintain CDR conformation. In addition, analysis of the sequences of the import and humanized antibodies and reference to the models can help to discern which framework residues are unusual and thereby might be involved in antigen binding or maintenance of proper antibody structure.

All of the humanized antibody models of this invention are based on a single three-dimensional computer graphics structure hereafter referred to as the consensus structure. This consensus structure is a key distinction from the approach of previous workers in the field, who typically begin by selecting a human antibody structure which has an amino acid sequence which is similar to the sequence of their import antibody.

The consensus structure of one embodiment of this invention was built in five steps as described below.

Step 1

Seven Fab X-ray crystal structures from the Brookhaven Protein Data Bank were used (entries 2FB4, 2RHE, 3FAB, and 1 REI which are human structures, and 2MCP, 1 FBJ, and 2HFL which are murine structures). For each structure, protein mainchain geometry and hydrogen bonding patterns were used to assign each residue to one of three secondary structure types: alpha-helix, beta-strand or other (i.e. non-helix and non-strand). The immunoglobulin residues used in superpositioning and those included in the consensus structure are shown in Table 1.

TABLE I

	Immunoglobulin Residues Used in Superpositioning and Those Included in the Consensus Structure							
Ig[a]	2FB4	2RHE	2MCP	3FAB	1FBJ	2HFL	1REI	Consensus[b]
				$V_L\kappa$ domain				
								2–11
	18–24	18–24	19–25	18–24	19–25	19–25	19–25	16–27
	32–37	34–39	39–44	32–37	32–37	32–37	33–38	33–39
								41–49
	60–66	62–68	67–72	53–66	60–65	60–65	61–66	59–77
	69–74	71–76	76–81	69–74	69–74	69–74	70–75	
	84–88	86–90	91–95	84–88	84–88	84–88	85–89	82–91
								101–105
RMS[c]		0.40	0.60	0.53	0.54	0.48	0.50	
				V_H domain				
								3–8
	18–25		18–25	18–25	18–25	18–25		17–23
	34–39		34–39	34–39	34–39	34–39		33–41
	46–52		46–52	46–52	46–52	46–52		45–51
	57–61		59–63	56–60	57–61	57–61		57–61
	68–71		70–73	67–70	68–71	68–71		66–71
	78–84		80–86	77–83	78–84	78–84		75–82
	92–99		94–101	91–98	92–99	92–99		88–94
								102–108

TABLE I-continued

	Immunoglobulin Residues Used in Superpositioning and Those Included in the Consensus Structure							
Ig[a]	2FB4	2RHE	2MCP	3FAB	1FBJ	2HFL	1REI	Consensus[b]
RMS[c]			0.43	0.85	0.62	0.91		
RMS[d]	0.91		0.73	0.77	0.92			

[a]Four-letter code for Protein Data Bank file.
[b]Residue numbers for the crystal structures are taken from the Protein Data Bank files. Residue numbers for the consensus structure are according to Kabat et al.
[c]Root-mean-square deviation in Å for (N, Cα, C) atoms superimposed on 2FB4.
[d]Root-mean-square deviation in Å for (N, Cα, C) atoms superimposed on 2HFL.

Step 2

Having identified the alpha-helices and beta-strands in each of the seven structures, the structures were superimposed on one another using the INSIGHT computer program (Biosym Technologies, San Diego, Calif.) as follows: The 2FB4 structure was arbitrarily chosen as the template (or reference) structure. The 2FB4 was held fixed in space and the other six structures rotated and translated in space so that their common secondary structural elements (i.e. alpha-helices and beta-strands) were oriented such that these common elements were as close in position to one another as possible. (This superpositioning was performed using accepted mathematical formulae rather than actually physically moving the structures by hand.)

Step 3

With the seven structures thus superimposed, for each residue in the template (2FB4) Fab one calculates the distance from the template alpha-carbon atom (Cα) to the analogous Cα atom in each of the other six superimposed structures. This results in a table of Cα-Cα distances for each residue position in the sequence. Such a table is necessary in order to determine which residue positions will be included in the consensus model. Generally, is if all Cα-Cα distances for a given residue position were ≦1.0 Å, that position was included in the consensus structure. If for a given position only one Fab crystal structure was >1.0 Å, the position was included but the outlying crystal structure was not included in the next step (for this position only). In general, the seven β-strands were included in the consensus structure while some of the loops connecting the β-strands, e.g. complementarity-determining regions (CDRs), were not included in view of Cα divergence.

Step 4

For each residue which was included in the consensus structure after step 3, the average of the coordinates for individual mainchain N, Cα, C, O and Cβ atoms were calculated. Due to the averaging procedure, as well as variation in bond length, bond angle and dihedral angle among the crystal structures, this "average" structure contained some bond lengths and angles which deviated from standard geometry. For purposes of this invention, "standard geometry" is understood to include geometries commonly accepted as typical, such as the compilation of bond lengths and angles from small molecule structures in Weiner, S. J. et. al., *J. Amer. Chem. Soc.,* 106: 765–784 (1984).

Step 5

In order to correct these deviations, the final step was to subject the "average" structure to 50 cycles of energy minimization (DISCOVER program, Biosym Technologies) using the AMBER (Weiner, S. J. et. al., *J. Amer. Chem. Soc.,* 106: 765–784 (1984)) parameter set with only the Ca coordinates fixed (i.e. all other atoms are allowed to move) (energy minimization is described below). This allowed any deviant bond lengths and angles to assume a standard (chemically acceptable) geometry. See Table II.

TABLE II

Average Bond Lengths and Angles for "Average" (Before) and Energy-Minimized Consensus (After 50 Cycles) Structures

	$V_L\kappa$ before (Å)	$V_L\kappa$ after (Å)	V_H before (Å)	V_H after (Å)	Standard Geometry (Å)
N—Cα	1.459(0.012)	1.451(0.004)	1.451(0.023)	1.452(0.004)	1.449
Cα-C	1.515(0.012)	1.523(0.005)	1.507(0.033)	1.542(0.005)	1.522
O=C	1.208(0.062)	1.229(0.003)	1.160(0.177)	1.231(0.003)	1.229
C—N	1.288(0.049)	1.337(0.002)	1.282(0.065)	1.335(0.004)	1.335
Cα-Cβ	1.508(0.026)	1.530(0.002)	1.499(0.039)	1.530(0.002)	1.526
	(*)	(*)	(*)	(*)	(*)
C—N—Cα	123.5(4.2)	123.8(1.1)	125.3(4.6)	124.0(1.1)	121.9
N—Cα-C	110.0(4.0)	109.5(1.9)	110.3(2.8)	109.5(1.6)	110.1
Cα-C—N	116.6(4.0)	116.6(1.2)	117.6(5.2)	116.6(0.8)	116.6
O=C—N	123.1(4.1)	123.4(0.6)	122.2(4.9)	123.3(0.4)	122.9
N—Cα-Cβ	110.3(2.1)	109.8(0.7)	110.6(2.5)	109.8(0.6)	109.5
Cβ-Cα-C	111.4(2.4)	111.1(0.7)	111.2(2.2)	111.1(0.6)	111.1

Values in parentheses are standard deviations. Note that while some bond length and angle averages did not change appreciably after energy-minimization, the corresponding standard deviations are reduced due to deviant geometries assuming standard values after energy-minimization. Standard geometry values are from the AMBER forcefield as implemented in DISCOVER (Biosym Technologies).

The consensus structure might conceivably be dependent upon which crystal structure was chosen as the template on which the others were superimposed. As a test, the entire procedure was repeated using the crystal structure with the worst superposition versus 2FB4, i.e. the 2HFL Fab structure, as the new template (reference). The two consensus structures compare favorably (root-mean-squared deviation of 0.11 Å for all N, Cα and C atoms).

Note that the consensus structure only includes mainchain (N, Cα, C, O, Cβ atoms) coordinates for only those residues which are part of a conformation common to all seven X-ray crystal structures. For the Fab structures, these include the common β-strands (which comprise two β-sheets) and a few non-CDR loops which connect these β-strands. The consensus structure does not include CDRs or sidechains, both of which vary in their conformation among the seven structures. Also, note that the consensus structure includes only the V_L and V_H domains.

This consensus structure is used as the archetype. It is not particular to any species, and has only the basic shape without side chains. Starting with this consensus structure

the model of any import, human, or humanized Fab can be constructed as follows. Using the amino acid sequence of the particular antibody V_L and V_H domains of interest, a computer graphics program (such as INSIGHT, Biosym Technologies) is used to add sidechains and CDRs to the consensus structure. When a sidechain is added, its conformation is chosen on the basis of known Fab crystal structures (see the Background section for publications of such crystal structures) and rotamer libraries (Ponder, J. W. & Richards, F. M., *J. Mol. Biol.* 193: 775–791 (1987)). The model also is constructed so that the atoms of the sidechain are positioned so as to not collide with other atoms in the Fab.

CDRs are added to the model (now having the backbone plus side chains) as follows. The size (i.e. number of amino acids) of each import CDR is compared to canonical CDR structures tabulated by Chothia et al., *Nature,* 342:877–883 (1989)) and which were derived from Fab crystals. Each CDR sequence is also reviewed for the presence or absence of certain specific amino acid residues which are identified by Chothia as structurally important: e.g. light chain residues 29 (CDR1) and 95 (CDR3), and heavy chain residues 26, 27, 29 (CDR1) and 55 (CDR2). For light chain CDR2, and heavy chain CDR3, only the size of the CDR is compared to the Chothia canonical structure. If the size and sequence (i.e. inclusion of the specific, structurally important residues as denoted by Chothia et al.) of the import CDR agrees in size and has the same structurally important residues as those of a canonical CDR, then the mainchain conformation of the import CDR in the model is taken to be the same as that of the canonical CDR. This means that the import sequence is assigned the structural configuration of the canonical CDR, which is then incorporated in the evolving model.

However, if no matching canonical CDR can be assigned for the import CDR, then one of two options can be exercised. First, using a program such as INSIGHT (Biosym Technologies), the Brookhaven Protein Data Bank can be searched for loops with a similar size to that of the import CDR and these loops can be evaluated as possible conformations for the import CDR in the model. Minimally, such loops must exhibit a conformation in which no loop atom overlaps with other protein atoms. Second, one can use available programs which calculate possible loop conformations, assuming a given loop size, using methods such as described by Bruccoleri et al., *Nature* 335: 564–568 (1988).

When all CDRs and sidechains have been added to the consensus structure to give the final model (import, human or humanized), the model is preferably subjected to energy minimization using programs which are available commercially (e.g. DISCOVER, Biosym Technologies). This technique uses complex mathematical formulae to refine the model by performing such tasks as checking that all atoms are within appropriate distances from one another and checking that bond lengths and angles are within chemically acceptable limits.

Models of a humanized, import or human antibody sequence are used in the practice of this invention to understand the impact of selected amino acid residues of the activity of the sequence being modeled. For example, such a model can show residues which may be important in antigen binding, or for maintaining the conformation of the antibody, as discussed in more detail below. Modeling can also be used to explore the potential impact of changing any amino acid residue in the antibody sequence.

Methods for Obtaining a Humanized Antibody Sequence

In the practice of this invention, the first step in humanizing an import antibody is deriving a consensus amino acid sequence into which to incorporate the import sequences. Next a model is generated for these sequences using the methods described above. In certain embodiments of this invention, the consensus human sequences are derived from the most abundant subclasses in the sequence compilation of Kabat et al. (Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)), namely V_L κ subgroup I and V_H group III, and have the sequences indicated in the definitions above.

While these steps may be taken in different order, typically a structure for the candidate humanized antibody is created by transferring the at least one CDR from the non-human, import sequence into the consensus human structure, after the entire corresponding human CDR has been removed. The humanized antibody may contain human replacements of the non-human import residues at positions within CDRs as defined by sequence variability (Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)) or as defined by structural variability (Chothia, C. & Lesk, A. M., *J. Mol. Biol.* 196:901–917 (1987)). For example, huMAb4D5 contains human replacements of the muMAb4D5 residues at three positions within CDRs as defined by sequence variability (Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)) but not as defined by structural variability (Chothia, C. & Lesk, A. M., *J. Mol Bol.* 196:901–917 (1987)): V_L-CDR1 K24R, V_L-CDR2 R54L and V_L-CDR2 T56S.

Differences between the non-human import and the human consensus framework residues are individually investigated to determine their possible influence on CDR conformation and/or binding to antigen. Investigation of such possible influences is desirably performed through modeling, by examination of the characteristics of the amino acids at particular locations, or determined experimentally through evaluating the effects of substitution or mutagenesis of particular amino acids.

In certain preferred embodiments of this invention, a humanized antibody is made comprising amino acid sequence of an import, non-human antibody and a human antibody, utilizing the steps of:

a. obtaining the amino acid sequences of at least a portion of an import antibody variable domain and of a consensus human variable domain;

b. identifying Complementarity Determining Region (CDR) amino acid sequences in the import and the human variable domain sequences;

c. substituting an import CDR amino acid sequence for the corresponding human CDR amino acid sequence;

d. aligning the amino acid sequences of a Framework Region (FR) of the import antibody and the corresponding FR of the consensus antibody;

e. identifying import antibody FR residues in the aligned FR sequences that are non-homologous to the corresponding consensus antibody residues;

f. determining if the non-homologous import amino acid residue is reasonably expected to have at least one of the following effects:

1. non-covalently binds antigen directly,

2. interacts with a CDR; or

3. participates in the V_L–V_H interface; and

g. for any non-homologous import antibody amino acid residue which is reasonably expected to have at least

one of these effects, substituting that residue for the corresponding amino acid residue in the consensus antibody FR sequence.

Optionally, one determines if any non-homologous residues identified in step (e) are exposed on the surface of the domain or buried within it, and if the residue is exposed but has none of the effects identified in step (f), one may retain the consensus residue.

Additionally, in certain embodiments the corresponding consensus antibody residues identified in step (e) above are selected from the group consisting of 4L, 35L, 36L, 38L, 43L, 44L, 46L, 58L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71 L, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 67H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H (utilizing the numbering system set forth in Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)).

In preferred embodiments, the method of this invention comprises the additional steps of searching either or both of the import, non-human and the consensus variable domain sequences for glycosylation sites, determining if the glycosylation is reasonably expected to be important for the desired antigen binding and biological activity of the antibody (i.e., determining if the glycosylation site binds to antigen or changes a side chain of an amino acid residue that binds to antigen, or if the glycosylation enhances or weakens antigen binding, or is important for maintaining antibody affinity). if the import sequence bears the glycosylation site, it is preferred to substitute that site for the corresponding residues in the consensus human sequence if the glycosylation site is reasonably expected to be important. If only the consensus sequence, and not the import, bears the glycosylation site, it is preferred to eliminate that glycosylation site or substitute therefor the corresponding amino acid residues from the import sequence.

Another preferred embodiment of the methods of this invention comprises aligning import antibody and the consensus antibody FR sequences, identifying import antibody FR residues which are non-homologous with the aligned consensus FR sequence, and for each such non-homologous import antibody FR residue, determining if the corresponding consensus antibody residue represents a residue which is highly conserved across all species at that site, and if it is so conserved, preparing a humanized antibody which comprises the consensus antibody amino acid residue at that site.

In certain alternate embodiments, one need not utilize the modeling and evaluation steps described above, and may instead proceed with the steps of obtaining the amino acid sequence of at least a portion of an import, non-human antibody variable domain having a CDR and a FR, obtaining the amino acid sequence of at least a portion of a consensus human antibody variable domain having a CDR and a FR, substituting the non-human CDR for the human CDR in the consensus human antibody variable domain, and then substituting an amino acid residue for the consensus amino acid residue at at least one of the following sites:

a. (in the FR of the variable domain of the light chain) 4L, 35L, 36L, 38L, 43L, 44L, 58L, 46L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, or

b. fin the FR of the variable domain of the heavy chain) 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 67H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H.

Preferably, the non-CDR residue substituted at the consensus FR site is the residue found at the corresponding location of the non-human antibody. If desired, one may utilize the other method steps described above for determining whether a particular amino acid residue can reasonably be expected to have undesirable effects, and remedying those effects.

If after making a humanized antibody according to the steps above and testing its activity one is not satisfied with the humanized antibody, one preferably reexamines the potential effects of the amino acids at the specific locations recited above. Additionally, it is desirable to reinvestigate any buried residues which are reasonably expected to affect the V_L–V_H interface but may not directly affect CDR conformation. It is also desirable to reevaluate the humanized antibody utilizing the steps of the methods claimed herein.

In certain embodiments of this invention, amino acid residues in the consensus human sequence are substituted for by other amino acid residues. In preferred embodiments, residues from a particular non-human import sequence are substituted, however there are circumstances where it is desired to evaluate the effects of other amino acids. For example, if after making a humanized antibody according to the steps above and testing its activity one is not satisfied with the humanized antibody, one may compare the sequences of other classes or subgroups of human antibodies, or classes or subgroups of antibodies from the particular non-human species, and determine which other amino acid side chains and amino acid residues are found at particular locations and substituting such other residues.

Antibodies

Certain aspects of this invention are directed to natural antibodies and to monoclonal antibodies, as illustrated in the Examples below and by antibody hybridomas deposited with the ATCC (as described below). Thus, the references throughout this description to the use of monoclonal antibodies are intended to include the use of natural or native antibodies as well as humanized and chimeric antibodies. As used herein, the term "antibody" includes the antibody variable domain and other separable antibody domains unless specifically excluded.

In accordance with certain aspects of this invention, antibodies to be humanized (import antibodies) are isolated from continuous hybrid cell lines formed by the fusion of antigen-primed immune lymphocytes with myeloma cells. in certain embodiments, the antibodies of this invention are obtained by routine screening. Polyclonal antibodies to an antigen generally are raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the antigen and an adjuvant. It may be useful to conjugate the antigen or a fragment containing the target amino acid sequence to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic is anhydride, $SOCl_2$, or $R^1N{=}C{=}NR$, where R and R^1 are different alkyl groups.

The route and schedule of the host animal or cultured antibody-producing cells therefrom are generally in keeping with established and conventional techniques for antibody stimulation and production. While mice are frequently employed as the test model, it is contemplated that any mammalian subject including human subjects or antibody-producing cells obtained therefrom can be manipulated according to the processes of this invention to serve as the basis for production of mammalian, including human, hybrid cell lines.

Animals are typically immunized against the immunogenic conjugates or derivatives by combining 1 mg or 1 μg of conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with ⅕ to ⅒ the original amount of conjugate in Freund's complete adjuvant (or other suitable adjuvant) by subcutaneous injection at multiple sites. 7 to 14 days later animals are bled and the serum is assayed for antigen titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different protein and/or through a different cross-linking agent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are used to enhance the immune response.

After immunization, monoclonal antibodiesare prepared by recovering immune lymphoid cells—typically spleen cells or lymphocytes from lymph node tissue—from immunized animals and immortalizing the cells in conventional fashion, e.g. by fusion with myeloma cells or by Epstein-Barr (EB)-virus transformation and screening for clones expressing the desired antibody. The hybridoma technique described originally by Kohler and Milstein, *Eur. J. Immunol.* 6:511 (1976) has been widely applied to produce hybrid cell lines that secrete high levels of monoclonal antibodies against many specific antigens.

It is possible to fuse cells of one species with another. However, it is preferable that the source of the immunized antibody producing cells and the myeloma be from the same species.

The hybrid cell lines can be maintained in culture in vitro in cell culture media. The cell lines of this invention can be selected and/or maintained in a composition comprising the continuous cell line in hypoxanthine-aminopterin thymidine (HAT) medium. In fact, once the hybridoma cell line is established, it can be maintained on a variety of nutritionally adequate media. Moreover, the hybrid cell lines can be stored. and preserved in any number of conventional ways, including freezing and storage under liquid nitrogen. Frozen cell lines can be revived and cultured indefinitely with resumed synthesis and secretion of monoclonal antibody. The secreted antibody is recovered from tissue culture supernatant by conventional methods such as precipitation, Ion exchange chromatography, affinity chromatography, or the like. The antibodies described herein are also recovered from hybridoma cell cultures by conventional methods for purification of IgG or IgM as the case may be that heretofore have been used to purify these immunoglobulins from pooled plasma, e.g. ethanol or polyethylene glycol precipitation procedures. The purified antibodies are sterile filtered, and optionally are conjugated to a detectable marker such as an enzyme or spin label for use in diagnostic assays of the antigen in test samples.

While routinely rodent monoclonal antibodies are used as the source of the import antibody, the invention is not limited to any species. Additionally, techniques developed for the production of chimeric antibodies (Morrison et al., *Proc. Natl. Acad. Sci.,* 81:6851 (1984); Neuberger et al., *Nature* 312:604 (1984); Takeda et al., *Nature* 314:452 (1985)) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity (such as ability to activate human complement and mediate ADCC) can be used; such antibodies are within the scope of this invention.

Techniques for creating recombinant DNA versions of the antigen-binding regions of antibody molecules (known as Fab fragments) which bypass the generation of monoclonal antibodies are encompassed within the practice of this invention. One extracts antibody-specific messenger ANA molecules from immune system cells taken from an immunized animal, transcribes these into complementary DNA (cDNA), and clones the CDNA into a bacterial expressions system. One example of such a technique suitable for the practice of this invention was developed by researchers at Scripps/Stratagene, and incorporates a proprietary bacteriophage lambda vector system which contains a leader sequence that causes the expressed Fab protein to migrate to the periplasmic space (between the bacterial cell membrane and the cell wall) or to be secreted. One can rapidly generate and screen great numbers of functional FAb fragments for those which bind the antigen. Such FAb fragments with specificity for the antigen are specifically encompassed within the term "antibody" as it is defined, discussed, and claimed herein.

Amino Acid Sequence Variants

Amino acid sequence variants of the antibodies and polypeptides of this invention (referred to in herein as the target polypeptide) are prepared by introducing appropriate nucleotide changes into the DNA encoding the target polypeptide, or by in vitro synthesis of the desired target polypeptide. Such variants include, for example, humanized variants of non-human antibodies, as well as deletions from, or insertions or substitutions of, residues within particular amino acid sequences. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the target polypeptide, such as changing the number or position of glycosylation sites, altering any membrane anchoring characteristics, and/or altering the intra-cellular location of the target polypeptide by inserting, deleting, or otherwise affecting any leader sequence of the native target polypeptide.

In designing amino acid sequence variants of target polypeptides, the location of the mutation site and the nature of the mutation will depend on the target polypeptide characteristics) to be modified. The sites for mutation can be modified individually or in series, e.g., by (1) substituting first with conservative amino acid choices and then with more radical selections depending upon the results achieved, (2) deleting the target residue, or (3) inserting residues of the same or a different class adjacent to the located site, or combinations of options 1–3. In certain embodiments, these choices are guided by the methods for creating humanized sequences set forth above.

A useful method for identification of certain residues or regions of the target polypeptide that are preferred locations for mutagenesis is called "alanine scanning mutagenesism" as described by Cunningham and Wells (*Science,* 244: 1081–1085 [1989). Here, a residue or group of target residues are identified (e.g., charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with the surrounding aqueous environment in or outside the cell. Those domains demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at or for the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation per se need not be predetermined. For example, to optimize the performance of a mutation at a given site, ala scanning or random mutagenesis may be conducted at the target codon or region and the

expressed target polypeptide variants are screened for the optimal combination of desired activity.

There are two principal variables in the construction of amino acid sequence variants: the location of the mutation site and the nature of the mutation. In general, the location and nature of the mutation chosen will depend upon the target polypeptide characteristic to be modified.

Amino acid sequence deletions of antibodies are generally not preferred, as maintaining the generally configuration of an antibody is believed to be necessary for its activity. Any deletions will be selected so as to preserve the structure of the target antibody.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Intrasequence insertions (i.e., insertions within the target polypeptide sequence) may range generally from about 1 to 10 residues, more preferably 1 to 5, most preferably 1 to 3. Examples of terminal insertions include the target polypeptide with an N-terminal methionyl residue, an artifact of the direct expression of target polypeptide in bacterial recombinant cell culture, and fusion of a heterologous N-terminal signal sequence to the N-terminus of the target polypeptide molecule to facilitate the secretion of the mature target polypeptide from recombinant host cells. Such signal sequences generally will be obtained from, and thus homologous to, the intended host cell species. Suitable sequences include STII or Ipp for *E. coli,* alpha factor for yeast, and viral signals such as herpes gD for mammalian cells.

Other insertional variants of the target polypeptide include the fusion to the N- or C-terminus of the target polypeptide of immunogenic polypeptides, e.g., bacterial polypeptides such as beta-lactamase or an enzyme encoded by the *E. coli* trp locus, or yeast protein, and C-terminal fusions with proteins having a long half-life such as immunoglobulin constant regions (or other immunoglobulin regions), albumin, or ferritin, as described in WO 89/02922 published Apr. 6, 1989.

Another group of variants are amino acid substitution variants. These variants have at least one amino acid residue in the target polypeptide molecule removed and a different residue inserted in its place. The sites of greatest interest for substitutional mutagenesis include sites identified as the active site(s) of the target polypeptide, and sites where the amino acids found in the target polypeptide from various species are substantially different in terms of side-chain bulk, charge, and/or hydrophobicity. Other sites for substitution are described infra, considering the effect of the substitution of the antigen binding, affinity and other characteristics of a particular target antibody.

Other sites of interest are those in which particular residues of the target polypeptides obtained from various species are identical. These positions may be important for the biological activity of the target polypeptide. These sites, especially those falling within a sequence of at least three other identically conserved sites, are substituted in a relatively conservative manner. If such substitutions result in a change in biological activity, then other changes are introduced and the products screened until the desired effect is obtained.

Substantial modifications in function or immunological identity of the target polypeptide are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, lb) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side chain properties:

(1) hydrophobic: norleucine, met, ala, val, leu, ile;

(2) neutral hydrophilic: cys, ser, thr;

(3) acidic: asp, glu;

(4) basic: asn, gin, his, lys, arg;

(5) residues that influence chain orientation: gly, pro; and

(6) aromatic: trp, tyr, phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another. Such substituted residues may be introduced into regions of the target polypeptide that are homologous with other antibodies of the same class or subclass, or, more preferably, into the non-homologous regions of the molecule.

Any cysteine residues not involved in maintaining the proper conformation of target polypeptide also may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking.

DNA encoding amino acid sequence variants of the target polypeptide is prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the target polypeptide. A particularly preferred method of gene conversion mutagenesis is described below in Example 1. These techniques may utilized target polypeptide nucleic acid (DNA or RNA), or nucleic acid complementary to the target polypeptide nucleic acid.

Oligonucleotide-mediated mutagenesis is a preferred method for preparing substitution, deletion, and insertion variants of target polypeptide DNA. This technique is well known in the art as described by Adelman et al., *DNA,* 2: 183 (1983). Briefly, the target polypeptide DNA is altered by hybridizing an oligonucleotide encoding the desired mutation to a DNA template, where the template is the single-stranded form of a plasmid or bacteriophage containing the unaltered or native DNA sequence of the target polypeptide. After hybridization, a DNA polymerase is used to synthesize an entire second complementary strand of the template that will thus incorporate the oligonucleotide primer, and will code for the selected alteration in the target polypeptide DNA.

Generally, oligonucleotides of at least 25 nucleotides in length are used. An optimal oligonucleotide will have 12 to 15 nucleotides that are completely complementary to the template on either side of the nucleotide(s) coding for the mutation. This ensures that the oligonucleotide will hybridize properly to the single-stranded DNA template molecule. The oligonucleotides are readily synthesized using techniques known in the art such as that described by Crea et al. (*Proc. Natl. Acad. Sci. USA,* 75: 5765 [1978]).

Single-stranded DNA template may also be generated by denaturing double-stranded plasmid (or other) DNA using standard techniques.

For alteration of the native DNA sequence (to generate amino acid sequence variants, for example), the oligonucleotide is hybridized to the single-stranded template under suitable hybridization conditions. A DNA polymerizing enzyme, usually the Klenow fragment of DNA polymerase 1, is then added to synthesize the complementary strand of the template using the oligonucleotide as a primer for

synthesis. A heteroduplex molecule is thus formed such that one strand of DNA encodes the mutated form of the target polypeptide, and the other strand (the original template) encodes the native, unaltered sequence of the target polypeptide. This heteroduplex molecule is then transformed into a suitable host cell, usually a prokaryote such as *E. coli* JM101. After the cells are grown, they are plated onto agarose plates and screened using the oligonucleotide primer radiolabeled with 32-phosphate to identify the bacterial colonies that contain the mutated DNA. The mutated region is then removed and placed in an appropriate vector for protein production, generally an expression vector of the type typically employed for transformation of an appropriate host.

The method described immediately above may be modified such that a homoduplex molecule is created wherein both strands of the plasmid contain the mutation(s). The modifications are as follows: The single-stranded oligonucleotide is annealed to the single-stranded template as described above. A mixture of three deoxyribonucleotides, deoxyriboadenosine (dATP), deoxyriboguanosine (dGTP), and deoxyribothymidine (dTTP), is combined with a modified thio-deoxyribocytosine called dCTP-(aS) (which can be obtained from Amersham Corporation). This mixture is added to the template-oligonucleotide complex. Upon addition of DNA polymerase to this mixture, a strand of DNA identical to the template except for the mutated bases is generated. In addition, this new strand of DNA will contain dCTP-(aS) instead of dCTP, which serves to protect it from restriction endonuclease digestion.

After the template strand of the double-stranded heteroduplex is nicked with an appropriate restriction enzyme, the template strand can be digested with ExoIII nuclease or another appropriate nuclease past the region that contains the site(s) to be mutagenized. The reaction is then stopped to leave a molecule that is only partially single-stranded. A complete double-stranded DNA homoduplex is then formed using DNA polymerase in the presence of all four deoxyribonucleotide triphosphates, ATP, and DNA ligase. This homoduplex molecule can then be transformed into a suitable host cell such as *E. coli* JM101, as described above.

DNA encoding target polypeptide variants with more than one amino acid to be substituted may be generated in one of several ways. If the amino acids are located close together in the polypeptide chain, they may be mutated simultaneously using one oligonucleotide that codes for all of the desired amino acid substitutions. If, however, the amino acids are located some distance from each other (separated by more than about ten amino acids), it is more difficult to generate a single oligonucleotide that encodes all of the desired changes. Instead, one of two alternative methods may be employed.

In the first method, a separate oligonucleotide is generated for each amino acid to be substituted. The oligonucleotides are then annealed to the single-stranded template DNA simultaneously, and the second strand of DNA that is synthesized from the template will encode all of the desired amino acid substitutions.

The alternative method involves two or more rounds of mutagenesis to produce the desired mutant. The first round is as described for the single mutants: wild-type DNA is used for the template, an oligonucleotide encoding the first desired amino acid substitution(s) is annealed to this template, and the heteroduplex DNA molecule is then generated. The second round of mutagenesis utilizes the mutated DNA produced in the first round of mutagenesis as the template. Thus, this template already contains one or more mutations. The oligonucleotide encoding the additional desired amino acid substitution(s) is then annealed to this template, and the resulting strand of DNA now encodes mutations from both the first and second rounds of mutagenesis. This resultant DNA can be used as a template in a third round of mutagenesis, and so on.

PCR mutagenesis is also suitable for making amino acid variants of target polypeptide. While the following discussion refers to DNA, it is understood that the technique also finds application with RNA. The PCR technique generally refers to the following procedure (see Erlich, supra, the chapter by R. Higuchi, p. 61–70): When small amounts of template DNA are used as starting material in a PCR, primers that differ slightly in sequence from the corresponding region in a template DNA can be used to generate relatively large quantities of a specific DNA fragment that differs from the template sequence only at the positions where the primers differ from the template. For introduction of a mutation into a plasmid DNA, one of the primers is designed to overlap the position of the mutation and to contain the mutation; the sequence of the other primer must be identical to a stretch of sequence of the opposite strand of the plasmid, but this sequence can be located anywhere along the plasmid DNA. It is preferred, however, that the sequence of the second primer is located within 200 nucleotides from that of the first, such that in the end the entire amplified region of DNA bounded by the primers can be easily sequenced. PCR amplification using a primer pair like the one just described results in a population of DNA fragments that differ at the position of the mutation specified by the primer, and possibly at other positions, as template copying is somewhat error-prone.

If the ratio of template to product material is extremely low, the vast majority of product DNA fragments incorporate the desired mutation(s). This product material is used to replace the corresponding region in the plasmid that served as PCR template using standard DNA technology. Mutations at separate positions can be introduced simultaneously by either using a mutant second primer, or performing a second PCR with different mutant primers and ligating the two resulting PCR fragments simultaneously to the vector fragment in a three (or more)-part ligation.

In a specific example of PCR mutagenesis, template plasmid DNA (1 μg) is linearized by digestion with a restriction endonuclease that has a unique recognition site in the plasmid DNA outside of the region to be amplified. Of this material, 100 ng is added to a PCR mixture containing PCR buffer, which contains the four deoxynucleotide triphosphates and is included in the GeneAmp® kits (obtained from Perkin-Elmer Cetus, Norwalk, Conn. and Emeryville, Calif.), and 25 pmole of each oligonucleotide primer, to a final volume of 50μl. The reaction mixture is overlayed with 35 μl mineral oil. The reaction is denatured for 5 minutes at 100° C., placed briefly on ice, and then 1 μl *Thermus aquaticus* (Taq) DNA polymerase (5 units/μl, purchased from Perkin-Elmer Cetus, Norwalk, Conn. and Emeryville, Calif.) is added below the mineral oil layer. The reaction mixture is then inserted into a DNA Thermal Cycler (purchased from Perkin-Elmer Cetus) programmed as follows: 2 min. at 55° C., then 30 sec. at 72° C., then 19 cycles of the following: 30 sec. at 94° C., 30 sec. at 55° C., and 30 sec. at 72° C.

At the end of the program, the reaction vial is removed from the thermal cycler and the aqueous phase transferred to a new vial, extracted with phenol/chloroform (50:50:vol), and ethanol precipitated, and the DNA is recovered by standard procedures. This material is subsequently subjected to the appropriate treatments for insertion into a vector.

Another method for preparing variants, cassette mutagenesis, is based on the technique described by Wells et al. (*Gene,* 34: 315 [1985]). The starting material is the plasmid (or other vector) comprising the target polypeptide DNA to be mutated. The codon(s) in the target polypeptide DNA to be mutated are identified. There must be a unique restriction endonuclease site on each side of the identified mutation site(s). If no such restriction sites exist, they may be generated using the above-described oligonucleotide-mediated mutagenesis method to introduce them at appropriate locations in the target polypeptide DNA. After the restriction sites have been introduced into the plasmid, the plasmid is cut at these sites to linearize it. A double-stranded oligonucleotide encoding the sequence of the DNA between the restriction sites but containing the desired mutation(s) is synthesized using standard procedures. The two strands are synthesized separately and then hybridized together using standard techniques. This double-stranded oligonucleotide is referred to as the cassette. This cassette is designed to have 3' and 5' ends that are compatible with the ends of the linearized plasmid, such that it can be directly ligated to the plasmid. This plasmid now contains the mutated target polypeptide DNA sequence.

Insertion of DNA into a Cloning Vehicle

The cDNA or genomic DNA encoding the target polypeptide is inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. Many vectors are available, and selection of the appropriate vector will depend on 1) whether it is to be used for DNA amplification or for DNA expression, 2) the size of the DNA to be inserted into the vector, and 3) the host cell to be transformed with the vector. Each vector contains various components depending on its function (amplification of DNA or expression of DNA) and the host cell for which it is compatible. The vector components generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence.

(a) Signal Sequence Component

In general, the signal sequence may be a component of the vector, or it may be a part of the target polypeptide DNA that is inserted into the vector.

The target polypeptides of this invention may be expressed not only directly, but also as a fusion with a heterologous polypeptide, preferably a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the vector, or it may be a part of the target polypeptide DNA that is inserted into the vector. Included within the scope of this invention are target polypeptides with any native signal sequence deleted and replaced with a heterologous signal sequence. The heterologous signal sequence selected should be one that is recognized and processed (i.e. cleaved by a signal peptidase) by the host cell. For prokaryotic host cells that do not recognize and process the native target polypeptide signal sequence, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin 11 leaders. For yeast secretion the native target polypeptide signal sequence may be substituted by the yeast invertase, alpha factor, or acid phosphatase leaders. In mammalian cell expression the native signal sequence is satisfactory, although other mammalian signal sequences may be suitable.

(b) Origin of Replication Component

Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Generally, in cloning vectors this sequence is one that enables the vector to replicate independently of the host chromosomal DNA, and includes origins of replication or autonomously replicating sequences. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2μ plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter).

Most expression vectors are "shuttle" vectors, i.e. they are capable of replication in at least one class of organisms but can be transfected into another organism for expression. For example, a vector is cloned in *E. coli* and then the same vector is transfected into yeast or mammalian cells for expression even though it is not capable of replicating independently of the host cell chromosome.

DNA may also be amplified by insertion into the host genome. This is readily accomplished using Bacillus species as hosts, for example, by including in the vector a DNA sequence that is complementary to a sequence found in Bacillus genomic DNA. Transfection of Bacillus with this vector results in homologous recombination with the genome and insertion of the target polypeptide DNA. However, the recovery of genomic DNA encoding the target polypeptide is more complex than that of an exogenously replicated vector because restriction enzyme digestion is required to excise the target polypeptide DNA.

(c) Selection Gene Component

Expression and cloning vectors should contain a selection gene, also termed a selectable marker. This gene encodes a protein necessary for the survival or growth of transformed host cells grown in a selective culture medium. Host cells not transformed with the vector containing the selection gene will not survive in the culture medium. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g. ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, e.g. the gene encoding D-alanine racemase for Bacilli.

One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene express a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin (Southern et al., *J. Molec. Appl. Genet.,* 1: 327 [1982]), mycophenolic acid (Mulligan et al., *Science=:* 1422 [1980]) or hygromycin (Sugden et al., *Mol. Cell. Biol.,* 5: 410–413 [1985]). The three examples given above employ bacterial genes under eukaryotic control to convey resistance to the appropriate drug G418 or neomycin (geneticin), xgpt (mycophenolic acid), or hygromycin, respectively.

Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the target polypeptide nucleic acid, such as dihydrofolate reductase (DHFR) or thymidine kinase. The mammalian cell transformants are placed under selection pressure which only the transformants are uniquely adapted to survive by virtue of having taken up the marker. Selection pressure is imposed by culturing the transformants

under conditions in which the concentration of selection agent in the medium is successively changed, thereby leading to amplification of both the selection gene and the DNA that encodes the target polypeptide. Amplification is the process by which genes in greater demand for the production of a protein critical for growth are reiterated in tandem within the chromosomes of successive generations of recombinant cells. Increased quantities of the target polypeptide are synthesized from the amplified DNA.

For example, cells transformed with the DHFR selection gene are first identified by culturing all of the transformants in a culture medium that contains methotrexate (Mtx), a competitive antagonist of DHFR. An appropriate host cell when wild-type DHFR is employed is the Chinese hamster ovary (CHO) cell line deficient in DHFR activity, prepared and propagated as described by Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA,* 77: 4216 [1980]. The transformed cells are then exposed to increased levels of methotrexate. This leads to the synthesis of multiple copies of the DHFR gene, and, concomitantly, multiple copies of other DNA comprising the expression vectors, such as the DNA encoding the target polypeptide. This amplification technique can be used with any otherwise suitable host, e.g., ATCC No. CCL61 CHO-K1, notwithstanding the presence of endogenous DHFR if, for example, a mutant DHFR gene that is highly resistant to Mtx is employed (EP 117,060). Alternatively, host cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding the target polypeptide, wild-type DHFR protein, and another selectable marker such as aminoglycoside 3' phosphotransferase (APH) can be selected by cell growth in medium containing a selection agent for the selectable marker such as an aminoglycosidic antibiotic, e.g., kanamycin, neomycin, or G418. See U.S. Pat. No. 4,965,199.

A suitable selection gene for use in yeast is the trp1 gene present in the yeast plasmid YRp7 (Stinchcomb et al., *Nature,* 22: 39 [1979]; Kingsman et al., *Gene,* 7: 141 [1979]; or Tschemper et al., *Gene,* 10: 157 [1980]). The trp1 gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1 (Jones, *Genetics,* 5: 12 [1977]). The presence of the trp1 lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan. Similarly, Leu2-deficient yeast strains (ATCC 20,622 or 38,626) are complemented by known plasmids bearing the Leu2 gene.

(d) Promoter Component

Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to the target polypeptide nucleic acid. Promoters are untranslated sequences located upstream (5') to the start codon of a structural gene (generally within about 100 to 1000 bp) that control the transcription and translation of a particular nucleic acid sequence, such as that encoding the target polypeptide, to which they are operably linked. Such promoters typically fall into two classes, inducible and constitutive. Inducible promoters are promoters that initiate increased levels of transcription from DNA under their control in response to some change in culture conditions, e.g. the presence or absence of a nutrient or a change in temperature. At this time a large number of promoters recognized by a variety of potential host cells are well known. These promoters are operably linked to DNA encoding the target polypeptide by removing the promoter from the source DNA by restriction enzyme digestion and inserting the isolated promoter sequence into the vector. Both the native target polypeptide promoter sequence and many heterologous promoters may be used to direct amplification and/or expression of the target polypeptide DNA. However, heterologous promoters are preferred, as they generally permit greater transcription and higher yields of expressed target polypeptide as compared to the native target polypeptide promoter.

Promoters suitable for use with prokaryotic hosts include the β-lactamase and lactose promoter systems (Chang et al., *Nature,* 275: 615 [1978]; and Goeddel et al., *Nature,* 281: 544 [1979]), alkaline phosphatase, a tryptophan (trp) promoter system (Goeddel, *Nucleic Acids Res.,* 8: 4057 [1980] and EP 36,776) and hybrid promoters such as the tao promoter (deBoer et al., *Proc. Natl. Acad. Sci, USA,* 80: 21–25 [1983]). However, other known bacterial promoters are suitable. Their nucleotide sequences have been published, thereby enabling a skilled worker operably to ligate them to DNA encoding the target polypeptide (Siebenlist et al., *Cell,* 20: 269 [1980]) using linkers or adaptors to supply any required restriction sites. Promoters for use in bacterial systems also generally will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding the target polypeptide.

Suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase (Hitzeman et al., *J. Biol. Chem.,* 255: 2073 [1980]) or other glycolytic enzymes (Hess et al., *J. Adv. Enzyme Reg.,* 2: 149 [1968]; and Holland, *Biochemistry,* 17: 4900 [1978]), such asenolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in Hitzeman et al., EP 73,657A. Yeast enhancers also ate advantageously used with yeast promoters.

Promoter sequences are known for eukaryotes. Virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CXCAAT region where X may be any nucleotide. At the 3' end of most eukaryotic genes is an AATAAA sequence that may be the signal for addition of the poly A tail to the 3' end of the coding sequence. All of these sequences are suitably inserted into mammalian expression vectors.

Target polypeptide transcription from vectors in mammalian host cells is controlled by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published Jul. 5, 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g. the actin promoter or an immunoglobulin promoter, from heat-shock promoters, and from the promoter normally associated with the target polypeptide sequence, provided such promoters are compatible with the host cell systems.

The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that

also contains the SV40 viral origin of replication. Fiers et al., *Nature,* 273:113 (1978); Mulligan and Berg, *Science,* 209: 1422–1427 (1980); Pavlakis et al., *Proc. Natl. Acad. Sci. USA,* 78: 7398–7402 (1981). The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment. Greenaway et al., *Gene,* 18: 355–360 (1982). A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. Pat. No. 4,419,446. A modification of this system is described in U.S. Pat. No. 4,601,978. See also Gray et al., *Nature,* 29: 503–508 (1982) on expressing cDNA encoding immune interferon in monkey cells; , Reyes et al., *Nature,* 297: 598–601 (1982) on expression of human β-interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus, Canaani and Berg, *Proc. Natl. Acad. Sci. USA,* 79: 5166–5170 (1982) on expression of the human interferon β1 gene in cultured mouse and rabbit cells, and Gorman et al., *Proc. Natl. Aced. Sci. USA,* 79: 6777–6781 (1982) on expression of bacterial CAT sequences in CV-1 monkey kidney cells, chicken embryo fibroblasts, Chinese hamster ovary cells, HeLa cells, and mouse NIH-3T3 cells using the Rous sarcoma virus long terminal repeat as a promoter.

(e) Enhancer Element Component

Transcription of DNA encoding the target polypeptide of this invention by higher eukaryotes is often increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10–300 bp, that act on a promoter to increase its transcription. Enhancers are relatively orientation and position independent having been found 5' (Laimins et al., *Proc. Natl. Acad. Sci. USA,* 78: 993 [1981]) and 3' (Lusky et al., *Mol. Cell Bio.* 3: 1108 [1983]) to the transcription unit, within an intron (Banerji et al., *Cell,* 33: 729 [1983]) as well as within the coding sequence itself (Osborne et al., *Mol. Cell Bio.,* 4: 1293 [1984]). Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100–270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, *Nature,* 297: 17–18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the target polypeptide DNA, but is preferably located at a site 5' from the promoter.

(f) Transcription Termination Component

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3' untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding the target polypeptide. The 3' untranslated regions also include transcription termination sites.

Construction of suitable vectors containing one or more of the above listed components the desired coding and control sequences employs standard ligation techniques. Isolated plasmids or DNA fragments are cleaved, tailored, and religated in the form desired to generate the plasmids required.

For analysis to confirm correct sequences in plasmids constructed, the ligation mixtures are used to transform *E. coli* K12 strain 294 (ATCC 31,446) and successful transformants selected by ampicillin or tetracycline resistance where appropriate. Plasmids from the transformants are prepared, analyzed by restriction endonuclease digestion, and/or sequenced is by the method of Messing et al., *Nucleic Acids Res.,* 9: 309 (1981) or by the method of Maxam et al., *Methods in Enzymology* 65: 499 (1980).

Particularly useful in the practice of this invention are expression vectors that provide for the transient expression in mammalian cells of DNA encoding the target polypeptide. In general, transient expression involves the use of an expression vector that is able to replicate efficiently in a host cell, such that the host cell accumulates many copies of the expression vector and, in turn, synthesizes high levels of a desired polypeptide encoded by the expression vector. Transient expression systems, comprising a suitable expression vector and a host cell, allow for the convenient positive identification of polypeptides encoded by cloned DNAs, as well as for the rapid screening of such polypeptides for desired biological or physiological properties. Thus, transient expression systems are particularly useful in the invention for purposes of identifying analogs and variants of the target polypeptide that have target polypeptide-like activity.

Other methods, vectors, and host cells suitable for adaptation to the synthesis of the target polypeptide in recombinant vertebrate cell culture are described in Gething et al., *Nature,* 293: 620–625 [1981]; Mantei et al., *Nature,* 281: 40–46 [1979]; Levinson et al.,; EP 117,060; and EP 117,058. A particularly useful plasmid for mammalian cell culture expression of the target polypeptide is pRK5 (EP pub. no. 307,247) or pSVI6B.

Selection and Transformation of Host Cells

Suitable host cells for cloning or expressing the vectors herein are the prokaryote, yeast, or higher eukaryote cells described above. Suitable prokaryotes include eubacteria, such as Gram-negative or Gram-positive organisms, for example, *E. coli* , Bacilli such as *B. subtilis*, Pseudomonas species such as *P. aeruginosa, Salmonella typhimurium,* or *Serratia marcescans*. One preferred *E. coli* cloning host is *E. coli* 294 (ATCC 31,446), although other strains such as *E. coli* B, *E. coli* X1776 (ATCC 31,537), and *E. coli* W3110 (ATCC 27,325) are suitable. These examples are illustrative rather than limiting. Preferably the host cell should secrete minimal amounts of proteolytic enzymes. Alternatively, in vitro methods of cloning, e.g. PCR or other nucleic acid polymerase reactions, are suitable.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable hosts for target polypeptide-encoding vectors. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *Schizosaccharomyces pombe* [Beach and Nurse, *Nature,* 290: 140 (1981); EP 139,383 published May 2, 1985], Kluyveromyces hosts (U.S. Pat. No. 4,943,529) such as, e.g., *K. lactis* [Louvencourt et al., *J. Bacteriol.,* 737 (1983)], *K. fragilis, K. bulgaricus, K. thermotolerans,* and *K. marxianus,* yarrowia [EP 402,226], *Pichia pastoris* [EP 183,070; Sreekrishna et al., *J. Basic Microbiol.,* 28: 265–278 (1988)], Candida, *Trichoderma reesia* [EP 244,2341], *Neurospora crassa* [Case et al., *Proc. Natl. Acad. Sci. USA,* 76: 5259–5263 (1979)], and filamentous fungi such as, e.g, Neurospora, Penicillium, Tolypocladium [WO 91/00357 published Jan. 10, 1991], and Aspergillus hosts such as *A. nidulans* [Ballance et al., *Biochem. Biophys. Res. Commun.* 112: 284–289 (1983); Tilburn et al., *Gene,* 26: 205–221 (1983); Yelton et al., *Proc. Natl. Acad. Sci. USA,* 81: 1470–1474 (1984)] and *A. niger* [Kelly and Hynes, *EMBO J.,* 4: 475–479 (1985)].

Suitable host cells for the expression of glycosylated target polypeptide are derived from multicellular organisms. Such host cells are capable of complex processing and glycosylation activities. In principle, any higher eukaryotic cell culture is workable, whether from vertebrate or invertebrate culture. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda* (caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosophila melanogaster* (fruitfly), and *Bombyx mori* host cells have been identified. See, e.g., Luckow et al., *Bio/Technologvy* 6: 47–55 (1988); Miller et al., in *Genetic Engineering* Setlow, J. K. et a., eds., Vol. 8 (Plenum Publishing, 1986), pp. 277–279; and Maeda et al., *Nature,* 315: 592–594 (1985). A variety of such viral strains are publicly available, e.g., the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells. Plant cell cutures of cotton, corn, potato, soybean, petunia, tomato, and tobacco can be utilized as hosts. Typically, plant cells are transfected by incubation with certain strains of the bacterium *Agrobacterium tumefaciens,* which has been previously manipulated to contain the target polypeptide DNA. During incubation of the plant cell culture with *A. tumefaciens,* the DNA encoding target polypeptide is transferred to the plant cell host such that it is transfected, and will, under appropriate conditions, express the target polypeptide DNA. In addition, regulatory and signal sequences compatible with plant cells are available, such as the nopaline synthase promoter and polyadenylation signal sequences. Depicker et al., *J. Mol. Appl. Gen.,* 1: 561 (1982). In addition, DNA segments isolated from the upstream region of the T-DNA 780 gene are capable of activating or increasing transcription levels of plant-expressible genes in recombinant DNA-containing plant tissue. See EP 321,196 published Jun. 21, 1989.

However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure in recent years [*Tissue Culture*, Academic Press, Kruse and Patterson, editors (1973)]. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., *J. Gen Virol.,* 36: 59 [1977]); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA,* 77: 4216 [1980]); mouse sertoli cells (TM4, Mather, *Biol. Reprod.,* 23: 243–251 [1980]); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HS 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather et al., *Annals N.Y. Acad. Sci.,* 383: 44–68 [1982]); MRC 5 cells; FS4 cells; and a human hepatoma cell line (Hep G2). Preferred host cells are human embryonic kidney 293 and Chinese hamster ovary cells.

Host cells are transfected and preferably transformed with the above-described expression or cloning vectors of this invention and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Transfection refers to the taking up of an expression vector by a host cell whether or not any coding sequences are in fact expressed. Numerous methods of transfection are known to the ordinarily skilled artisan, for example, $CaPO_4$ and electroporation. Successful transfection is generally recognized when any indication of the operation of this vector occurs within the host cell.

Transformation means introducing DNA into an organism so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integrant. Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in section 1.82 of Sambrook et al., supra, is generally used for prokaryotes or other cells that contain substantial cell-wall barriers. Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw et al., *Gene,* 23: 315 (1983) and WO 89/05859 published Jun. 29, 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method described in sections 16.30–16.37 of Sambrook et al., supra, is preferred. General aspects of mammalian cell host system transformations have been described by Axel in U.S. Pat. No. 4,399,216 issued Aug. 16, 1983. Transformations into yeast are typically carried out according to the method of Van Solingen et al., *J. Bact.,* 130: 946 (1977) and Hsiao et al., *Proc. Natl. Acad. Sci. (USA),* 76: 3829 (1979). However, other methods for introducing DNA into cells such as by nuclear injection, electroporation, or protoplast fusion may also be used.

Culturina the Host Cells

Prokaryotic cells used to produce the target polypeptide of this invention are cultured in suitable media as described generally in Sambrook et al., supra.

The mammalian host cells used to produce the target polypeptide of this invention may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ([MEM], Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ([DMEM], Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham and Wallace, *Meth. Enz.,* 58: 44 (1979), Barnes and Sato, *Anal. Biochem.* 102: 255 (1980), U.S. Pat. Nos. 4,767,704; 4,657,866; 4,927,762; or 4,560,655; WO 90/03430; WO 87/00195; U.S. Pat. No. Re. 30,985, may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleosides (such as adenosine and thymidine), antibiotics (such as Gentamycin™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

The host cells referred to in this disclosure encompass cells in in vitro culture as well as cells that are within a host animal.

It is further envisioned that the target polypeptides of this invention may be produced by homologous recombination, or with recombinant production methods utilizing control elements introduced into cells already containing DNA encoding the target polypeptide currently in use in the field.

For example, a powerful promoter/enhancer element, a suppressor, or an exogenous transcription modulatory element is inserted in the genome of the intended host cell in proximity and orientation sufficient to influence the transcription of DNA encoding the desired target polypeptide. The control element does not encode the target polypeptide of this invention, but the DNA is present in the host cell genome. One next screens for cells making the target polypeptide of this invention, or increased or decreased levels of expression, as desired.

Detecting Gene Amplification/Expression

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, northern blotting to quantitate the transcription of mRNA (Thomas, *Proc. Natl. Acad. Sci. USA,* 77: 5201–5205 [1980]), dot blotting (DNA analysis), or in situ hybridization, using an appropriately labeled probe, based on the sequences provided herein. Various labels may be employed, most commonly radioisotopes, particularly ^{32}P. However, other techniques may also be employed, such as using biotin-modified nucleotides for introduction into a polynucleotide. The biotin then serves as the site for binding to avidin or antibodies, which may be labeled with a wide variety of labels, such as radionuclides, fluorescers, enzymes, or the like. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. With immunohistochemical staining techniques, a cell sample is prepared, typically by dehydration and fixation, followed by reaction with labeled antibodies specific for the gene product coupled, where the labels are usually visually detectable, such as enzymatic labels, fluorescent labels, luminescent labels, and the like. A particularly sensitive staining technique suitable for use in the present invention is described by Hsu et al., *Am. J. Clin. Path.,* 75: 734–738 (1980).

Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared in any mammal. Conveniently, the antibodies may be prepared against a native target polypeptide or against a synthetic peptide based on the DNA sequences provided herein as described further in Section 4 below.

Purification of the Target Polypeptide

The target polypeptide preferably is recovered from the culture medium as a secreted polypeptide, although it also may be recovered from host cell lysates when directly expressed without a secretory signal.

When the target polypeptide is expressed in a recombinant cell other than one of human origin, the target polypeptide is completely free of proteins or polypeptides of human origin. However, it is necessary to purify the target polypeptide from recombinant cell proteins or polypeptides to obtain preparations that are substantially homogeneous as to the target polypeptide. As a first step, the culture medium or lysate is centrifuged to remove particulate cell debris. The membrane and soluble protein fractions are then separated. The target polypeptide may then be purified from the soluble protein fraction and from the membrane fraction of the culture lysate, depending on whether the target polypeptide is membrane bound. The following procedures are exemplary of suitable purification procedures: fractionation on immunoaffinity or ion-exchange columns; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-75; and protein A Sepharose columns to remove contaminants such as IgG.

Target polypeptide variants in which residues have been deleted, inserted or substituted are recovered in the same fashion, taking account of any substantial changes in properties occasioned by the variation. For example, preparation of a target polypeptide fusion with another protein or polypeptide, e.g. a bacterial or viral antigen, facilitates purification; an immunoaffinity column containing antibody to the antigen (or containing antigen, where the target polypeptide is an antibody) can be used to adsorb the fusion. Immunoaffinity columns such as a rabbit polyclonal anti-target polypeptide column can be employed to absorb the target polypeptide variant by binding it to at least one remaining immune epitope. A protease inhibitor such as phenyl methyl sulfonyl fluoride (PMSF) also may be useful to inhibit proteolytic degradation during purification, and antibiotics may be included to prevent the growth of adventitious contaminants. One skilled in the art will appreciate that purification methods suitable for native target polypeptide may require modification to account for changes in the character of the target polypeptide or its variants upon expression in recombinant cell culture.

Covalent Modifications of Target Polypeptides

Covalent modifications of target polypeptides are included within the scope of this invention. One type of covalent modification included within the scope of this invention is a target polypeptide fragment. Target polypeptide fragments having up to about 40 amino acid residues may be conveniently prepared by chemical synthesis, or by enzymatic or chemical cleavage of the full-length target polypeptide or variant target polypeptide. Other types of covalent modifications of the target polypeptide or fragments thereof are introduced into the molecule by reacting specific amino acid residues of the target polypeptide or fragments thereof with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues.

Cysteinyl residues most commonly are reacted with α-haloacetates (and corresponding amines), such as chloroacetic acid or chloroacetamide, to give carboxymethyl or carboxyamidomethyl derivatives. Cysteinyl residues also are derivatized by reaction with bromotrifluoroacetone, α-bromo-β-(5-imidozoyl)propionic acid, chloroacetyl phosphate, N-alkylmaleimides,3-nitro-2-pyridyl disulfide, methyl2-pyridyldisulfide, p-chloromercuribenzoate, 2-chloromercuri-4-nitrophenol, or chloro-7-nitrobenzo-2-oxa-1,3-diazole.

Histidyl residues are derivatized by reaction with diethylpyrocarbonate at pH 5.5–7.0 because this agent is relatively specific for the histidyl side chain. Para-bromophenacyl bromide also is useful; the reaction is preferably performed in 0.1M sodium cacodylate at pH 6.0.

Lysinyl and amino terminal residues are reacted with succinic or other carboxylic acid anhydrides. Derivatization with these agents has the effect of reversing the charge of the lysinyl residues. Other suitable reagents for derivatizing α-amino-containing residues include imidoesters such as methyl picolinimidate; pyridoxal phosphate; pyridoxal;

chloroborohydride; trinitrobenzenesulfonic acid; O-methylisourea; 2,4-pentanedione; and transaminase-catalyzed reaction with glyoxylate.

Arginyl residues are modified by reaction with one or several conventional reagents, among them phenylglyoxal, 2,3-butanedione, 1,2-cyclohexanedione, and ninhydrin. Derivatization of arginine residues requires that the reaction be performed in alkaline conditions because of the high pK_a of the guanidine functional group. Furthermore, these reagents may react with the groups of lysine as well as the arginine epsilon-amino group.

The specific modification of tyrosyl residues may be made, with particular interest in so introducing spectral labels into tyrosyl residues by reaction with aromatic diazonium compounds or tetranitromethane. Most commonly, N-acetylimidizole and tetranitromethane are used to form O-acetyl tyrosyl species and 3-nitro derivatives, respectively. Tyrosyl residues are iodinated using ^{125}I or ^{131}I to prepare labeled proteins for use in radioimmunoassay, the chloramine T method described above being suitable.

Carboxyl side groups (aspartyl or glutamyl) are selectively modified by reaction with carbodiimides (R'—N═C═N—R'), where R and R' are different alkyl groups, such as 1-cyclohexyl-3-(2-morpholinyl-4-ethyl) carbodiimide or 1-ethyl-3-(4-azonia-4,4-dimethylpentyl) carbodiimide. Furthermore, aspartyl and glutamyl residues are converted to asparaginyl and glutaminyl residues by reaction with ammonium ions.

Derivatization with bifunctional agents is useful for crosslinking target polypeptide to a water-insoluble support matrix or surface for use in the method for purifying anti-target polypeptide antibodies, and vice versa. Commonly used crosslinking agents include, e.g., 1,1-bis (diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis (succinimidylpropionate), and bifunctional maleimides such as bis-N-maleimido-1,8-octane. Derivatizing agents such as methyl-3-[(p-azidophenyl)dithio]propioimidate yield photoactivatable intermediates that are capable of forming crosslinks in the presence of light. Alternatively, reactive water-insoluble matrices such as cyanogen bromide-activated carbohydrates and the reactive substrates described in U.S. Pat. Nos. 3,969,287; 3,691,016; 4,195,128; 4,247,642; 4,229,537; and 4,330,440 are employed for protein immobilization.

Glutaminyl and asparaginyl residues are frequently deamidated to the corresponding glutamyl and aspartyl residues, respectively. Alternatively, these residues are deamidated under mildly acidic conditions. Either form of these residues falls within the scope of this invention.

Other modification include hydroxylation of proline and lysine, phophorylation of hydroxyl groups of seryl or threonyl resides, methylation of the α-amino groups of lysine, arginine, and histidine side chains, (T. E. Creighton, *Protein: Structure and Molecular Properties*, W. H. Freeman & Co., San Francisco, pp. 79–86 [1983]), acetylation of the N-terminal amine, and amidaatioon of any C-terminal carboxyl group.

Another type of covalent modification of the target polypeptide included within the scope of this invention comprises altering the native glucosylatuion pattern of the polypeptide. By altering is meant deleting one or more carbohydrate moieties found in the native target polypeptide, and/or adding one or more glycosylation sites that are not present in the native target polypeptide.

Gylcosylation of polypeptides is typically either N-linked or O-linked refers to the attachment of the carbonhydrate moiety to the side chain of an asparagine reisdue. The tri-peptide sequences asparagine-X-resine and asparagine-X-threonine, where X is any aminoe acid except proline, are the recoginition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tri-peptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgactosamine, galactose, or xylose, to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

Addition of glycosylation sites to the target polypeptide is conveniently accomplished by altering the amino acid sequence such that it contains one or more of hte above-described tri-peptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or theonine resides to the native target polypeptide sequence (for O-linked glycosylation sites). For ease, the target polypeptide amino acid sequences is preferably altered through changes at the DNA level, particularly by mutating the DNA encoding the target polypeptide at preselected bases such that condons are generated that will translate into the desired amino acids. The DNA mutation(s) may be made using methods described above under the heading of "Amino Acid Sequence Variants of Target Polypeptide".

Another means of increasing the number of carbohydrate moieties on the target polypeptide is by chemical or enzymatic coupling glycosides to the polypeptides. These procedures are advantageous in that they do not require production of the polypeptide in a host cell that has glycosylation capabilities for N- or O-linked glycosylation. Depending on the couple mode used, the sugar(s) may be attached to (a) arginine and histidine, (b) free carboxyl groups, (c) free sulfhydryl groups such as those of cysteine, (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline, (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan, or (f) the amide group of glutamine. These methods are described in WO 87/05330 published Sep. 11, 1987, and in Aplin and Wriston (*CRC Crit. Rev. Biochem., pp.* 259–306 [1981]).

Removal of carbohydrate moieties present on the native target polypeptide may be accomplished chemically or enzymatically. Chemical deglycosylation requires exposure of the polypeptide to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the polypeptide intact. Chemical deglycosylation is described by Hakimuddin et al. (*Arch. Biochem. Biophys.,* 259:52 [1987]) and by Edge et al. (*Anal. Biochem.,* 118:131 [1981]). Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura et al. (*Meth. Enzymol.* 138:350 [1987]).

Glycosylation at potential glycosylation sites may be prevented by the use of the compound tunicamycin as described by Duskin et al. (*J. Biol. Chem.,* 257:3105 [1982]). Tunicamycin blocks the formation of protein-N-glycoside linkages.

Another type of covalent modification of the target polypeptide comprises linking the target polypeptide to various nonproteinaceous polymers, e.g. polyethylene glycol, polypropylene glycol or polyoxyalkylenes, in the

manner set forth in U.S. Pat. Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337.

The target polypeptide also may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-microcapsules and poly-[methylmethacylate]microcapsules, respectively), in colloidal drug deliverysystems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), or in macroemulsions. Such techniques are disclosed in *Reminaton's Pharmaceutical Sciences,* 16th edition, Osol, A., Ed., (1980).

Target polypeptide preparations are also useful in generating antibodies, for screening for binding partners, as standards in assays for the target polypeptide (e.g. by labeling the target polypeptide for use as a standard in a radioimmunoassay, enzyme-linked immunoassay, or radioreceptor assay), in affinity purification techniques, and in competitive-type receptor binding assays when labeled with radioiodine, enzymes, fluorophores, spin labels, and the like.

Since it is often difficult to predict in advance the characteristics of a variant target polypeptide, it will be appreciated that some screening of the recovered variant will be needed to select the optimal variant. For example, a change in the immunological character of the target polypeptide molecule, such as affinity for a given antigen or antibody, is measured by a competitive-type immunoassay. The variant is assayed for changes in the suppression or enhancement of its activity by comparison to the activity observed for the target polypeptide in the same assay. Other potential modifications of protein or polypeptide properties such as redox or thermal stability, hydrophobicity, susceptibility to proteolytic degradation, stability in recombinant cell culture or in plasma, or the tendency to aggregate with carriers or into multimers are assayed by methods well known in the art.

Diagnostic and Related Uses of the Antibodies

The antibodies of this invention are useful in diagnostic assays for antigen expression in specific cells or tissues. The antibodies are detectably labeled and/or are immobilized on an insoluble matrix.

The antibodies of this invention find further use for the affinity purification of the antigen from recombinant cell culture or natural sources. Suitable diagnostic assays for the antigen and its antibodies depend on the particular antigen or antibody. Generally, such assays include competitive and sandwich assays, and steric inhibition assays. Competitive and sandwich methods employ a phase-separation step as an integral part of the method while steric inhibition assays are conducted in a single reaction mixture. Fundamentally, the same procedures are used for the assay of the antigen and for substances that bind the antigen, although certain methods will be favored depending upon the molecular weight of the substance being assayed. Therefore, the substance to be tested is referred to herein as an analyte, irrespective of its status otherwise as an antigen or antibody, and proteins that bind to the analyte are denominated binding partners, whether they be antibodies, cell surface receptors, or antigens.

Analytical methods for the antigen or its antibodies all use one or more of the following reagents: labeled analyte analogue, immobilized analyte analogue, labeled binding partner, immobilized binding partner and steric conjugates. The labeled reagents also are known as "tracers."

The label used (and this is also useful to label antigen nucleic acid for use as a probe) is any detectable functionality that does not interfere with the binding of analyte and its binding partner. Numerous labels are known for use in immunoassay, examples including moieties that may be detected directly, such as fluorochrome, chemiluminescent, and radioactive labels, as well as moieties, such as enzymes, that must be reacted or derivatized to be detected. Examples of such labels include the radioisotopes ^{32}P, ^{14}C, ^{125}I, ^{3}H, and ^{131}I, fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luceriferases, e.g., firefly luciferase and bacterial luciferase (U.S. Pat. No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, β-galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotintavidin, spin labels, bacteriophage labels, stable free radicals, and the like.

Conventional methods are available to bind these labels covalenily to proteins or polypeptides. For instance, coupling agents such as dialdehydes, carbodiimides, dimaleimides, bis-imidates, bis-diazotized benzidine, and the like may be used to tag the antibodies with the above-described fluorescent, chemiluminescent, and enzyme labels. See, for example, U.S. Pat. No. 3,940,475 (fluorimetry) and U.S. Pat. No. 3,645,090 (enzymes); Hunter et al., *Nature,* 144: 945 (1962); David et al., *Biochemistry,* 13: 1014–1021 (1974); Pain et al., *J. Immunol. Methods,* 40: 219–230 (1981); and Nygren, *J. Histochem. and Cytochem.,* 30: 407–412 (1982). Preferred labels herein are enzymes such as horseradish peroxidase and alkaline phosphatase.

The conjugation of such label, including the enzymes, to the antibody is a standard manipulative procedure for one of ordinary skill in immunoassay techniques. See, for example, O'Sullivan et al., "Methods for the Preparation of Enzyme-antibody Conjugates for Use in Enzyme Immunoassay," in *Methods in in Enzymology*, ed. J. J. Langone and H. Van Vunakis, Vol. 73 (Academic Press, New York, N.Y., 1981), pp. 147–166. Such bonding methods are suitable for use with the antibodies and polypeptides of this invention.

Immobilization of reagents is required for certain assay methods. Immobilization entails separating the binding partner from any analyte that remains free in solution. This conventionally is accomplished by either insolubilizing the binding partner or analyte analogue before the assay procedure, as by adsorption to a water-insoluble matrix or surface (Bennich et al., U.S. Pat. No. 3,720,760), by covalent coupling (for example, using glutaraldehyde cross-linking), or by insolubilizing the partner or analogue afterward, e.g., by immunoprecipitation.

Other assay methods, known as competitive or sandwich assays, are well established and widely used in the commercial diagnostics industry.

Competitive assays rely on the ability of a tracer analogue to compete with the test sample analyte for a limited number of binding sites on a common binding partner. The binding partner generally is insolubilized before or after the competition and then the tracer and analyte bound to the binding partner are separated from the unbound tracer and analyte. This separation is accomplished by decanting (where the binding partner was preinsolubilized) or by centrifuging (where the binding partner was precipitated after the competitive reaction). The amount of test sample analyte is inversely proportional to the amount of bound tracer as measured by the amount of marker substance. Dose-

response curves with known amounts of analyte are prepared and compared with the test results to quantitatively determine the amount of analyte present in the test sample. These assays are called ELISA systems when enzymes are used as the detectable markers.

Another species of competitive assay, called a "homogeneous" assay, does not require a phase separation. Here, a conjugate of an enzyme with the analyte is prepared and used such that when anti-analyte binds to the analyte the presence of the anti-analyte modifies the enzyme activity. In this case, the antigen or its immunologically active fragments are conjugated with a bifunctional organic bridge to an enzyme such as peroxidase. Conjugates are selected for use with antibody so that binding of the antibody inhibits or potentiates the enzyme activity of the label. This method per se is widely practiced under the name of EMIT.

Steric conjugates are used in steric hindrance methods for homogeneous assay. These conjugates are synthesized by covalently linking a low-molecular-weight hapten to a small analyte so that antibody to hapten substantially is unable to bind the conjugate at the same time as anti-analyte. Under this assay procedure the analyte present in the test sample will bind anti-analyte, thereby allowing anti-hapten to bind the conjugate, resulting in a change in the character of the conjugate hapten, e.g., a change in fluorescence when the hapten is a fluorophore.

Sandwich assays particularly are useful for the determination of antigen or antibodies. In sequential sandwich assays an immobilized binding partner is used to adsorb test sample analyte, the test sample is removed as by washing, the bound analyte is used to adsorb labeled binding partner, and bound material is then separated from residual tracer. The amount of bound tracer is directly proportional to test sample analyte. In "simultaneous" sandwich assays the test sample is not separated before adding the labeled binding partner. A sequential sandwich assay using an anti-antigen monoclonal antibody as one antibody and a polyclonal anti-antigen antibody as the other is useful in testing samples for particular antigen activity.

The foregoing are merely exemplary diagnostic assays for the import and humanized antibodies of this invention. Other methods now or hereafter developed for the determination of these analytes are included within the scope hereof, including the bioassays described above.

Immunotoxins

This invention is also directed to immunochemical derivatives of the antibodies of this invention such as immunotoxins (conjugates of the antibody and a cytotoxic moiety). Antibodies which carry the appropriate effector functions, such as with their constant domains, are also used to induce lysis through the natural complement process, and to interact with antibody dependent cytotoxic cells normally present.

For example, purified, sterile filtered antibodies are optionally conjugated to a cytotoxin such as ricin for use in AIDS therapy. U.S. patent application Ser. No. 07/350,895 illustrates methods for making and using immunotoxins for the treatment of HIV infection. The methods of this invention, for example, are suitable for obtaining humanized antibodies for use as immunotoxins for use in AIDS therapy.

The cytotoxic moiety of the immunotoxin may be a cytotoxic drug or an enzymatically active toxin of bacterial, fungal, plant or animal origin, or an enzymatically active fragment of such a toxin. Enzymatically active toxins and fragments thereof used are diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. In another embodiment, the antibodies are conjugated to small molecule anticancer drugs such as cis-platin or 5FU. Conjugates of the monoclonal antibody and such cytotoxic moieties are made using a variety of bifunctional protein coupling agents. Examples of such reagents are SPDP, IT, bifunctional derivatives of imidoesters such as dimethyl adipimidate HCl, active esters such as disuccinimidyl suberate, aldehydes such as glutaraldehyde, bis-azido compounds such as bis (p-azidobenzoyl) hexanediamine, bis-diazonium derivatives such as bis-(p-diazoniumbenzoyl)-ethylenediamine, diisocyanates such as tolylene 2,6-diisocyanate and bis-active fluorine compounds such as 1,5-difluoro-2,4-dinitrobenzene. The lysing portion of a toxin may be joined to the Fab fragment of the antibodies.

Immunotoxins can be made in a variety of ways, as discussed herein. Commonly known crosslinking reagents can be used to yield stable conjugates.

Advantageously, monoclonal antibodies specifically binding the domain of the antigen which is exposed on the infected cell surface, are conjugated to ricin A chain. Most advantageously the ricin A chain is deglycosylated and produced through recombinant means. An advantageous method of making the ricin immunotoxin is described in Vitetta et al., *Science* 238:1098 (1987).

When used to kill infected human cells in vitro for diagnostic purposes, the conjugates will typically be added to the cell culture medium at a concentration of at least about 10 nM. The formulation and mode of administration for in vitro use are not critical. Aqueous formulations that are compatible with the culture or perfusion medium will normally be used. Cytotoxicity may be read by conventional techniques.

Cytotoxic radiopharmaceuticals for treating infected cells may be made by conjugating radioactive isotopes (e.g. I, Y, Pr) to the antibodies. Advantageously alpha particle-emitting isotopes are used. The term "cytotoxic moiety" as used herein is intended to include such isotopes.

In a preferred embodiment, ricin A chain is deglycosylated or produced without oligosaccharides, to decrease its clearance by irrelevant clearance mechanisms (e.g., the liver). In another embodiment, whole ricin (A chain plus B chain) is conjugated to antibody if the galactose binding property of B-chain can be blocked ("blocked ricin").

In a further embodiment toxin-conjugates are made with Fab or $F(ab')_2$ fragments. Because of their relatively small size these fragments can better penetrate tissue to reach infected cells.

In another embodiment, fusogenic liposomes are filled with a cytotoxic drug and the liposomes are coated with antibodies specifically binding the particular antigen.

Antibody Dependent Cellular Cytotoxicity

Certain aspects of this invention involve antibodies which are (a) directed against a particular antigen and (b) belong to a subclass or isotype that is capable of mediating the lysis of cells to which the antibody molecule binds. More specifically, these antibodies should belong to a subclass or isotype that, upon complexing with cell surface proteins, activates serum complement and/or mediates antibody dependent cellular cytotoxicity (ADCC) by activating effector cells such as natural killer cells or macrophages.

Biological activity of antibodies is known to be determined, to a large extent, by the constant domains or Fc region of the antibody molecule (Uananue and Benacerraf,

Textbook of Immunology, 2nd Edition, Williams & Wilkins, p. 218 (1984)). This includes their ability to activate complement and to mediate antibody-dependent cellular cytotoxicity (ADCC) as effected by leukocytes. Antibodies of different classes and subclasses differ in this respect, as do antibodies from the same subclass but different species; according to the present invention, antibodies of those classes having the desired biological activity are prepared. Preparation of these antibodies involves the selection of antibody constant domains are their incorporation in the humanized antibody by known technique. For example, mouse immunoglobulins of the IgG3 and lgG2a class are capable of activating serum complement upon binding to the target cells which express the cognate antigen, and therefore humanized antibodies which incorporate IgG3 and lgG2a effector functions are desirable for certain therapeutic applications.

In general, mouse antibodies of the IgG2a and IgG3 subclass and occasionally IgG1 can mediate ADCC, and antibodies of the IgG3, IgG2a, and IgM subclasses bind and activate serum complement. Complement activation generally requires the binding of at least two IgG molecules in close proximity on the target cell. However, the binding of only one IgM molecule activates serum complement.

The ability of any particular antibody to mediate lysis of the target cell by complement activation and/or AOCC can be assayed. The cells of interest are grown and labeled in vitro; the antibody is added to the cell culture in combination with either serum complement or immune cells which may be activated by the antigen antibody complexes. Cytolysis of the target cells is detected by the release of label from the lysed cells. In fact, antibodies can be screened using the patient's own serum as a source of complement and/or immune cells. The antibody that is capable of activating complement or mediating ADCC in the in vitro test can then be used therapeutically in that particular patient.

This invention specifically encompasses consensus Fc antibody domains prepared and used according to the teachings of this invention.

Therapeutic and Other Uses of the Antibodies

When used in vivo for therapy, the antibodies of the subject invention are administered to the patient in therapeutically effective amounts (i.e. amounts that have desired therapeutic effect). They will normally be administered parenterally. The dose and dosage regimen will depend upon the degree of the infection, the characteristics of the particular antibody or immunotoxin used, e.g., its therapeutic index, the patient, and the patient's history. Advantageously the antibody or immunotoxin is administered continuously over a period of 1–2 weeks, intravenously to treat cells in the vasculature and subcutaneously and intraperitoneally to treat regional lymph nodes. Optionally, the administration is made during the course of adjunct therapy such as combined cycles of radiation, chemotherapeutic treatment, or administration of tumor necrosis factor, interferon or other cytoprotective or immunomodulatory agent.

For parenteral administration the antibodies will be formulated in a unit dosage injectable form (solution, suspension, emulsion) in association with a pharmaceutically acceptable parenteral vehicle. Such vehicles are inherently nontoxic, and non-therapeutic. Examples of such vehicles are water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Nonaqueous vehicles such as fixed oils and ethyl oleate can also be used. Liposomes may be used as carriers. The vehicle may contain minor amounts of additives such as substances that enhance isotonicity and chemical stability, e.g., buffers and preservatives. The antibodies will typically be formulated in such vehicles at concentrations of about 1 mg/ml to 10 mg/ml.

Use of IgM antibodies may be preferred for certain applications, however IgG molecules by being smaller may be more able than IgM molecules to localize to certain types of infected cells.

There is evidence that complement activation in vivo-leads to a variety of biological effects, including the induction of an inflammatory response and the activation of macrophages (Uananue and Benecerraf, *Textbook of Immunology,* 2nd Edition, Williams & Wilkins, p. 218 (1984)). The increased vasodilation accompanying inflammation may increase the ability of various agents to localize in infected cells. Therefore, antigen-antibody combinations of the type specified by this invention can be used therapeutically in many ways. Additionally, purified antigens (Hakomori, *Ann. Rev. Immunol.* 2:103 (1984)) or anti-idiotypic antibodies (Nepom et al., *Proc. Natl. Acad. Sci.* 81:2864 (1985); Koprowski et al., *Proc. Natl. Acad. Sci.* 81:216 (1984)) relating to such antigens could be used to induce an active immune response in human patients. Such a response includes the formation of antibodies capable of activating human complement and mediating ADCC and by such mechanisms cause infected cell destruction.

Optionally, the antibodies of this invention are useful in passively immunizing patients, as exemplified by the administration of humanized anti-HIV antibodies.

The antibody compositions used in therapy are formulated and dosages established in a fashion consistent with good medical practice taking into account the disorder to be treated, the condition of the individual patient, the site of delivery of the composition, the method of administration and other factors known to practitioners. The antibody compositions are prepared for administration according to the description of preparation of polypeptides for administration, infra.

Deposit of Materials

As described above, cultures of the muMAb4D5 have been deposited with the American Type Culture Collection, 10801 University Blvd., Mauassas, Va., USA (ATCC).

This deposit was made under the provisions of the Budapest Treaty on the international Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of viable cultures for 30 years from the date of the deposit. The organisms will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Genentech, Inc. and ATCC, which assures permanent and unrestricted availability of the progeny of the cultures to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures' availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 USC §122 and the Commissioner's rules pursuant thereto (including 37 CFR §1.12 with particular reference to 886 OG 638).

In respect of those designations in which a European patent is sought, a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample. (Rule 28(4) EPC)

The assignee of the present application has agreed that if the cultures on deposit should die or be lost or destroyed

when cultivated under suitable conditions, they will be promptly replaced on notification with a viable specimen of the same culture. Availability of the deposited strain is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by the constructs deposited, since the deposited embodiments are intended to illustrate only certain aspects of the invention and any constructs that are functionally equivalent are within the scope of this invention. The deposit of material herein does not constitute an admission that the written description herein contained is inadequate to enable the practice of any aspect of the invention, including the best mode thereof, nor is it to be construed as limiting the scope of the claims to the specific illustrations that they represent. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

It is understood that the application of the teachings of the present invention to a specific problem or situation will be within the capabilities of one having ordinary skill in the art in light of the teachings contained herein. Examples of the products of the present invention and representative processes for their isolation, use, and manufacture appear below, but should not be construed to limit the invention.

EXAMPLES

Example 1

Humanization of muMAb4D5

Here we report the chimerization of muMAb4D5 (chMAb4D5) and the rapid and simultaneous humanization of heavy (V_H) and light (V_L) chain variable region genes using a novel "gene conversion mutagenesis" strategy. Eight humanized variants (huMAb4D5) were constructed to probe the importance of several FR residues identified by our molecular modeling or previously proposed to be critical to the conformation of particular CDRs (see Chothia, C. & Lesk, A. M., *J. Mol. Biol.* 196:901–917 (1987); Chothia, C. et al., *Nature* 342:877–883 (1989); Tramontano, A. et al., *J. Mol. Biol.* 215:175–182 (1990)). Efficient transient expression of humanized variants in non-myeloma cells allowed us to rapidly investigate the relationship between binding affinity for $p185^{HER2}$ ECD and anti-proliferative activity against $p185^{HER2}$ overexpressing carcinoma cells.

Materials and Methods

Cloning of Variable Region Genes. The muMAb4D5 V_H and V_L genes were isolated by polymerase chain reaction (PCR) amplification of mRNA from the corresponding hybridoma (Fendly, B. M. et al., *Cancer Res.* 50:1550–1558 (1990)) as described by Orlandi et al. (Orlandi, R. et al., *Proc. Natl. Acad. Sci. USA* 86:3833–3837 (1989)). Amino terminal sequencing of muMAb4D5 V_L and V_H was used to design the sense strand PCR primers, whereas the anti-sense PCR primers were based upon consensus sequences of murine framework residues (Orlandi, R. et al., *Proc. Natl. Acad. Sci. USA* 86:3833–3837 (1989); Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)) incorporating restriction sites for directional cloning shown by underlining and listed after the sequences: V_L sense, 5'-TCC GATATCCAGCTGACCCAGTCTCCA-3' (SEQ. ID NO. 7), EcoRV; V_L anti-sense, 5'-GTTTGATCTCCAGCTT GGTACCHSCDCCGAA-3' (SEQ. ID NO. 8), Asp718; V_H sense, 5'-AGGTSMARCTGCAGSAGTCWGG-3' (SEQ. ID NO. 9), PstI and V_H anti-sense, 5'-TGAGGAGAC GGTGACCGTGGTCCCTTGGCCCCAG-3' (SEQ. ID. NO. 10), BstEII; where H=A or C or T, S=C or G, D=A or G or T, M=A or C, R=A or G and W=A or T. The PCR products were cloned into pUC119 (Vieira, J. & Messing, J., *Methods Enzymol.* 153:3–11 (1987)) and five clones for each variable domain sequenced by the dideoxy method (Sanger, F. et al., *Proc. Natl. Acad. Sci. USA* 74:5463–5467 (1977)).

Molecular Modelling. Models for muMAb4D5 V_H and V_L domains were constructed separately from consensus coordinates based upon seven Fab structures from the Brookhaven protein data bank (entries 1FB4, 2RHE, 2MCP, 3FAB, 1FBJ, 2HFL and 1REI). The Fab fragment KOL (Marquart, M. et al., *J. Mol. Biol.* 141:369–391 (1980)) was first chosen as a template for V_L and V_H domains and additional structures were then superimposed upon this structure using their main chain atom coordinates (INSIGHT program, Siosym Technologies). The distance from the template Cα to the analogous Cα in each of the superimposed structures was calculated for each residue position. If all (or nearly all) Cα—Cα distances for a given residue were ≦1 Å, then that position was included in the consensus structure. In most cases the β-sheet framework residues satisfied these criteria whereas the CDR loops did not. For each of these selected residues the average coordinates for individual N, Cα, C, O and Cβ atoms were calculated and then corrected for resultant deviations from non-standard bond geometry by 50 cycles of energy minimization using the DISCOVER program (Biosym Technologies) with the AMBER forcefield (Weiner, S. J. et al., *J. Amer. Chem. Soc.* 106:765–784 (1984)) and Cα coordinates fixed. The side chains of highly conserved residues, such as the disulfide-bridged cysteine residues, were then incorporated into the resultant consensus structure. Next the sequences of muMAb4D5 V_L and V_H were incorporated starting with the CDR residues and using the tabulations of CDR conformations from Chothia et al. (Chothia, C. et al., *Nature* 342:877–883 (1989)) as a guide. Side-chain conformations were chosen on the basis of Fab crystal structures, rotamer libraries (Ponder, J. W. & Richards, F. M., *J. Mol. Biol.* 193:775–791 (1987)) and packing considerations. Since V_H-COR3 could not be assigned a definite backbone conformation from these criteria, two models were created from a search of similar sized loops using the INSIGHT program. A third model was derived using packing and solvent exposure considerations. Each model was then subjected to 5000 cycles of energy minimization.

In humanizing muMAb4D5, consensus human sequences were first derived from the most abundant subclasses in the sequence compilation of Kabat et al. (Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)), namely V_L κ subgroup I and V_H group III, and a molecular model generated for these sequences using the methods described above. A structure for huMAb4D5 was created by transferring the CDRs from the muMAb4D5 model into the consensus human structure. All huMAb4D5 variants contain human replacements of muMAb4D5 residues at three positions within CDRs as defined by sequence variability (Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)) but notas defined by structural variability (Chothia, C. & Lesk, A. M., *J. Mol. Biol.* 196:901–917 (1987)):

V_L-CDR1 K24R, V_L-CDR2 R54L and V_L-CDR2 T56S. Differences between muMAb4D5 and the human consensus framework residues (FIG. 1) were individually modeled to investigate their possible influence on CDR conformation and/or binding to the $p185^{HER2}$ ECD.

Construction of Chimeric Genes. Genes encoding chMAb4D5 light and heavy chains were separately assembled in previously described phagemid vectors containing ihe human cytomegalovirus enhancer and promoter, a 5' intron and SV40 polyadenylation signal (Gorman, C. M. et al., *DNA & Prot. Engin. Tech.* 2:3–10 (1990)). Briefly, gene segments encoding muMAb4D5 V_L (FIG. 1A) and REI human κ_1 light chain C_L (Palm, W. & Hilschmann, N., *Z. Physiol. Chem.* 356:167–191 (1975)) were precisely joined as were genes for muMAb4D5 V_H (FIG. 1B) and human γ1 constant region (Capon, D. J. et al., *Nature* 337:525–531 (1989)) by simple subcloning (Boyle, A., in *Current Protocols in Molecular Biology,* Chapter 3 (F. A. Ausubel et al., eds., Greene Publishing & Wiley-Interscience, New York, 1990)) and site-directed mutagenesis (Carter, P., in *Mutagenesis: A Practical Approach*, Chapter 1 (IRL Press, Oxford, UK 1991)). The γ1 isotype was chosen as it has been found to be the preferred human isotype for supporting ADCC and complement dependent cytotoxicity using matched sets of chimeric (Brüggemann, M. et al., *J. Exp. Med.* 166:1351–1361 (1987)) or humanized antibodies (Riechmann, L. et al., *Nature* 332:323–327 (1988)). The PCR-generated V_L and V_H fragments (FIG. 1) were subsequently mutagenized so that they faithfully represent the sequence of muMAb4D5 determined at the protein level: V_H Q1E, V_L V_{104}L and T109A (variants are denoted by the amino acid residue and number followed by the replacement amino acid). The human γ1 constant regions are identical to those reported by Ellison et al. (Ellison, J. W. et al., *Nucleic Acids Res.* 13:4071–4079 (1982)) except for the mutations E359D and M361L (Eu numbering, as in Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)) which we installed to convert the antibody from the naturally rare A allotype to the much more common non-A allotype (Tramontano, A. et al., *J. Mol. Biol.* 215:175–182 (1990)). This was an attempt to reduce the risk of anti-allotype antibodies interfering with therapy.

Construction of Humanized Genes. Genes encoding chMAb4D5 light chain and heavy chain Fd fragment (V_H and C_H1 domains) were subcloned together into pUC119 (Vieira, J. & Messing, J., *Methods Enzymol.* 153:3–11 (1987)) to create pAK1 and simultaneously humanized in a single step (FIG. 2). Briefly, sets of 6 contiguous oligonucleotides were designed to humanize V_H and V_L (FIG. 1). These oligonucleotides are 28 to 83 nucleotides in length, contain zero to 19 mismatches to the murine antibody template and are constrained to have 8 or 9 perfectly matched residues at each end to promote efficient annealing and ligation of adjacent oligonucleotides. The sets of V_H and V_L humanization oligonucleotides (5 pmol each) were phosphorylated with either ATP or γ-^{32}P-ATP (Carter, P. *Methods Enzymol.* 154: 382–403 (1987)) and separately annealed with 3.7 pmol of pAK1 template in 40 μl 10 mM Tris-HCl (pH 8.0) and 10 mM $MgCl_2$ by cooling from 100° C. to room temperature over ~30 min. The annealed oligonucleotides were joined by incubation with T4 DNA ligase (12 units; New England Biolabs) in the presence of 2 μl 5 mM ATP and 2 μl 0.1 M DTT for 10 min at 14° C. After electrophoresis on a 6% acrylamide sequencing gel the assembled oligonucleotides were located by autoradiography and recovered by electroelution. The assembled oligonucleotides (~0.3 pmol each) were simultaneously annealed to 0.15 pmol single-stranded deoxyuridine-containing pAK1 prepared according to Kunkel et al. (Kunkel, T. A. et al., *Methods Enzymol.* 154:367–382 (1987)) in 10 μl 40 mM Tris-HCl (pH 7.5) and 16 mM $MgCl_2$ as above. Heteroduplex DNA was constructed by extending the primers with T7 DNA polymerase and transformed into *E. coli* BMH 71–18 mutL as previously described (Carter, P., in *Mutagenesis: A Practical Approach,* Chapter 1 (IRL Press, Oxford, UK 1991)). The resultant phagemid DNA pool was enriched first for huV_L by restriction purification using XhoI and then for huV_H by restriction selection using StuI as described in Carter, P., in *Mutagenesis: A Practical Approach,* Chapter 1 (IRL Press, Oxford, UK 1991); and in Wells, J. A. et al., *Phil. Trans. R. Soc. Lond., A* 317:415–423 (1986). Resultant clones containing both huV_L and huV_H genes were identified by nucleotide sequencing (Sanger, F. et al., *Proc. Natl. Acad. Sci. USA* 74:5463–5467 (1977)) and designated pAK2. Additional humanized variants were generated by site-directed mutagenesis (Carter, P., in *Mutagenesis: A Practical Approach,* Chapter 1 (IRL Press, Oxford, UK 1991)). The muMAb4D5 V_L and V_H gene segments in the transient expression vectors described above were then precisely replaced with their humanized versions.

Expression and Purification of MAb4D5 Variants. Appropriate MAb4D5 light and heavy chain cDNA expression vectors were co-transfected into an adenovirus transformed human embryonic kidney cell line, 293 (Graham, F. L. et al., *J. Gen. Virol.* 36:59–72 (1977)) using a high efficiency procedure (Gorman, C. M. et al., *DNA & Prot. Engin. Tech.* 2:3–10 (1990); Gorman, C., in *DNA Cloning,* vol II, pp 143–190 (D. M. Glover, ed., IRL Press, Oxford, UK 1985)). Media were harvested daily for up to 5 days and the cells re-fed with serum free media. Antibodies were recovered from the media and affinity purified on protein A sepharose CL-4B (Pharmacia) as described by the manufacturer. The eluted antibody was buffer-exchanged into phosphate-buffered saline by G25 gel filtration, concentrated by ultrafiltration (Centriprep-30 or Centricon-100, Amicon), sterile-filtered (Millex-GV, Millipore) and stored at 4° C. The concentration of antibody was determined by using both total immunoglobulin and antigen binding ELISAs. The standard used was huMAb4D5-5, whose concentration had been determined by amino acid composition analysis.

Cell Proliferation Assay. The effect of MAb4D5 variants upon proliferation of the human mammary adenocarcinoma cell line, SK-BR-3, was investigated as previously described (Fendly, B. M. et al., *Cancer Res.* 50:1550–1558 (1990)) using saturating MAb4D5 concentrations.

Affinity Measurements. The antigen binding affinity of MAb4D5 variants was determined using a secreted form of the $p185^{HER2}$ ECD prepared as described in Fendly, B. M. et al., *J. Biol. Resp. Mod.* 9:449–455 (1990). Briefly, antibody and $p185^{HER2}$ ECD were incubated in solution until equilibrium was found to be reached. The concentration of free antibody was then determined by ELISA using immobilized $p185^{HER2}$ ECD and used to calculate affinity (K_d) according to Friguet et al. (Friguet, B. et al., *J. Immunol. Methods* 77:305–319 (1985)).

Results

Humanization of muMAb4D5. The muMAb4D5 V_L and V_H gene segments were first cloned by PCR and sequenced (FIG. 1). The variable genes were then simultaneously humanized by gene conversion mutagenesis using preassembled oligonucleotides (FIG. 2). A 311-mer oligonucle-

otide containing 39 mismatches to the template directed 24 simultaneous amino acid changes required to humanize muMAb4D5 V_L. Humanization of muMAb4D5 V_H required 32 amino acid changes which were installed with a 361-mer containing 59 mismatches to the muMAb4D5 template. Two out of 8 clones sequenced precisely encode huMAb4D5-5, although one of these clones contained a single nucleotide imperfection. The 6 other clones were essentially humanized but contained a small number of errors: <3 nucleotide changes and <1 single nucleotide deletion per kilobase. Additional humanized variants (Table 3) were constructed by site-directed mutagenesis of huMAb4D5-5.

Expression levels of huMAb4D5 variants were in the range of 7 to 15 μg/ml as judged by ELISA using immobilized $p185^{HER2}$ ECD. Successive harvests of five 10 cm plates allowed 200 μg to 500 mg of each variant to be produced in a week. Antibodies affinity purified on protein A gave a single band on a Coomassie blue stained SDS polyacrylamide gel of mobility consistent with the expected M_r of ~150 kDa. Electrophoresis under reducing conditions gave 2 bands consistent with the expected M_r of free heavy (48 kDa) and light (23 kDa) chains (not shown). Amino terminal sequence analysis (10-cycles) gave the mixed sequence expected (see FIG. **1**) from an equimolar combination of light and heavy chains (not shown).

huMAb4D5 Variants. In general, the FR residues were chosen from consensus human sequences (Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)) and CDR residues from muMAb4D5. Additional variants were constructed by replacing selected human residues in huMAb4D5-1 with their muMAb4D5 counterparts. These are V_H residues 71, 73, 78, 93 plus 102 and V_L residues 55 plus 66 identified by our molecular modeling. V_H residue 71 has previously been proposed by others (Tramontano, A. et al., *J. Mol. Biol.* 215:175–182 (1990)) to be critical to the conformation of V_H-CDR2. Amino acid sequence differences between huMAb4D5 variant molecules are shown in Table 3, together with their $p185^{HER2}$ ECD binding affinity and maximal anti-proliferative activities against SK-BR-3 cells. Very similar K_d values were obtained for binding of MAb4D5 variants to either SK-BR-3 cells or to $p185^{HER2}$ ECD (Table 3). However, K_d estimates derived from binding of MAb4D5 variants to $p185^{HER2}$ ECD were more reproducible with smaller standard errors and consumed much smaller quantities of antibody than binding measurements with whole cells.

The most potent humanized variant designed by molecular modeling, huMAb4D5-8, contains 5 FR residues from muMAb4D5. This antibody binds the $p185^{HER2}$ ECD 3-fold more tightly than does muMAb4D5 itself (Table 3) and has comparable anti-proliferative activity with SK-BR-3 cells (FIG. **3**). In contrast, huMAb4D5-1 is the most humanized but least potent muMAb4D5 variant, created by simply installing the muMAb4D5 CDRs into the consensus human sequences. huMAb4D5-1 binds the $p185^{HER2}$ ECD 80-fold less tightly than does the murine antibody and has no detectable anti-proliferative activity at the highest antibody concentration investigated (16 μg/ml).

The anti-proliferative activity of huMAb4D5 variants against $p185^{HER2}$ overexpressing SK-BR-3 cells is not simply correlated with their binding affinity for the $p185^{HER2}$ ECD. For example, installation of three murine residues into the V_H domain of huMAb4D5-2 (D73T, L78A and A93S) to create huMAb4D5-3 does not change the antigen binding affinity but does confer significant anti-proliferative activity (Table 3).

The importance of V_H residue 71 (Tramontano, A. et al., *J. Mol. Biol.* 215:175–182 (1990)) is supported by the observed 5-fold increase in affinity for $p185^{HER2}$ ECD on replacement of R71 in huMAb4D5-1 with the corresponding murine residue, alanine (huMAb4D5-2). In contrast, replacing V_H L78 in huMAb4D5-4 with the murine residue, alanine (huMAb4D5-5), does not significantly change the affinity for the $p185^{HER2}$ ECD or change anti-proliferative activity, suggesting that residue 78 is not of critical functional significance to huMAb4D5 and its ability to interact properly with the extracellular domain of $p185^{HER2}$.

V_L residue 66 is usually a glycine in human and murine κ chain sequences (Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)) but an arginine occupies this position in the muMAb4D5 κ light chain. The side chain of residue 66 is likely to affect the conformation of V_L-CDR1 and V_L-CDR2 and the hairpin turn at 68–69 (FIG. **4**). Consistent with the importance of this residue, the mutation V_L G66R (huMAb4D5-3→huMAb4D5-5) increases the affinity for the $p185^{HER2}$ ECD by 4-fold with a concomitant increase in anti-proliferative activity.

From molecular modeling it appears that the tyrosyl side chain of muMAb4D5 V_L residue 55 may either stabilize the conformation of V_H-CDR3 or provide an interaction at the V_L–V_H interface. The latter function may be dependent upon the presence of V_HY102. In the context of huMAb4D5-5 the mutations V_L E55Y (huMAb4D5-6) and V_H V102Y (huMAb4D5-7) individually increase the affinity for $p185^{HER2}$ ECD by 5-fold and 2-fold respectively, whereas together (huMAb4D5-8) they increase the affinity by 11-fold. This is consistent with either proposed role of V_L Y55 and V_H Y102.

Secondary Immune Function of huMAb4D5-8. MuMAb4D5 inhibits the growth of human breast tumor cells which overexpress $p185^{HER2}$ (Hudziak, R. M. et al., *Molec. Cell. Biol.* 9:1165–1172 (1989)). The antibody, however, does not offer the possibility of direct tumor cytotoxic effects. This possibility does arise in huMAb4D5-8 as a result of its high affinity (Kd_d=0.1 μM) and its human IgG_1 subtype. Table 4 compares the ADCC mediated by huMAb4D5-8 with muMAb4D5 on a normal lung epithelial cell line, WI-38, which expresses a low level of $p185^{HER2}$ and on SK-BR-3, which expresses a high level of $p185^{HER2}$. The results demonstrate that: (1) huMAb4D5 has a greatly enhanced ability to carry out ADCC as compared with its murine parent; and (2) that this activity may be selective for cell types which overexpress $p185^{HER2}$.

Discussion

MuMAb4D5 is potentially useful for human therapy since it is cytostatic towards human breast and ovarian tumor lines overexpressing the HER2-encoded $p185^{HER2}$ receptor-like tyrosine kinase. Since both breast and ovarian carcinomas are chronic diseases it is anticipated that the optimal MAb4D5 variant molecule for therapy will have low immunogenicity and will be cytotoxic rather than solely cytostatic in effect. Humanization of muMAb4D5 should accomplish these goals. We have identified 5 different huMAb4D5 variants which bind tightly to $p185^{HER2}$ ECD ($K_d \leqq 1$ nM) and which have significant anti-proliferative activity (Table 3). Furthermore huMAb4D5-8 but not muMAb4D5 mediates ADCC against human tumor cell lines overexpressing $p185^{HER2}$ in the presence of human effector cells (Table 4) as anticipated for a human γ1 isotype (Brcüggemann, M. et al., *J. Exp. Med.* 166:1351–1361 (1987); Riechmann, L. et al., *Nature* 332:323–327 (1988)).

Rapid humanization of huMAb4D5 was facilitated by the gene conversion mutagenesis strategy developed here using long preassembled oligonucleotides. This method requires less than half the amount of synthetic DNA as does total gene synthesis and does not require convenient restriction sites in the target DNA. Our method appears to be simpler and more reliable than a variant protocol recently reported (Rostapshov, V. M. et al., *FEBS Lett.* 249: 379–382 (1989)). Transient expression of huMAb4D5 in human embryonic kidney 293 cells permitted the isolation of a few hundred micrograms of huMAb4D5 variants for rapid characterization by growth inhibition and antigen binding affinity assays. Furthermore, different combinations of light and heavy chain were readily tested by co-transfection of corresponding cDNA expression vectors.

The crucial role of molecular modeling in the humanization of muMAb4D5 is illustrated by the designed variant huMAb4D5-8 which binds the $p185^{HER2}$ ECD 250-fold more tightly than the simple CDR loop swap variant, huMAb4D5-1. It has previously been shown that the antigen binding affinity of a humanized antibody can be increased by mutagenesis based upon molecular modelling (Riechmann, L. et al., *Nature* 332:323–327 (1988); Queen, C. et al., *Proc. Natl. Acad. Sci. USA* 86:10029–10033 (1989)). Here we have extended this earlier work by others with a designed humanized antibody which binds its antigen 3-fold more tightly than the parent rodent antibody. While this result is gratifying, assessment of the success of the molecular modeling must await the outcome of X-ray structure determination. From analysis of huMAb4D5 variants (Table 3) it is apparent that their anti-proliferative activity is not a simple function of their binding affinity for $p185^{HER2}$ ECD. For example the huMAb4D5–8 variant binds $p185^{HER2}$ 3-fold more tightly than muMAb4D5 but the humanized variant is slightly less potent in blocking the proliferation of SK-SR-3 cells. Additional huMAb4D5 variants are currently being constructed in an attempt to identify residues triggering the anti-proliferative activity and in an attempt to enhance this activity.

In addition to retaining tight receptor binding and the ability to inhibit cell growth, the huMAb4D5-8 also confers a secondary immune function (ADCC). This allows for direct cytotoxic activity of the humanized molecule in the presence of human effector cells. The apparent selectivity of the cytotoxic activity for cell types which overexpress $p185^{HER2}$ allows for the evolution of a straightforward clinic approach to those human cancers characterized by overexpression of the HER2 protooncogene.

TABLE 3

$p185^{HER2}$ ECD binding affinity and anti-proliferative activities of MAb4D5 variants

	V_H Residue*					V_L Residue*			
MAb4D5 cell Variant proliferation‡	71 FR3	73 FR3	78 FR3	93 FR3	102 CDR3	55 CDR2	56 FR3	K_d† nM	Relative
huMAb4D5-1	R	D	L	A	V	E	G	25	102
huMAb4D5-2	Ala	D	L	A	V	E	G	4.7	101
huMAb4D5-3	Ala	Thr	Ala	Ser	V	E	G	4.4	66
huMAb4D5-4	Ala	Thr	L	Ser	V	E	Arg	0.82	56
huMAb4D5-5	Ala	Thre	Ala	Ser	V	E	Arg	1.1	48
huMAb4D5-6	Ala	Thr	Ala	Ser	V	Tyr	Arg	0.22	51
huMAb4D5-7	Ala	Thr	Ala	Ser	Tyr	E	Arg	0.62	53
huMAb4D5-8	Ala	Thr	Ala	Ser	Tyr	Tyr	Arg	0.10	54
muMAb4D5	Ala	Thr	Ala	Ser	Tyr	Tyr	Arg	0.30	37

*Human and murine residues are shown in one letter and three letter amino acid code respectively.
†K_d values for the $p185^{HER2}$ ECD were determined using the method of Friguet et al. (43) and the standard error of each estimate is ≦ ± 10%.
‡Proliferation of SK-BR-3 cells incubated for 96 hr with MAb4D5 variants shown as a percentage of the untreated control as described (Hudziak, R. M. et al., Molec. Cell. Biol. 9: 1165–1172 (1989)). Data represent the maximal anti-proliferative effect for each variant (see FIG. 3A) calculated as the mean of triplicate determinations at a MAb4D5 concentration of 8 μg/ml. Data are all taken from the same experiment with an estimated standard error of ≦ ± 15%.

TABLE 4

Selectivity of antibody dependent tumor cell cytotoxicity mediated by huMAb4D5-8

Effector:Target ratio†	WI-38* muMAb4D5	WI-38* huMAb4D5-8	SK-BR-3 muMAb4D5	SK-BR-3 huMAb4D5-8
A.‡				
25:1	<1.0	9.3	7.5	40.6
12.5:1	<1.0	11.1	4.7	36.8
6.25:1	<1.0	8.9	0.9	35.2
3.13:1	<1.0	8.5	4.6	19.6
B.				
25:1	<1.0	3.1	6.1	33.4
12.5:1	<1.0	1.7	5.5	26.2
6.25:1	1.3	2.2	2.0	21.0
3.13:1	<1.0	0.8	2.4	13.4

*Sensitivity to ADCC of two human cell lines (WI-38, normal lung epithelium; and SK-BR-3, human breast tumor cell line) are compared. WI-38 expresses a low level of $p185^{HER2}$ (0.6 pg per μg cell protein) and SK-BR-3 expresses a high level of $p185^{HER2}$ (64 pg $p185^{HER2}$ per μg cell protein), as determined by ELISA (Fendly et al., J. Biol. Resp. Mod. 9:449–455 (1990)).
†ADCC assays were carried out as described in Brüggemann et al., J. Exp. Med. 166:1351–1361 (1987). Effector to target ratios were of IL-2 activated human peripheral blood lymphocytes to either WI-38 fibroblasts or SK-BR-3 tumor cells in 96-well microtiter plates for 4 hours at 37° C. Values given represent percent specific cell lysis as determined by ^{51}Cr release. Estimated standard error in these quadruplicate determinations was ≦±10%.
‡Monoclonal antibody concentrations used were 0.1 μg/ml (A) and 0.1 μg/ml (B).

Example 2
Schematic Method for Humanizing an Antibody Sequence

This example illustrates one stepwise elaboration of the methods for creating a humanized sequence described

above. It will be understood that not all of these steps are essential to the claimed invention, and that steps may be taken in different order.

1. ascertain a consensus human variable domain amino acid sequence and prepare from it a consensus structural model.
2. prepare model of import (the non-human domain to be humanized) variable domain sequences and note structural differences with respect to consensus human model.
3. identify CDR sequences in human and in import, both by using Kabat (supra, 1987) and crystal structure criteria. If there is any difference in CDR identity from the different criteria, use of crystal structure definition of the CDR, but retain the Kabat residues as important framework residues to import.
4. substitute import CDR sequences for human CDR sequences to obtain initial "humanized" sequence.
5. compare import non-CDR variable domain sequence to the humanized sequence and note divergences.
6. Proceed through the following analysis for each amino acid residue where the import diverges from the humanized.
   a. If the humanized residue represents a residue which is generally highly conserved across all species, use the residue in the humanized sequence. If the residue is not conserved across all species, proceed with the analysis described in 6b.
   b. If the residue is not generally conserved across all species, ask if the residue is generally conserved in humans.
      i. If the residue is generally conserved in humans but the import residue differs, examine the structural models of the import and human sequences and determine if the import residue would be likely to affect the binding or biological activity of the CDRs by considering 1) could it bind antigen directly and 2) could it affect the conformation of the CDR. If the conclusion is that an affect on the CDRs is likely, substitute the import residue. If the conclusion is that a CDR affect is unlikely, leave the humanized residue unchanged.
      ii. If the residue is also not generally conserved in humans, examine the structural models of the import and human sequences and determine if the import residue would be likely to affect the binding or biological activity of the CDRs be considering 1) could it bind antigen directly and 2) could it affect the conformation of the CDR. If the conclusion is that an affect on the CDRs is likely, substitute the import residue. If the conclusion is that a CDR affect is unlikely, proceed to the next step.
         a) Examine the structural models of the import and human sequences and determine if the residue is exposed on the surface of the domain or is buried within. If the residue is exposed, use the residue in the humanized sequence. If the residue is buried, proceed to the next step.
            (i) Examine the structural models of the import and human sequences and determine if the residue is likely to affect the V_L–V_H interface. Residues involved with the interface include: 34L, 36L, 38L, 43L, 33L, 36L, 85L, 87L, 89L, 91L, 96L, 98L, 35H, 37H, 39H, 43H, 45H, 47H, 60H, 91H, 93H, 95H, 100H, and 103H. If no effect is likely, use the residue in the humanized sequence. If some affect is likely, substitute the import residue.
7. Search the import sequence, the consensus sequence and the humanized sequence for glycosylation sites outside the CDRs, and determine if this glycosylation site is likely to have any affect on antigen binding and/or biological activity. If no effect is likely, use the human sequence at that site; if some affect is likely, eliminate the glycosylation site or use the import sequence at that site.
8. After completing the above analysis, determine the planned humanized sequence and prepare and test a sample. If the sample does not bind well to the target antigen, examine the particular residues listed below, regardless of the question of residue identity between the import and humanized residues.
   a. Examine particular peripheral (non-CDR) variable domain residues that may, due to their position, possibly interact directly with a macromolecular antigen, including the following residues (where the * indicates residues which have been found to interact with antigen based on crystal structures):
      i. Variable light domain: 36, 46, 49*, 63–70
      ii. Variable heavy domain: 2, 47*, 68, 70, 73–76.
   b. Examine particular variable domain residues which could interact with, or otherwise affect, the conformation of variable domain CDRs, including the following (not including CDR residues themselves, since it is assumed that, because the CDRs interact with one another, any residue in one CDR could potentially affect the conformation of another CDR residue) (L=LIGHT, H=HEAVY, residues appearing in bold are indicated to be structurally important according the Chothia et al., Nature 342:877 (1989), and residues appearing in italic were altered during humanization by Queen et al. (PDL), Proc. Natl. Acad. Sci. USA 86:10029 (1989) and Proc. Natl. Acad. Sci. USA 88:2869 (1991).):
      i. Variable light domain:
         a) CDR-1 (residues 24L–34L): 2L, 4L, 66L-69L, 71L
         b) CDR-2 (residues 50L–56L): 35L, 46L, 47L, 48L, 49L, 58L, 62L, 64L–66L, 71L, 73L
         c) CDR-3 (residues 89L–97L): 2L, 4L, 36L, 98L, 37H, 45H, 47H, 58H, 60H
      ii. Variable heavy domain:
         a) CDR-1 (residues 26H–35H): 2H, 4H, 24H, 36H, 71H, 73H, 76H, 78H, 92H, 94H
         b) CDR-2 (residues 50H–55H): 49H, 69H, 69H, 71H, 73H, 78H
         c) CDR-3 (residues 95H–102H): examine all residues as possible interaction partners with this loop, because this loop varies in size and conformation much more than the other CDRs.
9. If after step 8 the humanized variable domain still is lacking in desired binding, repeat step 8. In addition, re-investigate any buried residues which might affect the V_L–V_H interface (but which would not directly affect CDR conformation). Additionally, evaluate the accessibility of non-CDR residues to solvent.

Example 3

Engineering a Humanized Bisnecific F(ab')$_2$ Fragment

This example demonstrates the construction of a humanized bispecific antibody (BsF(ab')$_2$v1 by separate *E. coli* expression of each Fab' arm followed by directed chemical coupling in vitro. BsF(ab')$_2$v1 (anti-CD3/anti-p185^{HER2}) was demonstrated to retarget the cytotoxic activity of human

$CD3^+$CTL in vitro against the human breast tumor cell line, SK-BR-3, which overexpresses the $p185^{HER2}$ product of the protooncogene HER2. This example demonstrates the minimalistic humanization strategy of installing as few murine residues as possible into a human antibody in order to recruit antigen-binding affinity and biological properties comparable to that of the murine parent antibody. This strategy proved very successful for the anti-$p185^{HER2}$arm of BsF $(ab')_2$v1. In contrast $BsF(ab')_2$ v1 binds to T cells via its anti-CD3 arm much less efficiently than does the chimeric $BsF(ab')_2$ which contains the variable domains of the murine parent anti-CD3 antibody. Here we have constructed additional $BsF(ab')_2$ fragments containing variant anti-CD3 arms with selected murine residues restored in an attempt to improve antibody binding to T cells. One such variant, Ss $F(ab')_2$v9, was created by replacing six residues in the second hypervariable loop of the anti-CD3 heavy chain variable domain of $BsF(ab')_2$v1 with their counterparts from the murine parent anti-CD3 antibody. $BsF(ab')_2$v9 binds to T cells (Jurkat) much more efficiently than does $BsF(ab')_2$v1 and almost as efficiently as the chimeric $BsF(ab')_2$. This improvement in the efficiency of T cell binding of the humanized $BsF(ab')_2$ is an important step in its development as a potential therapeutic agent for the treatment of $p185^{HER2}$-overexpressing cancers.

Bispecific antibodies (BsAbs) with specificities for tumor-associated antigens and surface markers on immune effector cells have proved effective for retargeting effector cells to kill tumor targets both in vitro and in vivo (reviewed by Fanger, M. W. et al., *Immunol. Today* 10: 92–99 (1989); Fanger, M. W. et al., *Immunol. Today* 12: 51–54 (1991); and Nelson, H., *Cancer Cells* 3: 163–172 (1991)). $BsF(ab')_2$ fragments have often been used in preference to intact BsAbs in retargeted cellular cytotoxicity to avoid the risk of killing innocent bystander cells binding to the Fc region of the antibody. An additional advantage of $BsF(ab')_2$ over intact BsAbs is that they are generally much simpler to prepare free of contaminating monospecific molecules (reviewed by Songsivilai, S. and Lachmann, P. J., *Clin. Exp. Immunol.* 79: 315–321 (1990) and Nolan, O. and O'Kennedy, R., *Biochim. Biophys. Acta* 1040: 1–11 (1990)).

$BsF(ab')_2$ fragments are traditionally constructed by directed chemical coupling of Fab' fragments obtained by limited proteolysis plus mild reduction of the parent rodent monoclonal Ab (Brennan, M. et al., *Science* 229, 81–83 (1985) and Glennie, M. J. et al., *J. Immunol.* 139: 2367–2375 (1987)). One such $BsF(ab')_2$ fragment (anti-glioma associated antigen/anti-CD3) was found to have clinical efficacy in glioma patients (Nitta, T. et al., *Lancet* 335: 368–371 (1990) and another $BsF(ab')_2$ (anti-indium chelate/anti-carcinoembryonic antigen) allowed clinical imaging of colorectal carcinoma (Stickney, D. R. et al., *Antibody, Immunoconj. Radiopharm.* 2: 1–13 (1989)). Future $SsF(ab')_2$ destined for clinical applications are likely to be constructed from antibodies which are either human or at least "humanized" (Riechmann, L. et al., *Nature* 332: 323–327 (1988) to reduce their immunogenicity (Hale, G. et al., *Lancet i:* 1394–1399 (1988)).

Recently a facile route to a fully humanized $BsF(ab')_2$ fragment designed for tumor immunotherapy has been demonstrated (Shalaby, M. R. et al., *J. Exp. Med.* 175: 217–225 (1992)). This approach involves separate *E. coli* expression of each Fab' arm followed by traditional directed chemical coupling in vitro to form the $BsF(ab')_2$. One arm of the $BsF(ab')_2$ was a humanized version (Carter, P. et al., *Proc. Natl. Aced. Sci. USA* (1992a) and Carter, P., et al., *Bio/Technology* 10: 163–167 (1992b)) of the murine monoclonal Ab 4D5 which is directed against the $p185^{HER2}$ product of the protooncogene HER2 (c-erbB-2) (Fendly, B. M. et al. *Cancer Res.* 50: 1550–1558 (1989)). The humanization of the antibody 4D5 is shown in Example 1 of this application. The second arm was a minimalistically humanized anti-CD3 antibody (Shalaby et al. supra) which was created by installing the CDR loops from the variable domains of the murine parent monoclonal Ab UCHT1 (Beverley, P. C. L. and Callard, R. E., *Eur. J. Immunol.* 11: 329–334 (1981)) into the humanized anti-$p185^{HER2}$ antibody. The $BsF(ab')_2$ fragment containing the most potent humanized anti-CD3 variant (v1) was demonstrated by flow cytometry to bind specifically to a tumor target overexpressing $p185^{HER2}$ and to human peripheral blood mononuclear cells carrying CD3. In addition, $BsF(ab')_2$v1 enhanced the cytotoxic effects of activated human CTL 4-fold against SK-SR-3 tumor cells overexpressing $p185^{HER2}$. The example descries efforts to improve the antigen binding affinity of the humanized anti-CD3 arm by the judicious recruitment of a small number of additional murine residues into the minimalistically humanized anti-CD3 variable domains.

Materials and Methods

Construction of Mutations in the Anti-CD3 Variable Region Genes

The construction of genes encoding humanized anti-CD3 variant 1 (v1) variable light (V_1) and heavy (V_H) chain domains in phagemid pUC119 has been described (Shalaby et al. supra). Additional anti-CD3 variants were generated using an efficient site-directed mutagenesis method (Carter, P., *Mutagenesis: a practical approach,* (M. J. McPherson, Ed.), Chapter 1, IRL Press, Oxford, UK (1991)) using mismatched oligonucleotides which either install or remove unique restriction sites. Oligonucleotides used are listed below using lowercase to indicate the targeted mutations. Corresponding coding changes are denoted by the starting amino acid in one letter code followed by the residue numbered according to Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest,* 5^{th} edition, National Institutes of Health, Bethesda, Md., USA (1991), then the replacement amino acid and finally the identity of the anti-CD3 variant:

HX11, 5' GTAGATAAATCCtctAACACAGCCTAtCTGCAAATG 3' (SEQ.ID. NO. 11) V_HK75S, v6;

HX12, 5' GTAGATAAATCCAAAtctACAGCCTAtCTGCAAATG 3' (SEQ.ID. NO. 12) V_H N76S, v7;

HX13, 5' GTAGATAAATCCtcttctACAGCCTAtCTGCAAATG 3' (SEQ.ID. NO. 13) V_H K75S:N76S, v8;

X14, 5' CTTATAAAGGTGTTtCcACCTATaaCcAgAaatTCAAGGatCGTTTCACgATAtcCGTAGATAAATCC 3' (SEO.ID.NO. 14) V_H T57S:A60N:D61Q:S62K:V63F:G65D, v9;

LX6, 5' CTATACCTCCCGTCTgcatTCTGGAGTCCC 3' (SEQ.ID. NO. 15) V_L E55H, v11.

Oliconucleotides HX11, HX12 and HX13 each remove a site for BspMI, whereas LX6 removes a site for XhoI and HX14 installs a site for EcoRV (bold). Anti-CD3 variant v10 was constructed from v9 by site-directed mutagenesis using oligonucleotide HX13. Mutants were verified by dideoxynucleotide sequencing (Sanger, F. et al., *Proc. Natl. Acad, Sci. USA* 74: 5463–5467 (1977)).

E. coli Expression of Fab' Fragments

The expression plasmid, pAK19, for the co-secretion of light chain and heavy chain Fd' fragment of the most preferred humanized anti-$p185^{HER2}$ variant, HuMAb4D5-8, is described in Carter et al., 1992b, supre. Briefly, the Fab' expression unit is bicistronic with both chains under the

transcriptional control of the ohoA promoter. Genes encoding humanized V_L and V_H domains are precisely fused on their 5' side to a gene segment encoding the heat-stable enterotoxin II signal sequence and on their 3' side to human k_1 C_L and IgG1C_H1 constant domain genes, respectively. The C_H1gene is immediately followed by a sequence encoding the hinge sequence CysAlaAla and followed by a bacteriophage λ t_o transcriptional terminator. Fab' expression plasmids for chimeric and humanized anti-CD3 variants (v1 to v4, Shalaby et al.,supra; v6 to v12, this study) were created from pAK19 by precisely replacing anti-p185^{HER2} V_L and V_H gene segments with those encoding murine and corresponding humanized variants of the anti-CD3 antibody, respectively, by sub-cloning and site-directed mutagenesis. The Fab' expression plasmid for the most potent humanized anti-CD3 variant identified in this study (v9) is designated pAK22. The anti-p185^{HER2} Fab' fragment was secreted from *E. coli* K12 strain 25F2 containing plasmid pAK19 grown for 32 to 40 hr at 37° C. in an aerated 10 liter fermentor. The final cell density was 120–150 OD_{550} and the titer of soluble and functional anti-p185^{HER2} Fab' was 1–2 g/liter as judged by antigen binding ELISA (Carter et al., 1992b, suora). Anti-CD3 Fab' variants were secreted from *E. coli* containing corresponding expression plasmids using very similar fermentation protocols. The highest expression titers of chimeric and humanized anti-CD3 variants were 200 mgaliter and 700 mgaliter, respectively, as judged by total immunoglobulin ELISA.

Construction of BsF(ab')$_2$ Fragments

Fab' fragments were directly recovered from *E. coli* fermentation pastes in the free thiol form (Fab'-SH) by affinity purification on Streptococcal protein G at pH 5 in the presence of EDTA (Carter et al., 1992b supra). Thioether linked BsF(ab')$_2$ fragments (anti-p185^{HER2}/anti-CD3) were constructed by the procedure of Glennie et al. supra with the following modifications. Anti-p185^{HER2} Fab'-SH in 100 mM Tris acetate, 5 mM EDTA (pH 5.0) was reacted with 0.1 vol of 40 mM N,N'-1,2-phenylenedimalemide (o-PDM) in dimethyl formamide for ~1.5 hr at 20° C. Excess o-PDM was removed by protein G purification of the Fab' maleimide derivative (Fab'-mal) followed by buffer exchange into 20 mM sodium acetate, 5 mM EDTA (pH 5.3) (coupling buffer) using centriprep-30 concentrators (Amicon). The total concentration of Fab' variants was estimated from the measured absorbance at 280 nm (HuMAb4D5-8 Fab' $e^{0.1}$%=1.56, Carter et al., 1992b, supra). The free thiol content of Fab' preparations was estimated by reaction with 5,5' -dithiobis (2-nitrobenzoic acid) as described by Creighton, T. E., *Protein structure: a practical approach,* (T. E. Creighton, Ed.), Chapter 7, IRL Press, Oxford, UK (1990). Equimolar amounts of anti-p185^{HER2} Fab'-mal (assuming quantitative reaction of Fab'-SH with o-PDM) and each anti-CD3 Fab'-SH variant were coupled together at a combined concentration of 1 to 2.5 mg/ml in the coupling buffer for 14 to 48 hr at 4° C. The coupling reaction was adjusted to 4 mM cysteine at pH 7.0 and incubated for 15 min at 20 ° C. to reduce any unwanted disulfide-linked F(ab')$_2$ formed. These reduction conditions are sufficient to reduce inter-heavy chain disulfide bonds with virtually no reduction of the disulfide between light and heavy chains. Any free thiols generated were then blocked with 50 mM iodoacetamide. BsF(ab')$_2$ was isolated from the coupling reaction by S100-HR (Pharmacia) size exclusion chromatography (2.5 cm×100 cm) in the presence of PBS. The BsF(ab')$_2$ samples were passed through a 0.2 mm filter flash frozen in liquid nitrogen and stored at −70° C.

Flow Cytometric Analysis of F(ab')$_2$Binding to Jurkat Cells

The Jurkat human acute T cell leukemia cell line was purchased from the American Type Culture Collection (Manassas Va.) (ATCC TIB 152) and grown as. recommended by the ATCC. Aliquots of 10^6 Jurkat cells were incubated with appropriate concentrations of BsF(ab')$_2$ (anti-p185^{HER2}/anti-CD3 variant) or control mono-specific anti-p185^{HER2} F(ab')$_2$ in PBS plus 0.1% (w/v) bovine serum albumin and 10 mM sodium azide for 45 min at 4° C. The cells were washed and then incubated with fluorescein-conjugated goat anti-human F(ab')$_2$ (Organon Teknika, West Chester, Pa.) for 45 min at 4° C. Cells were washed and analyzed on a FACScan® (Becton Dickinson and Co., Mountain View, Calif.). Cells (8×10^3) were acquired by list mode and gated by forward light scatter versus side light scatter excluding dead cells and debris.

Results

Design of Humanized anti-CD3 Variants

The most potent humanized anti-CD3 variant previously identified, v1, differs from the murine parent antibody, UCHT1 at 19 out of 107 amino acid residues within V_L and at 37 out of 122 positions within V_H (Shalaby et al.,supra) 1992). Here we recruited back additional murine residues into anti-CD3 v1 in an attempt to improve the binding affinity for CD3. The strategy chosen was a compromise between minimizing both the number of additional murine residues recruited and the number of anti-CD3 variants to be analyzed. We focused our attentions on a few CDR residues which were originally kept as human sequences in our minimalistic humanization regime. Thus human residues in V_H CDR2 of anti-CD3 v1 were replaced en bloc with their murine counterparts to give anti-CD3 v9: T57S:A60N:D61Q:S62K:V63F:G65D (SEQ ID NO:20). Similarly, the human residue E55 in V_L CDR2 of anti-CD3 v_1 was replaced with histidine from the murine anti-CD3 antibody to generate anti-CD3 v11. In addition, V_H framework region (FR) residues 75 and 76 in anti-CD3 v1 were also replaced with their murine counterparts to create anti-CD3 v8: K75S:N76S. V_H residues 75 and 76 are located in a loop close to V_H CDR1 and CDR2 and therefore might influence antigen binding. Additional variants created by combining mutations at these three sites are described below.

Preparation of BsF(ab')$_2$ Fragments

Soluble and functional anti-p185^{HER2} and anti-CD3 Fab' fragments were recovered directly from corresponding *E. coli* fermentation pastes with the single hinge cysteine predominantly in the free thiol form (75–100% Fab'-SH) by affinity purification on Streptococcal protein G at pH 5 in the presence of EDTA (Carter et al., 1992b, supra). Thioether-linked BsF(ab')$_2$ fragments were then constructed by directed coupling using o-PDM as described by Glennie et al., supra. One arm was always the most potent humanized anti-p185^{HER2} variant, HuMAb4D5-8 (Carter et al., 1992a, supra) and the other either a chimeric or humanized variant of the anti-CD3 antibody. Anti-p185^{HER2} Fab'-SH was reacted with o-PDM to form the maleimide derivative (Fab'-mal) and then coupled to the Fab'-SH for each anti-CD3 variant. F(ab')$_2$ was then purified away from unreacted Fab' by size exclusion chromatography as shown for a representative preparation (BsF(ab')$_2$ v8) in data not shown. The F(ab')$_2$ fragment represents ~54% of the total amount of antibody fragments (by mass) as judged by integration of the chromatograph peaks.

SDS-PAGE analysis of this BsF(ab')$_2$v8 preparation under non-reducing conditions gave one major band with the expected mobility (M, ~96 kD) as well as several very minor

bands (data not shown). Amino-terminal sequence analysis of the major band after electroblotting on to polyvinylidene difluoride 76 are located in a loop close to V_H CDR1 and CDR2 and therefore might membrane Matsudaira, P., *J. Biol. Chem.* 262: 10035–10038 (1987) gave the expected mixed sequence from a stoichiometric 1:1 mixture of light and heavy chains (V_L/V_H: D/E, I/V, Q/D, M/L, T/V, D/E, S/S) expected for BsF(ab')$_2$. The amino terminal region of both light chains are identical as are both heavy chains and correspond to consensus human FR sequences. We have previously demonstrated that F(ab')$_2$ constructed by directed chemical coupling carry both anti-p185^{HER2} and anti-CD3 antigen specificities (Shalaby et al., supra). The level of contamination of the BsF(ab')$_2$ with monospecific F(ab')$_2$ iS likely to be very low since mock coupling reactions with either anti-p185^{HER2} w Fab'-mal or anti-CD3 Fab'-SH alone did not yield detectable quantities of F(ab')$_2$. Furthermore the coupling reaction was subjected to a mild reduction step followed by alkylation to remove trace amounts of disulfide-linked F(ab')$_2$ that might be present. SDS-PAGE of the purified F(ab')$_2$ under reducing conditions gave two major bands with electrophoretic mobility and amino terminal sequence anticipated for free light chain and thioether-linked heavy chain dimers.

Scanning LASER densitometry of a o-PDM coupled F(ab')$_2$ preparation suggest that the minor species together represent ~10% of the protein. These minor contaminants were characterized by amino terminal sequence analysis and were tentatively identified on the basis of stoichiometry of light and heavy chain sequences and their electrophoretic mobility (data not shown). These data are consistent with the minor contaminants including imperfect F(ab')$_2$ in which the disulfide bond between light and heavy chains is missing in one or both arms, trace amounts of Fab' and heavy chain thioether-linked to light chain.

Binding of BsF(ab')$_2$ to Jurkat Cells

Binding of BsF(ab')$_2$ containing different anti-CD3 variants to Jurkat cells (human acute T cell leukemia) was investigated by flow cytometry (data not shown). BsF(ab')$_2$v9 binds much more efficiently to Jurkat cells than does our starting molecule, BsF(ab')$_2$vl, and almost as efficiently as the chimeric BsF(ab')$_2$. Installation of additional murine residues into anti-CD3 v9 to create v10 (V_HK75S:N76S) and v12 (V_HK75S:N76S plus V_L E55H) did not further improve binding of corresponding BsF(ab')$_2$to Jurkat cells. Nor did recruitment of these murine residues into anti-CD3 v1 improve Jurkat binding: V_HK75S (v6), V_HN76S (v7), V_HK75S:N76S (V8), V_LE55H (v11) (not shown). BsF(ab')$_2$v9 was chosen for future study since it is amongst the most efficient variants in binding to Jurkat cells and contains fewest murine residues in the humanized anti-CD3 arm. A monospecific anti-p185^{HEE2} F(ab')$_2$ did not show significant binding to Jurkat cells consistent with the interaction being mediated through the anti-CD3 arm.

Discussion

A minimalistic strategy was chosen to humanize the anti-p185^{HER2} (Carter et al., 1992a, supra) and anti-CD3 arms (Shalaby et al., supra) of the BsF(ab')$_2$ in this study in an attempt to minimize the potential immunogenicity of the resulting humanized antibody in the clinic. Thus we tried to install the minimum number of murine CDR and FR residues into the context of consensus human variable domain sequences as required to recruit antigen-binding affinity and biological properties comparable to the murine parent antibody. Molecular modeling was used firstly to predict the murine FR residues which might be important to antigen binding and secondly to predict the murine CDR residues that might not be required. A small number of humanized variants were then constructed to test these predictions.

Our humanization strategy was very successful for the anti-p185^{HER2} antibody where one out of eight humanized variants (HuMAb4D5-8, IgG1) was identified that bound the p185^{HER2} antigen ~3-fold more tightly than the parent murine antibody (Carter et al., 1992a, supra). HuMAb4D5-8 contains a total of five murine FR residues and nine murine CDR residues, including V_H CDR2 residues 60–65, were discarded in favor of human counterparts. In contrast, BsF(ab')$_2$v1 containing the most potent humanized anti-CD3 variant out of four originally constructed (Shalaby et al., supra) binds J6 cells with an affinity (K_d) of 140 nM which is ~70-fold weaker than that of the corresponding chimeric BsF(ab')$_2$.

Here we have restored T cell binding of the humanized anti-CD3 close to that of the chimeric variant by replacing six human residues in V_H CDR2 with their murine counterparts: T57S:A60N:D61Q:S62K:V63F:G65D (anti-CD3 v9, FIG. **5**). It appears more likely that these murine residues enhance antigen binding indirectly by influencing the conformation of residues in the N-terminal part of V_H CDR2 rather than by directly contacting antigen. Firstly, only N-terminal residues in V_H CDR2 (50–58) have been found to contact antigen in one or more of eight crystallographic structures of antibody/antigen complexes (Kabat et al., supra; and Mian, I. S. et al., *J. Mol. Biol* 217: 133–151 (1991), FIG. **5**). Secondly, molecular modeling suggests that residues in the C-terminal part of V_H CDR2 are at least partially buried (FIG. **5**). BsF(ab')$_2$v9 binds to SK-BR-3 breast tumor cells with equal efficiency to BsF(ab')$_2$v1 and chimeric BsF(ab')$_2$ as anticipated since the anti-p185^{HER2} arm is identical in all of these molecules (Shalaby et al., supra, not shown).

Our novel approach to the construction of BsF(ab')$_2$ fragments exploits an *E. coli* expression system which secretes humanized Fab' fragments at gram per liter titers and permits their direct recovery as Fab'-SH (Carter et al., 1992b, supra). Traditional directed chemical coupling of Fab'-SH fragments is then used to form BsF(ab')$_2$ in vitro (Brennan et al.,supra; and Glennie et al., supra). This route to Fab'-SH obviates problems which are inherent in their generation from intact antibodies: differences in susceptibility to proteolysis and nonspecific cleavage resulting in heterogeneity, low yield as well as partial reduction that is not completely selective for the hinge disulfide bonds. The strategy of using *E. coli*-derived Fab'-SH containing a single hinge cysteine abolishes some sources of heterogeneity in BsF(ab')$_2$ preparation such as intra-hinge disulfide formation and contamination with intact parent antibody whilst greatly diminishes others, eg. formation of F(ab')$_3$ fragments.

BsF(ab')2 fragments constructed here were thioether-linked as originally described by Glennie et al., supra with future in vivo testing of these molecules in mind. Thioether bonds, unlike disulfide bonds, are not susceptible to cleavage by trace amounts of thiol, which led to the proposal that thioether-linked F(ab')$_2$ may be more stable than disulfide-linked F(ab')$_2$ in vivo (Glennie et al., supra). This hypothesis is supported by our preliminary pharmacokinetic experiments in normal mice which suggest that thioether-linked BsF(ab')$_2$ v1 has a 3-fold longer plasma residence time than BsF(ab')$_2$ v1 linked by a single disulfide bond. Disulfide and thioether-linked chimeric BsF(ab')$_2$ were found to be indistinguishable in their efficiency of cell binding and in their retargeting of CTL cytotoxicity, which suggests that o-PDM directed coupling does not compromise binding of the

$BsF(ab')_2$ to either antigen (not shown). Nevertheless the nature of the linkage appears not to be critical since a disulfide-linked $BsF(ab')_2$ (murine anti-$p185^{HER2}$/murine anti-CD3) was recently shown by others (Nishimura et al., Int. *J. Cancer* 50: 800–804 (1992) to have potent anti-tumor activity in nude mice. Our previous study (Shalaby et al., supra) together with this one and that of Nishimura, T. et al., supra improve the potential for using $BsF(ab')_2$ in targeted immunotherapy of $p185^{HER2}$-overexpressing cancers in humans.

Example 4

Humanization of an anti-CD18 Antibody

A murine antibody directed against the leukocyte adhesion receptor β-chain (known as the H52 antibody) was humanized following the methods described above. FIGS. **6**A and **6**B provide amino acid sequence comparisons for the murine and humanized antibody light chains and heavy chains.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 26

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
  1               5                  10                  15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn
                 20                  25                  30

Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
                 35                  40                  45

Leu Leu Ile Tyr Ser Ala Ser Phe Leu Glu Ser Gly Val Pro Ser
                 50                  55                  60

Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile
                 65                  70                  75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
                 80                  85                  90

His Tyr Thr Thr Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu
                 95                 1 00                 1 05

Ile Lys Arg Thr
            109
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 120 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
  1               5                  10                  15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys
                 20                  25                  30

Asp Thr Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
                 35                  40                  45

Glu Trp Val Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr
                 50                  55                  60

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser
                 65                  70                  75
```

-continued

```
Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                 80                  85                  90

Thr Ala Val Tyr Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr
                 95                 1 00                 1 05

Ala Met Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
                110                 115                  120
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
  1               5                  10                  15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser
                 20                  25                  30

Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
                 35                  40                  45

Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser
                 50                  55                  60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
                 65                  70                  75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
                 80                  85                  90

Tyr Asn Ser Leu Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu
                 95                 1 00                 1 05

Ile Lys Arg Thr
            109
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 120 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
  1               5                  10                  15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
                 20                  25                  30

Asp Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
                 35                  40                  45

Glu Trp Val Ala Val Ile Ser Glu Asn Gly Ser Asp Thr Tyr Tyr
                 50                  55                  60

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser
                 65                  70                  75

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                 80                  85                  90

Thr Ala Val Tyr Tyr Cys Ala Arg Asp Arg Gly Gly Ala Val Ser
                 95                 1 00                 1 05

Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
                110                 115                  120
```

-continued

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Asp Ile Val Met Thr Gln Ser His Lys Phe Met Ser Thr Ser Val
1 5 10 15

Gly Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asp Val Asn
20 25 30

Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly His Ser Pro Lys
35 40 45

Leu Leu Ile Tyr Ser Ala Ser Phe Arg Tyr Thr Gly Val Pro Asp
50 55 60

Arg Phe Thr Gly Asn Arg Ser Gly Thr Asp Phe Thr Phe Thr Ile
65 70 75

Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln
80 85 90

His Tyr Thr Thr Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu
95 1 00 1 05

Ile Lys Arg Ala
109

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 120 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly
1 5 10 15

Ala Ser Leu Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys
20 25 30

Asp Thr Tyr Ile His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu
35 40 45

Glu Trp Ile Gly Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr
50 55 60

Asp Pro Lys Phe Gln Asp Lys Ala Thr Ile Thr Ala Asp Thr Ser
65 70 75

Ser Asn Thr Ala Tyr Leu Gln Val Ser Arg Leu Thr Ser Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr
95 1 00 1 05

Ala Met Asp Tyr Trp Gly Gln Gly Ala Ser Val Thr Val Ser Ser
110 115 120

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TCCGATATCC AGCTGACCCA GTCTCCA 27

-continued

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GTTTGATCTC CAGCTTGGTA CCHSCDCCGA A 31

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AGGTSMARCT GCAGSAGTCW GG 22

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TGAGGAGACG GTGACCGTGG TCCCTTGGCC CCAG 34

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GTAGATAAAT CCTCTAACAC AGCCTATCTG CAAATG 36

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GTAGATAAAT CCAAATCTAC AGCCTATCTG CAAATG 36

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GTAGATAAAT CCTCTTCTAC AGCCTATCTG CAAATG 36

-continued

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CTTATAAAGG TGTTTCCACC TATAACCAGA AATTCAAGGA TCGTTTCACG 50

ATATCCGTAG ATAAATCC 68

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CTATACCTCC CGTCTGCATT CTGGAGTCCC 30

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu
1 5 10 15

Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile Arg
20 25 30

Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys
35 40 45

Leu Leu Ile Tyr Tyr Thr Ser Arg Leu His Ser Gly Val Pro Ser
50 55 60

Lys Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile
65 70 75

Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln
80 85 90

Gly Asn Thr Leu Pro Trp Thr Phe Ala Gly Gly Thr Lys Leu Glu
95 1 00 1 05

Ile Lys
107

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile Arg
20 25 30

Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys

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                35                  40                  45

 Leu Leu Ile Tyr Tyr Thr Ser Arg Leu Glu Ser Gly Val Pro Ser
                50                  55                  60

 Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile
                65                  70                  75

 Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
                80                  85                  90

 Gly Asn Thr Leu Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu
                95                 1 00                 1 05

 Ile Lys
     107


(2) INFORMATION FOR SEQ ID NO:18:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 107 amino acids
          (B) TYPE: Amino Acid
          (D) TOPOLOGY: Linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

 Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
   1               5                  10                  15

 Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser
                20                  25                  30

 Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
                35                  40                  45

 Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser
                50                  55                  60

 Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
                65                  70                  75

 Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
                80                  85                  90

 Tyr Asn Ser Leu Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu
                95                 1 00                 1 05

 Ile Lys
     107


(2) INFORMATION FOR SEQ ID NO:19:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 122 amino acids
          (B) TYPE: Amino Acid
          (D) TOPOLOGY: Linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

 Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly
   1               5                  10                  15

 Ala Ser Met Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr
                20                  25                  30

 Gly Tyr Thr Met Asn Trp Val Lys Gln Ser His Gly Lys Asn Leu
                35                  40                  45

 Glu Trp Met Gly Leu Ile Asn Pro Tyr Lys Gly Val Ser Thr Tyr
                50                  55                  60

 Asn Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Val Asp Lys Ser
                65                  70                  75

 Ser Ser Thr Ala Tyr Met Glu Leu Leu Ser Leu Thr Ser Glu Asp
                80                  85                  90
```

-continued

```
Ser Ala Val Tyr Tyr Cys Ala Arg Ser Gly Tyr Tyr Gly Asp Ser
                 95                 1 00                 1 05

Asp Trp Tyr Phe Asp Val Trp Gly Ala Gly Thr Thr Val Thr Val
                110                 115                  120

Ser Ser
    122


(2) INFORMATION FOR SEQ ID NO:20:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 122 amino acids
          (B) TYPE: Amino Acid
          (D) TOPOLOGY: Linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
  1               5                  10                  15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Ser Phe Thr
                 20                  25                  30

Gly Tyr Thr Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
                 35                  40                  45

Glu Trp Val Ala Leu Ile Asn Pro Tyr Lys Gly Val Ser Thr Tyr
                 50                  55                  60

Asn Gln Lys Phe Lys Asp Arg Phe Thr Ile Ser Val Asp Lys Ser
                 65                  70                  75

Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                 80                  85                  90

Thr Ala Val Tyr Tyr Cys Ala Arg Ser Gly Tyr Tyr Gly Asp Ser
                 95                 1 00                 1 05

Asp Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val
                110                 115                  120

Ser Ser
    122


(2) INFORMATION FOR SEQ ID NO:21:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 122 amino acids
          (B) TYPE: Amino Acid
          (D) TOPOLOGY: Linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
  1               5                  10                  15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
                 20                  25                  30

Ser Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
                 35                  40                  45

Glu Trp Val Ser Val Ile Ser Gly Asp Gly Gly Ser Thr Tyr Tyr
                 50                  55                  60

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser
                 65                  70                  75

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                 80                  85                  90

Thr Ala Val Tyr Tyr Cys Ala Arg Gly Arg Val Gly Tyr Ser Leu
                 95                 1 00                 1 05

Ser Gly Leu Tyr Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val
                110                 115                  120
```

-continued

Ser Ser
122

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 454 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly
1 5 10 15

Ala Ser Val Lys Ile Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr
20 25 30

Glu Tyr Thr Met His Trp Met Lys Gln Ser His Gly Lys Ser Leu
35 40 45

Glu Trp Ile Gly Gly Phe Asn Pro Lys Asn Gly Gly Ser Ser His
50 55 60

Asn Gln Arg Phe Met Asp Lys Ala Thr Leu Ala Val Asp Lys Ser
65 70 75

Thr Ser Thr Ala Tyr Met Glu Leu Arg Ser Leu Thr Ser Glu Asp
80 85 90

Ser Gly Ile Tyr Tyr Cys Ala Arg Trp Arg Gly Leu Asn Tyr Gly
95 1 00 1 05

Phe Asp Val Arg Tyr Phe Asp Val Trp Gly Ala Gly Thr Thr Val
110 115 120

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
125 130 135

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
140 145 150

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
155 160 165

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
170 175 180

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
185 190 195

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
200 205 210

His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
215 220 225

Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
230 235 240

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
245 250 255

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
260 265 270

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
275 280 285

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
290 295 300

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
305 310 315

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
320 325 330

-continued

```
Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
                335                 340                 345

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
                350                 355                 360

Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
                365                 370                 375

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
                380                 385                 390

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
                395                 400                 405

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                410                 415                 420

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
                425                 430                 435

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
                440                 445                 450

Ser Pro Gly Lys
            454
```

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 469 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr
  1                 5                  10                  15

Gly Val His Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu
                 20                  25                  30

Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Thr Ser Gly
                 35                  40                  45

Tyr Thr Phe Thr Glu Tyr Thr Met His Trp Met Arg Gln Ala Pro
                 50                  55                  60

Gly Lys Gly Leu Glu Trp Val Ala Gly Ile Asn Pro Lys Asn Gly
                 65                  70                  75

Gly Thr Ser His Asn Gln Arg Phe Met Asp Arg Phe Thr Ile Ser
                 80                  85                  90

Val Asp Lys Ser Thr Ser Thr Ala Tyr Met Gln Met Asn Ser Leu
                 95                 1 00                 1 05

Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Trp Arg Gly
                110                 115                 120

Leu Asn Tyr Gly Phe Asp Val Arg Tyr Phe Asp Val Trp Gly Gln
                125                 130                 135

Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
                140                 145                 150

Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr
                155                 160                 165

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
                170                 175                 180

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
                185                 190                 195

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
                200                 205                 210
```

-continued

```
Val Val Thr Val Thr Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr
                215                 220                 225

Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr
                230                 235                 240

Val Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro
                245                 250                 255

Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
                260                 265                 270

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
                275                 280                 285

Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr
                290                 295                 300

Val Asp Gly Met Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
                305                 310                 315

Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr Val
                320                 325                 330

Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
                335                 340                 345

Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
                350                 355                 360

Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
                365                 370                 375

Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
                380                 385                 390

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
                395                 400                 405

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu
                410                 415                 420

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                425                 430                 435

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
                440                 445                 450

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
                455                 460                 465

Ser Pro Gly Lys
            469
```

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 214 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```
Asp Val Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu
  1               5                  10                  15

Gly Asp Arg Val Thr Ile Asn Cys Arg Ala Ser Gln Asp Ile Asn
                 20                  25                  30

Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asn Gly Thr Val Lys
                 35                  40                  45

Leu Leu Ile Tyr Tyr Thr Ser Thr Leu His Ser Gly Val Pro Ser
                 50                  55                  60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile
                 65                  70                  75
```

-continued

```
Ser Asn Leu Asp Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln
                 80                  85                  90

Gly Asn Thr Leu Pro Pro Thr Phe Gly Gly Gly Thr Lys Val Glu
                 95                 1 00                 1 05

Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
                110                 115                  120

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
                125                 130                  135

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val
                140                 145                  150

Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu
                155                 160                  165

Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr
                170                 175                  180

Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu
                185                 190                  195

Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn
                200                 205                  210

Arg Gly Glu Cys
            214
```

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 233 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr
  1                  5                  10                  15

Gly Val His Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu
                 20                  25                  30

Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser
                 35                  40                  45

Gln Asp Ile Asn Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly
                 50                  55                  60

Lys Ala Pro Lys Leu Leu Ile Tyr Tyr Thr Ser Thr Leu His Ser
                 65                  70                  75

Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr
                 80                  85                  90

Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
                 95                 1 00                 1 05

Tyr Cys Gln Gln Gly Asn Thr Leu Pro Pro Thr Phe Gly Gln Gly
                110                 115                  120

Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe
                125                 130                  135

Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser
                140                 145                  150

Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val
                155                 160                  165

Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
                170                 175                  180

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                185                 190                  195
```

-continued

```
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
                200                 205                 210

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr
                215                 220                 225

Lys Ser Phe Asn Arg Gly Glu Cys
                230          233


(2) INFORMATION FOR SEQ ID NO:26:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 122 amino acids
          (B) TYPE: Amino Acid
          (D) TOPOLOGY: Linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
  1               5                  10                  15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Ser Phe Thr
                20                  25                  30

Gly Tyr Thr Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
                35                  40                  45

Glu Trp Val Ala Leu Ile Asn Pro Tyr Lys Gly Val Thr Thr Tyr
                50                  55                  60

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Val Asp Lys Ser
                65                  70                  75

Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                80                  85                  90

Thr Ala Val Tyr Tyr Cys Ala Arg Ser Gly Tyr Tyr Gly Asp Ser
                95                 1 00                1 05

Asp Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val
                110                 115                 120

Ser Ser
    122
```

We claim:

1. A humanized antibody variable domain comprising non-human Complementarity Determining Region (CDR) amino acid residues which bind an antigen incorporated into a human antibody variable domain, and further comprising a Framework Region (FR) amino acid substitution at a site selected from the group consisting of: 4L, 38L, 43L, 44L, 58L, 62L, 65L, 66L, 67L, 68L, 69L, 73L, 85L, 98L, 2H, 4H, 36H, 39H, 43H, 45H, 69H, 70H, 74H, and 92H, utilizing the numbering system set forth in Kabat.

2. The humanized variable domain of claim **1** wherein the substituted residue is the residue found at the corresponding location of the non-human antibody from which the non-human CDR amino acid residues are obtained.

3. The humanized variable domain of claim **1** wherein no human Framework Region (FR) residue other than those set forth in the group has been substituted.

4. The humanized variable domain of claim **1** wherein the human antibody variable domain is a consensus human variable domain.

5. The humanized variable domain of claim **1** wherein the residue at site 4L has been substituted.

6. The humanized variable domain of claim **1** wherein the residue at site 38L has been substituted.

7. The humanized variable domain of claim **1** wherein the residue at site 43L has been substituted.

8. The humanized variable domain of claim **1** wherein the residue at site 44L has been substituted.

9. The humanized variable domain of claim **1** wherein the residue at site 58L has been substituted.

10. The humanized variable domain of claim **1** wherein the residue at site 62L has been substituted.

11. The humanized variable domain of claim **1** wherein the residue at site 65L has been substituted.

12. The humanized variable domain of claim **1** wherein the residue at site 66L has been substituted.

13. The humanized variable domain of claim **1** wherein the residue at site 67L has been substituted.

14. The humanized variable domain of claim **1** wherein the residue at site 68L has been substituted.

15. The humanized variable domain of claim **1** wherein the residue at site 69L has been substituted.

16. The humanized variable domain of claim **1** wherein the residue at site 73L has been substituted.

17. The humanized variable domain of claim **1** wherein the residue at site 85L has been substituted.

18. The humanized variable domain of claim **1** wherein the residue at site 98L has been substituted.

19. The humanized variable domain of claim **1** wherein the residue at site 2H has been substituted.

20. The humanized variable domain of claim **1** wherein the residue at site 4H has been substituted.

21. The humanized variable domain of claim 1 wherein the residue at site 36H has been substituted.

22. The humanized variable domain of claim 1 wherein the residue at site 39H has been substituted.

23. The humanized variable domain of claim 1 wherein the residue at site 43H has been substituted.

24. The humanized variable domain of claim 1 wherein the residue at site 45H has been substituted.

25. The humanized variable domain of claim 1 wherein the residue at site 69H has been substituted.

26. The humanized variable domain of claim 1 wherein the residue at site 70H has been substituted.

27. The humanized variable domain of claim 1 wherein the residue at site 74H has been substituted.

28. The humanized variable domain of claim 1 wherein the residue at site 92H has been substituted.

29. An antibody comprising the humanized variable domain of claim 1.

30. An antibody which binds $p185^{HER2}$ and comprises a humanized antibody variable domain, wherein the humanized antibody variable domain comprises non-human Complementarity Determining Region (CDR) amino acid residues which bind $p185^{HER2}$ incorporated into a human antibody variable domain, and further comprises a Framework Region (FR) amino acid substitution at a site selected from the group consisting of: 4L, 38L, 43L, 44L, 46L, 58L, 62L, 65L, 66L, 67L, 68L, 69L, 73L, 85L, 98L, 2H, 4H, 36H, 39H, 43H, 45H, 69H, 70H, 74H, 75H, 76H, 78H and 92H, utilizing the numbering system set forth in Kabat.

31. The antibody of claim 30 wherein the substituted residue is the residue found at the corresponding location of the non-human antibody from which the non-human CDR amino acid residues are obtained.

32. The antibody of claim 30 wherein no human Framework Region (FR) residue other than those set forth in the group has been substituted.

33. The antibody of claim 30 wherein the human antibody variable domain is a consensus human variable domain.

34. The antibody of claim 30 wherein the residue at site 4L has been substituted.

35. The antibody of claim 30 wherein the residue at site 38L has been substituted.

36. The antibody of claim 30 wherein the residue at site 43L has been substituted.

37. The antibody of claim 30 wherein the residue at site 44L has been substituted.

38. The antibody of claim 30 wherein the residue at site 46L has been substituted.

39. The antibody of claim 30 wherein the residue at site 58L has been substituted.

40. The antibody of claim 30 wherein the residue at site 62L has been substituted.

41. The antibody of claim 30 wherein the residue at site 65L has been substituted.

42. The antibody of claim 30 wherein the residue at site 66L has been substituted.

43. The antibody of claim 30 wherein the residue at site 67L has been substituted.

44. The antibody of claim 30 wherein the residue at site 68L has been substituted.

45. The antibody of claim 30 wherein the residue at site 69L has been substituted.

46. The antibody of claim 30 wherein the residue at site 73L has been substituted.

47. The antibody of claim 30 wherein the residue at site 85L has been substituted.

48. The antibody of claim 30 wherein the residue at site 98L has been substituted.

49. The antibody of claim 30 wherein the residue at site 2H has been substituted.

50. The antibody of claim 30 wherein the residue at site 4H has been substituted.

51. The antibody of claim 30 wherein the residue at site 36H has been substituted.

52. The antibody of claim 30 wherein the residue at site 39H has been substituted.

53. The antibody of claim 30 wherein the residue at site 43H has been substituted.

54. The antibody of claim 30 wherein the residue at site 45H has been substituted.

55. The antibody of claim 30 wherein the residue at site 69H has been substituted.

56. The antibody of claim 30 wherein the residue at site 70H has been substituted.

57. The antibody of claim 30 wherein the residue at site 74H has been substituted.

58. The antibody of claim 30 wherein the residue at site 75H has been substituted.

59. The antibody of claim 30 wherein the residue at site 76H has been substituted.

60. The antibody of claim 30 wherein the residue at site 78H has been substituted.

61. The antibody of claim 30 wherein the residue at site 92H has been substituted.

62. A humanized antibody variable domain comprising non-human Complementarity Determining Region (CDR) amino acid residues which bind an antigen incorporated into a consensus human variable domain, and further comprising an amino acid substitution at a site selected from the group consisting of: 4L, 38L, 43L, 44L, 46L, 58L, 62L, 65L, 66L, 67L, 68L, 69L, 73L, 85L, 98L, 2H, 4H, 36H, 39H, 43H, 45H, 69H, 70H, 74H, 75H, 76H, 78H and 92H, utilizing the numbering system set forth in Kabat.

63. A humanized antibody which lacks immunogenicity compared to a non-human parent antibody upon repeated administration to a human patient in order to treat a chronic disease in that patient, wherein the humanized antibody comprises non-human Complementarity Determining Region (CDR) amino acid residues which bind an antigen incorporated into a human antibody variable domain, and further comprises an amino acid substitution at a site selected from the group consisting of: 4L, 38L, 43L, 44L, 46L, 58L, 62L, 65L, 66L, 67L, 68L, 69L, 73L, 85L, 98L, 2H, 4H, 36H, 39H, 43H, 45H, 69H, 70H, 74H, 75H, 76H, 78H and 92H, utilizing the numbering system set forth in Kabat.

64. A humanized variant of a non-human parent antibody which binds an antigen and comprises a human variable domain comprising the most frequently occurring amino acid residues at each location in all human immunoglobulins of a human heavy chain immunoglobulin subgroup wherein amino acid residues forming Complementarity Determining Regions (CDRs) thereof comprise non-human antibody amino acid residues, and further comprises a Framework Region (FR) substitution where the substituted FR residue: (a) noncovalently binds antigen directly; (b) interacts with a CDR; (c) introduces a glycosylation site which affects the antigen binding or affinity of the antibody; or (d) participates in the V_L–V_H interface by affecting the proximity or orientation of the V_L and V_H regions with respect to one another.

65. The humanized variant of claim 63 which binds the antigen up to 3-fold more in the binding affinity than the parent antibody binds antigen.

66. A humanized antibody heavy chain variable domain comprising non-human Complementarity Determining

Region (CDR) amino acid residues which bind antigen incorporated into a human antibody variable domain, and further comprising a Framework Region (FR) amino acid substitution at a site selected from the group consisting of: 24H, 73H, 76H, 78H, and 93H, utilizing the numbering system set forth in Kabat.

67. The humanized variable domain of claim **66** wherein the substituted residue is the residue found at the corresponding location of the non-human antibody from which the non-human CDR amino acid residues are obtained.

68. The humanized variable domain of claim **66** wherein no human Framework Region (FR) residue other than those set forth in the group has been substituted.

69. The humanized variable domain of claim **66** wherein the human antibody variable domain is a consensus human variable domain.

70. The humanized variable domain of claim **66** wherein the residue at site 24H has been substituted.

71. The humanized variable domain of claim **66** wherein the residue at site 73H has been substituted.

72. The humanized variable domain of claim **66** wherein the residue at site 76H has been substituted.

73. The humanized variable domain of claim **66** wherein the residue at site 78H has been substituted.

74. The humanized variable domain of claim **66** wherein the residue at site 93H has been substituted.

75. The humanized variable domain of claim **66** which further comprises an amino acid substitution at site 71H.

76. The humanized variable domain of claim **66** which further comprises amino acid substitutions at sites 71H and 73H.

77. The humanized variable domain of claim **66** which further comprises amino acid substitutions at sites 71H, 73H and 78H.

78. An antibody comprising the humanized variable domain of claim **66**.

79. A humanized variant of a non-human parent antibody which binds an antigen, wherein the humanized variant comprises Complementarity Determining Region (CDR) amino acid residues of the non-human parent antibody incorporated into a human antibody variable domain, and further comprises Framework Region (FR) substitutions at heavy chain positions 71H, 73H, 78H and 93H, utilizing the numbering system set forth in Kabat.

80. A humanized antibody variable domain comprising non-human Complementarity Determining Region (CDR) amino acid residues which bind an antigen incorporated into a human antibody variable domain, and further comprising a Framework Region (FR) amino acid substitution where the substituted FR residue:

(a) noncovalently binds antigen directly;

(b) interacts with a CDR; or

(c) participates in the V_L–V_H interface by affecting the proximity or orientation of the V_L and V_H regions with respect to one another, and wherein the substituted FR residue is at a site selected from the group consisting of: 4L, 38L, 43L, 44L, 58L, 62L, 65L, 66L, 67L, 68L, 69L, 73L, 85L, 98L, 2H, 4H, 24H, 36H, 39H, 43H, 45H, 69H, 70H, 73H, 74H, 76H, 78H, 92H and 93H, utilizing the numbering system set forth in Kabat.

81. The humanized variable domain of claim **80** wherein the substituted residue is the residue found at the corresponding location of the non-human antibody from which the non-human CDR amino acid residues are obtained.

82. The humanized variable domain of claim **80** wherein no human Framework Region (FR) residue other than those set forth in the group has been substituted.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,407,213 B1
DATED : June 18, 2002
INVENTOR(S) : Carter et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 88,
Line 63, please delete "63" and insert therefor -- 79 --.

Signed and Sealed this

Third Day of December, 2002

JAMES E. ROGAN
Director of the United States Patent and Trademark Office

DATE FILED: 05/28/2010
DOCUMENT NO: 34

14042 U.S. PTO
112105

EV146601565US

PTO/SB/05 (05-05)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office. U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 C.F.R. 1.53(b))

Attorney Docket No.	CARP0001-112
First Inventor	John R. Adair et al.
Title	HUMANISED ANTIBODIES
Express Mail Label No.	EV146 601 565US

11/29/49 U.S. PTO
11/284261
112105

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

1. ☒ **Fee Transmittal Form (e.g., PTO/SB/17)**
   (Submit an original and a duplicate for fee processing)
2. ☐ **Applicant claims small entity status.**
   See 37 CFR 1.27.
3. ☒ **Specification** [*Total Pages* 70]
   Both the claims and abstract must start on a new page
   (For information on the preferred arrangement, see MPEP 608.01(a))
4. ☒ **Drawing(s)** (*35 U.S.C.113*) [*Total Sheets* 18]
5. **Oath or Declaration** [*Total Sheets* 03]
   a. ☐ Newly executed (original or copy)
   b. ☒ **Copy from a prior application (37 CFR 1.63 (d))**
   (for a continuation/divisional with Box 18 completed)
   i. ☐ DELETION OF INVENTOR(S)
   Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b).
6. ☒ **Application Data Sheet.** See 37 CFR 1.76
7. ☐ **CD-ROM or CD-R** in duplicate, large table or Computer Program *(Appendix)*
   ☐ Landscape Table on CD
8. **Nucleotide and/or Amino Acid Sequence Submission**
   (if applicable, items a.-c. are required)
   a. Computer Readable Form (CRF)
      i. ☐ Computer Readable Form (CRF)
      ii. ☒ **Transfer Request (37 CFR 1.821(e))**
   b. Specification Sequence Listing on:
      i. ☐ CD-ROM or CD-R (2 copies); or
      ii. ☒ **Paper Copy**
   c. ☒ **Statements verifying identity of above copies**

ADDRESS TO: **Commissioner for Patents**
P.O. Box 1450
Alexandria VA 22313-1450

ACCOMPANYING APPLICATIONS PARTS

9. ☐ **Assignment Papers** (cover sheet & document(s))
   Name of Assignee _____
10. ☐ **37 C.F.R. 3.73(b) Statement** *(when there is an assignee)* ☒ **Copy of Power of Attorney**
11. ☐ **English Translation Document** *(if applicable)*
12. ☐ **Information Disclosure Statement** (PTO/SB/08 or PTO-1449)
    ☐ Copies of foreign patent documents, publications & other information
13. ☒ **Preliminary Amendment and Request for Interference under 37 C.F.R. § 42.202**
14. ☒ **Return Receipt Postcard** (MPEP 503)
    (Should be specifically itemized)
15. ☒ **Certified Copy of Priority Document Was Received In Parent Application Serial No. 07/743,329, Filed September 17, 1991** *(if foreign priority is claimed)*
16. ☐ **Nonpublication Request** under 35 U.S.C. 122(b)(2)(B)(i). Applicant must attach form PTO/SB/35 or equivalent.
17. ☒ Other: Copy of Change of Correspondence Address – Application dated December 23, 2002, from Application Serial No. 08/846,658, Filed May 1, 1997.

18. If a CONTINUING APPLICATION, *check appropriate box, and supply the requisite information below and in the first sentence of the specification following the title, or in an Application Data Sheet under 37 CFR 1.76:*

☒ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior Application No. Serial No. 08/846,658, Filed May 1, 1997, which is a Continuation of Application Serial No. 08/303,569, Filed September 7, 1994, now U.S. Patent No. 5,859,205, Issued January 12, 1999, which is a File Wrapper Continuation of Application Serial No. 07/743,329, Filed September 17, 1991, Abandoned, which is a 35 U.S.C. 371 National Stage Application of International Application No. PCT/GB90/02017, International Filing Date of December 21, 1990, which claims priority to Great Britain Application No. 8928874.0, Filed December 21, 1989, all applications incorporated by reference herein in their entireties.

Prior application information: *Examiner* Minh Tam B. Davis *Art Unit:* 1642

19. CORRESPONDENCE ADDRESS

☒ The address associated with Customer Number 34132 *OR* ☐ Correspondence address below

Name					
Address					
City		*State*		*Zip Code*	
Country		*Telephone*		*Email address*	

Signature	Doreen Yatko Trujillo	Date	November 21, 2005
Name (Print/Type)	Doreen Yatko Trujillo	*Registration No. (Attorney/Agent)*	35,719

PTO/SB/17 (12-04v2)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

Effective on 12/08/2004.
Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).

FEE TRANSMITTAL for FY 2005

☐ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT	($) 1,000.00

Complete if Known	
Application Number	Not Yet Assigned
Filing Date	November 21, 2005
First Named Inventor	John R. Adair et al.
Examiner Name	Not Yet Assigned
Art Unit	Not Yet Assigned
Attorney Docket No.	CARP0001-112

METHOD OF PAYMENT (check all that apply)

☐ Check ☐ Credit Card ☐ Money Order ☐ None ☐ Other (please identify) : _____

☒ Deposit Account Deposit Account Number: **50-1275** Deposit Account Name: **Cozen O'Connor, P.C.**

For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

☒ Charge fee(s) indicated below ☐ Charge fee(s) indicated below, **except for the filing fee**

☒ Charge any additional fee(s) or underpayments of fee(s) Under 37 CFR 1.16 and 1.17 ☒ Credit any overpayments

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES

	FILING FEES		SEARCH FEES		EXAMINATION FEES		
		Small Entity		Small Entity		Small Entity	
Application Type	Fee ($)	Fee($)	Fee($)	Fee($)	Fee($)	Fee($)	Fees Paid ($)
Utility	300	150	500	250	200	100	$1,000.00
Design	200	100	100	50	130	65	____
Plant	200	100	300	150	160	80	____
Reissue	300	150	500	250	600	300	____
Provisional	200	100	0	0	0	0	____

2. EXCESS CLAIM FEES

Fee Description	Fee ($)	Small Entity Fee ($)
Each claim over 20 (including Reissues)	50	25
Each independent claim over 3 (including Reissues)	200	100
Multiple dependent claims	360	180

Total Claims	Extra Claims	Fee($)	Fee Paid ($)
02 - 20 or HP=	00	x ____	= ____

HP = highest number of total claims paid for, if greater than 20.

Indep. Claims	Extra Claims	Fee($)	Fee Paid ($)
01 - 03 or HP=	00	x ____	= ____

HP = highest number of independent claims paid for, if greater than 3.

Multiple Dependent Claims	
Fee ($)	Fee Paid ($)
____	____

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

Total Sheets	Extra Sheets	Number of each additional 50 or fraction thereof	Fee ($)	Fee Paid ($)
____	- ____ = ____ /50 =	____ (round up to a whole number) x	____ =	____

4. OTHER FEE(S)

	Fees Paid ($)
Non-English Specification, $130 fee (no small entity discount)	____
Other (e.g., late filing surcharge) : ____	____

SUBMITTED BY

Signature	Doreen Yatko Trujillo	Registration No. (Attorney/Agent) 35,719	Telephone (215) 665-5593
Name (Print/Type)	Doreen Yatko Trujillo		Date November 21, 2005

PTO/SB/17 (12-04v2)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

Effective on 12/08/2004.
Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).

FEE TRANSMITTAL for FY 2005

☐ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT	($) 1,000.00

Complete if Known	
Application Number	Not Yet Assigned
Filing Date	November 21, 2005
First Named Inventor	John R. Adair et al.
Examiner Name	Not Yet Assigned
Art Unit	Not Yet Assigned
Attorney Docket No.	CARP0001-112

METHOD OF PAYMENT (check all that apply)

☐ Check ☐ Credit Card ☐ Money Order ☐ None ☐ Other (please identify) : _____

☒ Deposit Account Deposit Account Number: **50-1275** Deposit Account Name: **Cozen O'Connor, P.C.**

For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

☒ Charge fee(s) indicated below ☐ Charge fee(s) indicated below, **except for the filing fee**

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FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES

	FILING FEES		SEARCH FEES		EXAMINATION FEES		
		Small Entity		Small Entity		Small Entity	
Application Type	Fee ($)	Fee($)	Fee($)	Fee($)	Fee($)	Fee($)	Fees Paid ($)
Utility	300	150	500	250	200	100	$1,000.00
Design	200	100	100	50	130	65	____
Plant	200	100	300	150	160	80	____
Reissue	300	150	500	250	600	300	____
Provisional	200	100	0	0	0	0	____

2. EXCESS CLAIM FEES

Fee Description	Fee ($)	Small Entity Fee ($)
Each claim over 20 (including Reissues)	50	25
Each independent claim over 3 (including Reissues)	200	100
Multiple dependent claims	360	180

Total Claims	Extra Claims	Fee($)	Fee Paid ($)
02 - 20 or HP=	00	x ____	= ____

HP = highest number of total claims paid for, if greater than 20.

Indep. Claims	Extra Claims	Fee($)	Fee Paid ($)
01 - 03 or HP=	00	x ____	= ____

HP = highest number of independent claims paid for, if greater than 3.

Multiple Dependent Claims	
Fee ($)	Fee Paid ($)
____	____

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

Total Sheets	Extra Sheets	Number of each additional 50 or fraction thereof	Fee ($)	Fee Paid ($)
____	- ____ = ____ /50 =	____ (round up to a whole number) x	____ =	____

4. OTHER FEE(S)

	Fees Paid ($)
Non-English Specification, $130 fee (no small entity discount)	____
Other (e.g., late filing surcharge) : ____	____

SUBMITTED BY

Signature	Doreen Yatko Trujillo	Registration No. (Attorney/Agent) 35,719	Telephone (215) 665-5593
Name (Print/Type)	Doreen Yatko Trujillo		Date November 21, 2005

HUMANISED ANTIBODIES

Field of the Invention

The present invention relates to humanised antibody molecules, to processes for their production using recombinant DNA technology, and to their therapeutic uses.

The term "humanised antibody molecule" is used to describe a molecule having an antigen binding site derived from an immunoglobulin from a non-human species, and remaining immunoglobulin-derived parts of the molecule being derived from a human immunoglobulin. The antigen binding site typically comprises complementarity determining regions (CDRs) which determine the binding specificity of the antibody molecule and which are carried on appropriate framework regions in the variable domains. There are 3 CDRs (CDR1, CDR2 and CDR3) in each of the heavy and light chain variable domains.

In the description, reference is made to a number of publications by number. The publications are listed in numerical order at the end of the description.

Background of the Invention

Natural immunoglobulins have been known for many years, as have the various fragments thereof, such as the Fab, $(Fab')_2$ and Fc fragments, which can be derived by enzymatic cleavage. Natural immunoglobulins comprise a generally Y-shaped molecule having an antigen-binding site towards the end of each upper arm. The remainder of the structure, and particularly the stem of the Y, mediates the effector functions associated with immunoglobulins.

Natural immunoglobulins have been used in assay, diagnosis and, to a more limited extent, therapy. However, such uses, especially in therapy, were hindered until recently by the polyclonal nature of natural immunoglobulins. A significant step towards the realisation of the potential

of immunoglobulins as therapeutic agents was the discovery of procedures for the production of monoclonal antibodies (MAbs) of defined specificity (1).

However, most MAbs are produced by hybridomas which are fusions of rodent spleen cells with rodent myeloma cells. They are therefore essentially rodent proteins. There are very few reports of the production of human MAbs.

Since most available MAbs are of rodent origin, they are naturally antigenic in humans and thus can give rise to an undesirable immune response termed the HAMA (Human Anti-Mouse Antibody) response. Therefore, the use of rodent MAbs as therapeutic agents in humans is inherently limited by the fact that the human subject will mount an immunological response to the MAb and will either remove it entirely or at least reduce its effectiveness. In practice, MAbs of rodent origin may not be used in patients for more than one or a few treatments as a HAMA response soon develops rendering the MAb ineffective as well as giving rise to undesirable reactions. For instance, OKT3 a mouse IgG2a/k MAb which recognises an antigen in the T-cell receptor-CD3 complex has been approved for use in many countries throughout the world as an immunosuppressant in the treatment of acute allograft rejection [Chatenoud et al (2) and Jeffers et al (3)]. However, in view of the rodent nature of this and other such MAbs, a significant HAMA response which may include a major anti-idiotype component, may build up on use. Clearly, it would be highly desirable to diminish or abolish this undesirable HAMA response and thus enlarge the areas of use of these very useful antibodies.

Proposals have therefore been made to render non-human MAbs less antigenic in humans. Such techniques can be generically termed "humanisation" techniques. These

techniques typically involve the use of recombinant DNA technology to manipulate DNA sequences encoding the polypeptide chains of the antibody molecule.

Early methods for humanising MAbs involved production of chimeric antibodies in which an antigen binding site comprising the complete variable domains of one antibody is linked to constant domains derived from another antibody. Methods for carrying out such chimerisation procedures are described in EP0120694 (Celltech Limited), EP0125023 (Genentech Inc. and City of Hope), EP-A-0 171496 (Res. Dev. Corp. Japan), EP-A-0 173 494 (Stanford University), and WO 86/01533 (Celltech Limited). This latter Celltech application (WO 86/01533) discloses a process for preparing an antibody molecule having the variable domains from a mouse MAb and the constant domains from a human immunoglobulin. Such humanised chimeric antibodies, however, still contain a significant proportion of non-human amino acid sequence, i.e. the complete non-human variable domains, and thus may still elicit some HAMA response, particularly if administered over a prolonged period [Begent et al (ref. 4)].

In an alternative approach, described in EP-A-0239400 (Winter), the complementarity determining regions (CDRs) of a mouse MAb have been grafted onto the framework regions of the variable domains of a human immunoglobulin by site directed mutagenesis using long oligonucleotides. The present invention relates to humanised antibody molecules prepared according to this alternative approach, i.e. CDR-grafted humanised antibody molecules. Such CDR-grafted humanised antibodies are much less likely to give rise to a HAMA response than humanised chimeric antibodies in view of the much lower proportion of non-human amino acid sequence which they contain.

The earliest work on humanising MAbs by CDR-grafting was carried out on MAbs recognising synthetic antigens, such as the NP or NIP antigens. However, examples in which a mouse MAb recognising lysozyme and a rat MAb recognising an antigen on human T-cells were humanised by CDR-grafting have been described by Verhoeyen et al (5) and Riechmann et al (6) respectively. The preparation of CDR-grafted antibody to the antigen on human T cells is also described in WO 89/07452 (Medical Research Council).

In Riechmann et al/Medical Research Council it was found that transfer of the CDR regions alone [as defined by Kabat refs. (7) and (8)] was not sufficient to provide satisfactory antigen binding activity in the CDR-grafted product. Riechmann et al found that it was necessary to convert a serine residue at position 27 of the human sequence to the corresponding rat phenylalanine residue to obtain a CDR-grafted product having improved antigen binding activity. This residue at position 27 of the heavy chain is within the structural loop adjacent to CDR1. A further construct which additionally contained a human serine to rat tyrosine change at position 30 of the heavy chain did not have a significantly altered binding activity over the humanised antibody with the serine to phenylalanine change at position 27 alone. These results indicate that changes to residues of the human sequence outside the CDR regions, in particular in the structural loop adjacent to CDR1, may be necessary to obtain effective antigen binding activity for CDR-grafted antibodies which recognise more complex antigens. Even so the binding affinity of the best CDR-grafted antibodies obtained was still significantly less than the original MAb.

Very recently Queen et al (9) have described the preparation of a humanised antibody that binds to the

interleukin 2 receptor, by combining the CDRs of a murine MAb (anti-Tac) with human immunoglobulin framework and constant regions. The human framework regions were chosen to maximise homology with the anti-Tac MAb sequence. In addition computer modelling was used to identify framework amino acid residues which were likely to interact with the CDRs or antigen, and mouse amino acids were used at these positions in the humanised antibody.

In WO 90/07861 Queen et al propose four criteria for designing humanised immunoglobulins. The first criterion is to use as the human acceptor the framework from a particular human immunoglobulin that is unusually homologous to the non-human donor immunoglobulin to be humanised, or to use a consensus framework from many human antibodies. The second criterion is to use the donor amino acid rather than the acceptor if the human acceptor residue is unusual and the donor residue is typical for human sequences at a specific residue of the framework. The third criterion is to use the donor framework amino acid residue rather than the acceptor at positions immediately adjacent to the CDRs. The fourth criterion is to use the donor amino acid residue at framework positions at which the amino acid is predicted to have a side chain atom within about 3 Å of the CDRs in a three-dimensional immunoglobulin model and to be capable of interacting with the antigen or with the CDRs of the humanised immunoglobulin. It is proposed that criteria two, three or four may be applied in addition or alternatively to criterion one, and may be applied singly or in any combination.

WO 90/07861 describes in detail the preparation of a single CDR-grafted humanised antibody, a humanised antibody having specificity for the p55 Tac protein of the

IL-2 receptor. The combination of all four criteria, as above, were employed in designing this humanised antibody, the variable region frameworks of the human antibody Eu (7) being used as acceptor. In the resultant humanised antibody the donor CDRs were as defined by Kabat et al (7 and 8) and in addition the mouse donor residues were used in place of the human acceptor residues, at positions 27, 30, 48, 66, 67, 89, 91, 94, 103, 104, 105 and 107 in the heavy chain and at positions 48, 60 and 63 in the light chain, of the variable region frameworks. The humanised anti-Tac antibody obtained is reported to have an affinity for p55 of 3×10^9 M^{-1}, about one-third of that of the murine MAb.

We have further investigated the preparation of CDR-grafted humanised antibody molecules and have identified a hierarchy of positions within the framework of the variable regions (i.e. outside both the Kabat CDRs and structural loops of the variable regions) at which the amino acid identities of the residues are important for obtaining CDR-grafted products with satisfactory binding affinity. This has enabled us to establish a protocol for obtaining satisfactory CDR-grafted products which may be applied very widely irrespective of the level of homology between the donor immunoglobulin and acceptor framework. The set of residues which we have identified as being of critical importance does not coincide with the residues identified by Queen et al (9).

Summary of the Invention

Accordingly, in a first aspect the invention provides a CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

In preferred embodiments, the heavy chain framework comprises donor residues at positions 23, 24, 49, 71, 73 and 78 or at positions 23, 24 and 49. The residues at positions 71, 73 and 78 of the heavy chain framework are preferably either all acceptor or all donor residues.

In particularly preferred embodiments the heavy chain framework additionally comprises donor residues at one, some or all of positions 6, 37, 48 and 94. Also it is particularly preferred that residues at positions of the heavy chain framework which are commonly conserved across species, i.e. positions 2, 4, 25, 36, 39, 47, 93, 103, 104, 106 and 107, if not conserved between donor and acceptor, additionally comprise donor residues. Most preferably the heavy chain framework additionally comprises donor residues at positions 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

In addition the heavy chain framework optionally comprises donor residues at one, some or all of positions:
1 and 3,
72 and 76,
69 (if 48 is different between donor and acceptor),
38 and 46 (if 48 is the donor residue),
80 and 20 (if 69 is the donor residue),
67,
82 and 18 (if 67 is the donor residue),
91,
88, and
any one or more of 9, 11, 41, 87, 108, 110 and 112.

In the first and other aspects of the present invention reference is made to CDR-grafted antibody products comprising acceptor framework and donor antigen binding regions. It will be appreciated that the invention is widely applicable to the CDR-grafting of antibodies in

general. Thus, the donor and acceptor antibodies may be derived from animals of the same species and even same antibody class or sub-class. More usually, however, the donor and acceptor antibodies are derived from animals of different species. Typically the donor antibody is a non-human antibody, such as a rodent MAb, and the acceptor antibody is a human antibody.

In the first and other aspects of the present invention, the donor antigen binding region typically comprises at least one CDR from the donor antibody. Usually the donor antigen binding region comprises at least two and preferably all three CDRs of each of the heavy chain and/or light chain variable regions. The CDRs may comprise the Kabat CDRs, the structural loop CDRs or a composite of the Kabat and structural loop CDRs and any combination of any of these. Preferably, the antigen binding regions of the CDR-grafted heavy chain variable domain comprise CDRs corresponding to the Kabat CDRs at CDR2 (residues 50-65) and CDR3 (residues 95-100) and a composite of the Kabat and structural loop CDRs at CDR1 (residues 26-35).

The residue designations given above and elsewhere in the present application are numbered according to the Kabat numbering [refs. (7) and (8)]. Thus the residue designations do not always correspond directly with the linear numbering of the amino acid residues. The actual linear amino acid sequence may contain fewer or additional amino acids than in the strict Kabat numbering corresponding to a shortening of, or insertion into, a structural component, whether framework or CDR, of the basic variable domain structure. For example, the heavy chain variable region of the anti-Tac antibody described by Queen et al (9) contains a single amino acid insert (residue 52a) after residue 52 of CDR2 and a three amino

acid insert (residues 82a, 82b and 82c) after framework residue 82, in the Kabat numbering. The correct Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

The invention also provides in a second aspect a CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 1 and/or 3 and 46 and/or 47. Preferably the CDR grafted light chain of the second aspect comprises donor residues at positions 46 and/or 47.

The invention also provides in a third aspect a CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, 58 and 71.

In a preferred embodiment of the third aspect, the framework comprises donor residues at all of positions 46, 48, 58 and 71.

In particularly preferred embodiments of the second and third aspects, the framework additionally comprises donor residues at positions 36, 44, 47, 85 and 87. Similarly positions of the light chain framework which are commonly conserved across species, i.e. positions 2, 4, 6, 35, 49, 62, 64-69, 98, 99, 101 and 102, if not conserved between donor and acceptor, additionally comprise donor residues. Most preferably the light chain framework additionally comprises donor residues at positions 2, 4, 6, 35, 36, 38, 44, 47, 49, 62, 64-69, 85, 87, 98, 99, 101 and 102.

In addition the framework of the second or third aspects optionally comprises donor residues at one, some or all of positions:
1 and 3,
63,
60 (if 60 and 54 are able to form at potential saltbridge),
70 (if 70 and 24 are able to form a potential saltbridge),
73 and 21 (if 47 is different between donor and acceptor),
37 and 45 (if 47 is different between donor and acceptor),
and
any one or more of 10, 12, 40, 80, 103 and 105.

Preferably, the antigen binding regions of the CDR-grafted light chain variable domain comprise CDRs corresponding to the Kabat CDRs at CDR1 (residue 24-34), CDR2 (residues 50-56) and CDR3 (residues 89-97).

The invention further provides in a fourth aspect a CDR-grafted antibody molecule comprising at least one CDR-grafted heavy chain and at least one CDR-grafted light chain according to the first and second or first and third aspects of the invention.

The humanised antibody molecules and chains of the present invention may comprise: a complete antibody molecule, having full length heavy and light chains; a fragment thereof, such as a Fab, $(Fab')_2$ or FV fragment; a light chain or heavy chain monomer or dimer; or a single chain antibody, e.g. a single chain FV in which heavy and light chain variable regions are joined by a peptide linker; or any other CDR-grafted molecule with the same specificity as the original donor antibody. Similarly the CDR-grafted heavy and light chain variable region may be combined with other antibody domains as appropriate.

Also the heavy or light chains or humanised antibody molecules of the present invention may have attached to them an effector or reporter molecule. For instance, it may have a macrocycle, for chelating a heavy metal atom, or a toxin, such as ricin, attached to it by a covalent bridging structure. Alternatively, the procedures of recombinant DNA technology may be used to produce an immunoglobulin molecule in which the Fc fragment or CH3 domain of a complete immunoglobulin molecule has been replaced by, or has attached thereto by peptide linkage, a functional non-immunoglobulin protein, such as an enzyme or toxin molecule.

Any appropriate acceptor variable region framework sequences may be used having regard to class/type of the donor antibody from which the antigen binding regions are derived. Preferably, the type of acceptor framework used is of the same/similar class/type as the donor antibody. Conveniently, the framework may be chosen to maximise/optimise homology with the donor antibody sequence particularly at positions close or adjacent to the CDRs. However, a high level of homology between donor and acceptor sequences is not important for application of the present invention. The present invention identifies a hierarchy of framework residue positions at which donor residues may be important or desirable for obtaining a CDR-grafted antibody product having satisfactory binding properties. The CDR-grafted products usually have binding affinities of at least 10^5 M^{-1}, preferably at least about 10^8 M^{-1}, or especially in the range 10^8-10^{12} M^{-1}. In principle, the present invention is applicable to any combination of donor and acceptor antibodies irrespective of the level of homology between their sequences. A protocol for applying the invention to any particular donor-acceptor antibody pair is given hereinafter. Examples of human frameworks which may be

used are KOL, NEWM, REI, EU, LAY and POM (refs. 4 and 5) and the like; for instance KOL and NEWM for the heavy chain and REI for the light chain and EU, LAY and POM for both the heavy chain and the light chain.

Also the constant region domains of the products of the invention may be selected having regard to the proposed function of the antibody in particular the effector functions which may be required. For example, the constant region domains may be human IgA, IgE, IgG or IgM domains. In particular, IgG human constant region domains may be used, especially of the IgG1 and IgG3 isotypes, when the humanised antibody molecule is intended for therapeutic uses, and antibody effector functions are required. Alternatively, IgG2 and IgG4 isotypes may be used when the humanised antibody molecule is intended for therapeutic purposes and antibody effector functions are not required, e.g. for simple blocking of lymphokine activity.

However, the remainder of the antibody molecules need not comprise only protein sequences from immunoglobulins. For instance, a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain is fused to a DNA sequence encoding the amino acid sequence of a functional polypeptide such as an effector or reporter molecule.

Preferably the CDR-grafted antibody heavy and light chain and antibody molecule products are produced by recombinant DNA technology.

Thus in further aspects the invention also includes DNA sequences coding for the CDR-grafted heavy and light chains, cloning and expression vectors containing the DNA sequences, host cells transformed with the DNA sequences

and processes for producing the CDR-grafted chains and antibody molecules comprising expressing the DNA sequences in the transformed host cells.

The general methods by which the vectors may be constructed, transfection methods and culture methods are well known per se and form no part of the invention. Such methods are shown, for instance, in references 10 and 11.

The DNA sequences which encode the donor amino acid sequence may be obtained by methods well known in the art. For example the donor coding sequences may be obtained by genomic cloning, or cDNA cloning from suitable hybridoma cell lines. Positive clones may be screened using appropriate probes for the heavy and light chain genes in question. Also PCR cloning may be used.

DNA coding for acceptor, e.g. human acceptor, sequences may be obtained in any appropriate way. For example DNA sequences coding for preferred human acceptor frameworks such as KOL, REI, EU and NEWM, are widely available to workers in the art.

The standard techniques of molecular biology may be used to prepare DNA sequences coding for the CDR-grafted products. Desired DNA sequences may be synthesised completely or in part using oligonucleotide synthesis techniques. Site-directed mutagenesis and polymerase chain reaction (PCR) techniques may be used as appropriate. For example oligonucleotide directed synthesis as described by Jones et al (ref. 20) may be used. Also oligonucleotide directed mutagenesis of a pre-exising variable region as, for example, described by Verhoeyen et al (ref. 5) or Riechmann et al (ref. 6) may be used. Also enzymatic filling in of gapped

oligonucleotides using T_4 DNA polymerase as, for example, described by Queen et al (ref. 9) may be used.

Any suitable host cell/vector system may be used for expression of the DNA sequences coding for the CDR-grafted heavy and light chains. Bacterial e.g. E. coli, and other microbial systems may be used, in particular for expression of antibody fragments such as FAb and $(Fab')_2$ fragments, and especially FV fragments and single chain antibody fragments e.g. single chain FVs. Eucaryotic e.g. mammalian host cell expression systems may be used for production of larger CDR-grafted antibody products, including complete antibody molecules. Suitable mammalian host cells include CHO cells and myeloma or hybridoma cell lines.

Thus, in a further aspect the present invention provides a process for producing a CDR-grafted antibody product comprising:

(a) producing in an expression vector an operon having a DNA sequence which encodes an antibody heavy chain according to the first aspect of the invention;

and/or

(b) producing in an expression vector an operon having a DNA sequence which encodes a complementary antibody light chain according to the second or third aspect of the invention;

(c) transfecting a host cell with the or each vector; and

(d) culturing the transfected cell line to produce the CDR-grafted antibody product.

The CDR-grafted product may comprise only heavy or light chain derived polypeptide, in which case only a heavy chain or light chain polypeptide coding sequence is used to transfect the host cells.
For production of products comprising both heavy and light chains, the cell line may be transfected with two vectors, the first vector may contain an operon encoding a light chain-derived polypeptide and the second vector containing an operon encoding a heavy chain-derived polypeptide. Preferably, the vectors are identical, except in so far as the coding sequences and selectable markers are concerned, so as to ensure as far as possible that each polypeptide chain is equally expressed. Alternatively, a single vector may be used, the vector including the sequences encoding both light chain- and heavy chain-derived polypeptides.

The DNA in the coding sequences for the light and heavy chains may comprise cDNA or genomic DNA or both. However, it is preferred that the DNA sequence encoding the heavy or light chain comprises at least partially, genomic DNA, preferably a fusion of cDNA and genomic DNA.

The present invention is applicable to antibodies of any appropriate specificity. Advantageously, however, the invention may be applied to the humanisation of non-human antibodies which are used for <u>in vivo</u> therapy or diagnosis. Thus the antibodies may be site-specific antibodies such as tumour-specific or cell surface-specific antibodies, suitable for use in <u>in vivo</u> therapy or diagnosis, e.g. tumour imaging. Examples of cell surface-specific antibodies are anti-T cell antibodies, such as anti-CD3, and CD4 and adhesion molecules, such as CR3, ICAM and ELAM. The antibodies may have specificity for interleukins (including lymphokines, growth factors and stimulating factors), hormones and other biologically active compounds, and receptors for any of these. For

example, the antibodies may have specificity for any of the following: Interferons α, β, γ or δ, IL1, IL2, IL3, or IL4, etc., TNF, GCSF, GMCSF, EPO, hGH, or insulin, etc.

The the present invention also includes therapeutic and diagnostic compositions comprising the CDR-grafted products of the invention and uses of such compositions in therapy and diagnosis.

Accordingly in a further aspect the invention provides a therapeutic or diagnostic composition comprising a CDR-grafted antibody heavy or light chain or molecule according to previous aspects of the invention in combination with a pharmaceutically acceptable carrier, diluent or excipient.

Accordingly also the invention provides a method of therapy or diagnosis comprising administering an effective amount of a CDR-grafted antibody heavy or light chain or molecule according to previous aspects of the invention to a human or animal subject.

A preferred protocol for obtaining CDR-grafted antibody heavy and light chains in accordance with the present invention is set out below together with the rationale by which we have derived this protocol. This protocol and rationale are given without prejudice to the generality of the invention as hereinbefore described and defined.

Protocol

It is first of all necessary to sequence the DNA coding for the heavy and light chain variable regions of the donor antibody, to determine their amino acid sequences. It is also necessary to choose appropriate acceptor heavy and light chain variable regions, of known amino acid sequence. The CDR-grafted chain is then designed

starting from the basis of the acceptor sequence. It will be appreciated that in some cases the donor and acceptor amino acid residues may be identical at a particular position and thus no change of acceptor framework residue is required.

1. As a first step donor residues are substituted for acceptor residues in the CDRs. For this purpose the CDRs are preferably defined as follows:

Heavy chain	-	CDR1:	residues 26-35
	-	CDR2:	residues 50-65
	-	CDR3:	residues 95-102
Light chain	-	CDR1:	residues 24-34
	-	CDR2:	residues 50-56
	-	CDR3:	residues 89-97

The positions at which donor residues are to be substituted for acceptor in the framework are then chosen as follows, first of all with respect to the heavy chain and subsequently with respect to the light chain.

2. Heavy Chain

2.1 Choose donor residues at all of positions 23, 24, 49, 71, 73 and 78 of the heavy chain or all of positions 23, 24 and 49 (71, 73 and 78 are always either all donor or all acceptor).

2.2 Check that the following have the same amino acid in donor and acceptor sequences, and if not preferably choose the donor: 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

2.3 To further optimise affinity consider choosing donor residues at one, some or any of:

i. 1, 3
ii. 72, 76
iii. If 48 is different between donor and acceptor sequences, consider 69
iv. If at 48 the donor residue is chosen, consider 38 and 46
v. If at 69 the donor residue is chosen, consider 80 and then 20
vi. 67
vii. If at 67 the donor residue is chosen, consider 82 and then 18
viii. 91
ix. 88
x. 9, 11, 41, 87, 108, 110, 112

3. Light Chain

3.1 Choose donor at 46, 48, 58 and 71

3.2 Check that the following have the same amino acid in donor and acceptor sequences, if not preferably choose donor:

2, 4, 6, 35, 38, 44, 47, 49, 62, 64-69 inclusive, 85, 87, 98, 99, 101 and 102

3.3 To further optimise affinity consider choosing donor residues at one, some or any of:

i. 1, 3
ii. 63

iii. 60, if 60 and 54 are able to form potential saltbridge
iv. 70, if 70 and 24 are able to form potential saltbridge
v. 73, and 21 if 47 is different between donor and acceptor
vi. 37, and 45 if 47 is different between donor and acceptor
vii. 10, 12, 40, 80, 103, 105

Rationale

In order to transfer the binding site of an antibody into a different acceptor framework, a number of factors need to be considered.

1. The extent of the CDRs

The CDRs (Complementary Determining Regions) were defined by Wu and Kabat (refs. 4 and 5) on the basis of an analysis of the variability of different regions of antibody variable regions. Three regions per domain were recognised. In the light chain the sequences are 24-34, 50-56, 89-97 (numbering according to Kabat (ref. 4), Eu Index) inclusive and in the heavy chain the sequences are 31-35, 50-65 and 95-102 inclusive.

When antibody structures became available it became apparent that these CDR regions corresponded in the main to loop regions which extended from the β barrel framework of the light and heavy variable domains. For H1 there was a discrepancy in that the loop was from 26 to 32 inclusive and for H2 the loop was 52 to 56 and for L2 from 50 to 53. However, with the exception of H1 the CDR regions encompassed the loop regions and extended into the β strand

frameworks. In H1 residue 26 tends to be a serine and 27 a phenylalanine or tyrosine, residue 29 is a phenylalanine in most cases. Residues 28 and 30 which are surface residues exposed to solvent might be involved in antigen-binding. A prudent definition of the H1 CDR therefore would include residues 26-35 to include both the loop region and the hypervariable residues 33-35.

It is of interest to note the example of Riechmann et al (ref. 3), who used the residue 31-35 choice for CDR-H1. In order to produce efficient antigen binding, residue 27 also needed to be recruited from the donor (rat) antibody.

2. Non-CDR residues which contribute to antigen binding

By examination of available X-ray structures we have identified a number of residues which may have an effect on net antigen binding and which can be demonstrated by experiment. These residues can be sub-divided into a number of groups.

2.1 Surface residues near CDR [all numbering as in Kabat et al (ref. 7)].

2.1.1. Heavy Chain - Key residues are 23, 71 and 73. Other residues which may contribute to a lesser extent are 1, 3 and 76. Finally 25 is usually conserved but the murine residue should be used if there is a difference.

2.1.2 Light Chain - Many residues close to the CDRs, e.g. 63, 65, 67 and 69 are conserved. If conserved none of the surface residues in the light chain are likely to have a major effect. However, if the murine residue at these positions

is unusual, then it would be of benefit to analyse the likely contribution more closely. Other residues which may also contribute to binding are 1 and 3, and also 60 and 70 if the residues at these positions and at 54 and 24 respectively are potentially able to form a salt bridge i.e. 60 + 54; 70 + 24.

2.2 Packing residues near the CDRs.

2.2.1. Heavy Chain - Key residues are 24, 49 and 78. Other key residues would be 36 if not a tryptophan, 94 if not an arginine, 104 and 106 if not glycines and 107 if not a threonine. Residues which may make a further contribution to stable packing of the heavy chain and hence improved affinity are 2, 4, 6, 38, 46, 67 and 69. 67 packs against the CDR residue 63 and this pair could be either both mouse or both human. Finally, residues which contribute to packing in this region but from a longer range are 18, 20, 80, 82 and 86. 82 packs against 67 and in turn 18 packs against 82. 80 packs against 69 and in turn 20 packs against 80. 86 forms an H bond network with 38 and 46. Many of the mouse-human differences appear minor e.g. Leu-Ile, but could have an minor impact on correct packing which could translate into altered positioning of the CDRs.

2.2.2. Light Chain - Key residues are 48, 58 and 71. Other key residues would be 6 if not glutamine, 35 if not tryptophan, 62 if not phenylalanine or tryosine, 64, 66, 68, 99 and 101 if not glycines and 102 if not a threonine. Residues which make a further contribution are 2, 4, 37, 45 and 47. Finally residues 73 and 21 and 19 may make long distance packing contributions of a minor nature.

2.3. Residues at the variable domain interface between heavy and light chains - In both the light and heavy chains most of the non-CDR interface residues are conserved. If a conserved residue is replaced by a residue of different character, e.g. size or charge, it should be considered for retention as the murine residue.

2.3.1. Heavy Chain - Residues which need to be considered are 37 if the residue is not a valine but is of larger side chain volume or has a charge or polarity. Other residues are 39 if not a glutamine, 45 if not a leucine, 47 if not a tryptophan, 91 if not a phenylalanine or tyrosine, 93 if not an alanine and 103 if not a tryptophan. Residue 89 is also at the interface but is not in a position where the side chain could be of great impact.

2.3.2. Light Chain - Residues which need to be considered are 36, if not a tyrosine, 38 if not a glutamine, 44 if not a proline, 46, 49 if not a tyrosine, residue 85, residue 87 if not a tyrosine and 98 if not a phenylalanine.

2.4. Variable-Constant region interface - The elbow angle between variable and constant regions may be affected by alterations in packing of key residues in the variable region against the constant region which may affect the position of V_L and V_H with respect to one another. Therefore it is worth noting the residues likely to be in contact with the constant region. In the heavy chain the surface residues potentially in contact with the variable region are conserved between mouse and human antibodies therefore the variable region contact residues may influence the V-C interaction. In the light chain the amino acids found at a number of the constant

region contact points vary, and the V & C regions are not in such close proximity as the heavy chain. Therefore the influences of the light chain V-C interface may be minor.

2.4.1. Heavy Chain - Contact residues are 7, 11, 41, 87, 108, 110, 112.

2.4.2. Light Chain - In the light chain potentially contacting residues are 10, 12, 40, 80, 83, 103 and 105.

The above analysis coupled with our considerable practical experimental experience in the CDR-grafting of a number of different antibodies have lead us to the protocol given above.

The present invention is now described, by way of example only, with reference to the accompanying Figures 1 - 13.

Brief Description of the Figures

Figure 1 shows DNA and amino acid sequences of the OKT3 light chain;

Figure 2 shows DNA and amino acid sequences of the OKT3 heavy chain;

Figure 3 shows the alignment of the OKT3 light variable region amino acid sequence with that of the light variable region of the human antibody REI;

Figure 4 shows the alignment of the OKT3 heavy variable region amino acid sequence with that of the heavy variable region of the human antibody KOL;

Figure 5 shows the heavy variable region amino acid sequences of OKT3, KOL and various corresponding CDR grafts;

Figure 6 shows the light variable region amino acid sequences of OKT3, REI and various corresponding CDR grafts;

Figure 7 shows a graph of binding assay results for various grafted OKT3 antibodies'

Figure 8 shows a graph of blocking assay results for various grafted OKT3 antibodies;

Figure 9 shows a similar graph of blocking assay results;

Figure 10 shows similar graphs for both binding assay and blocking assay results;

Figure 11 shows further similar graphs for both binding assay and blocking assay results;

Figure 12 shows a graph of competition assay results for a minimally grafted OKT3 antibody compared with the OKT3 murine reference standard, and

Figure 13 shows a similar graph of competition assay results comparing a fully grafted OKT3 antibody with the murine reference standard.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

EXAMPLE 1

CDR-GRAFTING OF OKT3

MATERIAL AND METHODS

1. INCOMING CELLS

Hybridoma cells producing antibody OKT3 were provided by Ortho (seedlot 4882.1) and were grown up in antibiotic free Dulbecco's Modified Eagles Medium (DMEM) supplemented with glutamine and 5% foetal calf serum, and divided to provide both an overgrown supernatant for evaluation and cells for extraction of RNA. The overgrown supernatant was shown to contain 250 ug/mL murine IgG2a/kappa antibody. The supernatant was negative for murine lambda light chain and IgG1, IgG2b, IgG3, IgA and IgM heavy chain. 20mL of supernatant was assayed to confirm that the antibody present was OKT3.

2. MOLECULAR BIOLOGY PROCEDURES

Basic molecular biology procedures were as described in Maniatis et al (ref. 9) with, in some cases, minor modifications. DNA sequencing was performed as described in Sanger et al (ref. 11) and the Amersham International Plc sequencing handbook. Site directed mutagenesis was as described in Kramer et al (ref. 12) and the Anglian Biotechnology Ltd. handbook. COS cell expression and metabolic labelling studies were as described in Whittle et al (ref. 13)

3. RESEARCH ASSAYS

3.1. ASSEMBLY ASSAYS

Assembly assays were performed on supernatants from transfected COS cells to determine the amount of intact IgG present.

3.1.1. COS CELLS TRANSFECTED WITH MOUSE OKT3 GENES

The assembly assay for intact mouse IgG in COS cell supernatants was an ELISA with the following format:

96 well microtitre plates were coated with F(ab')2 goat anti-mouse IgG Fc. The plates were washed in water and samples added for 1 hour at room temperature. The plates were washed and F(ab')2 goat anti-mouse IgG F(ab')2 (HRPO conjugated) was then added. Substrate was added to reveal the reaction. UPC10, a mouse IgG2a myeloma, was used as a standard.

3.1.2. COS AND CHO CELLS TRANSFECTED WITH CHIMERIC OR CDR-GRAFTED OKT3 GENES

The assembly assay for chimeric or CDR-grafted antibody in COS cell supernatants was an ELISA with the following format:

96 well microtitre plates were coated with F(ab')2 goat anti-human IgG Fc. The plates were washed and samples added and incubated for 1 hour at room temperature. The plates were washed and monoclonal mouse anti-human kappa chain was added for 1 hour at room temperature.

The plates were washed and F(ab')2 goat anti-mouse IgG Fc (HRPO conjugated) was added. Enzyme substrate was added to reveal the reaction. Chimeric B72.3 (IgG4) (ref. 13) was used as a standard. The use of a monoclonal anti-kappa chain in this assay allows grafted antibodies to be read from the chimeric standard.

3.2. ASSAY FOR ANTIGEN BINDING ACTIVITY

Material from COS cell supernatants was assayed for OKT3 antigen binding activity onto CD3 positive cells in a direct assay. The procedure was as follows:

HUT 78 cells (human T cell line, CD3 positive) were maintained in culture. Monolayers of HUT 78 cells were prepared onto 96 well ELISA plates using poly-L-lysine and glutaraldehyde. Samples were added to the monolayers for 1 hour at room temperature.

The plates were washed gently using PBS. F(ab')2 goat anti-human IgG Fc (HRPO conjugated) or F(ab')2 goat anti-mouse IgG Fc (HRPO conjugated) was added as appropriate for humanised or mouse samples. Substrate was added to reveal the reaction.

The negative control for the cell-based assay was chimeric B72.3. The positive control was mouse Orthomune OKT3 or chimeric OKT3, when available. This cell-based assay was difficult to perform, and an alternative assay was developed for CDR-grafted OKT3 which was more sensitive and easier to carry out.

In this system CDR-grafted OKT3 produced by COS cells was tested for its ability to bind to the CD3-positive HPB-ALL (human peripheral blood acute lymphocytic leukemia) cell line. It was also tested for its ability to block the binding of murine OKT3 to these cells. Binding was measured by the following procedure: HPB-ALL cells were harvested from tissue culture. Cells were incubated at 4°C for 1 hour with various dilutions of test antibody, positive control antibody, or negative control antibody. The cells were washed once and incubated at 4°C for 1 hour with an FITC-labelled goat anti-human IgG (Fc-

specific, mouse absorbed). The cells were washed twice and analysed by cytofluorography. Chimeric OKT3 was used as a positive control for direct binding. Cells incubated with mock- transfected COS cell supernatant, followed by the FITC-labelled goat anti-human IgG, provided the negative control. To test the ability of CDR-grafted OKT3 to block murine OKT3 binding, the HPB-ALL cells were incubated at 4°C for 1 hour with various dilutions of test antibody or control antibody. A fixed saturating amount of FITC OKT3 was added. The samples were incubated for 1 hour at 4°C, washed twice and analysed by cytofluorography. FITC-labelled OKT3 was used as a positive control to determine maximum binding. Unlabelled murine OKT3 served as a reference standard for blocking. Negative controls were unstained cells with or without mock-transfected cell supernatant. The ability of the CDR-grafted OKT3 light chain to bind CD3-positive cells and block the binding of murine OKT3 was initially tested in combination with the chimeric OKT3 heavy chain. The chimeric OKT3 heavy chain is composed of the murine OKT3 variable region and the human IgG4 constant region. The chimeric heavy chain gene is expressed in the same expression vector used for the CDR-grafted genes. The CDR-grafted light chain expression vector and the chimeric heavy chain expression vector were co-transfected into COS cells. The fully chimeric OKT3 antibody (chimeric light chain and chimeric heavy chain) was found to be fully capable of binding to CD3 positive cells and blocking the binding of murine OKT3 to these cells.

3.3 DETERMINATION OF RELATIVE BINDING AFFINITY

The relative binding affinities of CDR-grafted

anti-CD3 monoclonal antibodies were determined by competition binding (ref. 6) using the HPB-ALL human T cell line as a source of CD3 antigen, and fluorescein-conjugated murine OKT3 (Fl-OKT3) of known binding affinity as a tracer antibody. The binding affinity of Fl-OKT3 tracer antibody was determined by a direct binding assay in which increasing amounts of Fl-OKT3 were incubated with HPB-ALL ($5x10^5$) in PBS with 5% foetal calf serum for 60 min. at 4°C. Cells were washed, and the fluorescence intensity was determined on a FACScan flow cytometer calibrated with quantitative microbead standards (Flow Cytometry Standards, Research Triangle Park, NC). Fluorescence intensity per antibody molecule (F/P ratio) was determined by using microbeads which have a predetermined number of mouse IgG antibody binding sites (Simply Cellular beads, Flow Cytometry Standards). F/P equals the fluorescence intensity of beads saturated with Fl-OKT3 divided by the number of binding sites per bead. The amount of bound and free Fl-OKT3 was calculated from the mean fluorescence intensity per cell, and the ratio of bound/free was plotted against the number of moles of antibody bound. A linear fit was used to determine the affinity of binding (absolute value of the slope).

For competitive binding, increasing amounts of competitor antibody were added to a sub-saturating dose of Fl-OKT3 and incubated with $5x10^5$ HPB-ALL in 200 Ml of PBS with 5% foetal calf serum, for 60 min at 4°C. The fluorescence intensities of the cells were measured on a FACScan flow cytometer calibrated with quantitative microbead standards. The concentrations of bound and free Fl-OKT3 were calculated. The affinities of competing anti-

bodies were calculated from the equation [X]-[ORT3] = (1/Kx) - (1/Ka), where Ka is the affinity of murine ORT3, Kx is the affinity of competitor X, [ ] is the concentration of competitor antibody at which bound/free binding is R/2, and R is the maximal bound/free binding.

4. cDNA LIBRARY CONSTRUCTION

4.1. mRNA PREPARATION AND cDNA SYNTHESIS

ORT3 producing cells were grown as described above and 1.2 x 10^9 cells harvested and mRNA extracted using the guanidinium/LiCl extraction procedure. cDNA was prepared by priming from Oligo-dT to generate full length cDNA. The cDNA was methylated and EcoR1 linkers added for cloning.

4.2. LIBRARY CONSTRUCTION

The cDNA library was ligated to pSP65 vector DNA which had been EcoR1 cut and the 5′ phosphate groups removed by calf intestinal phosphatase (EcoR1/CIP). The ligation was used to transform high transformation efficiency Escherichia coli (E.coli) HB101. A cDNA library was prepared. 3600 colonies were screened for the light chain and 10000 colonies were screened for the heavy chain.

5. SCREENING

E.coli colonies positive for either heavy or light chain probes were identified by oligonucleotide screening using the oligonucleotides:
5′ TCCAGATGTTAACTGCTCAC for the light chain, which is complementary to a sequence in the mouse kappa constant region, and 5′ CAGGGGCCAGTGGATGGATAGAC for the heavy chain which is complementary to a sequence in the mouse IgG2a constant CH1 domain region. 12 light chain and 9 heavy chain clones

were identified and taken for second round screening. Positive clones from the second round of screening were grown up and DNA prepared. The sizes of the gene inserts were estimated by gel electrophoresis and inserts of a size capable of containing a full length cDNA were subcloned into M13 for DNA sequencing.

6. DNA SEQUENCING

Clones representing four size classes for both heavy and light chains were obtained in M13. DNA sequence for the 5′ untranslated regions, signal sequences, variable regions and 3′ untranslated regions of full length cDNAs [Figures 1(a) and 2(a)] were obtained and the corresponding amino acid sequences predicted [(Figures 1(b) and 2(b)]. In Figure 1(a) the untranslated DNA regions are shown in uppercase, and in both Figures 1 and 2 the signal sequences are underlined.

7. CONSTRUCTION OF cDNA EXPRESSION VECTORS

Celltech expression vectors are based on the plasmid pEE6hCMV (ref. 14). A polylinker for the insertion of genes to be expressed has been introduced after the major immediate early promoter/enhancer of the human Cytomegalovirus (hCMV). Marker genes for selection of the plasmid in transfected eukaryotic cells can be inserted as BamH1 cassettes in the unique BamH1 site of pEE6 hCMV; for instance, the neo marker to provide pEE6 hCMV neo. It is usual practice to insert the neo and gpt markers prior to insertion of the gene of interest, whereas the GS marker is inserted last because of the presence of internal EcoR1 sites in the cassette.

The selectable markers are expressed from the SV40 late promoter which also provides an origin of replication so that the vectors can be used for expression in the COS cell transient expression system.
The mouse sequences were excised from the M13 based vectors described above as EcoR1 fragments and cloned into either pEE6-hCMV-neo for the heavy chain and into EE6-hCMV-gpt for the light chain to yield vectors pJA136 and pJA135 respectively.

8. EXPRESSION OF cDNAS IN COS CELLS

Plasmids pJA135 and pJA136 were co-transfected into COS cells and supernatant from the transient expression experiment was shown to contain assembled antibody which bound to T-cell enriched lymphocytes. Metabolic labelling experiments using ^{35}S methionine showed expression and assembly of heavy and light chains.

9. CONSTRUCTION OF CHIMERIC GENES

Construction of chimeric genes followed a previously described strategy [Whittle et al (ref. 13)]. A restriction site near the 3′ end of the variable domain sequence is identified and used to attach an oligonucleotide adapter coding for the remainder of the mouse variable region and a suitable restriction site for attachment to the constant region of choice.

9.1. LIGHT CHAIN GENE CONSTRUCTION

The mouse light chain cDNA sequence contains an AvaI site near the 3′ end of the variable region [Fig. 1(a)]. The majority of the sequence of the variable region was isolated as a 396 bp. EcoR1-AvaI fragment. An oligonucleotide adapter was designed to replace the remainder of the 3′

region of the variable region from the AvaI site and to include the 5' residues of the human constant region up to and including a unique NarI site which had been previously engineered into the constant region.
A HindIII site was introduced to act as a marker for insertion of the linker.
The linker was ligated to the V_L fragment and the 413 bp EcoRI-NarI adapted fragment was purified from the ligation mixture.
The constant region was isolated as an NarI-BamHI fragment from an M13 clone NW361 and was ligated with the variable region DNA into an EcoRI/BamHI/CIP pSP65 treated vector in a three way reaction to yield plasmid JA143. Clones were isolated after transformation into E.coli and the linker and junction sequences were confirmed by the presence of the HindIII site and by DNA sequencing.

9.2 LIGHT CHAIN GENE CONSTRUCTION - VERSION 2

The construction of the first chimeric light chain gene produces a fusion of mouse and human amino acid sequences at the variable-constant region junction. In the case of the OKT3 light chain the amino acids at the chimera junction are:

.....Leu-Glu-Ile-Asn-Arg/ -/Thr-Val-Ala -Ala
VARIABLE CONSTANT

This arrangement of sequence introduces a potential site for Asparagine (Asn) linked (N-linked) glycosylation at the V-C junction. Therefore, a second version of the chimeric light chain oligonucleotide adapter was designed in which the threonine (Thr), the first amino acid of the human constant region, was replaced with the equivalent amino acid from the mouse constant region, Alanine (Ala).

An internal HindIII site was not included in this adapter, to differentiate the two chimeric light chain genes.
The variable region fragment was isolated as a 376 bp EcoR1-AvaI fragment. The oligonucleotide linker was ligated to NarI cut pNW36I and then the adapted 396bp constant region was isolated after recutting the modified pNW361 with EcoR1. The variable region fragment and the modified constant region fragment were ligated directly into EcoR1/C1P treated pEE6hCMVneo to yield pJA137. Initially all clones examined had the insert in the incorrect orientation. Therefore, the insert was re-isolated and recloned to turn the insert round and yield plasmid pJA141. Several clones with the insert in the correct orientation were obtained and the adapter sequence of one was confirmed by DNA sequencing

9.3. HEAVY CHAIN GENE CONSTRUCTION

9.3.1. CHOICE OF HEAVY CHAIN GENE ISOTYPE

The constant region isotype chosen for the heavy chain was human IgG4.

9.3.2. GENE CONSTRUCTION

The heavy chain cDNA sequence showed a BanI site near the 3′ end of the variable region [Fig. 2(a)]. The majority of the sequence of the variable region was isolated as a 426bp. EcoR1/C1P/BanI fragment. An oligonucleotide adapter was designated to replace the remainder of the 3′ region of the variable region from the BanI site up to and including a unique HindIII site which had been previously engineered into the first two amino acids of the constant region.
The linker was ligated to the V_H fragment and the EcoR1-HindIII adapted fragment was purified from the ligation mixture.

The variable region was ligated to the constant region by cutting pJA91 with EcoR1 and Hind111 removing the intron fragment and replacing it with the V_H to yield pJA142. Clones were isolated after transformation into E.coli JM101 and the linker and junction sequences were confirmed by DNA sequencing. (N.B. The Hind111 site is lost on cloning).

10. CONSTRUCTION OF CHIMERIC EXPRESSION VECTORS

10.1. neo AND gpt VECTORS

The chimeric light chain (version 1) was removed from pJA143 as an EcoR1 fragment and cloned into EcoR1/C1P treated pEE6hCMVneo expression vector to yield pJA145. Clones with the insert in the correct orientation were identified by restriction mapping.

The chimeric light chain (version 2) was constructed as described above.

The chimeric heavy chain gene was isolated from pJA142 as a 2.5Kbp EcoR1/BamH1 fragment and cloned into the EcoR1/Bc11/C1P treated vector fragment of a derivative of pEE6hCMVgpt to yield plasmid pJA144.

10.2. GS SEPARATE VECTORS

GS versions of pJA141 and pJA144 were constructed by replacing the neo and gpt cassettes by a BamH1/Sa11/C1P treatment of the plasmids, isolation of the vector fragment and ligation to a GS-containing fragment from the plasmid pRO49 to yield the light chain vector pJA179 and the heavy chain vector pJA180.

10.3. GS SINGLE VECTOR CONSTRUCTION

Single vector constructions containing the cL (chimeric light), cH (chimeric heavy) and GS genes on one plasmid in the order cL-cH-GS, or cH-cL-GS

and with transcription of the genes being head to tail e.g. cL>cH>GS were constructed. These plasmids were made by treating pJA179 or pJA180 with BamH1/ClP and ligating in a Bgl11/Hind111 hCMV promoter cassette along with either the Hind111/BamH1 fragment from pJA141 into pJA180 to give the cH-cL-GS plasmid pJA182 or the Hind111/BamH1 fragment from pJA144 into pJA179 to give the cL-cH-GS plasmid pJA181.

11. EXPRESSION OF CHIMERIC GENES

11.1. EXPRESSION IN COS CELLS

The chimeric antibody plasmid pJA145 (cL) and pJA144 (cH) were co-transfected into COS cells and supernatant from the transient expression experiment was shown to contain assembled antibody which bound to the HUT 78 human T-cell line. Metabolic labelling experiments using ^{35}S methionine showed expression and assembly of heavy and light chains. However the light chain mobility seen on reduced gels suggested that the potential glycosylation site was being glycosylated. Expression in COS cells in the presence of tunicamycin showed a reduction in size of the light chain to that shown for control chimeric antibodies and the OKT3 mouse light chain. Therefore JA141 was constructed and expressed. In this case the light chain did not show an aberrant mobility or a size shift in the presence or absence of tunicamycin. This second version of the chimeric light chain, when expressed in association with chimeric heavy (cH) chain, produced antibody which showed good binding to HUT 78 cells. In both cases antigen binding was equivalent to that of the mouse antibody.

11.2 EXPRESSION IN CHINESE HAMSTER OVARY (CHO) CELLS

Stable cell lines have been prepared from plasmids PJA141/pJA144 and from pJA179/pJA180, pJA181 and pJA182 by transfection into CHO cells.

12. CDR-GRAFTING

The approach taken was to try to introduce sufficient mouse residues into a human variable region framework to generate antigen binding activity comparable to the mouse and chimeric antibodies.

12.1. VARIABLE REGION ANALYSIS

From an examination of a small database of structures of antibodies and antigen-antibody complexes it is clear that only a small number of antibody residues make direct contact with antigen. Other residues may contribute to antigen binding by positioning the contact residues in favourable configurations and also by inducing a stable packing of the individual variable domains and stable interaction of the light and heavy chain variable domains.

The residues chosen for transfer can be identified in a number of ways:

(a) By examination of antibody X-ray crystal structures the antigen binding surface can be predominantly located on a series of loops, three per domain, which extend from the B-barrel framework.

(b) By analysis of antibody variable domain sequences regions of hypervariability [termed the Complementarity Determining Regions (CDRs) by Wu and Kabat (ref. 5)] can be identified. In the most but not all cases these CDRs correspond to, but extend a short way beyond, the loop regions noted above.

(c) Residues not identified by (a) and (b) may contribute to antigen binding directly or indirectly by affecting antigen binding site topology, or by inducing a stable packing of the individual variable domains and stabilising the inter-variable domain interaction. These residues may be identified either by superimposing the sequences for a given antibody on a known structure and looking at key residues for their contribution, or by sequence alignment analysis and noting "idiosyncratic" residues followed by examination of their structural location and likely effects.

12.1.1. LIGHT CHAIN

Figure 3 shows an alignment of sequences for the human framework region RE1 and the OKT3 light variable region. The structural loops (LOOP) and CDRs (KABAT) believed to correspond to the antigen binding region are marked. Also marked are a number of other residues which may also contribute to antigen binding as described in 13.1(c). Above the sequence in Figure 3 the residue type indicates the spatial location of each residue side chain, derived by examination of resolved structures from X-ray crystallography analysis. The key to this residue type designation is as follows:

N - near to CDR (From X-ray Structures)

P - Packing B - Buried Non-Packing

S - Surface E - Exposed

I - Interface * - Interface

- Packing/Part Exposed

? - Non-CDR Residues which may require to be left as Mouse sequence.

Residues underlined in Figure 3 are amino acids. RE1 was chosen as the human framework because the light chain is a kappa chain and the kappa variable regions show higher homology with the mouse sequences than a lambda light variable region, e.g. KOL (see below). RE1 was chosen in preference to another kappa light chain because the X-ray structure of the light chain has been determined so that a structural examination of individual residues could be made.

12.1.2. HEAVY CHAIN

Similarly Figure 4 shows an alignment of sequences for the human framework region KOL and the OKT3 heavy variable region. The structural loops and CDRs believed to correspond to the antigen binding region are marked. Also marked are a number of other residues which may also contribute to antigen binding as described in 12.1(c). The residue type key and other indicators used in Figure 4 are the same as those used in Figure 3. KOL was chosen as the heavy chain framework because the X-ray structure has been determined to a better resolution than, for example, NEWM and also the sequence alignment of OKT3 heavy variable region showed a slightly better homology to KOL than to NEWM.

12.2. DESIGN OF VARIABLE GENES

The variable region domains were designed with mouse variable region optimal codon usage [Grantham and Perrin (ref. 15)] and used the B72.3 signal sequences [Whittle et al (ref. 13)]. The sequences were designed to be attached to the constant region in the same way as for the chimeric genes described above. Some constructs contained the "Kozak consensus sequence" [Kozak (ref. 16)] directly linked to the 5' of the signal

sequence in the gene. This sequence motif is believed to have a beneficial role in translation initiation in eukaryotes.

12.3. GENE CONSTRUCTION

To build the variable regions, various strategies are available. The sequence may be assembled by using oligonucleotides in a manner similar to Jones et al (ref. 17) or by simultaneously replacing all of the CDRs or loop regions by oligonucleotide directed site specific mutagenesis in a manner similar to Verhoeyen et al (ref. 2). Both strategies were used and a list of constructions is set out in Tables 1 and 2 and Figures 4 and 5. It was noted in several cases that the mutagenesis approach led to deletions and rearrangements in the gene being remodelled, while the success of the assembly approach was very sensitive to the quality of the oligonucleotides.

13. CONSTRUCTION OF EXPRESSION VECTORS

Genes were isolated from M13 or SP65 based intermediate vectors and cloned into pEE6hCMVneo for the light chains and pEE6hCMVgpt for the heavy chains in a manner similar to that for the chimeric genes as described above.

TABLE 1 CDR-GRAFTED GENE CONSTRUCTS

CODE	MOUSE SEQUENCE CONTENT	METHOD OF CONSTRUCTION	KOZAK SEQUENCE -	KOZAK SEQUENCE +
LIGHT CHAIN	ALL HUMAN FRAMEWORK RE1			
121	26-32, 50-56, 91-96 inclusive	SDM and gene assembly	+	n.d.
121A	26-32, 50-56, 91-96 inclusive +1, 3, 46, 47	Partial gene assembly	n.d.	+
121B	26-32, 50-56, 91-96 inclusive + 46, 47	Partial gene assembly	n.d.	+
221	24-24, 50-56, 91-96 inclusive	Partial gene assembly	+	+
221A	24-34, 50-56, 91-96 inclusive +1, 3, 46, 47	Partial gene assembly	+	+
221B	24-34, 50-56, 91-96 inclusive +1, 3	Partial gene assembly	+	+
221C	24-34, 50-56, 91-96 inclusive	Partial gene assembly	+	+
HEAVY CHAIN	ALL HUMAN FRAMEWORK KOL			
121	26-32, 50-56, 95-100B inclusive	Gene assembly	n.d.	+
131	26-32, 50-58, 95-100B inclusive	Gene assembly	n.d.	+
141	26-32, 50-65, 95-100B inclusive	Partial gene assembly	+	n.d.
321	26-35, 50-56, 95-100B inclusive	Partial gene assembly	+	n.d.
331	26-35, 50-58, 95-100B inclusive	Partial gene assembly	+	
		Gene assembly		+
341	26-35, 50-65, 95-100B inclusive	SDM	+	
		Partial gene assembly		+
341A	26-35, 50-65, 95-100B inclusive +6, 23, 24, 48, 49, 71, 73, 76, 78, 88, 91 (+63 = human)	Gene assembly	n.d.	+
341B	26-35, 50-65, 95-100B inclusive + 48, 49, 71, 73, 76, 78, 88, 91 (+63 + human)	Gene assembly	n.d.	+

KEY

n.d.	not done
SDM	Site directed mutagenesis
Gene assembly	Variable region assembled entirely from oligonucleotides
Partial gene assembly	Variable region assembled by combination of restriction fragments either from other genes originally created by SDM and gene assembly or by oligonucleotide assembly of part of the variable region and reconstruction with restriction fragments from other genes originally created by SDM and gene assembly

14. EXPRESSION OF CDR-GRAFTED GENES

14.1. PRODUCTION OF ANTIBODY CONSISTING OF GRAFTED LIGHT (gL) CHAINS WITH MOUSE HEAVY (mH) OR CHIMERIC HEAVY (cH) CHAINS

All gL chains, in association with mH or cH produced reasonable amounts of antibody. Insertion of the Kozak consensus sequence at a position 5′ to the ATG (kgL constructs) however, led to a 2-5 fold improvement in net expression. Over an extended series of experiments expression levels were raised from approximately 200ng/ml to approximately 500 ng/ml for kgL/cH or kgL/mH combinations.

When direct binding to antigen on HUT 78 cells was measured, a construct designed to include mouse sequence based on loop length (gL121) did not lead to active antibody in association with mH or cH. A construct designed to include mouse sequence based on Kabat CDRs (gL221) demonstrated some weak binding in association with mH or cH. However, when framework residues 1, 3, 46, 47 were changed from the human to the murine OKT3 equivalents based on the arguments outlined in Section 12.1 antigen binding was demonstrated when both of the new constructs, which were termed 121A and 221A were co-expressed with cH. When the effects of these residues were examined in more detail, it appears that residues 1 and 3 are not major contributing residues as the product of the gL221B gene shows little detectable binding activity in association with cH. The light chain product of gL221C, in which mouse sequences are present at 46 and 47, shows good binding activity in association with cH.

14.2 PRODUCTION OF ANTIBODY CONSISTING OF GRAFTED HEAVY (gH) CHAINS WITH MOUSE LIGHT (mL) OR CHIMERIC LIGHT (cL) CHAINS

Expression of the gH genes proved to be more difficult to achieve than for gL. First, inclusion of the Kozak sequence appeared to have no marked effect on expression of gH genes. Expression appears to be slightly improved but not to the same degree as seen for the grafted light chain.

Also, it proved difficult to demonstrate production of expected quantities of material when the loop choice (amino acid 26-32) for CDR1 is used, e.g. gH121, 131, 141 and no conclusions can be drawn about these constructs.

Moreover, co-expression of the gH341 gene with cL or mL has been variable and has tended to produce lower amounts of antibody than the cH/cL or mH/mL combinations. The alterations to gH341 to produce gH341A and gH341B lead to improved levels of expression.

This may be due either to a general increase in the fraction of mouse sequence in the variable region, or to the alteration at position 63 where the residue is returned to the human amino acid Valine (Val) from Phenylalanine (Phe) to avoid possible internal packing problems with the rest of the human framework. This arrangement also occurs in gH331 and gH321.

When gH321 or gH331 were expressed in association with cL, antibody was produced but antibody binding activity was not detected.

When the more conservative gH341 gene was used antigen binding could be detected in association with cL or mL, but the activity was only marginally above the background level.

When further mouse residues were substituted based on the arguments in 12.1, antigen binding could be clearly demonstrated for the antibody produced when kgH341A and kgH341B were expressed in association with cL.

14.3 PRODUCTION OF FULLY CDR-GRAFTED ANTIBODY

The kgL221A gene was co-expressed with kgH341, kgH341A or kgH341B. For the combination kgH221A/kgH341 very little material was produced in a normal COS cell expression.

For the combinations kgL221A/kgH341A or kgH221A/kgH341B amounts of antibody similar to gL/cH was produced.

In several experiments no antigen binding activity could be detected with kgH221A/gH341 or kgH221A/kgH341 combinations, although expression levels were very low.

Antigen binding was detected when kgL221A/kgH341A or kgH221A/kgH341B combinations were expressed. In the case of the antibody produced from the kgL221A/kgH341A combination the antigen binding was very similar to that of the chimeric antibody.

An analysis of the above results is given below.

15. DISCUSSION OF CDR-GRAFTING RESULTS

In the design of the fully humanised antibody the aim was to transfer the minimum number of mouse amino acids that would confer antigen binding onto a human antibody framework.

15.1. LIGHT CHAIN

15.1.1. EXTENT OF THE CDRs

For the light chain the regions defining the loops known from structural studies of other antibodies to contain the antigen contacting residues, and

those hypervariable sequences defined by Kabat et al (refs. 4 and 5) as Complementarity Determining Regions (CDRs) are equivalent for CDR2. For CDR1 the hypervariable region extends from residues 24-34 inclusive while the structural loop extends from 26-32 inclusive. In the case of OKT3 there is only one amino acid difference between the two options, at amino acid 24, where the mouse sequence is a serine and the human framework RE1 has glutamine. For CDR3 the loop extends from residues 91-96 inclusive while the Kabat hypervariability extends from residues 89-97 inclusive. For OKT3 amino acids 89, 90 and 97 are the same between OKT3 and RE1 (Fig. 3). When constructs based on the loop choice for CDR1 (gL121) and the Kabat choice (gL221) were made and co-expressed with mH or cH no evidence for antigen binding activity could be found for gL121, but trace activity could be detected for the gL221, suggesting that a single extra mouse residue in the grafted variable region could have some detectable effect. Both gene constructs were reasonably well expressed in the transient expression system.

15.1.2. FRAMEWORK RESIDUES

The remaining framework residues were then further examined, in particular amino acids known from X-ray analysis of other antibodies to be close to the CDRs and also those amino acids which in OKT3 showed differences from the consensus framework for the mouse subgroup (subgroup VI) to which OKT3 shows most homology. Four positions 1, 3, 46 and 47 were identified and their possible contribution was examined by substituting the mouse amino acid for the human amino acid at each position. Therefore gL221A (gL221 + D1Q, Q3V, L46R, L47W,

see Figure 3 and Table 1) was made, cloned in EE6hCMVneo and co-expressed with cH (pJA144). The resultant antibody was well expressed and showed good binding activity. When the related genes gL221B (gL221 + D1Q, Q3V) and gL221C (gL221 + L46R, L47W) were made and similarly tested, while both genes produced antibody when co-expressed with cH, only the gL221C/cH combination showed good antigen binding. When the gL121A (gL121 + D1Q, Q3V, L46R, L47W) gene was made and co-expressed with cH, antibody was produced which also bound to antigen.

15.2. HEAVY CHAIN

15.2.1. EXTENT OF THE CDRs

For the heavy chain the loop and hypervariability analyses agree only in CDR3. For CDR1 the loop region extends from residues 26-32 inclusive whereas the Kabat CDR extends from residues 31-35 inclusive. For CDR2 the loop region is from 50-58 inclusive while the hypervariable region covers amino acids 50-65 inclusive. Therefore humanised heavy chains were constructed using the framework from antibody KOL and with various combinations of these CDR choices, including a shorter choice for CDR2 of 50-56 inclusive as there was some uncertainty as to the definition of the end point for the CDR2 loop around residues 56 to 58. The genes were co-expressed with mL or cL initially. In the case of the gH genes with loop choices for CDR1 e.g. gH121, gH131, gH141 very little antibody was produced in the culture supernatants. As no free light chain was detected it was presumed that the antibody was being made and assembled inside the cell but that the heavy chain was aberrant in some way, possibly incorrectly folded, and therefore the antibody was

being degraded internally. In some experiments trace amounts of antibody could be detected in ^{35}S labelling studies.
As no net antibody was produced, analysis of these constructs was not pursued further.
When, however, a combination of the loop choice and the Kabat choice for CDR1 was tested (mouse amino acids 26-35 inclusive) and in which residues 31 (Ser to Arg), 33 (Ala to Thr), and 35 (Tyr to His) were changed from the human residues to the mouse residue and compared to the first series, antibody was produced for gH321, kgH331 and kgH341 when co-expressed with cL. Expression was generally low and could not be markedly improved by the insertion of the Kozak consensus sequence 5′ to the ATG of the signal sequence of the gene, as distinct from the case of the gL genes where such insertion led to a 2-5 fold increase in net antibody production. However, only in the case of gH341/mL or kgH341/cL could marginal antigen binding activity be demonstrated. When the kgH341 gene was co-expressed with kgL221A, the net yield of antibody was too low to give a signal above the background level in the antigen binding assay.

15.2.2. FRAMEWORK RESIDUES

As in the case of the light chain the heavy chain frameworks were re-examined. Possibly because of the lower initial homology between the mouse and human heavy variable domains compared to the light chains, more amino acid positions proved to be of interest. Two genes kgH341A and kgH341B were constructed, with 11 or 8 human residues respectively substituted by mouse residues compared to gH341, and with the CDR2 residue 63 returned to the human amino acid potentially to

improve domain packing. Both showed antigen binding when combined with cL or kgL221A, the kgH341A gene with all 11 changes appearing to be the superior choice.

15.3 INTERIM CONCLUSIONS

It has been demonstrated, therefore, for OKT3 that to transfer antigen binding ability to the humanised antibody, mouse residues outside the CDR regions defined by the Kabat hypervariability or structural loop choices are required for both the light and heavy chains. Fewer extra residues are needed for the light chain, possibly due to the higher initial homology between the mouse and human kappa variable regions.

Of the changes seven (1 and 3 from the light chain and 6, 23, 71, 73 and 76 from the heavy chain) are predicted from a knowledge of other antibody structures to be either partly exposed or on the antibody surface. It has been shown here that residues 1 and 3 in the light chain are not absolutely required to be the mouse sequence; and for the heavy chain the gH341B heavy chain in combination with the 221A light chain generated only weak binding activity. Therefore the presence of the 6, 23 and 24 changes are important to maintain a binding affinity similar to that of the murine antibody. It was important, therefore, to further study the individual contribution of othe other 8 mouse residues of the kgH341A gene compared to kgH341.

16. FURTHER CDR-GRAFTING EXPERIMENTS

Additional CDR-grafted heavy chain genes were prepared substantially as described above. With reference to Table 2 the further heavy chain genes were based upon the gh341 (plasmid pJA178) and

gH341A (plasmid pJA185) with either mouse OKT3 or human KOL residues at 6, 23, 24, 48, 49, 63, 71, 73, 76, 78, 88 and 91, as indicated. The CDR-grafted light chain genes used in these further experiments were gL221, gL221A, gL221B and gL221C as described above.

TABLE 2

OKT3 HEAVY CHAIN CDR GRAFTS

1. gH341 and derivatives

RES NUM	6	23	24	48	49	63	71	73	76	78	88	91	
OKT3vh	Q	K	A	I	G	F	T	K	S	A	A	Y	
gH341	E	S	S	V	A	F	R	N	N	L	G	F	JA178
gH341A	Q	K	A	I	G	V	T	K	S	A	A	Y	JA185
gH341E	Q	K	A	I	G	V	T	K	S	A	G	G	JA198
gH341*	Q	K	A	I	G	V	T	K	N	A	G	F	JA207
gH341*	Q	K	A	I	G	V	R	N	N	A	G	F	JA209
gH341D	Q	K	A	I	G	V	T	K	N	L	G	F	JA197
gH341*	Q	K	A	I	G	V	R	N	N	L	G	F	JA199
gH341C	Q	K	A	V	A	F	R	N	N	L	G	F	JA184
gH341*	Q	S	A	I	G	V	T	K	S	A	A	Y	JA203
gH341*	E	S	A	I	G	V	T	K	S	A	A	Y	JA205
gH341B	E	S	S	I	G	V	T	K	S	A	A	Y	JA183
gH341*	Q	S	A	I	G	V	T	K	S	A	G	F	JA204
gH341*	E	S	A	I	G	V	T	K	S	A	G	F	JA206
gH341*	Q	S	A	I	G	V	T	K	N	A	G	F	JA208
KOL	E	S	S	V	A		R	N	N	L	G	F	

OKT3 LIGHT CHAIN CDR GRAFTS

2. gL221 and derivatives

RES NUM	1	3	46	47	
OKT3vl	Q	V	R	W	
GL221	D	Q	L	L	DA221
gL221A	Q	V	R	W	DA221A
gL221B	Q	V	L	L	DA221B
GL221C	D	Q	R	W	DA221C
REI	D	Q	L	L	

MURINE RESIDUES ARE UNDERLINED

The CDR-grafted heavy and light chain genes were co-expressed in COS cells either with one another in various combinations but also with the corresponding murine and chimeric heavy and light chain genes substantially as described above. The resultant antibody products were then assayed in binding and blocking assays with HPB-ALL cells as described above.

The results of the assays for various grafted heavy chains co-expressed with the gL221C light chain are given in Figures 7 and 8 (for the JA184, JA185, JA197 and JA198 constructs - see Table 2), in Figure 9 (for the JA183, JA184, JA185 and JA197 constructs) in Figure 10 (for the chimeric, JA185, JA199, JA204, JA205, JA207, JA208 and JA209 constructs) and in Figure 11 (for the JA183, JA184, JA185, JA198, JA203, JA205 and JA206 constructs).

The basic grafted product without any human to murine changes in the variable frameworks, i.e. gL221 co-expressed with gh341 (JA178), and also the "fully grafted" product, having most human to murine changes in the grafted heavy chain framework, i.e. gL221C co-expressed with gh341A (JA185), were assayed for relative binding affinity in a competition assay against murine OKT3 reference standard, using HPB-ALL cells. The assay used was as described above in section 3.3. The results obtained are given in Figure 12 for the basic grafted product and in Figure 13 for the fully grafted product. These results indicate that the basic grafted product has neglibible binding ability as compared with the OKT3 murine reference standard; whereas the "fully grafted" product has a binding ability very similar to that of the OKT3 murine reference standard.

The binding and blocking assay results indicate the following:

The JA198 and JA207 constructs appear to have the best binding characteristics and similar binding abilities, both substantially the same as the chimeric and fully grafted gH341A products. This indicates that positions 88 and 91 and position 76 are not highly critical for maintaining the OKT3 binding ability; whereas at least some of positions 6, 23, 24, 48, 49, 71, 73 and 78 are more important.

This is borne out by the finding that the JA209 and JA199, although of similar binding ability to one another, are of lower binding ability than the JA198 and JA207 constructs. This indicates the importance of having mouse residues at positions 71, 73 and 78, which are either completely or partially human in the JA199 and JA209 constructs respectively.

Moreover, on comparing the results obtained for the JA205 and JA183 constructs it is seen that there is a decrease in binding going from the JA205 to the JA183 constructs. This indicates the importance of retaining a mouse residue at position 23, the only position changed between JA205 and JA183.

These and other results lead us to the conclusion that of the 11 mouse framework residues used in the gH341A (JA185) construct, it is important to retain mouse residues at all of positions 6, 23, 24, 48 and 49, and possibly for maximum binding affinity at 71, 73 and 78.

Similar Experiments were carried out to CDR-graft a number of the rodent antibodies including antibodies having specificity for CD4 (OKT4), ICAM-1 (R6-5), TAG72 (B72.3), and TNFα(61E71, 101.4, hTNF1, hTNF2 and hTNF3).

EXAMPLE 2

CDR-GRAFTING OF A MURINE ANTI-CD4 T CELL RECEPTOR ANTIBODY, OKT4A

Anti OKT4A CDR-grafted heavy and light chain genes were prepared, expressed and tested substantially as described above in Example 1 for CDR-grafted OKT3. The CDR grafting of OKT4A is described in detail in Ortho patent application PCT/GB 90..... of even date herewith entitled "Humanised Antibodies". The disclosure of this Ortho patent application PCT/GB 90 ..... is incorporated herein by reference. A number of CDR-grafted OKT4 antibodies have been prepared. Presently the CDR-grafted OKT4A of choice is the combination of the grafted light chain LCDR2 and the grafted heavy chain HCDR10.

THE LIGHT CHAIN

The human acceptor framework used for the grafted light chains was RE1. The preferred LCDR2 light chain has human to mouse changes at positions 33, 34, 38, 49 and 89 in addition to the structural loop CDRs. Of these changed positions, positions 33, 34 and 89 fall within the preferred extended CDRs of the present invention (positions 33 and 34 in CDR1 and position 89 in CDR3). The human to murine changes at positions 38 and 49 corresponds to positions at which the amino acid residues are preferably donor murine amino acid residues in accordance with the present invention.

A comparison of the amino acid sequences of the donor murine light chain variable domain and the RE1 human acceptor light chain variable further reveals that the murine and human residues are identical at all of positions 46, 48 and 71 and at all of positions 2, 4, 6, 35, 36, 44, 47, 62, 64-69, 85, 87, 98, 99 and 101 and 102. However the amino acid residue at position 58 in LCDR2 is

the human RE1 framework residue not the mouse OKT4 residue as would be preferred in accordance with the present invention.

THE HEAVY CHAIN

The human acceptor framework used for the grafted heavy chains was KOL.

The preferred CDR graft HCDR10 heavy chain has human to mouse changes at positions 24, 35, 57, 58, 60, 88 and 91 in addition to the structural loop CDRs.

Of these positions, positions 35 (CDR1) and positions 57, 58 and 60 (CDR2) fall within the preferred extended CDRs of the present invention. Also the human to mouse change at position 24 corresponds to a position at which the amino acid residue is a donor murine residue in accordance with the present invention. Moreover, the human to mouse changes at positions 88 and 91 correspond to positions at which the amino acid residues are optionally donor murine residues.

Moreover, a comparison of the murine OKT4A and human KOL heavy chain variable amino acid sequences reveals that the murine and human residues are identical at all of positions 23, 49, 71, 73 and 78 and at all of positions 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

Thus the OKT4A CDR-grafted heavy chain HCDR10 corresponds to a particularly preferred embodiment according to the present invention.

EXAMPLE 3

CDR-GRAFTING OF AN ANTI-MUCIN SPECIFIC MURINE ANTIBODY, B72.3

The cloning of the genes coding for the anti-mucin specific murine monoclonal antibody B72.3 and the preparation of B72.3 mouse-human chimeric antibodies has been described previously (ref. 13 and WO 89/01783). CDR-grafted versions of B72.3 were prepared as follows.

(a) B72.3 Light Chain

CDR-grafting of this light chain was accomplished by direct transfer of the murine CDRs into the framework of the human light chain RE1.

The regions transferred were:

CDR Number	Residues
1	24-34
2	50-56
3	90-96

The activity of the resulting grafted light chain was assessed by co-expression in COS cells, of genes for the combinations:

B72.3 cH/B72.3 cL

and B72.3 cH/B72.3 gL

Supernatants were assayed for antibody concentration and for the ability to bind to microtitre plates coated with mucin. The results obtained indicated that, in combination with the B72.3 cH chain, B72.3 cL and B72.3 gL had similar binding properties.

Comparison of the murine B72.3 and REI light chain amino acid sequences reveals that the residues are identical at positions 46, 58 and 71 but are different at position 48.

Thus changing the human residue to the donor mouse residue at position 48 may further improve the binding characteristics of the CDR-grafted light chain, (B72.3 gL) in accordance with the present invention.

(b) B72.3 heavy chain

i. Choice of framework

At the outset it was necessary to make a choice of human framework. Simply put, the question was as follows: Was it necessary to use the framework regions from an antibody whose crystal structure was known or could the choice be made on some other criteria?

For B72.3 heavy chain, it was reasoned that, while knowledge of structure was important, transfer of the CDRs from mouse to human frameworks might be facilitated if the overall homology between the donor and receptor frameworks was maximised.

Comparison of the B72.3 heavy chain sequence with those in Kabat (ref. 4) for human heavy chains showed clearly that B72.3 had poor homology for KOL and NEWM (for which crystal structures are available) but was very homologous to the heavy chain for EU.

On this basis, EU was chosen for the CDR-grafting and the following residues transferred as CDRs.

CDR Number	Residues
1	27-36
2	50-63
3	93-102

Also it was noticed that the FR4 region of EU was unlike that of any other human (or mouse) antibody. Consequently, in the grafted heavy chain genes this was also changed to produce a "consensus" human sequence. (Preliminary experiments showed that grafted heavy chain genes containing the EU FR4 sequence expressed very poorly in transient expression systems.)

ii. Results with grafted heavy chain genes

Expression of grafted heavy chain genes containing all human framework regions with either gL or cL genes produced a grafted antibody with little ability to bind to mucin. The grafted antibody had about 1% the activity of the chimeric antibody.

In these experiments, however, it was noted that the activity of the grafted antibody could be increased to ~ 10% of B72.3 by exposure to pHs of 2-3.5.

This observation provided a clue as to how the activity of the grafted antibody could be improved without acid treatment. It was postulated that acid exposure brought about the protonation of an acidic residue (pKa of aspartic acid = 3.86 and of glutamine acid = 4.25) which in turn caused a change in structure of the CDR loops, or allowed better access of antigen.

From comparison of the sequences of B72.3 (ref. 13) and EU (refs. 4 and 5), it was clear that, in going from the mouse to human frameworks, only two positions had been changed in such a way that acidic residues had been introduced. These

positions are at residues 73 and 81, where K to E and Q to E changes had been made, respectively.
Which of these positions might be important was determined by examining the crystal structure of the KOL antibody. In KOL heavy chain, position 81 is far removed from either of the CDR loops.
Position 73, however, is close to both CDRs 1 and 3 of the heavy chain and, in this position it was possible to envisage that a K to E change in this region could have a detrimental effect on antigen binding.

iii. Framework changes in B72.3 gH gene
On the basis of the above analysis, E73 was mutated to a lysine (K). It was found that this change had a dramatic effect on the ability of the grafted Ab to bind to mucin. Further the ability of the grafted B72.3 produced by the mutated gH/gL combination to bind to mucin was similar to that of the B72.3 chimeric antibody.

iv. Other framework changes
In the course of the above experiments, other changes were made in the heavy chain framework regions. Within the accuracy of the assays used, none of the changes, either alone or together, appeared beneficial.

v. Other
All assays used measured the ability of the grafted Ab to bind to mucin and, as a whole, indicated that the single framework change at position 73 is sufficient to generate an antibody with similar binding properties to B72.3.

Comparison of the B72.3 murine and EU heavy chain sequences reveals that the mouse and human residues are identical at positions 23, 24, 71 and 78.
Thus the mutated CDR-grafted B72.3 heavy chain corresponds to a preferred embodiment of the present invention.

EXAMPLE 4

CDR-GRAFTING OF A MURINE ANTI-ICAM-1 MONOCLONAL ANTIBODY

A murine antibody, R6-5-D6 (EP 0314863) having specificity for Intercellular Adhesion Molecule 1 (ICAM-1) was CDR-grafted substantially as described above in previous examples. This work is described in greater detail in co-pending application, British Patent Application No. 9009549.8, the disclosure of which is incorporated herein by reference.

The human EU framework was used as the acceptor framework for both heavy and light chains. The CDR-grafted antibody currently of choice is provided by co-expression of grafted light chain gL221A and grafted heavy chain gH341D which has a binding affinity for ICAM 1 of about 75% of that of the corresponding mouse-human chimeric antibody.

LIGHT CHAIN

gL221A has murine CDRs at positions 24-34 (CDR1), 50-56 (CDR2) and 89-97 (CDR3). In addition several framework residues are also the murine amino acid. These residues were chosen after consideration of the possible contribution of these residues to domain packing and stability of the conformation of the antigen binding region. The residues which have been retained as mouse are at positions 2, 3, 48 (?), 60, 84, 85 and 87. Comparison of the murine anti-ICAM 1 and human EU light chain amino acid sequences reveals that the murine and human residues are identical at positions 46, 58 and 71.

HEAVY CHAIN

gH341D has murine CDRs at positions 26-35 (CDR1), 50-56 (CDR2) and94-100B (CDR3). In addition murine residues were used in gH341D at positions 24, 48, 69, 71, 73, 80, 88 and 91. Comparison of the murine anti-ICAM 1 and human EU heavy chain amino acid sequences are identical at positions 23, 49 and 78.

EXAMPLE 5

CDR-Grafting of murine anti-TNFα antibodies

A number of murine anti-TNFα monoclonal antibodies were CDR-grafted substantially as described above in previous examples. These antibodies include the murine monoclonal antibodies designated 61 E71, hTNF1, hTNF3 and 101.4 A brief summary of the CDR-grafting of each of these antibodies is given below.

61E71

A similar analysis as described above (Example 1, Section 12.1.) was done for 61E71 and for the heavy chain 10 residues were identified at 23, 24, 48, 49, 68, 69, 71, 73, 75 and 88 as residues to potentially retain as murine. The human frameworks chosen for CDR-grafting of this antibody, and the hTNF3 and 101.4 antibodies were RE1 for the light chain and KOL for the heavy chain.
Three genes were built, the first of which contained 23, 24, 48, 49, 71 and 73 [gH341(6)] as murine residues. The second gene also had 75 and 88 as murine residues [gH341(8)] while the third gene additionally had 68, 69, 75 and 88 as murine residues [gH341(10)]. Each was co-expressed with gL221, the minimum grafted light chain (CDRs only). The gL221/gH341(6) and gL221/gH341(8) antibodies both bound as well to TNF as murine 61E71. The gL221/gH341(10) antibody did not express and this combination was not taken further.
Subsequently the gL221/gH341(6) antibody was assessed in an L929 cell competition assay in which the antibody competes against the TNF receptor on L929 cells for binding to TNF in solution. In this assay the gL221/gH341(6) antibody was approximately 10% as active as murine 61E71.

hTNF1

hTNF1 is a monoclonal antibody which recognises an epitope on human TNF- . The EU human framework was used for CDR-grafting of both the heavy and light variable domains.

Heavy Chain

In the CDR-grafted heavy chain (ghTNF1) mouse CDRs were used at positions 26-35 (CDR1), 50-65 (CDR2) and 95-102 (CDR3). Mouse residues were also used in the frameworks at positions 48, 67, 69, 71, 73, 76, 89, 91, 94 and 108. Comparison of the TNF1 mouse and EU human heavy chain residues reveals that these are identical at positions 23, 24, 29 and 78.

Light Chain

In the CDR-grafted light chain (gLhTNF1) mouse CDRs wre used at positions 24-34 (CDR1), 50-56 (CDR2) and 89-97 (CDR3). In addition mouse residues were used in the frameworks at positions 3, 42, 48, 49, 83, 106 and 108. Comparison of the hTNF1 mouse and EU human light chain residues reveals that these are identical at positions 46, 58 and 71.

The grafted hTNF1 heavy chain was co-expressed with the chimeric light chain and the binding ability of the product compared with that of the chimeric light chain/chimeric heavy chain product in a TNF binding assay. The grafted heavy chain product appeared to have binding ability for TNF slightly better than the fully chimeric product.

Similarly, a grafted heavy chain/grafted light chain product was co-expressed and compared with the fully chimeric product and found to have closely similar binding properties to the latter product.

hTNF3

hTNF3 recognises an epitope on human TNF-α. The sequence of hTNF3 shows only 21 differences compared to 61E71 in the light and heavy chain variable regions, 10 in the light chain (2 in the CDRs at positions 50, 96 and 8 in the framework at 1, 19, 40, 45, 46, 76, 103 and 106) and 11 in the heavy chain (3 in the CDR regions at positions 52, 60 and 95 and 8 in the framework at 1, 10, 38, 40, 67, 73, 87 and 105). The light and heavy chains of the 61E71 and hTNF3 chimeric antibodies can be exchanged without loss of activity in the direct binding assay. However 61E71 is an order of magnitude less able to compete with the TNF receptor on L929 cells for TNF-a compared to hTNF3. Based on the 61E71 CDR grafting data gL221 and gH341(+23, 24, 48, 49 71 and 73 as mouse) genes have been built for hTNF3 and tested and the resultant grafted antibody binds well to TNF-a, but competes very poorly in the L929 assay. It is possible that in this case also the framework residues identified for OKT3 programme may improve the competitive binding ability of this antibody.

101.4

101.4 is a further murine monoclonal antibody able to recognise human TNF-a. The heavy chain of this antibody shows good homology to KOL and so the CDR-grafting has been based on RE1 for the light chain and KOL for the heavy chain. Several grafted heavy chain genes have been constructed with conservative choices for the CDR's (gH341) and which have one or a small number of non-CDR residues at positions 73, 78 or 77-79 inclusive, as the mouse amino acids. These have been co-expressed with cL or gL221. In all cases binding to TNF equivalent to the chimeric antibody is seen and when co-expressed with cL the resultant antibodies are able to compete well in the L929 assay. However, with gL221 the resultant antibodies

are at least an order of magnitude less able to compete for TNF against the TNF receptor on L929 cells.

Mouse residues at other positions in the heavy chain, for example, at 23 and 24 together or at 76 have been demonstrated to provide no improvement to the competitive ability of the grafted antibody in the L929 assay.

A number of other antibodies including antibodies having specificity for interleukins e.g. IL1 and cancer markers such as carcinoembryonic antigen (CEA) e.g. the monoclonal antibody A5B7 (ref. 21), have been successfully CDR-grafted according to the present invention.
It will be appreciated that the foregoing examples are given by way of illustration only and are not intended to limit the scope of the claimed invention. Changes and modifications may be made to the methods described whilst still falling within the spirit and scope of the invention.

References

1. Kohler & Milstein, Nature, 265, 295-497, 1975.

2. Chatenoud et al, (1986), J. Immunol. 137, 830-838.

3. Jeffers et al, (1986), Transplantation, 41, 572-578.

4. Begent et al, Br. J. Cancer 62: 487 (1990).

5. Verhoeyen et al, Science, 239, 1534-1536, 1988.

6. Riechmann et al, Nature, 332, 323-324, 1988.

7. Kabat, E.A., Wu, T.T., Reid-Miller, M., Perry, H.M., Gottesman, K.S., 1987, in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA.

8. Wu, T.T., and Kabat, E.A., 1970, J. Exp. Med. 132 211-250.

9. Queen et al, (1989), Proc. Natl. Acad. Sci. USA, 86, 10029-10033 and WO 90/07861

10. Maniatis et al, Molecular Cloning, Cold Spring Harbor, New York, 1989.

11. Primrose and Old, Principles of Gene Manipulation, Blackwell, Oxford, 1980.

12. Sanger, F., Nicklen, S., Coulson, A.R., 1977, Proc. Natl. Acad. Sci. USA, 74 5463

13. Kramer, W., Drutsa, V., Jansen, H.-W., Kramer, B., Plugfelder, M., Fritz, H.-J., 1984, Nucl. Acids Res. 12, 9441

14. Whittle, N., Adair, J., Lloyd, J.C., Jenkins, E., Devine, J., Schlom, J., Raubitshek, A., Colcher, D., Bodmer, M., 1987, Protein Engineering 1, 499.

15. Sikder, S.S., Akolkar, P.N., Kaledas, P.M., Morrison, S.L., Kabat, E.A., 1985, J. Immunol. 135, 4215.

16. Wallick, S.C., Kabat, E.A., Morrison, S.L., 1988, J. Exp. Med. 168, 1099

17. Bebbington, C.R., Published International Patent Application WO 89/01036.

18. Granthan and Perrin 1986, Immunology Today 7, 160.

19. Kozak, M., 1987, J. Mol. Biol. 196, 947.

20. Jones, T.P., Dear, P.H., Foote, J., Neuberger, M.S., Winter, G., 1986, Nature, 321, 522

21. Harwood et al, Br. J. Cancer, 54, 75-82 (1986).

CLAIMS

1. A CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

2. A CDR-grafted heavy chain according to Claim I comprising donor residues at positions 23, 24, 49, 71, 73 and 78, or at positions 23, 24 and 49.

3. A CDR-grafted heavy chain according to Claim 2 comprising donor residues at positions 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

4. A CDR-grafted heavy chain according to Claim 2 or 3, comprising donor residues at one, some or all of positions:
1 and 3,
69 (if 48 is different between donor and acceptor),
38 and 46 (if 48 is the donor residue),
67,
82 and 18 (if 67 is the donor residue),
91, and
any one or more of 9, 11, 41, 87, 108, 110 and 112.

5. A CDR-grafted heavy chain according to any of the preceding comprising donor CDRs at positions 26-35, 50-65 and 95-100.

6. A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 1 and/or 3 and 46 and/or 47.

7. A CDR-grafted light chain according to Claim 6 comprising donor residues at positions 46 and 47.

8. A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, 58 and 71.

9. A CDR-grafted light chain according to Claim 8 comprising donor residues at positions 46, 48, 58 and 71.

10. A CDR-grafted light chain according to Claim 8 or 9, comprising donor residues at positions 2, 4, 6, 35, 36, 38, 44, 47, 49, 62, 64-69, 85, 87, 98, 99, 101 and 102.

11. A CDR-grafted light chain according to Claim 9 or 10, comprising donor residues at one, some or all of positions:
1 and 3,
63,
60 (if 60 and 54 are able to form a potential saltbridge),
70 (if 70 and 24 are able to form a potential saltbridge),
73 and 21 (if 47 is different between donor and acceptor),
37 and 45 (if 47 if different between donor and acceptor), and
any one or more of 10, 12, 40, 83, 103 and 105.

12. A CDR-grafted light chain according to any one of Claims 6-11, comprising donor CDRs at positions 24-34, 50-56 and 89-97.

13. A CDR-grafted antibody molecule comprising at least one CDR-grafted heavy chain according to any one of Claims 1-5 and at least one CDR-grafted light chain according to any one of Claims 6-12.

14. A CDR-grafted antibody molecule according to Claim 13, which is a site-specific antibody molecule.

15. A CDR-grafted antibody molecule according to Claim 13 which has specificity for an interleukin, hormone or other biologically active compound or a receptor therefor.

16. A CDR-grafted antibody heavy or light chain or molecule according to any one of the preceding claims comprising human acceptor residues and non-human donor residues.

17. A DNA sequence which codes for a CDR-grafted heavy chain according to Claim 1 or a CDR-grafted light chain according to Claim 6 or Claim 8.

18. A cloning or expression vector containing a DNA sequence according to Claim 17.

19. A host cell transformed with a DNA sequence according to Claim 17.

20. A process for the production of a CDR-grafted antibody sequence according to Claim 17 in a transformed host cell.

21. A process for producing a CDR-grafted antibody product comprising:

(a) producing in an expression vector an operon having a DNA sequence which encodes an antibody heavy chain according to Claim 1;

and/or

(b) producing in an expression vector an operon having a DNA sequence which encodes a complementary antibody light chain according to Claim 6 or Claim 8;

(c) transfecting a host cell with the or each vector; and

(d) culturing the transfected cell line to produce the CDR-grafted antibody product.

22. A therapeutic or diagnostic composition comprising a CDR-grafted antibody heavy chain according to Claim 1, or a CDR-grafted light chain according to Claim 6 or Claim 8, or a CDR-grafted antibody molecule according to Claim 13 in combination with a pharmaceutically acceptable carrier, diluent or excipient.

23. A method of therapy or diagnosis comprising administering an effective amount of a CDR-grafted heavy chain according to Claim 1, or a CDR-grafted light chain according to Claim 6 or Claim 8, or a CDR-grafted antibody molecule according to Claim 13 to a human or animal subject.

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
C12P 21/08, C12N 15/13
A61K 39/395, C07K 15/06
C12N 5/10, 15/62

A1

(11) International Publication Number: WO 91/09967

(43) International Publication Date: 11 July 1991 (11.07.91)

(21) International Application Number: PCT/GB90/02017

(22) International Filing Date: 21 December 1990 (21.12.90)

(30) Priority data:
8928874.0 21 December 1989 (21.12.89) GB

(71) Applicant *(for all designated States except US)*: CELLTECH LIMITED [GB/GB]; 216 Bath Road, Slough, Berkshire SL1 4EN (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : ADAIR, John, Robert [GB/GB]; 23 George Road, Stokenchurch, High Wycombe, Buckinghamshire HP14 3RN (GB). ATHWAL, Diljeet, Singh [GB/GB]; Flat 35, Knollys House, Tavistock Square, London WC1 (GB). EMTAGE, John, Spencer [GB/GB]; 49 Temple Mill Island, Temple, Marlow, Buckinghamshire SL7 1SQ (GB).

(74) Agent: MERCER, Christopher, Paul; Carpmaels & Ransford, 43 Bloomsbury Square, London WC1A 2RA (GB).

(81) Designated States: AT, AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CF (OAPI patent), CG (OAPI patent), CH, CH (European patent), CM (OAPI patent), DE, DE (European patent), DK, FI, FR (European patent), GA (OAPI patent), GB, GB (European patent), GR, HU, IT (European patent), JP, KP, KR, LK, LU, LU (European patent), MC, MG, ML (OAPI patent), MR (OAPI patent), MW, NL, NL (European patent), NO, RO, SD, SE, SE (European patent), SN (OAPI patent), SU, TD (OAPI patent), TG (OAPI patent), US.

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.
With a request for rectification under Rule 91.1(f).

(54) Title: HUMANISED ANTIBODIES

(57) Abstract

CDR-grafted antibody heavy and light chains comprise acceptor framework and donor antigen binding regions, the heavy chains comprising donor residues at at least one of positions (6, 23) and/or (24, 48) and/or (49, 71) and/or (73, 75) and/or (76) and/or (78) and (88) and/or (91). The CDR-grafted light chains comprise donor residues at at least one of positions (1) and/or (3) and (46) and/or (47) or at at least one of positons (46, 48, 58) and (71). The CDR-grafted antibodies are preferably humanised antibodies, having non human, e.g. rodent, donor and human acceptor frameworks, and may be used for *in vivo* therapy and diagnosis. A generally applicable protocol is disclosed for obtaining CDR-grafted antibodies.

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```
  1  GAATTCCCAA AGACAAAatg gattttcaag tgcagatttt cagcttcctg
 51  ctaatcagtg cctcagtcat aatatccaga ggacaaattg ttctcaccca
101  gtctccagca atcatgtctg catctccagg ggagaaggtc accatgacct
151  gcagtgccag ctcaagtgta agttacatga actggtacca gcagaagtca
201  ggcacctccc ccaaaagatg gatttatgac acatccaaac tggcttctgg
251  agtccctgct cacttcaggg gcagtgggtc tgggacctct tactctctca
301  caatcagcgg catggaggct gaagatgctg ccacttatta ctgccagcag
351  tggagtagta  accattcac gttcggctcg gggacaaagt tggaaataaa
401  ccgggctgat actgcaccaa ctgtatccat cttcccacca tccagtgagc
451  agttaacatc tggaggtgcc tcagtcgtgt gcttcttgaa caacttctac
501  cccaaagaca tcaatgtcaa gtggaagatt gatggcagtg aacgacaaaa
551  tggcgtcctg aacagttgga ctgatcagga cagcaaagac agcacctaca
601  gcatgagcag caccctcacg ttgaccaagg acgagtatga acgacataac
651  agctatacct gtgaggccac tcacaagaca tcaacttcac ccattgtcaa
701  gagcttcaac aggaatgagt gtTAGAGACA AAGGTCCTGA GACGCCACCA
751  CCAGCTCCCA GCTCCATCCT ATCTTCCCTT CTAAGGTCTT GGAGGCTTCC
801  CCACAAGCGC tTACCACTGT TGCGGTGCTC TAAACCTCCT CCCACCTCCT
851  TCTCCTCCTC CTCCCTTTCC TTGGCTTTTA TCATGCTAAT ATTTGCAGAA
901  AATATTCAAT AAAGTGAGTC TTTGCCTTGA AAAAAAAAAA AAA
(SEQ ID NO:4)
```

FIG. 1a

```
  1  MDFQVQIFSF LLISASVIIS RGQIVLTQSP AIMSASPGEK VTMTCSASSS
 51  VSYMNWYQQK SGTSPKRWIY DTSKLASGVP AHFRGSGSGT  SYSLTISGME
101  AEDAATYYCQ QWSSNPFTFG SGTKLEINRA DTAPTVSIFP  PSSEQLTSGG
151  ASVVCFLNNF YPKDINVKWK IDGSERQNGV LNSWTDQDSK  DSTYSMSSTL
201  TLTKDEYERH NSYTCEATHK TSTSPIVKSF NRNEC* (SEQ ID NO:5)
```

FIG. 1b

```
   1  GAATTCCCCT CTCCACAGAC ACTGAAAACT CTGACTCAAC ATGGAAAGGC
  51  ACTGGATCTT TCTACTCCTG TTGTCAGTAA CTGCAGGTGT CCACTCCCAG
 101  GTCCAGCTGC AGCAGTCTGG GGCTGAACTG GCAAGACCTG GGGCCTCAGT
 151  GAAGATGTCC TGCAAGGCTT CTGGCTACAC CTTTACTAGG TACACGATGC
 201  ACTGGGTAAA ACAGAGGCCT GGACAGGGTC TGGAATGGAT TGGATACATT
 251  ATTCCTAGCC GTGGTTATAC TAATTACAAT CAGAAGTTCA AGGACAAGGC
 301  CACATTGACT ACAGACAAAT CCTCCAGCAC AGCCTACATG CAACTGAGCA
 351  GCCTGACATC TGAGGACTCT GCAGTCTATT ACTGTGCAAG ATATTATGAT
 401  GATCATTACT GCCTTGACTA CTGGGGCCAA GGCACCACTC TCACAGTCTC
 451  CTCAGCCAAA ACAACAGCCC CATCGGTCTA TCCACTGGCC CCTGTGTGTG
 501  GAGATACAAC TGGCTCCTCG GTGACTCTAG GATGCCTGGT CAAGGGTTAT
 551  TTCCCTGAGC CAGTGACCTT GACCTGGAAC TCTGGATCCC TGTCCAGTGG
 601  TGTGCACACC TTCCCAGCTG TCCTGCAGTC TGACCTCTAC ACCCTCAGCA
 651  GCTCAGTGAC TGTAACCTCG AGCACCTGGC CCAGCCAGTC CATCACCTGC
 701  AATGTGGCCC ACCCGGCAAG CAGCACCAAG GTGGACAAGA AAATTGAGCC
 801  ACCTCTTGGG TGGACCATCC GTCTTCATCT TCCCTCCAAA GATCAAGGAT
 851  GTACTCATGA TCTCCCTGAG CCCCATAGTC ACATGTGTGG TGGTGGATGT
 901  GAGCGAGGAT GACCCAGATG TCCAGATCAG CTGGTTTGTG AACAACGTGG
 951  AAGTACACAC AGCTCAGACA CAAACCCATA GAGAGGATTA CAACAGTACT
1001  CTCCGGGTGG TCAGTGCCCT CCCCATCCAG CACCAGGACT GGATGAGTGG
1051  CAAGGAGTTC AAATGCAAGG TCAACAACAA AGACCTCCCA GCGCCCATCG
1101  AGAGAACCAT CTCAAAACCC AAAGGGTCAG TAAGAGCTCC ACAGGTATAT
1151  GTCTTGCCTC CACCAGAAGA AGAGATGACT AAGAAACAGG TCACTCTGAC
1201  CTGCATGGTC ACAGACTTCA TGCCTGAAGA CATTTACGTG GAGTGGACCA
1251  ACAACGGGAA AACAGAGCTA AACTACAAGA ACACTGAACC AGTCCTGGAC
1301  TCTGATGGTT CTTACTTCAT GTACAGCAAG CTGAGAGTGG AAAAGAAGAA
1351  CTGGGTGGAA AGAAATAGCT ACTCCTGTTC AGTGGTCCAC GAGGGTCTGC
1401  ACAATCACCA CACGACTAAG AGCTTCTCCC GGACTCCGGG TAAATGAGCT
1451  CAGCACCCAC AAAACTCTCA GGTCCAAAGA GAGACCCACA CTCATCTCCA
1501  TGCTTCCCTT GTATAAATAA AGCACCCAGC AATGCCTGGG ACCATGTAAA
1551  AAAAAAAAAA AAAGGAATTC  (SEQ ID NO:6)
```

FIG. 2a

ΩKT 3 HEAVY CHAIN PROTEIN SEQUENCE DEDUCED FROM DNA SEQUENCE

```
  1  MERHWIFLLL LSVTAGVHSQ VQLQQSGAEL ARPGASVKMS CKASGYTFTR
 51  YTMHWVKQRP GQGLEWIGYI NPSRGYTNYN QKFKDKATLT TDKSSSTAYM
101  QLSSLTSEDS AVYYCARYYD DHYCLDYWGQ GTTLTVSSAK TTAPSVYPLA
151  PVCGDTTGSS VTLGCLVKGY FPEPVTLTWN SGSLSSGVHT FPAVLQSDLY
201  TLSSSVTVTS STWPSQSITC NVAHPASSTK VDKKIEPRGP TIKPCPPCKC
251  PAPNLLGGPS VFIFPPKIKD VLMISLSPIV TCVVVDVSED DPDVQISWFV
301  NNVEVHTAQT QTHREDYNST LRVVSALPIQ HQDWMSGKEF KCKVNNKDLP
351  APIERTISKP KGSVRAPQVY VLPPPEEEMT KKQVTLTCMV TDFMPEDIYV
401  EWTNNGKTEL NYKNTEPVLD SDGSYFMYSK LRVEKKNWVE RNSYSCSVVH
451  EGLHNHHTTK SFSRTPGK*  (SEQ ID NO:7)
```

FIG. 2b

```
                1                       23                      42
                NN      N                 N         N     N
RES TYPE        SBspSPESssBSbSsSssPSPSPsPSsse*s*p*Pi^ISsSe
Okt3vl          QIVLTQSPAIMSASPGEKVTMTCSASS.SVSYMNWYQQKSGT
REI             DIQMTQSPSSLSASVGDRVTITCQASQDIIKYLNWYQQIPGK
                ? ?
                  CDR1    (LOOP)          *******
                  CDR1    (KABAT)        **********


                          56                                    85
                N  NN
RES TYPE        *IsiPpleesesssSBEsePsPSBSSEsPspsPsseesSPePb
Okt3vl          SPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAAT
REI             APKLLIYEASNLQAGVPSRFSGSGSGTDYTFTISSLQPEDIAT (SEQ
ID NO:8)
                ?  ??                              ?  ?
                      *******  CDR2 (LOOP/KABAT)

                              102   108
RES TYPE        PiPIPies**iPIIsPPSPSPSS
Okt3vl          YYCQQWSSNPFTFGSGTKLEINR (SEQ ID NO:29)
REIvl           YYCQQYQSLPYTFGQGTKLQITR (SEQ ID NO:9)
                             ?    ?
                    ******       CDR3 (LOOP)
                  *********      CRD3(KABAT)
```

FIG. 3

```
          NN N                    23 26    32 35  N39  43
RES TYPE  SESPs^SBssS^sSSsSpSpSPsPSEbSBssBePi^PIpiesss
Okt3h     QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMNHWVKQRPGQ
KOL       QVQLVESGGGVVQPGRSLRLSCSSSGFIFSSYAMYWVRQAPGK
               ?                  ??
                                        ******     CDR1 (LOOP)
                                            *****  CDR1 (KABAT)

                    52a   60     65     N N  N   82abc     89
RES TYPE  IIeIppp^sssssssss^ps^pSSsbSpseSsSseSp^pSpsSBssS^ePb
Okt3vh    GLEWIGYINPSRGYTNTNQKFKRKATLTTDKSSSTAYMQLSSLTSEDSAV
KOL       GLEWVAIIWDDGSDQHYADSVKGRFTISRDNSKNTLFLQMDSLPPEDTGV
             ??                      ? ?  ?  ?  ?            ?
                  ***********         CDR2   (LOOP)
                  ******************  CDR2   (KABAT)

           92 N                   107    113
RES TYPE   PiPIEissssiiisssbibi*EIPIP*spSBSS
Okt3vh     YYCARYYDDHY.......CLDYWGQGTTLTVSS   (SEQ ID NO:30)
KOL        YFCARDGGHGFCSSASCFGPDYWGQGTPVTVSS   (SEQ ID NO:10)
               ***************  CRD4 (KABAT/LOOP)
```

FIG. 4

OKT 3 HEAVY CHAIN CDR GRAFTS

1. gh341 and derivatives

```
          1                                26        35  39  43
Okt3vh    QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQ
gH341     QVQLVESGGGVVQOGRSLRLSCSSSGYTFTRYTMHWVRQAPGK   JA178
gH341A    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA185

gH341E    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA198
gH341*    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA207
gH341*    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA209
gH341D    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA197
gH341*    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA199
gH341C    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA184

gH341*    QVQLVQSGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA203
gH341*    QVQLVESGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA205
gH341B    QVQLVESGGGVVQPGRSLRLSCSSSGYTFTRYTMHWVRQAPGK   JA183
gH341*    QVQLVQSGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA204
gH341*    QVQLVESGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA206
gH341*    QVQLVQSGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA208
KOL       QVQLVESGGGVVQPGRSLRLSCSSSGFIFSSYAMYWVRQAPGK
```

FIG. 5a

```
          44    50              65                  83
Okt3vh    GLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLT
gH341     GLEWVAYINPSRGYTNYNQKFKDRFTISRDNSKNTLFLQMDSLR   JA178
gH341A    GLEWIGYINPSRGYTNYNQKVKDRFTISIDKSKSTAFLQMDSLR   JA185

gH341E    GLEWIGYINPSRGYTNYNQKVKDRFTISIDKSKSTAFLQMDSLR   JA198
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISIDKSKNTAFLQMDSLR   JA207
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTAFLQMDSLR   JA209
gH341D    GLEWIGYINPSRGYTNYNQKVKDRFTISIDKSKNTLFLQMDSLR   JA197
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTLFLQMDSLR   JA199
gH341C    GLEWVAYINPSRGYTNYNQKFKDRFTISRDNSKNTLFLQMDSLR   JA184

gH341*    GLEWIGYINPSRGYTNYNOKVKDRFTISIDKSKSTAFLQMDSLR   JA207
gH341*    GLEWIGYINPSRGYTNYNOKVKDRFTISIDKSKSTAFLQMDSLR   JA205
gH341B    GLEWIGYINPSRGYTNYNOKVKDRFTISIDKSKSTAFLQMDSLR   JA183
gH341*    GLEWIGYINPSRGYTNYNOKVKDRFTISIDKSKSTAFLQMDSLR   JA204
gH341*    GLEWIGYINPSRGYTNYNOKVKDRFTISIDKSKSTAFLQMDSLR   JA206
gH341*    GLEWIGYINPSRGYTNYNOKVKDRFTISIDKSKNTAFLQMDSLR   JA208
KOL       GLEWVAIIWDDGSDQHYADSVKGRFTISRDNSKNTLFLQMDSLR
```

FIG. 5b

	84 95 102 113		SEQ ID NO:
Okt3vh	SEDSAVYYCARYYDDHY.....CLDYWGQGTTLTVSS		30
gH341	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA178	11
gH341A	PEDTAVYYCARYYDDHY.....CLDYWGQGTTLTVSS	JA185	12
gH341E	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA198	13
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA207	14
gH341D	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA197	15
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA209	16
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA199	17
gH341C	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA184	18
gH341*	PEDTAVYYCARYYDDHY.....CLDYWGQGTTLTVSS	JA203	19
gH341*	PEDTAVYYCARYYDDHY.....CLDYWGQGTTLTVSS	JA205	20
gH341B	PEDTAVYYCARYYDDHY.....CLDYWGQGTTLTVSS	JA183	21
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA204	22
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA206	23
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA208	24
KOL	PEDTGVYFCARDGGHGFCSSASCFGPDYWGQGTPVTVSS		10

FIG. 5c

OKT3 LIGHT CHAIN CDR GRAFTING

1. gL221 and derivatives

```
          1                        24        34      42
Okt3vl    QIVLTQSPAOMSASPGEKVTMTCSASS.SVSYMNWYQQKSGT
gL221     DIQMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
gL221A    QIVMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
gL221B    QIVMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
gL221C    DIQMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
REI       DIQMTQSPSSLSASVGDRVTITCQASQDIIKYLNWYQQTPGK

          43     50    56                          85
Okt3vl    SPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAAT
gL221     APKLLIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
gL221A    APKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
gL221B    APKLLIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
gL221C    APKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
REI       APKLLIYEASNLQAGVPSRFSGSGSGTDYTFTISSLQPEDIAT (SEQ ID NO:8)

          86   91   96         108
Okt3vl    YYCQQWSSNPFTFGSGTKLEINR     (SEQ ID NO:29)
gL221     YYCQQWSSNPETFGQGTKLQITR     (SEQ ID NO:25)
gL221A    YYCQQWSSNPETFGQGTKLQITR     (SEQ ID NO:26)
gL221B    YYCQQWSSNPETFGQGTKLQITR     (SEQ ID NO:27)
gL221C    YYCQQWSSNPETFGQGTKLQITR     (SEQ ID NO:28)
REI       YYCQQYQSLPYTFGQGTKLQITR     (SEQ ID NO:9)
```

CDR'S ARE UNDERLINED

FRAMEWORK RESIDUES INCLUDED IN THE GENE ARE DOUBLE UNDERLINED

FIG. 6

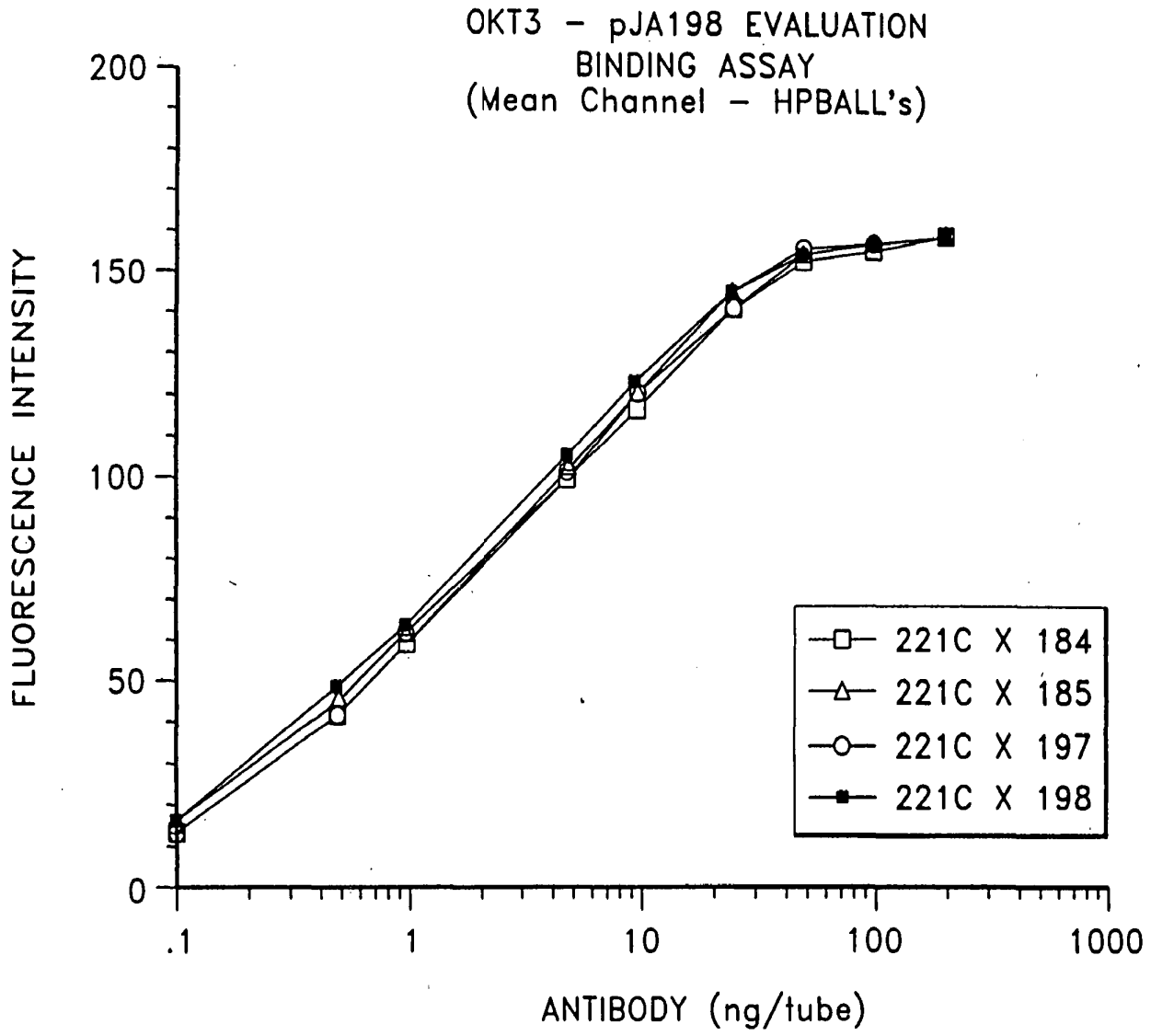

FIG. 7

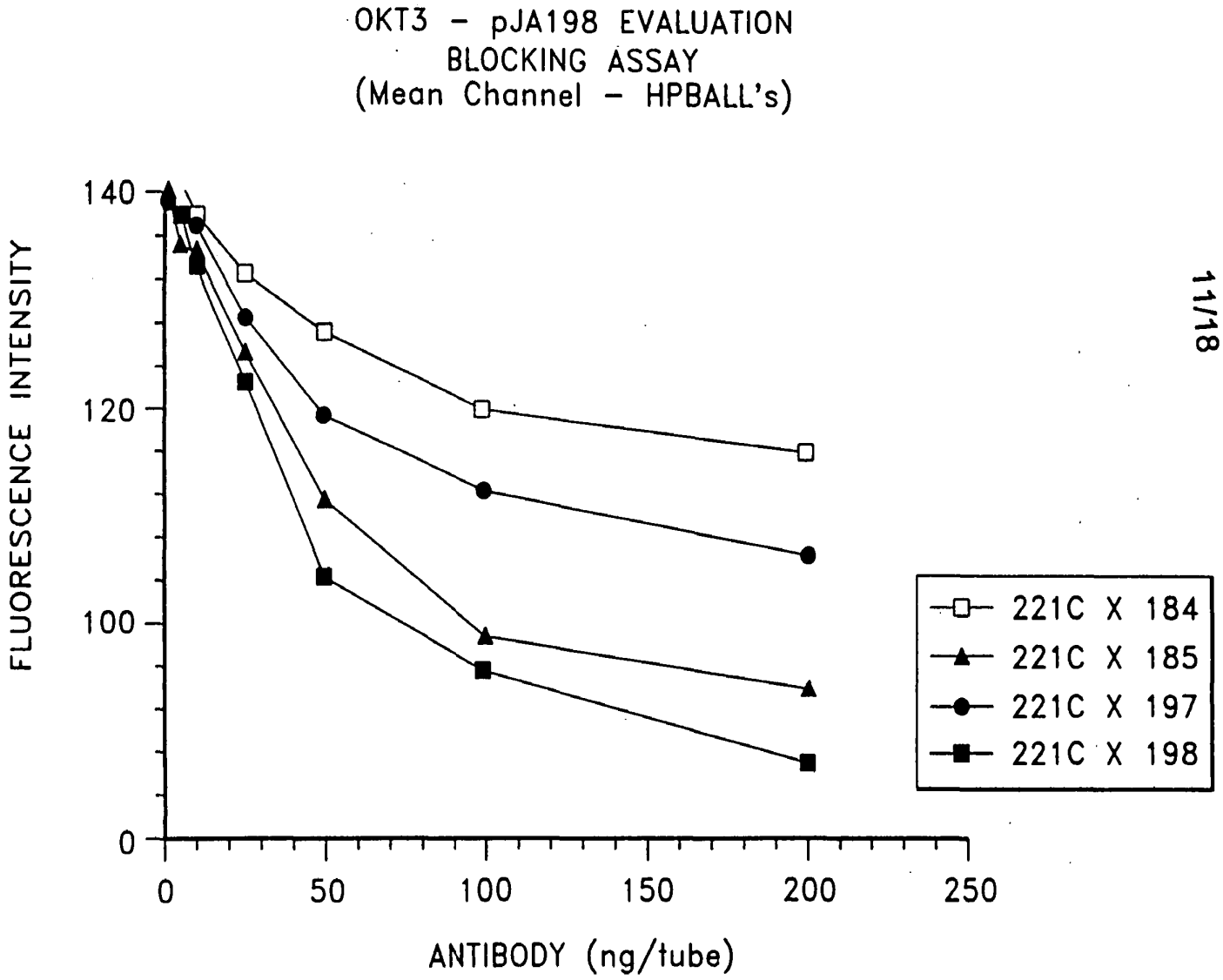

FIG. 8

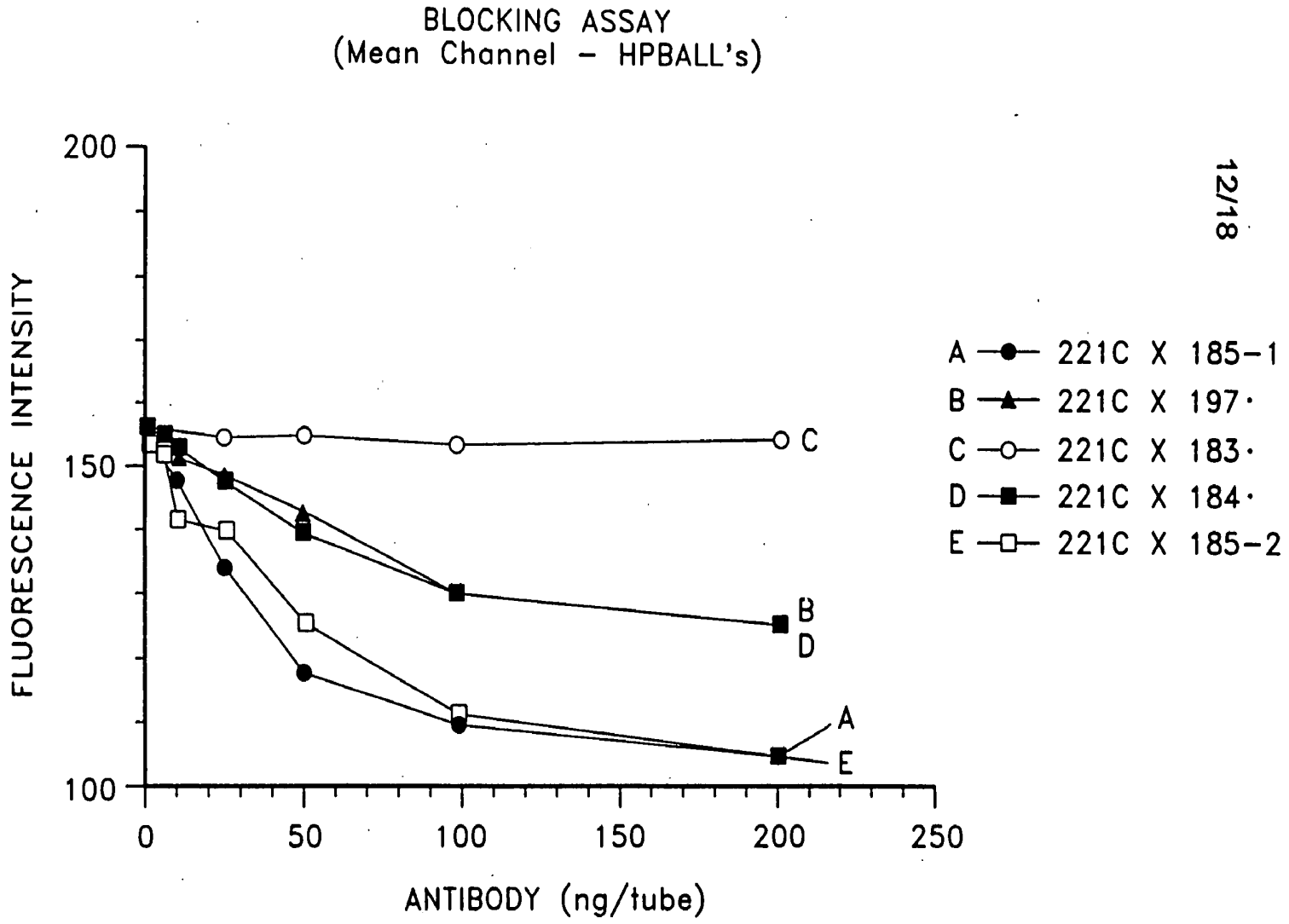

FIG. 9

FIG. 10a

OKT3 – GRAFTED HEAVY CHAINS
BINDING ASSAY
(Mean Channel – HPALL's)
FLUORESCENCE INTENSITY
0
50
100
150
200
.1
1
10
100
1000
ANTIBODY (ng/tube)
A
B
C
D
E
F
G
H
141 X 144
2210 X 185
2210 X 199
2210 X 204
2210 X 205
2210 X 207
2210 X 208
2210 X 209

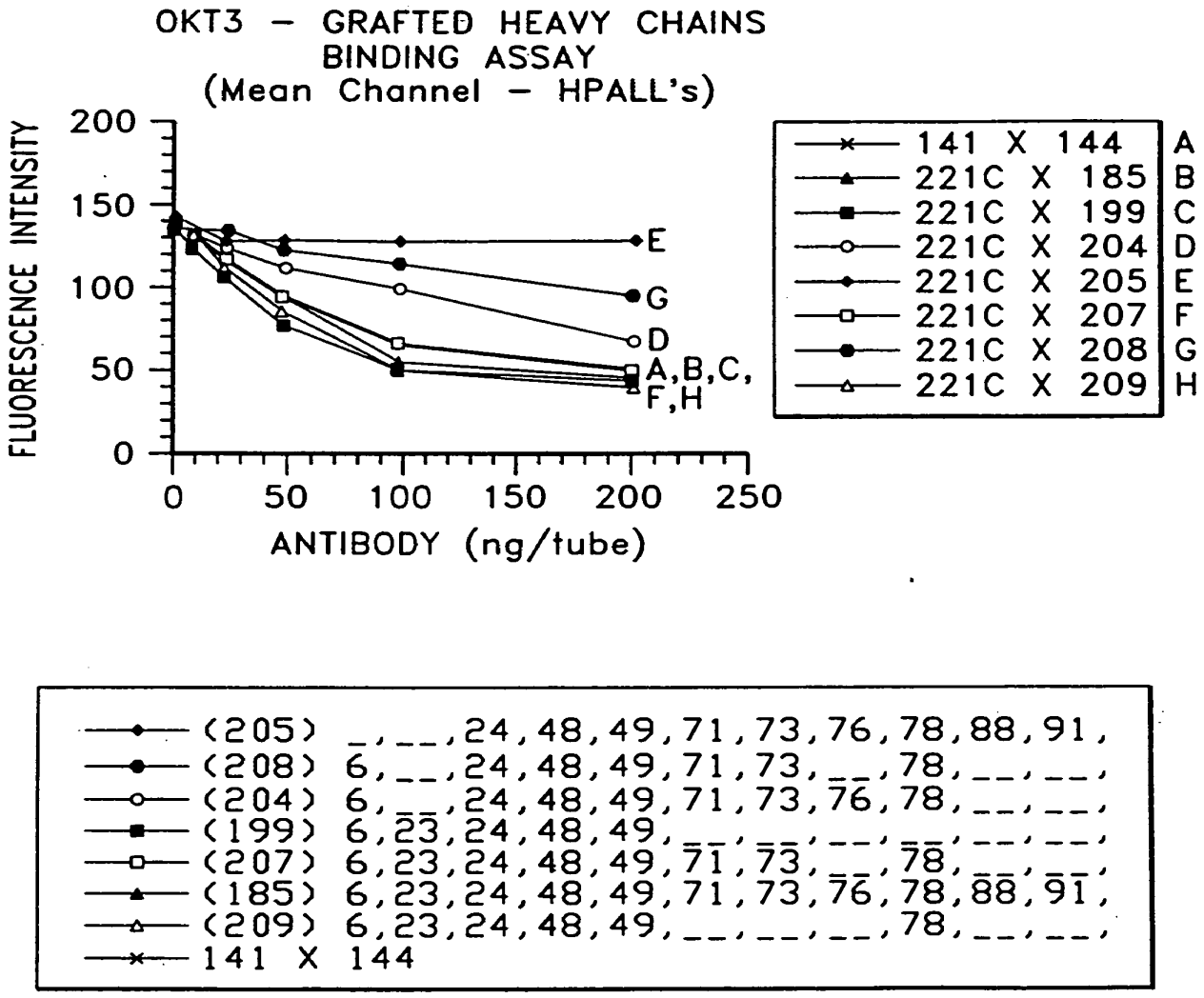
OKT3 – GRAFTED HEAVY CHAINS
BINDING ASSAY
(Mean Channel – HPALL's)
FLUORESCENCE INTENSITY
200
150
100
50
0
0
50
100
150
200
250
ANTIBODY (ng/tube)
E
G
D
A,B,C,
F,H
141 X 144 A
221C X 185 B
221C X 199 C
221C X 204 D
221C X 205 E
221C X 207 F
221C X 208 G
221C X 209 H
(205) _,__,24,48,49,71,73,76,78,88,91,
(208) 6,__,24,48,49,71,73,__,78,__,__,
(204) 6,__,24,48,49,71,73,76,78,__,__,
(199) 6,23,24,48,49,__,__,__,__,__,__,
(207) 6,23,24,48,49,71,73,__,78,__,__,
(185) 6,23,24,48,49,71,73,76,78,88,91,
(209) 6,23,24,48,49,__,__,__,78,__,__,
141 X 144

FIG. 10b

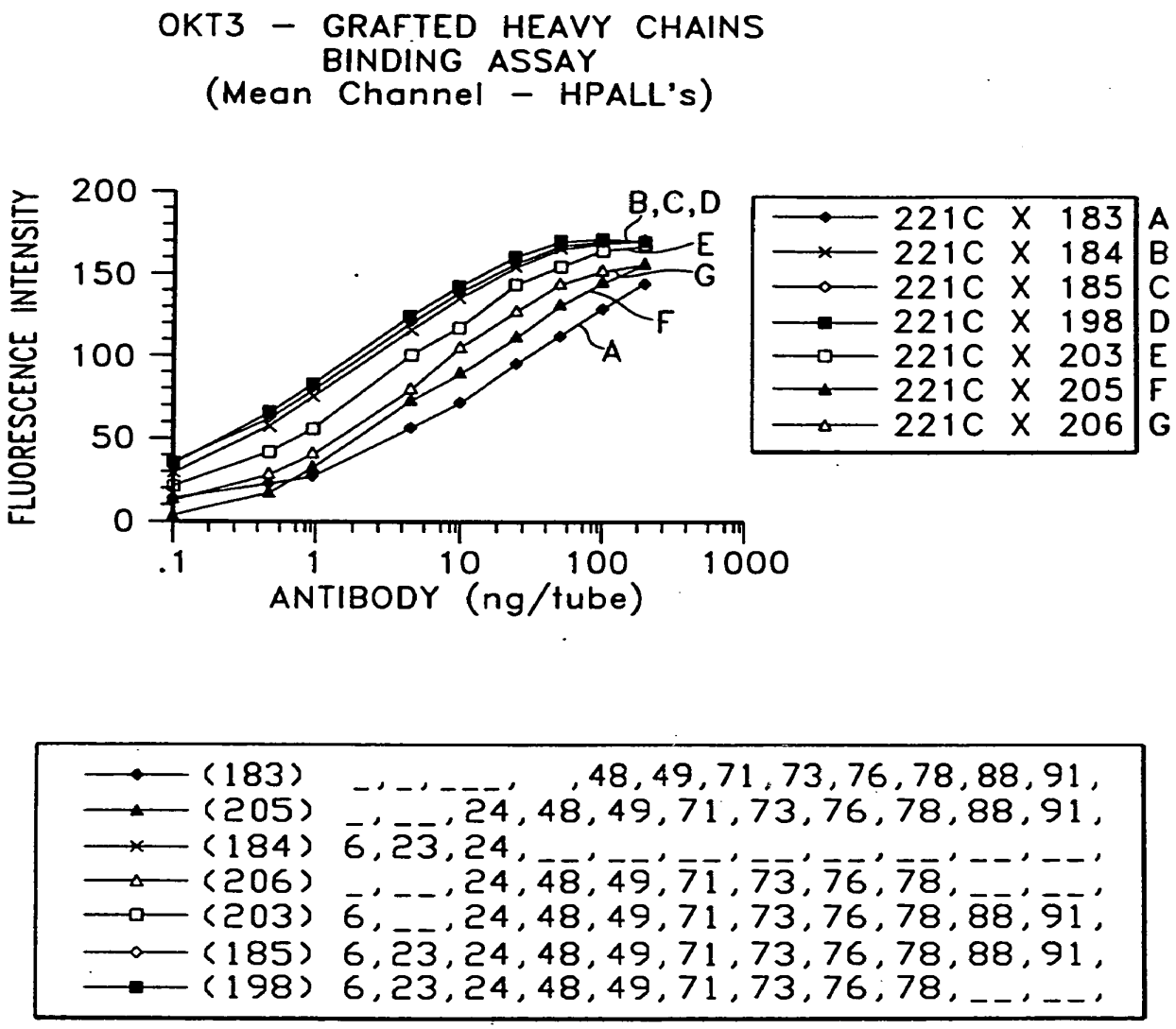
OKT3 – GRAFTED HEAVY CHAINS
BINDING ASSAY
(Mean Channel – HPALL's)
FLUORESCENCE INTENSITY
200
150
100
50
0
.1
1
10
100
1000
ANTIBODY (ng/tube)
B,C,D
E
G
F
A
221C X 183 A
221C X 184 B
221C X 185 C
221C X 198 D
221C X 203 E
221C X 205 F
221C X 206 G
(183) _,_,___, ,48,49,71,73,76,78,88,91,
(205) _,__,24,48,49,71,73,76,78,88,91,
(184) 6,23,24,__,__,__,__,__,__,__,__,
(206) _,__,24,48,49,71,73,76,78,__,__,
(203) 6,__,24,48,49,71,73,76,78,88,91,
(185) 6,23,24,48,49,71,73,76,78,88,91,
(198) 6,23,24,48,49,71,73,76,78,__,__,

FIG. 11a

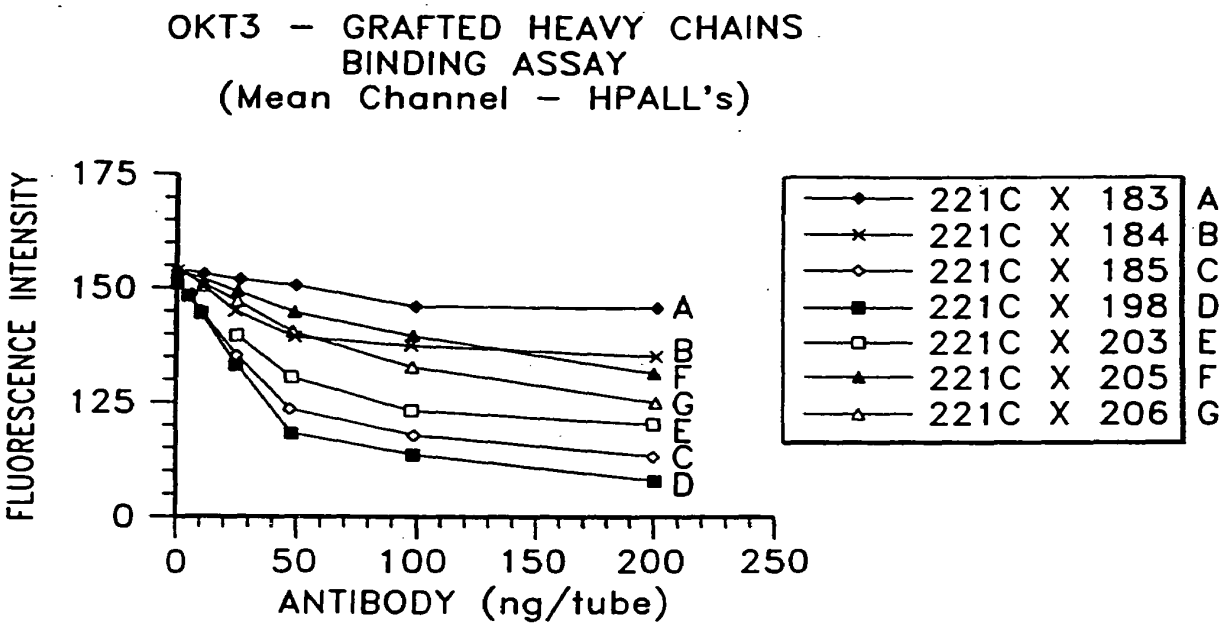
OKT3 – GRAFTED HEAVY CHAINS
BINDING ASSAY
(Mean Channel – HPALL's)
FLUORESCENCE INTENSITY
175
150
125
0
0
50
100
150
200
250
ANTIBODY (ng/tube)
A
B
F
G
E
C
D
221C X 183 A
221C X 184 B
221C X 185 C
221C X 198 D
221C X 203 E
221C X 205 F
221C X 206 G
(183) _,_,___, ,48,49,71,73,76,78,88,91,
(205) _,__,24,48,49,71,73,76,78,88,91,
(184) 6,23,24,__,__,__,__,__,__,__,__,
(206) _,__,24,48,49,71,73,76,78,__,__,
(203) 6,__,24,48,49,71,73,76,78,88,91,
(185) 6,23,24,48,49,71,73,76,78,88,91,
(198) 6,23,24,48,49,71,73,76,78,__,__,

FIG. 11b

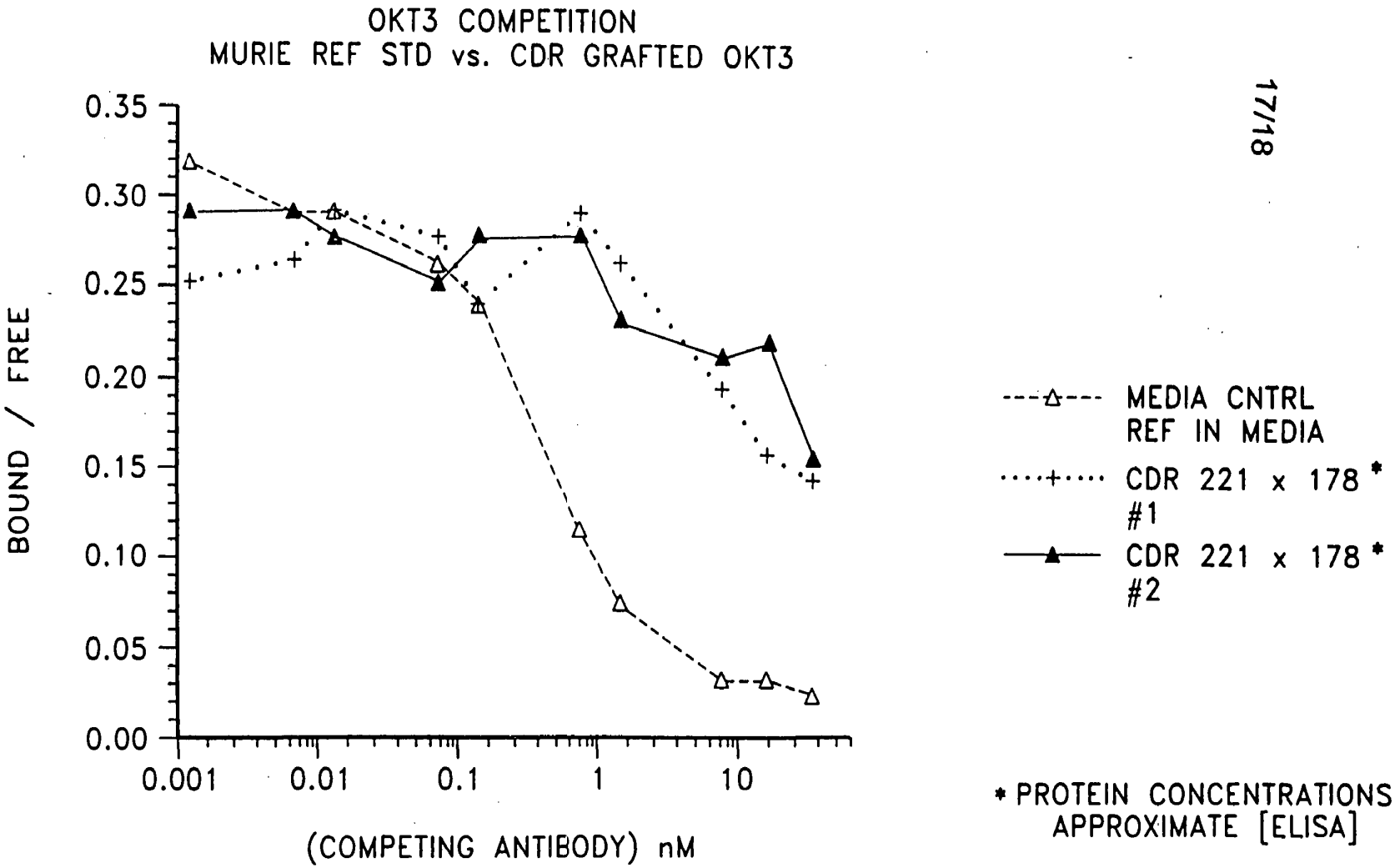

FIG. 12

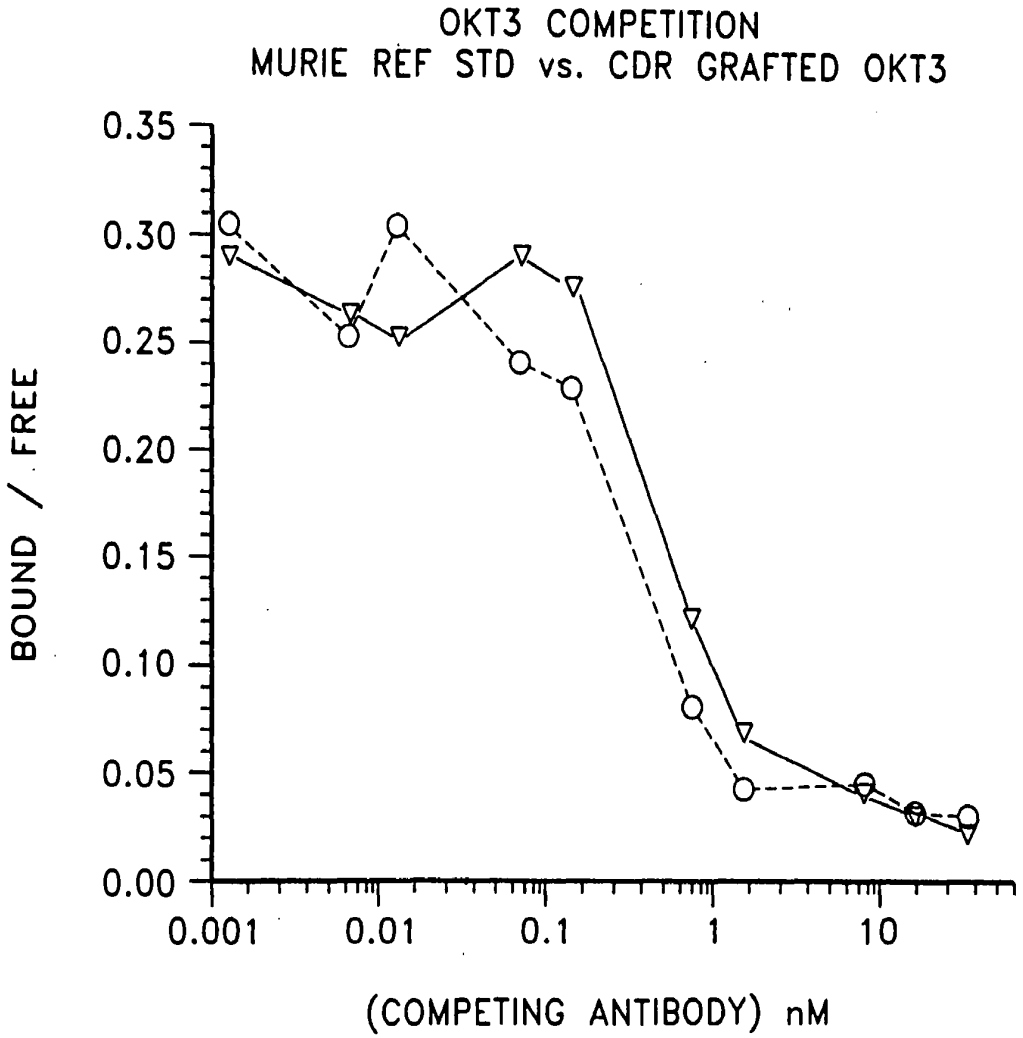

FIG. 13

DOCKET NO.: CARP-0009 PATENT

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name; and

I verily believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: HUMANISED ANTIBODIES the specification of which:

___ is attached hereto.

✓ International was filed on 21 December 1990 as Application Serial No. PCT/GB90/02017 and was amended on _____. (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with 37 CFR § 1.56(a).

I hereby claim foreign priority benefits under 35 U.S.C. § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of any application on which priority is claimed:

Country	Number	Date Filed	Priority Claimed
U.K.	8928874.0	21.12.89	yes
			___
			___

PTO-19

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose material information as defined in 37 CFR § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	Filing Date	Status (patented, pending)

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

Francis A. Paintin

Registration Nos. 19,386

of the firm of **WOODCOCK WASHBURN KURTZ MACKIEWICZ & NORRIS**, One Liberty Place - 46th Floor, Philadelphia, Pennsylvania 19103, and

Address all telephone calls and correspondence to:

Francis A. Paintin

WOODCOCK WASHBURN KURTZ MACKIEWICZ & NORRIS
One Liberty Place - 46th Floor
Philadelphia, PA 19103
Telephone No. **215-568-3100.**

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the

United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1	Full Name JOHN ROBERT ADAIR	Inventor's Signature John Robert Adair	Date 13/8/91
	Residence 23 George Road, Stokenchurch, High Wycombe, Buckinghamshire HP14 3RN, U.K.	Citizenship U.K.	
	Post Office Address 23 George Road, Stokenchurch, High Wycombe, Buckinghamshire HP14 3RN, U.K.		
2	Full Name DILJEET SINGH ATHWAL	Inventor's Signature	Date 13/8/91
	Residence Flat 35, Knollys House, Tavistock Square, London WC1, U.K.	Citizenship U.K.	
	Post Office Address Flat 35, Knollys House, Tavistock Square, London WC1, U.K.		
3	Full Name JOHN SPENCER EMTAGE	Inventor's Signature John Spencer Emtage	Date 13/8/91
	Residence 49 Temple Mill Island, Temple Marlow, Buckinghamshire, SL7 1SQ, U.K.	Citizenship U.K.	
	Post Office Address 49 Temple Mill Island, Temple, Marlow, Buckinghamshire SL7 1SQ, U.K.		
4	Full Name	Inventor's Signature	Date
	Residence	Citizenship	
	Post Office Address		
5	Full Name	Inventor's Signature	Date
	Residence	Citizenship	
	Post Office Address		

DOCKET NO.: CARP0001-112 **PATENT**
PRELIMINARY AMENDMENT AND REQUEST FOR INTERFERENCE UNDER 37 C.F.R. § 42.202 DATED NOVEMBER 21, 2005

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of: John R. Adair, Diljeet S. Athwal and John S. Emtage

Serial No.: Not Yet Assigned **Art Unit: Not Yet Assigned**

Filing Date: November 21, 2005 **Examiner: Not Yet Assigned**

For: HUMANISED ANTIBODIES

Customer No.: 34132

EXPRESS MAIL LABEL NO.: EV146 601 565US
DATE OF DEPOSIT: November 21, 2005

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

PRELIMINARY AMENDMENT
AND
REQUEST FOR INTERFERENCE UNDER 37 CFR § 42.202

Applicant respectfully requests entry of the following amendments prior to the calculation of filing fees:

Amendments To The Specification begin on page 2 of this paper.

Listing of the Claims, reflecting current amendments, begins on page 3 of this paper.

Remarks begin on page 4 of this paper.

The **Conclusion** is found on page 12.

Appendix A is found on page 14.

Appendix B is found on page 17.

Appendix C is found on page 19.

Appendix D is found on page 20.

Appendix E is found on page 21.

Carter Exhibit 2003
Carter v. Adair
Interference No. 105,744

AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph containing the cross-reference to related applications on page 1 of the specification with the following:

This application is a Continuation of U.S. Application Serial No. 08/846,658, Filed May 1, 1997, which is a Continuation of U.S. Application Serial No. 08/303,569, Filed September 7, 1994, now U.S. Patent No. 5,859,205, Issued January 12, 1999, which is a File Wrapper Continuation of U.S. Application Serial No. 07/743,329, Filed September 17, 1991, Abandoned, which is a 35 U.S.C. 371 National Stage Application of International Application No. PCT/GB90/02017, International Filing Date of December 21, 1990, which claims priority to Great Britain Application No. 8928874.0, Filed December 21, 1989, all applications are incorporated by reference herein in their entireties.

LISTING OF CLAIMS

This listing of claims represents the current status of the claims.

Claims 1-23 **(cancelled)**

Claim 24 **(new)** A humanised antibody heavy chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises an amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof, as numbered according to Kabat.

Claim 25 **(new)** A humanised antibody comprising the variable domain of claim 24.

REMARKS

Claims 24 to 25 are pending. Applicants hereby request an interference in accordance with 37 CFR § 42.202. It is noted that U.S. Patent No. 6,407,213, whose claims present the basis for an interference, is classified in Class 530, and was examined by Examiners Minh-Tam Davis and Anthony C. Caputa.

Compliance with 37 CFR § 42.202

Applicants respectfully submit that all requirements of 37 CFR § 42.202 have been met and respectfully request examination of the present application and declaration of an interference.

(a) Identifying The Patent

Applicants request that an interference be declared between Applicants' above-identified application and U.S. Patent No. 6,407,213 B1 (hereinafter the 213 patent), issued to Carter et al. on June 18, 2002, a copy of which is enclosed herewith.

(b) Compliance with 35 USC § 135(b)

Although the present rules do not require a showing of compliance under 35 USC § 135(b), Applicants submit the following to advance the examination of the present application to allowability. The present application is a Continuation of U.S. Application Serial No. 08/846,658, Filed May 1, 1997, which is a Continuation of U.S. Application Serial No. 08/303,569, Filed September 7, 1994, now U.S. Patent No. 5,859,205, Issued January 12, 1999, which is a File Wrapper Continuation of U.S. Application Serial No. 07/743,329, Filed September 17, 1991, Abandoned, which is a 35 U.S.C. 371 National Stage Application of International Application No. PCT/GB90/02017, International Filing Date of December 21, 1990 (hereinafter "the PCT application," a copy of which is enclosed herewith), which claims priority to Great Britain Application No. 8928874.0, Filed December 21, 1989. Claims 1-23 as filed in the PCT application are attached as Appendix A.

Under 35 USC § 135(b)(1), Applicants must show that they had a claim to the same, or substantially the same, subject mater as a claim of the 213 patent within one year of the issuance of the 213 patent, or June 18, 2003. The 213 patent issued on June 18, 2002. The PCT application was filed on December 21, 1990, over 10 years earlier than the 213 patent issued. The time limit of Section 135(b)(1) has been complied with fully. See *Corbett v. Chisholm*, 196 USPQ 337 (CCPA 1977).

To meet the "same or substantially the same invention" requirement of Section 135(b)(1), Applicants must show that their claim contained all material limitations, i.e. limitations necessary to patentability, of the claim of the 213 patent alleged to be to the same, or substantially the same, invention. *Corbett v. Chisholm*, 196 USPQ 337 (C.C.P.A. 1977), *citing Wetmore v. Miller*, 477 F.2d 960, 177 USPQ 699 (C.C.P.A. 19730).

As is evident from Appendix A, Applicants made a claim for the same, or substantially the same, subject matter as a claim of the 213 patent well before the issuance of the 213 patent. Claim 16 of the PCT application, as depending from claim 8, is to substantially the same subject matter as at least claim 1 of the 213 patent. For the Office's convenience, all three claims are duplicated below.

> **Claim 8 of the PCT application**: A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, **58** and 71.
>
> **Claim 16 of the PCT application**: A CDR-grafted antibody heavy or **light chain** or molecule according to any one of the preceding claims comprising human acceptor residues and non-human donor residues.
>
> **Claim 1 of the 213 patent**: A humanized antibody variable domain comprising non-human Complementarity Determining Region (CDR) amino acid residues

which bind an antigen incorporated into a human antibody variable domain, and further comprising a Framework Region (FR) amino acid substitution at a site selected from the group consisting of: 4L, 38L, 43L, 44L, **58L**, 62L, 65L, 66L, 67L, 68L, 69L, 73L, 85L, 98L, 2H, 4H, 36H, 39H, 43H, 45H, 69H, 70H, 74H, and 92H, utilizing the numbering system set forth in Kabat.

Both claim 16 of the PCT application, as it depends from claim 8, and claim 1 of the 213 patent are directed to variable domains comprising non-human Complementarity Determining Regions ("CDRs") that bind antigen, i.e., antigen binding regions, human framework regions, and a non-human framework amino acid at residue 58 of the light chain, using Kabat numbering. Both claims also comprise a human framework region; claim 1 of the 213 patent simply recites it differently -- if one incorporates non-human CDR amino acid residues into a human antibody variable domain, one ends up with a human framework region. Further, the 213 patent defines "humanized antibody" as an antibody having a framework region "having substantially the amino acid sequence of a human immunoglobulin," i.e., a human framework region (see column 8, lines 11-17, of the 213 patent). "Antigen binding regions," as recited in claim 8 of the PCT application, refers to CDRs (see page 8, lines 10-13, of the PCT application). As indicated on page 8, lines 22-24 of the PCT application, all numbering is according to Kabat. Claim 16 implicitly contains the recitation that the amino acid be substituted. As indicated on page 17, lines 1-19 of the PCT application, substitution of the non-human framework residues for the human framework residues is required when the donor and framework residue at any of the recited positions differ. While Applicants' claim recites a "light **chain**" and claim 1 of the 213 patent recites a "variable domain," Applicants submit that this is merely a difference in scope, and not a material difference. *See Corbett.*

(c) Presentation Of A Proposed Count

Applicants present in Appendix B, attached hereto, a proposed count that is presented in the "alternative" format as claim 30 or claim 80 of the 213 patent or Applicants' claim 24. All alternatives are to the same patentable invention.

All alternatives recite that the variable domain comprises non-human complementarity determining region amino acid residues which bind an antigen. All alternatives also comprise a human framework region. As discussed above for compliance with Section 135(b), the 213 patent defines "humanized antibody" as an antibody having a framework region "having substantially the amino acid sequence of a human immunoglobulin," i.e., a human framework region (see column 8, lines 11-17, of the 213 patent). And, if one incorporates non-human CDR amino acid residues into a human antibody variable domain, as recited in claims 30 and 80, one ends up with a human framework region. Further, all alternatives require only that one of the listed framework residues be substituted. Claims 30 and 80 of the 213 patent and Applicants' claim 24 recite substituting, *inter alia*, residue 78 of the heavy chain.

Although claim 30 of the 213 patent and Applicants' claim 24 do not recite the alternative recitations (a) through (c) of claim 80 of the 213 patent, e.g., "noncovalently binds antigen directly," such limitations are clearly implicit for all the framework residue substitutions recited in claim 80.

Although claims 30 and 80 of the 213 patent do not recite that the variable domain is from the light chain, it clearly must be the case when a light chain framework residue, i.e., one designated with an "L," is to be substituted.

Claim 30 of the 213 patent recites an "antibody" while the remaining alternatives recite a "variable domain." The antibody recitation is anticipated by and/or obvious over the recitation of variable domain and *vice versa*. "Humanized antibody" is defined in the 213 patent to comprise, *inter alia*, at least one variable domain (see column 8, lines 26-28, of the 213 patent).

Claim 30 of the 213 patent further recites a specific antibody target. The alternatives of the count that do not recite a particular target are clearly anticipated thereby. Considering the target, i.e., the tyrosine kinase receptor for HER2, $p185^{HER2}$, Applicants submit that claim 30 is clearly obvious over the other alternatives of the count for the reasons that follow.

The import of $p185^{HER2}$ to cancer, particularly breast cancer, had been well documented well before the priority date of the 213 patent. See, for example, Slamon, D.J. et al., *Science*, 235:177-182,1987. The development of antibodies against $p185^{HER2}$ for use in therapy had also

been well documented, as was the use of antibodies other than hybridoma-produced monoclonal antibodies. Applicants respectfully submit that, in view of the knowledge of the art at the time, a humanized antibody against $p185^{HER2}$ would have been obvious over the remaining alternatives of the count.

(d) Identification of claims corresponding to the count

Applicants identify all of the 213 patent claims, claims 1-82, and all of Applicants' pending claims, claims 24-25, as corresponding to the proposed count. All of said claims are either anticipated by, or obvious over, the proposed count as required by 37 CFR § 41.207(b)(2).

More specifically, the alternatives of the proposed count recite framework residue substitutions recited in claims 1, 5-28, 34-63, 66, 70-77, and 79 of the 213 patent.

Claims 2, 31, 67, and 81 of the 213 patent recite that the substituted residue is the residue found at the corresponding location of the non-human antibody from which the non-human CDR amino acids are obtained. Such a claim is anticipated by the proposed count – the goal of substituting framework residues is to improve affinity to approach that of the antibody from which the CDRs are obtained. See, for example, Riechmann, et al., *Nature*, 332:323-327, 1988.

Claims 3, 32, 68, and 82 of the 213 patent recite that no human framework residue other than those set forth in the claim from which they depend has been substituted. Such claims are anticipated by, or clearly obvious over, the proposed count in view of the use of the Markush language "selected from the group consisting of." The transitional phrase "consisting of" is closed, meaning that it excludes any element not listed. See MPEP 2111.03.

Claims 29 and 78 recite an antibody comprising the humanized variable domains of the claims from which they depend. Such claims are clearly anticipated by claim 30 of the proposed count.

While claims 4, 33, 62 and 69 of the 213 patent further recite that the variable domain is a "consensus" human variable domain, use of such framework regions in making humanized antibodies was known by the priority date of the 213 patent. See, for example, column 13, lines

6-9, of U.S. Patent No. 5,530,101, issued to Queen et al., filed on December 19, 1990. Claims 62 and 69 are, thus, obvious in view of the proposed count and the art.

The recitation in claim 63 that the humanized antibody lacks immunogenicity when compared to the non-human parent upon repeated administration is the very goal of humanization and is, thus, implicit. The motivation for preparing humanized antibodies was to reduce immunogenicity. *See* column 1, lines 51-58, of the 213 patent, discussing the "Background of the Invention." References from the early 1980's are cited therein disclosing the anti-globulin response to rodent monoclonal antibodies. Claim 63 is, at a minimum, obvious over the proposed count and the art.

The alternatives recited in claim 64 include the alternatives recited in claim 80 of the count. Claim 64 is, thus, anticipated by the proposed count.

The recitation in claim 65 that the variant of claim 63 binds the antigen "**up to** 3-fold more in the binding affinity than the parent antibody binds antigen" broadly includes variants that have binding affinities equal to **and** less than the parent. As recognized in the "Background of the Invention" section of the 213 patent, at column 3, lines 50-55, humanizing antibody while **retaining** high affinity for antigen was difficult to achieve; achieving lower affinity was not. This recitation, thus, is also either anticipated by, or obvious over, the proposed count in view of the art.

All of Applicants claims, claims 24-25, are anticipated by or obvious over the proposed count. Claim 25 is anticipated by claim 30 of the count.

(e) Interference-In-Fact

"An interference-in-fact exists if the subject matter of a claim of one party would, if prior art, have anticipated or rendered obvious the subject matter of a claim of the opposing party and vice versa." 37 CFR § 41.203(a) (2004). Applicants set forth in attached Appendix C a comparison of claim 66 of the 213 patent with Applicants' claim 24, both of which correspond to the present count.

As is clear from Appendix C, each claim anticipates and/or renders obvious the other. Both claims recite a humanized variable domain. Both claims are directed to a heavy chain

variable domain. Both claims also recite that the variable domain comprises non-human complementarity determining region amino acid residues which bind an antigen. Both claims also comprise a human framework region; claim 66 of the 213 patent simply recites it differently -- if one incorporates non-human CDR amino acid residues into a human antibody variable domain, one ends up with a human framework region. Indeed, as discussed above, the 213 patent defines "humanized antibody" as an antibody having a framework region "having substantially the amino acid sequence of a human immunoglobulin," i.e., a human framework region (see column 8, lines 11-17, of the 213 patent). Both claims recite a single amino acid substitution in the framework region to be selected from a Markush group listed thereafter; both recite that residue 24 of the heavy chain is to be substituted. Finally, both claims recite that numbering is according to Kabat.

(f) Support for Applicants Claims

In attached Appendix D, Applicants illustrate the representative support in their disclosure for the limitations of their claims 24 to 25. There is, of course, additional support in Applicants' application omitted herein for the sake of brevity. In Appendix E, Applicants show support for their claim 24, filed December 21, 1989. Methods for preparing exemplary antibodies having framework substitutions are described, *inter alia*, on pages 18-23, Sections 13.1.1 through 15.3, of the GB priority application.

(g) Applicants Will Prevail on Priority

US Serial No. 08/146,206, which issued as the 213 patent, was filed on June 15, 1992 as a PCT continuation-in-part of U.S. Serial No. 07/715,272, filed June 14, 1991.

The present application is a continuation of Application Serial No. 08/846,658, filed May 1, 1997, which is a continuation of Application Serial No. 08/303,569, filed September 7, 1994, now U.S. Patent No. 5,859,205, which is a continuation of Application Serial No. 07/743,329, filed as PCT/GB90/02017, filed December 21, 1990 and which claims priority benefit of GB 8928874.0, filed December 21, 1989. Applicants' earliest constructive reduction to practice date to which they are entitled is at least as early as December 21, 1989, but not later than December

21, 1990. Regardless, both dates are earlier than the earliest filing date of the 213 patent , or June 14, 1991.

CONCLUSION

The Requested Interference Should Be Declared

Early consideration and indication of allowability of all pending claims is respectfully requested. For an interference to be declared, however, only one claim needs to be allowable. MPEP § 2307.02. Should the present examination involve rejections of applicant's claims that would have been equally applicable against the 213 patent claims, applicants respectfully note MPEP § 2307.02, which requires the approval of the Group Director for such a rejection. Applicants are presumptively the prior inventors of the claimed subject matter and only desire an interference to prove that they are the actual prior inventors. Their opportunity to do so should not be unduly delayed. In view of the foregoing, Applicants respectfully request that an interference be declared between the present application and the 213 patent.

To assist the Examiner, Applicants note the following.

(1) the proposed count for the interference should be the Proposed Count set forth in Appendix B.

(2) the claims of the 213 patent which should be designated as corresponding to the count are claims 1-82, all of the issued claims of the patent.

(3) the claims of Applicants that should be designated as corresponding to the count are claims 24-25, all pending claims of the Applicants' above-identified application.

(4) no claims of either party should be designated as not corresponding to the count since none are directed to a separate patentable invention when considering the proposed count.

The examiner is requested to contact the undersigned attorney if an interview, telephonic or personal, would facilitate allowance of the claims or declaration of an interference.

Respectfully submitted,

Doreen Yatko Trujillo
Registration No. 35,719

Date: November 21, 2005

COZEN O'CONNOR, P.C.
1900 Market Street, 6th Floor
Philadelphia, PA 19103-3508
(215) 665-5593 - Telephone
(215) 701-2005 - Facsimile

Attachments: **Appendices A-E**
Copy of U.S. Patent No. 6,407,213 B1
Copy of PCT Publication WO 91/09967

APPENDIX A

1. A CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

2. A CDR-grafted heavy chain according to claim 1 comprising donor residues at positions 23, 24, 49, 71, 73 and 78, or at positions 23, 24 and 49.

3. A CDR-grafted heavy chain according to claim 2 comprising donor residues at positions 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

4. A CDR-grafted heavy chain according to claim 2 or 3, comprising donor residues at one, some or all of positions: 1 and 3, 69 (if 48 is different between donor and acceptor), 38 and 46 (if 48 is the donor residue), 67, 82 and IS (if 67 is the donor residue), 91, and any one or more of 9, 11, 41, 87, 108, 110 and 112.

5. A CDR-grafted heavy chain according to any of the preceding comprising donor CDRs at positions 26-35, 50-65 and 95-100.

6. A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 1 and/or 3 and 46 and/or 47.

7. A CDR-grafted light chain according to claim 6 comprising donor residues at positions 46 and 47.

8. A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor

residues at at least one of positions 46, 48, 58 and 71.

9. A CDR-grafted light chain according to claim 8 comprising donor residues at positions 46, 48, 58 and 71.

10. A CDR-grafted light chain according to claim 8 or 9, comprising donor residues at positions 2, 4, 6, 35, 36, 38, 44, 47, 49, 62, 64-69, 85, 87, 98, 99, 101 and 102.

11. A CDR-grafted light chain according to claim 9 or 10, comprising donor residues at one, some or all of positions: 1 and 3, 63, 60 (if 60 and 54 are able to form a potential saltbridge), 70 (if 70 and 24 are able to form a potential saltbridge), 73 and 21 (if 47 is different between donor and acceptor), 37 and 45 (if 47 if different between donor and acceptor), and any one or more of 10, 12, 40, 83, 103 and 105.

12. A CDR-grafted light chain according to any one of claims 6-11, comprising donor CDRs at positions 24-34, 50-56 and 89-97.

13. A CDR-grafted antibody molecule comprising at least one CDR-grafted heavy chain according to any one of claims 1-5 and at least one CDR-grafted light chain according to any one of claims 6-12.

14. A CDR-grafted antibody molecule according to claim 13, which is a site-specific antibody molecule.

15. A CDR-grafted antibody molecule according to claim 13 which has specificity for an interleukin, hormone or other biologically active compound or a receptor therefor.

16. A CDR-grafted antibody heavy or light chain or molecule according to any one of the preceding claims comprising human acceptor residues and non-human donor residues.

17. A DNA sequence which codes for a CDR-grafted heavy chain according to claim 1 or a

CDR-grafted light chain according to claim 6 or claim 8.

18. A cloning or expression vector containing a DNA sequence according to claim 17.

19. A host cell transformed with a DNA sequence according to claim 17.

20. A process for the production of a CDR-grafted antibody sequence according to claim 17 in a transformed host cell.

21. A process for producing a CDR-grafted antibody product comprising: (a) producing in an expression vector an operon having a DNA sequence which encodes an antibody heavy chain according to claim 1; and/or (b) producing in an expression vector an operon having a DNA sequence which encodes a complementary antibody light chain according to claim 6 or claim 8; (c) transfecting a host cell with the or each vector; and (d) culturing the transfected cell line to produce the CDR-grafted antibody product.

22. A therapeutic or diagnostic composition comprising a CDR-grafted antibody heavy chain according to claim 1, or a CDR-grafted light chain according to claim 6 or claim 8, or a CDR-grafted antibody molecule according to claim 13 in combination with a pharmaceutically acceptable carrier, diluent or excipient.

23. A method of therapy or diagnosis comprising administering an effective amount of a CDR-grafted heavy chain according to claim 1, or a CDR-grafted light chain according to claim 6 or claim 8, or a CDR-grafted antibody molecule according to claim 13 to a human or animal subject.

APPENDIX B

Proposed Count for interference

Claim 30 of the 213 patent

An antibody which binds $p185^{HER2}$ and comprises a humanized antibody variable domain, wherein the humanized antibody variable domain comprises non-human Complementarity Determining Region (CDR) amino acid residues which bind $p185^{HER2}$ incorporated into a human antibody variable domain, and further comprises a Framework Region (FR) amino acid substitution at a site selected from the group consisting of: 4L, 38L, 43L, 44L, 46L, 58L, 62L, 65L, 66L, 67L, 68L, 69L, 73L, 85L, 98L, 2H, 4H, 36H, 39H, 43H, 45H, 69H, 70H, 74H, 75H, 76H, **78H** and 92H, utilizing the numbering system set forth in Kabat.

OR

Claim 80 of the 213 patent

A humanized antibody variable domain comprising non-human Complementarity Determining Region (CDR) amino acid residues which bind an antigen incorporated into a human antibody variable domain, and further comprising a Framework Region (FR) amino acid substitution where the substituted FR residue:

(a) noncovalently binds antigen directly;

(b) interacts with a CDR; or

(c) participates in the V_L - V_H interface by affecting the proximity or orientation of the V_L and V_H regions with respect to one another, and wherein the substituted FR residue is at a site selected from the group consisting of: 4L, 38L, 43L, 44L, 58L, 62L, 65L, 66L, 67L, 68L, 69L, 73L, 85L, 98L, 2H, 4H, 24H, 36H, 39H, 43H, 45H, 69H, 70H, 73H, 74H, 76H, **78H**, 92H and 93H, utilizing the numbering system set forth in Kabat.

OR

Applicants' claim 24

A humanised antibody **heavy chain** variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises an amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and **78**, and combinations thereof, as numbered according to Kabat.

APPENDIX C

Comparison of Applicants' claim 24 and claim 66 of the 213 patent

Applicants' Claim 24	213 patent Claim 66
Claim 24 – A humanised antibody heavy chain variable domain comprising	A humanized antibody heavy chain variable domain comprising
non-human complementarity determining region amino acid residues which bind an antigen and	non-human Complementarity Determining Region (CDR) amino acid residues which bind an antigen
a human framework region wherein said framework region comprises an amino acid substitution at a residue selected from the group consisting of	incorporated into a human antibody variable domain, and further comprising a Framework Region (FR) amino acid substitution at a site selected from the group consisting of:
23, **24**, 49, 71, 73, and 78, and combinations thereof,	**24H**, 73H, 76H, 78H, and 93H
as numbered according to Kabat.	utilizing the numbering system as set forth in Kabat.

APPENDIX D

Support for Applicants' claims in Applicants' Present Specification

Claim	Present Specification
Claim 24 -- A humanised antibody heavy chain variable domain comprising	page 6, lines 29-31
non-human complementarity determining region amino acid residues which bind an antigen and	page 7, line 29, through page 8, line 7 and page 17, lines 6-7
a human framework region wherein said framework region comprises an amino acid substitution at a residue selected from the group consisting of	page 7, line 29, through page 8, line 7 and page 17, lines 1-19
23, 24, 49, 71, 73, and 78, and combinations thereof,	page 7, lines 1-3
as numbered according to Kabat.	page 8, lines 22-24
Claim 25 -- A humanised antibody comprising the variable domain of claim 24.	see above support for claim 24

APPENDIX E

Support For Applicants' Claims in the GB Application, filed December 21, 1989

Claim	1989 GB Application
Claim 24 – A humanised antibody heavy chain variable domain comprising	page 5, lines 8-10
non-human complementarity determining region amino acid residues which bind an antigen and	page 5, lines 8-21
a human framework region wherein said framework region comprises an amino acid substitution at a residue selected from the group consisting of	page 5, line 10 and page 26, lines 31-33
23, 24, 49, 71, 73, and 78, and combinations thereof,	page 6, lines 8-10 and Table 1
as numbered according to Kabat.	page 6, lines 5-7

DOCKET NO.: CARP0001-112 **PATENT**
APPLICATION SERIAL NO. 11/284,261

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re application of: **John R. Adair et al.** Confirmation No. **5305**

Serial No.: **11/284,261** Art Unit: **1643**

Filed: **November 21, 2005** Examiner: **Anne Gussow**

Title: **HUMANISED ANTIBODIES**

Customer No.: **34132**

Via EFS Web:
DATE FILED: September 9, 2009

MAIL STOP AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR RECONSIDERATION

Dear Sir:

This paper is being filed in response to the Non-Final Office Action dated as mailed March 9, 2009. Applicants hereby petition for a three-month extension of time to respond to the Non-Final Office Action and request the Commissioner to charge Deposit Account 50-3111 the appropriate extension of time fee.

Carter Exhibit 2004
Carter v. Adair
Interference No. 105,744

LISTING OF THE CLAIMS

Claims 1-23 (**cancelled**)

Claim 24 (**currently amended**): A humanised antibody comprising a heavy chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises a non-human amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof, as numbered according to Kabat.

Claim 25 (**cancelled**)

REMARKS

Claims 24 and 25 were pending. All pending claims were rejected in the Non-Final Rejection. In view of the foregoing amendments and arguments that follow, Applicants respectfully request withdrawal of all rejections upon reconsideration.

Applicants acknowledge with appreciation the Office's withdrawal of the objections to claim 24 under 35 U.S.C. 112, second paragraph, as being indefinite.

Rejection Under 35 U.S.C. § 112, First Paragraph

Claim 24 was amended with the RCE filing and was again rejected as allegedly being indefinite. The Office alleges that the specification is enabling for a humanized antibody comprising a heavy chain variable domain and a light chain variable domain, with all 6 CDRs, and does not provide enablement for a humanized antibody heavy chain variable domain alone. The Office is clearly disregarding the fact that the CDR-grafted chains can be combined with other chains, as disclosed in the specification, including chimeric and mouse chains. Thus, it is not necessary for the claims to recite both chains. Applicants traverse this rejection but have amended claim 24 to recite a humanized antibody comprising a heavy chain variable domain.

Applicants respectfully submit that this rejection has been overcome.

Rejection Under 35 U.S.C. 102(e)

Claims 24 and 25 were rejected under 35 U.S.C. 102(e) as allegedly being anticipated by Queen, et al US Patent 5,585,089 the "'089 patent". Claim 25 has been cancelled. Applicants traverse this rejection as it applies to claim 24.

With all due respect, the Office has apparently misread the claims. Initially, the office states that the claims recite that the framework region comprises a **non-human** amino acid

substitution at a residue selected from 23, 24, 49, 71, 73, and 78, and combinations thereof. The Office then asserts that the "'089 patent" teaches an antibody comprising a humanized heavy chain variable domain comprising human residues at positions except the CDRs, and the following framework positions—27, 93, 95, 98, 107-109, 11, 30, 67, 48, and 68. The Office concludes, thus, that all the remaining framework positions are the human antibody allegedly taught in the "'089 patent". The Office then states that, since the claims recite substitution to **human** residues in the heavy chain in residues 23, 24, 49, 71, 73, and 78, and the "'089 patent" teaches human residues in all of those positions, the claim limitations are met. As the Office previously acknowledged, however, these residues are **non-human** residues in claim 24. The "'089 patent", thus, does not anticipate the Applicants invention.

Applicants respectfully request that this rejection be withdrawn.

CONCLUSION

Applicants respectfully submit that claim 24 is allowable and early allowance of the same. If a telephonic conversation with Applicants' attorney would help expedite the prosecution of the above-identified application, the Examiner is urged to call the undersigned at 215-665-5593.

Respectfully submitted,

/Doreen Yatko Trujillo/

Doreen Yatko Trujillo
Registration No. 35,719

Date: September 9, 2009

COZEN O'CONNOR, P.C.
1900 Market Street, 4th Floor
Philadelphia, PA 19103-3508
Telephone: (215) 665-5593
Facsimile: (215) 701-2005

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C12P 21/08, C12N 15/13 A61K 39/395, C07K 15/06 C12N 5/10, 15/62	A1	(11) International Publication Number: WO 91/09967 (43) International Publication Date: 11 July 1991 (11.07.91)

(21) International Application Number: PCT/GB90/02017

(22) International Filing Date: 21 December 1990 (21.12.90)

(30) Priority data:
8928874.0 21 December 1989 (21.12.89) GB

(71) Applicant *(for all designated States except US)*: CELLTECH LIMITED [GB/GB]; 216 Bath Road, Slough, Berkshire SL1 4EN (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : ADAIR, John, Robert [GB/GB]; 23 George Road, Stokenchurch, High Wycombe, Buckinghamshire HP14 3RN (GB). ATHWAL, Diljeet, Singh [GB/GB]; Flat 35, Knollys House, Tavistock Square, London WC1 (GB). EMTAGE, John, Spencer [GB/GB]; 49 Temple Mill Island, Temple, Marlow, Buckinghamshire SL7 1SQ (GB).

(74) Agent: MERCER, Christopher, Paul; Carpmaels & Ransford, 43 Bloomsbury Square, London WC1A 2RA (GB).

(81) Designated States: AT, AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CF (OAPI patent), CG (OAPI patent), CH, CH (European patent), CM (OAPI patent), DE, DE (European patent), DK, FI, FR (European patent), GA (OAPI patent), GB, GB (European patent), GR, HU, IT (European patent), JP, KP, KR, LK, LU, LU (European patent), MC, MG, ML (OAPI patent), MR (OAPI patent), MW, NL, NL (European patent), NO, RO, SD, SE, SE (European patent), SN (OAPI patent), SU, TD (OAPI patent), TG (OAPI patent), US.

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.
With a request for rectification under Rule 91.1(f).

(54) Title: HUMANISED ANTIBODIES

(57) Abstract

CDR-grafted antibody heavy and light chains comprise acceptor framework and donor antigen binding regions, the heavy chains comprising donor residues at at least one of positions (6, 23) and/or (24, 48) and/or (49, 71) and/or (73, 75) and/or (76) and/or (78) and (88) and/or (91). The CDR-grafted light chains comprise donor residues at at least one of positions (1) and/or (3) and (46) and/or (47) or at at least one of positons (46, 48, 58) and (71). The CDR-grafted antibodies are preferably humanised antibodies, having non human, e.g. rodent, donor and human acceptor frameworks, and may be used for *in vivo* therapy and diagnosis. A generally applicable protocol is disclosed for obtaining CDR-grafted antibodies.

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HUMANISED ANTIBODIES

Field of the Invention

The present invention relates to humanised antibody molecules, to processes for their production using recombinant DNA technology, and to their therapeutic uses.

The term "humanised antibody molecule" is used to describe a molecule having an antigen binding site derived from an immunoglobulin from a non-human species, and remaining immunoglobulin-derived parts of the molecule being derived from a human immunoglobulin. The antigen binding site typically comprises complementarity determining regions (CDRs) which determine the binding specificity of the antibody molecule and which are carried on appropriate framework regions in the variable domains. There are 3 CDRs (CDR1, CDR2 and CDR3) in each of the heavy and light chain variable domains.

In the description, reference is made to a number of publications by number. The publications are listed in numerical order at the end of the description.

Background of the Invention

Natural immunoglobulins have been known for many years, as have the various fragments thereof, such as the Fab, $(Fab')_2$ and Fc fragments, which can be derived by enzymatic cleavage. Natural immunoglobulins comprise a generally Y-shaped molecule having an antigen-binding site towards the end of each upper arm. The remainder of the structure, and particularly the stem of the Y, mediates the effector functions associated with immunoglobulins.

Natural immunoglobulins have been used in assay, diagnosis and, to a more limited extent, therapy. However, such uses, especially in therapy, were hindered until recently by the polyclonal nature of natural immunoglobulins. A significant step towards the realisation of the potential

of immunoglobulins as therapeutic agents was the discovery of procedures for the production of monoclonal antibodies (MAbs) of defined specificity (1).

However, most MAbs are produced by hybridomas which are fusions of rodent spleen cells with rodent myeloma cells. They are therefore essentially rodent proteins. There are very few reports of the production of human MAbs.

Since most available MAbs are of rodent origin, they are naturally antigenic in humans and thus can give rise to an undesirable immune response termed the HAMA (Human Anti-Mouse Antibody) response. Therefore, the use of rodent MAbs as therapeutic agents in humans is inherently limited by the fact that the human subject will mount an immunological response to the MAb and will either remove it entirely or at least reduce its effectiveness. In practice, MAbs of rodent origin may not be used in patients for more than one or a few treatments as a HAMA response soon develops rendering the MAb ineffective as well as giving rise to undesirable reactions. For instance, OKT3 a mouse IgG2a/k MAb which recognises an antigen in the T-cell receptor-CD3 complex has been approved for use in many countries throughout the world as an immunosuppressant in the treatment of acute allograft rejection [Chatenoud et al (2) and Jeffers et al (3)]. However, in view of the rodent nature of this and other such MAbs, a significant HAMA response which may include a major anti-idiotype component, may build up on use. Clearly, it would be highly desirable to diminish or abolish this undesirable HAMA response and thus enlarge the areas of use of these very useful antibodies.

Proposals have therefore been made to render non-human MAbs less antigenic in humans. Such techniques can be generically termed "humanisation" techniques. These

techniques typically involve the use of recombinant DNA technology to manipulate DNA sequences encoding the polypeptide chains of the antibody molecule.

Early methods for humanising MAbs involved production of chimeric antibodies in which an antigen binding site comprising the complete variable domains of one antibody is linked to constant domains derived from another antibody. Methods for carrying out such chimerisation procedures are described in EP0120694 (Celltech Limited), EP0125023 (Genentech Inc. and City of Hope), EP-A-0 171496 (Res. Dev. Corp. Japan), EP-A-0 173 494 (Stanford University), and WO 86/01533 (Celltech Limited). This latter Celltech application (WO 86/01533) discloses a process for preparing an antibody molecule having the variable domains from a mouse MAb and the constant domains from a human immunoglobulin. Such humanised chimeric antibodies, however, still contain a significant proportion of non-human amino acid sequence, i.e. the complete non-human variable domains, and thus may still elicit some HAMA response, particularly if administered over a prolonged period [Begent et al (ref. 4)].

In an alternative approach, described in EP-A-0239400 (Winter), the complementarity determining regions (CDRs) of a mouse MAb have been grafted onto the framework regions of the variable domains of a human immunoglobulin by site directed mutagenesis using long oligonucleotides. The present invention relates to humanised antibody molecules prepared according to this alternative approach, i.e. CDR-grafted humanised antibody molecules. Such CDR-grafted humanised antibodies are much less likely to give rise to a HAMA response than humanised chimeric antibodies in view of the much lower proportion of non-human amino acid sequence which they contain.

The earliest work on humanising MAbs by CDR-grafting was carried out on MAbs recognising synthetic antigens, such as the NP or NIP antigens. However, examples in which a mouse MAb recognising lysozyme and a rat MAb recognising an antigen on human T-cells were humanised by CDR-grafting have been described by Verhoeyen et al (5) and Riechmann et al (6) respectively. The preparation of CDR-grafted antibody to the antigen on human T cells is also described in WO 89/07452 (Medical Research Council).

In Riechmann et al/Medical Research Council it was found that transfer of the CDR regions alone [as defined by Kabat refs. (7) and (8)] was not sufficient to provide satisfactory antigen binding activity in the CDR-grafted product. Riechmann et al found that it was necessary to convert a serine residue at position 27 of the human sequence to the corresponding rat phenylalanine residue to obtain a CDR-grafted product having improved antigen binding activity. This residue at position 27 of the heavy chain is within the structural loop adjacent to CDR1. A further construct which additionally contained a human serine to rat tyrosine change at position 30 of the heavy chain did not have a significantly altered binding activity over the humanised antibody with the serine to phenylalanine change at position 27 alone. These results indicate that changes to residues of the human sequence outside the CDR regions, in particular in the structural loop adjacent to CDR1, may be necessary to obtain effective antigen binding activity for CDR-grafted antibodies which recognise more complex antigens. Even so the binding affinity of the best CDR-grafted antibodies obtained was still significantly less than the original MAb.

Very recently Queen et al (9) have described the preparation of a humanised antibody that binds to the

interleukin 2 receptor, by combining the CDRs of a murine MAb (anti-Tac) with human immunoglobulin framework and constant regions. The human framework regions were chosen to maximise homology with the anti-Tac MAb sequence. In addition computer modelling was used to identify framework amino acid residues which were likely to interact with the CDRs or antigen, and mouse amino acids were used at these positions in the humanised antibody.

In WO 90/07861 Queen et al propose four criteria for designing humanised immunoglobulins. The first criterion is to use as the human acceptor the framework from a particular human immunoglobulin that is unusually homologous to the non-human donor immunoglobulin to be humanised, or to use a consensus framework from many human antibodies. The second criterion is to use the donor amino acid rather than the acceptor if the human acceptor residue is unusual and the donor residue is typical for human sequences at a specific residue of the framework. The third criterion is to use the donor framework amino acid residue rather than the acceptor at positions immediately adjacent to the CDRs. The fourth criterion is to use the donor amino acid residue at framework positions at which the amino acid is predicted to have a side chain atom within about 3 Å of the CDRs in a three-dimensional immunoglobulin model and to be capable of interacting with the antigen or with the CDRs of the humanised immunoglobulin. It is proposed that criteria two, three or four may be applied in addition or alternatively to criterion one, and may be applied singly or in any combination.

WO 90/07861 describes in detail the preparation of a single CDR-grafted humanised antibody, a humanised antibody having specificity for the p55 Tac protein of the

IL-2 receptor. The combination of all four criteria, as above, were employed in designing this humanised antibody, the variable region frameworks of the human antibody Eu (7) being used as acceptor. In the resultant humanised antibody the donor CDRs were as defined by Kabat et al (7 and 8) and in addition the mouse donor residues were used in place of the human acceptor residues, at positions 27, 30, 48, 66, 67, 89, 91, 94, 103, 104, 105 and 107 in the heavy chain and at positions 48, 60 and 63 in the light chain, of the variable region frameworks. The humanised anti-Tac antibody obtained is reported to have an affinity for p55 of 3×10^9 M^{-1}, about one-third of that of the murine MAb.

We have further investigated the preparation of CDR-grafted humanised antibody molecules and have identified a hierarchy of positions within the framework of the variable regions (i.e. outside both the Kabat CDRs and structural loops of the variable regions) at which the amino acid identities of the residues are important for obtaining CDR-grafted products with satisfactory binding affinity. This has enabled us to establish a protocol for obtaining satisfactory CDR-grafted products which may be applied very widely irrespective of the level of homology between the donor immunoglobulin and acceptor framework. The set of residues which we have identified as being of critical importance does not coincide with the residues identified by Queen et al (9).

Summary of the Invention

Accordingly, in a first aspect the invention provides a CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

In preferred embodiments, the heavy chain framework comprises donor residues at positions 23, 24, 49, 71, 73 and 78 or at positions 23, 24 and 49. The residues at positions 71, 73 and 78 of the heavy chain framework are preferably either all acceptor or all donor residues.

In particularly preferred embodiments the heavy chain framework additionally comprises donor residues at one, some or all of positions 6, 37, 48 and 94. Also it is particularly preferred that residues at positions of the heavy chain framework which are commonly conserved across species, i.e. positions 2, 4, 25, 36, 39, 47, 93, 103, 104, 106 and 107, if not conserved between donor and acceptor, additionally comprise donor residues. Most preferably the heavy chain framework additionally comprises donor residues at positions 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

In addition the heavy chain framework optionally comprises donor residues at one, some or all of positions:
1 and 3,
72 and 76,
69 (if 48 is different between donor and acceptor),
38 and 46 (if 48 is the donor residue),
80 and 20 (if 69 is the donor residue),
67,
82 and 18 (if 67 is the donor residue),
91,
88, and
any one or more of 9, 11, 41, 87, 108, 110 and 112.

In the first and other aspects of the present invention reference is made to CDR-grafted antibody products comprising acceptor framework and donor antigen binding regions. It will be appreciated that the invention is widely applicable to the CDR-grafting of antibodies in

general. Thus, the donor and acceptor antibodies may be derived from animals of the same species and even same antibody class or sub-class. More usually, however, the donor and acceptor antibodies are derived from animals of different species. Typically the donor antibody is a non-human antibody, such as a rodent MAb, and the acceptor antibody is a human antibody.

In the first and other aspects of the present invention, the donor antigen binding region typically comprises at least one CDR from the donor antibody. Usually the donor antigen binding region comprises at least two and preferably all three CDRs of each of the heavy chain and/or light chain variable regions. The CDRs may comprise the Kabat CDRs, the structural loop CDRs or a composite of the Kabat and structural loop CDRs and any combination of any of these. Preferably, the antigen binding regions of the CDR-grafted heavy chain variable domain comprise CDRs corresponding to the Kabat CDRs at CDR2 (residues 50-65) and CDR3 (residues 95-100) and a composite of the Kabat and structural loop CDRs at CDR1 (residues 26-35).

The residue designations given above and elsewhere in the present application are numbered according to the Kabat numbering [refs. (7) and (8)]. Thus the residue designations do not always correspond directly with the linear numbering of the amino acid residues. The actual linear amino acid sequence may contain fewer or additional amino acids than in the strict Kabat numbering corresponding to a shortening of, or insertion into, a structural component, whether framework or CDR, of the basic variable domain structure. For example, the heavy chain variable region of the anti-Tac antibody described by Queen et al (9) contains a single amino acid insert (residue 52a) after residue 52 of CDR2 and a three amino

acid insert (residues 82a, 82b and 82c) after framework residue 82, in the Kabat numbering. The correct Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

The invention also provides in a second aspect a CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 1 and/or 3 and 46 and/or 47. Preferably the CDR grafted light chain of the second aspect comprises donor residues at positions 46 and/or 47.

The invention also provides in a third aspect a CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, 58 and 71.

In a preferred embodiment of the third aspect, the framework comprises donor residues at all of positions 46, 48, 58 and 71.

In particularly preferred embodiments of the second and third aspects, the framework additionally comprises donor residues at positions 36, 44, 47, 85 and 87. Similarly positions of the light chain framework which are commonly conserved across species, i.e. positions 2, 4, 6, 35, 49, 62, 64-69, 98, 99, 101 and 102, if not conserved between donor and acceptor, additionally comprise donor residues. Most preferably the light chain framework additionally comprises donor residues at positions 2, 4, 6, 35, 36, 38, 44, 47, 49, 62, 64-69, 85, 87, 98, 99, 101 and 102.

In addition the framework of the second or third aspects optionally comprises donor residues at one, some or all of positions:
1 and 3,
63,
60 (if 60 and 54 are able to form at potential saltbridge),
70 (if 70 and 24 are able to form a potential saltbridge),
73 and 21 (if 47 is different between donor and acceptor),
37 and 45 (if 47 is different between donor and acceptor), and
any one or more of 10, 12, 40, 80, 103 and 105.

Preferably, the antigen binding regions of the CDR-grafted light chain variable domain comprise CDRs corresponding to the Kabat CDRs at CDR1 (residue 24-34), CDR2 (residues 50-56) and CDR3 (residues 89-97).

The invention further provides in a fourth aspect a CDR-grafted antibody molecule comprising at least one CDR-grafted heavy chain and at least one CDR-grafted light chain according to the first and second or first and third aspects of the invention.

The humanised antibody molecules and chains of the present invention may comprise: a complete antibody molecule, having full length heavy and light chains; a fragment thereof, such as a Fab, $(Fab')_2$ or FV fragment; a light chain or heavy chain monomer or dimer; or a single chain antibody, e.g. a single chain FV in which heavy and light chain variable regions are joined by a peptide linker; or any other CDR-grafted molecule with the same specificity as the original donor antibody. Similarly the CDR-grafted heavy and light chain variable region may be combined with other antibody domains as appropriate.

Also the heavy or light chains or humanised antibody molecules of the present invention may have attached to them an effector or reporter molecule. For instance, it may have a macrocycle, for chelating a heavy metal atom, or a toxin, such as ricin, attached to it by a covalent bridging structure. Alternatively, the procedures of recombinant DNA technology may be used to produce an immunoglobulin molecule in which the Fc fragment or CH3 domain of a complete immunoglobulin molecule has been replaced by, or has attached thereto by peptide linkage, a functional non-immunoglobulin protein, such as an enzyme or toxin molecule.

Any appropriate acceptor variable region framework sequences may be used having regard to class/type of the donor antibody from which the antigen binding regions are derived. Preferably, the type of acceptor framework used is of the same/similar class/type as the donor antibody. Conveniently, the framework may be chosen to maximise/optimise homology with the donor antibody sequence particularly at positions close or adjacent to the CDRs. However, a high level of homology between donor and acceptor sequences is not important for application of the present invention. The present invention identifies a hierarchy of framework residue positions at which donor residues may be important or desirable for obtaining a CDR-grafted antibody product having satisfactory binding properties. The CDR-grafted products usually have binding affinities of at least 10^5 M^{-1}, preferably at least about 10^8 M^{-1}, or especially in the range 10^8-10^{12} M^{-1}. In principle, the present invention is applicable to any combination of donor and acceptor antibodies irrespective of the level of homology between their sequences. A protocol for applying the invention to any particular donor-acceptor antibody pair is given hereinafter. Examples of human frameworks which may be

used are KOL, NEWM, REI, EU, LAY and POM (refs. 4 and 5) and the like; for instance KOL and NEWM for the heavy chain and REI for the light chain and EU, LAY and POM for both the heavy chain and the light chain.

Also the constant region domains of the products of the invention may be selected having regard to the proposed function of the antibody in particular the effector functions which may be required. For example, the constant region domains may be human IgA, IgE, IgG or IgM domains. In particular, IgG human constant region domains may be used, especially of the IgG1 and IgG3 isotypes, when the humanised antibody molecule is intended for therapeutic uses, and antibody effector functions are required. Alternatively, IgG2 and IgG4 isotypes may be used when the humanised antibody molecule is intended for therapeutic purposes and antibody effector functions are not required, e.g. for simple blocking of lymphokine activity.

However, the remainder of the antibody molecules need not comprise only protein sequences from immunoglobulins. For instance, a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain is fused to a DNA sequence encoding the amino acid sequence of a functional polypeptide such as an effector or reporter molecule.

Preferably the CDR-grafted antibody heavy and light chain and antibody molecule products are produced by recombinant DNA technology.

Thus in further aspects the invention also includes DNA sequences coding for the CDR-grafted heavy and light chains, cloning and expression vectors containing the DNA sequences, host cells transformed with the DNA sequences

and processes for producing the CDR-grafted chains and antibody molecules comprising expressing the DNA sequences in the transformed host cells.

The general methods by which the vectors may be constructed, transfection methods and culture methods are well known per se and form no part of the invention. Such methods are shown, for instance, in references 10 and 11.

The DNA sequences which encode the donor amino acid sequence may be obtained by methods well known in the art. For example the donor coding sequences may be obtained by genomic cloning, or cDNA cloning from suitable hybridoma cell lines. Positive clones may be screened using appropriate probes for the heavy and light chain genes in question. Also PCR cloning may be used.

DNA coding for acceptor, e.g. human acceptor, sequences may be obtained in any appropriate way. For example DNA sequences coding for preferred human acceptor frameworks such as KOL, REI, EU and NEWM, are widely available to workers in the art.

The standard techniques of molecular biology may be used to prepare DNA sequences coding for the CDR-grafted products. Desired DNA sequences may be synthesised completely or in part using oligonucleotide synthesis techniques. Site-directed mutagenesis and polymerase chain reaction (PCR) techniques may be used as appropriate. For example oligonucleotide directed synthesis as described by Jones et al (ref. 20) may be used. Also oligonucleotide directed mutagenesis of a pre-exising variable region as, for example, described by Verhoeyen et al (ref. 5) or Riechmann et al (ref. 6) may be used. Also enzymatic filling in of gapped

oligonucleotides using T_4 DNA polymerase as, for example, described by Queen et al (ref. 9) may be used.

Any suitable host cell/vector system may be used for expression of the DNA sequences coding for the CDR-grafted heavy and light chains. Bacterial e.g. E. coli, and other microbial systems may be used, in particular for expression of antibody fragments such as FAb and $(Fab')_2$ fragments, and especially FV fragments and single chain antibody fragments e.g. single chain FVs. Eucaryotic e.g. mammalian host cell expression systems may be used for production of larger CDR-grafted antibody products, including complete antibody molecules. Suitable mammalian host cells include CHO cells and myeloma or hybridoma cell lines.

Thus, in a further aspect the present invention provides a process for producing a CDR-grafted antibody product comprising:

(a) producing in an expression vector an operon having a DNA sequence which encodes an antibody heavy chain according to the first aspect of the invention;

and/or

(b) producing in an expression vector an operon having a DNA sequence which encodes a complementary antibody light chain according to the second or third aspect of the invention;

(c) transfecting a host cell with the or each vector; and

(d) culturing the transfected cell line to produce the CDR-grafted antibody product.

The CDR-grafted product may comprise only heavy or light chain derived polypeptide, in which case only a heavy chain or light chain polypeptide coding sequence is used to transfect the host cells.
For production of products comprising both heavy and light chains, the cell line may be transfected with two vectors, the first vector may contain an operon encoding a light chain-derived polypeptide and the second vector containing an operon encoding a heavy chain-derived polypeptide. Preferably, the vectors are identical, except in so far as the coding sequences and selectable markers are concerned, so as to ensure as far as possible that each polypeptide chain is equally expressed. Alternatively, a single vector may be used, the vector including the sequences encoding both light chain- and heavy chain-derived polypeptides.

The DNA in the coding sequences for the light and heavy chains may comprise cDNA or genomic DNA or both. However, it is preferred that the DNA sequence encoding the heavy or light chain comprises at least partially, genomic DNA, preferably a fusion of cDNA and genomic DNA.

The present invention is applicable to antibodies of any appropriate specificity. Advantageously, however, the invention may be applied to the humanisation of non-human antibodies which are used for in vivo therapy or diagnosis. Thus the antibodies may be site-specific antibodies such as tumour-specific or cell surface-specific antibodies, suitable for use in in vivo therapy or diagnosis, e.g. tumour imaging. Examples of cell surface-specific antibodies are anti-T cell antibodies, such as anti-CD3, and CD4 and adhesion molecules, such as CR3, ICAM and ELAM. The antibodies may have specificity for interleukins (including lymphokines, growth factors and stimulating factors), hormones and other biologically active compounds, and receptors for any of these. For

example, the antibodies may have specificity for any of the following: Interferons α, β, γ or δ, IL1, IL2, IL3, or IL4, etc., TNF, GCSF, GMCSF, EPO, hGH, or insulin, etc.

The the present invention also includes therapeutic and diagnostic compositions comprising the CDR-grafted products of the invention and uses of such compositions in therapy and diagnosis.

Accordingly in a further aspect the invention provides a therapeutic or diagnostic composition comprising a CDR-grafted antibody heavy or light chain or molecule according to previous aspects of the invention in combination with a pharmaceutically acceptable carrier, diluent or excipient.

Accordingly also the invention provides a method of therapy or diagnosis comprising administering an effective amount of a CDR-grafted antibody heavy or light chain or molecule according to previous aspects of the invention to a human or animal subject.

A preferred protocol for obtaining CDR-grafted antibody heavy and light chains in accordance with the present invention is set out below together with the rationale by which we have derived this protocol. This protocol and rationale are given without prejudice to the generality of the invention as hereinbefore described and defined.

Protocol

It is first of all necessary to sequence the DNA coding for the heavy and light chain variable regions of the donor antibody, to determine their amino acid sequences. It is also necessary to choose appropriate acceptor heavy and light chain variable regions, of known amino acid sequence. The CDR-grafted chain is then designed

starting from the basis of the acceptor sequence. It will be appreciated that in some cases the donor and acceptor amino acid residues may be identical at a particular position and thus no change of acceptor framework residue is required.

1. As a first step donor residues are substituted for acceptor residues in the CDRs. For this purpose the CDRs are preferably defined as follows:

Heavy chain	-	CDR1:	residues 26-35
	-	CDR2:	residues 50-65
	-	CDR3:	residues 95-102
Light chain	-	CDR1:	residues 24-34
	-	CDR2:	residues 50-56
	-	CDR3:	residues 89-97

The positions at which donor residues are to be substituted for acceptor in the framework are then chosen as follows, first of all with respect to the heavy chain and subsequently with respect to the light chain.

2. Heavy Chain

2.1 Choose donor residues at all of positions 23, 24, 49, 71, 73 and 78 of the heavy chain or all of positions 23, 24 and 49 (71, 73 and 78 are always either all donor or all acceptor).

2.2 Check that the following have the same amino acid in donor and acceptor sequences, and if not preferably choose the donor: 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

2.3 To further optimise affinity consider choosing donor residues at one, some or any of:

i. 1, 3
ii. 72, 76
iii. If 48 is different between donor and acceptor sequences, consider 69
iv. If at 48 the donor residue is chosen, consider 38 and 46
v. If at 69 the donor residue is chosen, consider 80 and then 20
vi. 67
vii. If at 67 the donor residue is chosen, consider 82 and then 18
viii. 91
ix. 88
x. 9, 11, 41, 87, 108, 110, 112

3. Light Chain

3.1 Choose donor at 46, 48, 58 and 71

3.2 Check that the following have the same amino acid in donor and acceptor sequences, if not preferably choose donor:

2, 4, 6, 35, 38, 44, 47, 49, 62, 64-69 inclusive, 85, 87, 98, 99, 101 and 102

3.3 To further optimise affinity consider choosing donor residues at one, some or any of:

i. 1, 3
ii. 63

iii. 60, if 60 and 54 are able to form potential saltbridge
iv. 70, if 70 and 24 are able to form potential saltbridge
v. 73, and 21 if 47 is different between donor and acceptor
vi. 37, and 45 if 47 is different between donor and acceptor
vii. 10, 12, 40, 80, 103, 105

Rationale

In order to transfer the binding site of an antibody into a different acceptor framework, a number of factors need to be considered.

1. The extent of the CDRs

The CDRs (Complementary Determining Regions) were defined by Wu and Kabat (refs. 4 and 5) on the basis of an analysis of the variability of different regions of antibody variable regions. Three regions per domain were recognised. In the light chain the sequences are 24-34, 50-56, 89-97 (numbering according to Kabat (ref. 4), Eu Index) inclusive and in the heavy chain the sequences are 31-35, 50-65 and 95-102 inclusive.

When antibody structures became available it became apparent that these CDR regions corresponded in the main to loop regions which extended from the β barrel framework of the light and heavy variable domains. For H1 there was a discrepancy in that the loop was from 26 to 32 inclusive and for H2 the loop was 52 to 56 and for L2 from 50 to 53. However, with the exception of H1 the CDR regions encompassed the loop regions and extended into the β strand

frameworks. In H1 residue 26 tends to be a serine and 27 a phenylalanine or tyrosine, residue 29 is a phenylalanine in most cases. Residues 28 and 30 which are surface residues exposed to solvent might be involved in antigen-binding. A prudent definition of the H1 CDR therefore would include residues 26-35 to include both the loop region and the hypervariable residues 33-35.

It is of interest to note the example of Riechmann et al (ref. 3), who used the residue 31-35 choice for CDR-H1. In order to produce efficient antigen binding, residue 27 also needed to be recruited from the donor (rat) antibody.

2. Non-CDR residues which contribute to antigen binding

By examination of available X-ray structures we have identified a number of residues which may have an effect on net antigen binding and which can be demonstrated by experiment. These residues can be sub-divided into a number of groups.

2.1 Surface residues near CDR [all numbering as in Kabat et al (ref. 7)].

2.1.1. Heavy Chain - Key residues are 23, 71 and 73. Other residues which may contribute to a lesser extent are 1, 3 and 76. Finally 25 is usually conserved but the murine residue should be used if there is a difference.

2.1.2 Light Chain - Many residues close to the CDRs, e.g. 63, 65, 67 and 69 are conserved. If conserved none of the surface residues in the light chain are likely to have a major effect. However, if the murine residue at these positions

is unusual, then it would be of benefit to analyse the likely contribution more closely. Other residues which may also contribute to binding are 1 and 3, and also 60 and 70 if the residues at these positions and at 54 and 24 respectively are potentially able to form a salt bridge i.e. 60 + 54; 70 + 24.

2.2 Packing residues near the CDRs.

2.2.1. Heavy Chain - Key residues are 24, 49 and 78. Other key residues would be 36 if not a tryptophan, 94 if not an arginine, 104 and 106 if not glycines and 107 if not a threonine. Residues which may make a further contribution to stable packing of the heavy chain and hence improved affinity are 2, 4, 6, 38, 46, 67 and 69. 67 packs against the CDR residue 63 and this pair could be either both mouse or both human. Finally, residues which contribute to packing in this region but from a longer range are 18, 20, 80, 82 and 86. 82 packs against 67 and in turn 18 packs against 82. 80 packs against 69 and in turn 20 packs against 80. 86 forms an H bond network with 38 and 46. Many of the mouse-human differences appear minor e.g. Leu-Ile, but could have an minor impact on correct packing which could translate into altered positioning of the CDRs.

2.2.2. Light Chain - Key residues are 48, 58 and 71. Other key residues would be 6 if not glutamine, 35 if not tryptophan, 62 if not phenylalanine or tryosine, 64, 66, 68, 99 and 101 if not glycines and 102 if not a threonine. Residues which make a further contribution are 2, 4, 37, 45 and 47. Finally residues 73 and 21 and 19 may make long distance packing contributions of a minor nature.

2.3. Residues at the variable domain interface between heavy and light chains - In both the light and heavy chains most of the non-CDR interface residues are conserved. If a conserved residue is replaced by a residue of different character, e.g. size or charge, it should be considered for retention as the murine residue.

2.3.1. Heavy Chain - Residues which need to be considered are 37 if the residue is not a valine but is of larger side chain volume or has a charge or polarity. Other residues are 39 if not a glutamine, 45 if not a leucine, 47 if not a tryptophan, 91 if not a phenylalanine or tyrosine, 93 if not an alanine and 103 if not a tryptophan. Residue 89 is also at the interface but is not in a position where the side chain could be of great impact.

2.3.2. Light Chain - Residues which need to be considered are 36, if not a tyrosine, 38 if not a glutamine, 44 if not a proline, 46, 49 if not a tyrosine, residue 85, residue 87 if not a tyrosine and 98 if not a phenylalanine.

2.4. Variable-Constant region interface - The elbow angle between variable and constant regions may be affected by alterations in packing of key residues in the variable region against the constant region which may affect the position of V_L and V_H with respect to one another. Therefore it is worth noting the residues likely to be in contact with the constant region. In the heavy chain the surface residues potentially in contact with the variable region are conserved between mouse and human antibodies therefore the variable region contact residues may influence the V-C interaction. In the light chain the amino acids found at a number of the constant

region contact points vary, and the V & C regions are not in such close proximity as the heavy chain. Therefore the influences of the light chain V-C interface may be minor.

2.4.1. Heavy Chain - Contact residues are 7, 11, 41, 87, 108, 110, 112.

2.4.2. Light Chain - In the light chain potentially contacting residues are 10, 12, 40, 80, 83, 103 and 105.

The above analysis coupled with our considerable practical experimental experience in the CDR-grafting of a number of different antibodies have lead us to the protocol given above.

The present invention is now described, by way of example only, with reference to the accompanying Figures 1 - 13.

Brief Description of the Figures

Figure 1 shows DNA and amino acid sequences of the OKT3 light chain;

Figure 2 shows DNA and amino acid sequences of the OKT3 heavy chain;

Figure 3 shows the alignment of the OKT3 light variable region amino acid sequence with that of the light variable region of the human antibody REI;

Figure 4 shows the alignment of the OKT3 heavy variable region amino acid sequence with that of the heavy variable region of the human antibody KOL;

Figure 5 shows the heavy variable region amino acid sequences of OKT3, KOL and various corresponding CDR grafts;

Figure 6 shows the light variable region amino acid sequences of OKT3, REI and various corresponding CDR grafts;

Figure 7 shows a graph of binding assay results for various grafted OKT3 antibodies'

Figure 8 shows a graph of blocking assay results for various grafted OKT3 antibodies;

Figure 9 shows a similar graph of blocking assay results;

Figure 10 shows similar graphs for both binding assay and blocking assay results;

Figure 11 shows further similar graphs for both binding assay and blocking assay results;

Figure 12 shows a graph of competition assay results for a minimally grafted OKT3 antibody compared with the OKT3 murine reference standard, and

Figure 13 shows a similar graph of competition assay results comparing a fully grafted OKT3 antibody with the murine reference standard.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

EXAMPLE 1

CDR-GRAFTING OF OKT3

MATERIAL AND METHODS

1. INCOMING CELLS

Hybridoma cells producing antibody OKT3 were provided by Ortho (seedlot 4882.1) and were grown up in antibiotic free Dulbecco's Modified Eagles Medium (DMEM) supplemented with glutamine and 5% foetal calf serum, and divided to provide both an overgrown supernatant for evaluation and cells for extraction of RNA. The overgrown supernatant was shown to contain 250 ug/mL murine IgG2a/kappa antibody. The supernatant was negative for murine lambda light chain and IgG1, IgG2b, IgG3, IgA and IgM heavy chain. 20mL of supernatant was assayed to confirm that the antibody present was OKT3.

2. MOLECULAR BIOLOGY PROCEDURES

Basic molecular biology procedures were as described in Maniatis et al (ref. 9) with, in some cases, minor modifications. DNA sequencing was performed as described in Sanger et al (ref. 11) and the Amersham International Plc sequencing handbook. Site directed mutagenesis was as described in Kramer et al (ref. 12) and the Anglian Biotechnology Ltd. handbook. COS cell expression and metabolic labelling studies were as described in Whittle et al (ref. 13)

3. RESEARCH ASSAYS

3.1. ASSEMBLY ASSAYS

Assembly assays were performed on supernatants from transfected COS cells to determine the amount of intact IgG present.

3.1.1. COS CELLS TRANSFECTED WITH MOUSE OKT3 GENES

The assembly assay for intact mouse IgG in COS cell supernatants was an ELISA with the following format:

96 well microtitre plates were coated with F(ab')2 goat anti-mouse IgG Fc. The plates were washed in water and samples added for 1 hour at room temperature. The plates were washed and F(ab')2 goat anti-mouse IgG F(ab')2 (HRPO conjugated) was then added. Substrate was added to reveal the reaction. UPC10, a mouse IgG2a myeloma, was used as a standard.

3.1.2. COS AND CHO CELLS TRANSFECTED WITH CHIMERIC OR CDR-GRAFTED OKT3 GENES

The assembly assay for chimeric or CDR-grafted antibody in COS cell supernatants was an ELISA with the following format:

96 well microtitre plates were coated with F(ab')2 goat anti-human IgG Fc. The plates were washed and samples added and incubated for 1 hour at room temperature. The plates were washed and monoclonal mouse anti-human kappa chain was added for 1 hour at room temperature.

The plates were washed and F(ab')2 goat anti-mouse IgG Fc (HRPO conjugated) was added. Enzyme substrate was added to reveal the reaction. Chimeric B72.3 (IgG4) (ref. 13) was used as a standard. The use of a monoclonal anti-kappa chain in this assay allows grafted antibodies to be read from the chimeric standard.

3.2. ASSAY FOR ANTIGEN BINDING ACTIVITY

Material from COS cell supernatants was assayed for OKT3 antigen binding activity onto CD3 positive cells in a direct assay. The procedure was as follows:

HUT 78 cells (human T cell line, CD3 positive) were maintained in culture. Monolayers of HUT 78 cells were prepared onto 96 well ELISA plates using poly-L-lysine and glutaraldehyde. Samples were added to the monolayers for 1 hour at room temperature.

The plates were washed gently using PBS. F(ab')2 goat anti-human IgG Fc (HRPO conjugated) or F(ab')2 goat anti-mouse IgG Fc (HRPO conjugated) was added as appropriate for humanised or mouse samples. Substrate was added to reveal the reaction.

The negative control for the cell-based assay was chimeric B72.3. The positive control was mouse Orthomune OKT3 or chimeric OKT3, when available. This cell-based assay was difficult to perform, and an alternative assay was developed for CDR-grafted OKT3 which was more sensitive and easier to carry out.

In this system CDR-grafted OKT3 produced by COS cells was tested for its ability to bind to the CD3-positive HPB-ALL (human peripheral blood acute lymphocytic leukemia) cell line. It was also tested for its ability to block the binding of murine OKT3 to these cells. Binding was measured by the following procedure: HPB-ALL cells were harvested from tissue culture. Cells were incubated at 4°C for 1 hour with various dilutions of test antibody, positive control antibody, or negative control antibody. The cells were washed once and incubated at 4°C for 1 hour with an FITC-labelled goat anti-human IgG (Fc-

specific, mouse absorbed). The cells were washed twice and analysed by cytofluorography. Chimeric OKT3 was used as a positive control for direct binding. Cells incubated with mock- transfected COS cell supernatant, followed by the FITC-labelled goat anti-human IgG, provided the negative control. To test the ability of CDR-grafted OKT3 to block murine OKT3 binding, the HPB-ALL cells were incubated at 4°C for 1 hour with various dilutions of test antibody or control antibody. A fixed saturating amount of FITC OKT3 was added. The samples were incubated for 1 hour at 4°C, washed twice and analysed by cytofluorography. FITC-labelled OKT3 was used as a positive control to determine maximum binding. Unlabelled murine OKT3 served as a reference standard for blocking. Negative controls were unstained cells with or without mock-transfected cell supernatant. The ability of the CDR-grafted OKT3 light chain to bind CD3-positive cells and block the binding of murine OKT3 was initially tested in combination with the chimeric OKT3 heavy chain. The chimeric OKT3 heavy chain is composed of the murine OKT3 variable region and the human IgG4 constant region. The chimeric heavy chain gene is expressed in the same expression vector used for the CDR-grafted genes. The CDR-grafted light chain expression vector and the chimeric heavy chain expression vector were co-transfected into COS cells. The fully chimeric OKT3 antibody (chimeric light chain and chimeric heavy chain) was found to be fully capable of binding to CD3 positive cells and blocking the binding of murine OKT3 to these cells.

3.3 DETERMINATION OF RELATIVE BINDING AFFINITY

The relative binding affinities of CDR-grafted

anti-CD3 monoclonal antibodies were determined by competition binding (ref. 6) using the HPB-ALL human T cell line as a source of CD3 antigen, and fluorescein-conjugated murine OKT3 (Fl-OKT3) of known binding affinity as a tracer antibody. The binding affinity of Fl-OKT3 tracer antibody was determined by a direct binding assay in which increasing amounts of Fl-OKT3 were incubated with HPB-ALL ($5x10^5$) in PBS with 5% foetal calf serum for 60 min. at 4°C. Cells were washed, and the fluorescence intensity was determined on a FACScan flow cytometer calibrated with quantitative microbead standards (Flow Cytometry Standards, Research Triangle Park, NC). Fluorescence intensity per antibody molecule (F/P ratio) was determined by using microbeads which have a predetermined number of mouse IgG antibody binding sites (Simply Cellular beads, Flow Cytometry Standards). F/P equals the fluorescence intensity of beads saturated with Fl-OKT3 divided by the number of binding sites per bead. The amount of bound and free Fl-OKT3 was calculated from the mean fluorescence intensity per cell, and the ratio of bound/free was plotted against the number of moles of antibody bound. A linear fit was used to determine the affinity of binding (absolute value of the slope).

For competitive binding, increasing amounts of competitor antibody were added to a sub-saturating dose of Fl-OKT3 and incubated with $5x10^5$ HPB-ALL in 200 Ml of PBS with 5% foetal calf serum, for 60 min at 4°C. The fluorescence intensities of the cells were measured on a FACScan flow cytometer calibrated with quantitative microbead standards. The concentrations of bound and free Fl-OKT3 were calculated. The affinities of competing anti-

bodies were calculated from the equation [X]-[OKT3] = (1/Kx) - (1/Ka), where Ka is the affinity of murine OKT3, Kx is the affinity of competitor X, [ ] is the concentration of competitor antibody at which bound/free binding is R/2, and R is the maximal bound/free binding.

4. cDNA LIBRARY CONSTRUCTION

4.1. mRNA PREPARATION AND cDNA SYNTHESIS

OKT3 producing cells were grown as described above and 1.2 x 10^9 cells harvested and mRNA extracted using the guanidinium/LiCl extraction procedure. cDNA was prepared by priming from Oligo-dT to generate full length cDNA. The cDNA was methylated and EcoR1 linkers added for cloning.

4.2. LIBRARY CONSTRUCTION

The cDNA library was ligated to pSP65 vector DNA which had been EcoR1 cut and the 5′ phosphate groups removed by calf intestinal phosphatase (EcoR1/CIP). The ligation was used to transform high transformation efficiency Escherichia coli (E.coli) HB101. A cDNA library was prepared. 3600 colonies were screened for the light chain and 10000 colonies were screened for the heavy chain.

5. SCREENING

E.coli colonies positive for either heavy or light chain probes were identified by oligonucleotide screening using the oligonucleotides:
5′ TCCAGATGTTAACTGCTCAC for the light chain, which is complementary to a sequence in the mouse kappa constant region, and 5′ CAGGGGCCAGTGGATGGATAGAC for the heavy chain which is complementary to a sequence in the mouse IgG2a constant CH1 domain region. 12 light chain and 9 heavy chain clones

were identified and taken for second round screening. Positive clones from the second round of screening were grown up and DNA prepared. The sizes of the gene inserts were estimated by gel electrophoresis and inserts of a size capable of containing a full length cDNA were subcloned into M13 for DNA sequencing.

6. DNA SEQUENCING

Clones representing four size classes for both heavy and light chains were obtained in M13. DNA sequence for the 5′ untranslated regions, signal sequences, variable regions and 3′ untranslated regions of full length cDNAs [Figures 1(a) and 2(a)] were obtained and the corresponding amino acid sequences predicted [(Figures 1(b) and 2(b)]. In Figure 1(a) the untranslated DNA regions are shown in uppercase, and in both Figures 1 and 2 the signal sequences are underlined.

7. CONSTRUCTION OF cDNA EXPRESSION VECTORS

Celltech expression vectors are based on the plasmid pEE6hCMV (ref. 14). A polylinker for the insertion of genes to be expressed has been introduced after the major immediate early promoter/enhancer of the human Cytomegalovirus (hCMV). Marker genes for selection of the plasmid in transfected eukaryotic cells can be inserted as BamH1 cassettes in the unique BamH1 site of pEE6 hCMV; for instance, the neo marker to provide pEE6 hCMV neo. It is usual practice to insert the neo and gpt markers prior to insertion of the gene of interest, whereas the GS marker is inserted last because of the presence of internal EcoR1 sites in the cassette.

The selectable markers are expressed from the SV40 late promoter which also provides an origin of replication so that the vectors can be used for expression in the COS cell transient expression system.
The mouse sequences were excised from the M13 based vectors described above as EcoR1 fragments and cloned into either pEE6-hCMV-neo for the heavy chain and into EE6-hCMV-gpt for the light chain to yield vectors pJA136 and pJA135 respectively.

8. EXPRESSION OF cDNAS IN COS CELLS

Plasmids pJA135 and pJA136 were co-transfected into COS cells and supernatant from the transient expression experiment was shown to contain assembled antibody which bound to T-cell enriched lymphocytes. Metabolic labelling experiments using ^{35}S methionine showed expression and assembly of heavy and light chains.

9. CONSTRUCTION OF CHIMERIC GENES

Construction of chimeric genes followed a previously described strategy [Whittle et al (ref. 13)]. A restriction site near the 3′ end of the variable domain sequence is identified and used to attach an oligonucleotide adapter coding for the remainder of the mouse variable region and a suitable restriction site for attachment to the constant region of choice.

9.1. LIGHT CHAIN GENE CONSTRUCTION

The mouse light chain cDNA sequence contains an AvaI site near the 3′ end of the variable region [Fig. 1(a)]. The majority of the sequence of the variable region was isolated as a 396 bp. EcoR1-AvaI fragment. An oligonucleotide adapter was designed to replace the remainder of the 3′

region of the variable region from the AvaI site and to include the 5′ residues of the human constant region up to and including a unique NarI site which had been previously engineered into the constant region.

A HindIII site was introduced to act as a marker for insertion of the linker.

The linker was ligated to the V_L fragment and the 413 bp EcoR1-NarI adapted fragment was purified from the ligation mixture.

The constant region was isolated as an NarI-BamH1 fragment from an M13 clone NW361 and was ligated with the variable region DNA into an EcoR1/BamH1/C1P pSP65 treated vector in a three way reaction to yield plasmid JA143. Clones were isolated after transformation into E.coli and the linker and junction sequences were confirmed by the presence of the HindIII site and by DNA sequencing.

9.2 LIGHT CHAIN GENE CONSTRUCTION - VERSION 2

The construction of the first chimeric light chain gene produces a fusion of mouse and human amino acid sequences at the variable-constant region junction. In the case of the OKT3 light chain the amino acids at the chimera junction are:

.....Leu-Glu-Ile-Asn-Arg/ -/Thr-Val-Ala -Ala

VARIABLE CONSTANT

This arrangement of sequence introduces a potential site for Asparagine (Asn) linked (N-linked) glycosylation at the V-C junction. Therefore, a second version of the chimeric light chain oligonucleotide adapter was designed in which the threonine (Thr), the first amino acid of the human constant region, was replaced with the equivalent amino acid from the mouse constant region, Alanine (Ala).

An internal Hind111 site was not included in this adapter, to differentiate the two chimeric light chain genes.

The variable region fragment was isolated as a 376 bp EcoR1-Aval fragment. The oligonucleotide linker was ligated to Nar1 cut pNW361 and then the adapted 396bp constant region was isolated after recutting the modified pNW361 with EcoR1. The variable region fragment and the modified constant region fragment were ligated directly into EcoR1/C1P treated pEE6hCMVneo to yield pJA137. Initially all clones examined had the insert in the incorrect orientation. Therefore, the insert was re-isolated and recloned to turn the insert round and yield plasmid pJA141. Several clones with the insert in the correct orientation were obtained and the adapter sequence of one was confirmed by DNA sequencing

9.3. HEAVY CHAIN GENE CONSTRUCTION

9.3.1. CHOICE OF HEAVY CHAIN GENE ISOTYPE

The constant region isotype chosen for the heavy chain was human IgG4.

9.3.2. GENE CONSTRUCTION

The heavy chain cDNA sequence showed a Ban1 site near the 3′ end of the variable region [Fig. 2(a)]. The majority of the sequence of the variable region was isolated as a 426bp. EcoR1/C1P/Ban1 fragment. An oligonucleotide adapter was designated to replace the remainder of the 3′ region of the variable region from the Ban1 site up to and including a unique HindIII site which had been previously engineered into the first two amino acids of the constant region.

The linker was ligated to the V_H fragment and the EcoR1-Hind111 adapted fragment was purified from the ligation mixture.

The variable region was ligated to the constant region by cutting pJA91 with EcoR1 and Hind111 removing the intron fragment and replacing it with the V_H to yield pJA142. Clones were isolated after transformation into E.coli JM101 and the linker and junction sequences were confirmed by DNA sequencing. (N.B. The Hind111 site is lost on cloning).

10. CONSTRUCTION OF CHIMERIC EXPRESSION VECTORS

10.1. neo AND gpt VECTORS

The chimeric light chain (version 1) was removed from pJA143 as an EcoR1 fragment and cloned into EcoR1/C1P treated pEE6hCMVneo expression vector to yield pJA145. Clones with the insert in the correct orientation were identified by restriction mapping.

The chimeric light chain (version 2) was constructed as described above.

The chimeric heavy chain gene was isolated from pJA142 as a 2.5Kbp EcoR1/BamH1 fragment and cloned into the EcoR1/Bcl1/C1P treated vector fragment of a derivative of pEE6hCMVgpt to yield plasmid pJA144.

10.2. GS SEPARATE VECTORS

GS versions of pJA141 and pJA144 were constructed by replacing the neo and gpt cassettes by a BamH1/Sa11/C1P treatment of the plasmids, isolation of the vector fragment and ligation to a GS-containing fragment from the plasmid pRO49 to yield the light chain vector pJA179 and the heavy chain vector pJA180.

10.3. GS SINGLE VECTOR CONSTRUCTION

Single vector constructions containing the cL (chimeric light), cH (chimeric heavy) and GS genes on one plasmid in the order cL-cH-GS, or cH-cL-GS

and with transcription of the genes being head to tail e.g. cL>cH>GS were constructed. These plasmids were made by treating pJA179 or pJA180 with BamH1/C1P and ligating in a Bgl11/Hind111 hCMV promoter cassette along with either the Hind111/BamH1 fragment from pJA141 into pJA180 to give the cH-cL-GS plasmid pJA182 or the Hind111/BamH1 fragment from pJA144 into pJA179 to give the cL-cH-GS plasmid pJA181.

11. EXPRESSION OF CHIMERIC GENES

11.1. EXPRESSION IN COS CELLS

The chimeric antibody plasmid pJA145 (cL) and pJA144 (cH) were co-transfected into COS cells and supernatant from the transient expression experiment was shown to contain assembled antibody which bound to the HUT 78 human T-cell line. Metabolic labelling experiments using ^{35}S methionine showed expression and assembly of heavy and light chains. However the light chain mobility seen on reduced gels suggested that the potential glycosylation site was being glycosylated. Expression in COS cells in the presence of tunicamycin showed a reduction in size of the light chain to that shown for control chimeric antibodies and the OKT3 mouse light chain. Therefore JA141 was constructed and expressed. In this case the light chain did not show an aberrant mobility or a size shift in the presence or absence of tunicamycin. This second version of the chimeric light chain, when expressed in association with chimeric heavy (cH) chain, produced antibody which showed good binding to HUT 78 cells. In both cases antigen binding was equivalent to that of the mouse antibody.

11.2 EXPRESSION IN CHINESE HAMSTER OVARY (CHO) CELLS

Stable cell lines have been prepared from plasmids PJA141/pJA144 and from pJA179/pJA180, pJA181 and pJA182 by transfection into CHO cells.

12. CDR-GRAFTING

The approach taken was to try to introduce sufficient mouse residues into a human variable region framework to generate antigen binding activity comparable to the mouse and chimeric antibodies.

12.1. VARIABLE REGION ANALYSIS

From an examination of a small database of structures of antibodies and antigen-antibody complexes it is clear that only a small number of antibody residues make direct contact with antigen. Other residues may contribute to antigen binding by positioning the contact residues in favourable configurations and also by inducing a stable packing of the individual variable domains and stable interaction of the light and heavy chain variable domains.

The residues chosen for transfer can be identified in a number of ways:

(a) By examination of antibody X-ray crystal structures the antigen binding surface can be predominantly located on a series of loops, three per domain, which extend from the B-barrel framework.

(b) By analysis of antibody variable domain sequences regions of hypervariability [termed the Complementarity Determining Regions (CDRs) by Wu and Kabat (ref. 5)] can be identified. In the most but not all cases these CDRs correspond to, but extend a short way beyond, the loop regions noted above.

(c) Residues not identified by (a) and (b) may contribute to antigen binding directly or indirectly by affecting antigen binding site topology, or by inducing a stable packing of the individual variable domains and stabilising the inter-variable domain interaction. These residues may be identified either by superimposing the sequences for a given antibody on a known structure and looking at key residues for their contribution, or by sequence alignment analysis and noting "idiosyncratic" residues followed by examination of their structural location and likely effects.

12.1.1. LIGHT CHAIN

Figure 3 shows an alignment of sequences for the human framework region RE1 and the OKT3 light variable region. The structural loops (LOOP) and CDRs (KABAT) believed to correspond to the antigen binding region are marked. Also marked are a number of other residues which may also contribute to antigen binding as described in 13.1(c). Above the sequence in Figure 3 the residue type indicates the spatial location of each residue side chain, derived by examination of resolved structures from X-ray crystallography analysis. The key to this residue type designation is as follows:

N - near to CDR (From X-ray Structures)

P - Packing B - Buried Non-Packing

S - Surface E - Exposed

I - Interface * - Interface - Packing/Part Exposed

? - Non-CDR Residues which may require to be left as Mouse sequence.

Residues underlined in Figure 3 are amino acids. RE1 was chosen as the human framework because the light chain is a kappa chain and the kappa variable regions show higher homology with the mouse sequences than a lambda light variable region, e.g. KOL (see below). RE1 was chosen in preference to another kappa light chain because the X-ray structure of the light chain has been determined so that a structural examination of individual residues could be made.

12.1.2. HEAVY CHAIN

Similarly Figure 4 shows an alignment of sequences for the human framework region KOL and the OKT3 heavy variable region. The structural loops and CDRs believed to correspond to the antigen binding region are marked. Also marked are a number of other residues which may also contribute to antigen binding as described in 12.1(c). The residue type key and other indicators used in Figure 4 are the same as those used in Figure 3. KOL was chosen as the heavy chain framework because the X-ray structure has been determined to a better resolution than, for example, NEWM and also the sequence alignment of OKT3 heavy variable region showed a slightly better homology to KOL than to NEWM.

12.2. DESIGN OF VARIABLE GENES

The variable region domains were designed with mouse variable region optimal codon usage [Grantham and Perrin (ref. 15)] and used the B72.3 signal sequences [Whittle et al (ref. 13)]. The sequences were designed to be attached to the constant region in the same way as for the chimeric genes described above. Some constructs contained the "Kozak consensus sequence" [Kozak (ref. 16)] directly linked to the 5′ of the signal

sequence in the gene. This sequence motif is believed to have a beneficial role in translation initiation in eukaryotes.

12.3. GENE CONSTRUCTION

To build the variable regions, various strategies are available. The sequence may be assembled by using oligonucleotides in a manner similar to Jones et al (ref. 17) or by simultaneously replacing all of the CDRs or loop regions by oligonucleotide directed site specific mutagenesis in a manner similar to Verhoeyen et al (ref. 2). Both strategies were used and a list of constructions is set out in Tables 1 and 2 and Figures 4 and 5. It was noted in several cases that the mutagenesis approach led to deletions and rearrangements in the gene being remodelled, while the success of the assembly approach was very sensitive to the quality of the oligonucleotides.

13. CONSTRUCTION OF EXPRESSION VECTORS

Genes were isolated from M13 or SP65 based intermediate vectors and cloned into pEE6hCMVneo for the light chains and pEE6hCMVgpt for the heavy chains in a manner similar to that for the chimeric genes as described above.

TABLE 1 CDR-GRAFTED GENE CONSTRUCTS

CODE	MOUSE SEQUENCE CONTENT	METHOD OF CONSTRUCTION	KOZAK SEQUENCE -	KOZAK SEQUENCE +
LIGHT CHAIN	ALL HUMAN FRAMEWORK RE1			
121	26-32, 50-56, 91-96 inclusive	SDM and gene assembly	+	n.d.
121A	26-32, 50-56, 91-96 inclusive +1, 3, 46, 47	Partial gene assembly	n.d.	+
121B	26-32, 50-56, 91-96 inclusive + 46, 47	Partial gene assembly	n.d.	+
221	24-24, 50-56, 91-96 inclusive	Partial gene assembly	+	+
221A	24-34, 50-56, 91-96 inclusive +1, 3, 46, 47	Partial gene assembly	+	+
221B	24-34, 50-56, 91-96 inclusive +1, 3	Partial gene assembly	+	+
221C	24-34, 50-56, 91-96 inclusive	Partial gene assembly	+	+
HEAVY CHAIN	ALL HUMAN FRAMEWORK KOL			
121	26-32, 50-56, 95-100B inclusive	Gene assembly	n.d.	+
131	26-32, 50-58, 95-100B inclusive	Gene assembly	n.d.	+
141	26-32, 50-65, 95-100B inclusive	Partial gene assembly	+	n.d.
321	26-35, 50-56, 95-100B inclusive	Partial gene assembly	+	n.d.
331	26-35, 50-58, 95-100B inclusive	Partial gene assembly	+	
		Gene assembly		+
341	26-35, 50-65, 95-100B inclusive	SDM	+	
		Partial gene assembly		+
341A	26-35, 50-65, 95-100B inclusive +6, 23, 24, 48, 49, 71, 73, 76, 78, 88, 91 (+63 = human)	Gene assembly	n.d.	+
341B	26-35, 50-65, 95-100B inclusive + 48, 49, 71, 73, 76, 78, 88, 91 (+63 + human)	Gene assembly	n.d.	+

KEY

n.d.	not done
SDM	Site directed mutagenesis
Gene assembly	Variable region assembled entirely from oligonucleotides
Partial gene assembly	Variable region assembled by combination of restriction fragments either from other genes originally created by SDM and gene assembly or by oligonucleotide assembly of part of the variable region and reconstruction with restriction fragments from other genes originally created by SDM and gene assembly

14. EXPRESSION OF CDR-GRAFTED GENES

14.1. PRODUCTION OF ANTIBODY CONSISTING OF GRAFTED LIGHT (gL) CHAINS WITH MOUSE HEAVY (mH) OR CHIMERIC HEAVY (cH) CHAINS

All gL chains, in association with mH or cH produced reasonable amounts of antibody.

Insertion of the Kozak consensus sequence at a position 5′ to the ATG (kgL constructs) however, led to a 2-5 fold improvement in net expression. Over an extended series of experiments expression levels were raised from approximately 200ng/ml to approximately 500 ng/ml for kgL/cH or kgL/mH combinations.

When direct binding to antigen on HUT 78 cells was measured, a construct designed to include mouse sequence based on loop length (gL121) did not lead to active antibody in association with mH or cH. A construct designed to include mouse sequence based on Kabat CDRs (gL221) demonstrated some weak binding in association with mH or cH. However, when framework residues 1, 3, 46, 47 were changed from the human to the murine OKT3 equivalents based on the arguments outlined in Section 12.1 antigen binding was demonstrated when both of the new constructs, which were termed 121A and 221A were co-expressed with cH. When the effects of these residues were examined in more detail, it appears that residues 1 and 3 are not major contributing residues as the product of the gL221B gene shows little detectable binding activity in association with cH. The light chain product of gL221C, in which mouse sequences are present at 46 and 47, shows good binding activity in association with cH.

14.2 PRODUCTION OF ANTIBODY CONSISTING OF GRAFTED HEAVY (gH) CHAINS WITH MOUSE LIGHT (mL) OR CHIMERIC LIGHT (cL) CHAINS

Expression of the gH genes proved to be more difficult to achieve than for gL. First, inclusion of the Kozak sequence appeared to have no marked effect on expression of gH genes. Expression appears to be slightly improved but not to the same degree as seen for the grafted light chain.

Also, it proved difficult to demonstrate production of expected quantities of material when the loop choice (amino acid 26-32) for CDR1 is used, e.g. gH121, 131, 141 and no conclusions can be drawn about these constructs.

Moreover, co-expression of the gH341 gene with cL or mL has been variable and has tended to produce lower amounts of antibody than the cH/cL or mH/mL combinations. The alterations to gH341 to produce gH341A and gH341B lead to improved levels of expression.

This may be due either to a general increase in the fraction of mouse sequence in the variable region, or to the alteration at position 63 where the residue is returned to the human amino acid Valine (Val) from Phenylalanine (Phe) to avoid possible internal packing problems with the rest of the human framework. This arrangement also occurs in gH331 and gH321.

When gH321 or gH331 were expressed in association with cL, antibody was produced but antibody binding activity was not detected.

When the more conservative gH341 gene was used antigen binding could be detected in association with cL or mL, but the activity was only marginally above the background level.

When further mouse residues were substituted based on the arguments in 12.1, antigen binding could be clearly demonstrated for the antibody produced when kgH341A and kgH341B were expressed in association with cL.

14.3 PRODUCTION OF FULLY CDR-GRAFTED ANTIBODY

The kgL221A gene was co-expressed with kgH341, kgH341A or kgH341B. For the combination kgH221A/kgH341 very little material was produced in a normal COS cell expression.

For the combinations kgL221A/kgH341A or kgH221A/kgH341B amounts of antibody similar to gL/cH was produced.

In several experiments no antigen binding activity could be detected with kgH221A/gH341 or kgH221A/kgH341 combinations, although expression levels were very low.

Antigen binding was detected when kgL221A/kgH341A or kgH221A/kgH341B combinations were expressed. In the case of the antibody produced from the kgL221A/kgH341A combination the antigen binding was very similar to that of the chimeric antibody.

An analysis of the above results is given below.

15. DISCUSSION OF CDR-GRAFTING RESULTS

In the design of the fully humanised antibody the aim was to transfer the minimum number of mouse amino acids that would confer antigen binding onto a human antibody framework.

15.1. LIGHT CHAIN

15.1.1. EXTENT OF THE CDRs

For the light chain the regions defining the loops known from structural studies of other antibodies to contain the antigen contacting residues, and

those hypervariable sequences defined by Kabat et al (refs. 4 and 5) as Complementarity Determining Regions (CDRs) are equivalent for CDR2. For CDR1 the hypervariable region extends from residues 24-34 inclusive while the structural loop extends from 26-32 inclusive. In the case of OKT3 there is only one amino acid difference between the two options, at amino acid 24, where the mouse sequence is a serine and the human framework RE1 has glutamine. For CDR3 the loop extends from residues 91-96 inclusive while the Kabat hypervariability extends from residues 89-97 inclusive. For OKT3 amino acids 89, 90 and 97 are the same between OKT3 and RE1 (Fig. 3). When constructs based on the loop choice for CDR1 (gL121) and the Kabat choice (gL221) were made and co-expressed with mH or cH no evidence for antigen binding activity could be found for gL121, but trace activity could be detected for the gL221, suggesting that a single extra mouse residue in the grafted variable region could have some detectable effect. Both gene constructs were reasonably well expressed in the transient expression system.

15.1.2. FRAMEWORK RESIDUES

The remaining framework residues were then further examined, in particular amino acids known from X-ray analysis of other antibodies to be close to the CDRs and also those amino acids which in OKT3 showed differences from the consensus framework for the mouse subgroup (subgroup VI) to which OKT3 shows most homology. Four positions 1, 3, 46 and 47 were identified and their possible contribution was examined by substituting the mouse amino acid for the human amino acid at each position. Therefore gL221A (gL221 + D1Q, Q3V, L46R, L47W,

see Figure 3 and Table 1) was made, cloned in EE6hCMVneo and co-expressed with cH (pJA144). The resultant antibody was well expressed and showed good binding activity. When the related genes gL221B (gL221 + D1Q, Q3V) and gL221C (gL221 + L46R, L47W) were made and similarly tested, while both genes produced antibody when co-expressed with cH, only the gL221C/cH combination showed good antigen binding. When the gL121A (gL121 + D1Q, Q3V, L46R, L47W) gene was made and co-expressed with cH, antibody was produced which also bound to antigen.

15.2. HEAVY CHAIN

15.2.1. EXTENT OF THE CDRs

For the heavy chain the loop and hypervariability analyses agree only in CDR3. For CDR1 the loop region extends from residues 26-32 inclusive whereas the Kabat CDR extends from residues 31-35 inclusive. For CDR2 the loop region is from 50-58 inclusive while the hypervariable region covers amino acids 50-65 inclusive. Therefore humanised heavy chains were constructed using the framework from antibody KOL and with various combinations of these CDR choices, including a shorter choice for CDR2 of 50-56 inclusive as there was some uncertainty as to the definition of the end point for the CDR2 loop around residues 56 to 58. The genes were co-expressed with mL or cL initially. In the case of the gH genes with loop choices for CDR1 e.g. gH121, gH131, gH141 very little antibody was produced in the culture supernatants. As no free light chain was detected it was presumed that the antibody was being made and assembled inside the cell but that the heavy chain was aberrant in some way, possibly incorrectly folded, and therefore the antibody was

being degraded internally. In some experiments trace amounts of antibody could be detected in ^{35}S labelling studies.
As no net antibody was produced, analysis of these constructs was not pursued further.
When, however, a combination of the loop choice and the Kabat choice for CDR1 was tested (mouse amino acids 26-35 inclusive) and in which residues 31 (Ser to Arg), 33 (Ala to Thr), and 35 (Tyr to His) were changed from the human residues to the mouse residue and compared to the first series, antibody was produced for gH321, kgH331 and kgH341 when co-expressed with cL. Expression was generally low and could not be markedly improved by the insertion of the Kozak consensus sequence 5′ to the ATG of the signal sequence of the gene, as distinct from the case of the gL genes where such insertion led to a 2-5 fold increase in net antibody production. However, only in the case of gH341/mL or kgH341/cL could marginal antigen binding activity be demonstrated. When the kgH341 gene was co-expressed with kgL221A, the net yield of antibody was too low to give a signal above the background level in the antigen binding assay.

15.2.2. FRAMEWORK RESIDUES

As in the case of the light chain the heavy chain frameworks were re-examined. Possibly because of the lower initial homology between the mouse and human heavy variable domains compared to the light chains, more amino acid positions proved to be of interest. Two genes kgH341A and kgH341B were constructed, with 11 or 8 human residues respectively substituted by mouse residues compared to gH341, and with the CDR2 residue 63 returned to the human amino acid potentially to

improve domain packing. Both showed antigen binding when combined with cL or kgL221A, the kgH341A gene with all 11 changes appearing to be the superior choice.

15.3 INTERIM CONCLUSIONS

It has been demonstrated, therefore, for OKT3 that to transfer antigen binding ability to the humanised antibody, mouse residues outside the CDR regions defined by the Kabat hypervariability or structural loop choices are required for both the light and heavy chains. Fewer extra residues are needed for the light chain, possibly due to the higher initial homology between the mouse and human kappa variable regions.

Of the changes seven (1 and 3 from the light chain and 6, 23, 71, 73 and 76 from the heavy chain) are predicted from a knowledge of other antibody structures to be either partly exposed or on the antibody surface. It has been shown here that residues 1 and 3 in the light chain are not absolutely required to be the mouse sequence; and for the heavy chain the gH341B heavy chain in combination with the 221A light chain generated only weak binding activity. Therefore the presence of the 6, 23 and 24 changes are important to maintain a binding affinity similar to that of the murine antibody. It was important, therefore, to further study the individual contribution of othe other 8 mouse residues of the kgH341A gene compared to kgH341.

16. FURTHER CDR-GRAFTING EXPERIMENTS

Additional CDR-grafted heavy chain genes were prepared substantially as described above. With reference to Table 2 the further heavy chain genes were based upon the gh341 (plasmid pJA178) and

gH341A (plasmid pJA185) with either mouse OKT3 or human KOL residues at 6, 23, 24, 48, 49, 63, 71, 73, 76, 78, 88 and 91, as indicated. The CDR-grafted light chain genes used in these further experiments were gL221, gL221A, gL221B and gL221C as described above.

TABLE 2

OKT3 HEAVY CHAIN CDR GRAFTS

1. gH341 and derivatives

RES NUM	6	23	24	48	49	63	71	73	76	78	88	91	
OKT3vh	Q	K	A	I	G	F	T	K	S	A	A	Y	
gH341	E	S	S	V	A	F	R	N	N	L	G	F	JA178
gH341A	Q	K	A	I	G	V	T	K	S	A	A	Y	JA185
gH341E	Q	K	A	I	G	V	T	K	S	A	G	G	JA198
gH341*	Q	K	A	I	G	V	T	K	N	A	G	F	JA207
gH341*	Q	K	A	I	G	V	R	N	N	A	G	F	JA209
gH341D	Q	K	A	I	G	V	T	K	N	L	G	F	JA197
gH341*	Q	K	A	I	G	V	R	N	N	L	G	F	JA199
gH341C	Q	K	A	V	A	F	R	N	N	L	G	F	JA184
gH341*	Q	S	A	I	G	V	T	K	S	A	A	Y	JA203
gH341*	E	S	A	I	G	V	T	K	S	A	A	Y	JA205
gH341B	E	S	S	I	G	V	T	K	S	A	A	Y	JA183
gH341*	Q	S	A	I	G	V	T	K	S	A	G	F	JA204
gH341*	E	S	A	I	G	V	T	K	S	A	G	F	JA206
gH341*	Q	S	A	I	G	V	T	K	N	A	G	F	JA208
KOL	E	S	S	V	A		R	N	N	L	G	F	

OKT3 LIGHT CHAIN CDR GRAFTS

2. gL221 and derivatives

RES NUM	1	3	46	47	
OKT3vl	Q	V	R	W	
GL221	D	Q	L	L	DA221
gL221A	Q	V	R	W	DA221A
gL221B	Q	V	L	L	DA221B
GL221C	D	Q	R	W	DA221C
RE1	D	Q	L	L	

MURINE RESIDUES ARE UNDERLINED

The CDR-grafted heavy and light chain genes were co-expressed in COS cells either with one another in various combinations but also with the corresponding murine and chimeric heavy and light chain genes substantially as described above. The resultant antibody products were then assayed in binding and blocking assays with HPB-ALL cells as described above.

The results of the assays for various grafted heavy chains co-expressed with the gL221C light chain are given in Figures 7 and 8 (for the JA184, JA185, JA197 and JA198 constructs - see Table 2), in Figure 9 (for the JA183, JA184, JA185 and JA197 constructs) in Figure 10 (for the chimeric, JA185, JA199, JA204, JA205, JA207, JA208 and JA209 constructs) and in Figure 11 (for the JA183, JA184, JA185, JA198, JA203, JA205 and JA206 constructs).

The basic grafted product without any human to murine changes in the variable frameworks, i.e. gL221 co-expressed with gh341 (JA178), and also the "fully grafted" product, having most human to murine changes in the grafted heavy chain framework, i.e. gL221C co-expressed with gh341A (JA185), were assayed for relative binding affinity in a competition assay against murine OKT3 reference standard, using HPB-ALL cells. The assay used was as described above in section 3.3. The results obtained are given in Figure 12 for the basic grafted product and in Figure 13 for the fully grafted product. These results indicate that the basic grafted product has neglibible binding ability as compared with the OKT3 murine reference standard; whereas the "fully grafted" product has a binding ability very similar to that of the OKT3 murine reference standard.

The binding and blocking assay results indicate the following:

The JA198 and JA207 constructs appear to have the best binding characteristics and similar binding abilities, both substantially the same as the chimeric and fully grafted gH341A products. This indicates that positions 88 and 91 and position 76 are not highly critical for maintaining the OKT3 binding ability; whereas at least some of positions 6, 23, 24, 48, 49, 71, 73 and 78 are more important.

This is borne out by the finding that the JA209 and JA199, although of similar binding ability to one another, are of lower binding ability than the JA198 and JA207 constructs. This indicates the importance of having mouse residues at positions 71, 73 and 78, which are either completely or partially human in the JA199 and JA209 constructs respectively.

Moreover, on comparing the results obtained for the JA205 and JA183 constructs it is seen that there is a decrease in binding going from the JA205 to the JA183 constructs. This indicates the importance of retaining a mouse residue at position 23, the only position changed between JA205 and JA183.

These and other results lead us to the conclusion that of the 11 mouse framework residues used in the gH341A (JA185) construct, it is important to retain mouse residues at all of positions 6, 23, 24, 48 and 49, and possibly for maximum binding affinity at 71, 73 and 78.

Similar Experiments were carried out to CDR-graft a number of the rodent antibodies including antibodies having specificity for CD4 (OKT4), ICAM-1 (R6-5), TAG72 (B72.3), and TNFα(61E71, 101.4, hTNF1, hTNF2 and hTNF3).

EXAMPLE 2

CDR-GRAFTING OF A MURINE ANTI-CD4 T CELL RECEPTOR ANTIBODY, OKT4A

Anti OKT4A CDR-grafted heavy and light chain genes were prepared, expressed and tested substantially as described above in Example 1 for CDR-grafted OKT3. The CDR grafting of OKT4A is described in detail in Ortho patent application PCT/GB 90..... of even date herewith entitled "Humanised Antibodies". The disclosure of this Ortho patent application PCT/GB 90 ..... is incorporated herein by reference. A number of CDR-grafted OKT4 antibodies have been prepared. Presently the CDR-grafted OKT4A of choice is the combination of the grafted light chain LCDR2 and the grafted heavy chain HCDR10.

THE LIGHT CHAIN

The human acceptor framework used for the grafted light chains was RE1. The preferred LCDR2 light chain has human to mouse changes at positions 33, 34, 38, 49 and 89 in addition to the structural loop CDRs. Of these changed positions, positions 33, 34 and 89 fall within the preferred extended CDRs of the present invention (positions 33 and 34 in CDR1 and position 89 in CDR3). The human to murine changes at positions 38 and 49 corresponds to positions at which the amino acid residues are preferably donor murine amino acid residues in accordance with the present invention.

A comparison of the amino acid sequences of the donor murine light chain variable domain and the RE1 human acceptor light chain variable further reveals that the murine and human residues are identical at all of positions 46, 48 and 71 and at all of positions 2, 4, 6, 35, 36, 44, 47, 62, 64-69, 85, 87, 98, 99 and 101 and 102. However the amino acid residue at position 58 in LCDR2 is

the human RE1 framework residue not the mouse OKT4 residue as would be preferred in accordance with the present invention.

THE HEAVY CHAIN

The human acceptor framework used for the grafted heavy chains was KOL.

The preferred CDR graft HCDR10 heavy chain has human to mouse changes at positions 24, 35, 57, 58, 60, 88 and 91 in addition to the structural loop CDRs.

Of these positions, positions 35 (CDR1) and positions 57, 58 and 60 (CDR2) fall within the preferred extended CDRs of the present invention. Also the human to mouse change at position 24 corresponds to a position at which the amino acid residue is a donor murine residue in accordance with the present invention. Moreover, the human to mouse changes at positions 88 and 91 correspond to positions at which the amino acid residues are optionally donor murine residues.

Moreover, a comparison of the murine OKT4A and human KOL heavy chain variable amino acid sequences reveals that the murine and human residues are identical at all of positions 23, 49, 71, 73 and 78 and at all of positions 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

Thus the OKT4A CDR-grafted heavy chain HCDR10 corresponds to a particularly preferred embodiment according to the present invention.

EXAMPLE 3

CDR-GRAFTING OF AN ANTI-MUCIN SPECIFIC MURINE ANTIBODY, B72.3

The cloning of the genes coding for the anti-mucin specific murine monoclonal antibody B72.3 and the preparation of B72.3 mouse-human chimeric antibodies has been described previously (ref. 13 and WO 89/01783). CDR-grafted versions of B72.3 were prepared as follows.

(a) B72.3 Light Chain

CDR-grafting of this light chain was accomplished by direct transfer of the murine CDRs into the framework of the human light chain RE1. The regions transferred were:

CDR Number	Residues
1	24-34
2	50-56
3	90-96

The activity of the resulting grafted light chain was assessed by co-expression in COS cells, of genes for the combinations:

B72.3 cH/B72.3 cL

and B72.3 cH/B72.3 gL

Supernatants were assayed for antibody concentration and for the ability to bind to microtitre plates coated with mucin. The results obtained indicated that, in combination with the B72.3 cH chain, B72.3 cL and B72.3 gL had similar binding properties.

Comparison of the murine B72.3 and REI light chain amino acid sequences reveals that the residues are identical at positions 46, 58 and 71 but are different at position 48.

Thus changing the human residue to the donor mouse residue at position 48 may further improve the binding characteristics of the CDR-grafted light chain, (B72.3 gL) in accordance with the present invention.

(b) B72.3 heavy chain

i. Choice of framework

At the outset it was necessary to make a choice of human framework. Simply put, the question was as follows: Was it necessary to use the framework regions from an antibody whose crystal structure was known or could the choice be made on some other criteria?

For B72.3 heavy chain, it was reasoned that, while knowledge of structure was important, transfer of the CDRs from mouse to human frameworks might be facilitated if the overall homology between the donor and receptor frameworks was maximised.

Comparison of the B72.3 heavy chain sequence with those in Kabat (ref. 4) for human heavy chains showed clearly that B72.3 had poor homology for KOL and NEWM (for which crystal structures are available) but was very homologous to the heavy chain for EU.

On this basis, EU was chosen for the CDR-grafting and the following residues transferred as CDRs.

CDR Number	Residues
1	27-36
2	50-63
3	93-102

Also it was noticed that the FR4 region of EU was unlike that of any other human (or mouse) antibody. Consequently, in the grafted heavy chain genes this was also changed to produce a "consensus" human sequence. (Preliminary experiments showed that grafted heavy chain genes containing the EU FR4 sequence expressed very poorly in transient expression systems.)

ii. Results with grafted heavy chain genes

Expression of grafted heavy chain genes containing all human framework regions with either gL or cL genes produced a grafted antibody with little ability to bind to mucin. The grafted antibody had about 1% the activity of the chimeric antibody.

In these experiments, however, it was noted that the activity of the grafted antibody could be increased to ~ 10% of B72.3 by exposure to pHs of 2-3.5.

This observation provided a clue as to how the activity of the grafted antibody could be improved without acid treatment. It was postulated that acid exposure brought about the protonation of an acidic residue (pKa of aspartic acid = 3.86 and of glutamine acid = 4.25) which in turn caused a change in structure of the CDR loops, or allowed better access of antigen.

From comparison of the sequences of B72.3 (ref. 13) and EU (refs. 4 and 5), it was clear that, in going from the mouse to human frameworks, only two positions had been changed in such a way that acidic residues had been introduced. These

positions are at residues 73 and 81, where K to E and Q to E changes had been made, respectively.

Which of these positions might be important was determined by examining the crystal structure of the KOL antibody. In KOL heavy chain, position 81 is far removed from either of the CDR loops.

Position 73, however, is close to both CDRs 1 and 3 of the heavy chain and, in this position it was possible to envisage that a K to E change in this region could have a detrimental effect on antigen binding.

iii. Framework changes in B72.3 gH gene

On the basis of the above analysis, E73 was mutated to a lysine (K). It was found that this change had a dramatic effect on the ability of the grafted Ab to bind to mucin. Further the ability of the grafted B72.3 produced by the mutated gH/gL combination to bind to mucin was similar to that of the B72.3 chimeric antibody.

iv. Other framework changes

In the course of the above experiments, other changes were made in the heavy chain framework regions. Within the accuracy of the assays used, none of the changes, either alone or together, appeared beneficial.

v. Other

All assays used measured the ability of the grafted Ab to bind to mucin and, as a whole, indicated that the single framework change at position 73 is sufficient to generate an antibody with similar binding properties to B72.3.

Comparison of the B72.3 murine and EU heavy chain sequences reveals that the mouse and human residues are identical at positions 23, 24, 71 and 78.
Thus the mutated CDR-grafted B72.3 heavy chain corresponds to a preferred embodiment of the present invention.

EXAMPLE 4

CDR-GRAFTING OF A MURINE ANTI-ICAM-1 MONOCLONAL ANTIBODY

A murine antibody, R6-5-D6 (EP 0314863) having specificity for Intercellular Adhesion Molecule 1 (ICAM-1) was CDR-grafted substantially as described above in previous examples. This work is described in greater detail in co-pending application, British Patent Application No. 9009549.8, the disclosure of which is incorporated herein by reference.

The human EU framework was used as the acceptor framework for both heavy and light chains. The CDR-grafted antibody currently of choice is provided by co-expression of grafted light chain gL221A and grafted heavy chain gH341D which has a binding affinity for ICAM 1 of about 75% of that of the corresponding mouse-human chimeric antibody.

LIGHT CHAIN

gL221A has murine CDRs at positions 24-34 (CDR1), 50-56 (CDR2) and 89-97 (CDR3). In addition several framework residues are also the murine amino acid. These residues were chosen after consideration of the possible contribution of these residues to domain packing and stability of the conformation of the antigen binding region. The residues which have been retained as mouse are at positions 2, 3, 48 (?), 60, 84, 85 and 87. Comparison of the murine anti-ICAM 1 and human EU light chain amino acid sequences reveals that the murine and human residues are identical at positions 46, 58 and 71.

HEAVY CHAIN

gH341D has murine CDRs at positions 26-35 (CDR1), 50-56 (CDR2) and94-100B (CDR3). In addition murine residues were used in gH341D at positions 24, 48, 69, 71, 73, 80, 88 and 91. Comparison of the murine anti-ICAM 1 and human EU heavy chain amino acid sequences are identical at positions 23, 49 and 78.

EXAMPLE 5

CDR-Grafting of murine anti-TNFα antibodies

A number of murine anti-TNFα monoclonal antibodies were CDR-grafted substantially as described above in previous examples. These antibodies include the murine monoclonal antibodies designated 61 E71, hTNF1, hTNF3 and 101.4 A brief summary of the CDR-grafting of each of these antibodies is given below.

61E71

A similar analysis as described above (Example 1, Section 12.1.) was done for 61E71 and for the heavy chain 10 residues were identified at 23, 24, 48, 49, 68, 69, 71, 73, 75 and 88 as residues to potentially retain as murine. The human frameworks chosen for CDR-grafting of this antibody, and the hTNF3 and 101.4 antibodies were RE1 for the light chain and KOL for the heavy chain.
Three genes were built, the first of which contained 23, 24, 48, 49, 71 and 73 [gH341(6)] as murine residues. The second gene also had 75 and 88 as murine residues [gH341(8)] while the third gene additionally had 68, 69, 75 and 88 as murine residues [gH341(10)]. Each was co-expressed with gL221, the minimum grafted light chain (CDRs only). The gL221/gH341(6) and gL221/gH341(8) antibodies both bound as well to TNF as murine 61E71. The gL221/gH341(10) antibody did not express and this combination was not taken further.
Subsequently the gL221/gH341(6) antibody was assessed in an L929 cell competition assay in which the antibody competes against the TNF receptor on L929 cells for binding to TNF in solution. In this assay the gL221/gH341(6) antibody was approximately 10% as active as murine 61E71.

hTNF1

hTNF1 is a monoclonal antibody which recognises an epitope on human TNF- . The EU human framework was used for CDR-grafting of both the heavy and light variable domains.

Heavy Chain

In the CDR-grafted heavy chain (ghTNF1) mouse CDRs were used at positions 26-35 (CDR1), 50-65 (CDR2) and 95-102 (CDR3). Mouse residues were also used in the frameworks at positions 48, 67, 69, 71, 73, 76, 89, 91, 94 and 108. Comparison of the TNF1 mouse and EU human heavy chain residues reveals that these are identical at positions 23, 24, 29 and 78.

Light Chain

In the CDR-grafted light chain (gLhTNF1) mouse CDRs wre used at positions 24-34 (CDR1), 50-56 (CDR2) and 89-97 (CDR3). In addition mouse residues were used in the frameworks at positions 3, 42, 48, 49, 83, 106 and 108. Comparison of the hTNF1 mouse and EU human light chain residues reveals that these are identical at positions 46, 58 and 71.

The grafted hTNF1 heavy chain was co-expressed with the chimeric light chain and the binding ability of the product compared with that of the chimeric light chain/chimeric heavy chain product in a TNF binding assay. The grafted heavy chain product appeared to have binding ability for TNF slightly better than the fully chimeric product.

Similarly, a grafted heavy chain/grafted light chain product was co-expressed and compared with the fully chimeric product and found to have closely similar binding properties to the latter product.

hTNF3

hTNF3 recognises an epitope on human TNF-α. The sequence of hTNF3 shows only 21 differences compared to 61E71 in the light and heavy chain variable regions, 10 in the light chain (2 in the CDRs at positions 50, 96 and 8 in the framework at 1, 19, 40, 45, 46, 76, 103 and 106) and 11 in the heavy chain (3 in the CDR regions at positions 52, 60 and 95 and 8 in the framework at 1, 10, 38, 40, 67, 73, 87 and 105). The light and heavy chains of the 61E71 and hTNF3 chimeric antibodies can be exchanged without loss of activity in the direct binding assay. However 61E71 is an order of magnitude less able to compete with the TNF receptor on L929 cells for TNF-a compared to hTNF3. Based on the 61E71 CDR grafting data gL221 and gH341(+23, 24, 48, 49 71 and 73 as mouse) genes have been built for hTNF3 and tested and the resultant grafted antibody binds well to TNF-a, but competes very poorly in the L929 assay. It is possible that in this case also the framework residues identified for OKT3 programme may improve the competitive binding ability of this antibody.

101.4

101.4 is a further murine monoclonal antibody able to recognise human TNF-a. The heavy chain of this antibody shows good homology to KOL and so the CDR-grafting has been based on RE1 for the light chain and KOL for the heavy chain. Several grafted heavy chain genes have been constructed with conservative choices for the CDR's (gH341) and which have one or a small number of non-CDR residues at positions 73, 78 or 77-79 inclusive, as the mouse amino acids. These have been co-expressed with cL or gL221. In all cases binding to TNF equivalent to the chimeric antibody is seen and when co-expressed with cL the resultant antibodies are able to compete well in the L929 assay. However, with gL221 the resultant antibodies

are at least an order of magnitude less able to compete for TNF against the TNF receptor on L929 cells.

Mouse residues at other positions in the heavy chain, for example, at 23 and 24 together or at 76 have been demonstrated to provide no improvement to the competitive ability of the grafted antibody in the L929 assay.

A number of other antibodies including antibodies having specificity for interleukins e.g. IL1 and cancer markers such as carcinoembryonic antigen (CEA) e.g. the monoclonal antibody A5B7 (ref. 21), have been successfully CDR-grafted according to the present invention.
It will be appreciated that the foregoing examples are given by way of illustration only and are not intended to limit the scope of the claimed invention. Changes and modifications may be made to the methods described whilst still falling within the spirit and scope of the invention.

References

1. Kohler & Milstein, Nature, 265, 295-497, 1975.

2. Chatenoud et al, (1986), J. Immunol. 137, 830-838.

3. Jeffers et al, (1986), Transplantation, 41, 572-578.

4. Begent et al, Br. J. Cancer 62: 487 (1990).

5. Verhoeyen et al, Science, 239, 1534-1536, 1988.

6. Riechmann et al, Nature, 332, 323-324, 1988.

7. Kabat, E.A., Wu, T.T., Reid-Miller, M., Perry, H.M., Gottesman, K.S., 1987, in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA.

8. Wu, T.T., and Kabat, E.A., 1970, J. Exp. Med. 132 211-250.

9. Queen et al, (1989), Proc. Natl. Acad. Sci. USA, 86, 10029-10033 and WO 90/07861

10. Maniatis et al, Molecular Cloning, Cold Spring Harbor, New York, 1989.

11. Primrose and Old, Principles of Gene Manipulation, Blackwell, Oxford, 1980.

12. Sanger, F., Nicklen, S., Coulson, A.R., 1977, Proc. Natl. Acad. Sci. USA, 74 5463

13. Kramer, W., Drutsa, V., Jansen, H.-W., Kramer, B., Plugfelder, M., Fritz, H.-J., 1984, Nucl. Acids Res. 12, 9441

14. Whittle, N., Adair, J., Lloyd, J.C., Jenkins, E., Devine, J., Schlom, J., Raubitshek, A., Colcher, D., Bodmer, M., 1987, Protein Engineering 1, 499.

15. Sikder, S.S., Akolkar, P.N., Kaledas, P.M., Morrison, S.L., Kabat, E.A., 1985, J. Immunol. 135, 4215.

16. Wallick, S.C., Kabat, E.A., Morrison, S.L., 1988, J. Exp. Med. 168, 1099

17. Bebbington, C.R., Published International Patent Application WO 89/01036.

18. Granthan and Perrin 1986, Immunology Today 7, 160.

19. Kozak, M., 1987, J. Mol. Biol. 196, 947.

20. Jones, T.P., Dear, P.H., Foote, J., Neuberger, M.S., Winter, G., 1986, Nature, 321, 522

21. Harwood et al, Br. J. Cancer, 54, 75-82 (1986).

CLAIMS

1. A CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

2. A CDR-grafted heavy chain according to Claim 1 comprising donor residues at positions 23, 24, 49, 71, 73 and 78, or at positions 23, 24 and 49.

3. A CDR-grafted heavy chain according to Claim 2 comprising donor residues at positions 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

4. A CDR-grafted heavy chain according to Claim 2 or 3, comprising donor residues at one, some or all of positions:
1 and 3,
69 (if 48 is different between donor and acceptor),
38 and 46 (if 48 is the donor residue),
67,
82 and 18 (if 67 is the donor residue),
91, and
any one or more of 9, 11, 41, 87, 108, 110 and 112.

5. A CDR-grafted heavy chain according to any of the preceding comprising donor CDRs at positions 26-35, 50-65 and 95-100.

6. A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 1 and/or 3 and 46 and/or 47.

7. A CDR-grafted light chain according to Claim 6 comprising donor residues at positions 46 and 47.

8. A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, 58 and 71.

9. A CDR-grafted light chain according to Claim 8 comprising donor residues at positions 46, 48, 58 and 71.

10. A CDR-grafted light chain according to Claim 8 or 9, comprising donor residues at positions 2, 4, 6, 35, 36, 38, 44, 47, 49, 62, 64-69, 85, 87, 98, 99, 101 and 102.

11. A CDR-grafted light chain according to Claim 9 or 10, comprising donor residues at one, some or all of positions:
1 and 3,
63,
60 (if 60 and 54 are able to form a potential saltbridge),
70 (if 70 and 24 are able to form a potential saltbridge),
73 and 21 (if 47 is different between donor and acceptor),
37 and 45 (if 47 if different between donor and acceptor), and
any one or more of 10, 12, 40, 83, 103 and 105.

12. A CDR-grafted light chain according to any one of Claims 6-11, comprising donor CDRs at positions 24-34, 50-56 and 89-97.

13. A CDR-grafted antibody molecule comprising at least one CDR-grafted heavy chain according to any one of Claims 1-5 and at least one CDR-grafted light chain according to any one of Claims 6-12.

14. A CDR-grafted antibody molecule according to Claim 13, which is a site-specific antibody molecule.

15. A CDR-grafted antibody molecule according to Claim 13 which has specificity for an interleukin, hormone or other biologically active compound or a receptor therefor.

16. A CDR-grafted antibody heavy or light chain or molecule according to any one of the preceding claims comprising human acceptor residues and non-human donor residues.

17. A DNA sequence which codes for a CDR-grafted heavy chain according to Claim 1 or a CDR-grafted light chain according to Claim 6 or Claim 8.

18. A cloning or expression vector containing a DNA sequence according to Claim 17.

19. A host cell transformed with a DNA sequence according to Claim 17.

20. A process for the production of a CDR-grafted antibody sequence according to Claim 17 in a transformed host cell.

21. A process for producing a CDR-grafted antibody product comprising:

(a) producing in an expression vector an operon having a DNA sequence which encodes an antibody heavy chain according to Claim 1;

and/or

(b) producing in an expression vector an operon having a DNA sequence which encodes a complementary antibody light chain according to Claim 6 or Claim 8;

(c) transfecting a host cell with the or each vector; and

(d) culturing the transfected cell line to produce the CDR-grafted antibody product.

22. A therapeutic or diagnostic composition comprising a CDR-grafted antibody heavy chain according to Claim 1, or a CDR-grafted light chain according to Claim 6 or Claim 8, or a CDR-grafted antibody molecule according to Claim 13 in combination with a pharmaceutically acceptable carrier, diluent or excipient.

23. A method of therapy or diagnosis comprising administering an effective amount of a CDR-grafted heavy chain according to Claim 1, or a CDR-grafted light chain according to Claim 6 or Claim 8, or a CDR-grafted antibody molecule according to Claim 13 to a human or animal subject.

```
   1  GAATTCCCAA AGACAAAatg gattttcaag tgcagatttt cagcttcctg
  51  ctaatcagtg cctcagtcat aatatccaga ggacaaattg ttctcaccca
 101  gtctccagca atcatgtctg catctccagg ggagaaggtc accatgacct
 151  gcagtgccag ctcaagtgta agttacatga actggtacca gcagaagtca
 201  ggcacctccc ccaaaagatg gatttatgac acatccaaac tggcttctgg
 251  agtccctgct cacttcaggg gcagtgggtc tgggacctct tactctctca
 301  caatcagcgg catggaggct gaagatgctg ccacttatta ctgccagcag
 351  tggagtagta acccattcac gttcggctcg gggacaaagt tggaaataaa
 401  ccgggctgat actgcaccaa ctgtatccat cttcccacca tccagtgagc
 451  agttaacatc tggaggtgcc tcagtcgtgt gcttcttgaa caacttctac
 501  cccaaagaca tcaatgtcaa gtggaagatt gatggcagtg aacgacaaaa
 551  tggcgtcctg aacagttgga ctgatcagga cagcaaagac agcacctaca
 601  gcatgagcag caccctcacg ttgaccaagg acgagtatga acgacataac
 651  agctatacct gtgaggccac tcacaagaca tcaacttcac ccattgtcaa
 701  gagcttcaac aggaatgagt gtTAGAGACA AAGGTCCTGA GACGCCACCA
 751  CCAGCTCCCA GCTCCATCCT ATCTTCCCTT CTAAGGTCTT GGAGGCTTCC
 801  CCACAAGCGC tTACCACTGT TGCGGTGCTC tAAACCTCCT CCCACCTCCT
 851  TCTCCTCCTC CTCCCTTTCC TTGGCTTTTA TCATGCTAAT ATTTGCAGAA
 901  AATATTCAAT AAAGTGAGTC TTTGCCTTGA AAAAAAAAAA AAA
```

Fig. 1(a)

```
   1  MDFOVOIFSF LLISASVIIS RGQIVLTQSP AIMSASPGEK VTMTCSASSS
  51  VSYMNWYQQK SGTSPKRWIY DTSKLASGVP AHFRGSGSGT SYSLTISGME
 101  AEDAATYYCQ QWSSNPFTFG SGTKLEINRA DTAPTVSIFP PSSEQLTSGG
 151  ASVVCFLNNF YPKDINVKWK IDGSERQNGV LNSWTDQDSK DSTYSMSSTL
 201  TLTKDEYERH NSYTCEATHK TSTSPIVKSF NRNEC*
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Fig. 1(b)

SUBSTITUTE SHEET

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   1 GAATTCCCCT CTCCACAGAC ACTGAAAACT CTGACTCAAC ATGGAAAGGC
  51 ACTGGATCTT TCTACTCCTG TTGTCAGTAA CTGCAGGTGT CCACTCCCAG
 101 GTCCAGCTGC AGCAGTCTGG GGCTGAACTG GCAAGACCTG GGGCCTCAGT
 151 GAAGATGTCC TGCAAGGCTT CTGGCTACAC CTTTACTAGG TACACGATGC
 201 ACTGGGTAAA ACAGAGGCCT GGACAGGGTC TGGAATGGAT TGGATACATT
 251 AATCCTAGCC GTGGTTATAC TAATTACAAT CAGAAGTTCA AGGACAAGGC
 301 CACATTGACT ACAGACAAAT CCTCCAGCAC AGCCTACATG CAACTGAGCA
 351 GCCTGACATC TGAGGACTCT GCAGTCTATT ACTGTGCAAG ATATTATGAT
 401 GATCATTACT GCCTTGACTA CTGGGGCCAA GGCACCACTC TCACAGTCTC
 451 CTCAGCCAAA ACAACAGCCC CATCGGTCTA TCCACTGGCC CCTGTGTGTG
 501 GAGATACAAC TGGCTCCTCG GTGACTCTAG GATGCCTGGT CAAGGGTTAT
 551 TTCCCTGAGC CAGTGACCTT GACCTGGAAC TCTGGATCCC TGTCCAGTGG
 601 TGTGCACACC TTCCCAGCTG TCCTGCAGTC TGACCTCTAC ACCCTCAGCA
 651 GCTCAGTGAC TGTAACCTCG AGCACCTGGC CCAGCCAGTC CATCACCTGC
 701 AATGTGGCCC ACCCGGCAAG CAGCACCAAG GTGGACAAGA AAATTGAGCC
 751 CAGAGGGCCC ACAATCAAGC CCTGTCCTCC ATGCAAATGC CCAGCACCTA
 801 ACCTCTTGGG TGGACCATCC GTCTTCATCT TCCCTCCAAA GATCAAGGAT
 851 GTACTCATGA TCTCCCTGAG CCCCATAGTC ACATGTGTGG TGGTGGATGT
 901 GAGCGAGGAT GACCCAGATG TCCAGATCAG CTGGTTTGTG AACAACGTGG
 951 AAGTACACAC AGCTCAGACA CAAACCCATA GAGAGGATTA CAACAGTACT
1001 CTCCGGGTGG TCAGTGCCCT CCCCATCCAG CACCAGGACT GGATGAGTGG
1051 CAAGGAGTTC AAATGCAAGG TCAACAACAA AGACCTCCCA GCGCCCATCG
1101 AGAGAACCAT CTCAAAACCC AAAGGGTCAG TAAGAGCTCC ACAGGTATAT
1151 GTCTTGCCTC CACCAGAAGA AGAGATGACT AAGAAACAGG TCACTCTGAC
1201 CTGCATGGTC ACAGACTTCA TGCCTGAAGA CATTTACGTG GAGTGGACCA
1251 ACAACGGGAA AACAGAGCTA AACTACAAGA ACACTGAACC AGTCCTGGAC
1301 TCTGATGGTT CTTACTTCAT GTACAGCAAG CTGAGAGTGG AAAAGAAGAA
1351 CTGGGTGGAA AGAAATAGCT ACTCCTGTTC AGTGGTCCAC GAGGGTCTGC
1401 ACAATCACCA CACGACTAAG AGCTTCTCCC GGACTCCGGG TAAATGAGCT
1451 CAGCACCCAC AAAACTCTCA GGTCCAAAGA GACACCCACA CTCATCTCCA
1501 TGCTTCCCTT GTATAAATAA AGCACCCAGC AATGCCTGGG ACCATGTAAA
1551 AAAAAAAAAA AAAGGAATTC
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Fig. 2(a)

OKT 3 HEAVY CHAIN PROTEIN SEQUENCE DEDUCED FROM DNA SEQUENCE

```
  1  MERHWIFLLL LSVTAGVHSQ VQLQQSGAEL ARPGASVKMS CKASGYTFTR
 51  YTMHWVKQRP GQGLEWIGYI NPSRGYTNYN QKFKDKATLT TDKSSSTAYM
101  QLSSLTSEDS AVYYCARYYD DHYCLDYWGQ GTTLTVSSAK TTAPSVYPLA
151  PVCGDTTGSS VTLGCLVKGY FPEPVTLTWN SGSLSSGVHT FPAVLQSDLY
201  TLSSSVTVTS STWPSQSITC NVAHPASSTK VDKKIEPRGP TIKPCPPCKC
251  PAPNLLGGPS VFIFPPKIKD VLMISLSPIV TCVVVDVSED DPDVQISWFV
301  NNVEVHTAQT QTHREDYNST LRVVSALPIQ HQDWMSGKEF KCKVNNKDLP
351  APIERTISKP KGSVRAPQVY VLPPPEEEMT KKQVTLTCMV TDFMPEDIYV
401  EWTNNGKTEL NYKNTEPVLD SDGSYFMYSK LRVEKKNWVE RNSYSCSVVH
451  EGLHNHHTTK SFSRTPGK*
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Fig. 2(b)

```
                1                       23                      42
                NN      N                 N        N    N
RES TYPE        SBspSPESssBSbSsSssPSPSPsPSsse*s*p*Pi^ISsSe
Okt3vl          QIVLTQSPAIMSASPGEKVTMTCSASS.SVSYMNWYQQKSGT
REI             DIQMTQSPSSLSASVGDRVTITCQASQDIIKYLNWYQQTPGK
                ? ?
                  CDR1    (LOOP)          *******
                  CDR1    (KABAT)        ***********


                          56                              85
                N  NN
RES TYPE        *IsiPpIeesesssSBEsePsPSBSSEsPspsPsseesSPePb
Okt3vl          SPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAAT
REI             APKLLIYEASNLQAGVPSRFSGSGSGTDYTFTISSLQPEDIAT
                ?  ??                        ?  ?
                      *******  CDR2 (LOOP/KABAT)


                             102   108
RES TYPE        PiPIPies**iPIIsPPSPSPSS
Okt3vl          YYCQQWSSNPFTFGSGTKLEINR
REIvl           YYCQQYQSLPYTFGQGTKLQITR
                             ?    ?
                   ******        CDR3 (LOOP)
                 *********       CRD3 (KABAT)
```

Fig. 3

```
          NN N                  23 26    32 35 N39  43
RES TYPE  SESPs^SBssS^sSSsSpSpSPsPSEbSBssBePiPIpiesss
Okt3h     QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQ
KOL       QVQLVESGGGVVQPGRSLRLSCSSSGFIFSSYAMYWVRQAPGK
               ?                ??
                                     ******    CDR1 (LOOP)
                                         ***** CDR1 (KABAT)

                  52a      60  65     N N  N    82abc     89
RES TYPE IIeIppp^sssssssss^ps^pSSsbSpseSsSseSp^pSpsSBssS^ePb
Okt3vh   GLEWIGYINPSRGYTNTNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAV
KOL      GLEWVAIIWDDGSDQHYADSVKGRFTISRDNSKNTLFLQMDSLRPEDTGV
            ??                    ? ?  ?  ?             ?
               ************          CDR2  (LOOP)
               ********************  CDR2  (KABAT)

           92 N                   107    113
RES TYPE  PiPIEissssiiisssbibi*EIPIP*spSBSS
Okt3vh    YYCARYYDDHY.......CLDYWGQGTTLTVSS
KOL       YFCARDGGHGFCSSASCFGPDYWGQGTPVTVSS
               ***************** CRD3 (KABAT/LOOP)
```

Fig. 4

OKT 3 HEAVY CHAIN CDR GRAFTS

1. gh341 and derivatives

```
          1                          26        35  39  43
Okt3vh    QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQ
gH341     QVQLVESGGGVVQPGRSLRLSCSSSGYTFTRYTMHWVRQAPGK   JA178
gH341A    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA185

gH341E    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA198
gH341*    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA207
gH341*    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA209
gH341D    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA197
gH341*    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA199
gH341C    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA184

gH341*    QVQLVQSGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA203
gH341*    QVQLVESGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA205
gH341B    QVQLVESGGGVVQPGRSLRLSCSSSGYTFTRYTMHWVRQAPGK   JA183
gH341*    QVQLVQSGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA204
gH341*    QVQLVESGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA206
gH341*    QVQLVQSGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA208
KOL       QVQLVESGGGVVQPGRSLRLSCSSSGFIFSSYAMYWVRQAPGK
```

Fig. 5(i)

```
          44     50                  65                        83
Okt3vh    GLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLT
gH341     GLEWVAYINPSRGYTNYNQKFKDRFTISRDNSKNTLFLQMDSLR JA178
gH341A    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR JA185

gH341E    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR JA198
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKNTAFLQMDSLR JA207
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTAFLQMDSLR JA209
gH341D    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKNTLFLQMDSLR JA197
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTLFLQMDSLR JA199
gH341C    GLEWVAYINPSRGYTNYNQKFKDRFTISRDNSKNTLFLQMDSLR JA184

gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR JA207
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR JA205
gH341B    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR JA183
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR JA204
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR JA206
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKNTAFLQMDSLR JA208
KOL       GLEWVAIIWDDGSDQHYADSVKGRFTISRDNSKNTLFLQMDSLR
```

Fig. 5(ii)

```
          84          95              102         113
Okt3vh    SEDSAVYYCARYYDDHY.......CLDYWGQGTTLTVSS
gH341     PEDTGVYFCARYYDDHY.......CLDYWGQGTTLTVSS    JA178
gH341A    PEDTAVYYCARYYDDHY.......CLDYWGQGTTLTVSS    JA185

gH341E    PEDTGVYFCARYYDDHY.......CLDYWGQGTTLTVSS    JA198
gH341*    PEDTGVYFCARYYDDHY.......CLDYWGQGTTLTVSS    JA207
gH341D    PEDTGVYFCARYYDDHY.......CLDYWGQGTTLTVSS    JA197
gH341*    PEDTGVYFCARYYDDHY.......CLDYWGQGTTLTVSS    JA209
gH341*    PEDTGVYFCARYYDDHY.......CLDYWGQGTTLTVSS    JA199
gH341C    PEDTGVYFCARYYDDHY.......CLDYWGQGTTLTVSS    JA184

gH341*    PEDTAVYYCARYYDDHY.......CLDYWGQGTTLTVSS    JA203
gH341*    PEDTAVYYCARYYDDHY.......CLDYWGQGTTLTVSS    JA205
gH341B    PEDTAVYYCARYYDDHY.......CLDYWGQGTTLTVSS    JA183
gH341*    PEDTGVYFCARYYDDHY.......CLDYWGQGTTLTVSS    JA204
gH341*    PEDTGVYFCARYYDDHY.......CLDYWGQGTTLTVSS    JA206
gH341*    PEDTGVYFCARYYDDHY.......CLDYWGQGTTLTVSS    JA208
KOL       PEDTGVYFCARDGGHGFCSSASCFGPDYWGQGTPVTVSS
```

Fig. 5(iii)

OKT3 LIGHT CHAIN CDR GRAFTING

1. gL221 and derivatives

```
          1                        24        34       42
Okt3vl    QIVLTQSPAIMSASPGEKVTMTCSASS.SVSYMNWYQQKSGT
gL221     DIQMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
gL221A    QIVMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
gL221B    QIVMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
gL221C    DIQMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
REI       DIQMTQSPSSLSASVGDRVTITCQASQDIIKYLNWYQQTPGK

          43     50    56                           85
Okt3vl    SPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAAT
gL221     APKLLIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
gL221A    APKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
gL221B    APKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
gL221C    APKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
REI       APKLLIYEASNLQAGVPSRFSGSGSGTDYTFTISSLQPEDIAT

          86   91   96          108
Okt3vl    YYCQQWSSNPFTFGSGTKLEINR
gL221     YYCQQWSSNPFTFGQGTKLQITR
gL221A    YYCQQWSSNPFTFGQGTKLQITR
gL221B    YYCQQWSSNPFTFGQGTKLQITR
gL221C    YYCQQWSSNPFTFGQGTKLQITR
REI       YYCQQYQSLPYTFGQGTKLQITR
```

CDR'S ARE UNDERLINED

FRAMEWORK RESIDUES INCLUDED IN THE GENE ARE DOUBLE UNDERLINED

Fig. 6

OKT3 - pJA198 EVALUATION
BINDING ASSAY
(Mean Channel - HPBALL's)

Fig. 7

OKT3 - pJA198 EVALUATION
BLOCKING ASSAY
(Mean Channel -HPBALL's)

Fig. 8

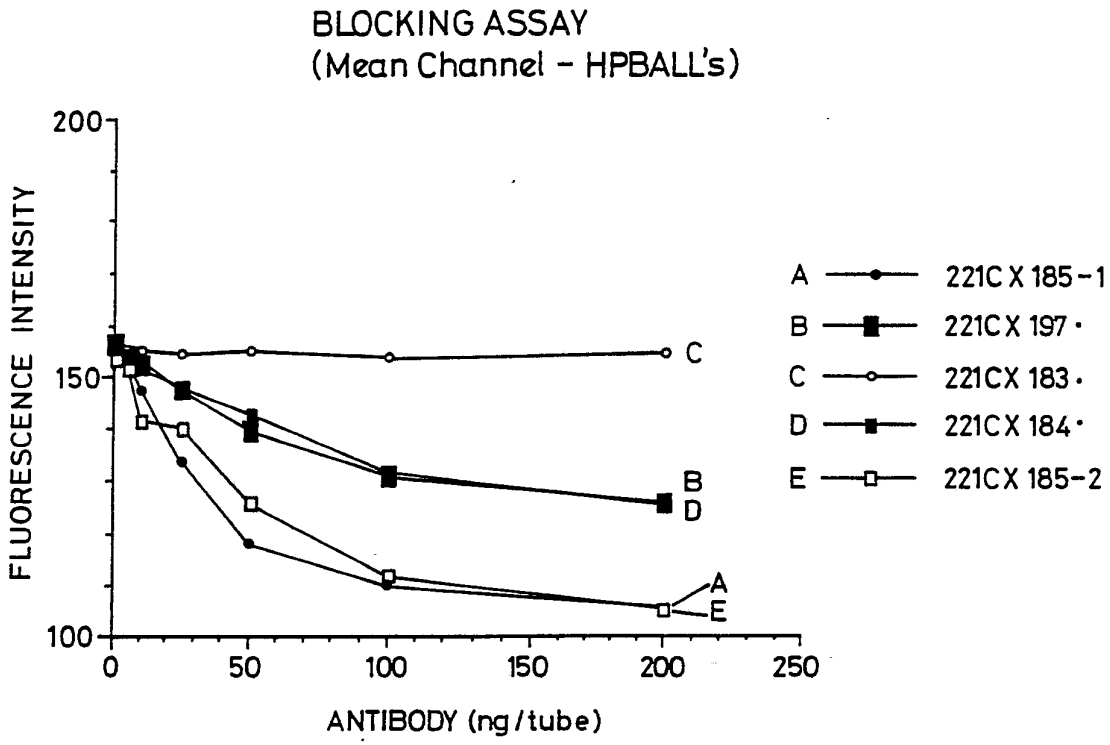
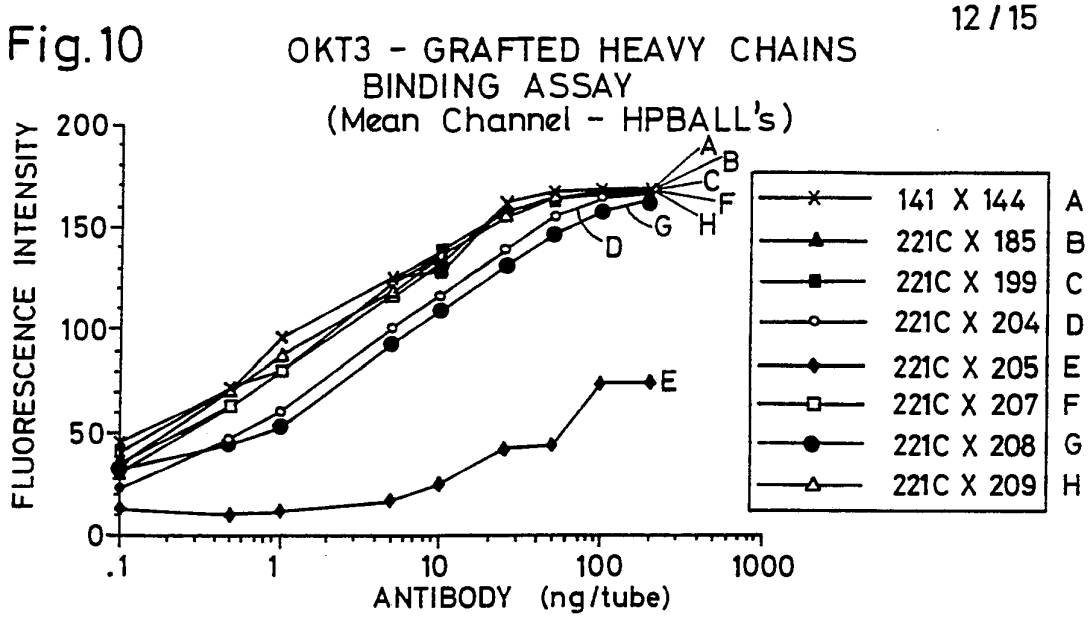
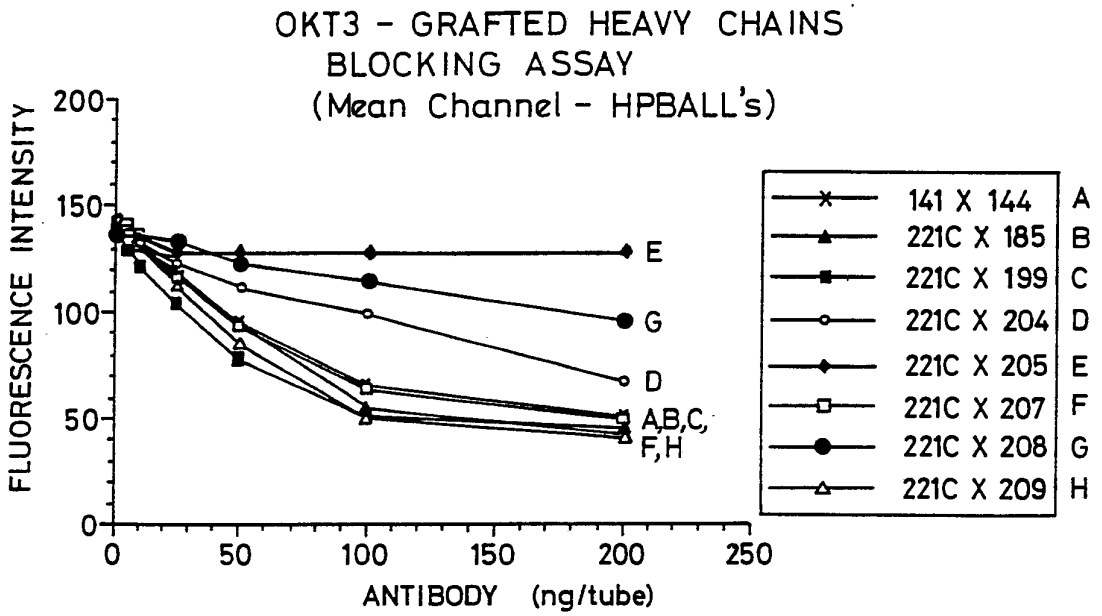

BLOCKING ASSAY
(Mean Channel - HPBALL's)
FLUORESCENCE INTENSITY
200
150
100
0
50
100
150
200
250
ANTIBODY (ng/tube)
C
B
D
A
E
A 221C X 185-1
B 221C X 197
C 221C X 183
D 221C X 184
E 221C X 185-2

Fig. 9

Fig.10

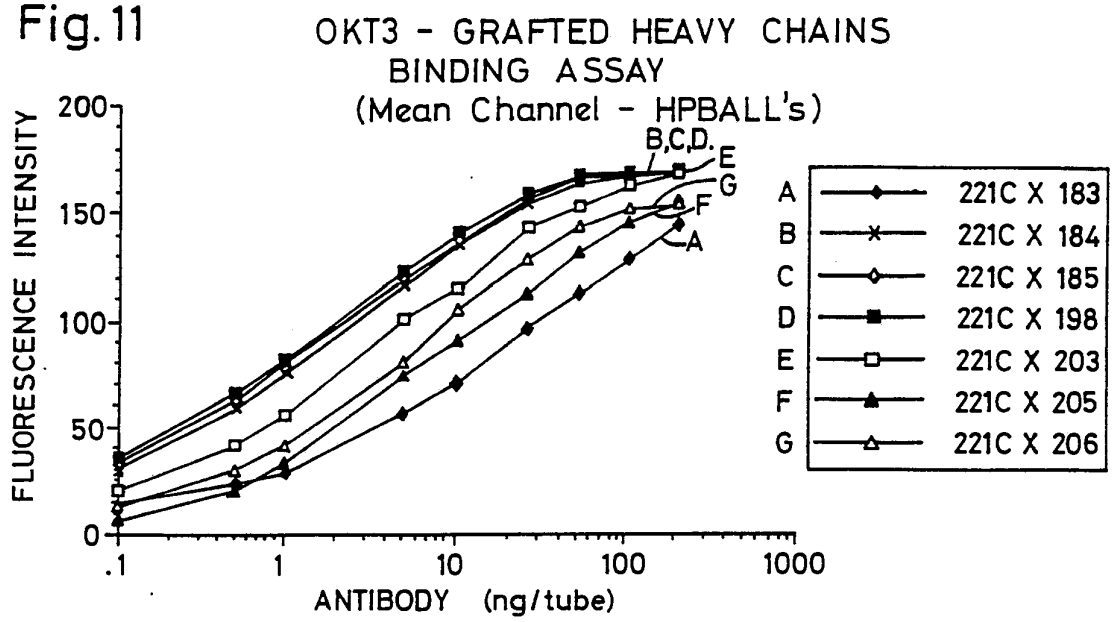


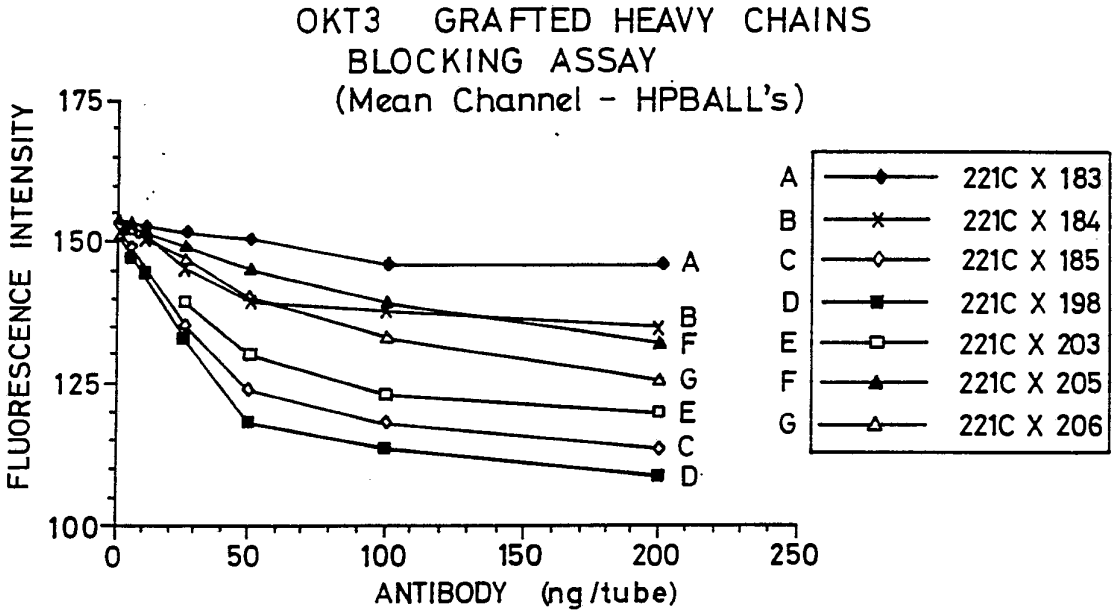


(205) _,__,24,48,49,71,73,76,78,88,91,
(208) 6,__,24,48,49,71,73,__,78,__,__,
(204) 6,__,24,48,49,71,73,76,78,__,__,
(199) 6,23,24,48,49,__,__,__,__,__,__,
(207) 6,23,24,48,49,71,73,__,78,__,__,
(185) 6,23,24,48,49,71,73,76,78,88,91,
(209) 6,23,24,48,49,__,__,__,78,__,__,
141 X 144

SUBSTITUTE SHEET

Fig. 11

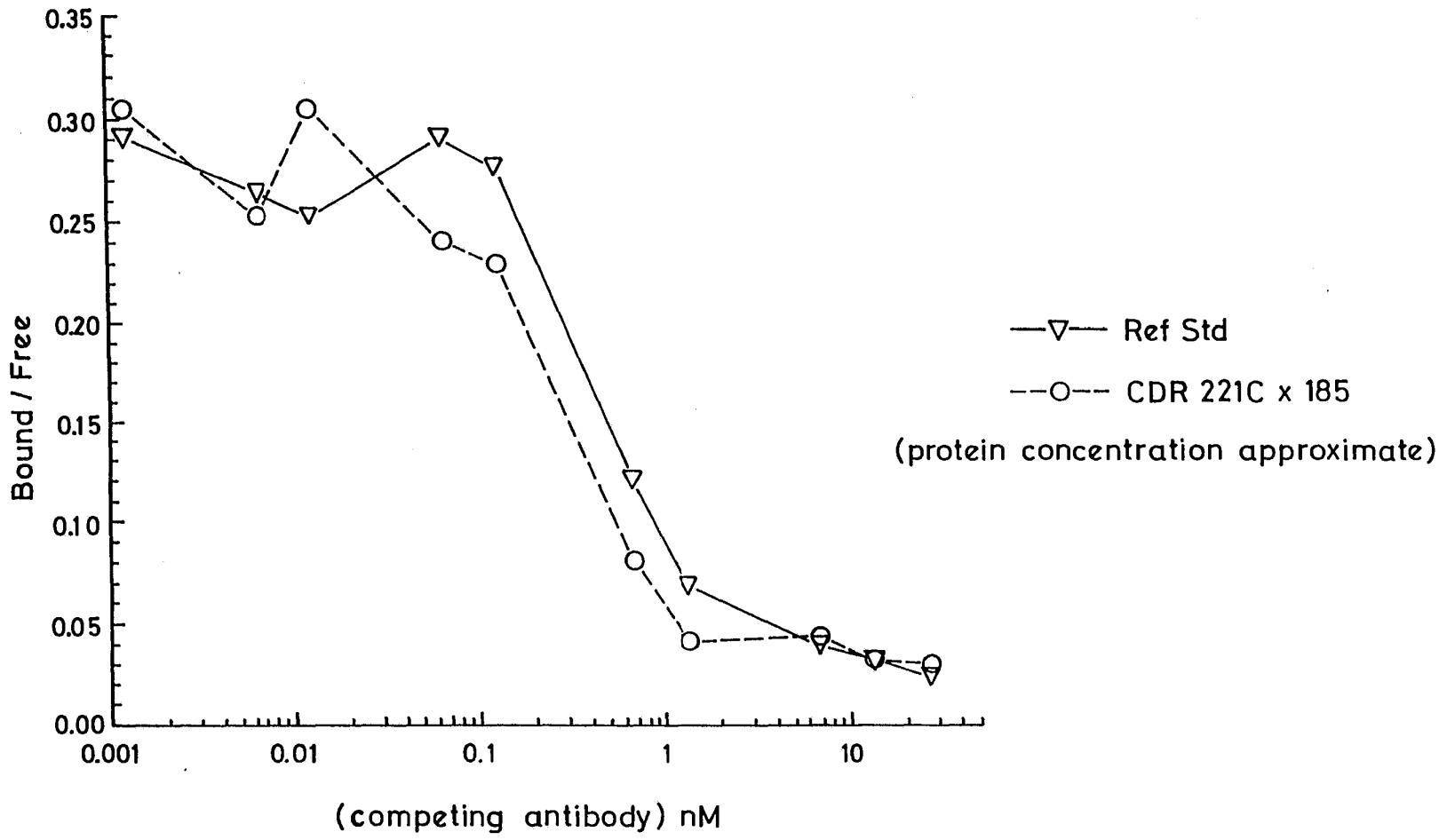




◆	(183)	_,__,__,48,49,71,73,76,78,88,91,
▲	(205)	_,__,24,48,49,71,73,76,78,88,91,
×	(184)	6,23,24,__,__,__,__,__,__,__,__,
△	(206)	_,__,24,48,49,71,73,76,78,__,__,
□	(203)	6,__,24,48,49,71,73,76,78,88,91,
◇	(185)	6,23,24,48,49,71,73,76,78,88,91,
■	(198)	6,23,24,48,49,71,73,76,78,__,__,

Fig. 12

Fig. 13



INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 90/02017

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: C 12 P 21/08, C 12 N 15/13, A 61 K 39/395, C 07 K 15/06
C 12 N 5/10, 15/62

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	C 12 P; C 12 N; A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
P,X	EP, A1, 0403156 (GENZYME CORPORATION ET AL.) 19 December 1990, see examples 8-12 and corresponding tables	1,6,8, 13,14-22
Y	Proc. Natl. Acad. Sci. USA, vol. 86, December 1989, C. Queen et al.: "A humanized antibody that binds to the interleukin 2 receptor ", see page 10029- page 10033 see the whole document and in particular page 10031 right col. - page 10032, left col. and page 10033 left col.	1,6,8, 13,14-22
Y	EP, A1, 0328404 (MEDICAL RESEARCH COUNCIL ET AL.) 16 August 1989, see pages 1-3, page 9, lines 49-54 and the claims	1,6,8, 13,14-22

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
11th April 1991	17.05.91
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	F.W. HECK

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	Nature, vol. 332, March 1988, L. Riechmann et al.: "Reshaping human antibodies for therapy ", see page 323 - page 327 see in particular page 327, right col.	1,6,8, 13,144-22
A	Nature, vol. 321, May 1986, P.T. Jones et al.: "Replacing the complementarity-determining regions in a human antibody with those from a mouse ", see page 522 - page 525 see the whole document	1-22
A	Nature, vol. 328, August 1987, S. Roberts et al.: "Generation of an antibody with enhanced affinity and specificity for its antigen by protein engineering ", see page 731 - page. 734 see the whole document	1,6
A	Science, vol. 239, 1988, M. Verhoeyen et al.: "Reshaping Human Antibodies: Grafting an Antilysozyme Activity ", see page 1534 - page 1536 see the whole document	1,6
A	EP, A2, 0239400 (WINTER, GREGORY PAUL) 30 September 1987, see the whole document	1,6,17-22
A	EP, A1, 0323806 (CIBA-GEIGY AG) 12 July 1989, see pages 2-6	1,6,17-22
A	Nature, vol. 341, October 1989, E.S. Ward et al.: "Binding activities of a repertoire of single immunoglobulin variable domains secreted from Escherichia coli ", see page 544 - page 546	1,6

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. PCT/GB 90/02017

SA 43080

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 28/02/91
The European Patent office is in no way liable for theseparticulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A1- 0403156	19/12/90	NONE		
EP-A1- 0328404	16/08/89	AU-D-	3062689	06/09/89
		GB-A-	2216126	04/10/89
		WO-A-	89/07452	24/08/89
EP-A2- 0239400	30/09/87	GB-A-B-	2188638	07/10/87
		JP-A-	62296890	24/12/87
EP-A1- 0323806	12/07/89	AU-D-	2759588	06/07/89
		JP-A-	2154696	14/06/90

For more details about this annex : see Official Journal of the European patent Office, No. 12/82

15
Int 1

HUMANISED ANTIBODIES

Field of the Invention

The present invention relates to humanised antibody molecules, to processes for their production using recombinant DNA technology, and to their therapeutic uses.

The term "humanised antibody molecule" is used to describe a molecule having an antigen binding site derived from an immunoglobulin from a non-human species, and remaining immunoglobulin-derived parts of the molecule being derived from a human immunoglobulin. The antigen binding site typically comprises complementarity determining regions (CDRs) which determine the binding specificity of the antibody molecule and which are carried on appropriate framework regions in the variable domains. There are 3 CDRs (CDR1, CDR2 and CDR3) in each of the heavy and light chain variable domains.

In the description, reference is made to a number of publications by number. The publications are listed in numerical order at the end of the description.

Background of the Invention

Natural immunoglobulins have been known for many years, as have the various fragments thereof, such as the Fab, $(Fab')_2$ and Fc fragments, which can be derived by enzymatic cleavage. Natural immunoglobulins comprise a generally Y-shaped molecule having an antigen-binding site towards the end of each upper arm. The remainder of the structure, and particularly the stem of the Y, mediates the effector functions associated with immunoglobulins.

Natural immunoglobulins have been used in assay, diagnosis and, to a more limited extent, therapy. However, such uses, especially in therapy, were hindered until recently by the polyclonal nature of natural immunoglobulins. A significant step towards the realisation of the potential

2

of immunoglobulins as therapeutic agents was the discovery of procedures for the production of monoclonal antibodies (MAbs) of defined specificity (1).

However, most MAbs are produced by hybridomas which are fusions of rodent spleen cells with rodent myeloma cells. They are therefore essentially rodent proteins. There are very few reports of the production of human MAbs.

Since most available MAbs are of rodent origin, they are naturally antigenic in humans and thus can give rise to an undesirable immune response termed the HAMA (Human Anti-Mouse Antibody) response. Therefore, the use of rodent MAbs as therapeutic agents in humans is inherently limited by the fact that the human subject will mount an immunological response to the MAb and will either remove it entirely or at least reduce its effectiveness. In practice, MAbs of rodent origin may not be used in patients for more than one or a few treatments as a HAMA response soon develops rendering the MAb ineffective as well as giving rise to undesirable reactions. For instance, OKT3 a mouse IgG2a/k MAb which recognises an antigen in the T-cell receptor-CD3 complex has been approved for use in many countries throughout the world as an immunosuppressant in the treatment of acute allograft rejection [Chatenoud et al (2) and Jeffers et al (3)]. However, in view of the rodent nature of this and other such MAbs, a significant HAMA response which may include a major anti-idiotype component, may build up on use. Clearly, it would be highly desirable to diminish or abolish this undesirable HAMA response and thus enlarge the areas of use of these very useful antibodies.

Proposals have therefore been made to render non-human MAbs less antigenic in humans. Such techniques can be generically termed "humanisation" techniques. These

techniques typically involve the use of recombinant DNA technology to manipulate DNA sequences encoding the polypeptide chains of the antibody molecule.

Early methods for humanising MAbs involved production of chimeric antibodies in which an antigen binding site comprising the complete variable domains of one antibody is linked to constant domains derived from another antibody. Methods for carrying out such chimerisation procedures are described in EP0120694 (Celltech Limited), EP0125023 (Genentech Inc. and City of Hope), EP-A-0 171496 (Res. Dev. Corp. Japan), EP-A-0 173 494 (Stanford University), and WO 86/01533 (Celltech Limited). This latter Celltech application (WO 86/01533) discloses a process for preparing an antibody molecule having the variable domains from a mouse MAb and the constant domains from a human immunoglobulin. Such humanised chimeric antibodies, however, still contain a significant proportion of non-human amino acid sequence, i.e. the complete non-human variable domains, and thus may still elicit some HAMA response, particularly if administered over a prolonged period [Begent et al (ref. 4)].

In an alternative approach, described in EP-A-0239400 (Winter), the complementarity determining regions (CDRs) of a mouse MAb have been grafted onto the framework regions of the variable domains of a human immunoglobulin by site directed mutagenesis using long oligonucleotides. The present invention relates to humanised antibody molecules prepared according to this alternative approach, i.e. CDR-grafted humanised antibody molecules. Such CDR-grafted humanised antibodies are much less likely to give rise to a HAMA response than humanised chimeric antibodies in view of the much lower proportion of non-human amino acid sequence which they contain.

The earliest work on humanising MAbs by CDR-grafting was carried out on MAbs recognising synthetic antigens, such as the NP or NIP antigens. However, examples in which a mouse MAb recognising lysozyme and a rat MAb recognising an antigen on human T-cells were humanised by CDR-grafting have been described by Verhoeyen et al (5) and Riechmann et al (6) respectively. The preparation of CDR-grafted antibody to the antigen on human T cells is also described in WO 89/07452 (Medical Research Council).

In Riechmann et al/Medical Research Council it was found that transfer of the CDR regions alone [as defined by Kabat refs. (7) and (8)] was not sufficient to provide satisfactory antigen binding activity in the CDR-grafted product. Riechmann et al found that it was necessary to convert a serine residue at position 27 of the human sequence to the corresponding rat phenylalanine residue to obtain a CDR-grafted product having improved antigen binding activity. This residue at position 27 of the heavy chain is within the structural loop adjacent to CDR1. A further construct which additionally contained a human serine to rat tyrosine change at position 30 of the heavy chain did not have a significantly altered binding activity over the humanised antibody with the serine to phenylalanine change at position 27 alone. These results indicate that changes to residues of the human sequence outside the CDR regions, in particular in the structural loop adjacent to CDR1, may be necessary to obtain effective antigen binding activity for CDR-grafted antibodies which recognise more complex antigens. Even so the binding affinity of the best CDR-grafted antibodies obtained was still significantly less than the original MAb.

Very recently Queen et al (9) have described the preparation of a humanised antibody that binds to the

interleukin 2 receptor, by combining the CDRs of a murine MAb (anti-Tac) with human immunoglobulin framework and constant regions. The human framework regions were chosen to maximise homology with the anti-Tac MAb sequence. In addition computer modelling was used to identify framework amino acid residues which were likely to interact with the CDRs or antigen, and mouse amino acids were used at these positions in the humanised antibody.

In WO 90/07861 Queen et al propose four criteria for designing humanised immunoglobulins. The first criterion is to use as the human acceptor the framework from a particular human immunoglobulin that is unusually homologous to the non-human donor immunoglobulin to be humanised, or to use a consensus framework from many human antibodies. The second criterion is to use the donor amino acid rather than the acceptor if the human acceptor residue is unusual and the donor residue is typical for human sequences at a specific residue of the framework. The third criterion is to use the donor framework amino acid residue rather than the acceptor at positions immediately adjacent to the CDRs. The fourth criterion is to use the donor amino acid residue at framework positions at which the amino acid is predicted to have a side chain atom within about 3 Å of the CDRs in a three-dimensional immunoglobulin model and to be capable of interacting with the antigen or with the CDRs of the humanised immunoglobulin. It is proposed that criteria two, three or four may be applied in addition or alternatively to criterion one, and may be applied singly or in any combination.

WO 90/07861 describes in detail the preparation of a single CDR-grafted humanised antibody, a humanised antibody having specificity for the p55 Tac protein of the

IL-2 receptor. The combination of all four criteria, as above, were employed in designing this humanised antibody, the variable region frameworks of the human antibody Eu (7) being used as acceptor. In the resultant humanised antibody the donor CDRs were as defined by Kabat et al (7 and 8) and in addition the mouse donor residues were used in place of the human acceptor residues, at positions 27, 30, 48, 66, 67, 89, 91, 94, 103, 104, 105 and 107 in the heavy chain (SEQ ID NO:31) and at positions 48, 60 and 63 in the light chain, of the variable region frameworks. The humanised anti-Tac antibody obtained is reported to have an affinity for p55 of 3 x 10^9 M^{-1}, about one-third of that of the murine MAb.

We have further investigated the preparation of CDR-grafted humanised antibody molecules and have identified a hierarchy of positions within the framework of the variable regions (i.e. outside both the Kabat CDRs and structural loops of the variable regions) at which the amino acid identities of the residues are important for obtaining CDR-grafted products with satisfactory binding affinity. This has enabled us to establish a protocol for obtaining satisfactory CDR-grafted products which may be applied very widely irrespective of the level of homology between the donor immunoglobulin and acceptor framework. The set of residues which we have identified as being of critical importance does not coincide with the residues identified by Queen et al (9).

Summary of the Invention

Accordingly, in a first aspect the invention provides a CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

In preferred embodiments, the heavy chain framework comprises donor residues at positions 23, 24, 49, 71, 73 and 78 or at positions 23, 24 and 49. The residues at positions 71, 73 and 78 of the heavy chain framework are preferably either all acceptor or all donor residues.

In particularly preferred embodiments the heavy chain framework additionally comprises donor residues at one, some or all of positions 6, 37, 48 and 94. Also it is particularly preferred that residues at positions of the heavy chain framework which are commonly conserved across species, i.e. positions 2, 4, 25, 36, 39, 47, 93, 103, 104, 106 and 107, if not conserved between donor and acceptor, additionally comprise donor residues. Most preferably the heavy chain framework additionally comprises donor residues at positions 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

In addition the heavy chain framework optionally comprises donor residues at one, some or all of positions:
1 and 3,
72 and 76,
69 (if 48 is different between donor and acceptor),
38 and 46 (if 48 is the donor residue),
80 and 20 (if 69 is the donor residue),
67,
82 and 18 (if 67 is the donor residue),
91,
88, and
any one or more of 9, 11, 41, 87, 108, 110 and 112.

In the first and other aspects of the present invention reference is made to CDR-grafted antibody products comprising acceptor framework and donor antigen binding regions. It will be appreciated that the invention is widely applicable to the CDR-grafting of antibodies in

general. Thus, the donor and acceptor antibodies may be derived from animals of the same species and even same antibody class or sub-class. More usually, however, the donor and acceptor antibodies are derived from animals of different species. Typically the donor antibody is a non-human antibody, such as a rodent MAb, and the acceptor antibody is a human antibody.

In the first and other aspects of the present invention, the donor antigen binding region typically comprises at least one CDR from the donor antibody. Usually the donor antigen binding region comprises at least two and preferably all three CDRs of each of the heavy chain and/or light chain variable regions. The CDRs may comprise the Kabat CDRs, the structural loop CDRs or a composite of the Kabat and structural loop CDRs and any combination of any of these. Preferably, the antigen binding regions of the CDR-grafted heavy chain variable domain comprise CDRs corresponding to the Kabat CDRs at CDR2 (residues 50-65) and CDR3 (residues 95-100) and a composite of the Kabat and structural loop CDRs at CDR1 (residues 26-35).

The residue designations given above and elsewhere in the present application are numbered according to the Kabat numbering [refs. (7) and (8)]. Thus the residue designations do not always correspond directly with the linear numbering of the amino acid residues. The actual linear amino acid sequence may contain fewer or additional amino acids than in the strict Kabat numbering corresponding to a shortening of, or insertion into, a structural component, whether framework or CDR, of the basic variable domain structure. For example, the heavy chain variable region of the anti-Tac antibody described by Queen et al (9) contains a single amino acid insert (residue 52a) after residue 52 of CDR2 and a three amino

acid insert (residues 82a, 82b and 82c) after framework residue 82, in the Kabat numbering. The correct Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

The invention also provides in a second aspect a CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 1 and/or 3 and 46 and/or 47. Preferably the CDR grafted light chain of the second aspect comprises donor residues at positions 46 and/or 47.

The invention also provides in a third aspect a CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, 58 and 71.

In a preferred embodiment of the third aspect, the framework comprises donor residues at all of positions 46, 48, 58 and 71.

In particularly preferred embodiments of the second and third aspects, the framework additionally comprises donor residues at positions 36, 44, 47, 85 and 87. Similarly positions of the light chain framework which are commonly conserved across species, i.e. positions 2, 4, 6, 35, 49, 62, 64-69, 98, 99, 101 and 102, if not conserved between donor and acceptor, additionally comprise donor residues. Most preferably the light chain framework additionally comprises donor residues at positions 2, 4, 6, 35, 36, 38, 44, 47, 49, 62, 64-69, 85, 87, 98, 99, 101 and 102.

In addition the framework of the second or third aspects optionally comprises donor residues at one, some or all of positions:
1 and 3,
63,
60 (if 60 and 54 are able to form at potential saltbridge),
70 (if 70 and 24 are able to form a potential saltbridge),
73 and 21 (if 47 is different between donor and acceptor),
37 and 45 (if 47 is different between donor and acceptor),
and
any one or more of 10, 12, 40, 80, 103 and 105.

Preferably, the antigen binding regions of the CDR-grafted light chain variable domain comprise CDRs corresponding to the Kabat CDRs at CDR1 (residue 24-34), CDR2 (residues 50-56) and CDR3 (residues 89-97).

The invention further provides in a fourth aspect a CDR-grafted antibody molecule comprising at least one CDR-grafted heavy chain and at least one CDR-grafted light chain according to the first and second or first and third aspects of the invention.

The humanised antibody molecules and chains of the present invention may comprise: a complete antibody molecule, having full length heavy and light chains; a fragment thereof, such as a Fab, $(Fab')_2$ or FV fragment; a light chain or heavy chain monomer or dimer; or a single chain antibody, e.g. a single chain FV in which heavy and light chain variable regions are joined by a peptide linker; or any other CDR-grafted molecule with the same specificity as the original donor antibody. Similarly the CDR-grafted heavy and light chain variable region may be combined with other antibody domains as appropriate.

Also the heavy or light chains or humanised antibody molecules of the present invention may have attached to them an effector or reporter molecule. For instance, it may have a macrocycle, for chelating a heavy metal atom, or a toxin, such as ricin, attached to it by a covalent bridging structure. Alternatively, the procedures of recombinant DNA technology may be used to produce an immunoglobulin molecule in which the Fc fragment or CH3 domain of a complete immunoglobulin molecule has been replaced by, or has attached thereto by peptide linkage, a functional non-immunoglobulin protein, such as an enzyme or toxin molecule.

Any appropriate acceptor variable region framework sequences may be used having regard to class/type of the donor antibody from which the antigen binding regions are derived. Preferably, the type of acceptor framework used is of the same/similar class/type as the donor antibody. Conveniently, the framework may be chosen to maximise/optimise homology with the donor antibody sequence particularly at positions close or adjacent to the CDRs. However, a high level of homology between donor and acceptor sequences is not important for application of the present invention. The present invention identifies a hierarchy of framework residue positions at which donor residues may be important or desirable for obtaining a CDR-grafted antibody product having satisfactory binding properties. The CDR-grafted products usually have binding affinities of at least 10^5 M^{-1}, preferably at least about 10^8 M^{-1}, or especially in the range 10^8-10^{12} M^{-1}. In principle, the present invention is applicable to any combination of donor and acceptor antibodies irrespective of the level of homology between their sequences. A protocol for applying the invention to any particular donor-acceptor antibody pair is given hereinafter. Examples of human frameworks which may be

used are KOL, NEWM, REI, EU, LAY and POM (refs. 4 and 5) and the like; for instance KOL and NEWM for the heavy chain and REI for the light chain and EU, LAY and POM for both the heavy chain and the light chain.

Also the constant region domains of the products of the invention may be selected having regard to the proposed function of the antibody in particular the effector functions which may be required. For example, the constant region domains may be human IgA, IgE, IgG or IgM domains. In particular, IgG human constant region domains may be used, especially of the IgG1 and IgG3 isotypes, when the humanised antibody molecule is intended for therapeutic uses, and antibody effector functions are required. Alternatively, IgG2 and IgG4 isotypes may be used when the humanised antibody molecule is intended for therapeutic purposes and antibody effector functions are not required, e.g. for simple blocking of lymphokine activity.

However, the remainder of the antibody molecules need not comprise only protein sequences from immunoglobulins. For instance, a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain is fused to a DNA sequence encoding the amino acid sequence of a functional polypeptide such as an effector or reporter molecule.

Preferably the CDR-grafted antibody heavy and light chain and antibody molecule products are produced by recombinant DNA technology.

Thus in further aspects the invention also includes DNA sequences coding for the CDR-grafted heavy and light chains, cloning and expression vectors containing the DNA sequences, host cells transformed with the DNA sequences

and processes for producing the CDR-grafted chains and antibody molecules comprising expressing the DNA sequences in the transformed host cells.

The general methods by which the vectors may be constructed, transfection methods and culture methods are well known per se and form no part of the invention. Such methods are shown, for instance, in references 10 and 11.

The DNA sequences which encode the donor amino acid sequence may be obtained by methods well known in the art. For example the donor coding sequences may be obtained by genomic cloning, or cDNA cloning from suitable hybridoma cell lines. Positive clones may be screened using appropriate probes for the heavy and light chain genes in question. Also PCR cloning may be used.

DNA coding for acceptor, e.g. human acceptor, sequences may be obtained in any appropriate way. For example DNA sequences coding for preferred human acceptor frameworks such as KOL, REI, EU and NEWM, are widely available to workers in the art.

The standard techniques of molecular biology may be used to prepare DNA sequences coding for the CDR-grafted products. Desired DNA sequences may be synthesised completely or in part using oligonucleotide synthesis techniques. Site-directed mutagenesis and polymerase chain reaction (PCR) techniques may be used as appropriate. For example oligonucleotide directed synthesis as described by Jones et al (ref. 20) may be used. Also oligonucleotide directed mutagenesis of a pre-exising variable region as, for example, described by Verhoeyen et al (ref. 5) or Riechmann et al (ref. 6) may be used. Also enzymatic filling in of gapped

oligonucleotides using T_4 DNA polymerase as, for example, described by Queen et al (ref. 9) may be used.

Any suitable host cell/vector system may be used for expression of the DNA sequences coding for the CDR-grafted heavy and light chains. Bacterial e.g. E. coli, and other microbial systems may be used, in particular for expression of antibody fragments such as FAb and $(Fab')_2$ fragments, and especially FV fragments and single chain antibody fragments e.g. single chain FVs. Eucaryotic e.g. mammalian host cell expression systems may be used for production of larger CDR-grafted antibody products, including complete antibody molecules. Suitable mammalian host cells include CHO cells and myeloma or hybridoma cell lines.

Thus, in a further aspect the present invention provides a process for producing a CDR-grafted antibody product comprising:

(a) producing in an expression vector an operon having a DNA sequence which encodes an antibody heavy chain according to the first aspect of the invention;

and/or

(b) producing in an expression vector an operon having a DNA sequence which encodes a complementary antibody light chain according to the second or third aspect of the invention;

(c) transfecting a host cell with the or each vector; and

(d) culturing the transfected cell line to produce the CDR-grafted antibody product.

The CDR-grafted product may comprise only heavy or light chain derived polypeptide, in which case only a heavy chain or light chain polypeptide coding sequence is used to transfect the host cells.
For production of products comprising both heavy and light chains, the cell line may be transfected with two vectors, the first vector may contain an operon encoding a light chain-derived polypeptide and the second vector containing an operon encoding a heavy chain-derived polypeptide. Preferably, the vectors are identical, except in so far as the coding sequences and selectable markers are concerned, so as to ensure as far as possible that each polypeptide chain is equally expressed. Alternatively, a single vector may be used, the vector including the sequences encoding both light chain- and heavy chain-derived polypeptides.

The DNA in the coding sequences for the light and heavy chains may comprise cDNA or genomic DNA or both. However, it is preferred that the DNA sequence encoding the heavy or light chain comprises at least partially, genomic DNA, preferably a fusion of cDNA and genomic DNA.

The present invention is applicable to antibodies of any appropriate specificity. Advantageously, however, the invention may be applied to the humanisation of non-human antibodies which are used for in vivo therapy or diagnosis. Thus the antibodies may be site-specific antibodies such as tumour-specific or cell surface-specific antibodies, suitable for use in in vivo therapy or diagnosis, e.g. tumour imaging. Examples of cell surface-specific antibodies are anti-T cell antibodies, such as anti-CD3, and CD4 and adhesion molecules, such as CR3, ICAM and ELAM. The antibodies may have specificity for interleukins (including lymphokines, growth factors and stimulating factors), hormones and other biologically active compounds, and receptors for any of these. For

example, the antibodies may have specificity for any of the following: Interferons α, β, γ or δ, IL1, IL2, IL3, or IL4, etc., TNF, GCSF, GMCSF, EPO, hGH, or insulin, etc.

The the present invention also includes therapeutic and diagnostic compositions comprising the CDR-grafted products of the invention and uses of such compositions in therapy and diagnosis.

Accordingly in a further aspect the invention provides a therapeutic or diagnostic composition comprising a CDR-grafted antibody heavy or light chain or molecule according to previous aspects of the invention in combination with a pharmaceutically acceptable carrier, diluent or excipient.

Accordingly also the invention provides a method of therapy or diagnosis comprising administering an effective amount of a CDR-grafted antibody heavy or light chain or molecule according to previous aspects of the invention to a human or animal subject.

A preferred protocol for obtaining CDR-grafted antibody heavy and light chains in accordance with the present invention is set out below together with the rationale by which we have derived this protocol. This protocol and rationale are given without prejudice to the generality of the invention as hereinbefore described and defined.

Protocol

It is first of all necessary to sequence the DNA coding for the heavy and light chain variable regions of the donor antibody, to determine their amino acid sequences. It is also necessary to choose appropriate acceptor heavy and light chain variable regions, of known amino acid sequence. The CDR-grafted chain is then designed

starting from the basis of the acceptor sequence. It will be appreciated that in some cases the donor and acceptor amino acid residues may be identical at a particular position and thus no change of acceptor framework residue is required.

1. As a first step donor residues are substituted for acceptor residues in the CDRs. For this purpose the CDRs are preferably defined as follows:

Heavy chain	-	CDR1:	residues 26-35
	-	CDR2:	residues 50-65
	-	CDR3:	residues 95-102
Light chain	-	CDR1:	residues 24-34
	-	CDR2:	residues 50-56
	-	CDR3:	residues 89-97

The positions at which donor residues are to be substituted for acceptor in the framework are then chosen as follows, first of all with respect to the heavy chain and subsequently with respect to the light chain.

2. Heavy Chain

2.1 Choose donor residues at all of positions 23, 24, 49, 71, 73 and 78 of the heavy chain or all of positions 23, 24 and 49 (71, 73 and 78 are always either all donor or all acceptor).

2.2 Check that the following have the same amino acid in donor and acceptor sequences, and if not preferably choose the donor: 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

2.3 To further optimise affinity consider choosing donor residues at one, some or any of:

i. 1, 3
ii. 72, 76
iii. If 48 is different between donor and acceptor sequences, consider 69
iv. If at 48 the donor residue is chosen, consider 38 and 46
v. If at 69 the donor residue is chosen, consider 80 and then 20
vi. 67
vii. If at 67 the donor residue is chosen, consider 82 and then 18
viii. 91
ix. 88
x. 9, 11, 41, 87, 108, 110, 112

3. Light Chain

3.1 Choose donor at 46, 48, 58 and 71

3.2 Check that the following have the same amino acid in donor and acceptor sequences, if not preferably choose donor:

2, 4, 6, 35, 38, 44, 47, 49, 62, 64-69 inclusive, 85, 87, 98, 99, 101 and 102

3.3 To further optimise affinity consider choosing donor residues at one, some or any of:

i. 1, 3
ii. 63

iii. 60, if 60 and 54 are able to form potential saltbridge
iv. 70, if 70 and 24 are able to form potential saltbridge
v. 73, and 21 if 47 is different between donor and acceptor
vi. 37, and 45 if 47 is different between donor and acceptor
vii. 10, 12, 40, 80, 103, 105

Rationale

In order to transfer the binding site of an antibody into a different acceptor framework, a number of factors need to be considered.

1. The extent of the CDRs

The CDRs (Complementary Determining Regions) were defined by Wu and Kabat (refs. 4 and 5) on the basis of an analysis of the variability of different regions of antibody variable regions. Three regions per domain were recognised. In the light chain the sequences are 24-34, 50-56, 89-97 (numbering according to Kabat (ref. 4), Eu Index) inclusive and in the heavy chain the sequences are 31-35, 50-65 and 95-102 inclusive.

When antibody structures became available it became apparent that these CDR regions corresponded in the main to loop regions which extended from the β barrel framework of the light and heavy variable domains. For H1 there was a discrepancy in that the loop was from 26 to 32 inclusive and for H2 the loop was 52 to 56 and for L2 from 50 to 53. However, with the exception of H1 the CDR regions encompassed the loop regions and extended into the β strand

frameworks. In H1 residue 26 tends to be a serine and 27 a phenylalanine or tyrosine, residue 29 is a phenylalanine in most cases. Residues 28 and 30 which are surface residues exposed to solvent might be involved in antigen-binding. A prudent definition of the H1 CDR therefore would include residues 26-35 to include both the loop region and the hypervariable residues 33-35.

It is of interest to note the example of Riechmann et al (ref. 3), who used the residue 31-35 choice for CDR-H1. In order to produce efficient antigen binding, residue 27 also needed to be recruited from the donor (rat) antibody.

2. Non-CDR residues which contribute to antigen binding

By examination of available X-ray structures we have identified a number of residues which may have an effect on net antigen binding and which can be demonstrated by experiment. These residues can be sub-divided into a number of groups.

2.1 Surface residues near CDR [all numbering as in Kabat et al (ref. 7)].

2.1.1. Heavy Chain - Key residues are 23, 71 and 73. Other residues which may contribute to a lesser extent are 1, 3 and 76. Finally 25 is usually conserved but the murine residue should be used if there is a difference.

2.1.2 Light Chain - Many residues close to the CDRs, e.g. 63, 65, 67 and 69 are conserved. If conserved none of the surface residues in the light chain are likely to have a major effect. However, if the murine residue at these positions

is unusual, then it would be of benefit to analyse the likely contribution more closely. Other residues which may also contribute to binding are 1 and 3, and also 60 and 70 if the residues at these positions and at 54 and 24 respectively are potentially able to form a salt bridge i.e. 60 + 54; 70 + 24.

2.2 Packing residues near the CDRs.

2.2.1. Heavy Chain - Key residues are 24, 49 and 78. Other key residues would be 36 if not a tryptophan, 94 if not an arginine, 104 and 106 if not glycines and 107 if not a threonine. Residues which may make a further contribution to stable packing of the heavy chain and hence improved affinity are 2, 4, 6, 38, 46, 67 and 69. 67 packs against the CDR residue 63 and this pair could be either both mouse or both human. Finally, residues which contribute to packing in this region but from a longer range are 18, 20, 80, 82 and 86. 82 packs against 67 and in turn 18 packs against 82. 80 packs against 69 and in turn 20 packs against 80. 86 forms an H bond network with 38 and 46. Many of the mouse-human differences appear minor e.g. Leu-Ile, but could have an minor impact on correct packing which could translate into altered positioning of the CDRs.

2.2.2. Light Chain - Key residues are 48, 58 and 71. Other key residues would be 6 if not glutamine, 35 if not tryptophan, 62 if not phenylalanine or tryosine, 64, 66, 68, 99 and 101 if not glycines and 102 if not a threonine. Residues which make a further contribution are 2, 4, 37, 45 and 47. Finally residues 73 and 21 and 19 may make long distance packing contributions of a minor nature.

2.3. Residues at the variable domain interface between heavy and light chains - In both the light and heavy chains most of the non-CDR interface residues are conserved. If a conserved residue is replaced by a residue of different character, e.g. size or charge, it should be considered for retention as the murine residue.

2.3.1. Heavy Chain - Residues which need to be considered are 37 if the residue is not a valine but is of larger side chain volume or has a charge or polarity. Other residues are 39 if not a glutamine, 45 if not a leucine, 47 if not a tryptophan, 91 if not a phenylalanine or tyrosine, 93 if not an alanine and 103 if not a tryptophan. Residue 89 is also at the interface but is not in a position where the side chain could be of great impact.

2.3.2. Light Chain - Residues which need to be considered are 36, if not a tyrosine, 38 if not a glutamine, 44 if not a proline, 46, 49 if not a tyrosine, residue 85, residue 87 if not a tyrosine and 98 if not a phenylalanine.

2.4. Variable-Constant region interface - The elbow angle between variable and constant regions may be affected by alterations in packing of key residues in the variable region against the constant region which may affect the position of V_L and V_H with respect to one another. Therefore it is worth noting the residues likely to be in contact with the constant region. In the heavy chain the surface residues potentially in contact with the variable region are conserved between mouse and human antibodies therefore the variable region contact residues may influence the V-C interaction. In the light chain the amino acids found at a number of the constant

region contact points vary, and the V & C regions are not in such close proximity as the heavy chain. Therefore the influences of the light chain V-C interface may be minor.

2.4.1. Heavy Chain - Contact residues are 7, 11, 41, 87, 108, 110, 112.

2.4.2. Light Chain - In the light chain potentially contacting residues are 10, 12, 40, 80, 83, 103 and 105.

The above analysis coupled with our considerable practical experimental experience in the CDR-grafting of a number of different antibodies have lead us to the protocol given above.

The present invention is now described, by way of example only, with reference to the accompanying Figures 1 - 13.

Brief Description of the Figures

Figure 1 shows DNA and amino acid sequences of the OKT3 light chain;

Figure 2 shows DNA and amino acid sequences of the OKT3 heavy chain;

Figure 3 shows the alignment of the OKT3 light variable region amino acid sequence with that of the light variable region of the human antibody REI;

Figure 4 shows the alignment of the OKT3 heavy variable region amino acid sequence with that of the heavy variable region of the human antibody KOL;

Figure 5 shows the heavy variable region amino acid sequences of OKT3, KOL and various corresponding CDR grafts;

Figure 6 shows the light variable region amino acid sequences of OKT3, REI and various corresponding CDR grafts;

Figure 7 shows a graph of binding assay results for various grafted OKT3 antibodies'

Figure 8 shows a graph of blocking assay results for various grafted OKT3 antibodies;

Figure 9 shows a similar graph of blocking assay results;

Figure 10a and b shows similar graphs for both binding assay and blocking assay results;

Figure 11a and b shows further similar graphs for both binding assay and blocking assay results;

Figure 12 shows a graph of competition assay results for a minimally grafted OKT3 antibody compared with the OKT3 murine reference standard, and

Figure 13 shows a similar graph of competition assay results comparing a fully grafted OKT3 antibody with the murine reference standard.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

EXAMPLE 1

CDR-GRAFTING OF OKT3

MATERIAL AND METHODS

1. INCOMING CELLS

Hybridoma cells producing antibody OKT3 were provided by Ortho (seedlot 4882.1) and were grown up in antibiotic free Dulbecco's Modified Eagles Medium (DMEM) supplemented with glutamine and 5% foetal calf serum, and divided to provide both an overgrown supernatant for evaluation and cells for extraction of RNA. The overgrown supernatant was shown to contain 250 ug/mL murine IgG2a/kappa antibody. The supernatant was negative for murine lambda light chain and IgG1, IgG2b, IgG3, IgA and IgM heavy chain. 20mL of supernatant was assayed to confirm that the antibody present was OKT3.

2. MOLECULAR BIOLOGY PROCEDURES

Basic molecular biology procedures were as described in Maniatis et al (ref. 9) with, in some cases, minor modifications. DNA sequencing was performed as described in Sanger et al (ref. 11) and the Amersham International Plc sequencing handbook. Site directed mutagenesis was as described in Kramer et al (ref. 12) and the Anglian Biotechnology Ltd. handbook. COS cell expression and metabolic labelling studies were as described in Whittle et al (ref. 13)

3. RESEARCH ASSAYS

3.1. ASSEMBLY ASSAYS

Assembly assays were performed on supernatants from transfected COS cells to determine the amount of intact IgG present.

3.1.1. COS CELLS TRANSFECTED WITH MOUSE OKT3 GENES

The assembly assay for intact mouse IgG in COS cell supernatants was an ELISA with the following format:

96 well microtitre plates were coated with F(ab')2 goat anti-mouse IgG Fc. The plates were washed in water and samples added for 1 hour at room temperature. The plates were washed and F(ab')2 goat anti-mouse IgG F(ab')2 (HRPO conjugated) was then added. Substrate was added to reveal the reaction. UPC10, a mouse IgG2a myeloma, was used as a standard.

3.1.2. COS AND CHO CELLS TRANSFECTED WITH CHIMERIC OR CDR-GRAFTED OKT3 GENES

The assembly assay for chimeric or CDR-grafted antibody in COS cell supernatants was an ELISA with the following format:

96 well microtitre plates were coated with F(ab')2 goat anti-human IgG Fc. The plates were washed and samples added and incubated for 1 hour at room temperature. The plates were washed and monoclonal mouse anti-human kappa chain was added for 1 hour at room temperature.

The plates were washed and F(ab')2 goat anti-mouse IgG Fc (HRPO conjugated) was added. Enzyme substrate was added to reveal the reaction. Chimeric B72.3 (IgG4) (ref. 13) was used as a standard. The use of a monoclonal anti-kappa chain in this assay allows grafted antibodies to be read from the chimeric standard.

3.2. ASSAY FOR ANTIGEN BINDING ACTIVITY

Material from COS cell supernatants was assayed for OKT3 antigen binding activity onto CD3 positive cells in a direct assay. The procedure was as follows:

HUT 78 cells (human T cell line, CD3 positive) were maintained in culture. Monolayers of HUT 78 cells were prepared onto 96 well ELISA plates using poly-L-lysine and glutaraldehyde. Samples were added to the monolayers for 1 hour at room temperature.

The plates were washed gently using PBS. F(ab')2 goat anti-human IgG Fc (HRPO conjugated) or F(ab')2 goat anti-mouse IgG Fc (HRPO conjugated) was added as appropriate for humanised or mouse samples. Substrate was added to reveal the reaction.

The negative control for the cell-based assay was chimeric B72.3. The positive control was mouse Orthomune OKT3 or chimeric OKT3, when available. This cell-based assay was difficult to perform, and an alternative assay was developed for CDR-grafted OKT3 which was more sensitive and easier to carry out.

In this system CDR-grafted OKT3 produced by COS cells was tested for its ability to bind to the CD3-positive HPB-ALL (human peripheral blood acute lymphocytic leukemia) cell line. It was also tested for its ability to block the binding of murine OKT3 to these cells. Binding was measured by the following procedure: HPB-ALL cells were harvested from tissue culture. Cells were incubated at 4°C for 1 hour with various dilutions of test antibody, positive control antibody, or negative control antibody. The cells were washed once and incubated at 4°C for 1 hour with an FITC-labelled goat anti-human IgG (Fc-

specific, mouse absorbed). The cells were washed twice and analysed by cytofluorography. Chimeric OKT3 was used as a positive control for direct binding. Cells incubated with mock- transfected COS cell supernatant, followed by the FITC-labelled goat anti-human IgG, provided the negative control. To test the ability of CDR-grafted OKT3 to block murine OKT3 binding, the HPB-ALL cells were incubated at 4°C for 1 hour with various dilutions of test antibody or control antibody. A fixed saturating amount of FITC OKT3 was added. The samples were incubated for 1 hour at 4°C, washed twice and analysed by cytofluorography. FITC-labelled OKT3 was used as a positive control to determine maximum binding. Unlabelled murine OKT3 served as a reference standard for blocking. Negative controls were unstained cells with or without mock-transfected cell supernatant. The ability of the CDR-grafted OKT3 light chain to bind CD3-positive cells and block the binding of murine OKT3 was initially tested in combination with the chimeric OKT3 heavy chain. The chimeric OKT3 heavy chain is composed of the murine OKT3 variable region and the human IgG4 constant region. The chimeric heavy chain gene is expressed in the same expression vector used for the CDR-grafted genes. The CDR-grafted light chain expression vector and the chimeric heavy chain expression vector were co-transfected into COS cells. The fully chimeric OKT3 antibody (chimeric light chain and chimeric heavy chain) was found to be fully capable of binding to CD3 positive cells and blocking the binding of murine OKT3 to these cells.

3.3 DETERMINATION OF RELATIVE BINDING AFFINITY

The relative binding affinities of CDR-grafted

anti-CD3 monoclonal antibodies were determined by competition binding (ref. 6) using the HPB-ALL human T cell line as a source of CD3 antigen, and fluorescein-conjugated murine OKT3 (Fl-OKT3) of known binding affinity as a tracer antibody. The binding affinity of Fl-OKT3 tracer antibody was determined by a direct binding assay in which increasing amounts of Fl-OKT3 were incubated with HPB-ALL ($5x10^5$) in PBS with 5% foetal calf serum for 60 min. at 4°C. Cells were washed, and the fluorescence intensity was determined on a FACScan flow cytometer calibrated with quantitative microbead standards (Flow Cytometry Standards, Research Triangle Park, NC). Fluorescence intensity per antibody molecule (F/P ratio) was determined by using microbeads which have a predetermined number of mouse IgG antibody binding sites (Simply Cellular beads, Flow Cytometry Standards). F/P equals the fluorescence intensity of beads saturated with Fl-OKT3 divided by the number of binding sites per bead. The amount of bound and free Fl-OKT3 was calculated from the mean fluorescence intensity per cell, and the ratio of bound/free was plotted against the number of moles of antibody bound. A linear fit was used to determine the affinity of binding (absolute value of the slope).

For competitive binding, increasing amounts of competitor antibody were added to a sub-saturating dose of Fl-OKT3 and incubated with $5x10^5$ HPB-ALL in 200 Ml of PBS with 5% foetal calf serum, for 60 min at 4°C. The fluorescence intensities of the cells were measured on a FACScan flow cytometer calibrated with quantitative microbead standards. The concentrations of bound and free Fl-OKT3 were calculated. The affinities of competing anti-

bodies were calculated from the equation [X]-[OKT3] = (1/Kx) - (1/Ka), where Ka is the affinity of murine OKT3, Kx is the affinity of competitor X, [ ] is the concentration of competitor antibody at which bound/free binding is R/2, and R is the maximal bound/free binding.

4. cDNA LIBRARY CONSTRUCTION

4.1. mRNA PREPARATION AND cDNA SYNTHESIS

OKT3 producing cells were grown as described above and 1.2 x 10^9 cells harvested and mRNA extracted using the guanidinium/LiCl extraction procedure. cDNA was prepared by priming from Oligo-dT to generate full length cDNA. The cDNA was methylated and EcoR1 linkers added for cloning.

4.2. LIBRARY CONSTRUCTION

The cDNA library was ligated to pSP65 vector DNA which had been EcoR1 cut and the 5′ phosphate groups removed by calf intestinal phosphatase (EcoR1/CIP). The ligation was used to transform high transformation efficiency Escherichia coli (E.coli) HB101. A cDNA library was prepared. 3600 colonies were screened for the light chain and 10000 colonies were screened for the heavy chain.

5. SCREENING

E.coli colonies positive for either heavy or light chain probes were identified by oligonucleotide screening using the oligonucleotides:

5′ TCCAGATGTTAACTGCTCAC (SEQ ID NO:1) for the light chain, which is complementary to a sequence in the mouse kappa constant region, and 5′ CAGGGGCCAGTGGATGGATAGAC (SEQ ID NO:2) for the heavy chain which is complementary to a sequence in the mouse IgG2a constant CH1 domain region. 12 light chain and 9 heavy chain clones

were identified and taken for second round screening. Positive clones from the second round of screening were grown up and DNA prepared. The sizes of the gene inserts were estimated by gel electrophoresis and inserts of a size capable of containing a full length cDNA were subcloned into M13 for DNA sequencing.

6. DNA SEQUENCING

Clones representing four size classes for both heavy and light chains were obtained in M13. DNA sequence for the 5' untranslated regions, signal sequences, variable regions and 3' untranslated regions of full length cDNAs [Figures 1(a) (SEQ ID NO:4) and 2(a) (SEQ ID NO:6)] were obtained and the corresponding amino acid sequences predicted [(Figures 1(b) (SEQ ID NO:8) and 2(b) (SEQ ID NO:9)]. In Figure 1(a) the untranslated DNA regions are shown in uppercase, and in both Figures 1 and 2 (SEQ ID NO:4+5) the signal sequences are underlined.

7. CONSTRUCTION OF cDNA EXPRESSION VECTORS

Celltech expression vectors are based on the plasmid pEE6hCMV (ref. 14). A polylinker for the insertion of genes to be expressed has been introduced after the major immediate early promoter/enhancer of the human Cytomegalovirus (hCMV). Marker genes for selection of the plasmid in transfected eukaryotic cells can be inserted as BamH1 cassettes in the unique BamH1 site of pEE6 hCMV; for instance, the neo marker to provide pEE6 hCMV neo. It is usual practice to insert the neo and gpt markers prior to insertion of the gene of interest, whereas the GS marker is inserted last because of the presence of internal EcoR1 sites in the cassette.

The selectable markers are expressed from the SV40 late promoter which also provides an origin of replication so that the vectors can be used for expression in the COS cell transient expression system.

The mouse sequences were excised from the M13 based vectors described above as EcoR1 fragments and cloned into either pEE6-hCMV-neo for the heavy chain and into EE6-hCMV-gpt for the light chain to yield vectors pJA136 and pJA135 respectively.

8. EXPRESSION OF cDNAS IN COS CELLS

Plasmids pJA135 and pJA136 were co-transfected into COS cells and supernatant from the transient expression experiment was shown to contain assembled antibody which bound to T-cell enriched lymphocytes. Metabolic labelling experiments using ^{35}S methionine showed expression and assembly of heavy and light chains.

9. CONSTRUCTION OF CHIMERIC GENES

Construction of chimeric genes followed a previously described strategy [Whittle et al (ref. 13)]. A restriction site near the 3′ end of the variable domain sequence is identified and used to attach an oligonucleotide adapter coding for the remainder of the mouse variable region and a suitable restriction site for attachment to the constant region of choice.

9.1. LIGHT CHAIN GENE CONSTRUCTION

The mouse light chain cDNA sequence contains an AvaI site near the 3′ end of the variable region [Fig. 1(a)] (SEQ ID NO:4). The majority of the sequence of the variable region was isolated as a 396 bp. EcoR1-AvaI fragment. An oligonucleotide adapter was designed to replace the remainder of the 3′

region of the variable region from the AvaI site and to include the 5' residues of the human constant region up to and including a unique NarI site which had been previously engineered into the constant region.
A HindIII site was introduced to act as a marker for insertion of the linker.
The linker was ligated to the V_L fragment and the 413 bp EcoR1-NarI adapted fragment was purified from the ligation mixture.
The constant region was isolated as an NarI-BamHI fragment from an M13 clone NW361 and was ligated with the variable region DNA into an EcoR1/BamH1/C1P pSP65 treated vector in a three way reaction to yield plasmid JA143. Clones were isolated after transformation into E.coli and the linker and junction sequences were confirmed by the presence of the HindIII site and by DNA sequencing.

9.2 LIGHT CHAIN GENE CONSTRUCTION - VERSION 2

The construction of the first chimeric light chain gene produces a fusion of mouse and human amino acid sequences at the variable-constant region junction. In the case of the OKT3 light chain the amino acids at the chimera junction are: (SEQ ID NO:3)

.....Leu-Glu-Ile-Asn-Arg/ -/Thr-Val-Ala -Ala

VARIABLE CONSTANT

This arrangement of sequence introduces a potential site for Asparagine (Asn) linked (N-linked) glycosylation at the V-C junction. Therefore, a second version of the chimeric light chain oligonucleotide adapter was designed in which the threonine (Thr), the first amino acid of the human constant region, was replaced with the equivalent amino acid from the mouse constant region, Alanine (Ala).

An internal HindIII site was not included in this adapter, to differentiate the two chimeric light chain genes.

The variable region fragment was isolated as a 376 bp EcoR1-AvaI fragment. The oligonucleotide linker was ligated to NarI cut pNW361 and then the adapted 396bp constant region was isolated after recutting the modified pNW361 with EcoR1. The variable region fragment and the modified constant region fragment were ligated directly into EcoR1/C1P treated pEE6hCMVneo to yield pJA137. Initially all clones examined had the insert in the incorrect orientation. Therefore, the insert was re-isolated and recloned to turn the insert round and yield plasmid pJA141. Several clones with the insert in the correct orientation were obtained and the adapter sequence of one was confirmed by DNA sequencing

9.3. HEAVY CHAIN GENE CONSTRUCTION

9.3.1. CHOICE OF HEAVY CHAIN GENE ISOTYPE

The constant region isotype chosen for the heavy chain was human IgG4.

9.3.2. GENE CONSTRUCTION

The heavy chain cDNA sequence showed a BanI site (SEQ ID NO:6) near the 3′ end of the variable region [Fig. 2(a)]. The majority of the sequence of the variable region was isolated as a 426bp. EcoR1/C1P/BanI fragment. An oligonucleotide adapter was designated to replace the remainder of the 3′ region of the variable region from the BanI site up to and including a unique HindIII site which had been previously engineered into the first two amino acids of the constant region.

The linker was ligated to the V_H fragment and the EcoR1-HindIII adapted fragment was purified from the ligation mixture.

The variable region was ligated to the constant region by cutting pJA91 with EcoR1 and Hind111 removing the intron fragment and replacing it with the V_H to yield pJA142. Clones were isolated after transformation into E.coli JM101 and the linker and junction sequences were confirmed by DNA sequencing. (N.B. The Hind111 site is lost on cloning).

10. CONSTRUCTION OF CHIMERIC EXPRESSION VECTORS

10.1. neo AND gpt VECTORS

The chimeric light chain (version 1) was removed from pJA143 as an EcoR1 fragment and cloned into EcoR1/C1P treated pEE6hCMVneo expression vector to yield pJA145. Clones with the insert in the correct orientation were identified by restriction mapping.

The chimeric light chain (version 2) was constructed as described above.

The chimeric heavy chain gene was isolated from pJA142 as a 2.5Kbp EcoR1/BamH1 fragment and cloned into the EcoR1/Bc11/C1P treated vector fragment of a derivative of pEE6hCMVgpt to yield plasmid pJA144.

10.2. GS SEPARATE VECTORS

GS versions of pJA141 and pJA144 were constructed by replacing the neo and gpt cassettes by a BamH1/Sa11/C1P treatment of the plasmids, isolation of the vector fragment and ligation to a GS-containing fragment from the plasmid pRO49 to yield the light chain vector pJA179 and the heavy chain vector pJA180.

10.3. GS SINGLE VECTOR CONSTRUCTION

Single vector constructions containing the cL (chimeric light), cH (chimeric heavy) and GS genes on one plasmid in the order cL-cH-GS, or cH-cL-GS

and with transcription of the genes being head to tail e.g. cL>cH>GS were constructed. These plasmids were made by treating pJA179 or pJA180 with BamH1/C1P and ligating in a Bgl11/Hind111 hCMV promoter cassette along with either the Hind111/BamH1 fragment from pJA141 into pJA180 to give the cH-cL-GS plasmid pJA182 or the Hind111/BamH1 fragment from pJA144 into pJA179 to give the cL-cH-GS plasmid pJA181.

11. EXPRESSION OF CHIMERIC GENES

11.1. EXPRESSION IN COS CELLS

The chimeric antibody plasmid pJA145 (cL) and pJA144 (cH) were co-transfected into COS cells and supernatant from the transient expression experiment was shown to contain assembled antibody which bound to the HUT 78 human T-cell line. Metabolic labelling experiments using ^{35}S methionine showed expression and assembly of heavy and light chains. However the light chain mobility seen on reduced gels suggested that the potential glycosylation site was being glycosylated. Expression in COS cells in the presence of tunicamycin showed a reduction in size of the light chain to that shown for control chimeric antibodies and the OKT3 mouse light chain. Therefore JA141 was constructed and expressed. In this case the light chain did not show an aberrant mobility or a size shift in the presence or absence of tunicamycin. This second version of the chimeric light chain, when expressed in association with chimeric heavy (cH) chain, produced antibody which showed good binding to HUT 78 cells. In both cases antigen binding was equivalent to that of the mouse antibody.

11.2 EXPRESSION IN CHINESE HAMSTER OVARY (CHO) CELLS

Stable cell lines have been prepared from plasmids PJA141/pJA144 and from pJA179/pJA180, pJA181 and pJA182 by transfection into CHO cells.

12. CDR-GRAFTING

The approach taken was to try to introduce sufficient mouse residues into a human variable region framework to generate antigen binding activity comparable to the mouse and chimeric antibodies.

12.1. VARIABLE REGION ANALYSIS

From an examination of a small database of structures of antibodies and antigen-antibody complexes it is clear that only a small number of antibody residues make direct contact with antigen. Other residues may contribute to antigen binding by positioning the contact residues in favourable configurations and also by inducing a stable packing of the individual variable domains and stable interaction of the light and heavy chain variable domains.

The residues chosen for transfer can be identified in a number of ways:

(a) By examination of antibody X-ray crystal structures the antigen binding surface can be predominantly located on a series of loops, three per domain, which extend from the B-barrel framework.

(b) By analysis of antibody variable domain sequences regions of hypervariability [termed the Complementarity Determining Regions (CDRs) by Wu and Kabat (ref. 5)] can be identified. In the most but not all cases these CDRs correspond to, but extend a short way beyond, the loop regions noted above.

(c) Residues not identified by (a) and (b) may contribute to antigen binding directly or indirectly by affecting antigen binding site topology, or by inducing a stable packing of the individual variable domains and stabilising the inter-variable domain interaction. These residues may be identified either by superimposing the sequences for a given antibody on a known structure and looking at key residues for their contribution, or by sequence alignment analysis and noting "idiosyncratic" residues followed by examination of their structural location and likely effects.

12.1.1. LIGHT CHAIN

Figure 3 shows an alignment of sequences for the human framework region REI and the OKT3 light variable region. The structural loops (LOOP) and CDRs (KABAT) believed to correspond to the antigen binding region are marked. Also marked are a number of other residues which may also contribute to antigen binding as described in 13.1(c). Above the sequence in Figure 3 the residue type indicates the spatial location of each residue side chain, derived by examination of resolved structures from X-ray crystallography analysis. The key to this residue type designation is as follows:

N - near to CDR (From X-ray Structures)
P - Packing
B - Buried Non-Packing
S - Surface
E - Exposed
I - Interface
* - Interface
- Packing/Part Exposed
? - Non-CDR Residues which may require to be left as Mouse sequence.

Residues underlined in Figure 3 are amino acids. RE1 was chosen as the human framework because the light chain is a kappa chain and the kappa variable regions show higher homology with the mouse sequences than a lambda light variable region, e.g. KOL (see below). RE1 was chosen in preference to another kappa light chain because the X-ray structure of the light chain has been determined so that a structural examination of individual residues could be made.

12.1.2. HEAVY CHAIN

Similarly Figure 4 shows an alignment of sequences for the human framework region KOL and the OKT3 heavy variable region. The structural loops and CDRs believed to correspond to the antigen binding region are marked. Also marked are a number of other residues which may also contribute to antigen binding as described in 12.1(c). The residue type key and other indicators used in Figure 4 are the same as those used in Figure 3. KOL was chosen as the heavy chain framework because the X-ray structure has been determined to a better resolution than, for example, NEWM and also the sequence alignment of OKT3 heavy variable region showed a slightly better homology to KOL than to NEWM.

12.2. DESIGN OF VARIABLE GENES

The variable region domains were designed with mouse variable region optimal codon usage [Grantham and Perrin (ref. 15)] and used the B72.3 signal sequences [Whittle et al (ref. 13)]. The sequences were designed to be attached to the constant region in the same way as for the chimeric genes described above. Some constructs contained the "Kozak consensus sequence" [Kozak (ref. 16)] directly linked to the 5′ of the signal

sequence in the gene. This sequence motif is believed to have a beneficial role in translation initiation in eukaryotes.

12.3. GENE CONSTRUCTION

To build the variable regions, various strategies are available. The sequence may be assembled by using oligonucleotides in a manner similar to Jones et al (ref. 17) or by simultaneously replacing all of the CDRs or loop regions by oligonucleotide directed site specific mutagenesis in a manner similar to Verhoeyen et al (ref. 2). Both strategies were used and a list of constructions is set out in Tables 1 and 2 and Figures 4 and 5a-c. It was noted in several cases that the mutagenesis approach led to deletions and rearrangements in the gene being remodelled, while the success of the assembly approach was very sensitive to the quality of the oligonucleotides.

13. CONSTRUCTION OF EXPRESSION VECTORS

Genes were isolated from M13 or SP65 based intermediate vectors and cloned into pEE6hCMVneo for the light chains and pEE6hCMVgpt for the heavy chains in a manner similar to that for the chimeric genes as described above.

TABLE 1 CDR-GRAFTED GENE CONSTRUCTS

CODE	MOUSE SEQUENCE CONTENT	METHOD OF CONSTRUCTION	KOZAK SEQUENCE -	KOZAK SEQUENCE +
LIGHT CHAIN	ALL HUMAN FRAMEWORK REl			
121	26-32, 50-56, 91-96 inclusive	SDM and gene assembly	+	n.d.
121A	26-32, 50-56, 91-96 inclusive +1, 3, 46, 47	Partial gene assembly	n.d.	+
121B	26-32, 50-56, 91-96 inclusive + 46, 47	Partial gene assembly	n.d.	+
221	24-24, 50-56, 91-96 inclusive	Partial gene assembly	+	+
221A	24-34, 50-56, 91-96 inclusive +1, 3, 46, 47	Partial gene assembly	+	+
221B	24-34, 50-56, 91-96 inclusive +1, 3	Partial gene assembly	+	+
221C	24-34, 50-56, 91-96 inclusive	Partial gene assembly	+	+
HEAVY CHAIN	ALL HUMAN FRAMEWORK KOL			
121	26-32, 50-56, 95-100B inclusive	Gene assembly	n.d.	+
131	26-32, 50-58, 95-100B inclusive	Gene assembly	n.d.	+
141	26-32, 50-65, 95-100B inclusive	Partial gene assembly	+	n.d.
321	26-35, 50-56, 95-100B inclusive	Partial gene assembly	+	n.d.
331	26-35, 50-58, 95-100B inclusive	Partial gene assembly	+	
		Gene assembly		+
341	26-35, 50-65, 95-100B inclusive	SDM	+	
		Partial gene assembly		+
341A	26-35, 50-65, 95-100B inclusive +6, 23, 24, 48, 49, 71, 73, 76, 78, 88, 91 (+63 = human) (SEQ ID NO:8-28)	Gene assembly	n.d.	+
341B	26-35, 50-65, 95-100B inclusive + 48, 49, 71, 73, 76, 78, 88, 91 (+63 + human)	Gene assembly	n.d.	+

KEY

n.d.	not done
SDM	Site directed mutagenesis
Gene assembly	Variable region assembled entirely from oligonucleotides
Partial gene assembly	Variable region assembled by combination of restriction fragments either from other genes originally created by SDM and gene assembly or by oligonucleotide assembly of part of the variable region and reconstruction with restriction fragments from other genes originally created by SDM and gene assembly

14. EXPRESSION OF CDR-GRAFTED GENES

14.1. PRODUCTION OF ANTIBODY CONSISTING OF GRAFTED LIGHT (gL) CHAINS WITH MOUSE HEAVY (mH) OR CHIMERIC HEAVY (cH) CHAINS

All gL chains, in association with mH or cH produced reasonable amounts of antibody. Insertion of the Kozak consensus sequence at a position 5' to the ATG (kgL constructs) however, led to a 2-5 fold improvement in net expression. Over an extended series of experiments expression levels were raised from approximately 200ng/ml to approximately 500 ng/ml for kgL/cH or kgL/mH combinations.

When direct binding to antigen on HUT 78 cells was measured, a construct designed to include mouse sequence based on loop length (gL121) did not lead to active antibody in association with mH or cH. A construct designed to include mouse sequence based on Kabat CDRs (gL221 (SEQ ID NO:26)) demonstrated some weak binding in association with mH or cH. However, when framework residues 1, 3, 46, 47 were changed from the human to the murine OKT3 equivalents based on the arguments outlined in Section 12.1 antigen binding was demonstrated when both of the new constructs, which were termed 121A and 221A were co-expressed with cH. When the effects of these residues were examined in more detail, it appears that residues 1 and 3 are not major contributing residues as the product of the gL221B gene (SEQ ID NO:27) shows little detectable binding activity in association with cH. The light chain product of gL221C (SEQ ID NO:28), in which mouse sequences are present at 46 and 47, shows good binding activity in association with cH.

14.2 PRODUCTION OF ANTIBODY CONSISTING OF GRAFTED HEAVY (gH) CHAINS WITH MOUSE LIGHT (mL) OR CHIMERIC LIGHT (cL) CHAINS

Expression of the gH genes proved to be more difficult to achieve than for gL. First, inclusion of the Kozak sequence appeared to have no marked effect on expression of gH genes. Expression appears to be slightly improved but not to the same degree as seen for the grafted light chain.

Also, it proved difficult to demonstrate production of expected quantities of material when the loop choice (amino acid 26-32) for CDR1 is used, e.g. gH121, 131, 141 and no conclusions can be drawn about these constructs.

Moreover, co-expression of the gH341 gene (SEQ ID NO:11) with cL or mL has been variable and has tended to produce lower amounts of antibody than the cH/cL or mH/mL combinations. The alterations to gH341 (SEQ ID NO:11) to produce gH341A (SEQ ID NO:12) and gH341B (SEQ ID NO:21) lead to improved levels of expression.

This may be due either to a general increase in the fraction of mouse sequence in the variable region, or to the alteration at position 63 where the residue is returned to the human amino acid Valine (Val) from Phenylalanine (Phe) to avoid possible internal packing problems with the rest of the human framework. This arrangement also occurs in gH331 and gH321.

When gH321 or gH331 were expressed in association with cL, antibody was produced but antibody binding activity was not detected.

When the more conservative gH341 gene (SEQ ID NO:11) was used antigen binding could be detected in association with cL or mL, but the activity was only marginally above the background level.

When further mouse residues were substituted based on the arguments in 12.1, antigen binding could be clearly demonstrated for the antibody produced when kgH341A and kgH341B were expressed in association with cL.

14.3 PRODUCTION OF FULLY CDR-GRAFTED ANTIBODY

The kgL221A gene was co-expressed with kgH341, kgH341A or kgH341B. For the combination kgH221A/kgH341 very little material was produced in a normal COS cell expression.

For the combinations kgL221A/kgH341A or kgH221A/kgH341B amounts of antibody similar to gL/cH was produced.

In several experiments no antigen binding activity could be detected with kgH221A/gH341 or kgH221A/kgH341 combinations, although expression levels were very low.

Antigen binding was detected when kgL221A/kgH341A or kgH221A/kgH341B combinations were expressed. In the case of the antibody produced from the kgL221A/kgH341A combination the antigen binding was very similar to that of the chimeric antibody.

An analysis of the above results is given below.

15. DISCUSSION OF CDR-GRAFTING RESULTS

In the design of the fully humanised antibody the aim was to transfer the minimum number of mouse amino acids that would confer antigen binding onto a human antibody framework.

15.1. LIGHT CHAIN

15.1.1. EXTENT OF THE CDRs

For the light chain the regions defining the loops known from structural studies of other antibodies to contain the antigen contacting residues, and

those hypervariable sequences defined by Kabat et al (refs. 4 and 5) as Complementarity Determining Regions (CDRs) are equivalent for CDR2. For CDR1 the hypervariable region extends from residues 24-34 inclusive while the structural loop extends from 26-32 inclusive. In the case of OKT3 (SEQ ID NO:5) there is only one amino acid difference between the two options, at amino acid 24, where the mouse sequence is a serine and the human framework RE1 has glutamine. For CDR3 the loop extends from residues 91-96 inclusive while the Kabat hypervariability extends from residues 89-97 inclusive. For OKT3 amino acids 89, 90 and 97 are the same between OKT3 and RE1 (Fig. 3) (SEQ ID NO. 8 AND 9). When constructs based on the loop choice for CDR1 (gL121) and the Kabat choice (gL221) were made and co-expressed with mH or cH no evidence for antigen binding activity could be found for gL121, but trace activity could be detected for the gL221, suggesting that a single extra mouse residue in the grafted variable region could have some detectable effect. Both gene constructs were reasonably well expressed in the transient expression system.

15.1.2. FRAMEWORK RESIDUES

The remaining framework residues were then further examined, in particular amino acids known from X-ray analysis of other antibodies to be close to the CDRs and also those amino acids which in OKT3 showed differences from the consensus framework for the mouse subgroup (subgroup VI) to which OKT3 shows most homology. Four positions 1, 3, 46 and 47 were identified and their possible contribution was examined by substituting the mouse amino acid for the human amino acid at each position. Therefore gL221A (gL221 + D1Q, Q3V, L46R, L47W,

see Figure 3 and Table 1) was made, cloned in EE6hCMVneo and co-expressed with cH (pJA144). The resultant antibody was well expressed and showed good binding activity. When the related genes gL221B (SEQ ID NO:28) (gL221 + D1Q, Q3V) and gL221C (gL221 + L46R, L47W) were made and similarly tested, while both genes produced antibody when co-expressed with cH, only the gL221C/cH combination showed good antigen binding. When the gL121A (gL121 + D1Q, Q3V, L46R, L47W) gene was made and co-expressed with cH, antibody was produced which also bound to antigen.

15.2. HEAVY CHAIN

15.2.1. EXTENT OF THE CDRs

For the heavy chain the loop and hypervariability analyses agree only in CDR3. For CDR1 the loop region extends from residues 26-32 inclusive whereas the Kabat CDR extends from residues 31-35 inclusive. For CDR2 the loop region is from 50-58 inclusive while the hypervariable region covers amino acids 50-65 inclusive. Therefore humanised heavy chains were constructed using the framework from antibody KOL and with various combinations of these CDR choices, including a shorter choice for CDR2 of 50-56 inclusive as there was some uncertainty as to the definition of the end point for the CDR2 loop around residues 56 to 58. The genes were co-expressed with mL or cL initially. In the case of the gH genes with loop choices for CDR1 e.g. gH121, gH131, gH141 very little antibody was produced in the culture supernatants. As no free light chain was detected it was presumed that the antibody was being made and assembled inside the cell but that the heavy chain was aberrant in some way, possibly incorrectly folded, and therefore the antibody was

being degraded internally. In some experiments trace amounts of antibody could be detected in ^{35}S labelling studies.

As no net antibody was produced, analysis of these constructs was not pursued further.

When, however, a combination of the loop choice and the Kabat choice for CDR1 was tested (mouse amino acids 26-35 inclusive) and in which residues 31 (Ser to Arg), 33 (Ala to Thr), and 35 (Tyr to His) were changed from the human residues to the mouse residue and compared to the first series, antibody was produced for gH321, kgH331 and kgH341 when co-expressed with cL. Expression was generally low and could not be markedly improved by the insertion of the Kozak consensus sequence 5′ to the ATG of the signal sequence of the gene, as distinct from the case of the gL genes where such insertion led to a 2-5 fold increase in net antibody production. However, only in the case of gH341/mL or kgH341/cL could marginal antigen binding activity be demonstrated. When the kgH341 gene was co-expressed with kgL221A (SEQ ID NO: 26), the net yield of antibody was too low to give a signal above the background level in the antigen binding assay.

15.2.2. FRAMEWORK RESIDUES

As in the case of the light chain the heavy chain frameworks were re-examined. Possibly because of the lower initial homology between the mouse and human heavy variable domains compared to the light chains, more amino acid positions proved to be of interest. Two genes kgH341A and kgH341B were constructed, with 11 or 8 human residues respectively substituted by mouse residues compared to gH341, and with the CDR2 residue 63 returned to the human amino acid potentially to

improve domain packing. Both showed antigen binding when combined with cL or kgL221A, the kgH341A gene with all 11 changes appearing to be the superior choice.

15.3 INTERIM CONCLUSIONS

It has been demonstrated, therefore, for OKT3 that to transfer antigen binding ability to the humanised antibody, mouse residues outside the CDR regions defined by the Kabat hypervariability or structural loop choices are required for both the light and heavy chains. Fewer extra residues are needed for the light chain, possibly due to the higher initial homology between the mouse and human kappa variable regions.

Of the changes seven (1 and 3 from the light chain and 6, 23, 71, 73 and 76 from the heavy chain) are predicted from a knowledge of other antibody structures to be either partly exposed or on the antibody surface. It has been shown here that residues 1 and 3 in the light chain are not absolutely required to be the mouse sequence; and for the heavy chain the gH341B heavy chain in combination with the 221A light chain generated only weak binding activity. Therefore the presence of the 6, 23 and 24 changes are important to maintain a binding affinity similar to that of the murine antibody. It was important, therefore, to further study the individual contribution of othe other 8 mouse residues of the kgH341A gene compared to kgH341.

16. FURTHER CDR-GRAFTING EXPERIMENTS

Additional CDR-grafted heavy chain genes were prepared substantially as described above. With reference to Table 2 the further heavy chain genes were based upon the gh341 (plasmid pJA178) and

gH341A (plasmid pJA185) with either mouse OKT3 or human KOL residues at 6, 23, 24, 48, 49, 63, 71, 73, 76, 78, 88 and 91, as indicated. The CDR-grafted light chain genes used in these further experiments were gL221, gL221A, gL221B and gL221C as described above.

TABLE 2

OKT3 HEAVY CHAIN CDR GRAFTS

1. gH341 and derivatives

RES NUM	6	23	24	48	49	63	71	73	76	78	88	91	
OKT3vh	Q	K	A	I	G	F	T	K	S	A	A	Y	
gH341	E	S	S	V	A	F	R	N	N	L	G	F	JA178
gH341A	Q	K	A	I	G	V	T	K	S	A	A	Y	JA185
gH341E	Q	K	A	I	G	V	T	K	S	A	G	G	JA198
gH341*	Q	K	A	I	G	V	T	K	N	A	G	F	JA207
gH341*	Q	K	A	I	G	V	R	N	N	A	G	F	JA209
gH341D	Q	K	A	I	G	V	T	K	N	L	G	F	JA197
gH341*	Q	K	A	I	G	V	R	N	N	L	G	F	JA199
gH341C	Q	K	A	V	A	F	R	N	N	L	G	F	JA184
gH341*	Q	S	A	I	G	V	T	K	S	A	A	Y	JA203
gH341*	E	S	A	I	G	V	T	K	S	A	A	Y	JA205
gH341B	E	S	S	I	G	V	T	K	S	A	A	Y	JA183
gH341*	Q	S	A	I	G	V	T	K	S	A	G	F	JA204
gH341*	E	S	A	I	G	V	T	K	S	A	G	F	JA206
gH341*	Q	S	A	I	G	V	T	K	N	A	G	F	JA208
KOL	E	S	S	V	A		R	N	N	L	G	F	

A J (SEQ ID NO: 7, 10 and 11-24)

OKT3 LIGHT CHAIN CDR GRAFTS

2. gL221 and derivatives

RES NUM	1	3	46	47	
OKT3v1	Q	V	R	W	
GL221	D	Q	L	L	DA221
gL221A	Q	V	R	W	DA221A
gL221B	Q	V	L	L	DA221B
GL221C	D	Q	R	W	DA221C
RE1	D	Q	L	L	

K J (SEQ ID NO: 8, 8, 9 and 25-28)

MURINE RESIDUES ARE UNDERLINED

The CDR-grafted heavy and light chain genes were co-expressed in COS cells either with one another in various combinations but also with the corresponding murine and chimeric heavy and light chain genes substantially as described above. The resultant antibody products were then assayed in binding and blocking assays with HPB-ALL cells as described above.

The results of the assays for various grafted heavy chains co-expressed with the gL221C light chain (SEQ ID NO:28) are given in Figures 7 and 8 (for the JA184, JA185, JA197 and JA198 constructs - see Table 2), in Figure 9 (for the JA183, JA184, JA185 and JA197 constructs) in Figure 10 a and b (for the chimeric, JA185, JA199, JA204, JA205, JA207, JA208 and JA209 constructs) and in Figure 11 a and b (for the JA183, JA184, JA185, JA198, JA203, JA205 and JA206 constructs).

The basic grafted product without any human to murine changes in the variable frameworks, i.e. gL221 (SEQ ID NO:25) co-expressed with gh341 (JA178) (SEQ ID NO. 11), and also the "fully grafted" product, having most human to murine changes in the grafted heavy chain framework, i.e. gL221C (SEQ ID NO:28) co-expressed with gh341A (JA185) (SEQ ID NO:12), were assayed for relative binding affinity in a competition assay against murine OKT3 reference standard, using HPB-ALL cells. The assay used was as described above in section 3.3. The results obtained are given in Figure 12 for the basic grafted product and in Figure 13 for the fully grafted product. These results indicate that the basic grafted product has neglibible binding ability as compared with the OKT3 murine reference standard; whereas the "fully grafted" product has a binding ability very similar to that of the OKT3 murine reference standard.

The binding and blocking assay results indicate the following:

The JA198 and JA207 constructs appear to have the best binding characteristics and similar binding abilities, both substantially the same as the chimeric and fully grafted gH341A products. This indicates that positions 88 and 91 and position 76 are not highly critical for maintaining the OKT3 binding ability; whereas at least some of positions 6, 23, 24, 48, 49, 71, 73 and 78 are more important.

This is borne out by the finding that the JA209 and JA199, although of similar binding ability to one another, are of lower binding ability than the JA198 and JA207 constructs. This indicates the importance of having mouse residues at positions 71, 73 and 78, which are either completely or partially human in the JA199 and JA209 constructs respectively.

Moreover, on comparing the results obtained for the JA205 and JA183 constructs it is seen that there is a decrease in binding going from the JA205 to the JA183 constructs. This indicates the importance of retaining a mouse residue at position 23, the only position changed between JA205 and JA183.

These and other results lead us to the conclusion that of the 11 mouse framework residues used in the gH341A (JA185) construct, it is important to retain mouse residues at all of positions 6, 23, 24, 48 and 49, and possibly for maximum binding affinity at 71, 73 and 78.

Similar Experiments were carried out to CDR-graft a number of the rodent antibodies including antibodies having specificity for CD4 (OKT4), ICAM-1 (R6-5), TAG72 (B72.3), and TNFα(61E71, 101.4, hTNF1, hTNF2 and hTNF3).

EXAMPLE 2

CDR-GRAFTING OF A MURINE ANTI-CD4 T CELL RECEPTOR ANTIBODY, OKT4A

Anti OKT4A CDR-grafted heavy and light chain genes were prepared, expressed and tested substantially as described above in Example 1 for CDR-grafted OKT3. The CDR grafting of OKT4A is described in detail in Ortho patent application PCT/GB 90..... of even date herewith entitled "Humanised Antibodies". The disclosure of this Ortho patent application PCT/GB 90 ..... is incorporated herein by reference. A number of CDR-grafted OKT4 antibodies have been prepared. Presently the CDR-grafted OKT4A of choice is the combination of the grafted light chain LCDR2 and the grafted heavy chain HCDR10.

THE LIGHT CHAIN

The human acceptor framework used for the grafted light chains was RE1. (SEQ ID NO: 8 and 9) The preferred LCDR2 light chain has human to mouse changes at positions 33, 34, 38, 49 and 89 in addition to the structural loop CDRs. Of these changed positions, positions 33, 34 and 89 fall within the preferred extended CDRs of the present invention (positions 33 and 34 in CDR1 and position 89 in CDR3). The human to murine changes at positions 38 and 49 corresponds to positions at which the amino acid residues are preferably donor murine amino acid residues in accordance with the present invention.

A comparison of the amino acid sequences of the donor murine light chain variable domain and the RE1 human acceptor light chain variable further reveals that the murine and human residues are identical at all of positions 46, 48 and 71 and at all of positions 2, 4, 6, 35, 36, 44, 47, 62, 64-69, 85, 87, 98, 99 and 101 and 102. However the amino acid residue at position 58 in LCDR2 is

the human RE1 framework residue not the mouse OKT4 residue as would be preferred in accordance with the present invention.

THE HEAVY CHAIN

The human acceptor framework used for the grafted heavy chains was KOL (SEQ ID NO: 10)

The preferred CDR graft HCDR10 heavy chain has human to mouse changes at positions 24, 35, 57, 58, 60, 88 and 91 in addition to the structural loop CDRs.

Of these positions, positions 35 (CDR1) and positions 57, 58 and 60 (CDR2) fall within the preferred extended CDRs of the present invention. Also the human to mouse change at position 24 corresponds to a position at which the amino acid residue is a donor murine residue in accordance with the present invention. Moreover, the human to mouse changes at positions 88 and 91 correspond to positions at which the amino acid residues are optionally donor murine residues.

Moreover, a comparison of the murine OKT4A and human KOL heavy chain variable amino acid sequences reveals that the murine and human residues are identical at all of positions 23, 49, 71, 73 and 78 and at all of positions 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

Thus the OKT4A CDR-grafted heavy chain HCDR10 corresponds to a particularly preferred embodiment according to the present invention.

EXAMPLE 3

CDR-GRAFTING OF AN ANTI-MUCIN SPECIFIC MURINE ANTIBODY, B72.3

The cloning of the genes coding for the anti-mucin specific murine monoclonal antibody B72.3 and the preparation of B72.3 mouse-human chimeric antibodies has been described previously (ref. 13 and WO 89/01783). CDR-grafted versions of B72.3 were prepared as follows.

(a) B72.3 Light Chain

CDR-grafting of this light chain was accomplished by direct transfer of the murine CDRs into the framework of the human light chain RE1. The regions transferred were:

CDR Number	Residues
1	24-34
2	50-56
3	90-96

The activity of the resulting grafted light chain was assessed by co-expression in COS cells, of genes for the combinations:

B72.3 cH/B72.3 cL

and B72.3 cH/B72.3 gL

Supernatants were assayed for antibody concentration and for the ability to bind to microtitre plates coated with mucin. The results obtained indicated that, in combination with the B72.3 cH chain, B72.3 cL and B72.3 gL had similar binding properties.

Comparison of the murine B72.3 and REI (SEQ ID NO:8 and 9) light chain amino acid sequences reveals that the residues are identical at positions 46, 58 and 71 but are different at position 48.

Thus changing the human residue to the donor mouse residue at position 48 may further improve the binding characteristics of the CDR-grafted light chain, (B72.3 gL) in accordance with the present invention.

(b) B72.3 heavy chain

i. Choice of framework

At the outset it was necessary to make a choice of human framework. Simply put, the question was as follows: Was it necessary to use the framework regions from an antibody whose crystal structure was known or could the choice be made on some other criteria?

For B72.3 heavy chain, it was reasoned that, while knowledge of structure was important, transfer of the CDRs from mouse to human frameworks might be facilitated if the overall homology between the donor and receptor frameworks was maximised. Comparison of the B72.3 heavy chain sequence with those in Kabat (ref. 4) for human heavy chains showed clearly that B72.3 had poor homology for KOL (SEQ ID NO:10) and NEWM (for which crystal structures are available) but was very homologous to the heavy chain for EU.

On this basis, EU was chosen for the CDR-grafting and the following residues transferred as CDRs.

CDR Number	Residues
1	27-36
2	50-63
3	93-102

Also it was noticed that the FR4 region of EU was unlike that of any other human (or mouse) antibody. Consequently, in the grafted heavy chain genes this was also changed to produce a "consensus" human sequence. (Preliminary experiments showed that grafted heavy chain genes containing the EU FR4 sequence expressed very poorly in transient expression systems.)

ii. Results with grafted heavy chain genes

Expression of grafted heavy chain genes containing all human framework regions with either gL or cL genes produced a grafted antibody with little ability to bind to mucin. The grafted antibody had about 1% the activity of the chimeric antibody.

In these experiments, however, it was noted that the activity of the grafted antibody could be increased to ~ 10% of B72.3 by exposure to pHs of 2-3.5.

This observation provided a clue as to how the activity of the grafted antibody could be improved without acid treatment. It was postulated that acid exposure brought about the protonation of an acidic residue (pKa of aspartic acid = 3.86 and of glutamine acid = 4.25) which in turn caused a change in structure of the CDR loops, or allowed better access of antigen.

From comparison of the sequences of B72.3 (ref. 13) and EU (refs. 4 and 5), it was clear that, in going from the mouse to human frameworks, only two positions had been changed in such a way that acidic residues had been introduced. These

positions are at residues 73 and 81, where K to E and Q to E changes had been made, respectively.

Which of these positions might be important was determined by examining the crystal structure of the KOL antibody. In KOL heavy chain (SEQ ID NO:10), position 81 is far removed from either of the CDR loops.

Position 73, however, is close to both CDRs 1 and 3 of the heavy chain and, in this position it was possible to envisage that a K to E change in this region could have a detrimental effect on antigen binding.

iii. Framework changes in B72.3 gH gene

On the basis of the above analysis, E73 was mutated to a lysine (K). It was found that this change had a dramatic effect on the ability of the grafted Ab to bind to mucin. Further the ability of the grafted B72.3 produced by the mutated gH/gL combination to bind to mucin was similar to that of the B72.3 chimeric antibody.

iv. Other framework changes

In the course of the above experiments, other changes were made in the heavy chain framework regions. Within the accuracy of the assays used, none of the changes, either alone or together, appeared beneficial.

v. Other

All assays used measured the ability of the grafted Ab to bind to mucin and, as a whole, indicated that the single framework change at position 73 is sufficient to generate an antibody with similar binding properties to B72.3.

Comparison of the B72.3 murine and EU heavy chain sequences reveals that the mouse and human residues are identical at positions 23, 24, 71 and 78.
Thus the mutated CDR-grafted B72.3 heavy chain corresponds to a preferred embodiment of the present invention.

EXAMPLE 4

CDR-GRAFTING OF A MURINE ANTI-ICAM-1 MONOCLONAL ANTIBODY

A murine antibody, R6-5-D6 (EP 0314863) having specificity for Intercellular Adhesion Molecule 1 (ICAM-1) was CDR-grafted substantially as described above in previous examples. This work is described in greater detail in co-pending application, British Patent Application No. 9009549.8, the disclosure of which is incorporated herein by reference.

The human EU framework was used as the acceptor framework for both heavy and light chains. The CDR-grafted antibody currently of choice is provided by co-expression of grafted light chain gL221A (SEQ ID NO: 26) and grafted heavy chain gH341D (SEQ ID NO: 16) which has a binding affinity for ICAM 1 of about 75% of that of the corresponding mouse-human chimeric antibody.

LIGHT CHAIN

gL221A has murine CDRs at positions 24-34 (CDR1), 50-56 (CDR2) and 89-97 (CDR3). In addition several framework residues are also the murine amino acid. These residues were chosen after consideration of the possible contribution of these residues to domain packing and stability of the conformation of the antigen binding region. The residues which have been retained as mouse are at positions 2, 3, 48 (?), 60, 84, 85 and 87. Comparison of the murine anti-ICAM 1 and human EU light chain amino acid sequences reveals that the murine and human residues are identical at positions 46, 58 and 71.

HEAVY CHAIN

gH341D has murine CDRs at positions 26-35 (CDR1), 50-56 (CDR2) and94-100B (CDR3). In addition murine residues were used in gH341D at positions 24, 48, 69, 71, 73, 80, 88 and 91. Comparison of the murine anti-ICAM 1 and human EU heavy chain amino acid sequences are identical at positions 23, 49 and 78.

EXAMPLE 5

CDR-Grafting of murine anti-TNFα antibodies

A number of murine anti-TNFα monoclonal antibodies were CDR-grafted substantially as described above in previous examples. These antibodies include the murine monoclonal antibodies designated 61 E71, hTNF1, hTNF3 and 101.4 A brief summary of the CDR-grafting of each of these antibodies is given below.

61E71

A similar analysis as described above (Example 1, Section 12.1.) was done for 61E71 and for the heavy chain 10 residues were identified at 23, 24, 48, 49, 68, 69, 71, 73, 75 and 88 as residues to potentially retain as murine. The human frameworks chosen for CDR-grafting of this antibody, and the hTNF3 and 101.4 antibodies were RE1 for the light chain and KOL for the heavy chain.
Three genes were built, the first of which contained 23, 24, 48, 49, 71 and 73 [gH341(6)] as murine residues. The second gene also had 75 and 88 as murine residues [gH341(8)] while the third gene additionally had 68, 69, 75 and 88 as murine residues [gH341(10)]. Each was co-expressed with gL221, the minimum grafted light chain (CDRs only). The gL221/gH341(6) and gL221/gH341(8) antibodies both bound as well to TNF as murine 61E71. The gL221/gH341(10) antibody did not express and this combination was not taken further.
Subsequently the gL221/gH341(6) antibody was assessed in an L929 cell competition assay in which the antibody competes against the TNF receptor on L929 cells for binding to TNF in solution. In this assay the gL221/gH341(6) antibody was approximately 10% as active as murine 61E71.

hTNF1

hTNF1 is a monoclonal antibody which recognises an epitope on human TNF- . The EU human framework was used for CDR-grafting of both the heavy and light variable domains.

Heavy Chain

In the CDR-grafted heavy chain (ghTNF1) mouse CDRs were used at positions 26-35 (CDR1), 50-65 (CDR2) and 95-102 (CDR3). Mouse residues were also used in the frameworks at positions 48, 67, 69, 71, 73, 76, 89, 91, 94 and 108. Comparison of the TNF1 mouse and EU human heavy chain residues reveals that these are identical at positions 23, 24, 29 and 78.

Light Chain

In the CDR-grafted light chain (gLhTNF1) mouse CDRs wre used at positions 24-34 (CDR1), 50-56 (CDR2) and 89-97 (CDR3). In addition mouse residues were used in the frameworks at positions 3, 42, 48, 49, 83, 106 and 108. Comparison of the hTNF1 mouse and EU human light chain residues reveals that these are identical at positions 46, 58 and 71.

The grafted hTNF1 heavy chain was co-expressed with the chimeric light chain and the binding ability of the product compared with that of the chimeric light chain/chimeric heavy chain product in a TNF binding assay. The grafted heavy chain product appeared to have binding ability for TNF slightly better than the fully chimeric product.

Similarly, a grafted heavy chain/grafted light chain product was co-expressed and compared with the fully chimeric product and found to have closely similar binding properties to the latter product.

hTNF3

hTNF3 recognises an epitope on human TNF-α. The sequence of hTNF3 shows only 21 differences compared to 61E71 in the light and heavy chain variable regions, 10 in the light chain (2 in the CDRs at positions 50, 96 and 8 in the framework at 1, 19, 40, 45, 46, 76, 103 and 106) and 11 in the heavy chain (3 in the CDR regions at positions 52, 60 and 95 and 8 in the framework at 1, 10, 38, 40, 67, 73, 87 and 105). The light and heavy chains of the 61E71 and hTNF3 chimeric antibodies can be exchanged without loss of activity in the direct binding assay. However 61E71 is an order of magnitude less able to compete with the TNF receptor on L929 cells for TNF-a compared to hTNF3. Based on the 61E71 CDR grafting data gL221 and gH341(+23, 24, 48, 49 71 and 73 as mouse) genes have been built for hTNF3 and tested and the resultant grafted antibody binds well to TNF-a, but competes very poorly in the L929 assay. It is possible that in this case also the framework residues identified for OKT3 programme may improve the competitive binding ability of this antibody.

101.4

101.4 is a further murine monoclonal antibody able to recognise human TNF-a. The heavy chain of this antibody shows good homology to KOL and so the CDR-grafting has been based on RE1 for the light chain and KOL for the heavy chain. Several grafted heavy chain genes have been constructed with conservative choices for the CDR's (gH341) (SEQ ID NO:11) and which have one or a small number of non-CDR residues at positions 73, 78 or 77-79 inclusive, as the mouse amino acids. These have been co-expressed with cL or gL221. In all cases binding to TNF equivalent to the chimeric antibody is seen and when co-expressed with cL the resultant antibodies are able to compete well in the L929 assay. However, with gL221 the resultant antibodies

are at least an order of magnitude less able to compete for TNF against the TNF receptor on L929 cells.

Mouse residues at other positions in the heavy chain, for example, at 23 and 24 together or at 76 have been demonstrated to provide no improvement to the competitive ability of the grafted antibody in the L929 assay.

A number of other antibodies including antibodies having specificity for interleukins e.g. IL1 and cancer markers such as carcinoembryonic antigen (CEA) e.g. the monoclonal antibody A5B7 (ref. 21), have been successfully CDR-grafted according to the present invention.
It will be appreciated that the foregoing examples are given by way of illustration only and are not intended to limit the scope of the claimed invention. Changes and modifications may be made to the methods described whilst still falling within the spirit and scope of the invention.

References

1. Kohler & Milstein, Nature, 265, 295-497, 1975.

2. Chatenoud et al, (1986), J. Immunol. 137, 830-838.

3. Jeffers et al, (1986), Transplantation, 41, 572-578.

4. Begent et al, Br. J. Cancer 62: 487 (1990).

5. Verhoeyen et al, Science, 239, 1534-1536, 1988.

6. Riechmann et al, Nature, 332, 323-324, 1988.

7. Kabat, E.A., Wu, T.T., Reid-Miller, M., Perry, H.M., Gottesman, K.S., 1987, in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA.

8. Wu, T.T., and Kabat, E.A., 1970, J. Exp. Med. 132 211-250.

9. Queen et al, (1989), Proc. Natl. Acad. Sci. USA, 86, 10029-10033 and WO 90/07861

10. Maniatis et al, Molecular Cloning, Cold Spring Harbor, New York, 1989.

11. Primrose and Old, Principles of Gene Manipulation, Blackwell, Oxford, 1980.

12. Sanger, F., Nicklen, S., Coulson, A.R., 1977, Proc. Natl. Acad. Sci. USA, 74 5463

13. Kramer, W., Drutsa, V., Jansen, H.-W., Kramer, B., Plugfelder, M., Fritz, H.-J., 1984, Nucl. Acids Res. 12, 9441

14. Whittle, N., Adair, J., Lloyd, J.C., Jenkins, E., Devine, J., Schlom, J., Raubitshek, A., Colcher, D., Bodmer, M., 1987, Protein Engineering 1, 499.

15. Sikder, S.S., Akolkar, P.N., Kaledas, P.M., Morrison, S.L., Kabat, E.A., 1985, J. Immunol. 135, 4215.

16. Wallick, S.C., Kabat, E.A., Morrison, S.L., 1988, J. Exp. Med. 168, 1099

17. Bebbington, C.R., Published International Patent Application WO 89/01036.

18. Granthan and Perrin 1986, Immunology Today 7, 160.

19. Kozak, M., 1987, J. Mol. Biol. 196, 947.

20. Jones, T.P., Dear, P.H., Foote, J., Neuberger, M.S., Winter, G., 1986, Nature, 321, 522

21. Harwood et al, Br. J. Cancer, 54, 75-82 (1986).

CLAIMS

1. A CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

2. A CDR-grafted heavy chain according to Claim 1 comprising donor residues at positions 23, 24, 49, 71, 73 and 78, or at positions 23, 24 and 49.

3. A CDR-grafted heavy chain according to Claim 2 comprising donor residues at positions 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

4. A CDR-grafted heavy chain according to Claim 2 or 3, comprising donor residues at one, some or all of positions:
1 and 3,
69 (if 48 is different between donor and acceptor),
38 and 46 (if 48 is the donor residue),
67,
82 and 18 (if 67 is the donor residue),
91, and
any one or more of 9, 11, 41, 87, 108, 110 and 112.

5. A CDR-grafted heavy chain according to any of the preceding comprising donor CDRs at positions 26-35, 50-65 and 95-100.

6. A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 1 and/or 3 and 46 and/or 47.

7. A CDR-grafted light chain according to Claim 6 comprising donor residues at positions 46 and 47.

8. A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, 58 and 71.

9. A CDR-grafted light chain according to Claim 8 comprising donor residues at positions 46, 48, 58 and 71.

10. A CDR-grafted light chain according to Claim 8 or 9, comprising donor residues at positions 2, 4, 6, 35, 36, 38, 44, 47, 49, 62, 64-69, 85, 87, 98, 99, 101 and 102.

11. A CDR-grafted light chain according to Claim 9 or 10, comprising donor residues at one, some or all of positions:
1 and 3,
63,
60 (if 60 and 54 are able to form a potential saltbridge),
70 (if 70 and 24 are able to form a potential saltbridge),
73 and 21 (if 47 is different between donor and acceptor),
37 and 45 (if 47 if different between donor and acceptor), and
any one or more of 10, 12, 40, 83, 103 and 105.

12. A CDR-grafted light chain according to any one of Claims 6-11, comprising donor CDRs at positions 24-34, 50-56 and 89-97.

13. A CDR-grafted antibody molecule comprising at least one CDR-grafted heavy chain according to any one of Claims 1-5 and at least one CDR-grafted light chain according to any one of Claims 6-12.

14. A CDR-grafted antibody molecule according to Claim 13, which is a site-specific antibody molecule.

15. A CDR-grafted antibody molecule according to Claim 13 which has specificity for an interleukin, hormone or other biologically active compound or a receptor therefor.

16. A CDR-grafted antibody heavy or light chain or molecule according to any one of the preceding claims comprising human acceptor residues and non-human donor residues.

17. A DNA sequence which codes for a CDR-grafted heavy chain according to Claim 1 or a CDR-grafted light chain according to Claim 6 or Claim 8.

18. A cloning or expression vector containing a DNA sequence according to Claim 17.

19. A host cell transformed with a DNA sequence according to Claim 17.

20. A process for the production of a CDR-grafted antibody sequence according to Claim 17 in a transformed host cell.

21. A process for producing a CDR-grafted antibody product comprising:

(a) producing in an expression vector an operon having a DNA sequence which encodes an antibody heavy chain according to Claim 1;

and/or

(b) producing in an expression vector an operon having a DNA sequence which encodes a complementary antibody light chain according to Claim 6 or Claim 8;

(c) transfecting a host cell with the or each vector; and

(d) culturing the transfected cell line to produce the CDR-grafted antibody product.

22. A therapeutic or diagnostic composition comprising a CDR-grafted antibody heavy chain according to Claim 1, or a CDR-grafted light chain according to Claim 6 or Claim 8, or a CDR-grafted antibody molecule according to Claim 13 in combination with a pharmaceutically acceptable carrier, diluent or excipient.

23. A method of therapy or diagnosis comprising administering an effective amount of a CDR-grafted heavy chain according to Claim 1, or a CDR-grafted light chain according to Claim 6 or Claim 8, or a CDR-grafted antibody molecule according to Claim 13 to a human or animal subject.

ABSTRACT

CDR-grafted antibody heavy and light chains comprise acceptor framework and donor antigen binding regions, the heavy chains comprising donor residues at at least one of positions (6, 23) and/or (24, 48) and/or (49, 71) and/or (73, 75) and/or (76) and/or (78) and (88) and/or (91). The CDR-grafted light chains comprise donor residues at at least one of positions (1) and/or (3) and (46) and/or (47) or at at least one of positions (46, 48, 58) and (71). The CDR-grafted antibodies are preferably humanised antibodies, having non human, e.g. rodent, donor and human acceptor frameworks, and may be used for *in vivo* therapy and diagnosis. A generally applicable protocol is disclosed for obtaining CDR-grafted antibodies.

```
   1  GAATTCCCAA AGACAAAatg gattttcaag tgcagatttt cagcttcctg
  51  ctaatcagtg cctcagtcat aatatccaga ggacaaattg ttctcaccca
 101  gtctccagca atcatgtctg catctccagg ggagaaggtc accatgacct
 151  gcagtgccag ctcaagtgta agttacatga actggtacca gcagaagtca
 201  ggcacctccc ccaaaagatg gatttatgac acatccaaac tggcttctgg
 251  agtccctgct cacttcaggg gcagtgggtc tgggacctct tactctctca
 301  caatcagcgg catggaggct gaagatgctg ccacttatta ctgccagcag
 351  tggagtagta acccattcac gttcggctcg gggacaaagt tggaaataaa
 401  ccgggctgat actgcaccaa ctgtatccat cttcccacca tccagtgagc
 451  agttaacatc tggaggtgcc tcagtcgtgt gcttcttgaa caacttctac
 501  cccaaagaca tcaatgtcaa gtggaagatt gatggcagtg aacgacaaaa
 551  tggcgtcctg aacagttgga ctgatcagga cagcaaagac agcacctaca
 601  gcatgagcag caccctcacg ttgaccaagg acgagtatga acgacataac
 651  agctatacct gtgaggccac tcacaagaca tcaacttcac ccattgtcaa
 701  gagcttcaac aggaatgagt gtTAGAGACA AAGGTCCTGA GACGCCACCA
 751  CCAGCTCCCA GCTCCATCCT ATCTTCCCTT CTAAGGTCTT GGAGGCTTCC
 801  CCACAAGCGC tTACCACTGT TGCGGTGCTC tAAACCTCCT CCCACCTCCT
 851  TCTCCTCCTC CTCCCTTTCC TTGGCTTTTA TCATGCTAAT ATTTGCAGAA
 901  AATATTCAAT AAAGTGAGTC TTTGCCTTGA AAAAAAAAAA AAA
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Fig. 1(a)

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   1  MDFQVQIFSF LLISASVIIS RGQIVLTQSP AIMSASPGEK VTMTCSASSS
  51  VSYMNWYQQK SGTSPKRWIY DTSKLASGVP AHFRGSGSGT SYSLTISGME
 101  AEDAATYYCQ QWSSNPFTFG SGTKLEINRA DTAPTVSIFP PSSEQLTSGG
 151  ASVVCFLNNF YPKDINVKWK IDGSERQNGV LNSWTDQDSK DSTYSMSSTL
 201  TLTKDEYERH NSYTCEATHK TSTSPIVKSF NRNEC*
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Fig. 1(b)

SUBSTITUTE SHEET

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   1 GAATTCCCCT CTCCACAGAC ACTGAAAACT CTGACTCAAC ATGGAAAGGC
  51 ACTGGATCTT TCTACTCCTG TTGTCAGTAA CTGCAGGTGT CCACTCCCAG
 101 GTCCAGCTGC AGCAGTCTGG GGCTGAACTG GCAAGACCTG GGGCCTCAGT
 151 GAAGATGTCC TGCAAGGCTT CTGGCTACAC CTTTACTAGG TACACGATGC
 201 ACTGGGTAAA ACAGAGGCCT GGACAGGGTC TGGAATGGAT TGGATACATT
 251 AATCCTAGCC GTGGTTATAC TAATTACAAT CAGAAGTTCA AGGACAAGGC
 301 CACATTGACT ACAGACAAAT CCTCCAGCAC AGCCTACATG CAACTGAGCA
 351 GCCTGACATC TGAGGACTCT GCAGTCTATT ACTGTGCAAG ATATTATGAT
 401 GATCATTACT GCCTTGACTA CTGGGGCCAA GGCACCACTC TCACAGTCTC
 451 CTCAGCCAAA ACAACAGCCC CATCGGTCTA TCCACTGGCC CCTGTGTGTG
 501 GAGATACAAC TGGCTCCTCG GTGACTCTAG GATGCCTGGT CAAGGGTTAT
 551 TTCCCTGAGC CAGTGACCTT GACCTGGAAC TCTGGATCCC TGTCCAGTGG
 601 TGTGCACACC TTCCCAGCTG TCCTGCAGTC TGACCTCTAC ACCCTCAGCA
 651 GCTCAGTGAC TGTAACCTCG AGCACCTGGC CCAGCCAGTC CATCACCTGC
 701 AATGTGGCCC ACCCGGCAAG CAGCACCAAG GTGGACAAGA AAATTGAGCC
 751 CAGAGGGCCC ACAATCAAGC CCTGTCCTCC ATGCAAATGC CCAGCACCTA
 801 ACCTCTTGGG TGGACCATCC GTCTTCATCT TCCCTCCAAA GATCAAGGAT
 851 GTACTCATGA TCTCCCTGAG CCCCATAGTC ACATGTGTGG TGGTGGATGT
 901 GAGCGAGGAT GACCCAGATG TCCAGATCAG CTGGTTTGTG AACAACGTGG
 951 AAGTACACAC AGCTCAGACA CAAACCCATA GAGAGGATTA CAACAGTACT
1001 CTCCGGGTGG TCAGTGCCCT CCCCATCCAG CACCAGGACT GGATGAGTGG
1051 CAAGGAGTTC AAATGCAAGG TCAACAACAA AGACCTCCCA GCGCCCATCG
1101 AGAGAACCAT CTCAAAACCC AAAGGGTCAG TAAGAGCTCC ACAGGTATAT
1151 GTCTTGCCTC CACCAGAAGA AGAGATGACT AAGAAACAGG TCACTCTGAC
1201 CTGCATGGTC ACAGACTTCA TGCCTGAAGA CATTTACGTG GAGTGGACCA
1251 ACAACGGGAA AACAGAGCTA AACTACAAGA ACACTGAACC AGTCCTGGAC
1301 TCTGATGGTT CTTACTTCAT GTACAGCAAG CTGAGAGTGG AAAAGAAGAA
1351 CTGGGTGGAA AGAAATAGCT ACTCCTGTTC AGTGGTCCAC GAGGGTCTGC
1401 ACAATCACCA CACGACTAAG AGCTTCTCCC GGACTCCGGG TAAATGAGCT
1451 CAGCACCCAC AAAACTCTCA GGTCCAAAGA GACACCCACA CTCATCTCCA
1501 TGCTTCCCTT GTATAAATAA AGCACCCAGC AATGCCTGGG ACCATGTAAA
1551  AAAAAAAAAA AAAGGAATTC
```

Fig. 2(a)

SUBSTITUTE SHEET

OKT 3 HEAVY CHAIN PROTEIN SEQUENCE DEDUCED FROM DNA SEQUENCE

```
  1  MERHWIFLLL LSVTAGVHSQ VQLQQSGAEL ARPGASVKMS CKASGYTFTR
 51  YTMHWVKQRP GQGLEWIGYI NPSRGYTNYN QKFKDKATLT TDKSSSTAYM
101  QLSSLTSEDS AVYYCARYYD DHYCLDYWGQ GTTLTVSSAK TTAPSVYPLA
151  PVCGDTTGSS VTLGCLVKGY FPEPVTLTWN SGSLSSGVHT FPAVLQSDLY
201  TLSSSVTVTS STWPSQSITC NVAHPASSTK VDKKIEPRGP TIKPCPPCKC
251  PAPNLLGGPS VFIFPPKIKD VLMISLSPIV TCVVVDVSED DPDVQISWFV
301  NNVEVHTAQT QTHREDYNST LRVVSALPIQ HQDWMSGKEF KCKVNNKDLP
351  APIERTISKP KGSVRAPQVY VLPPPEEEMT KKQVTLTCMV TDFMPEDIYV
401  EWTNNGKTEL NYKNTEPVLD SDGSYFMYSK LRVEKKNWVE RNSYSCSVVH
451  EGLHNHHTTK SFSRTPGK*
```

Fig. 2(b)

```
                1                       23                  42
                NN      N                 N         N     N
RES TYPE        SBspSPESssBSbSsSssPSPSPsPSsse*s*p*Pi^ISsSe
Okt3v1          QIVLTQSPAIMSASPGEKVTMTCSASS.SVSYMNWYQQKSGT
REI             DIQMTQSPSSLSASVGDRVTITCQASQDIIKYLNWYQQTPGK
                ? ?
                  CDR1    (LOOP)          *******
                  CDR1    (KABAT)        ***********

                          56                              85
                N  NN
RES TYPE        *IsiPpIeesesssSBEsePsPSBSSEsPspsPsseesSPePb
Okt3v1          SPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAAT
REI             APKLLIYEASNLQAGVPSRFSGSGSGTDYTFTISSLQPEDIAT
                ?  ??                          ?  ?
                      *******  CDR2 (LOOP/KABAT)

                               102   108
RES TYPE        PiPIPies**iPIIsPPSPSPSS
Okt3v1          YYCQQWSSNPFTFGSGTKLEINR
REIv1           YYCQQYQSLPYTFGQGTKLQITR
                              ?    ?
                    ******        CDR3 (LOOP)
                  *********       CRD3 (KABAT)
```

Fig. 3

SUBSTITUTE SHEET

```
          NN N                  23 26    32 35 N39  43
RES TYPE  SESPs^SBssS^sSSsSpSpSPsPSEbSBssBePiPIpiesss
Okt3h     QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQ
KOL       QVQLVESGGGVVQPGRSLRLSCSSSGFIFSSYAMYWVRQAPGK
               ?              ??
                                      ******    CDR1 (LOOP)
                                     ***** CDR1 (KABAT)

                  52a      60  65     N N  N    82abc    89
RES TYPE IIeIppp^sssssssss^ps^pSSsbSpseSsSseSp^pSpsSBssS^ePb
Okt3vh   GLEWIGYINPSRGYTNTNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAV
KOL      GLEWVAIIWDDGSDQHYADSVKGRFTISRDNSKNTLFLQMDSLRPEDTGV
           ??                      ? ?  ?  ?            ?
              ************          CDR2  (LOOP)
              ********************  CDR2  (KABAT)

           92 N                  107    113
RES TYPE  PiPIEisssiiisssbibi*EIPIP*spSBSS
Okt3vh    YYCARYYDDHY.......CLDYWGQGTTLTVSS
KOL       YFCARDGGHGFCSSASCFGPDYWGQGTPVTVSS
               ***************** CRD3 (KABAT/LOOP)
```

Fig. 4

SUBSTITUTE SHEET

OKT 3 HEAVY CHAIN CDR GRAFTS

1. gh341 and derivatives

	1 26 35 39 43	
Okt3vh	QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQ	
gH341	QVQLVESGGGVVQPGRSLRLSCSSSGYTFTRYTMHWVRQAPGK	JA178
gH341A	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK	JA185
gH341E	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK	JA198
gH341*	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK	JA207
gH341*	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK	JA209
gH341D	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK	JA197
gH341*	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK	JA199
gH341C	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK	JA184
gH341*	QVQLVQSGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK	JA203
gH341*	QVQLVESGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK	JA205
gH341B	QVQLVESGGGVVQPGRSLRLSCSSSGYTFTRYTMHWVRQAPGK	JA183
gH341*	QVQLVQSGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK	JA204
gH341*	QVQLVESGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK	JA206
gH341*	QVQLVQSGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK	JA208
KOL	QVQLVESGGGVVQPGRSLRLSCSSSGFIFSSYAMYWVRQAPGK	

Fig. 5(i)

SUBSTITUTE SHEET

```
          44    50              65                  83
Okt3vh    GLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLT
gH341     GLEWVAYINPSRGYTNYNQKFKDRFTISRDNSKNTLFLQMDSLR JA178
gH341A    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR JA185

gH341E    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR JA198
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKNTAFLQMDSLR JA207
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTAFLQMDSLR JA209
gH341D    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKNTLFLQMDSLR JA197
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTLFLQMDSLR JA199
gH341C    GLEWVAYINPSRGYTNYNQKFKDRFTISRDNSKNTLFLQMDSLR JA184

gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR JA207
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR JA205
gH341B    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR JA183
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR JA204
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR JA206
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKNTAFLQMDSLR JA208
KOL       GLEWVAIIWDDGSDQHYADSVKGRFTISRDNSKNTLFLQMDSLR
```

Fig. 5(ii)

SUBSTITUTE SHEET

	84 95 102 113	
Okt3vh	SEDSAVYYCARYYDDHY.....CLDYWGQGTTLTVSS	
gH341	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA178
gH341A	PEDTAVYYCARYYDDHY.....CLDYWGQGTTLTVSS	JA185
gH341E	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA198
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA207
gH341D	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA197
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA209
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA199
gH341C	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA184
gH341*	PEDTAVYYCARYYDDHY.....CLDYWGQGTTLTVSS	JA203
gH341*	PEDTAVYYCARYYDDHY.....CLDYWGQGTTLTVSS	JA205
gH341B	PEDTAVYYCARYYDDHY.....CLDYWGQGTTLTVSS	JA183
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA204
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA206
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA208
KOL	PEDTGVYFCARDGGHGFCSSASCFGPDYWGQGTPVTVSS	

Fig. 5(iii)

OKT3 LIGHT CHAIN CDR GRAFTING

1. gL221 and derivatives

```
          1                        24        34       42
Okt3vl    QIVLTQSPAIMSASPGEKVTMTCSASS.SVSYMNWYQQKSGT
gL221     DIQMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
gL221A    QIVMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
gL221B    QIVMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
gL221C    DIQMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
REI       DIQMTQSPSSLSASVGDRVTITCQASQDIIKYLNWYQQTPGK

          43      50     56                          85
Okt3vl    SPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAAT
gL221     APKLLIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
gL221A    APKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
gL221B    APKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
gL221C    APKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
REI       APKLLIYEASNLQAGVPSRFSGSGSGTDYTFTISSLQPEDIAT

          86   91   96          108
Okt3vl    YYCQQWSSNPFTFGSGTKLEINR
gL221     YYCQQWSSNPFTFGQGTKLQITR
gL221A    YYCQQWSSNPFTFGQGTKLQITR
gL221B    YYCQQWSSNPFTFGQGTKLQITR
gL221C    YYCQQWSSNPFTFGQGTKLQITR
REI       YYCQQYQSLPYTFGQGTKLQITR
```

CDR'S ARE UNDERLINED

FRAMEWORK RESIDUES INCLUDED IN THE GENE ARE DOUBLE UNDERLINED

Fig. 6

SUBSTITUTE SHEET

OKT3 - pJA198 EVALUATION
BINDING ASSAY
(Mean Channel - HPBALL's)

Fig. 7

OKT3 - pJA198 EVALUATION
BLOCKING ASSAY
(Mean Channel -HPBALL's)

Fig. 8

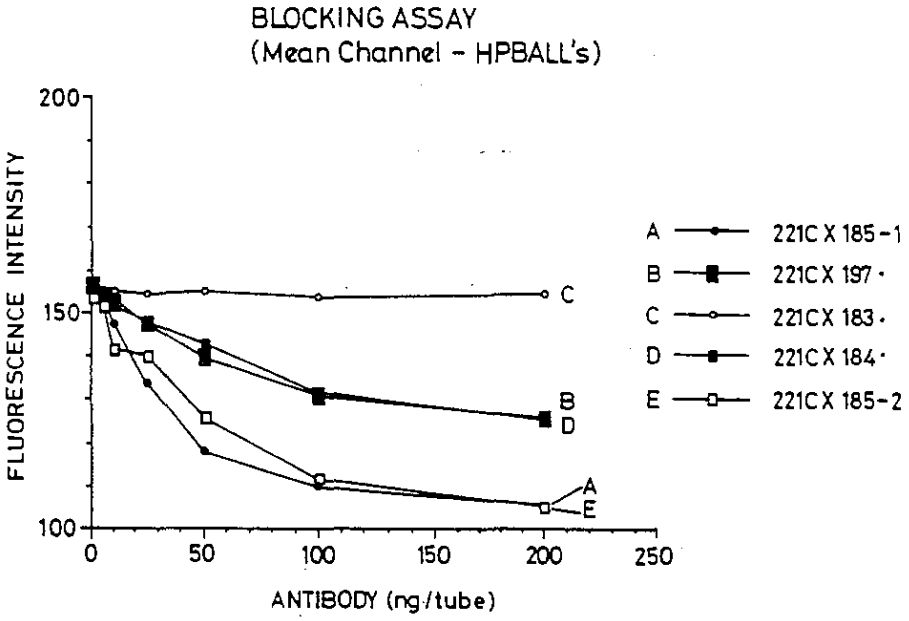
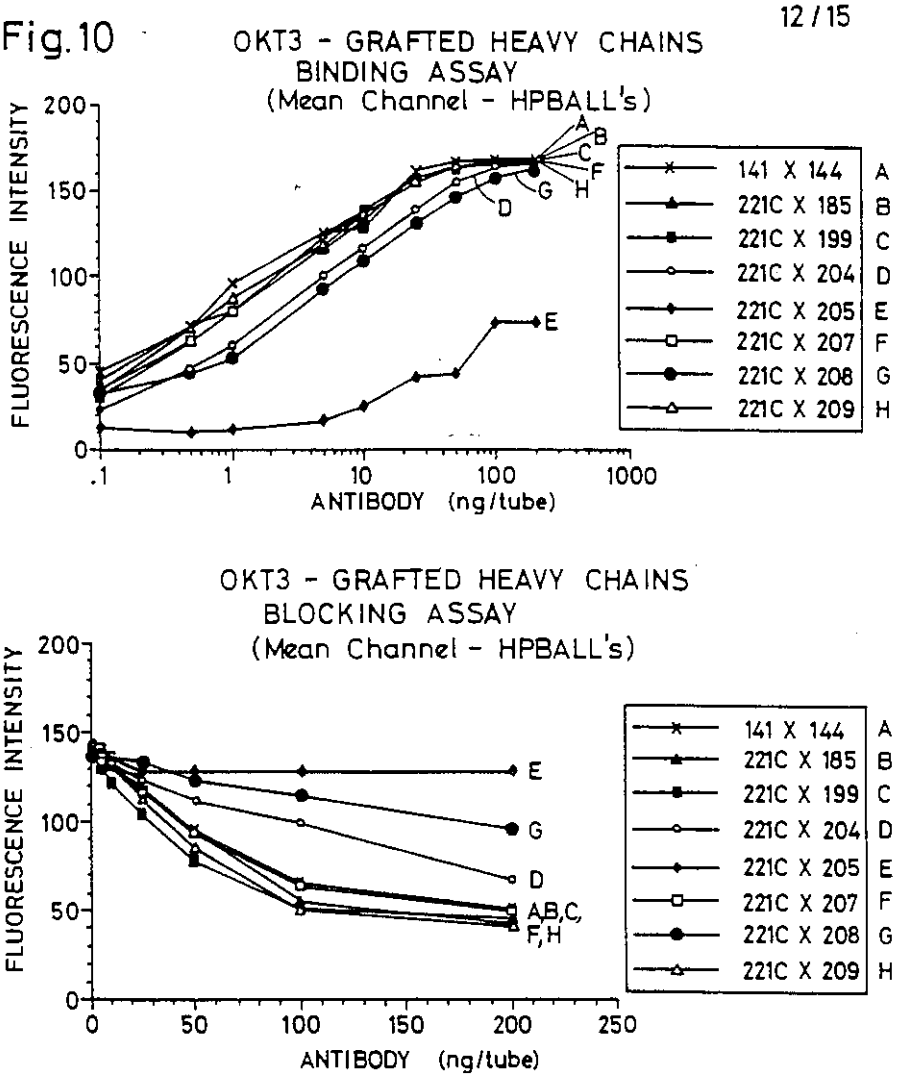
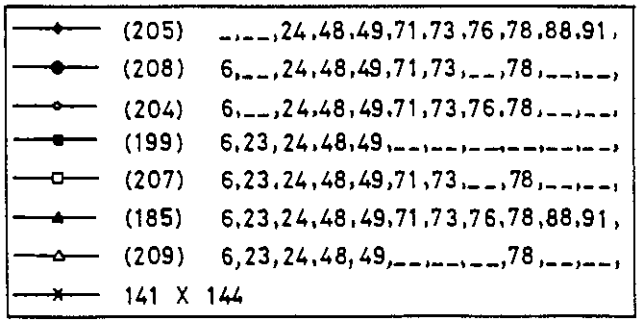


Fig. 9

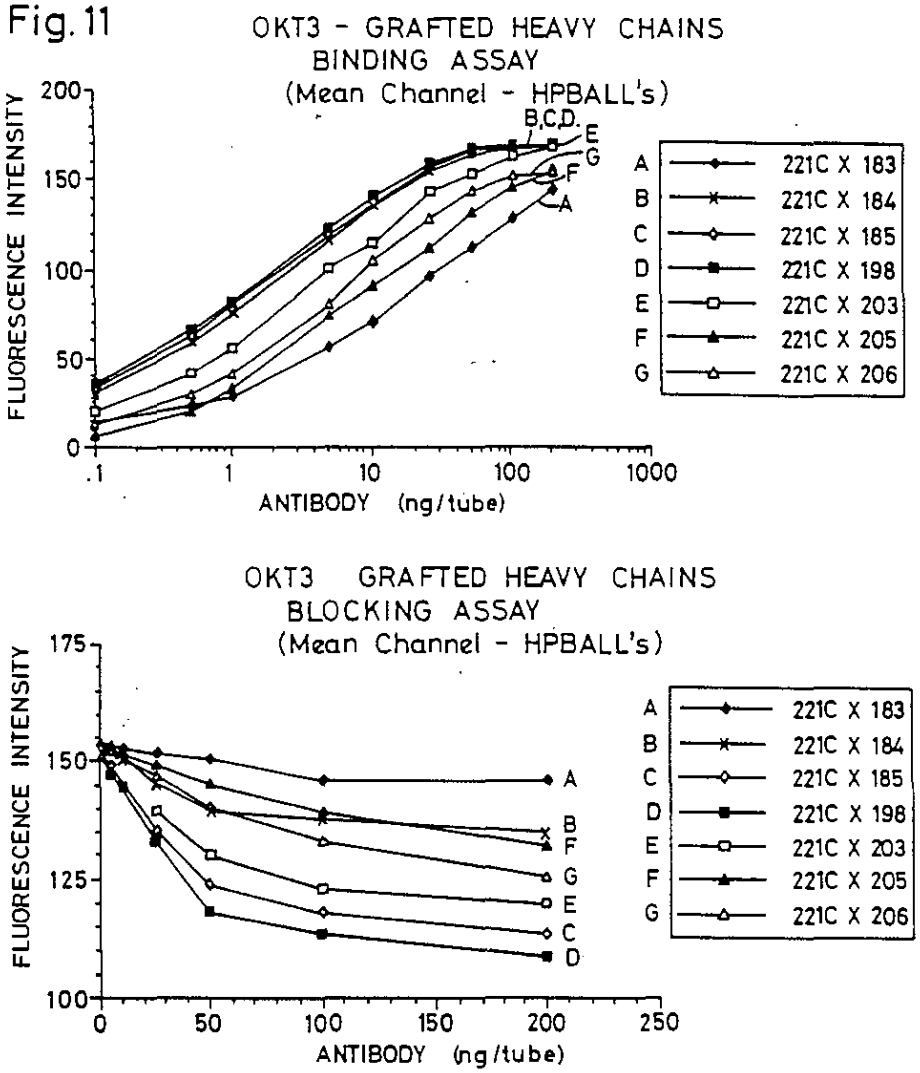
Fig.10





SUBSTITUTE SHEET

Fig. 11



(183)	_,_,_,_,_,48,49,71,73,76,78,88,91,
(205)	_,_,_,_,24,48,49,71,73,76,78,88,91,
(184)	6,23,24,_,_,_,_,_,_,_,_,_,_,_,_,_,
(206)	_,_,_,_,24,48,49,71,73,76,78,_,_,_,_,
(203)	6,_,_,_,24,48,49,71,73,76,78,88,91,
(185)	6,23,24,48,49,71,73,76,78,88,91,
(198)	6,23,24,48,49,71,73,76,78,_,_,_,_,

SUBSTITUTE SHEET

Fig. 12

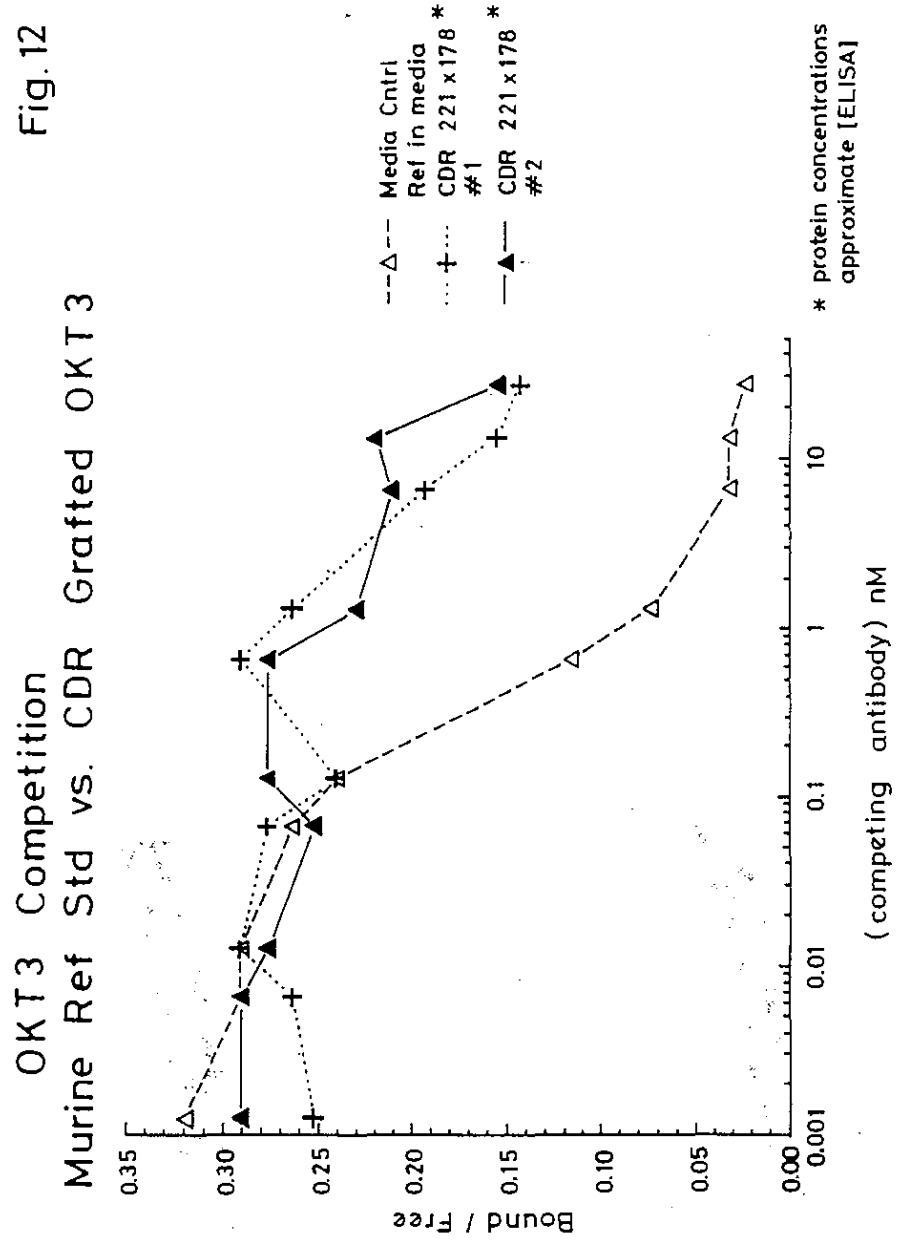
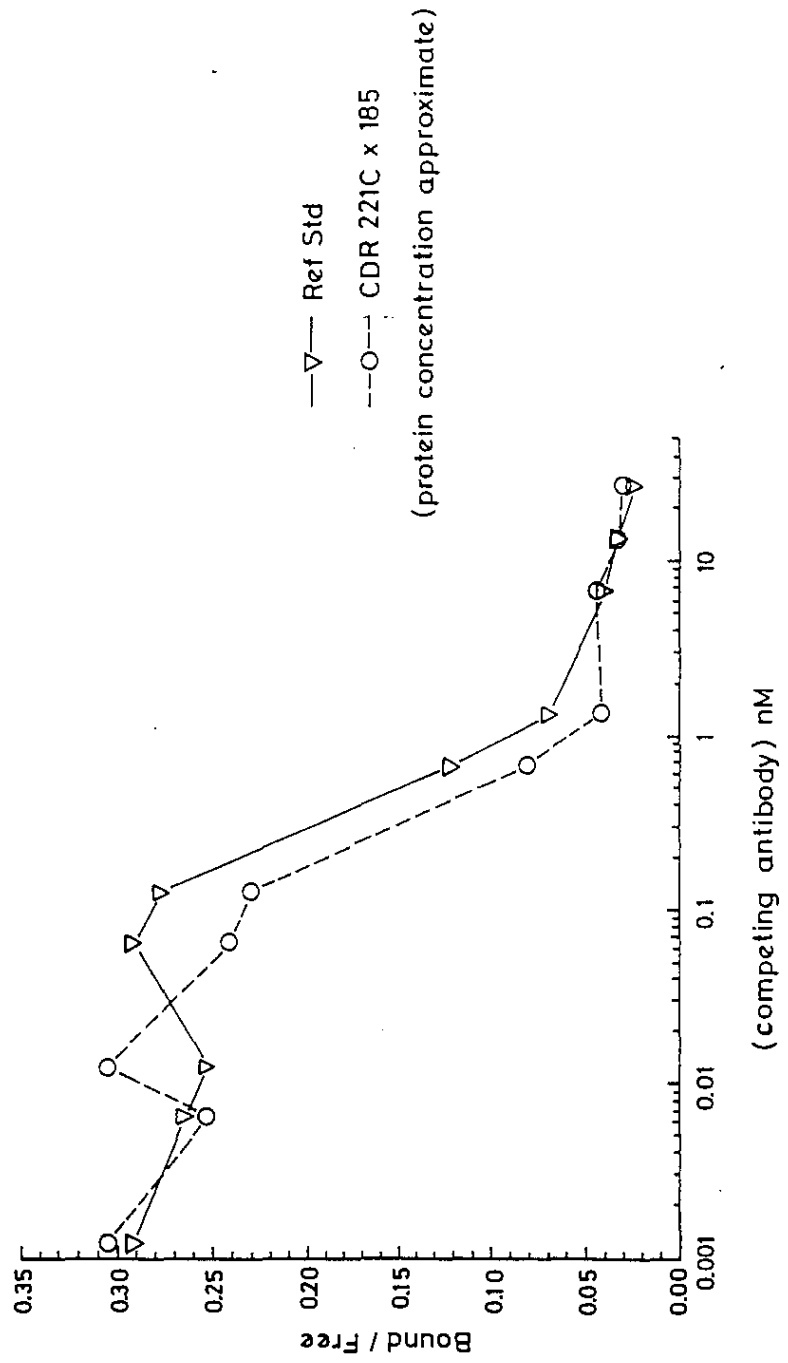

OKT3 Competition
Murine Ref Std vs. CDR Grafted OKT3
Media Cntrl
Ref in media
CDR 221 x178 * #1
CDR 221 x178 * #2
* protein concentrations approximate [ELISA]
Bound / Free
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0.001
0.01
0.1
1
10
(competing antibody) nM

Fig. 13

OKT3 Competition
Murine Ref Std vs. CDR Grafted OKT3



#4

FORM PTO-1390 (REV. 5-87) — U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)

ATTORNEY'S DOCKET NUMBER: CARP-0009

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB90/02017	21 December 1990	21 December 1989

TITLE OF INVENTION: HUMANISED ANTIBODIES

APPLICANT(S) FOR DO/EO/US: ADAIR, John, Robert, ATHWAL, Diljeet, Singh, and EMTAGE, John, Spencer

Applicant herewith submits to the United States Designated/ Elected Office (DO/EO/US) the following items under 35 U.S.C. 371:

1. ☐ This express request to immediately begin national examination procedures (35 U.S.C. 371(f)).
2. ☑ The U.S. National Fee (35 U.S.C. 371(c)(1)) and other fees as follows:

CLAIMS	(1) FOR	(2) NUMBER FILED	(3) NUMBER EXTRA	(4) RATE	(5) CALCULATIONS
	TOTAL CLAIMS	-20-		X$ 20.00	$
	INDEPENDENT CLAIMS	-3-		X$ 60.00	
	MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+$ 200.00	
	BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(4)): ☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) ..... $ 330 ☐ No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445 (a)(2)) ..... $ 370 ☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO..... $ 500 ☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2) to (4)..... $ 50				
	Surcharge of $120 for furnishing the National fee or oath or declaration later than ☑20 ☐30 mos. from the earliest claimed priority date (37 CFR 1.492(e)).				$120.00
			TOTAL OF ABOVE CALCULATIONS		-$120.00
	Reduction by 1/2 for filing by small entity, if applicable. Affidavit musts be filed also. (Note 37 CFR 1.9, 1.27, 1.28.)				$60.00
			SUBTOTAL		+$60.00
	Processing fee of $30 for furnishing the English Translation later than ☐20 ☐30 mos. from the earliest claimed priority date (37 CFR 1.492(f)).				
			TOTAL NATIONAL FEE		$ 60.00
	Fee for recording the enclosed assignment (37 CFR 1.21(h)).				+
			TOTAL FEES ENCLOSED		$ 60.00

a. ☒ A check in the amount of $ 60.00 to cover the above fees is enclosed.
b. ☐ Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.
c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 23-3050. A duplicate copy of this sheet is enclosed.

10/16/91X060 * 00238-00257*

01U3329

25-9 60

"EXPRESS MAIL" Mailing Label No. RB567260177
Date of Deposit September 17, 1991
I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231

Diane M. Kushner
(Typed)
Diane M. Kushner
(Signature)

ATTORNEY'S DOCKET NUMBER
CARP-0009

3. A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
   c. ☐ has been transmitted by the International Bureau.
4. ☐ A translation of the International Application into English (35 U.S.C. 371 (c)(2)).
5. Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
6. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
7. ☒ An oath or declaration of the inventor (35 U.S.C. 371(c)(4)).
8. ☐ A translation of the Annexes to the International Preliminary Examination Report under PCT Article 36(35 U.S.C. 371(c)(5)).

Other document(s) or information included:

9. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
10. ☐ An assignment document for recording.
    Please mail the recorded assignment document to:
    a. ☐ the person whose signature, name & address appears at the bottom of this page.
    b. ☐ the following:

11. The above checked items are being transmitted
    a. ☐ before the 18th month publication.
    b. ☐ after publication and the Article 20 communication but before 20 months from the priority date.
    c. ☒ after 20 months but before 22 months (surcharge and/or processing fee included).
    d. ☐ after 22 months (surcharge and/or processing fee included).
       Note: Petition to revive (37 CFR 1.137(a) or (b)) is necessary if 35 U.S.C. 371 requirements submitted after 22 months *and no proper demand for International Preliminary Examination was made by 19 months from the earliest claimed priority date.*
    e. ☐ by 30 months and a proper demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
    f. ☐ after 30 months but before 32 months and a proper demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date (surcharge and/or processing fee included).
    g. ☐ after 32 months (surcharge and/or processing fee included).
       Note: Petition to revive (37 CFR 1.137(a) or (b)) is necessary if 35 U.S.C. 371 requirements submitted after 32 months and a proper demand for International Preliminary Examination was made by 19 months from the earliest claimed priority date.
12. At the time of transmittal, the time limit for amending claims under Article 19
    a. ☐ has expired and no amendments were made.
    b. ☐ has not yet expired.
13. ☐ Certain requirements under 35 U.S.C. 371 were previously submitted by the applicant on _____ (date), namely:
14. Submission of Verified Statement Claiming Small Entity Status and Request for Refund

Francis A. Paintin
NAME

Woodcock Washburn Kurtz Mackiewicz & Norris
ADDRESS One Liberty Place - 46th Floor

Philadelphia, PA 19103

SIGNATURE 19,386 REGISTRATION NUMBER

Form PTO-1390 (Rev. 8-87) USCOMM-DC-87-3805

#54

DOCKET NO.: CARP-0009 **PATENT**

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name; and

I verily believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: HUMANISED ANTIBODIES the specification of which:

___ is attached hereto.

✓ International was filed on 21 December 1990 as Application Serial No. PCT/GB90/02017 and was amended on _____. (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with 37 CFR § 1.56(a).

I hereby claim foreign priority benefits under 35 U.S.C. § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of any application on which priority is claimed:

Country	Number	Date Filed	Priority Claimed
U.K.	8928874.0	21.12.89	yes
			___
			___

PTO-19

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose material information as defined in 37 CFR § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	**Filing Date**	**Status (patented, pending)**

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

Francis A. Paintin

Registration Nos. 19,386

of the firm of **WOODCOCK WASHBURN KURTZ MACKIEWICZ & NORRIS**, One Liberty Place - 46th Floor, Philadelphia, Pennsylvania 19103, and

Address all telephone calls and correspondence to:

Francis A. Paintin

WOODCOCK WASHBURN KURTZ MACKIEWICZ & NORRIS
One Liberty Place - 46th Floor
Philadelphia, PA 19103
Telephone No. **215-568-3100.**

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the

United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-00	**Full Name** JOHN ROBERT ADAIR	**Inventor's Signature** John Robert Adair	**Date** 13/8/91
1	**Residence** 23 George Road, Stokenchurch, High Wycombe, Buckinghamshire HP14 3RN, U.K. GBX	**Citizenship** U.K.	
	Post Office Address 23 George Road, Stokenchurch, High Wycombe, Buckinghamshire HP14 3RN, U.K.		
2-00	**Full Name** DILJEET SINGH ATHWAL	**Inventor's Signature**	**Date** 13/8/91
2	**Residence** Flat 35, Knollys House, Tavistock Square, London WC1, U.K. GBX	**Citizenship** U.K.	
	Post Office Address Flat 35, Knollys House, Tavistock Square, London WC1, U.K.		
3-00	**Full Name** JOHN SPENCER EMTAGE	**Inventor's Signature** John Spencer Emtage	**Date** 13/8/91
3	**Residence** 49 Temple Mill Island, Temple Marlow, Buckinghamshire, SL7 1SQ, U.K. GBX	**Citizenship** U.K.	
	Post Office Address 49 Temple Mill Island, Temple, Marlow, Buckinghamshire SL7 1SQ, U.K.		
4	**Full Name**	**Inventor's Signature**	**Date**
	Residence	**Citizenship**	
	Post Office Address		
5	**Full Name**	**Inventor's Signature**	**Date**
	Residence	**Citizenship**	
	Post Office Address		

#15

DOCKET NO.: CARP-0009 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

John R. Adair et al.

Serial No.: 07/743,329 Group No.: 1807

Filed: August 16, 1991 Examiner: L. Bennett

For: HUMANISED ANTIBODIES

Certificate of Facsimile Transmission

I hereby certify that this paper is being facsimile transmitted to the Patent and Trademark Office on the date shown below.

On January 19, 1993

Dawn L. McMahon

Commissioner of Patents & Trademarks
Washington, DC 20231

Sir:

RESPONSE TO OFFICE ACTION

This is in response to the Office Action of November 18, 1992, the time for a response to which is set to expire on January 18, 1993. January 18, 1993 is a Federal Holiday in the District of Columbia (observance of Martin Luther King, Jr.'s birthday). This response is timely filed under 37 CFR §1.7 since it is filed on January 19, 1993, the next succeeding day that is not a Saturday, Sunday or Federal Holiday within the District of Columbia. Please amend the above-identified patent application as follows.

In the claims:

21. (amended) A process for producing [a CDR-grafted antibody product] an antigen-binding molecule comprising:

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(a) producing in an expression vector an operon having a DNA sequence which encodes a heavy chain according to [Claim 1] Claim 24 or Claim 25;

[and/or]

(b) producing in an expression vector an operon having a DNA sequence which encodes a complementary light chain according to Claim [6] 42 or Claim [8] 43;

(c) transfecting a host cell with the [or each vector] expression vectors of steps (a) and (b) to form a transfected cell containing said expression vectors;

and

(d) culturing [the] said transfected cell [line] to produce [the CDR-grafted antibody product] said antigen binding molecule.

Please cancel claims 1-20, 22 and 23 without prejudice and substitute therefor the following new claims.

24. An antigen-binding molecule having affinity for a predetermined antigen, comprising a heavy chain and a complementary light chain, said heavy chain having a variable domain comprising framework regions from an acceptor antibody heavy chain and antigen binding regions from the heavy chain of a donor antibody which has affinity for said predetermined antigen, wherein amino acid residues 23, 24, 31 to 35, 49 to 58 and 95 to

102, according to the Kabat numbering system, of said heavy chain are donor antibody residues.

25. The antigen binding molecule of claim 24 wherein residues 71, 73 and 78, according to the Kabat numbering system, in the heavy chain are additionally donor antibody residues.

26. The antigen-binding molecule of claim 24, wherein residues 26 to 30, according to the Kabat numbering system, in the heavy chain are additionally donor antibody residues.

27. The antigen-binding molecule of claim 25, wherein residues 26 to 30, according to the Kabat numbering system, in the heavy chain are additionally donor antibody residues.

B2 cont.

28. The antigen-binding molecule of claim 24, wherein residues 59 to 65, according to the Kabat numbering system, in the heavy chain are additionally donor antibody residues.

29. The antigen-binding molecule of claim 25, wherein residues 59 to 65, according to the Kabat numbering system, in the heavy chain are additionally donor antibody residues.

30. The antigen-binding molecule of claim 24, wherein residues 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107, according to the Kabat numbering system, in the heavy chain are additionally donor antibody residues.

31. The antigen-binding molecule of claim 25, wherein residues 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106

and 107, according to the Kabat numbering system, in the heavy chain are additionally donor antibody residues.

32. The antigen-binding molecule of claim 30, wherein, according to the Kabat numbering system, if residue 48 in the donor antibody heavy chain is different from residue 48 in the acceptor antibody heavy chain, then residue 69 in the heavy chain is additionally a donor antibody residue.

33. The antigen-binding molecule of claim 31, wherein, according to the Kabat numbering system, if residue 48 in the donor antibody heavy chain is different from residue 48 in the acceptor antibody heavy chain, then residue 69 in the heavy chain is additionally a donor antibody residue.

B2 cont.

34. The antigen-binding molecule of claim 30, wherein residues 38 and 46, according to the Kabat numbering system, in the heavy chain are additionally donor antibody residues.

35. The antigen-binding molecule of claim 31, wherein residues 38 and 46, according to the Kabat numbering system, in the heavy chain are additionally donor antibody residues.

36. The antigen-binding molecule of claim 32, wherein residues 20 and 80, according to the Kabat numbering system, in the heavy chain are additionally donor antibody residues.

37. The antigen-binding molecule of claim 33, wherein residues 20 and 80, according to the Kabat numbering system, in the heavy chain are donor antibody residues.

38. The antigen-binding molecule of claim 30, wherein residues 18, 67 and 82, according to the Kabat numbering system, in the heavy chain are donor antibody residues.

39. The antigen-binding molecule of claim 31, wherein residues 18, 67 and 82, according to the Kabat numbering system, in the heavy chain are donor antibody residues.

40. The antigen-binding molecule of claim 30, wherein residues 1, 3, 9, 11, 41, 72, 76, 87, 88, 91, 108, 110 and 112, according to the Kabat numbering system, in the heavy chain are donor antibody residues.

B2 cont.

41. The antigen-binding molecule of claim 31, wherein residues 1, 3, 9, 11, 41, 72, 76, 87, 88, 91, 108, 110 and 112, according to the Kabat numbering system, in the heavy chain are donor antibody residues.

42. An antigen-binding molecule having affinity for a predetermined antigen, comprising a light chain and a complementary heavy chain, the light chain having a variable domain comprising framework regions from an acceptor antibody light chain and antigen binding regions from the light chain of a donor antibody which has affinity for said predetermined antigen, wherein amino acid residues 24 to 34, 46, 48, 50 to 56, 58, 71 and 89 to 97, according to the Kabat numbering system, are donor antibody residues.

43. The antigen-binding molecule of claim 42, wherein residues 2, 4, 6, 35, 38, 44, 47, 49, 64 to 69, 85, 87, 98, 99, 101 and 102, according to the Kabat numbering system, in the heavy chain are donor antibody residues.

44. The antigen-binding molecule of claim 42, wherein, according to the Kabat numbering system, if residue 60 in the light chain can form a salt bridge with residue 54 in the light chain, then residue 60 in the light chain is a donor antibody residue.

B2 cont. 45. The antigen-binding molecule of claim 42, wherein, according to the Kabat numbering system, if residue 70 in the light chain can form a salt bridge with residue 24 in the light chain, then residue 70 in the light chain is a donor antibody residue.

46. The antigen-binding molecule of claim 43, wherein, according to the Kabat numbering system, residues 21 and 73 in the light chain are additionally donor antibody residues.

47. The antigen-binding molecule of claim 43, wherein, according to the Kabat numbering system, residues 37 and 45 in the light chain are additionally donor antibody residues.

48. The antigen-binding molecule of claim 43, wherein residues 1, 3, 10, 12, 40, 63, 80, 103 and 105, according to the Kabat numbering system, in the light chain are additionally donor antibody residues.

49. An antigen-binding molecule having affinity for a predetermined antigen, comprising a heavy chain and a complementary light chain:

the heavy chain having a variable domain comprising framework regions from an acceptor antibody heavy chain and antigen binding regions from the heavy chain of a donor antibody which has affinity for said predetermined antigen, wherein amino acid residues 23, 24, 31 to 35, 49 to 58 and 95 to 102, accordingly to the Kabat numbering system, are donor antibody residues; and

B2 Cont.

the complementary light chain having a variable domain comprising framework regions from an acceptor antibody light chain and antigen binding regions from said donor antibody light chain wherein amino acid residues 24 to 34, 46, 48, 50 to 56, 58, 71 and 89 to 97 according to the Kabat numbering system are donor antibody residues.

50. The antigen-binding molecule of claim 49 wherein residues 71, 73, 78 and 95 to 102, according to the Kabat numbering system, of said heavy chain are additionally donor antibody residues.

51. The antigen-binding molecule of claim 49 or claim 50, which is a site specific antibody molecule.

52. The antigen-binding molecule of claim 49 or claim 50, which has specificity for an antigen selected from the group

consisting of interleukins, hormones, biologically active compounds and receptors therefor.

53. A DNA molecule which encodes an antigen-binding molecule heavy chain having a variable domain comprising framework regions from the heavy chain of a donor antibody which has affinity for said predetermined antigen wherein amino acid residues 23, 24, 31 to 35, 49 to 58 and 95 to 102 of said heavy chain, according to the Kabat numbering system, are donor antibody residues.

54. The DNA molecule of claim 53 wherein residues 71, 73 and 78, according to the Kabat numbering system, of said heavy chain are additionally donor antibody residues.

53 cont.

55. A DNA molecule which encodes an antigen-binding molecule light chain having a variable domain comprising framework regions from an acceptor antibody light chain and antigen binding regions from the light chain of an antibody having affinity for a predetermined antigen wherein amino acid residues 24 to 34, 46, 48, 50 to 56, 58, 71 and 89 to 97, according to the Kabat numbering system, of said light are donor antibody residues.

56. A cloning vector comprising a DNA molecule according to claims 53, 54 or 55.

57. An expression vector comprising a DNA molecule according to claims 53, 54 or 55.

58. A host cell transformed with a DNA molecule according to claims 53, 54 or 55.

59. A therapeutic composition comprising a therapeutically effective amount of an antigen-binding molecule according to claims 24, 25 or 42 in combination with a pharmaceutically acceptable carrier.

60. A therapeutic composition comprising a therapeutically effective amount of an antigen-binding molecule according to claim 49 or claim 50 in combination with a pharmaceutically acceptable carrier.

B2 Cont.

61. A diagnostic composition comprising a diagnostically effective amount of an antigen-binding molecule according to claims 24, 25 or 42 in combination with a diagnostically acceptable carrier.

62. A diagnostic composition comprising a diagnostically effective amount of an antigen-binding molecule according to claim 49 or claim 50 in combination with a diagnostically acceptable carrier.

63. A method of therapy comprising administering to a human or animal subject an antigen-binding molecule according to claims 24, 25 or 42.

64. A method of diagnosis comprising administering to a human or animal subject a composition according to claims 24, 25 or 42.

65. A method for producing an antigen-binding molecule having affinity for a predetermined antigen comprising the steps of:

(1) providing a heavy chain for an antigen-binding molecule, said heavy chain having acceptor framework regions and donor antigen binding regions wherein amino acid residues 23, 24, 31 to 35, 49 to 58 and 95 to 102 according to the Kabat numbering system are donor antibody residues;

(2) associating the heavy chain of step (1) with a complementary light chain to form an antigen-binding molecule;

(3) determining the affinity of the antigen-binding molecule formed in step (2) for said predetermined antigen;

B2 cont.

(4) if the affinity determined in step (3) is not equivalent to that of the donor antibody, providing a heavy chain as described in step (1) in which amino acid residues 71, 73 and 78, according to the Kabat numbering system, are additionally donor antibody residues;

(5) associating the heavy chain of step (4) with a complementary light chain to form an antigen-binding molecule;

(6) determining the affinity of the antigen-binding molecule formed in step (5) for said predetermined antigen;

(7) if the affinity determined in step (6) is not equivalent to that of the donor antibody, providing a heavy chain as described in step (4) in which amino acid residues 26 to 30,

according to the Kabat numbering system, are additionally donor antibody residues;

(8) associating the heavy chain of step (7) with a complementary light chain to form an antigen-binding molecule;

(9) determining the affinity of the antigen-binding molecule formed in step (8) for said predetermined antigen;

(10) if the affinity determined in step (9) is not equivalent to that of the donor antibody, providing a heavy chain as described in step (7) in which amino acid residues 59 to 65, according to the Kabat numbering system, are additionally donor antibody residues;

B2 Cont.

(11) associating the heavy chain of step (10) with a complementary light chain to form an antigen-binding molecule;

(12) determining the affinity of the antigen-binding molecule formed in step (11) for said predetermined antigen;

(13) if the affinity determined in step (12) is not equivalent to that of the donor antibody, providing a heavy chain as described in step (10) in which amino acid residues 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107, according to the Kabat numbering system, are additionally donor antibody residues; and

(14) associating the heavy chain produced in step (13) with a complementary light chain to form an antigen-binding molecule.

66. A method for producing an antigen-binding molecule having affinity for a predetermined antigen comprising the steps of:

(1) providing a light chain for an antigen-binding molecule, said light chain having acceptor framework regions and donor antigen binding regions wherein amino acid residues 24 to 34, 46, 48, 50 to 56, 58, 71 and 89 to 97 according to the Kabat numbering system are donor antibody residues;

(2) associating the light chain of step (1) with a complementary heavy chain to form an antigen-binding molecule;

(3) determining the affinity of the antigen-binding molecule formed in step (2) for said predetermined antigen;

B2 Cont.

(4) if the affinity determined in step (3) is not equivalent to that of the donor antibody, providing a light chain as described in step (1) in which amino acid residues 2, 4, 6, 35, 38, 44, 47, 49, 64 to 69, 85, 87, 98, 99, 101 and 102, according to the Kabat numbering system, are additionally donor antibody residues;

(5) associating the light chain of step (4) with a complementary heavy chain to form an antigen-binding molecule;

(6) determining the affinity of the antigen-binding molecule formed in step (5) for said predetermined antigen;

(7) if the affinity determined in step (6) is not equivalent to that of the donor antibody, providing a light chain

as described in step (4) in which amino acid residues 21, 37, 45, 60, 70 and 73, accordingly to the Kabat numbering system, are additionally donor antibody residues;

(8) associating the light chain of step (7) with a complementary heavy chain to form an antigen-binding molecule;

(9) determining the affinity of the antigen-binding molecule formed in step (8) for said predetermined antigen;

(10) if the affinity determined in step (9) is not equivalent to that of the donor antibody, providing a light chain as described in step (7) in which amino acid residues 1, 3, 10, 12, 40, 63, 80, 103 and 105, according to Kabat numbering system, are additionally donor antibody residues; and

(11) associating the light chain of step (10) with a complementary heavy chain to form an antigen-binding molecule.

REMARKS

The Office Action of November 18, 1992 has been carefully considered and this response prepared. Claims 1-20, 22 and 23 have been cancelled without prejudice and replaced with new claims 24-66. Claim 21 has been amended to more clearly point out aspects of the claimed method. Support for the newly submitted claims can be found throughout the specification, particularly at pages 17 and 18, page 19, line 23, page 41, Table 1 (each relating to the heavy chain), pages 17-19 (relating to

the light chain), and pages 17-23 (relating to methods for production of antigen-binding molecules).

At paragraph 15 of the Office Action, the Examiner objected to the disclosure, stating that various words in the specification are misspelled.

It is respectfully submitted that the spellings referred to by the Examiner are not incorrect. The use of an "s" instead of a "z" in such words as "recognise" is acceptable English spelling. In fact, the use of "s" is the original English spelling and the use of "z" has only more recently been recognized as being acceptable. In this respect, the Examiner is referred to the enclosed page from Chambers 20th Century Dictionary (1973 Edition) which illustrates this point. Therefore, no amendments have been made. Withdrawal of this objection to the specification is respectfully requested.

At paragraph 16 of the Office Action, the Examiner objected to claims 5, 11-16, 22 and 23 under 37 CFR §1.75(c) as being in improper form because a multiple dependent claims cannot depend from any other multiple dependent claim.

Claims 5, 11-16, 22 and 23 have been cancelled without prejudice and substituted with new claims having proper dependent form. Withdrawal of this objection to the specification is respectfully requested.

At paragraph 17 of the Office Action, the Examiner objected to claims 1-23 stating that the term "CDR-grafted" is improper because abbreviations and acronyms are not appropriate in claim language.

The term CDR-grafted has been deleted from the claims. Withdrawal of this objection to the claims is respectfully requested.

At paragraph 18 of the Office Action, the Examiner rejected claims 1-12 under 35 USC §101. This rejection is divided into four parts which will be answered separately.

In Part A of this section 101 rejection, claims 1-12 were rejected as being inoperative and thus lacking utility. It was stated that the claims are drawn to single heavy or light chains and that there is no evidence in the specification indicating that heavy and light chains alone have activity.

Applicants respectfully traverse this rejection.

It is respectfully submitted that there is no reason to show that the isolated antibody heavy and light chains of the present invention have utility in binding to antigen. As the Examiner has correctly pointed out, the assembled antibodies of the invention have been shown to be able to bind to antigen. What has also been shown is that the isolated heavy and light chains have utility in that they can be used to form the antigen-binding antibody. Thus, the isolated chains have patentable

utility as intermediates for use in CDR-grafted antibodies. There is no requirement that the claimed invention be the ultimate product.

Nonetheless, in order to reduce the number of issues to be dealt with, claims 1-12 have been cancelled without prejudice and new claims have been submitted that are drawn to antibody-binding molecules having heavy and light chains. However, the Applicants reserve the right to file a divisional application relating to the isolated chains.

In Part B of this section 101 rejection, claim 17 was rejected as being drawn to non-statutory subject matter. The Examiner stated that claim 17 is drawn to a DNA sequence coding for a CDR-grafted heavy of light chain, but that "DNA sequences" are not patentable because they are algorithms. The Examiner suggested amending the claims to recite "DNA molecule" instead of "DNA sequence".

Applicants respectfully traverse this rejection. An algorithm is a procedure for solving a given type of mathematical problem. DNA sequences are chemical compounds. Thus the prohibition of section 101 against granting patents on mathematical algorithms does not apply to DNA sequences.

Applicants respectfully submit that the term DNA sequence is proper claim language. This language appears in the claims of issued patents as well as the rules issued by the.

Patent and Trademark Office for applications containing nucleic acid and/or amino acid sequences (see 37 CFR §§1.821 - 1.825). In order to advance prosecution of this application, however, the term "DNA molecule" has been substituted for "DNA sequence" in the claims.

In part C of this section 101 rejection, the Examiner rejected claims 22-23 as inoperative and therefor lacking utility. The Examiner stated that the specification fails to establish the utility of the claim method and pharmaceutical composition using a CDR-grafted antibody in humans or any other animal. The Examiner further stated that the specification does not present any *in vivo* or *in vitro* data to support the claims, and that pharmaceutical therapy with reshaped monoclonal antibodies is unpredictable in the absence of *in vivo* clinical data.

Applicants respectfully traverse this rejection. The Examiner asserts that because the application does not contain any *in vivo* data, it is not possible for a person of ordinary skill in the art to use the humanized antibodies shown in the application in human therapy and diagnosis *in vivo*. It is submitted that at the priority date of the application a person of ordinary skill, having read the application, would have had every reason to expect that a humanized antibody, for instance

one directed against the CD3 antigen, would be useful diagnostically or therapeutically *in vivo*.

For any antibody to be useful *in vivo* in therapy or diagnosis, there are two main factors to consider. The first is the affinity of the antibody for the antigen. The second is the bioavailability of the antibody after administration. As regards the affinity, clearly the antibody would be of no use if its affinity were so low that it did not bind to the antigen, however much of the antibody was available to be bound. Similarly, if the antibody can never reach the antigen, for instance because it is degraded too fast, it will be of no use, however strongly it binds to the antigen.

In the present case, it is clearly shown that the humanized antibodies according to the invention have equivalent binding affinity to that of the prototype murine antibody such as OKT3, OKT4, 61E71 or B72.3. It is therefore plain to anyone with reasonable knowledge in this field that, if the humanized antibody can reach its site of action, it will be able to bind effectively to the appropriate antigen. The *in vitro* data given in the application clearly show that the humanized antibody would be expected to be equally effective as the prototype antibody once it reaches the relevant site.

As to bioavailability, it is plain to anyone with reasonable knowledge of the art that the humanized antibodies of

the present invention are predominantly human in appearance to the human immune system. The only part which may appear foreign is the antigen binding site. However, in this respect, the humanized antibody is no different from a natural human antibody. The antigen binding site in a natural human antibody is also, eventually, regarded as being foreign and the body mounts an immune response against it. This is called an anti-idiotype response and may be involved in the regulation of the immune system.

In view of the close, overall similarity between the humanized antibodies of the present invention and a natural human antibody, anyone with reasonable knowledge in the field would expect that the humanized antibody would have about the same bioavailability as a normal human antibody. The knowledgeable person would therefore expect that the humanized antibody would have very good bioavailability.

The skilled person would certainly expect that the humanized antibody would have better bioavailability than the prototype murine antibody. The prototype antibody is entirely of mouse origin and would therefore be regarded as being foreign by a human immune system. The human immune system would therefore

set up a response against the murine antibody which would lead to its more rapid clearance from the body.

The knowledgeable person would therefore have expected the humanized antibody of the present invention to have good bioavailability and would have seen from the *in vitro* data in the application that it has acceptable affinity. There would therefore have been every reason to expect that the humanized antibody would have good therapeutic and diagnostic uses *in vivo*.

The skilled person would certainly have known that OKT3, even though it is a totally murine antibody, is a highly effective therapeutic agent, especially in the treatment of acute organ transplant rejection episodes. This is very well documented in the prior art. On the basis of the data given in the present application, it would be expected that the humanized antibody could be used in the same way as is OKT3 to treat the same conditions.

The skilled person would also have known that OKT4, 61E71 and B72.3, while not approved for general use, have been used therapeutically and diagnostically in certain limited circumstances, demonstrating their utility. It would again be expected that this same utility could be demonstrated by the humanized versions of these murine antibodies.

The knowledgeable person would not need any data correlating the *in vitro* activity with *in vivo* use to come to

this conclusion. There is nothing in the art which would lead him in any way to expect that the *in vitro* data did not give a reasonable basis for a confident prediction of *in vivo* utility. Certainly, the Applicants are not aware of any studies which show that *in vitro* results with antibodies cannot be extrapolated to *in vivo* results.

The Examiner raises various possible reasons why the humanized antibodies might not work. However, the Examiner has provided no documentary evidence to support these suggestions. In the absence of such documentary evidence, it is respectfully submitted that these reasons represent no more than speculation. In any event, the speculations are unfounded. As to (1), there is no reason why a humanized antibody should be any more subject to proteolytic degradation than the murine prototype antibodies or normal human antibodies. Both murine and normal human antibodies are subjected to such degradation in the human body, in order to clear unwanted antibodies from the system. However, since the rate of clearance is generally of the order of a week or two, such degradation will not prevent their efficacious use. As to (2), it has been shown that murine antibodies reach the required target site. Why should a humanized antibody not reach the target site also? As to (3), the data in the specification show that acceptable affinity can be obtained. The specification also shows how the choice of the heavy chain constant domains

leads to desired effector functions. Thus, there is no basis for the Examiner's assertions. As to (4), all antibodies are eventually recognized as foreign and cleared from circulation. There is no reason to expect a rejection reaction, as discussed above, since the antibodies are predominantly human in appearance.

It is therefore submitted that there is no force in any of the arguments put forward by the Examiner. Accordingly, one skilled in the art is adequately taught by the specification how to practice the claimed invention. Withdrawal of this 35 USC §101 rejection is respectfully requested.

At paragraph 19 of the Office Action, the Examiner objected to the specification and rejected claims 1-12 under 37 USC §112, first paragraph as failing to adequately teach how to make and use the claimed invention. The Examiner stated that isolated heavy and light chains appear to be inoperable for binding antigen and as a consequence undue experimentation would be required of the skilled artisan in order to practice the invention.

It is respectfully submitted that the points made in response to the section 101 rejection above also apply here. The isolated chains have patent utility as intermediates in the preparation of the antibodies which have been demonstrated as having end use utility. The point is now moot, however, in view

of the cancellation of claims 1 through 12 and the submission of new claims drawn to antigen-binding molecule having both heavy and light chains. Withdrawal of this 35 USC §112, first paragraph rejection is respectfully requested.

At paragraph 20 of the Office Action, the Examiner objected to the specification and rejected claims 22 and 23 under 35 USC §112, first paragraph as failing to adequately teach how to make and use the claimed invention. The Examiner stated that the claimed invention appears to be inoperable without supporting *in vivo* data for the reasons discussed in the rejection made under 35 USC §101, and therefore undue experimentation would be required of the skilled artisan in order to practice the claimed invention.

Applicants respectfully traverse this rejection.

A person of ordinary skill in the art could, without the need for undue experimentation, practice the invention disclosed in the present application. It should be borne in mind that the use of murine monoclonal antibody OKT3 for therapy and murine monoclonal antibodies OKT3, OKT4, 61E71 and B72.3 for diagnosis has been reported in the prior art. Thus, therapeutic treatment regimes, amounts used per treatment, intervals between treatments, routes of administration, etc. for OKT3 and diagnostic regimes for all four antibodies are well known in the art. Since the *in vitro* results show that the humanized

antibodies of the present invention have the same affinity as the prototype antibodies and since it can reasonably be predicted that the humanized antibodies will have as good, if not better, bioavailability as the prototype antibodies, the knowledgeable person will confidently adopt the same sort of treatment regimes as are adopted for the prototype antibodies.

It is therefore submitted that the invention claimed in the present application can readily be put in to effect by the skilled person. Withdrawal of this 35 USC §112, first paragraph rejection is respectfully requested.

At paragraph 21 of the Office Action, the Examiner rejected claims 13-16 under 35 USC §112, first paragraph stating that the disclosure is enabling only for claims limited to specific CDR-grafted antibodies disclosed in the specification as having effective binding affinities for their specific antibody, i.e. which are similar to the non-humanized donor antibodies.

Applicants respectfully traverse this rejection. The Examiner contends that the present specification is only enabling in respect of the exemplified humanized antibodies because, in the Examiner's view, it would require undue experimentation to adapt the teaching in the present application to other antibodies. This view appears to be based on an analysis of the prior art rather than an analysis of the present invention.

It is indeed true that in the prior art, there is very little guidance as to how to go about producing a recombinant antibody which has the same antigen binding affinity as the prototype antibody. The most guidance is provided by Queen et al. (PNAS-USA, 86, 10029-10033, 1989). However, the amount of guidance provided is small. Although it is not explicitly stated in Queen et al., it is clear that it is necessary before beginning the process described therein to determine the amino acid sequence of the antibody chain to be recombinant. The skilled person reading Queen et al. is told firstly to select a human chain which is as closely comparable to the murine chain as possible. It is then necessary to carry out computer modelling of the chain to determine which residues outside the Kabat CDRs may be important for antigen binding or retaining the appropriate shape in the antigen binding region. This computer modelling is by no means trivial as it requires the modeler to make a number of critical choices of parameters will lead to a different model and may well lead to a different determination of non-CDR residues.

By carrying out this procedure, Queen et al. identified a number of residues outside the CDR which are altered in order to improve the affinity of the recombinant antibody. However, Queen et al. provides no guidance as to which residues are critical for improving affinity in the particular case referred

to by Queen et al. (the anti-TAC antibody). Moreover, there is no indication that it might be possible to change the same residues in a different antibody in the expectation of achieving good affinity.

It is also to be noted that Queen et al. does not use the Kabat numbering for the amino acid residues. The sequences are merely numbered in a linear fashion. It is thus not possible readily to determine which residues according to the Kabat numbering system were altered. This makes the teaching in Queen et al. even more specific to the particular antibody shown by Queen et al.

Thus, the teaching of Queen et al. is that, in order to reshape any antibody, it is necessary to treat each antibody individually and to carry out for each antibody the steps of sequence determination, acceptor sequence selection and computer modelling. Clearly, this requires undue experimentation to apply the teaching of Queen et al. to other antibodies.

In contrast, the teaching in the present application can be applied without any undue experimentation to any antibody. All that is required is experimentation following a protocol which is clearly set out in the description, in particular at page 16, line 30 to page 19, line 9. In order to follow this protocol, as a first step, it is necessary to determine the amino acid sequence of the donor chain. The sequence of the acceptor

chain will already be known, for instance from a sequence data base.

There is then no need to carry out computer modelling to determine which donor residues to substitute into the acceptor sequence. The protocol in the present application provides the teaching directly. It instructs the skilled person to compare the two sequences and change certain specified residues in the acceptor sequence to donor residues.

Moreover, the present application provides a hierarchical structure of residues which can be considered. Thus, if changing the residues identified at the top of the structure does not provide adequate affinity, then a lower level of residues are considered, and so on until acceptable affinity is obtained.

The manipulation of an acceptor sequence using recombinant DNA technology is a matter of routine. For instance, a known sequence can be altered using site directed mutagenesis. Alternatively, a complete sequence can be synthesized from isolated nucleotides. The chain having the altered amino acid sequence can be tested for affinity using assays of the type described in the application. Thus, producing recombinant chains and testing them for affinity merely involves routine experimentation following a protocol which is clearly defined in the application.

It is submitted that this identifies where the present invention makes a significant departure from the prior art. The prior art indicates that each antibody has to be treated individually. In contrast, the present invention teaches that, by following the protocol set forth in the present application, it is possible to reshape any antibody.

The Examiner pointed to the fact that the present application refers to only three antibodies (OKT3, OKT4 and anti-ICAM) and contends that this is no basis for predicting that the protocol is generally application. It is respectfully submitted that the Examiner is incorrect. In the first place, the application refers to nine different antibodies (OKT3, OKT4, RG-5, B72.3, 61R71, 101.4, hTNF1, hTNF2 and hTNF3) (see page 52, last paragraph) which have been humanized successfully using the protocol set out in the application. It therefore cannot be seen how it can be contended that the concept underlying the present claims has not been fully developed. Since it has proved possible to humanize all nine antibodies, it is submitted that a reasonably skilled person would readily predict that the concept is applicable to other antibodies.

The Examiner refers to the absence from the application of binding affinity values and alleges that the description of binding affinity is only qualitative. It is submitted that this is not the case. Figures 7 to 13 clearly show data. On page 60,

second paragraph, the binding affinity of the humanized anti-ICAM antibody is given a quantitative value. On page 62, quantitative statements regarding binding activity are also given. Thus, the application does contain quantitative data showing that the concept is applicable to produce a useful antigen-binding molecule.

It is therefore submitted that the description is fully enabling without the need for undue experimentation. Withdrawal of this 35 USC §112 rejection is therefore respectfully requested.

At paragraph 22 of the Office Action, the Examiner rejected claims 1-23 under 35 USC §112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. This rejection is in several parts which will answered separately.

In Part A of the rejection, the Examiner stated that claims 1-5 are indefinite due to the language of the claims relating to donor positions and because the position numbers are arbitrary.

Claims 1-5 have been cancelled without prejudice. The newly submitted claims do not contain the language found objectionable by the Examiner. The residue numbers in the newly submitted claims refers to the numbering system devised by Kabat.

This is a standard numbering system used to enable comparisons of antibody chain amino acid sequences to be made.

The Kabat numbering system was developed by studying the amino acid sequences of a large number of heavy and light chains. It was discovered that, within the variable domains, there were some residues which are highly conserved, some areas in which sequence variation is low and some areas in which the sequence variation is high. On the basis of the highly conserved residues and the low variability areas, it proved to be possible to assign numbers to residues in all heavy and light chains.

In some cases, it was found that there had been deletions in the sequence. In other cases, there were additional residues. Generally, these additions or deletions occurred in the highly variable areas. In order to allow for this, in some sequences the numbering has a jump in it to account for a deletion and in other sequences there are a number of residues with the same number, followed by a, b, c, etc., to account for insertions. Nonetheless, it is still readily possible to align antibody chain sequences on the basis of the Kabat numbering. This is a system which is widely used and commonly recognized in the art. In this respect, reference can be made to Riechmann et al., page 325, first sentence after "Strategy".

It is therefore believed that the language now used in the claims is in accordance with the requirements of section 112.

In Part B of the rejection, the Examiner stated that claims 6-12 are also indefinite due to the language of the claims relating to donor positions and because the position numbers are arbitrary.

Claims 6-12 have been cancelled without prejudice. The newly submitted claims do not contain the language found objectionable by the Examiner. The position numbers of the donor residues refer to the numbering system devised by Kabat, as discussed in the response to Part A of this rejection.

In Part C of the rejection, the Examiner stated that claims 4 and 11 are indefinite and confusing because they are in an improper Markush listing.

Claims 4 and 11 have been cancelled without prejudice. This rejection is now moot.

In Part D of the rejection, the Examiner stated that claim 21 is indefinite. Claim 21 has been amended to correct an inadvertent clerical in step (c), so that step (c) now recites "transfecting a host cell with the expression vectors of steps (a) and (b) to form a transfected cell containing said expression vectors". Step (c) indicates that the expression vectors are transfected into one host cell. The amendment to step (c) also provides an antecedent basis for "the transfected cell line" in step (d).

The use of the term "complementary" in claim 21 does not render the claim indefinite. It is submitted that this is clear to the person skilled in the art. It is well known that it is possible for almost any heavy chain to associate to some extent with almost any light chain. However, randomly associated heavy/light chain dimers will generally not have any antigen binding activity. Antigen binding activity will only be obtainable if the heavy and light chains are of such sequence and configuration as to provide antigen binding regions which fit together to form a site which binds to a desired antigen. It is well known to the art that a heavy and a light chain which, when associated, form a binding site for a desired antigen are said to be complementary. Thus, in the present claims, "complementary" is used in the way it is normally understood by those skilled in the art and does not introduce any lack of clarity in the claims. Withdrawal of this entire 35 USC §112, second paragraph rejection is respectfully requested.

At paragraph 23 of the Office Action the Examiner rejected the claims under 35 USC §102(b). This rejection is divided into two parts with will be answered separately.

In Part A of this rejection, claims 1, 5, 6-8, and 12-22 were rejected as anticipated by Riechmann et al. The Examiner stated that claim 1 and claim 6 were interpreted to mean that the framework has donor residues in at least one of any of positions

6, 23, 24, 48, 49, 71, 73, 75, 76, 78, 88, or 91 in the heavy chain and (1, 3, 46, or 47) or 46, 48, 58, or 71) in the light chain, and thus, the teachings of Riechmann et al. anticipate the invention as claimed.

The Examiner contends that the original claims lacked novelty over Riechmann et al. Claims 1, 5, 6-8, 12 and 22 have been cancelled without prejudice and submitted as new claims that more distinctly point out certain aspects of the present invention.

In present claims 24 and 25, it is specified that residues 23 and 24 in the heavy chain should be donor residues. However, as can be seen from Fig. 1, panel (a) in Riechmann et al., in the recombinant antibody shown there, residues 23 and 24 are acceptor residues.

In the present claim 42, it is specified that residue 58 in the light chain should be a donor residue. However, as can be seen from Fig. 1, panel (b) in Riechmann et al., in the recombinant antibody shown there, residue 58 is an acceptor residue. Applicants' claimed antigen-binding molecules are thus not anticipated by Riechmann et al.

In Part B of the rejection, the Examiner rejected claims 1-6 and 12-22 as anticipated by Queen et al.

Claims 1-6, 12-20 and 22 have been cancelled without prejudice and submitted as new claims that more distinctly point out certain aspects of the present invention.

In present claims 24 and 25, it is specified that residues 48, 66, 67, 68, 93, 103 to 108 and 110 should all be acceptor residues. However, in Queen et al., as can be seen from Fig. 2B, in these positions Queen et al. uses donor, rather than acceptor, residues. It should again be borne in mind that Queen et al. does not use the Kabat numbering and it is therefore necessary to look carefully at the disclosure in Queen et al. before it is possible to come to any final conclusion.

In present claim 38, it is specified that residue 71 should be a donor residue. However, as can be seen from Fig. 2A of Queen et al., in that position Queen et al. uses an acceptor, rather than a donor residue.

Applicants' claimed antigen-binding molecules are thus not anticipated by Queen et al. Withdrawal of this entire 35 USC §102(b) rejection is respectfully requested.

At paragraph 24 of the Office Action, the Examiner rejected claims 1-21 under 35 USC §103 as being obvious over Riechmann et al. in view of Queen et al. The Examiner states

> It would have been *prima facie* obvious to one of ordinary skill in the art in the art at the time the invention was made to use the guidelines taught by Riechmann et al. and Queen et al. to reshape any given antibody to

> "humanize" that antibody by making changes in the framework regions of the human acceptor to the donor residue when those residues are close to the CDR's and when those amino acids affect the conformation of the CDR's.

Applicants respectfully traverse this rejection.

The Examiner contended that all the previous claims lacked an inventive step over either Riechmann et al. or Queen et al. It is submitted, for the following reasons, that the Examiner's contention is unfounded.

With respect to Riechmann et al., it is submitted that this document does not go much beyond the original idea of Winter et al. (see WO-A) 89/07452 referred to on page 4 of the present application) of transferring only the CDRs to a human framework. Thus, Riechmann et al. shows transferring the hypervariable regions identified according to Kabat (i.e. the Kabat CDRs) to a human framework (see Riechmann et al., page 325, after "Strategy"). This basic concept was to a certain extent modified for the heavy chain on the basis of the difference between Kabat CDR1 (as defined by sequence) and CDR1 as defined by structural studies.

On the basis of specific sequence differences in structural CDR1 between the rat (donor) and human (acceptor) sequences, Riechmann et al. decided to change residues 27 and 30 in the acceptor sequence to the equivalent residues in the donor sequence. It is made clear that these sequence changes were made

because the rat (donor) sequence was unusual. It is also to be noted that Riechmann et al. made no other changes outside the CDRs in the heavy chain.

As regards the light chain, all that Riechmann et al. did was to transfer the rat CDRs (as determined by Kabat) to the human acceptor framework. No residue changes outside the CDRs were made.

It can thus be seen that, at best, Riechmann et al. teaches the skilled person to transfer the six CDRs and to look at the possibility of transferring a composite CDR1 comprising a combination of the Kabat and structural CDR1, but only if there are any unusual residues in the area of the structural CDR1 not covered by the Kabat CDR1. Even this teaching, however, is specific to the particular antibody considered by Riechmann et al. There is nothing in Riechmann et al. to suggest that this teaching is generally applicable to other antibodies.

In any event, Riechmann et al. does not in any way suggest that altering residues remote from the CDRs might be effective in improving affinity. Certainly, Riechmann et al. in no way suggest that there might be a hierarchy of residues which should be considered if improvements in affinity are to be sought. Still less does Riechmann et al. provide any teaching which would allow the skilled person to identify the hierarchy of residues as set forth in the present application.

It is therefore submitted that the subject matter of present claims is in no way suggested by Riechmann et al. Thus, the present invention is not at all obvious over Riechmann et al.

As regards Queen et al., as the Examiner has noted, the teaching there is quite different from that in the present application. Queen et al. teaches that the amino acid sequence of the antibody chain in question (donor) should be determined and then compared to that of known acceptor chains. An acceptor chain should then be chosen which is as homologous as possible to the donor chain.

The next step is to carry out a computer modelling exercise to determine which residues might be involved in antigen binding or in ensuring that the antigen binding site adopts the correct conformation. It is again to be pointed out that computer modelling can lead to different results, depending on the parameter choices made. Thus, following Queen et al. may not always lead to the same results. That this is the case can be seen from page 10031, paragraph 3, lines 13 to 15, which shows that an earlier model differed from a later model.

It is also to be pointed out that the criteria for making the choices are indeterminate. All that Queen et al. indicates is that the residues need to be "close enough" to the CDR's (see page 10031, paragraph 3). There is no indication as to how close is "close enough". It is therefore difficult for

the skilled person to follow the teaching of Queen et al. without having to make critical decisions on which no guidance is given.

Once these exercises have been carried out, the acceptor sequence is altered so that not only the Kabat CDR's but also all the other residues outside the CDRs identified by the modelling procedure are changed to donor residues.

The fact that the donor sequence is compared to a number of possible acceptor sequences and that a computer model of the donor antibody must be made shows that the whole procedure in Queen et al. is specific to one antibody at a time. Underlying these facts is the assumption that each antibody is different from other antibodies and that it is not possible to predict from work carried on one antibody how to deal with another antibody.

It is no doubt true that implication of Queen et al. is that carrying out the procedure disclosed therein will enable one to produce a recombinant antibody having at least some of the affinity of the prototype antibody. (In the case shown in Queen et al. the affinity of the recombinant antibody was only one third that of the prototype antibody.) However, there is no suggestion in Queen et al. that changing the same residues as were changed when reshaping the anti-TAC antibody could be expected to provide acceptable binding affinity in another recombinant antibody.

Even if (which is denied) a skilled person did try to change residues in accordance with the numbers determined by Queen et al., this still does not lead to the present invention. As has been discussed above, Queen et al. changes residues which, according to the present invention, need not be changed or leaves as acceptor residues ones which should be changed. Thus, following the Queen et al. procedure cannot lead to the production of an antibody as now claimed.

The Queen et al. procedure can be contrasted with the protocol set forth in the present application. In the case of the present invention, there is no need to compare the donor sequence with a number of possible acceptor sequences. Any acceptor sequence can be used. Moreover, there is no need to carry out any computer modelling. All that is required is for the skilled person to go through the hierarchy of residue changes set forth in the application, beginning at page 16, and to make the minimum number of changes required to obtain acceptable activity.

It is submitted that there is nothing in Queen et al. which in any way suggests that this simple hierarchical protocol can be adopted in the expectation of being able to reshape any desired antibody. Queen et al. teaches that each antibody requires its own reshaping procedure. The present invention

teaches that one reshaping procedure can be applied to any antibody.

In order to establish a *prima facie* case of obviousness, it must be shown that the prior art would have suggested to those or ordinary skill in the art that they should make the claimed composition or device, or carry out the claimed process; and that the prior art would also have revealed that in so making or carrying out, those of ordinary skill would have a reasonable expectation of success. Both the suggestion and the reasonable expectation of success must be founded in the prior art, not in the Applicants' disclosure.

As discussed above, at best, Riechmann et al. teaches the skilled person to transfer the six CDRs and to look at the possibility of transferring a composite CDR1 comprising of the Kabat and structural CDR1, but only if there are any unusual residues in the area of the structural CDR1 not covered by the Kabat CDR1. Riechmann et al. does not in any way suggest that altering residues remote from the CDR's might be effective in improving affinity of the CDR-grafted antibody. Additionally, Riechmann et al. in no way suggests that there might be a hierarchy of residues which should be considered if improvements in affinity are to be sought. Still less does Riechmann et al. provide any teaching which would allow the skilled person to

identify the hierarchy of residues as set forth in the present application.

Regarding Queen et al., the fact that the donor sequence is compared to a number of possible acceptor sequences and that a computer model of the donor antibody must be made shows that the whole procedure in Queen et al. is specific to one antibody at a time. Underlying these facts is the assumption that it is not possible to predict from work carried out on one antibody how to deal with another antibody. There is no suggestion in Queen et al. that changing the same residues as were changed when reshaping the anti-TAC antibody could be expected to provide acceptable binding affinity in another recombinant antibody. Queen et al. thus teaches that each antibody requires its own reshaping procedure thereby teaching away from the present invention.

In summary, there is neither a suggestion in the prior art to make the claimed CDR-grafted antibodies, nor does the prior art show a reasonable expectation of success of making the claimed CDR-grafted antibodies. Applicants' claims are thus not *prima facie* obvious over Riechmann et al. and Queen et al. Withdrawal of this 35 USC §103 rejection is respectfully requested.

DOCKET NO.: CARP-0009 **PATENT**

In view of the above, the present application is believed to be in a condition ready for allowance. Reconsideration of the application is respectfully requested and an early Notice of Allowance is earnestly solicited.

Respectfully submitted,

Date: January 19, 1993

Signature
Liza D. Hohenschutz
Registration No. 33,712

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS
One Liberty Place - 46th Floor
Philadelphia, PA 19103
(215) 568-3100

JAN 19 1993

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B. White
4-21-93

Docket No: CARP-0009 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: John R. ADAIR et al.
Serial No.: 07/743 329
Group Art Unit: 1807
Filed: 17th September 1991
Examiner: L. Bennett
For: Humanised Antibodies

Honorable Commissioner of Patents and Trademarks
Washington DC 20231.

Dear Sir,

AMENDMENT

1. This amendment is filed following the interview with Examiners Bennett and Chambers on 27th January 1993.

2. Amendments

Please cancel all the present claims and replace them with the new claims as follows.

67. An antibody molecule having affinity for a predetermined antigen and comprising a "composite" heavy chain and a complementary light chain, said composite heavy chain having a variable domain comprising human acceptor antibody heavy chain framework residues and donor antibody heavy chain antigen-binding residues, said donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain, amino acid residues 5, 8, 10, 12 to 17, 19, 21, 22, 40, 42 to 44, 66, 68, 70, 74, 77, 79, 81, 83 to 85, 90, 92, 105, 109, 111 and 113 at least are acceptor residues and amino acid residues 23, 24,

31 to 35, 49 to 58, 71, 73, 78 and 95 to 102 at least are donor residues.

68. The antibody molecule of claim 67, wherein amino acid residues 26 to 30 and 59 to 65 in said composite heavy chain are additionally donor residues.

69. The antibody molecule of claim 67, wherein at least one of amino acid residues 1, 3, and 76 in said composite heavy chain are additionally donor residues.

70. The antibody molecule of claim 67, wherein at least one of amino acid residues 36, 94, 104, 106 and 107 in said composite heavy chain are additionally donor residues.

71. The antibody molecule of claim 70, wherein at least one of amino acid residues 2, 4, 6, 38, 48, 67 and 69 in said composite heavy chain are additionally donor residues.

72. The antibody molecule of claim 67, wherein amino acid residues 7, 9, 11, 18, 20, 25, 37, 39, 41, 45, 47, 48, 72, 75, 80, 82, 86 to 89, 91, 93, 103, 108, 110 and 112 in said composite heavy chain are additionally acceptor residues.

73. A tumour-specific antibody molecule having affinity for a predetermined tumour antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain comprising human acceptor antibody heavy chain framework residues and donor antibody heavy chain antigen-binding residues, said donor antibody having affinity for said predetermined tumour antigen, wherein, according to the Kabat numbering system, in said composite heavy chain, amino acid residues 5, 8, 10, 12 to 17, 19, 21, 22, 40, 42 to 44, 66, 68, 70, 74, 77, 79, 81, 83 to 85, 90, 92, 105, 109, 111 and 113 at least are acceptor residues and amino acid residues 23, 24, 31 to 35, 49 to 58 and 95 to 102 at least are donor residues.

74. The antibody molecule of claim 73, wherein amino acid residues 71, 73 and 78 in said composite heavy chain are additionally donor residues.

75. The antibody molecule of claim 73, wherein amino acid residues 26 to 30 and 59 to 65 in said composite heavy chain are additionally donor residues.

76. The antibody molecule of claim 73, wherein at least one of amino acid residues 1, 3, and 76 in said composite heavy chain are additionally donor residues.

77. The antibody molecule of claim 73, wherein at least one of amino acid residues 36, 94, 104, 106 and 107 in said composite heavy chain are additionally donor residues.

78. The antibody molecule of claim 77, wherein at least one of amino acid residues 2, 4, 6, 38, 48, 67 and 69 in said composite heavy chain are additionally donor residues.

79. The antibody molecule of claim 73, wherein amino acid residues 7, 9, 11, 18, 20, 25, 37, 39, 41, 45, 47, 48, 72, 75, 80, 82, 86 to 89, 91, 93, 103, 108, 110 and 112 in said composite heavy chain are additionally acceptor residues.

80. An interleukin-specific antibody molecule having affinity for a predetermined interleukin and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain comprising human acceptor antibody heavy chain framework residues and donor antibody heavy chain antigen-binding residues, said donor antibody having affinity for said predetermined interleukin wherein, according to the Kabat numbering system, in said composite heavy chain, amino acid residues 5, 8, 10, 12 to 17, 19, 21, 22, 40, 42 to 44, 66, 68, 70, 74, 77, 79, 81, 83 to 85, 90, 92, 105, 109, 111 and 113 at least are acceptor residues and amino acid residues 23, 24, 31 to 35, 49 to 58 and 95 to 102 at least are donor residues.

81. The antibody molecule of claim 80, wherein amino acid residues 71, 73 and 78 in said composite heavy chain are additionally donor residues.

82. The antibody molecule of claim 80, wherein amino acid residues 26 to 30 and 59 to 65 in said composite heavy chain are additionally donor residues.

83. The antibody molecule of claim 80, wherein at least one of amino acid residues 1, 3 and 76 in said composite heavy chain are additionally donor residues.

84. The antibody molecule of claim 80, wherein at least one of amino acid residues 36, 94, 104, 106 and 107 in said composite heavy chain are additionally donor residues.

85. The antibody molecule of claim 84, wherein at least one of amino acid residues 2, 4, 6, 38, 48, 67 and 69 in said composite heavy chain are additionally donor residues.

86. The antibody molecule of claim 80, wherein amino acid residues 7, 9, 11, 18, 20, 25, 37, 39, 41, 45, 47, 48, 72, 75, 80, 82, 86 to 89, 91, 93, 103, 108, 110 and 112 in said composite heavy chain are additionally acceptor residues.

87. An anti-CD3 antibody molecule having affinity for the CD3 antigen comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain comprising human acceptor antibody heavy chain framework residues and donor antibody heavy chain antigen-binding residues from a donor antibody heavy chain, said donor antibody having affinity for said CD3 antigen, wherein, according to the Kabat numbering system, in said composite heavy chain, amino acid residues 5, 8, 10, 12 to 17, 19, 21, 22, 40, 42 to 44, 66, 68, 70, 74, 77, 79, 81, 83 to 85, 90, 92, 105, 109, 111 and 113 at least are acceptor residues and amino acid residues 23, 24, 31 to 35, 49 to 58 and 95 to 102 at least are donor residues.

88. The antibody molecule of claim 87, wherein amino acid residues 71, 73 and 78 in said composite heavy chain are additionally donor residues.

89. The antibody molecule of claim 87, wherein amino acid residues 26 to 30 and 59 to 65 in said composite heavy chain are additionally donor residues.

90. The antibody molecule of claim 87, wherein at least one of amino acid residues 1, 3, and 76 in said composite heavy chain are additionally donor residues.

91. The antibody molecule of claim 87, wherein at least one of amino acid residues 36, 94, 104, 106 and 107 in said composite heavy chain are additionally donor residues.

92. The antibody molecule of claim 91, wherein at least one of amino acid residues 2, 4, 6, 38, 48, 67 and 69 in said composite heavy chain are additionally donor residues.

93. The antibody molecule of claim 87, wherein amino acid residues 7, 9, 11, 18, 20, 25, 37, 39, 41, 45, 47, 48, 72, 75, 80, 82, 86 to 89, 91, 93, 103, 108, 110 and 112 in said composite heavy chain are additionally acceptor residues.

94. An anti-CD4 antibody molecule having affinity for the CD4 antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain comprising human acceptor antibody heavy chain framework residues and donor antibody heavy chain antigen-binding residues, said donor antibody having affinity for said CD4 antigen, wherein, according to the Kabat numbering system, in said composite heavy chain, amino acid residues 5, 8, 10, 12 to 17, 19, 21, 22, 40, 42 to 44, 66, 68, 70, 74, 77, 79, 81, 83 to 85, 90, 92, 105, 109, 111 and 113 at least are acceptor residues and amino acid residues 23, 24, 31 to 35, 49 to 58 and 95 to 102 at least are donor residues.

95. The antibody molecule of claim 94, wherein amino acid residues 71, 73 and 78 in said composite heavy chain are additionally donor residues.

96. The antibody molecule of claim 94, wherein amino acid residues 26 to 30 and 59 to 65 in said composite heavy chain are additionally donor residues.

97. The antibody molecule of claim 94, wherein at least one of amino acid residues 1, 3, and 76 in said composite heavy chain are additionally donor residues.

98. The antibody molecule of claim 94, wherein at least one of amino acid residues 36, 94, 104, 106 and 107 in said composite heavy chain are additionally donor residues.

99. The antibody molecule of claim 98, wherein at least one of amino acid residues 2, 4, 6, 38, 48, 67 and 69 in said composite heavy chain are additionally donor residues.

100. The antibody molecule of claim 94, wherein amino acid residues 7, 9, 11, 18, 20, 25, 37, 39, 41, 45, 47, 48, 72, 75, 80, 82, 86 to 89, 91, 93, 103, 108, 110 and 112 in said composite heavy chain are additionally acceptor residues.

101. An anti-adhesion molecule antibody molecule having affinity for an adhesion molecule and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain comprising human acceptor antibody heavy chain framework residues and donor antibody heavy chain antigen-binding residues from a donor antibody heavy chain, said donor antibody having affinity for said adhesion molecule wherein, according to the Kabat numbering system, in said composite heavy chain, amino acid residues 5, 8, 10, 12 to 17, 19, 21, 22, 40, 42 to 44, 66, 68, 70, 74, 77, 79, 81, 83 to 85, 90, 92, 105, 109, 111 and 113 at least are acceptor residues and amino acid residues 23, 24, 31 to 35, 49 to 58 and 95 to 102 at least are donor residues.

102. The antibody molecule of claim 101, wherein amino acid residues 71, 73 and 78 in said composite heavy chain are additionally donor residues.

103. The antibody molecule of claim 101, wherein amino acid residues 26 to 30 and 59 to 65 in said composite heavy chain are additionally donor residues.

104. The antibody molecule of claim 101, wherein at least one of amino acid residues 1, 3, and 76 in said composite heavy chain are additionally donor residues.

105. The antibody molecule of claim 101, wherein at least one of amino acid residues 36, 94, 104, 106 and 107 in said composite heavy chain are additionally donor residues.

106. The antibody molecule of claim 105, wherein at least one of amino acid residues 2, 4, 6, 38, 48, 67 and 69 in said composite heavy chain are additionally donor residues.

107. The antibody molecule of claim 101, wherein amino acid residues 7, 9, 11, 18, 20, 25, 37, 39, 41, 45, 47, 48, 72, 75, 80, 82, 86 to 89, 91, 93, 103, 108, 110 and 112 in said composite heavy chain are additionally acceptor residues.

108. The antibody molecule of claim 67, wherein said complementary light chain is a composite light chain having a variable domain comprising human acceptor antibody light chain framework residues and donor antibody light chain antigen-binding residues, said donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite light chain, amino acid residues 5, 7 to 9, 11, 13 to 18, 20, 22, 23, 39, 41 to 43, 57, 59, 61, 72, 74 to 79, 81, 82, 84, 86, 88, 100, 104, 106 and 107 at least are acceptor residues and amino acid residues 24 to 34, 46, 48, 50 to 56, 58, 71 and 89 to 97 at least are donor residues.

109. The antibody molecule of claim 73, wherein said complementary light chain is a composite light chain having a variable domain comprising human acceptor antibody light chain framework residues and donor antibody light chain antigen-binding residues, said donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite light chain, amino acid residues 5, 7 to 9, 11, 13 to 18, 20, 22, 23, 39, 41 to 43, 57, 59, 61, 72, 74 to 79, 81, 82, 84, 86, 88, 100, 104, 106 and 107 at least are acceptor residues and amino acid residues 24 to 34, 46, 48, 50 to 56, 58, 71 and 89 to 97 at least are donor residues.

110 . The antibody molecule of claim 80, wherein said complementary light chain is a composite light chain having a variable domain comprising human acceptor antibody light chain framework residues and donor antibody light chain antigen-binding residues, said donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite light chain, amino acid residues 5, 7 to 9, 11, 13 to 18, 20, 22, 23, 39, 41 to 43, 57, 59, 61, 72, 74 to 79, 81, 82, 84, 86, 88, 100, 104, 106 and 107 at least are acceptor residues and amino acid residues 24 to 34, 46, 48, 50 to 56, 58, 71 and 89 to 97 at least are donor residues.

111. The antibody molecule of claim 87, wherein said complementary light chain is a composite light chain having a variable domain comprising human acceptor antibody light chain framework residues and donor antibody light chain antigen-binding residues, said donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite light chain, amino acid residues 5, 7 to 9, 11, 13 to 18, 20, 22, 23, 39, 41 to 43, 57, 59, 61, 72, 74 to 79, 81, 82, 84, 86, 88, 100, 104, 106 and 107 at least are acceptor residues and amino acid residues 24 to 34, 46, 48, 50 to 56, 58, 71 and 89 to 97 at least are donor residues.

112. The antibody molecule of claim 94, wherein said complementary light chain is a composite light chain having a variable domain comprising human acceptor antibody light chain framework residues and donor antibody light chain antigen-binding residues, said donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite light chain, amino acid residues 5, 7 to 9, 11, 13 to 18, 20, 22, 23, 39, 41 to 43, 57, 59, 61, 72, 74 to 79, 81, 82, 84, 86, 88, 100, 104, 106 and 107 at least are acceptor residues and amino acid residues 24 to 34, 46, 48, 50 to 56, 58, 71 and 89 to 97 at least are donor residues.

113. The antibody molecule of claim 101, wherein said complementary light chain is a composite light chain having a variable domain comprising human acceptor antibody light chain framework residues and donor antibody light chain antigen-binding residues, said donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite light chain, amino acid residues 5, 7 to 9, 11, 13 to 18, 20, 22, 23, 39, 41 to 43, 57, 59, 61, 72, 74 to 79, 81, 82, 84, 86, 88, 100, 104, 106 and 107 at least are acceptor residues and amino acid residues 24 to 34, 46, 48, 50 to 56, 58, 71 and 89 to 97 at least are donor residues.

114. The antibody molecule of claim 108, wherein amino acid residues 1, 3 and 47 in said composite light chain are additionally donor residues.

115. The antibody molecule of claim 108, wherein amino acid residues 36, 44, 47, 85 and 87 in said composite light chain are additionally donor residues.

116. The antibody molecule of claim 108, wherein at least one of amino acid residues 2, 4, 6, 49, 62, 64 to 69, 98, 99, 101 and 102 in said composite lightly chain are additionally donor residues.

117. The antibody molecule of claim 108, wherein at least one of amino acid residues 1, 3, 10, 12, 21, 40, 60, 63, 70, 73, 80, 103 and 105 in said composite light chain are additionally donor residues.

118. A method for producing a recombinant antigen binding molecule having affinity for a predetermined antigen comprising the steps of:

[1] determining the amino acid sequence of the variable domain of the heavy chain of a donor antibody which has affinity for said predetermined antigen;

[2] determining the amino acid sequence of the variable domain of the heavy chain of a non-specific acceptor antibody;

[3] providing a composite heavy chain for an antibody molecule, said composite heavy chain having acceptor framework residues and donor antigen binding residues wherein, according to the Kabat numbering system, amino acid residues 5, 8, 10, 12 to 17, 19, 21, 22, 40, 42 to 44, 66, 68, 70, 74, 77, 79, 81, 83 to 85, 90, 92, 105, 109, 111 and 113 at least are acceptor residues and amino acid residues 23, 24, 31 to 35, 49 to 58 and 95 to 102 at least are donor residues;

[4] associating the heavy chain produced in step [3] with a complementary light chain to form an antibody molecule;

[5] determining the affinity of the antibody molecule formed in step [4] for said predetermined antigen;

[6] if the affinity determined in step [5] is not equivalent to that of the donor antibody, providing a heavy chain as described in [3] above but in which amino acid residues 71, 73 and 78 are additionally donor residues;

[7] associating the heavy chain produced in step [6] with a complementary light chain to form an antibody molecule;

[8] determining the affinity of the antibody molecule formed in step [7] for said predetermined antigen;

[9] if the affinity determined in step [8] is not equivalent to that of the donor antibody, providing a heavy chain as described in [6] above but in which amino acid residues 26 to 30 are additionally donor residues;

[10] associating the heavy chain produced in step [9] with a complementary light chain to form an antibody molecule;

[11] determining the affinity of the antibody molecule formed in step [10] for said predetermined antigen;

[12] if the affinity determined in step [11] is not equivalent to that of the donor antibody, providing a heavy chain as described in [9] above but in which at least one of amino acid residues 1, 3, and 76 are additionally donor residues;

[13] associating the heavy chain produced in step [12] with a complementary light chain to form an antibody molecule;

[14] determining the affinity of the antibody molecule formed in step [13] for said predetermined antigen;

[15] if the affinity determined in step [14] is not equivalent to that of the donor antibody, providing a heavy chain as described in [12] above but in which at least one of amino acid residues 36, 94, 104, 106, 107 are additionally donor residues;

[16] associating the heavy chain produced in step [15] with a complementary light chain to form an antibody molecule.

[17] determining the affinity of the antibody molecule formed in step [16] for said predetermined antigen;

[18] if the affinity determined in step [17] is not equivalent to that of the donor antibody, providing a heavy chain as described in [15] above but in which at least one of amino acid residues 2, 4, 6, 38, 48, 67 and 69 are additionally donor residues; and

[19] associating the heavy chain produced in step [18] with a complementary light chain to form an antibody molecule.

119. The method of claim **118**, further comprising the steps of:

[1] determining the amino acid sequence of the variable domain of the light chain of said donor antibody which has affinity for said predetermined antigen;

[2] determining the amino acid sequence of the variable domain of the light chain of a non-specific acceptor antibody;

[3] providing a composite light chain for an antibody molecule, said composite light chain having acceptor framework residues and donor antigen binding residues wherein, according to the Kabat numbering system, amino acid residues 5, 7 to 9, 11, 13 to 18,

20, 22, 23, 39, 41 to 43, 57, 59, 61, 72, 74 to 79 to 79, 81, 82, 84, 86, 88, 100, 104 and 106 to 109 at least are acceptor residues and amino acid residues 24 to 34, 46, 48, 50 to 56, 58, 71 and 89 to 97 at least are donor residues;

[4] associating the light chain produced in step [3] with a complementary heavy chain to form an antibody molecule;

[5] determining the affinity of the antibody molecule formed in step [4] for said predetermined antigen;

[6] if the affinity determined in step [5] is not equivalent to that of the donor antibody, providing a light chain as described in [3] above but in which amino acid residues 1, 2, 3 and 47 are additionally donor residues;

[7] associating the light chain produced in step [6] with a complementary heavy chain to form an antigen-binding molecule;

[8] determining the affinity of the antigen-binding molecule formed in step [7] for said predetermined antigen;

[9] if the affinity determined in step [8] is not equivalent to that of the donor antibody, providing a light chain as described in [6] above but in which amino acid residues 36, 44, 47, 85 and 87 are additionally donor residues;

[10] associating the light chain produced in step [9] with a complementary heavy chain to form an antibody molecule;

[11] determining the affinity of the antibody molecule formed in step [10] for said predetermined antigen;

[12] if the affinity determined in step [11] is not equivalent to that of the donor antibody, providing a light chain as described in [9] above but in which at least one of amino acid residues 2, 4, 6, 49, 62, 64 to 69, 98, 99, 101 are additionally donor residues; and

[13] associating the light chain produced in step [9] with a complementary heavy chain to form an antibody molecule.

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3. Remarks

The undersigned would like to thank Examiners Lisa Bennett and Scott Chambers for the very cordial and helpful interview on January 27, 1993 relating to this application. The amendments to the claims reflect the discussions of the interview as recorded in the Examiner

Interview Summary Record.

Having considered the Examiner's concerns that the language of the claims might be indefinite, because it was not clear whether the specified residues were the only or the minimum number of residues to be donor residues, the Applicants have amended the claims. In all the claims it is made clear that there is a minimum number of residues which have to be donor residues and a minimum number which have to be acceptor residues. Those residues which are not specified in the claims may be either donor or acceptor.

The claims have been amended to delete "antigen-binding molecule" and recite instead "antibody molecule". In order to reduce issues, the claims have also been amended recite that the acceptor antibody heavy or light chain is a human acceptor antibody heavy or light chain. These amendments to the claims address the Examiner's concern relating to utility and enablement of the claims. In view of the great conservation of structure between antibodies of different species, however, Applicants believe the acceptor and donor antibodies can come from any species.

In order to reduce the issues further, the claims have been limited to antibodies in which the heavy chain has been "superhumanised". Claims referring to the light chain have been made dependent on the claims referring to the heavy chain.

Regarding claim 67, it can be seen from the description (see page 6 line 29 to page 7, line 28; page 17, lines 9 to 11, Sections 2.1 to 2.3 bridging pages 17 and 18; Section 1 bridging pages 19 and 20; Sections 2.1.1, 2.2.1, 2.3.1 and 2.4.1 in the passage bridging pages 20 to 23; and Section 15.2.1 on pages 46 and 47) that a number of residues are mentioned which can be considered for changing from acceptor to donor residues. It follows that if a residue has not been considered for changing, it must remain as in the acceptor chain. In order to make this clear in the claim, it has been specified in claim 67 that all the unmentioned residues must be acceptor residues.

As regards the recitation of the donor residues in claim 67, this comprises a combination of the minimum residues need to define the CDR loops or the Kabat CDRs. For CDR1, this comprises residues 31 to 35 (see page 46, line 18) and for CDR2 this comprises residues 50 to 58 (see page 46, line 20). For CDR3, the Kabat definition of residues 95 to 102 was used (see page 17, line 11).

In addition claim 67 recites as donor residues those identified on page 20, line 25 and page 21, line 9 as being key residues outside the CDRs.

In claim 67, it has been specified that residues 71, 73 and 78 are all donor residues in order to ensure that claim 67 is novel over the anti-TAC antibody disclosed by Queen. This anti-TAC antibody has an acceptor residue at residue 73. However, as can be seen from page 7, lines 1 to 5, the Applicant considers that in general, residues 71, 73 and 78 can be either all donor or all acceptor.

Claim 68 increases the sizes of CDR1 and CDR2 to the prudent definition given on page 17, line 9 and page 20, lines 6 to 9.

Claim 69 is based on the passage at page 20, lines 26 and 27. Claim 70 is based on the passage at page 21, lines 10 to 12. Claim 71 is based on the passage on page 21, lines 13 to 16.

Claim 72 is derived by taking all the donor residues mentioned in claims 67 to 71 and specifying that all apart from these residues are acceptor residues.

Claim 73 is based on page 15, line 27 and pages 55 to 59 of the description. Claim 80 is based on page 15, line 33 of the description. Claim 87 is based on page 15, line 31 and pages 25 to 52 of the description. Claim 94 is based on page 15, line 31 and pages 53 and 54 of the description. Claim 101 is based on page 15, line 32 and pages 60 and 61 of the description.

None of the prior art CDR-grafted antibodies has specificity for a tumour-specific antigen, an interleukin, the CD3 or CD4 antigen, or an adhesion molecule. It is therefore submitted that claims 73, 80, 87, 94 and 101 are all novel.

It is stated on page 7, lines 1 to 5 that residues 71, 73 and 78 should all be either acceptor or donor. Claims 73, 80, 87, 94 and 101 cover the first alternative and claims 74, 81, 88, 95 and 102 cover the second alternative.

Claims 75 to 90, 82 to 86, 89 to 93, 96 to 100 and 103 to 107 are equivalent to claims 68 to 72.

Claims 108 to 113 are based on previously filed claim 42 and is derived in the same way that present claim 67 was derived. The residues which can be donor residues are listed on page 9, line 7 to page 10, line 15; page 17, lines 12 to 14; Section 3 bridging pages 18 and 19; Section 2.1.2 on page 20; Section 2.2.2 on page 21; Section 2.3.2 on page 22 and Section 2.4.2 on page 23.

Claims 114 to 117 are based on the passage from page 9, line 7 to page 10, line 11.

Claims 118 and 119 are based on previous claims 65 and 66 but with amendments to make them consistent with claims 67 onwards.

It is therefore believed that all the claims are fully supported by the description.

Although all the previous claims have been cancelled, the applicants reserve the right to file divisional applications relating to the deleted subject matter.

At the interview, the Examiner expressed concerns that the claims may lack novelty and that the method disclosed in the application might not be universally applicable. In an effort to resolve these questions, the applicants send herewith three Tables relating to a

number of antibodies which have been "superhumanised" by the method of the invention. The first Table relates to the heavy chain and the second Table relates to the light chain. On the third Table is set out the degree of affinity recovered as a percentage of the affinity of the original monoclonal antibody. There is also provided on the Tables a comparison with antibodies which have been produced by prior art proposals.

On each of the Tables, the top line shows the residue numbering according to the Kabat scheme. It can be seen that this includes, for the heavy chain, residues 52a-52c, 82a-82c and 100a to 100i.

Underneath the residue numbering is set out schematically the residue assignment for each of the antibodies referred to. This is schematic in that it does not give the actual residue but instead indicates whether it is a donor (D), acceptor (A) or common (c) residue. "Common" means that the same residue was present in both the donor and the acceptor sequences. Some of the common residues are also highly or completely conserved, but this has not been marked on the Tables.

In the heavy chain Table, the first five residues of 39D10 are marked by a question mark. This is because, due to the cloning method used, the first five residues in the donor chain were not determined. Thus, no comparison can be made.

At the end of each line is given the name of the acceptor sequence used to produce the chain.

It should be noted that for the antibodies 61E71 and hTNF3, the projects were terminated before the method had been fully applied. The applicants are confident that, had they had the funds to complete the work on these antibodies, good recovery of affinity would have been obtained.

In the Tables, the B1.8, D1.3, CAMPATH and anti-TAC entries represent prior art antibodies. There is no entry in the light chain Table for

B1.8 because the B1.8 light chain was never CDR-grafted.

B1.8 recognises an artificial antigen, the nitrophenyl hapten. Its heavy chain has acceptor residues at positions 23, 24, 73 and 78.

D1.3 recognises lysosyme. Its heavy chain has acceptor residues at positions 71, 73 and 78. Its light chain has acceptor residues at positions 48 and 71.

CAMPATH recognises the CD52 differentiation antigen found on various leukocytes. Its heavy chain has acceptor residues at positions 23, 24, 71, 73 and 78. Its light chain has an acceptor residue at position 58.

Anti-TAC is the antibody described by Queen. It recognises an epitope on a surface receptor for IL-2. (It does not recognise IL-2 itself). Its heavy chain has an acceptor residue at position 73. Its light chain has an acceptor residue at position 71.

Looking at the heavy chain Table, it can be seen that in all the successfully "superhumanised" antibodies produced by the applicants, in the heavy chain residues 23, 24, 31 to 35, 49 to 58 and 95 to 102 are all donor residues. Also in each case residues 26 to 30 are donor residues. Moreover, in most cases, residues 59 to 65 are also donor residues.

In one chain, OKT3 209, residues 71 and 73 were left as acceptor. For this particular antibody, it was shown that as long as residue 78 is donor, it does not make much difference whether residues 71 and 73 are donor or acceptor. This seems to be a peculiarity of this antibody, but which highlights the importance of residue 78.

In the heavy chains for 61E71 and hTNF3, residue 78 remained as acceptor. In both cases, the donor residue is alanine and the acceptor residue is leucine. Since leucine is more bulky than alanine, this may explain why the affinity of these chains was lower than desirable.

In hTNF1 a number of residues (12, 66 and 83) are donor residues whereas claim 67 specifies them as being acceptor residues. This antibody was prepared as the first in a series and the aim was to ensure that it worked. Therefore, any residues which were in the slightest unusual were changed to donor residues, even though it was suspected that some of the changes were unnecessary. As can be seen from, for instance 101/4, a later antibody in the series, if residues 12, 66 and 83 remain as acceptor, reasonable affinity is recovered. It is submitted that this is evidence that hTNF1 was overengineered.

In P67.6, residue 44 is a donor residue whereas claim 67 specifies that it should be an acceptor residue. In the donor antibody in this case, residue 44 was a very unusual amino acid for that position and it was therefore felt that, in that particular case, the unusual amino acid should be adopted.

At residue 77, which is specified as an acceptor residue, 101/4 and 39D10 have the donor residue. The change here in both cases is of a surface residue and is from leucine to valine. These are both hydrophobic residues and it is unlikely that this change will significantly affect the recovery of activity.

In 101/4 residue 79 is donor whereas claim 67 specifies it as acceptor. The donor residue is tyrosine and the acceptor is phenylalanine. This is a very conservative change and it is highly unlikely that this change will significantly affect the recovery of affinity.

For residue 105, which should be acceptor, B1RR0001, CTM01 and P67.6 all have donor residues. B1RR0001 gave very low recovery of affinity and it is believed that retaining the acceptor sequence at residue 105 would have improve the recovery of affinity. For the other two antibodies, the change is asparagine for glutamine, a very conservative change. It is highly unlikely that this change will have had any significant effect on the recovery of affinity.

Heavy chain residue 78 is specified as donor. In 61E71 and hTNF3, it is acceptor. However, these antibodies have low recovery of affinity. The projects on these antibodies were terminated at an early stage. If residue 78 had been changed to donor, it is believed that better recovery of affinity would have been obtained.

It is submitted that the data given in the three Tables amply demonstrate that successful "superhumanisation" can be achieved by following the method of the invention.

The Examiners expressed some concern that the language of the claims could read on naturally-occurring antibody heavy or light chains. The claims recite a "donor" antibody and an "acceptor" antibody as sources of residues in the heavy or light chains. Such an antibody, as claimed, cannot exist in nature. Therefore, the claims cannot read on a naturally-occurring antibody.

It is submitted that the present claims define inventive subject matter, for the reasons set forth in the Amendment mailed 19th January 1993.

In view of the above amendments and discussion, the present application is believed to be in a condition ready for allowance. Reconsideration of the application is respectfully requested and an early Notice of Allowance is respectfully requested.

Respectfully submitted,

Date: April 7, 1993

Signature
Francis A. Paintin
Registration No. 19,386

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS
One Liberty Place - 46th Floor
Philadelphia, PA 19103
(215) 568-3100

HEAVY CHAIN

RESNUM	1 2 3 4 5 6 7 8 9 10 11 11	
	1234567890123456789012345678901234567890123456789012abc345678901234567890123456789012abc34567890123456789 0abcde1234567890123	ACCEPTOR
B72.3	ccccAccAccAAcccAcccAccccccDccDDDDDDcAAcAcAcccccAcDDDDDDDDDDDDDDDDDDDAAcAcccDcAAccccAcAccAcccAcAcccDDDDDD........DDDcDAAcccc	EU
OKT3 209	ccccADccAAAAAccAcAAAccDDccDDcDDDDDDccAcAccAccccDDDDDDDDDDDDDDDDDDDADDAAcAAAcAcAAcDAAcAAccAAccAAccAcccDDDDDDDD...DDcccccAAcccc	KOL
OKT3 207	ccccADccAAAAAccAcAAAccDDccDDcDDDDDDccAcAccAccccDDDDDDDDDDDDDDDDDDDADDAAcAADcDcAAcDAAcAAccAAccAAccAcccDDDDDDDD...DDcccccAAcccc	KOL
OKT4A	AcAccccccAAcAccAccAccccDcccDccDDDDDccccAcAcAcccccDDDDDDDDDDDDDDDDDDDccccccccAccccAcccAcccAcccDAcDcccDDDDDDDDD..DDcccccAAcccc	KOL
BIRR0001	ccccAcccAcAAAccAcccAcccDccDccDDDDDDccAAAAAAAcccDcDDDDDDDDDDDDDDDDDDDDDAAcDcDcDcAccccDccAAcAcccAcDcDccDDDDDDDDD...DDDDDcDDAcccc	EU
A5B7	DcAcAccccccccccccccccccDcccccDDDDDDccccAcccAcccDDDDDDDDDDDDDDDDDDDDDDccccccDDcADAccccccDcAccDccAcccDcDDDDDDDD...DDcccccAAcccc	LAY
CTM01	cDccAcccAcAAccçAcccAccccccDccDDDDDDcDAcAcccccccDcDDDDDDDDDDDDDDDDDDDADcDcDcDcAAccccAccccAcccccAccccDDDDDDDDDD..DDDDDcDAccccA	EU
P67.6	DcccAcccAccAcccAcccAcccccccDcDDDDDDDccAcADcADcccDcDDDDDDDDDDDDDDDDDDDDDADcDcDcDDAAcccccccAccAcccAcAcDcDDDDDDD.....DDDDDcDcccccc	EU
A33	DcAcAcccccccAcccccAccccccccDccDDDDDccccAcAcAccccccDDDDDDDDDDDDDDDDDDDDccccccDDAAcccccccADcAAcDAcAccccDDDDDDD.....DDcccccccccccA	LAY
61E71	cAcccAccAAAAAccAAAAAAccDDccDDcDDDDDDccAcAcccccAcDDDDDDDDDDDDDDDDDDDDDDcAAAcDADcAAcAAccAAAcAAccAAAAccccDDDDDDD....DDcccccAAcccc	KOL
hTNF3	AAcccAccAAAAAccAAAAAccDDccDDcDDDDDDccAcccccccAcDDDDDDDDDDDDDDDDDDDDDDccAAcDADcAAcAAccAAAcAAcccAAcccccDDDDDDD....DDcccccAAcccc	KOL
101/4	AcADAcccccDccccAcAAcccDDcccDccDDDDDccccAcAccccccccDDDDDDDDDDDDDDDDDDDDcccccccDccADDDcccAAcAAccccAcDcDDD..........DDcccccDccccA	KOL
hTNF1	AcAcAcccAcADcccAcccAAcccccDccDDDDDccccAAcAAcDcDcDDDDDDDDDDDDDDDDDDDDDDcDcDcDcADccccccAccDcccccDcDccDDDDDDDDDDDD.DDDDAcDDccccc	EU
39D10	?????AAcccAcccAAAcAcAcDDccDDDDDDDDDcDccAcccccccADDDDDDDDDDDDDDDDDDDDDcAAccccDccDDDADAccccAAccccAcAcccDDDDDD.....DDcccccAccccc	HIL
L243	cAccccccAcAccccAAccAccccccDccDDDDDDccAccccAccAcDcDDDDDDDDDDDDDDDDDDDDDcDADAcDccDDcccAAAAAcAAccccAcAccDDDDDDDDDDD.DDcccccAAcccc	SGI consensus
HLT29	cDccccccccccccAccAcccAcccccDDDDDDDcAcccccccccccDDDDDDDDDDDDDDDDDDDDDccccccccccDccccccccccAccccAcAcccDDDDDDDDDDDDDccccAAAcccc	SGIII consensus
B1.8	cccccAAcAAccAcAAAAAcAcAAccAccADDDDDccAcAcccccccccDDDDDDDDDDDDDDDDDDDAAcAAccAAAAAAAAAcccAcAAcAccccccccDDDDDDD....DDccccAAAcccc	NEW
D1.3	ccccAccccccccAcccAccAccccccAAAADDDDcccccccAccccAcDDDDDDDDDDDDDDDDDDDDcAAAAAcAccAcAAcAAAcAAAAcccAcccccDDDDDD.....DDccccAAAcccc	NEW
CAMPATH	AcAcAcccAcccAcAAAAAcAcAAccDccDDDDDDcAcccAcAAAccAcDDDDDDDDDDDDDDDDDDDDcAcAAAcAAAcAAAcAAAAAAcAcccAcccccDDDDDDD.....DDccccAAAcccc	NEW
Anti-TAC	ccccAcccccAAcccAcccAcccccccDccDDDDDDccAcAcccccccDcDDDDDDDDDDDDDDDDDDDDDDcAcccAcAAccccAccccAAccAcccccccDDDDDD......DDDccccAAcccc	EU(PDL)
	1234567890123456789012345678901234567890123456789012abc34567890123456789012345678901 2abc34567890123456789 0abcde1234567890123	

c CONSERVED BETWEEN DONOR AND ACCEPTOR

D DONOR RESIDUE IN HUMANIZED FORM

A ACCEPTOR RESIDUE IN HUMANIZED FORM

SGI consensus means the acceptor sequence was the consensus sequence of the human antibodies in subgroup I of the Kabat et al 1987 compendium

SGIII consensus means the acceptor sequence was the consensus sequence of the human antibodies in subgroup III of the Kabat et al 1987 compendium

EU(PDL)) is a modified form of the EU sequence in which certain residues have been altered to make the sequence more homologous to the consensus sequence for the subgroup.

```
LIGHT CHAIN                                                                                                        1   1
                   1         2         3               4         5         6         7         8         9         0   0
          1234567890123456789012345678901234567890abcdef1234567890123456789012345678901234567890123456789012345678901234567    ACCEPTOR

B72.3     ccccccccAcccAcccAAccccccDDDDDDD......DDDDccccAAccAcAccAcDDDDDDDcccccccccccccAcAAAcAcccAccAAAcccDDDDDDDDDccAccAcAcA    REI
OKT3      AcAAccccAAAcccAcAAccAccDDDDDD.......DDDDccccAAcAAccDDccDDDDDDDcccAAcAccccccAcAAcccAAAAccAcccccDDDDDDDDDccAccccAcA    REI
OKT4a     ccccccccccccccAcAAcccAcDDDDDDD......DDDDcccDAcccAcAcccDDDDDDDDcAcccccccccccAccAcAccAcAcccccccccDDDDDDDDDccAccccAcA    REI
BIRR0001  cDDcccccAAcAAcAccAAAcAcDDDDDDDDDDDD.DDDDccAccAcAcccccDcDDDDDDDcccDccAccccccDcccAccAAAAAcADDcDcDDDDDDDDDccAcccAcAc    EU
A5B7      DDDDAcccAAccAcAcAAccAccDDDDDD.......DDDDcccccccAAccDAccDDDDDDDcccAcccccccccADAAcccAAAAccAcccccDDDDDDDDDccAcccAcAc    LAY
CTM01     ccAcccAAAAAAAAAcAAAAcAcDDDDDDDDDDDD.DDDDcDAcAccAAcAccAcDDDDDDDcccAccDccccccAcccAAAAAAAAcAAAcccDDDDDDDDDccAcccAcAc    EU
P67.6     ccADccccAAcAAcAcAcAccAcDDDDDDDDDDD..DDDDcDccAccAAccccDcDDDDDDDcccAccDcccccccccAcAcAAAAAccAcAcAcDDDDDDDDDccAcccAcAc    EU
A33       ccAccccAAAAcAccccccAcccDDDDDDD......DDDDcccccccAAccDcccDDDDDDDcccAccAcccccccccAcccAAcAccDcAcDcDDDDDDDDDccAcccAccc    LAY
61E71     AcAcccAcAAcAAcAcccccccccDDDDDDD......DDDDccccAAcAAccAcccDDDDDDDcccAccAcccAcccAccccAAAcAccAcAcAcDDDDDDDDDccAccccAcA    REI
hTNF3     AcAcccAcAAcAAcAcccAccccDDDDDDD......DDDDccccAccAAcAccccDDDDDDDcccAccAcccAcccAccccAAAcAccAcAcAcDDDDDDDDDccAccAcAcA    REI
101/4     DcDDccccAAAcccAcAAccAccDDDDDDD......DDDDccccDccAAcAccccDDDDDDDcccAcccccccccAcAccccAAAAccAcccccDDDDDDDDDccAcccADcD    REI
hTNF1     ccDcAccccAcAAcccAAccAAcDDDDDDDDDDDDDDDDDccccccccDAccccDDDDDDDDDcccAccAcccccccAccccccccAAAAcAcAcccDDDDDDDDDccAcAcAcDA    REI
39D10     ccccccccAcccccAcAAAAcAcDDDDDDD......DDDDccccccccAAcAccccDDDDDDDccccccccccccAcADAAAcccAccccAAAcAcDDDDDDDDDccAcccAcAc    LAY
L243      ccccccccAcccAcccAAccccccDDDDDDD......DDDDccAccAccAcDccADDDDDDDDDcccccccccccccDDAcAcAcccAcccAAcccDDDDDDDDDccAccAAccc    SGI consensus
HLT29     ccccccccccccAccAcccccccccDDDDDDD......DDDDcDccAcccAcDcDccDDDDDDDcccccccccccccDccAcccAccAAccAcAcccDDDDDDDDDccAcccAccc    SGI consensus

D1.3      ccccccccAccccccccAAccccccDDDDDDD......DDDDcccccAccAcAccAcDDDDDDDcccccccccccccAAAAAcAcccccccAAAcccDDDDDDDDDccAcccAccc    REI(LMB)
CAMPATH-1 ccAccccccAccccccccccccAAcDDDDDDD......DDDDcccccAcAAccccccDDDDDDDcAccccccccccccccccAccccccccccAcccAcDDDDDDDDDccAcccAcAc    REI(LMB)
anti-TAC  AcAAccccAAAcccAcAAccccccDDDDDD.......DDDDcAcccccAAcccAccDDDDDDDccccccccccccccAAAccccAAAAAcAcccccDDDDDDDDDccAcccAcAc    EU(PDL)
          1234567890123456789012345678901234567890abcdef1234567890123456789012345678901234567890123456789012345678901234567
```

c CONSERVED BETWEEN DONOR AND ACCEPTOR

D DONOR RESIDUE IN HUMANIZED FORM

A ACCEPTOR RESIDUE IN HUMANIZED FORM

SGI consensus means the acceptor sequence was the consensus sequence of the human antibodies in subgroup I of the Kabat et al 1987 compendium

REI(LMB) is a modified form of the REI sequence in which certain residues have been altered to make the sequence more homologous to the consensus sequence for the subgroup

EU(PDL)) is a modified form of the EU sequence in which certain residues have been altered to make the sequence more homologous to the consensus sequence for the subgroup.

TABLE HUMANIZED ANTIBODIES

ANTIBODY	SPECIFICITY	POTENCY[1] RELATIVE TO RODENT ANTIBODY %
ANTI-TUMOUR		
B72.3	Tumour associated glycoprotein 72 (TAG72)	30
P67.6	CD33	85
CTM01	Polymorphic epithelial mucin (PEM)	> 100
A5B7	Carcinoembryonic antigen (CEA)	50
A33	Epithelial antigen	75
ANTI-CYTOKINE		
HTNF1	Tumour necrosis factor (TNF) α	100[2]
101/4	TNFα	30[2]
61E71	TNFα	100; < 1[2]
HTNF3	TNFα	100; < 1[2]
HLT29	Lymphotoxin (LT)	> 70[2]
39D10	Interleukin-5	> 80
ANTI-LYMPHOCYTE		
OKT3	CD3	100
OKT4	CD4	68
L243	MHC CLASS II	60
ANTI-ADHESION MOLECULE		
BIRR-001	ICAM-1	25

1. POTENCY IS MEASURED AS RELATIVE ABILITY TO COMPETE WITH THE RODENT ANTIBODY FOR BINDING TO ANTIGEN

2. CYTOKINE NEUTRALIZATION ASSAY. ANTIBODY BINDS TO CYTOKINE AND THE COMPLEX IS TESTED FOR ABILITY TO AFFECT THE GROWTH OF L929 CELLS.

TOTAL P.02

35

CARPMAELS & RANSFORD

CHARTERED PATENT ATTORNEYS EUROPEAN PATENT ATTORNEYS TRADE MARK AGENTS

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TELEPHONE 071 242 8692
TELEX 267209
FACSIMILE 071 405 4166
071 831 8501

43 BLOOMSBURY SQUARE
LONDON · WC1A 2RA
AND AT MUNICH

European Patent Office,
Munich.

-7 21747

9th September 1993

YOUR REF

OUR REF P07856EP/CPM/EK

Dear Sirs,

Re: European Patent Application No. 91901433.2 2116
Celltech Limited

1. Accelerated Prosecution

1.1. It is hereby requested that the above-referenced application be subjected to accelerated prosecution. The Examination Fee was paid on 23rd May 1991. It is requested that the first communication on Substantive Examination be issued as soon as possible.

1.2. The present application relates to "humanised" antibody molecules and to methods for producing them. In order to "humanise" an antibody, a donor antibody, generally a mouse monoclonal antibody, having a desired specificity is produced. The sequence of the variable domain in each of the heavy and light chains of the donor antibody is then determined. An acceptor antibody, usually a human antibody, is taken and the sequences of its variable domains are changed so that the complementarity determining regions (CDRs) and a number of the framework residues in the original acceptor antibody correspond to the equivalent residues in the donor antibody. In this way, the antibody thus produced, which is a hybrid of donor and acceptor, has donor antigen-binding properties but mainly acceptor non-antigen-binding functions.

1.3. The technique of humanising antibodies is growing in importance in the medical field. It is anticipated that the market for humanized antibodies for use in medical treatments will grow considerably over the next few years.

1.4. The Applicant believes that the present application covers a pioneering invention in this field as it allows any combination of donor and acceptor antibody to be used to produce the hybrid antibody. Moreover, it allows the production of a hybrid antibody having about the same (or in some cases better) affinity for the target antigen as the donor antibody. It is believed that these possibilities have not previously been available.

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1.5. The Applicant is aware that there are a number of companies which are offering antibody humanization services. It is likely that the antibodies they produce will infringe valid claims to be granted on the present application. As evidence of the existence of such companies, I enclose copies of three extracts from SCRIP (of: 9/8/91, page 14; 19/2/92, page 15; and 12/2/93, page 11). These relate to one company which, the Applicant believes, is intending to commercialize at least one of its humanized antibodies in the near future.

1.6. The Applicant would like to be in a position to take action against the company mentioned in SCRIP, and any other similar company, as soon as possible in order to prevent its legitimate rights from being infringed. For the above reasons, it is submitted that the request for Accelerated Prosecution is justified.

2. Amendments

2.1. In order to assist in accelerating the prosecution of this application, it is requested that the present claims be deleted and that new claims 1 to 20 (on new pages 67 to 74) (enclosed in triplicate) be used as the basis for substantive examination.

2.2. At present, no amendments to the description are offered. However, it is appreciated that it will be necessary to amend the description. It is suggested that it would be more efficient to agree on an allowable set of claims. Thereafter, the description can be amended to bring it into conformity with the agreed claims.

2.3. On review of the Application, it was felt that the language of the claims might be imprecise, because it was not clear whether the specified residues were the only or the minimum number of residues to be donor residues. The Applicants have therefore amended the claims to make it clear that there is a minimum number of residues which have to be donor residues and a minimum number which have to be acceptor residues. Those residues which are not specified in the claims may be either donor or acceptor.

2.4. In order to limit the issues raised in this case, the main independent claims have been limited to antibodies comprising both a heavy chain and a complementary light chain. Claims to the separate chains have been cancelled. Also, claims relating to changes in the light chain have been made dependent on the main independent claims.

2.5. Further, claims to DNA sequences, vectors, transformed host cells and methods of culturing such cells have been cancelled. Claim 23 has also been cancelled, in view of Article 52(4) EPC.

2.6. Since claims have been cancelled in order to limit the issues in the present application, the Applicant reserves the right to file divisional applications relating to the deleted subject matter.

2.7. Regarding new claim 1, it can be seen from the description (see page 6, line 29 to page 7, line 28; page 17, lines 9 to 11,

Sections 2.1 to 2.3 bridging pages 17 and 18; Section 1 bridging pages 19 and 20; Sections 2.1.1, 2.2.1, 2.3.1 and 2.4.1 in the passage bridging pages 20 to 23; and Section 15.2.1 on pages 46 and 47) that a number of residues are mentioned which can be considered for changing from acceptor to donor residues. It follows that if a residue has not been considered for changing, it must remain as in the acceptor chain. In order to make this clear, it has been specified in new claim 1 that all the unmentioned residues must be acceptor residues.

2.8. As regards the recitation of the donor residues in new claim 1, this comprises a combination of the minimum residues need to define the CDR loops or the Kabat CDRs. For CDR1, this comprises residues 31 to 35 (see page 46, line 18) and for CDR2 this comprises residues 50 to 58 (see page 46, line 20). For CDR3, the Kabat definition of residues 95 to 102 was used (see page 17, line 11).

2.9. In addition, new claim 1 recites as donor residues those identified on page 20, line 25 and page 21, line 9 as being key residues outside the CDRs.

2.10. In new claim 1, it has been specified that residues 71, 73 and 78 are all donor residues in order to ensure that new claim 1 is novel over the anti-TAC antibody disclosed in PNAS-USA, 86, 10029-10033, 1989 (Queen) (cited in the International Search Report). This anti-TAC antibody has an acceptor residue at residue 73. However, as can be seen from page 7, lines 1 to 5, the Applicant considers that in general, residues 71, 73 and 78 can be either all donor or all acceptor.

2.11. Claim 2 is based on page 15, line 27 and pages 55 to 59 of the description. Claim 3 is based on page 15, line 33 of the description. Claim 4 is based on page 15, line 31 and pages 25 to 52 of the description. Claim 5 is based on page 15, line 31 and pages 53 and 54 of the description. Claim 6 is based on page 15, line 32 and pages 60 and 61 of the description.

2.12. It is stated on page 7, lines 1 to 5 that residues 71, 73 and 78 should all be either acceptor or donor. Claims 2 to 6 cover the first alternative and claim 7 covers the second alternative.

2.13. Claim 8 increases the sizes of CDR1 and CDR2 to the prudent definition given on page 17, line 9 and page 20, lines 6 to 9.

2.14. Claim 9 is based on the passage at page 20, lines 26 and 27. Claim 10 is based on the passage at page 21, lines 10 to 12. Claim 11 is based on the passage on page 21, lines 13 to 16.

2.15. Claim 12 is derived by taking all the donor residues mentioned in claims 1 and 7 to 11 and specifying that all apart from these residues are acceptor residues.

2.16. Claim 13 is derived in the same way that present claim 1 was derived. The residues which can be donor residues are listed on page 9, line 7 to page 10, line 15; page 17, lines 12 to 14; Section 3 bridging pages 18 and 19; Section 2.1.2 on page 20; Section 2.2.2 on

page 21; Section 2.3.2 on page 22 and Section 2.4.2 on page 23.

2.17. Claims 14 to 17 are based on the passage from page 9, line 7 to page 10, line 11.

2.18. Claim 18 is equivalent to original claim 22.

2.19. Claims 19 and 20 put into claim format the Protocol set forth on pages 16 to 23 of the description in a way which is consistent with new claims 1 to 17.

2.20. It is therefore believed that all the claims are fully supported by the description.

3. The Prior art

3.1. In the International Search Report, the first cited document is EP-A-0 403 156 (Genzyme). The present application claims a priority date of 21st December 1989. The Genzyme application was published on 19th December 1990 and therefore cannot be used as a citation under Article 54(2) EPC. It can be used as a citation under Article 54(3) EPC, but only as long as the relevant disclosures in the citation are entitled to a priority date earlier than that of the present application.

3.2. The Genzyme application claims priority from US Patent Applications Nos. 362 549 and 529 979. The '549 application was filed before the priority date of the present application but the '979 application was not. Therefore, only subject matter which is supported by the '549 application can be cited against the present application. We enclose a copy of the '549 application.

3.3. Reference is made to Examples 8 to 12 of the Genzyme application. Examples 8 to 11, at least as far as page 7, line 55 of the Genzyme application, are equivalent to Examples 8 to 11 in the '549 application. However, these Examples relate solely to the production of chimeric antibodies, that is an antibody in which the whole variable domain is derived from a donor antibody. There is no disclosure of any antibody in which the variable domains comprise a mixture of donor and acceptor residues. Thus, these Examples do not deprive the present claims of novelty.

3.4. Example 12 and Tables 6A, 6B, 7A and 7B of the Genzyme application do not appear in the '549 application and thus cannot be cited against the present claims.

3.5. It is therefore believed that the present claims are novel over the parts of the Genzyme application which are entitled to the date of the '549 application.

3.6. Regarding the Queen article referred to above, this discloses a CDR-grafted antibody which has an acceptor residue at position 73 in the heavy chain whereas new claim 1 requires a donor residue at this position. Thus, it is submitted that new claim 1 is novel over Queen.

3.7. The antibody disclosed in the Queen article is directed against the p55 chain of the human interleukin 2 receptor. It does not have specificity for a tumour-specific antigen, an interleukin, the CD3 or CD4 antigen, or an adhesion molecule. It is therefore not specific for any of the types of antigen specified in claims 2 to 6. It is therefore submitted that all these claims are novel over Queen.

3.8. Since claims 7 to 18 are dependent on claims 1 to 6, it is submitted that all these claims are novel over Queen.

3.9. The Queen article discloses a method for "humanising" antibodies which involves molecular modelling and selection of residues to be changed to donor residues on the basis of comparisons of molecular models. This is an entirely different process from the one set forth in claims 19 and 20. Thus, these claims are novel over Queen.

3.10. In fact, there is nothing in the Queen paper which even suggests that it might be possible to determine a hierarchy of residues to be changed to donor which will work for any antibody with any framework. The disclosure in Queen suggests that each donor antibody needs to be treated as an individual case in comparison with the acceptor antibody. There is no suggestion that results obtained with one pair of donor and acceptor antibodies could be applied successfully to a different pair of antibodies. In contrast, the present invention shows that it is possible to apply general rules to any pair of antibodies in the expectation of success.

3.11. It is therefore submitted that the present claims are both novel and inventive over Queen.

3.12. EP-A-0 239 400 (Winter) is also cited in the International Search Report. The basic disclosure in the Winter application is limited to grafting only the CDRs from one antibody to another. All the Examples in the Winter application relate to this. There is a passage in the Winter application which indicates that it may be necessary to alter some framework residues. However, there is no guidance at all as to which residues should be changed or as to how such residues should be selected. There is certainly no suggestion that there is a hierarchy of residues which should be considered. Thus, it is submitted that the present claims are both novel and inventive over the Winter application.

3.13. It is believed that none of the other documents cited in the International Search Report is more relevant to the present claims than the three documents referred to above. Therefore, no discussion of any of these documents is provided. However, the Applicant would be pleased to address any concern the Examiner may have in this respect.

4. Procedure

4.1. It is suggested that, once the Examiner has had an opportunity to study the file and familiarize himself with the case, it would be useful to hold an interview to address any concerns that the Examiner may have. If, after consideration of the file, the Examiner agrees

with this suggestion, it is requested that the Examiner sends out a Communication identifying any areas of concern and any documents which need to be discussed so that the Applicant can address those points fully.

4.2. If the Examiner feels that prosecution could be expedited in any other way, he is requested to telephone the undersigned to discuss the matter.

4.3. As a precautionary measure, the Applicant hereby requests Oral Proceedings, in the event that the Examining Division should feel minded to refuse the application.

Your faithfully,

MERCER, CHRISTOPHER PAUL

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CLAIMS

1. An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain comprising acceptor antibody heavy chain framework residues and donor antibody heavy chain antigen-binding residues, said donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain, amino acid residues 5, 8, 10, 12 to 17, 19, 21, 22, 40, 42 to 44, 66, 68, 70, 74, 77, 79, 81, 83 to 85, 90, 92, 105, 109, 111 and 113 at least are acceptor residues and amino acid residues 23, 24, 31 to 35, 49 to 58, 71, 73, 78 and 95 to 102 at least are donor residues.

2. A tumour-specific antibody molecule having affinity for a predetermined tumour antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain comprising acceptor antibody heavy chain framework residues and donor antibody heavy chain antigen-binding residues, said donor antibody having affinity for said predetermined tumour antigen, wherein, according to the Kabat numbering system, in said composite heavy chain, amino acid residues 5, 8, 10, 12 to 17, 19, 21, 22, 40, 42 to 44, 66, 68, 70, 74, 77, 79, 81, 83 to 85, 90, 92, 105, 109, 111 and 113 at least are acceptor residues and amino acid residues 23, 24, 31 to 35, 49 to 58 and 95 to 102 at least are donor residues.

3. An interleukin-specific antibody molecule having affinity for a predetermined interleukin and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain comprising acceptor antibody heavy chain framework residues and donor antibody heavy chain antigen-binding residues, said donor antibody having affinity for said predetermined interleukin wherein, according to the Kabat numbering system, in said

composite heavy chain, amino acid residues 5, 8, 10, 12 to 17, 19, 21, 22, 40, 42 to 44, 66, 68, 70, 74, 77, 79, 81, 83 to 85, 90, 92, 105, 109, 111 and 113 at least are acceptor residues and amino acid residues 23, 24, 31 to 35, 49 to 58 and 95 to 102 at least are donor residues.

4. An anti-CD3 antibody molecule having affinity for the CD3 antigen comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain comprising acceptor antibody heavy chain framework residues and donor antibody heavy chain antigen-binding residues, said donor antibody having affinity for said CD3 antigen, wherein, according to the Kabat numbering system, in said composite heavy chain, amino acid residues 5, 8, 10, 12 to 17, 19, 21, 22, 40, 42 to 44, 66, 68, 70, 74, 77, 79, 81, 83 to 85, 90, 92, 105, 109, 111 and 113 at least are acceptor residues and amino acid residues 23, 24, 31 to 35, 49 to 58 and 95 to 102 at least are donor residues.

5. An anti-CD4 antibody molecule having affinity for the CD4 antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain comprising acceptor antibody heavy chain framework residues and donor antibody heavy chain antigen-binding residues, said donor antibody having affinity for said CD4 antigen, wherein, according to the Kabat numbering system, in said composite heavy chain, amino acid residues 5, 8, 10, 12 to 17, 19, 21, 22, 40, 42 to 44, 66, 68, 70, 74, 77, 79, 81, 83 to 85, 90, 92, 105, 109, 111 and 113 at least are acceptor residues and amino acid residues 23, 24, 31 to 35, 49 to 58 and 95 to 102 at least are donor residues.

6. An anti-adhesion molecule antibody molecule having affinity for an adhesion molecule and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain comprising human

acceptor antibody heavy chain framework residues and donor antibody heavy chain antigen-binding residues, said donor antibody having affinity for said adhesion molecule wherein, according to the Kabat numbering system, in said composite heavy chain, amino acid residues 5, 8, 10, 12 to 17, 19, 21, 22, 40, 42 to 44, 66, 68, 70, 74, 77, 79, 81, 83 to 85, 90, 92, 105, 109, 111 and 113 at least are acceptor residues and amino acid residues 23, 24, 31 to 35, 49 to 58 and 95 to 102 at least are donor residues.

7. The antibody molecule of any one of claims 2 to 6 wherein amino acid residues 71, 73 and 78 in said composite heavy chain are additionally donor residues.

8. The antibody molecule of any one of claims 1 to 7, wherein amino acid residues 26 to 30 and 59 to 65 in said composite heavy chain are additionally donor residues.

9. The antibody molecule of any one of claims 1 to 8, wherein at least one of amino acid residues 1, 3, and 76 in said composite heavy chain are additionally donor residues.

10. The antibody molecule of any one of claims 1 to 9, wherein at least one of amino acid residues 36, 94, 104, 106 and 107 in said composite heavy chain are additionally donor residues.

11. The antibody molecule of claim 10, wherein at least one of amino acid residues 2, 4, 6, 38, 48, 67 and 69 in said composite heavy chain are additionally donor residues.

12. The antibody molecule of any one of claims 1 to 11 wherein amino acid residues 7, 9, 11, 18, 20, 25, 37, 39, 41, 45, 47, 48, 72, 75, 80, 82, 86 to 89, 91, 93, 103, 108, 110 and 112 in said composite heavy chain are additionally acceptor residues.

13. The antibody molecule of any one of claims 1 to 12,

wherein said complementary light chain is a composite light chain having a variable domain comprising acceptor antibody light chain framework residues and donor antibody light chain antigen-binding residues, said donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite light chain, amino acid residues 5, 7 to 9, 11, 13 to 18, 20, 22, 23, 39, 41 to 43, 57, 59, 61, 72, 74 to 79, 81, 82, 84, 86, 88, 100, 104, 106 and 107 at least are acceptor residues and amino acid residues 24 to 34, 46, 48, 50 to 56, 58, 71 and 89 to 97 at least are donor residues.

14. The antibody molecule of claim 13, wherein amino acid residues 1, 3 and 47 in said composite light chain are additionally donor residues.

15. The antibody molecule of claim 13 or claim 14, wherein amino acid residues 36, 44, 47, 85 and 87 in said composite light chain are additionally donor residues.

16. The antibody molecule of any one of claims 13 to 15, wherein at least one of amino acid residues 2, 4, 6, 49, 62, 64 to 69, 98, 99, 101 and 102 in said composite lightly chain are additionally donor residues.

17. The antibody molecule of any one of claims 13 to 16, wherein at least one of amino acid residues 1, 3, 10, 12, 21, 40, 60, 63, 70, 73, 80, 103 and 105 in said composite light chain are additionally donor residues.

18. A therapeutic or diagnostic composition comprising the antibody molecule of any one of claims 1 to 17 in combination with a pharmaceutically acceptable carrier, diluent or excipient.

19. A method for producing a recombinant antigen binding molecule having affinity for a predetermined antigen comprising the steps of:

[1] determining the amino acid sequence of the variable domain of the heavy chain of a donor antibody which has affinity for said predetermined antigen;

[2] determining the amino acid sequence of the variable domain of the heavy chain of a non-specific acceptor antibody;

[3] providing a composite heavy chain for an antibody molecule, said composite heavy chain having acceptor framework residues and donor antigen binding residues wherein, according to the Kabat numbering system, amino acid residues 5, 8, 10, 12 to 17, 19, 21, 22, 40, 42 to 44, 66, 68, 70, 74, 77, 79, 81, 83 to 85, 90, 92, 105, 109, 111 and 113 at least are acceptor residues and amino acid residues 23, 24, 31 to 35, 49 to 58 and 95 to 102 at least are donor residues;

[4] associating the heavy chain produced in step [3] with a complementary light chain to form an antibody molecule;

[5] determining the affinity of the antibody molecule formed in step [4] for said predetermined antigen;

[6] if the affinity determined in step [5] is not equivalent to that of the donor antibody, providing a heavy chain as described in [3] above but in which amino acid residues 71, 73 and 78 are additionally donor residues;

[7] associating the heavy chain produced in step [6] with a complementary light chain to form an antibody molecule;

[8] determining the affinity of the antibody molecule formed in step [7] for said predetermined antigen;

[9] if the affinity determined in step [8] is not equivalent to that of the donor antibody, providing a heavy chain as described in [6] above but in which amino acid residues 26 to 30 are additionally donor residues;

[10] associating the heavy chain produced in step [9] with a complementary light chain to form an antibody molecule;

[11] determining the affinity of the antibody molecule formed in step [10] for said predetermined antigen;

[12] if the affinity determined in step [11] is not equivalent to that of the donor antibody, providing a heavy chain as described in [9] above but in which at least one of amino acid residues 1, 3, and 76 are additionally donor residues;

[13] associating the heavy chain produced in step [12] with a complementary light chain to form an antibody molecule;

[14] determining the affinity of the antibody molecule formed in step [13] for said predetermined antigen;

[15] if the affinity determined in step [14] is not equivalent to that of the donor antibody, providing a heavy chain as described in [12] above but in which at least one of amino acid residues 36, 94, 104, 106, 107 are additionally donor residues;

[16] associating the heavy chain produced in step [15] with a complementary light chain to form an antibody molecule.

[17] determining the affinity of the antibody molecule formed in step [16] for said predetermined antigen;

[18] if the affinity determined in step [17] is not equivalent to that of the donor antibody, providing a heavy chain as described in [15] above but in which at least one of amino acid residues 2, 4, 6, 38, 48, 67 and 69 are additionally donor residues; and

[19] associating the heavy chain produced in step [18] with a complementary light chain to form an antibody molecule.

20. The method of claim 19, further comprising the steps of:

[1] determining the amino acid sequence of the variable domain of the light chain of said donor antibody which has affinity for said predetermined antigen;

[2] determining the amino acid sequence of the variable domain of the light chain of a non-specific acceptor antibody;

[3] providing a composite light chain for an

antibody molecule, said composite light chain having acceptor framework residues and donor antigen binding residues wherein, according to the Kabat numbering system, amino acid residues 5, 7 to 9, 11, 13 to 18, 20, 22, 23, 39, 41 to 43, 57, 59, 61, 72, 74 to 79 to 79, 81, 82, 84, 86, 88, 100, 104 and 106 to 109 at least are acceptor residues and amino acid residues 24 to 34, 46, 48, 50 to 56, 58, 71 and 89 to 97 at least are donor residues;

[4] associating the light chain produced in step [3] with a complementary heavy chain to form an antibody molecule;

[5] determining the affinity of the antibody molecule formed in step [4] for said predetermined antigen;

[6] if the affinity determined in step [5] is not equivalent to that of the donor antibody, providing a light chain as described in [3] above but in which amino acid residues 1, 2, 3 and 47 are additionally donor residues;

[7] associating the light chain produced in step [6] with a complementary heavy chain to form an antigen-binding molecule;

[8] determining the affinity of the antigen-binding molecule formed in step [7] for said predetermined antigen;

[9] if the affinity determined in step [8] is not equivalent to that of the donor antibody, providing a light chain as described in [6] above but in which amino acid residues 36, 44, 47, 85 and 87 are additionally donor residues;

[10] associating the light chain produced in step [9] with a complementary heavy chain to form an antibody molecule;

[11] determining the affinity of the antibody molecule formed in step [10] for said predetermined antigen;

[12] if the affinity determined in step [11] is not equivalent to that of the donor antibody, providing a light chain as described in [9] above but in which at least one of amino acid residues 2, 4, 6, 49, 62, 64 to 69, 98, 99, 101 are additionally donor residues; and

[13] associating the light chain produced in step [9]

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with a complementary heavy chain to form an antibody molecule.

DOCKET NO.: CARP-0000 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

John R. Adair, et al.

Serial No.: 07/743,329 Group Art Unit: 1807

Filed: September 17, 1991 Examiner: L. Bennett

For: HUMANISED ANTIBODIES

I, Doreen Yatko Trujillo, Registration No. 35,719 certify that this correspondence is being deposited with the U.S. Postal Service as First Class mail in an envelope addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231.

On February 7, 1994

Doreen Yatko Trujillo

Doreen Yatko Trujillo, Reg. No. 35,719

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Dear Sir:

AMENDMENT

This amendment is filed in response to the Office Action mailed September 7, 1993. A petition for extension of time and the appropriate fee is attached.

In the claims:

Please cancel claims 73 to 107, 109 to 113 and 115 to 119, without prejudice.

Please amend claims 67 and 71 as follows:

67. (Amended) An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain comprising human acceptor antibody heavy chain [framework] residues and donor antibody heavy chain [antigen-binding] residues, said donor antibody having affinity for said predetermined antigen, said variable domain further comprising complementarity determining regions, wherein, according to the Kabat numbering system, in said composite heavy chain, said complementarity determining regions comprise donor residues at least at residues 31 to 35, 50 to 58 and 95 to 102; amino acid residues 5, 8, 10, 12 to 17, 19, 21, 22, 40, 42 to 44, 66, 68, 70, 74, 77, 79, 81, 83 to 85, 90, 92, 105, 109, 111 and 113 at least are acceptor residues; and amino acid residues 23, 24, [31 to 35,] 49 [to 58], 71, 73[,] and 78 [and 95 to 102] at least are donor residues.

Claim 71, line 2, please delete "48" and insert --46--.

REMARKS

Claims 67-72, 108 and 114 are pending. The number of claims pending in the present application has been reduced in order to expedite the prosecution of the case. The deletion of some claims should not be taken to be an admission that the subject

matter of the deleted claims is unpatentable. The Applicants reserve the right to file continuation applications directed to the deleted subject matter. The Examiner is thanked for bringing the typographical error in Claim 71 to the Applicants' attention.

To the extent the rejections are maintained against amended claim 67, and the remaining claims, Applicants respectfully request reconsideration for the reasons set forth below.

Rejections Under 35 U.S.C. § 112, First Paragraph

In paragraph 16 of the present Office Action, the Examiner contends that the application does not contain any support for the recitation of acceptor residues in the light or heavy chains. It is submitted, for the following reasons, that the Examiner's contention is incorrect.

At a very helpful interview held at the beginning of 1993, there was some discussion of the word "comprising" as used in the claims under consideration at that time. In those claims, it was only specified that certain residues should be donor residues. It was considered that it was not clear whether these were the only residues which could be donor residues. The alternative view was that these were only the minimum number of residues which must be donor but that any of the other residues could also be donor.

If the second line of interpretation were taken, the claims could be read to cover a situation in which all except one of the residues in the variable domain were donor residues. In

this case, the claims could then be interpreted to cover a structure similar to a "chimeric" antibody comprising a donor variable domain and a human constant region. Such chimeric antibodies were already well known at the priority date.

It plainly is not the intention of the Applicants to claim chimeric antibodies or any similar structures. As can be seen from the description, the superhumanised antibodies of the present invention are compared to the prior art chimeric antibodies. Moreover, the present invention was intended to deal with the problem of chimeric antibodies in that chimeric antibodies were believed to be too "foreign" because of the presence of the complete donor variable domain.

For the above reasons, it is clear that the wording of the claims needed to be changed so that the Applicants' intention of excluding chimeric antibodies was made effective. The language now present in the claims puts this intention clearly into effect.

As to support for this wording, the Examiner is referred firstly to page 16, under the heading "Protocol". It can be seen from this paragraph that the first step in the process involves the choice of an appropriate acceptor chain variable domain. This acceptor domain must be of known sequence. Thus, the protocol starts with a variable domain in which all the residues are acceptor residues. In the sentence bridging pages 16 and 17, it is stated that:

> "The CDR-grafted chain is then designed starting from the basis of the acceptor sequence".

On page 17, in the middle paragraph, it is stated that:

> "The positions at which donor residues are to be substituted for acceptor in the framework are then chosen as follows...."

This again shows that, unless a residue is chosen for substitution, it will remain as in the acceptor sequence.

It must also be borne in mind that the purpose of the invention is to obviate some of the disadvantages of prior art proposals. The proposal of using chimeric antibodies had the disadvantage that they were more "foreign" than desirable. The problem of making CDR-grafted antibodies was that they generally did not provide good recovery of affinity. Thus, the aim of the present invention was to minimise as far as possible the "foreign" nature of the antibody while maximising as far as possible its affinity.

Bearing the passages referred to above and the aim of the invention in mind, it would have been abundantly clear to the skilled person reading the application that as many residues as possible should remain as acceptor residues. If this were not the case, it could hardly be said that the composite chain is based on the acceptor sequence.

The skilled person reading the application can plainly see that certain residues have been considered for changing from acceptor to donor. These are clearly set out in the description.

It would be plain to the skilled person that all other residues should not be considered for changing at all. It would therefore be obvious that any residue which is not specified as being under consideration for changing must remain as in the acceptor chain.

It may be that there is no explicit statement in the description that the specified residues should remain as in the acceptor chain. However, the disclosure in a specification is not limited to the explicit disclosure but also includes that which is implicit. It is implicit, in the recitation that the chain is based on the acceptor and that only certain residues are considered for changing, that all non-specified residues must remain as acceptor residues. Subject matter which might be fairly deduced from the disclosure is not new matter. *Acme Highway Products Corp. v. D.S. Brown Co.*, 431 F.2d 1074, 1080, 167 U.S.P.Q. 129, 132-133 (6th Cir. 1970), *cert denied*, 401 U.S. 956 (1971).

Another way to look at it is to consider a different way in which the claim could be drafted. It could be specified that in the composite chain, at least a certain minimum number of residues are donor residues (as in the present claims) and at most a certain maximum number of residues are donor residues. The maximum number would be derived by listing all the residues which are considered for changing. Such an amendment would have clear explicit basis in the description because all those residues are mentioned as such. However, the effect of such an amendment would be to produce claims of exactly the same scope as the present claims. It can thus be

seen that the present claims do not add subject matter but are plainly properly based on the disclosure in the description.

It is therefore submitted that the claims are fully supported by the description, are commensurate in scope with the disclosure in the description, and are properly delimited over the prior art.

The rejections of Claims 73-107, 109-113, and 115-119 under 35 U.S.C. §112 has been rendered moot by their withdrawal.

In paragraph 26 of the Office Action, the Examiner maintains the rejection of the claims for lack of enablement. It is submitted that this rejection cannot stand for the following reasons.

The Examiner contends that the description does not provide a "representative" number of Examples falling within the scope of the claims. Even if this were the case (and it is not, for reasons set out below) this does not provide a proper basis for rejection under 35 U.S.S. §112. A "representative" number of Examples is not required to obtain a patent. All that is required is that the disclosure be enabling. Enablement does not depend on the number of examples provided. Sufficient disclosure can be provided by illustrative examples or terminology. Further, "It is well settled that patent applications are not required to disclose every species encompassed by their claims, even in an unpredictable art." *In re Vaeck,* 20 U.S.P.Q.2d 1438, 1445 (Fed. Cir. 1991).

The Examiner also appears to be arguing that it may be that some antibodies will not be susceptible to the protocol of the present invention, i.e. that not all embodiments will work. Even if this were the case (and it is not, for reasons set out below). this also does not provide a proper basis for rejection under 35 U.S.C. §112. That inoperative embodiments may be encompassed is not detrimental. "It is not the function of claims or the specification to exclude all inoperative substances." *Ex parte Janin,* 209 U.S.P.Q. 761, 763 (Bd. of App. 1979). "The mere fact that a claim embraces undisclosed or inoperative species or embodiments does not necessarily render it unduly broad." *Horton v. Stevens,* 7 U.S.P.Q.2d 1245, 1247 (Bd. of Pat. App. & Int. 1988).

Apart from the legal points made above, it is submitted that the Examiner is incorrect on the technical facts. Before expanding on this, however, it would be worthwhile to make a few points concerning affinity. There is no absolute value which can be set which defines good affinity. Affinity can be measured, for instance in reciprocal moles (M^{-1}). In this measurement system, affinity can vary from 10^6 to 10^{12}.

Natural antibodies, as produced *in vivo,* do not all have the same affinity, even for the same antigen. Thus, in the normal polyclonal antiserum produced on challenge by an antigen, the body will produce a variety of antibodies having affinities within the range given above. Monoclonal antibodies, as produced by hybridoma

technology, also have varying affinities, again within the range given above. The variation in the affinity may in part be due to the structure of the antibody and in part to the structure of the antigen. It may therefore be that a good antibody directed against antigen X has an affinity of only 10^7 whereas a good antibody against antigen Y may have an affinity of 10^{12}. These are both good antibodies, even though they have very different affinities.

It can be seen that if an engineered antibody is produced against antigen X with an affinity of 10^8, this will be regarded as being exceptional, in that the affinity has gone up 10 fold compared to the good antibody. However, if an engineered antibody recognizing antigen Y is produced with an affinity of 10^8, this will be regarded as being an awful result as the affinity will have been reduced 10,000 fold.

Thus, the only sensible way to determine whether an engineered antibody is successful is to compare its affinity with that of the prototype antibody from which it is derived. It is pointless to look at the absolute value of the affinity because this does not tell you anything about the success or failure of the engineering operation. It is for this reason that the Applicants have provided such qualitative evidence of the success of the protocol described in the application.

Further, in some cases, a residue which is selected for changing according to the protocol described in the application may

not need to be changed. It may be that, fortuitously, it is the same in the donor and acceptor chains. This does not mean that, if the residues had been different, it would not have been changed. It merely means that, in effect, the change had already been made.

As to the number of antibodies which have been shown to have been successfully superhumanised using the protocol of the present invention, the Examiner is requested to look at the sheets attached to the previous response submitted April 7, 1993. Although Applicants are not required to provide a "representative number of examples", the provision of so many antibodies in these attachments should have satisfied any doubts on the part of the Examiner. Yet the Examiner makes no reference to these attachments and the evidence they provide.

The Examiner is also referred to the passage beginning on page 17 through page 19 of the last response. This shows in detail that a representative number of antibodies falling within the terms of the present claims were superhumanised successfully. Again the Examiner has not even referred to these pages. The Examiner has not provided any reasoning as to why these pages are not persuasive. It is submitted that mere allegation is not enough. The Examiner must also provide references or, if based upon personal knowledge, an affidavit, in support of the Examiner's allegations. MPEP § 706.02.

As has been shown by the third sheet attached to the previous response, the successful antibodies are representative not

only in number, but also as regards to antigens recognized. The antigens include cell surface antigens found on both healthy and cancerous cells, soluble cytokines and adhesion molecules. These are all very different in structure and function, yet antibodies against each of them have been successfully superhumanised using the protocol of the present invention.

It is no doubt the case that some of the antibodies referred to in the sheets were more successfully humanised than others. However, the reasons for this were clearly set out in the previous response. Thus, evidence that the replacement scheme is not generally applicable has not been provided.

The Examiner places much reliance on the prior art as, in her view, showing that there would have been no reasonable expectation of success. The Applicants agree that, if there were only the prior art to go on, then there would have been no reasonable expectation of success. However, the skilled person trying to put the present invention into practice does not have to rely on only the prior art. The skilled person has available the teaching of the present application. It is specifically stated in the application that the present protocol represents a departure from the procedures of Reichmann and Queen, at least. Thus, the skilled person would not rely on Reichmann and Queen as teachings relevant to whether the present description is enabling.

It is submitted that the skilled person would rely on the clear teaching given in the application and find that it is

enabling. The specification plainly sets out what actions need to be taken. It is presumed that the Examiner agrees that the skilled person could have taken those actions. The application also sets out that, contrary to the teachings of Reichmann and Queen, the protocol is generally applicable. The application further shows that it had been successfully implemented. Thus, it is submitted that the skilled person would find that the present application is properly enabled the full extent of the claims.

Rejections Under 35 U.S.C. § 103

The Examiner rejected all the claims as being obvious over Reichmann and Queen. However, this rejection appears contrary to her previous assertions. When attacking the enablement of the claims, the Examiner stated that:

> "... in light of the prior art (for instance, Reichmann et al., Queen et al., and Chothia et al.) such a universal property appears to be unpredictable... The prior art does not teach that standardized principle... is possible."

(emphasis added)

The Applicants agree with the Examiner that the prior art provides no predictability of success and certainly no expectation that a generally applicable principle can be devised. It is submitted that this is a clear indication that the surprising

discovery that there is a generally applicable principle involves an invention.

The Examiner indicated that the arguments previously presented by the Applicants were deemed to be non-persuasive because they did not address the combined effect of Reichmann and Queen. This, of course, assumes that the skilled artisan would have combined Reichmann and Queen in the first place. The Examiner has shown no reason why Reichmann and Queen would have been combined. It is submitted that there is no reason why they should be combined.

The earlier publication is Reichmann. This shows a relatively simple procedure in which the six CDRs from a rat antibody against a leukocyte cell surface antigen are transferred onto human frameworks. The only additional residue change is in the heavy chain at residue 27. The reason that this residue is changed is because it was atypical in the human (acceptor) chain. The change was to replace residue 27 with the more normal acceptor residue. Thus, the teaching of Reichmann is that, as long as you have normal human (acceptor) chain, all that is needed is for the CDRs to be changed.

Queen does in fact refer to Reichmann. Reichmann is reference 24 in Queen. However, this is only referred to in passing on page 10029 as being an example of the work of Winter and his colleagues. The teaching of Queen clearly goes beyond that of

Reichmann. Thus, there is no incentive to try to combine the teachings of Reichmann and Queen.

Even if one could find some motivation for combining Reichmann and Queen, it is submitted that Reichmann would not add to Queen such that Applicants invention would be rendered obvious. Reichmann teaches the skilled person to use a normal acceptor sequence and merely to change the CDRs. If the acceptor sequence is not normal, then the abnormal residues are to be changed to normal acceptor residues. This is all disclosed in Queen. Since, at best, Queen incorporates all the teaching of Reichmann, even if Reichmann and Queen are combined, the total teaching is no more than teaching of Queen by itself. If the Examiner is of the contrary view, she is requested to point out specifically the teaching in Reichmann which is not present in Queen and why she believes this additional teaching, when combined with the teaching in Queen, renders the present claims obvious.

For the reasons set forth in the previous response, it is submitted that Queen, and therefore also a combination of Queen and Reichmann, does not render the present claims obvious.

The Examiner noted that the previously presented arguments concentrated on the process aspects of the disclosure in Queen and contended that this is irrelevant as far as the product claims in the present application are concerned. It is submitted that this is not correct. The only specifically disclosed product in Queen does not fall within the terms of the present claims. It

is therefore necessary for the Examiner to show that a product falling within the terms of the claims would have been produced using the teachings of Queen, alone or in combination with the prior art, or obvious variants thereof. If there is no expectation that such a product would have been produced, then the product claims are not obvious.

To maintain otherwise is to employ impermissible hindsight. Queen only describes the replacement of some acceptor residues outside the CDR for a "specific" antibody. Queen does not disclose, or even suggest, a "general" approach for replacing acceptor residues outside the CDRs with donor residues. In fact, the absence of such disclosure in Queen, or in any of the prior art cited, was previously emphasized in the Examiner's rejection under § 112 for enablement. Therefore, it is respectfully submitted that the Examiner would not have even contemplated that Queen discloses, or suggests, a general superhumanised antibody as claimed without the benefit of Applicants' disclosure. Neither would one skilled in the art.

Further, it is submitted that the Examiner has not established a *prima facie* case of obviousness. The teachings from the prior art *itself* should appear to have suggested the claimed subject matter to a person of ordinary skill in the art to establish a *prima facie* case. *In re Rijckaert*, 28 U.S.P.Q. 2d 1955 (Fed. Cir. 1993). As already admitted by the Examiner in the rejections under § 112, the expectation that a generalized approach

is feasible is nowhere present in the prior art. Thus, the claims as drafted, covering superhumanised antibodies in general (i.e., not limited to a particular antibody) are not obvious over the prior art.

The Examiner also asserts that how the residues are identified is irrelevant. It is submitted that this is not the case when the rejection is one for obviousness. The references do not disclose the residues claimed by Applicants. To render Applicants' claims obvious, the Examiner needs to show that the prior art could lead one skilled in the art to identify the same residues as are identified in the present claims. The Examiner has not shown any reason why the skilled person, carrying out the method described by Queen, alone or in combination with Reichmann, would have expected to identify the specific sets of residues identified in the present claims. In this regard, it is noted that a rejection for obviousness was not levied against the method claims in the Office Action--i.e., claims 118 and 119.

It is again to be pointed out that the present claims cannot be generalized to "an antibody in which the CDRs and some, (unspecified) framework residues have been changed." The present claims relate to "an antibody in which the CDRs and only certain, specific framework residues have been changed." Nothing in the prior art, whatever combination is used, leads the skilled person to the specific set of residues set forth in the present claims.

It is therefore submitted that the present claims are not at all obvious over Queen, combined with Reichmann, or on its own.

Paragraphs 18 to 25 and 27 to 29

The contents of paragraphs 18 to 25 and 27 to 29 are noted with appreciation.

The foregoing represents a bona fide attempt to advance the case to allowance. Applicants respectfully request that all presently pending claims be allowed.

Respectfully submitted,

Doreen Yatko Trujillo

Doreen Yatko Trujillo
Registration No. 35,719

Date: February 7, 1994

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS
One Liberty Place - 46th Floor
Philadelphia, PA 19103
(215) 568-3100

different paths, and by ensuring that an azimuthally uniform coverage of stations is used in the averaging calculation. To compensate for other factors, such as focal depth, fault geometry and corner frequency would require such a detailed knowledge of the earthquake source that the M_s measurement itself would be redundant.

The results of this analysis can be summarized in five points.

(1) A global average moment-magnitude relationship M_s has been defined which can be used to predict M_0 over a wide range of magnitudes and scalar moments.

(2) The variance of surface wave measurements for an event of a particular scalar moment is ~0.2 magnitude units.

(3) Large regional biases in M_s exist.

(4) Differences in source scaling may explain some of the differences. Specifically, observations show that the transition from a slope of unity to a smaller value occurs at large moments for continental events than for ridge and fracture zone events, suggesting systematic differences in stress drop.

(5) Other systematic factors affecting the calculation of M_s also appear to contribute to the observed regional bias.

We thank Professor J. H. Woodhouse for reading and correcting the manuscript and Professor H. Kanamori for constructive criticism throughout our work on this subject. This work was supported by the NSF.

Received 20 October 1987; accepted 4 February 1988.

1. Richter, C. F. *Bull. seism. Soc. Am.* **25**, 1–32 (1935).
2. Vanek, J. *et al. Izv. akad. Nauk. USSR, Ser. Geophys.* **2**, 153–158 (1962).
3. Aki, K. *Bull Earthqu. Res. Inst. Tokyo Univ.* **44**, 23–88 (1966).
4. Agnew, D., Berger, J., Buland, R., Farrell, W. & Gilbert, F. *Eos* **57**, 280–288 (1976).
5. Peterson, J., Butler, H. M., Holcomb, L. G. & Hutt, C. R. *Bull seism. Soc. Am.* **66**, 2049–2068 (1976).
6. Kanamori, H. & Given, J. W. *Phys. Earth planet. Inter.* **27**, 8–31 (1981).
7. Dziewonski, A. M., Chou, T. A. & Woodhouse, J. H. *J. geophys. Res.* **86**, 2825–2852 (1981).
8. Woodhouse, J. H. & Dziewonski, A. M. *J. geophys. Res.* **88**, 3247–3271 (1983).
9. Woodhouse, J. H. & Dziewonski, A. M. *J. geophys. Res.* **89**, 5953–5986 (1984).
10. Dziewonski, A. M., Franzen, J. E. & Woodhouse, J. H. *Phys. Earth planet. Inter.* **34**, 209–219 (1984).
11. Dziewonski, A. M., Ekström, G., Franzen, J. E. & Woodhouse, J. H. *Phys. Earth planet. Inter.* **45**, 11–36 (1987).
12. Dziewonski, A. M., Ekström, G., Woodhouse, J. H. & Zwart, G. *Phys. Earth planet. Inter.* (in the press).
13. Kanamori, H. *J. geophys. Res.* **82**, 2981–2987 (1977).
14. Richter, C. F. *Elementary Seismology* (W. H. Freeman, San Fransisco, 1958).
15. Lienkamper, J. J. *Bull. seism. Soc. Am.* **74**, 2357–2368 (1984).
16. Kanamori, H. Anderson, D. L. *Bull. seism. Soc. Am.* **65**, 1073–1095 (1975).
17. Ekström, G. & Dziewonski, A. M. *Bull. seism. Soc. Am.* **75**, 23–39 (1985).
18. Sipkin, S. A. *Bull. seism. Soc. Am.* **76**, 1515–1541 (1986).
19. Harkrider, D. G. *Bull. seism. Soc. Am.* **54**, 627–679 (1964).
20. Gutenberg, B. & Richter, C. F. *Gerlands Beitr. z. Geophysik* **47**, 73–131 (1936).
21. Gutenberg, B. *Bull. seism. Soc. Am.* **35**, 3–12 (1945).
22. Von Seggern, D. *Bull. seism. Soc. Am.* **60**, 503–516 (1970).
23. Nuttli, O. *Tectonophysics* **118**, 161–174 (1985).
24. Kanamori, H. & Allen, C. R. in *Maurice Ewing Series Vol. 6, Earthquake Source Mechanics* (American Geophysical Union, Washington, DC, 1986).
25. Zhou, T. & Kanamori, H. *Bull. seism. Soc. Am.* **77**, 514–529 (1987).

Reshaping human antibodies for therapy

Lutz Riechmann†, Michael Clark*, Herman Waldmann* & Greg Winter‡

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK
* Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QP, UK

A human IgG1 antibody has been reshaped for serotherapy in humans by introducing the six hypervariable regions from the heavy- and light-chain variable domains of a rat antibody directed against human lymphocytes. The reshaped human antibody is as effective as the rat antibody in complement and is more effective in cell-mediated lysis of human lymphocytes.

IN 1890 it was shown that resistance to diphtheria toxin could be transferred from one animal to another by the transfer of serum. It was concluded that the immune serum contained an anti-toxin, later called an antibody[1]. For many years animal antisera were used in the treatment of microbial infections and for the neutralization of toxins in man[2]. More recently rodent monoclonal antibodies (mAbs)[3] have been used as 'magic bullets'[4] to kill and to image tumours[5,6]. The foreign immunoglobulin, however, can elicit an anti-globulin response which may interefere with therapy[7] or cause allergic or immune complex hypersensitivity[8]. Thus ideally human antibodies would be used. Human immunoglobulins are widely used as both prophylactic and microbicidal agents[9], but it would be far better to have available human mAbs of the desired specificity. It has proven difficult, however, to make such mAbs by the conventional route of immortalization of human antibody-producing cells[9].

There is an alternative approach. Antibody genes have been transfected into lymphoid cells, and the encoded antibodies expressed and secreted; by shuffling genomic exons, simple chimaeric antibodies with mouse variable regions and human constant regions have been made[10–12]. Such chimaeric antibodies have at least two advantages over mouse antibodies. First, the effector functions can be selected or tailored as desired. For example, of the human IgG isotypes, IgG1 and IgG3 appear to be the most effective for complement and cell-mediated lysis[13–15], and therefore for killing tumour cells. Second, the use of human rather than mouse isotypes should minimize the anti-globulin responses during therapy[16,17] by avoiding anti-isotypic antibodies. The extent to which anti-idiotypic responses to rodent antibodies in therapy are dictated by foreign components of the variable versus the constant region is not known, but the use of human isotypes should reduce the anti-idiotypic response. For example, when mice were made tolerant to rat immunoglobulin constant-region determinants, administration of rat anti-lymphocyte antibodies did evoke anti-idiotypic responses, but these were delayed and weaker than in animals that had not been made tolerant[18]. Nevertheless, it is likely that a chimaeric antibody would provoke a greater immune response than a human mAb.

We have attempted to build rodent antigen binding sites directly into human antibodies by transplanting only the antigen binding site, rather than the entire variable domain, from a rodent antibody. The antigen binding site is essentially encoded by the hypervariable loops at one end of the β-sheet framework. The hypervariable regions of the heavy chain of mouse antibodies against a hapten[19] or a protein antigen[20] were previously transplanted into a human heavy chain, and, in association with the mouse light chain, the antigen binding site was retained.

† Address from April 1988: Department of Molecular Biology, The Research Institute of Scripps Clinic, North Torrey Pines Road, La Jolla, California 92037, USA
‡ To whom correspondence should be addressed.

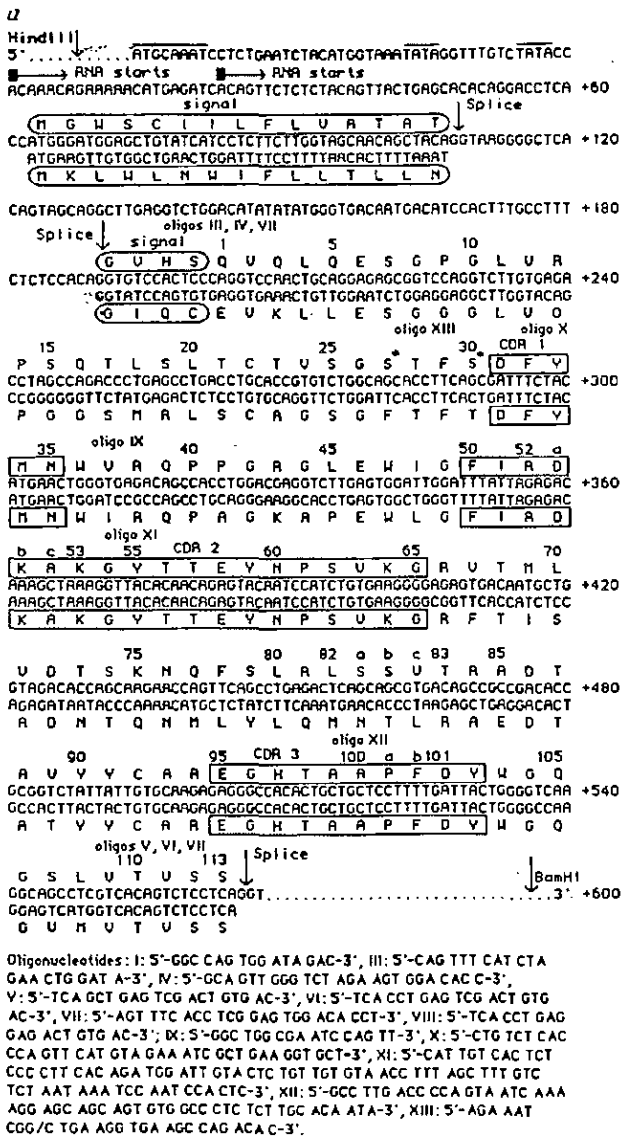


Fig. 1 Heavy-chain (*a*) and light-chain (*b*) sequences of the variable domains of reshaped (upper line) or rat YTH 34.5HL (lower line) antibodies. The reshaped heavy-chain variable domain HuVHCAMP was based on the HuVHNP gene[12,19], with the framework regions of human NEW (see note) alternating with the hypervariable regions of rat YTH 34.5HL. The reshaped light-chain variable domain HuVLCAMP is a similar construct, except with the framework regions of the human myeloma protein REI, with the C-terminal and the 3' non-coding sequence taken from a human J_κ-region sequence[36]. The sequences of oligonucleotide primers are given and their locations on the genes are marked.

Methods. Messenger mRNA was purified[37] from the hybridoma clone YTH 34.5HL (γ2a, κ^b). First strand cDNA was synthesized by priming with oligonucleotides complementary to the 5' end of the CH1 (oligonucleotide I) and the Cκ exons (oligonucleotide II), and then cloned and sequenced as described previously[18,19]. Two restriction sites (*Xba*I and *Sal*I) were introduced at each end of the rat heavy-chain variable region RaVHCAMP cDNA clone in M13 using mutagenic oligonucleotides III and V respectively, and the *Xba*I–*Sal*I fragment was excised. The corresponding sites were introduced into the M13–HuVHNP gene using oligonucleotides IV and VI, and the region between the sites was then exchanged. The sequence at the junctions was corrected with oligonucleotides VII and VIII, and an internal *Bam*HI site removed using the oligonucleotide IX, to create the M13–RaVHCAMP gene. The encoded sequence of the mature domain is thus identical to that of YTH 34.5HL. The reshaped heavy-chain variable domain (HuVHCAMP) was constructed in an M13 vector by priming with three long oligonucleotides simultaneously on the single strand containing the M13–HuVHNP gene[12,19]. Each oligonucleotide (X, XI and XII) was designed to replace each of the hypervariable regions with the corresponding region from the heavy chain of the YTH 34.5HL antibody. Colony blots were probed initially with the oligonucleotide X and hybridization positives were sequenced: the overall yield of the triple mutant was 5%. The (Ser27 → Phe) and (Ser27 → Phe, Ser30 → Thr) mutants of M13mp8–HuVHCAMP were made with the mixed oligonucleotide XIII. The reshaped light-chain variable domain (HuVLCAMP) was constructed in M13 from a gene with framework regions based on human REI (J. Foote, unpublished data). As above, three long oligonucleotides (XIV, XV and XVI) were used to introduce the hypervariable regions of the YTH 34.5HL light chain.

Note: There are discrepancies involving the first framework region and the first hypervariable loop of the NEW heavy chain between the published sequence[27] used here and the sequence deposited in the Brookhaven data base (in parentheses): Ser27 (→Thr), Thr28 (→Ser) and Ser30 (→Asp). Neither version is definitive (R. J. Poljak, personal communication) and the discrepancies do not affect our interpretations.

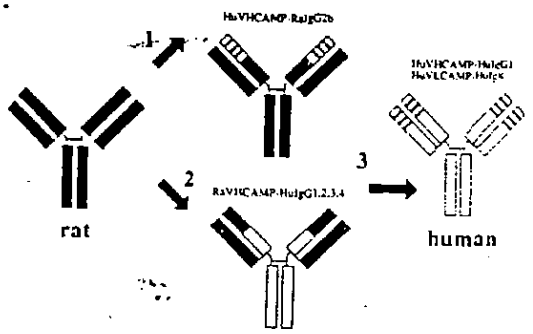


Fig. 2 Strategy for reshaping a human antibody for therapy. Sequences of rat origin are marked in black, and those of human origin in white. The recombinant heavy and light chains are also marked using a systematic nomenclature. See text for description of stages 1, 2 and 3. The genes encoding the variable domains were excised from the M13 vectors as *Hin*dIII–*Bam*HI fragments, and recloned into pSV2gpt[29] (heavy chains) or pSV2neo[30] (light chains), expression vectors containing the immunoglobulin enhancer[12]. The human γ1 (ref. 40), γ2 (ref. 41), γ3 (ref. 42), γ4 (ref. 41) and κ (ref. 36) and the rat γ2b (ref. 43) constant domains were introduced as *Bam*HI fragments. The following plasmids were constructed and transfected into lymphoid cell lines by electroporation[44]. In stage 1, the pSVgpt plasmids HuVHCAMP-RaIgG2B, HuVHCAMP(Ser→Phe)-RaIgG2B, HuVHCAMP-(Ser27→Phe, Ser30→Thr)-RaIgG2B were introduced into the heavy chain loss variant of YTH 34.5HL. In stage 2, the pSVgpt plasmids RaVHCAMP-RaIgG2B, RaVHCAMP-HuIgG1, RaVHCAMP-HuIgG2, RaVHCAMP-HuIgG3, RaVHCAMP-HuIgG4 were transfected as above. In stage 3, the pSV-gpt plasmid Hu(Ser27→Phe, Ser30→Thr)VHCAMP-HuIgG1 was co-transfected with the pSV-neo plasmid HuVLCAMP-HuIgK into the rat myeloma cell line Y0 (Y B2/3.0 Ag 20 (ref. 31). In each of the three stages, clones resistant to mycophenolic acid were selected and screened for antibody production by ELISA assays. Clones secreting antibody were subcloned by limiting dilution (for Y0) or the soft agar method (for the loss variant) and assayed again before 1 litre growth in roller bottles.

Since, to a first approximation, the sequences of hypervariable regions do not contain characteristic rodent or human motifs, such 'reshaped' antibodies should be indistinguishable in sequence from human antibodies.

There are mAbs to many cell-type-specific differentiation antigens, but only a few have therapeutic potential. Of particular interest is a group of rat mAbs directed against an antigen, the 'CAMPATH-1' antigen, which is strongly expressed on virtually all human lymphocytes and monocytes, but is absent from other blood cells including the haemopoietic stem cells[20]. The CAMPATH-1 series contains rat mAb of IgM, IgG2a and IgG2c isotypes[21], and more recently IgG1 and IgG2b isotypes which were isolated as class-switch variants from the IgG2a-secreting cell line YTH 34.5HL[22]. All of these antibodies, except the rat IgG2c isotype, are able to lyse human lymphocytes efficiently with human complement. Also the IgG2b antibody YTH 34.5HL-G2b, but not the other isotypes, is effective in antibody-dependent cell-mediated cytotoxicity (ADCC) with human effector cells[22]. These rat mAbs have important applications in problems of immunosuppression: for example control of graft-versus-host disease in bone-marrow transplantation[20]; the management of organ rejection[23]; the prevention of marrow rejection; and the treatment of various lymphoid malignancies (ref. 24 and M. J. Dyer, Hale, G., Hayhoe, F. G. J. and Waldmann, H., unpublished observations). The IgG2b antibody YTH 34.5HL-G2b seems to be the most effective at depleting lymphocytes *in vivo* but the use of all of these antibodies is limited by the anti-globulin response which can occur within two weeks of the initiation of treatment[24]. Here we describe the reshaping of human heavy and light chains towards binding the CAMPATH-1 antigen and the selection of human effector functions to match the lytic potential of the rat IgG2b isotype.

Table 1 Reshaping the heavy-chain variable domain

Heavy chain variable domain	Concentration of antibody in μg ml^{-1} at 50% antigen binding	50% complement lysis
RaVHCAMP	0.7	2.1
HuVHCAMP	27.3	*
HuVHCAMP (Ser27→Phe)	1.8	16.3
HuVHCAMP (Ser 27→Phe, Ser 30→Thr)	2.0	17.6

Antibodies with the heavy-chain variable domains listed above, rat IgG2b constant domains and rat light chains were collected from supernatants of cells at stationary phase and concentrated by precipitation with ammonium sulphate, followed by ion exchange chromatography on a Pharmacia MonoQ column. The yields of antibody were measured by an enzyme-linked immunosorbent assay (ELISA) directed against the rat IgG2b isotype, and each was adjusted to the same concentration[35]. To measuring binding to antigen, partially purified CAMPATH-1 antigen was coated onto microtitre wells and bound antibody was detected via a biotin-labelled anti-rat IgG2b mAb[35], developed with a streptavidin-peroxidase conjugate (Amersham). Complement lysis of human lymphocytes was with human serum as the complement source[21]. For both binding and complement assays, antibody titres were determined by fitting the data to a sigmoid curve by at least squares iterative procedure[21].

* Complement lysis with the HuVHCAMP variable domain was too weak for the estimation of lytic titre.

Strategy

The amino-acid sequences of the heavy- and light-chain variable domains of the rat IgG2a CAMPATH-1 antibody YTH 34.5HL were determined from the cloned complementary DNA (Fig. 1), and the hypervariable regions were identified according to Kabat[25]. In the heavy-chain variable domain there is an unusual feature in the framework region. In most known heavy-chain sequences Pro41 and Leu45 are highly conserved: Pro41 helps turn a loop distant from the antigen binding site and Leu45 is in the β bulge which forms part of the conserved packing between heavy- and light-chain variable domains[26]. In YTH 34.5HL these residues are replaced by Ala41 and Pro45 and presumably this could have some effect on the packing of the heavy- and light-chain variable domains. Working at the level of the gene and using three large mutagenic oligonucleotides for each variable domain, the rat hypervariable regions were mounted in a single step on the human heavy- or light-chain framework regions taken from the crystallographically solved proteins NEW[27] and REI[28] respectively (Fig. 1). The REI light chain was used because there is a deletion at the beginning of the third framework region in NEW. The reshaped human heavy- and light-chain variable domains were then assembled with constant domains in three stage (Fig. 2). This permits a step-wise check on the reshaping of the heavy-chain variable domain (stage 1), the selection of the human isotype (stage 2), and the reshaping of the light-chain variable domain and the assembly of human antibody (stage 3). The plasmid constructions were genomic, with the sequences encoding variable domains cloned as *Hin*dIII–*Bam*HI fragments and those encoding the constant domains as *Bam*HI–*Bam*HI fragments in either pSVgpt (heavy chain)[29] or pSVneo (light chain)[30] vectors. The heavy-chain enhancer sequence was included on the 5′ side of the variable domain, and expression of both light and heavy chains was driven from the heavy-chain promoter and the heavy-chain signal sequence.

Heavy-chain variable domain

In stage 1, the reshaped heavy-chain variable domain (HuVHCAMP) was attached to constant domains of the rat

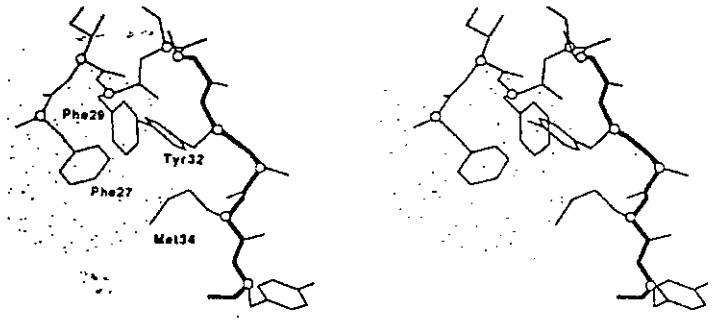


Fig. 3 Loop Phe27 to Tyr35 in the heavy-chain variable domain of the human myeloma protein KOL, which has been solved crystallographically[45]. The backbone of the hypervariable region according to Kabat[25] is highlighted, and a 200% van der Waal surface is thrown around Phe 27 to show the interactions with Tyr 32 and Met 34 of the Kabat hypervariable region. In the rat YTH 34.5HL heavy chain, these three side chains are conserved in character, but in HuVHCAMP, Phe27 is replaced by Ser.

isotype IgG2b and transfected into a heavy-chain loss variant of the YTH 34.5 hybridoma. This variant carries two light chains, one derived from the Y3 fusion partner[31]. The cloned rat heavy-chain variable domain (RaVHCAMP) was also expressed as above, and the antibodies were purified and quantified (Table 1). The HuVHCAMP and RaVHCAMP antibodies, each of the rat IgG2b isotype, were compared to the CAMPATH-1 antigen in a direct binding assay and in complement lysis of human lymphocytes (Table 1). Compared with the original rat antibody, or the engineered equivalent, the antibody with the reshaped heavy-chain domain bound poorly to the CAMPATH-1 antigen and was weakly lytic. This suggested an error in the design of the reshaped domain.

There are several assumptions underlying the transfer of hypervariable loops from one antibody to another[47], in particular the assumption that the antigen binds mainly to the hypervariable regions. These are defined as regions of sequence[25] or structural[32] hypervariability, the locations of hypervariable regions being similar by both criteria except for the first hypervariable loop of the heavy chain. By sequence the first hypervariable loop extends from residues 31–35 (ref. 25) whereas by structure it extends from residues 26–32 (ref. 32). Residues 29 and 30 form part of the surface loop, and residue 27, which is phenylalanine or tyrosine in most sequences, including YTH 35.5HL, helps pack against residues 32 and 34 (Fig. 3). Unlike most human heavy chains, in NEW (see note in Fig. 1) the phenylalanine is replaced by serine, which would be unable to pack in the same way. To restore the packing of the loop, we made both a Ser 27→Phe mutation, and a Ser 27→Phe, Ser 30→Thr double mutation in HuVHCAMP. These two mutants showed a significant increase in binding to CAMPATH-1 antigen and lysed human lymphocytes with human complement (Table 1). Thus the affinity of the reshaped antibody could be restored by a single Ser 27→Phe mutation, possibly as a consequence of an altered packing between the hypervariable regions and the framework. This suggests that alterations in the 'Kabat' framework region can enhance the affinity of the antibody and extends previous work in which an engineered change in the hypervariable region yielded an antibody with increased affinity[33].

Heavy-chain constant domains

In stage 2 (Fig. 2), the rat heavy-chain variable domain was attached to constant domains of the human isotypes IgG1, 2, 3 and 4, and transfected into the heavy-chain loss variant of the YTH 34.5 hybridoma. In complement lysis (Fig. 4*a*), the human IgG1 isotype proved similar to the YTH 34.5HL-G2b, with the human IgG3 isotype being less effective. The human IgG2 isotype was only weakly lytic and the IgG4 isotype was non-lytic. In ADCC (Fig. 4*b*) the human IgG1 was more lytic than the YTH 34.5HL-G2b antibody. The decrease in lysis at higher concentrations of the rat IgG2b and the human IgG1 antibody is due to an excess of antibody, which causes the lysis of effector cells. The human IgG3 antibody was weakly lytic, and the IgG2 and IgG4 isotypes were non-lytic.

We therefore selected the human IgG1 isotype for the reshaped antibody. Other recent work also favours the use of IgG1 isotype for therapeutic application. When the effector functions of human isotypes were compared using a set of chimaeric antibodies with an anti-hapten variable domain, the IgG1 isotype appeared superior to the IgG3 in both complement and cell-mediated lysis[15]. Also, of two mouse chimaeric antibodies with human IgG1 or IgG3 isotypes directed against cell surface antigens as tumour cell markers, only the IgG1 isotype mediated complement lysis[13,14].

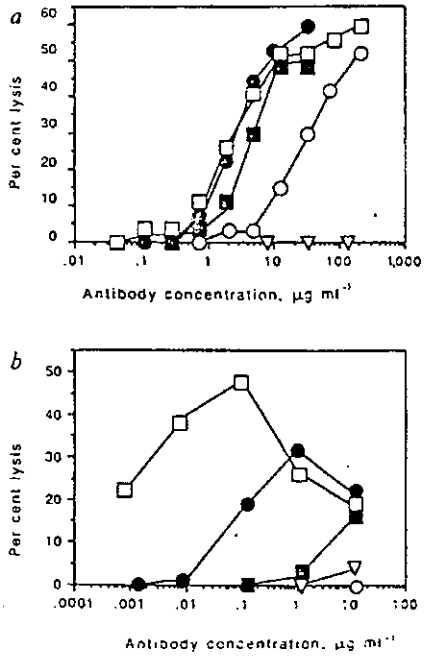


Fig. 4 *a*, Complement lysis and *b*, ADCC for antibodies with rat light-chain and rat heavy-chain variable domain attached to human IgG1 (□), IgG2 (○), IgG3 (■), or IgG4 (▽) isotypes. Lysis with the YTH 34.5HL antibody (●) is also shown.
Methods. Antibody was collected from cells in stationary phase, concentrated by precipitation with ammonium sulphate and desalted into phosphate buffered saline (PBS). Antibodies bound to the CAMPATH-1 antigen-coated on microtitre plates, were assayed in ELISA directed against the rat κ light chain[35], and each adjusted to the same concentration. The antibodies were assayed in complement lysis (Table 1) and ADCC with activated human peripheral blood mononuclear cells[35,46]. Briefly, 5×10^4 human peripheral blood cells were labelled with ^{51}Cr and incubated for 30 min at room temperature with different concentrations of antibody. Excess antibody was removed and a 20-fold excess of activated cells added as effectors. After 4 h at 37 °C cell death was estimated by ^{51}Cr release.

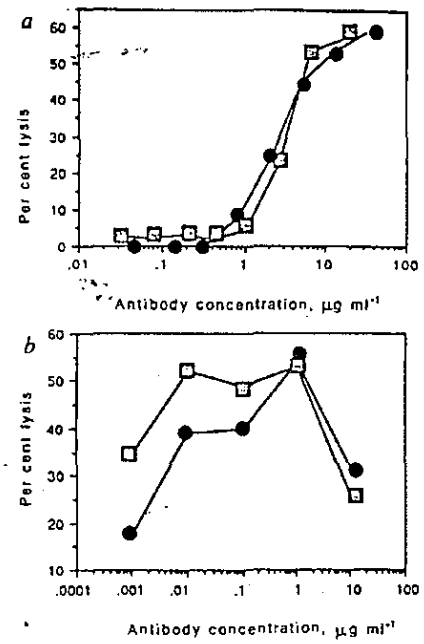


Fig. 5 *a*, Complement lysis and *b*, ADCC of the reshaped human (▨) and rat YTH 34.5HL (●) antibodies. Antibody HuVHCAMP (Ser27→Phe, Thr30→Ser)-HuIGG1, HuVLCAMP-HuIGK was purified from supernatants of cells in stationary phase by affinity chromotography on protein-A Sepharose. The yield (about 10 mg l^{-1}) was measured spectrophotometrically. Complement and ADCC assays were performed as in Fig. 4.

Light chain

In stage 3 (Fig. 2), the reshaped heavy chain was completed by attaching the reshaped HuVHCAMP (Ser27→Phe, Ser30→Thr) domain to the human IgG1 isotype. The reshaped light-chain domain HuVLCAMP was attached to the human Cκ domain. The two clones were co-transfected into the non-secreting rat Y0 myeloma line. The resultant antibody, bound to CAMPATH-1 antigen (data not shown), and proved almost identical to the YTH 34.5HL-G2b antibody in complement lysis (Fig. 5*a*). In cell-mediated lysis the reshaped human antibody was more effective than the rat antibody (Fig. 5*b*). Similar results were obtained with three different donors of target and effector cells (data not shown). Also, the antibody was as effective as YTH 34.5HL-G2b in killing leukaemic cells from three patients with B-cell lymphocytic leukaemia by complement-mediated lysis with human serum. Thus, by transplanting the hypervariable regions from a rodent to a human antibody of the IgG1 subtype, we have reshaped the antibody for therapeutic application.

Prospects

The availability of a reshaped human antibody with specificity for the CAMPATH-1 antigen should permit a full analysis of the *in vivo* potency and immunogenicity of an anti-lymphocyte antibody with wide therapeutic potential. Even if anti-idiotypic responses are eventually observed, considerable therapeutic benefit could be derived from an extended course of treatment. Also, it should be possible to circumvent an anti-globulin response restricted to idiotype by using a series of antibodies with different idiotypes[34]. In principle, the idiotype of the reshaped CAMPATH-1 could be changed by altering the hypervariable regions or the framework regions—evidence from a reshaped antibody specific for the hapten nitrophenyl acetate suggests that recognition by anti-idiotypic antisera and anti-idiotypic mAbs is influenced by residues in the framework region[19]. Thus, recycling the hypervariable regions on different human framework regions should change the idiotype, although ultimately it might focus the response directly onto the binding site for the CAMPATH-1 antigen. Although such focusing would be undesirable for CAMPATH-1 antibodies, it could be an advantage for the development of anti-idiotypic vaccines. It is likely that the answers to some of these questions will emerge from the use of this reshaped antibody in therapy.

We thank J. Foote for the synthetic gene encoding the framework regions of a human light chain; P. Leder, T. R. Rabbitts, T. Honjo, M. P. Lefranc respectively for clones encoding the constant regions of human κ chain, human IgG2 and IgG4, human IgG1, human IgG3; G. Hale for CAMPATH-1 antigen and for advice; M. Bruggemann and M. S. Neuberger for subclones of the heavy-chain constant regions[15] and for advice; M. Frewin for technical assistance and C. Milstein for encouragement. L.R. is supported by a fellowship from the German 'Sonderprogramm Gentechnologie des DAAD'. The work was supported by the Medical Research Council and by Wellcome Biotechnology Ltd. 'CAMPATH' is a trademark of The Wellcome Foundation Ltd.

Received 3 December 1987; accepted 12 February 1988.

1. Behring, E. von & Kitasato, S. *Dtsch. Med. Wochenschr.* 16, 1113-1114 (1890).
2. Ratner, B. *Allergy Anaphylaxis and Immunotherapy* (Williams and Wilkins, Baltimore, 1943).
3. Köhler, G. & Milstein, C. *Nature* 256, 495-497 (1975).
4. Ehrlich, P. in *Immunology and Cancer Research* vol. 2 (ed. Himmelweit, F.) (Pergamon, London, 1957).
5. Deland, F. H. & Goldenberg, D. M. in Radionuclide Imaging (ed. Kuhl, D. E.) 289-307 (Pergamon, Paris, 1982).
6. Levy, R. & Miller, R. A. *Ann. Rev. Med.* 34, 107-116 (1983).
7. Miller, R. A., Oseroff, A. R., Stratte, P. T. & Levy, R. *Blood* 62, 988-995 (1983).
8. Leung, D. Y. M., Rosen, & Geha, R. S. in *Primary and Secondary Immunodeficiency Disorders* (ed. Chandra, R. K.) 44-61 (Churchill Livingstone, New York, 1983).
9. Carson, D. A. & Fremark, B. D. *Adv. Immun.* 38, 275-311 (1986).
10. Morrison, S. L., Johnson, M. J., Herzenberg, S. A. & Oi, V. T. *Proc. natn. Acad. Sci. U.S.A.* 81, 6851-6855 (1984).
11. Boulianne, G. L., Hozumi, N. & Shulman, M. J. *Nature* 312, 643-646 (1984).
12. Neuberger, M. S. *et al. Nature* 314, 268-270 (1985).
13. Liu, A. Y. *et al. Proc. natn. Acad. Sci. U.S.A.* 84, 3439-3443 (1987).
14. Shaw, D. R. *et al. J. Immun.* 138, 4534-4538 (1987).
15. Bruggemann, M. *et al. J. exp. Med.* 166, 1351-1361 (1987).
16. Hale, G., Swirsky, D. M., Hayhoe, F. G. J. & Waldmann, H. *Molec. biol. Med.* 1, 321-334 (1983).
17. Giorgi, J. V. *et al. Transplantn Proc.* 15, 639-642 (1983).
18. Benjamin, R., Cobbold, S. P., Clark, M. R. & Waldmann, H. *J. exp. Med.* 163, 1539-1552 (1986).
19. Jones, P. T., Dear, P. H., Foote, J., Neuberger, M. S. & Winter, G. *Nature* 321, 522-525 (1986).
20. Hale, G. *et al. Blood* 62, 873-882 (1983).
21. Hale, G., Hoang, T., Prospero, T., Watt, S. M. & Waldmann, H. *Molec. biol. Med.* 1, 305-319 (1983).
22. Hale, G. *et al. J. Immun. Meth.* 103, 59-67 (1987).
23. Hale, G., Waldmann, H., Friend, P. & Calne, R. *Transplantation* 42, 308-311 (1986).
24. Hale, G., Swirsky, O. M., Hayhoe, F. G. J. & Waldmann, H. *Molec. biol. Med.* 1, 321-334 (1983).
25. Kabat, E. A., Wu, T. T., Reid-Miller, M., Perry, H. M. & Gottesman, K. S. in *Sequences of Proteins of Immunological Interest* (US Dept. of Health and Human Services, US Government Printing Office, 1987).
26. Chothia, C., Novotny, J., Bruccoleri, R. & Karplus, M. *J. J. molec. Biol.* 186, 651-663 (1985).
27. Saul, F. A., Amzel, M. & Poljak, R. J. *J. biol. Chem.* 253, 585-597 (1978).
28. Epp, O. *et al. Eur. J. Biochem.* 45, 513-524 (1974).
29. Mulligan, R. C. & Berg, P. *Proc. natn. Acad. Sci. U.S.A.* 78, 2072-2076 (1981).
30. Southern, P. J. & Berg, P. *J. molec. appl. Genetics* 1, 327-341 (1981).
31. Galfre, G. & Milstein, C. *Meth. Enzym.* 73, 1-46 (1981).
32. Chothia, C. & Lesk, A. *J. Mol. Biol.* 196, 901-917 (1987).
33. Roberts, S., Cheetham, J. C. & Rees, A. R. *Nature* 328, 731-734 (1987).
34. Junker, M. & den Hink, J. H. A. M. *Eur. J. Immun.* 17, 1547-1552 (1987).
35. Clark, M. & Waldmann, H. *J. Exp. Med.* 1593-1598 (1987).
36. Hieter, P. A., Max, E. E., Seidman, J. G., Maizel, J. V. Jr & Leder, P. *Cell* 22, 197-207 (1980).
37. Kaartinen, M. *et al. J. Immun.* 130, 320-324 (1983).
38. Gubler, U. & Hoffmann, B. J. *Gene* 25, 263-269 (1983).
39. Sanger, F., Nicklen, S. A. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* 74, 5463-5467 (1977).
40. Takahashi, N., Ueda, N. S., Obata, M., Nikaido, T. & Honno, T. *Cell* 29, 671-679 (1982).
41. Flanagan, J. G. & Rabbits, T. H. *Nature* 300, 709-713 (1982).
42. Huck, S., Fort, P., Crawford, D. H., Lefranc, M.-P. & Lefranc, G. *Nucleic Acids Res.* 14, 1779-1789 (1986).
43. Bruggemann, M. *et al. Proc. natn. Acad. Sci. U.S.A.* 83, 6075-6079 (1986).
44. Potter, H., Weir, L. & Leder, P. *Proc. natn. Acad. Sci. U.S.A.* 81, 7161-7165 (1984).
45. Marquardt, M., Deisenhofer, J., Huber, R. & Palm, W. *J. molec. Biol.* 141, 369-391 (1980).
46. Hale, G., Clark, M. & Waldmann, H. *J. Immun.* 123, 3056-3061 (1985).
47. Verhoeyen, M., Milstein, C. & Winter, G. *Science* (in the press).

5-18-94 DrShore

27/E B.White 5-20-94 (NE)

DOCKET NO.: CARP-0009 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

John R. Adair, et al.

Serial No.: 07/743,329 Group Art Unit: 1807

Filed: September 17, 1991 Examiner: L. Bennett Arthur

For: HUMANISED ANTIBODIES

I, Doreen Yatko Trujillo, Registration No. 35,719 certify that this correspondence is being deposited with the U.S. Postal Service as First Class mail in an envelope addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231.

On May 9, 1994

Doreen Yatko Trujillo

Doreen Yatko Trujillo, Reg. No. 35,719

K Foerster 5-23-94 B.White 9/28/94

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

RECEIVED
MAY 13 1994
GROUP 1800

Dear Sir:

RESPONSE TO ADVISORY ACTION

In the claims:

Please amend claim 67 as follows:

In claim 67, line 4, after "chain", please insert --framework--.

REMARKS

This paper is being filed in response to the Advisory Action mailed February 25, 1994, pursuant to Examiner Arthur's suggestion during a teleconference. This response is being filed in order to reduce the number of issues to be addressed upon

appeal. It is believed that no petition or extension fee is required, since the response is being filed within two months of the "Notice of Appeal". To the extent this belief is erroneous, please charge any fees due to Deposit Account No. 23-3050.

In the Advisory Action, Examiner Arthur stated that the deletion of the recitation "acceptor antibody heavy chain [framework] resides [sic]" changes the scope of the claimed invention to more broadly encompass composite antibodies in which the acceptor contributes more than just the framework. During a teleconference, the Examiner clarified that she was referring to the deletion of the word "framework", and not suggesting that the entire recitation in quotes had been deleted. Accordingly, Applicants have amended claim 67 to reinsert the word "framework" where it had been previously deleted.

The Examiner also stated, in the Advisory Action, that the deletion changed the scope such that the "...donor antibody is no longer limited to donating the antigen binding residues. The specification does not appear to support this broader concept." The Examiner, therefore, asserted that the amendment raised new matter and a new scope rejection under 35 U.S.C. § 112, first paragraph.

During the teleconference, the undersigned explained that the donor antibody was *never* limited to contributing the antigen

binding residues. The Examiner suggested submission of the present response to clarify this point and make the foregoing amendment.

On page 17, line 15, of the application as filed, the following is stated: "The positions at which donor residues are to be substituted for *acceptor* residues are then chosen as follows." (Emphasis added.) The text following this passage discloses which residues in the **framework** are to be changed, in addition to those changes to be made in the CDRs.

In paragraph 2.1 on the same page, residues 23, 24, 49, 71, 73, and 78 in the heavy chain variable domain are listed to be changed. In a previous paragraph, numbered 1, the residues of the CDRs (i.e. antigen binding residues) are listed for the heavy chain. As can be seen, the residues to be changed do not occur in the CDRs and are, therefore, in the framework region. Claim 1 as originally filed recited changing these residues to donor. Contrary to the Examiner's interpretation, the claimed invention was never limited to changes to donor only within the antigen binding residues.

Neither the Advisory Action, nor the teleconference, specifically addressed the remaining rejections. It appears, however, that the rejections stand "for the reasons of record." (See item #4, Advisory Action.) Applicants do not wish to reiterate the whole Amendment here, but would like to emphasize

some points in the interest of possibly reducing issues to be addressed on appeal.

The first point concerns the specification of acceptor residues in the claims. It was previously believed, as a result of an interview, that the Examiner thought the claims as drafted covered the situation in which all the variable domain residues are donor -- i.e., chimeric antibodies. Thus, the Applicants specified residues which are to remain as acceptors. As discussed in the Amendment, this is implicit, if not explicit, from the application as filed.

Further, the claims specify that the variable domain comprises donor and acceptor sequences. This specification distinguishes the antibodies of the claims from chimerics. In chimerics, the variable domain is comprised entirely of donor residues.

Now, however, it appears the Examiner thinks the claims had previously been limited to antibodies in which the residues in the CDRs are donor, and the remaining residues are acceptor. This is inconsistent with the specification and claims as well as the prior Office Actions and suggests further discussion is merited.

Applicants would also like to clarify a point regarding the Queen reference. Panels A and B on page 10003 of Queen refer to the light and heavy chains, respectively, of the acceptor antibody (upper sequences) and the humanized anti-TAC antibody

(lower sequences). The attached panels correspond to Panels A and B of Queen. The upper and lower sequences are further separated in the attached, the lower sequence representing the humanized antibody. The numbers above the sequences utilize the linear numbering system. The numbers below utilize Kabat numbering. Queen donor residues are indicated in red. Applicants' donor residues are indicated in blue. As can be seen, the two approaches are very different particularly for the heavy chain.

During the teleconference, the Examiner also indicated that she would be amenable to conducting an interview with the Applicants upon return from her upcoming leave. It was further indicated that her return would be sometime in May. Applicants still wish to conduct an interview and respectfully request a prompt communication thereafter regarding scheduling so that appropriate arrangements can be made.

Respectfully submitted,

Doreen Yatko Trujillo

Doreen Yatko Trujillo
Registration No. **35,719**

Date: **May 9, 1994**

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS
One Liberty Place - 46th Floor
Philadelphia, PA 19103
(215) 568-3100

+8352

A

QUEEN ET AL - SEQUENCES FROM FIGURE 2 - NUMBERING IN PAPER ABOVE
NUMBERING ACCORDING TO KABAT BELOW

PANEL A - UPPER SEQUENCE

```
1                 10                  20                  30
D I Q M T Q S P S T L S A S V G D R V T I T C R A S Q S I N T W L A
1                 10                  20  23              30

         40                  50                  60
W Y Q Q K P G K A P K L L M Y K A S S L E S G V P S R F I G S G S G
         40                  50                  60

 70                  80                  90                  100
T E F T L T I S S L Q P D D F A T Y Y C Q Q Y N S D S K M F G Q G I
 70                  80                  88  90              100

      107
K V E V K
      107
```

PANEL A - LOWER SEQUENCE

```
1                 10                  20                  30
Q I V L T Q S P A I M S A S P G E K V T I T C S A S S S I S Y M H W
1                                                 23              35

         40                  50                  60
F Q Q K P G T S P K L W I Y T T S N L A S G V P A R F S G S G S G T
         40                  50                  60

 70                  80                  90                  100
S Y S L T I S R M E A E D A A T Y Y C H Q R S T Y P L T F G S G T K
70                  80                  88  90              100

    106
L E L K
    107
```

B

PANEL B - UPPER SEQUENCE

```
1                 10                  20                  30
Q V Q L V Q S G A E V K K P G S S V K V S C K A S G G T F S R S A I
1                 10                  20 22               30

        40                  50                  60
I W V R Q A P G Q G L E W M G G I V P M F G P P N Y A Q K F Q G R V
 36

 70                  80                  90                  100
T I T A D E S T N T A Y M E L S S L R S E D T A F Y F C A G G Y G I
                          82 a b c            90 92

           110          117
Y S P E E Y N G G L V T V S S
100                 110  113
```

PANEL B - LOWER SEQUENCE

```
1                 10                  20                  30
Q V Q L Q Q S G A E L A K P G A S V K M S C K A S G Y T F T S Y R M
                                                22 - -

        40                  50                  60
H W V K Q R P G Q G L E W I G Y I N P S T G Y T E Y N Q K F K D K A
                            52 a                60

 70                  80                  90                  100
T L T A D K S S S T A Y M Q L S S L T F E D S A V Y Y C A R G G G V
                          82 a b c            92  94

          110          116
F D Y W G Q G T T L T V S S
103                 113
```

(There is a deletion in the Kabat system between F and D at beginning of last line.)

DOCKET NO.: CARP-0032 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

35/M
DJ
11/17/94

In re application of:

John Robert Adair, Diljeet Singh Athwal and John Spencer Emtage

Serial No.: N/A Group Art Unit: 1807

Filed: Herewith Examiner: B. Sisson

For: HUMANISED ANTIBODIES

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Dear Sir:

PRELIMINARY AMENDMENT

Pursuant to 37 C.F.R. § 1.115, prior to examination of the above-identified patent application, please amend the claims as follows.

In the claims:

Please cancel claims 67 - 72, 108, and 114 without prejudice.

Please add claims 120 - 127, as follows:

-- 120. An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in

a donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues, ~~provided that the antibody does not have affinity for the p55 chain of the human interleukin 2 receptor~~.

121. The antibody molecule of claim 120, wherein amino acid residues 26 to 30 and 59 to 65 in said composite heavy chain are additionally donor residues.

122. The antibody molecule of claim 120, wherein amino acid residues 71, 73, and 78 in said composite heavy chain are additionally donor residues.

123. The antibody molecule of claim 120, wherein at least one of amino acid residues 1, 3, and 76 in said composite heavy chain are additionally donor residues.

124. The antibody molecule of claim 120, wherein at least one of amino acid residues 36, 94, 104, 106, and 107 in said composite heavy chain are additionally donor residues.

~~125~~. The antibody molecule of claim ~~124~~, wherein at least one of amino acid residues 2, ~~4~~, 6, 38, 46, 67, and 69 in said composite heavy chain are additionally donor residues.

~~126~~. The antibody molecule of claim ~~120~~, wherein said complementary light chain is a composite light chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody light chain framework residues, the remaining light chain residues corresponding to the equivalent residues in a donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite light chain; said CDRs comprise donor residues at least at residues 24 to 34, 50 to 56, and 89 to 97; and amino acid residues 46, 48, 58, and 71 at least are donor residues.

~~127~~. The antibody molecule of claim ~~126~~, wherein amino acid residues 1, 3, 60 (if this residue can form a salt bridge with residue 54), and 70 (if this residue can form a salt bridge with residue 24) in said composite light chain are additionally donor residues.--

REMARKS

The present application is a continuation of U.S. Application Serial No. 07/743,329 (hereinafter the "'329 application") under 37 C.F.R. § 1.62.

Claims 67 - 72, 108, and 114 were pending in the '329 application. Those claims have been cancelled in the present amendment. Applicants reserve the right to pursue the subject matter of the cancelled claims in continuation or divisional applications, as may be appropriate.

Claims 120 - 127 are being submitted in the present amendment. Support for the amendments is found in the application as originally filed as indicated below. In claims 120 and 126, the specification that the variable domain "predominantly" comprises "human acceptor framework residues" is supported by, *inter alia*, page 3, lines 16 - 21, of the application as originally filed. The problems attendant to chimerics are therein described. It is also clear from the number of framework residues discussed as being changed to donor that the framework residues remain, predominantly, acceptor. Support for the residues specified as donor in claim 120 can be found, *inter alia*, on page 7, first full paragraph, page 19, Section 1, and page 46, Section 15.2.1. Support for the residues specified as donor in claim 126 can be found, *inter alia*, on page 17, lines 12 to 14 and page 18, Section 3.1.

As to the remaining claims, support for the residues specified as donor in claim 121 can be found, *inter alia*, on page 8, middle paragraph and page 17, Section 1. Support for the residues specified as donor in claim 122 can be found, *inter alia*, on page 7, first full paragraph. Support for the residues specified as donor in claim 123 can be found, *inter alia*, on page 20, Section 2.1.1. Support for the residues specified as donor in claim 124 can be found, *inter alia*, on page 21, lines 10 to 12. Support for the residues specified as donor in claim 125 can be found, *inter alia*, on page 21, lines 13 to 16. Support for the residues specified as donor in claim 127 can be found, *inter alia*, on page 21, lines 3 to 7.

In a helpful telephonic discussion on June 2, 1994 between Examiner Sisson and the undersigned, Examiner Sisson expressed his reservations regarding specification of acceptor residues in the claims. The present claims do not specify acceptor residues. It is expected that this concern has been obviated.

Alternatively, the present claims specify that the variable domain comprises "**predominantly** human acceptor antibody heavy chain framework residues". It is, thus, asserted that any concerns regarding the claims encompassing chimeric antibodies in which the entire variable domain is of donor origin have been obviated.

The Examiner also indicated during that discussion that his concerns were more directed to issues of scope and that, if the claims contained critical limitations not taught in the art, the art rejections would not be a problem.

Applicants believe that, in light of the amendments, the application is now in condition for allowance and request early notification of the same. To the extent this belief is erroneous, Applicants request that the Examiner contact the undersigned at (215) 564-8352 to discuss the same.

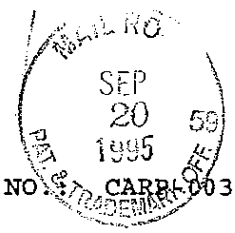
Respectfully submitted,

Doreen Yatko Trujillo

Doreen Yatko Trujillo
Registration No. 35,719

Date: **September 7, 1994**

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS
One Liberty Place - 46th Floor
Philadelphia, PA 19103
(215) 568-3100



DOCKET NO.: CARR-0032 PATENT

39/H
B. Denny
10/20/95

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

John Robert Adair, Diljeet Singh Athwal, and John Spencer Emtage

Serial No.: 08/303,569 Group Art Unit: 1806

Filed: 9/07/94 Examiner: D. Adams

For: HUMANISED ANTIBODIES

I, Doreen Yatko Trujillo, Registration No. 35,719 certify that this correspondence is being deposited with the U.S. Postal Service as First Class mail in an envelope addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231.

On September 18, 1995

Doreen Yatko Trujillo

Doreen Yatko Trujillo, Esquire Reg. No. 35,719

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Dear Sir:

AMENDMENT

Please amend the application as follows.

In the Specification:

Page 1, line 2, after "filed September 17, 1991", please insert -- , now abandoned --.

Please insert new page 94.

In the Claims:

Claim 120, line 12, please delete, ", provided that the antibody does not have affinity for the P55 chain of the human interleukin 2 receptor."

REMARKS

This paper is being filed in response to the Office Action mailed on May 16, 1995. The following comments use the section numbering set forth in the Office Action. Applicants respectfully request reconsideration and withdrawal of all rejections.

CLAIM AMENDMENT

As will be shown below, a reference which the Examiner initially asserted as prior art was not published before the priority date. The "provided that .... " clause in claim 120 was inserted in view of this reference. Since the rejection over this reference is not proper, claim 120 has been amended accordingly.

Sections 15 to 18

The contents of these sections are noted. No action is required.

Section 19

It is believed that this point has been taken care of by the amendment to the description.

Section 20

The Abstract has been added in the required form on new page 94. The text of the abstract is found on the cover page of the PCT publication from which the present application derived and, therefore, does not represent new matter.

Sections 21 to 23

In these sections, the Examiner raises objections to the description and claims under 35 USC § 112, first paragraph. The Examiner alleges that the specification fails to provide an enabling disclosure. However, it appears that the Examiner is actually questioning whether the invention will work over the entire of the scope of the claims.

As regards enablement, the first paragraph of § 112 requires that a person skilled in the art should be able to make and use the invention. In order to make and use the invention, the skilled person needs to be able to carry out the following steps, all of which are clearly set out in the specification.

1. Obtain a donor antibody having affinity for a predetermined antibody. This can best be done either by obtaining a hybridoma, for instance from a culture collection such as the ATCC, or by producing a hybridoma, using the well established Kohler-Milstein procedure.

2. Determine the sequences of the variable domains of the heavy and light chains of the donor antibody. As shown in the specification, this can best be achieved by isolating cDNA from the hybridoma, sequencing the cDNA and decoding the cDNA to give

the amino acid sequence.

3. Number the amino acid sequences from the donor antibody according to the Kabat numbering system. For any person of skill in the art, this is a simple matter, as the Kabat numbering system was well established at the priority date of the application. Thus, the skilled person would readily be able to identify the donor residues specified in the present claims.

4. Determine the amino acid sequences of the variable domains of a human antibody. This is again a simple matter as the sequences of a large number of human antibodies have been published, for instance by Kabat. Thus, this merely requires the skilled person to look in a readily available reference book. Alternatively, the skilled person would look in any one of a large number of papers disclosing the sequences of human antibodies.

5. Number the human amino acid sequences. Quite often, this had already been done. In any event, the comments in 3 above apply.

6. Determine the composite sequence(s) in accordance with the instructions in the claims. This merely requires comparison of the numbered sequences of steps 3 and 5.

7. Produce DNA molecule(s) encoding the composite sequence(s) determined in step 6. This is a matter of standard recombinant DNA technology. The DNA molecule(s) could be produced by total synthesis, partial synthesis or mutagenesis techniques, all of which were well know in the art at the priority date.

8. Transform a suitable host cell with the DNA sequence(s) produced in step 7 such that the host cell expresses the DNA sequence(s). This again is a matter of standard recombinant DNA technology.

It is to be noted that the present specification discloses copious details how to carry out all these steps. For instance, on page 25, there is a description of how to grow

hybridomas. On page 30, there is a description of cDNA preparation and screening, with details of probes to be used. Page 31 discloses details of DNA sequencing and production of expression vectors. Page 36 gives details of expression. Further on in the specification, even more detailed instructions are provided for the production of antibodies as defined in the present claims.

The procedures disclosed in the present specification could have been applied by any skilled person at the priority date for any available donor antibody or for any donor antibody the skilled person could have produced. The skilled person is told exactly what to do and how it can be done. The skilled person is given a number of examples to follow. It cannot be seen that this would require "undue experimentation".

The Examiner has not pointed to any step in the disclosed processes which could not have been carried out by a person of skill in the art. If the Examiner believes that any particular one or more of the steps could not have been carried out by a person of skill in the art, it is requested that the Examiner provide evidence of the same or, if in the Examiner's knowledge, provide an affidavit, both pursuant to 37 C.F.R. §1.107.

It is submitted that, in the absence of any evidence to the contrary, it must be accepted that the skilled person could have started with any donor antibody and followed the instructions in the specification to produce an antibody as now claimed.

The Examiner's real point appears to be that he does not believe that carrying out the steps referred to above would always lead to the production of a useful antibody. However, the Examiner has provided no evidence to support his belief; nor is this a requirement for enablement. *In re Sarrett*, 140 U.S.P.Q. 474 (C.C.P.A., 1964), *reh'g denied*. ". . . the mere *possibility* of inclusion of inoperative substances does not prevent *allowance* of broad claims." (emphasis in original) *Id.*, 140 U.S.P.Q. at 486.

Regardless, as the Examiner has pointed out in Section 22 (A) of the Office Action, the present specification provides a number of examples in which the procedure described in the specification has been applied successfully. The specification has examples relating to OKT3, OKT4A, B72.3 (an anti-mucin MAb), R6-5-D6 (an anti-ICAM-1 MAb) and 61E71 hTNF1, hTNF3 and 101.4 (all anti-TNF MAbs). Thus, the specification by itself provides examples in which MAbs against a variety of different antigens have been successfully humanised.

The Examiner is also referred to the enclosed Declaration executed by Dr. G. T. Yarranton (who the Examiner met during interviews on other cases last year). This declaration provides further evidence that the Applicants' employers have successfully used the procedure disclosed in the specification to humanise 17 antibodies.

The Examiner cannot point to a single example where anyone has tried to use the procedure set forth in the present specification and has failed to produce a useful antibody. The Examiner has merely referred to three papers which do not even try to put the Applicants' invention into effect. It is submitted that this does not provide any evidence that the procedure set forth in the present specification cannot be applied to any antibody. It is the *claimed invention* which is relevant to an analysis of enablement. *Ex parte Ehrlich*, 3 U.S.P.Q. 2d 1011 (Bd. Pat. App's. Int. 1987).

The first paper to which the Examiner refers is Reichmann et al. (Nature, 372, 323-327, 1988). This paper shows the results of "reshaping" the rat monoclonal antibody YTH34.5HL. As can be seen from Table 1 in the right hand column on page 325, Reichmann made four heavy chain constructs. The first (RaVHCAMP) is a chimeric heavy chain having the rat heavy chain domain fused to a human constant region. In the other three constructs, the heavy chain variable domain is predominantly of human origin but all three CDRs (residues 31 to 35, 50 to 58 and 95 to 102) correspond to those of the rat antibody. In the second construct, only the CDRs are rat residues. In the third construct, in addition to changing the CDRs, residue 27 has been changed to a more usual human residues (Ser → Phe). In the fourth construct, in addition to changing the CDRS, residues 27 and 30 have been changed to more

usual human residues (27 Ser → Phe, 30 Ser → Thr). In the light chain construct only the CDRs are changed to rat.

It can thus be seen that Reichmann does not disclose a procedure which leads to an antibody as defined in the present claims. As far as the heavy chain is concerned, Reichmann does not even mention Kabat residues 23, 24 and 49, let alone change them to the rat residues. Reichmann did not carry out a procedure as set forth in the present specification and thus Reichmann does not provide any evidence which shows or suggests that the present procedure cannot be put into practice for any antibody.

The second paper to which the Examiner refers is Queen et al. (PNAS-USA, 86 10029-10033, 1989). However, as is discussed in more detail below, Queen et al. is not prior art. Nonetheless, to the extent Queen et al. is contemporaneous, it will be discussed. Queen adopts an entirely different approach than that set forth in the present specification. This can be seen from the passage in the right hand column on page 10031 headed "Construction of a Humanised Anti-Tac Antibody". The first step in this approach is to select human acceptor sequences which are *as homologous as possible* to the mouse donor antibody. The second step is to use the donor CDRs in the human acceptor sequence. The third step is to carry out molecular modelling and then to select donor residues, to be put into the acceptor sequences, on the basis of various criteria. This leads to a composite variable domain sequence which

contains a large number of donor residues. Although the procedure used by Queen is different from that disclosed in the present specification, in the heavy chain, the CDRs and residues 23, 24 and 49 all correspond to those residues in the donor mouse antibody. Thus, the humanised antibody of Queen is, to a certain extent, in accordance with the present invention.

However, the foregoing residues were not the only residues transformed to donor in Queen. This point was acknowledged by the Examiner on page 4, lines 35-36, of the Office Action. It is to be noted that Queen's humanised antibody has only one third of the affinity of the donor mouse antibody. This is not a particularly good result as the aim of any humanisation procedure is to recover the same affinity as that of the donor antibody. It is believed that the reason for such a low recovery of affinity by Queen is due to there being too many donor residues in the acceptor frameworks. It is believed that, had the procedure of the present specification been adopted, certain of these residues would not have been changed, and improved recovery of affinity would have been obtained.

Nevertheless, given the above explanation of the low recovery affinity in Queen, it can be seen that, if anything, Queen shows that following the procedure of the present specification will lead to the production of useful antibodies

In passing, it is to be noted that the Examiner appears to be laboring under a misconception. The Examiner refers in Section 22 (A) to the changes resulting "in increased antigen binding affinity". The present Applicants do not claim that using the procedure of the specification will result in increased affinity. The aim of the invention is to provide an antibody having equivalent affinity to that of the donor antibody, but with increased human compatibility. The problem with mouse or rat antibodies is not that they have low affinity. They generally have very good affinity. The problem is that they are not compatible with humans. Thus, the problem is to retain the affinity but to remove human incompatibility.

It is no doubt true that, in some cases, it is possible to increase the affinity of the antibody by using the procedure of the present specification. However, this is a bonus effect and is not the object of the invention. Thus, while recovering one third of affinity may not be acceptable, recovering close to 100% of activity is more than enough. It is therefore suggested that the Examiner should look at the present invention in terms of recovering, not increasing, affinity. Nonetheless, an increase in affinity is an unexpected result relevant to nonobviousness.

Returning to the main theme of this Section the Examiner finally relies on Chothia et al. (J. Mol. Biol., 196, 901917, 1987). However, this has no bearing at all on whether the present

invention is applicable to any donor antibody.

Chothia describes theoretical work carried out on the structures and sequences of known antibodies or antibodylike molecules. These are all "natural" molecules in that they have not been in any way engineered. Chothia studied the resolved crystallographic structures of some of these molecules and also compared the sequences of the molecules. Most of the work was concerned with the conformation of the antigen-binding loops (L1 to L3 and H1 to H3). Chothia arranged the loops into groups, called "canonical structures".

Chothia also looked at the framework regions to a certain extent and identified certain framework residues which appeared to be involved in positioning the loops. However, Chothia appears to believe that these residues are specific to the loops with which they are associated. There is no indication that it is possible to make any predictions on the basis of these observations.

It can thus be seen that Chothia did not make any composite antibody chains, nor did Chothia produce any antibody chains by recombinant DNA technology. Most importantly, Chothia did not even attempt to produce a composite antibody chain using the procedure of the present specification. Thus, Chothia provides absolutely no evidence that one skilled in the art could not apply the teachings given in the present specification to any donor antibody.

It is submitted that the disclosures in Reichmann and Queen are relevant in one sense, in that they clearly show that, at the priority date, the skilled person was able to carry out all the necessary steps, for instance using recombinant DNA technology, to produce composite antibody chains. Thus, Reichmann and Queen support the Applicants' view that the present specification provides an enabling disclosure.

It is submitted that the Examiner's objection under Section 112 confuses the requirement for an enabling disclosure with the requirement for the invention to be nonobvious. As to enablement, the question to be asked is whether the skilled person, given the teaching in the specification, could have put that teaching into effect. In the present case, it is clear that the skilled person was able to carry out the necessary steps to produce an antibody according to the claims, using any donor antibody. Moreover, the present specification clearly shows that the procedure disclosed therein had been used successfully to produce a number of humanised antibodies having affinity equivalent to that of the donor antibody. Thus, on the basis of the teaching in the specification, the skilled person had every reason to expect that the procedure would be applicable to any donor antibody. As has been shown above, the prior art does not provide any evidence to suggest otherwise.

It is no doubt true, as the Examiner stated in Section 22 (A), that the prior art does not teach that a standardized principle is possible. However, this has no relevance to the question of enablement, because the prior art does not include the disclosure in the present specification. Absent the disclosure in the present specification, the skilled person would not have been taught that there was a standardized principle. This merely shows that the claimed subject matter is non-obvious. It does not show that the teaching in the present description is non-enabling.

In summary, it is submitted that:

(i) the present specification provides all the instructions necessary to enable the procedure disclosed therein to be put into effect for any donor antibody;

(ii) Reichmann and Queen confirm that, at the priority date, the skilled person was able to carry out the disclosed procedure;

(iii) the present specification shows that the disclosed procedure had been applied successfully to a number of donor antibodies, thus providing the skilled person with a reasonable expectation that the procedure is applicable to any donor antibody;

(iv) there is no evidence to show that the procedure is unsuccessful; and

(v) the prior art is not relevant to the skilled person's expectation of success because it does not include the disclosure in the present specification.

It is therefore submitted that the present specification provides an enabling disclosure for the whole scope of the claims.

For the above reasons, it is respectfully requested that the rejection under 35 USC § 112 against the specification and claims be withdrawn.

Section 25

The Applicants confirm the Examiner's presumption that the subject matter of all the claims was commonly owned at the time the inventions covered by the claims were made.

Sections 26 and 27

The Examiner's raising of a provisional obviousness-type double patenting rejection is acknowledged. This will be dealt with, probably by use of a terminal disclaimer, once the Examiner has acknowledged that the claimed subject matters in this and the co-pending application are patentable.

Section 28

In Section 28, the Examiner rejected all the claims as allegedly being obvious over Reichmann (see above), Queen (see above) and Waldmann (sic) (EP-A-0 239 400). It is presumed that the Examiner correctly cited the European patent application number (EP-A-0 239 400) for the third reference. If this is the case, the applicant is Winter. The Applicants therefore assume that the name for the third reference should be Winter. If this is not the case,

the Examiner is requested to identify the Waldmann paper to which he refers.

As a preliminary point, it is submitted that Queen et al. is not part of the prior art. The attached Declaration by the undersigned attorney shows that Queen was not published before the priority date of the present application. Thus, Queen cannot be used to attack the present claims.

The present application is a national phase filing of a PCT application claiming a foreign priority date of December 21, 1989. As indicated in the attached Declaration of the undersigned, the journal volume in which the Queen et al. reference appeared was not mailed until December 20, 1989. The journal was mailed by second class mail. Accordingly, no addressee could have received the journal *before* December 21, 1989.

As the Examiner is aware, magazines are effective as of the date they are received, not the date they are mailed. M.P.E.P. § 715.01(c). As no addressee could have received the reference before the foreign priority date, no addressee could have been in possession of the reference such that the subject matter sought to be patented as a whole would have been obvious "at the time the invention was made."[1] Indeed, in *Protein Foundation, Inc. v. Brenner*, 151 U.S.P.Q. 561 (D.D.C. 1966), the court took judicial

[1] In that regard, the time differential is also emphasized. The priority application was filed in the United Kingdom. The reference volume was mailed in the United States.

notice of the fact that second class mail does not travel and is not distributed as fast as first class mail. The court concluded that no magazine was delivered two days after being mailed in bulk. *Id.*, at 562. Regardless, it is emphasized that Queen et al. discloses that only 1/3 of the affinity of the murine antibody was recovered in their slightly less "human" antibody, and that "further work" was needed.

Since Queen is not part of the prior art, it is presumed that the Examiner's obviousness rejection can only be based on Reichmann and Winter. Before discussing those references in details, the Examiner is referred to Section 22 (A) of the Office Action, wherein the Examiner himself states that:

> The prior art does not teach that a standardized principle of which amino acids must always be changed is possible, but instead appears to teach that three dimensional structures of the antibodies and an understanding of protein folding properties is necessary to reasonable (sic) predict which amino acids will be effective in retaining antigen binding ability for a particular antibody.

It is submitted that this statement completely undermines the Examiner's position on obviousness. If the skilled person is not taught that a standardized principle is possible, it cannot be seen how the present invention, directed to a standardized principle, can be obvious.

It is also to be pointed out that the present invention, as set forth in the claims, is not that it will be necessary to

change some (unspecified) framework residues. The invention is that it will be necessary to change certain, carefully specified framework residues. Thus, the Examiner has to go beyond showing that a standardized principle was possible and has to show that the skilled person would inevitably have been led to the residue changes set forth in the present claims. As the Examiner admits in Section 28 of the Office Action:

> Neither Reichmann et al. or Queen et al. teach all of the exact mutations found in the claimed antibodies.

It thus cannot be seen how it can be obvious to get to the present invention.

This conclusion is reinforced if the prior art references cited by the Examiner not including Queen are properly considered. It is firstly to be pointed out that Winter was published in 1987, whereas Reichmann was published in 1988. Moreover, one of the authors of Reichmann is Dr. Winter, the sole inventor of Winter. It can thus be clearly seen that Reichmann follows on from the work of Winter. It is therefore believed that it is appropriate to discuss Winter first and then Reichmann as this reflects the way in which the art developed.

Winter teaches the basic concept of CDR-grafting. Thus, Winter clearly teaches the production of a grafted antibody in which only the CDRs (as defined by Kabat) from a donor antibody are grafted into an acceptor antibody in place of its natural CDRs.

There are examples in the citation showing this CDR grafting procedure. It is to be noted that in the examples, it is only the CDRs which are changed. No changes at all are made in the framework regions.

It is no doubt true that Winter contains a passage from page 7, line 25 to page 8, line 18 which refers to the possibility that framework residues may need to be changed. However, this passage is entirely devoid of any practical teaching. It does not mention a single residue number, nor does it even mention possible locations for such residues. Thus, Winter provides absolutely no suggestion, much less guidance, as to where to look for framework residues which may need changing.

The Examiner has asserted in Section 22 (A) of the Office Action that:

> ... it would require undue experimentation for a person of ordinary skill in the art to practice applicant's claimed invention from what has been disclosed in the specification.

Although the Applicants believe, for the reasons set forth above, that this is not true of the present specification, it is submitted that the Examiner's assertion applies with full force to Winter. The passage in Winter is merely an invitation to carry out further experiments without providing any directions or even hints as to how to carry out such experiments. It is therefore

submitted that the present claims are not at all obvious over Winter.

Turning now to Reichmann, it can be seen that this follows on from Winter. The first construct made by Reichmann is one in which only the donor CDRs are grafted into the acceptor frameworks. This construct is HUVHCAMP. As can be seen from Table 1, the first construct was nearly 40 times worse at binding antigen than the original rat antibody and was immeasurably worse in a complement binding assay. Reichmann therefore produced two more constructs. HuVHCAMP (Ser 27 → Phe, Ser 30 → Phe) and HuVHCAMP (Ser 27 → Phe, Ser 30 → Thr). The further changes in these constructs significantly improved both antigen binding and complement activation, but not to the level of the rat antibody.

The reason for making the further mutations at positions 27 and 30 is set forth in the left-hand column on page 326 of Reichmann. It can be seen that, at residue 27, the human acceptor sequence was unusual. Residue 27 was therefore changed to the more usual human residue. The change at residue 30 was made to bring the extended CDR, including the surface loop (residues 26 to 32) as well as the Kabat CDRs (residues 31 to 35) into conformity with those of the rat antibody. Thus, one mutation has the effect of making the grafted antibody look more human. The other mutation has the effect of extending CDR1.

It cannot be seen how this can in any way suggest the present invention. At best, it suggests that it would be better to define CDR1 as being residues 26 to 35 in the heavy chain. However, this certainly does not teach a general principle of changing framework residues and in particular it does not suggest that the particular framework residues of the present claims should be altered.

The passage on page 326 of Reichmann ends with the following sentence.

> This suggests that alterations in the 'Kabat' framework region can enhance the affinity of the antibody and extends previous work in which an engineered change in the hypervariable region yielded an antibody with increased affinity. (emphasis added).

Again, this is merely a suggestion for further work. It does not provide any hint or direction as to how the work should proceed. The only teaching is to extend CDR1 to include the surface loop. Thus, this certainly does not even remotely suggest the specific residue changes of the present claims.

The Examiner asserts that:

> ..... it would have been prima facie obvious ..... to apply the teachings of Winter to those of Reichmann .....

However, the Examiner appears to have failed to appreciate that the teaching of Winter is already contained in Reichmann. In fact, the teaching of Reichmann is based on the teaching of Winter. The

teaching of Winter was also published in Jones et al. (Nature, 321, 522-525, 1986), of which Winter is a co-author. Jones is reference 19 in Reichmann and is referred to in the paragraph bridging pages 323 and 325 of Reichmann. This paragraph makes it clear that Jones (and thus Winter) provided the basis for the work reported by Reichmann. Thus, the obvious combination of Winter and Reichmann had already been made in the prior art. However, despite having the expertise of Winter to call on, Reichmann comes nowhere near the present invention. It is therefore submitted that Reichmann by itself proves that a combination of Winter and Reichmann does not lead to the present invention.

Accordingly, Applicants submit that the Examiner has not established a *prima facie* case of nonobviousness of Applicants invention, with or without the Queen et al. reference. In fact, the Examiner's arguments under 35 U.S.C. §112 support this. Alternatively, to the extent a *prima facie case* is believed to be established, it is rebutted by the unexpected results of increased affinity.

For the above reasons, it is requested that the rejection under 35 U.S.C. § 103 be withdrawn.

Section 29

A copy of document AT is enclosed.

Summary

It is submitted that the remarks set forth above and the evidence sent herewith clearly show that the present application is in order for allowance which is hereby respectfully requested.

Respectfully submitted,

Doreen Yatko Trujillo

Doreen Yatko Trujillo
Registration No. 35,719

Date: September 18, 1995

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS
One Liberty Place - 46th Floor
Philadelphia, PA 19103
(215) 568-3100
DYT/mb
Enclosures

DOCKET NO.: CARP-0046 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of:

Adair, et al.

Serial No.: **08/485,686** Group No.: **1806**

Filed: **June 7, 1995** Examiner: **Not Yet Assigned.**

For: **Humanised Antibodies**

CERTIFICATE OF FACSIMILE TRANSMISSION
I, Doreen Yatko Trujillo, Registration No. 35,719 certify that this correspondence is being facsimile transmitted to Examiner Cech at (703) 308-4242 at the U.S. Patent and Trademark Office., Washington, D.C. 20231.

On August 23, 1996
Doreen Yatko Trujillo
Doreen Yatko Trujillo, Reg. No. 35,719

Assistant Commissioner for Patents
Washington DC 20231

Sir:

PRELIMINARY AMENDMENT

Prior to examination, please amend the above-identified application as follows:

In the Claims:

Please cancel claims 1-23.

Please add the following claims:

24. Antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues corresponding to the equivalent residues in a donor

Carter Exhibit 2015
Carter v. Adair
Interference No. 105,744

antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 100; and amino acid residues 23, 24, 49, 71, 73 and 78 at least are donor residues.

25. An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in a donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 26 to 35, 50 to 65, and 95 to 102; and amino acid residues 23, 24 and 49 at least are donor residues.

26. An antibody according to claim 24 wherein one or more of residues 1, 3, 46, 48, 58, and 71 are additionally donor residues.

27. An antibody according to claim 25 wherein one or more of residues 1, 3, 46, 47, 48, 58, and 71 are additionally donor residues.

28. An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in a donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat

Cont

numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 100; and amino acid residues 23, 24 and 49 at least are donor residues, provided that the antibody does not have affinity for the p55 chain of the human interleukin 2 receptor.

29. An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in a donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 100; and amino acid residues 23, 24, 49, 71, 73 and 78 at least are donor residues, provided that the antibody does not have affinity for the p55 chain of the human interleukin 2 receptor.

30. An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in a donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 26 to 35, 50 to 65, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues, provided that the antibody does not have affinity for the p55 chain of the human interleukin 2 receptor.

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31. An antibody according to claim 28 wherein one or more of residues 1, 3, 46, 47, 48, 58, and 71 are additionally donor residues.

32. An antibody according to claim 29 wherein one or more of residues 1, 3, 46, 47, 48, 58, and 71 are additionally donor residues.

33. An antibody according to claim 30 wherein one or more of residues 1, 3, 46, 47, 48, 58, and 71 are additionally donor residues.

34. An antibody molecule having affinity for a T-cell antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in a donor antibody having affinity for a T-cell antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

35. An antibody molecule having affinity for a lymphokine and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in a donor antibody having affinity for a lymphokine, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

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36. An antibody molecule having affinity for a growth factor and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in a donor antibody having affinity for a growth factor, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

37. An antibody molecule having affinity for a stimulating factor and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in a donor antibody having affinity for a stimulating factor, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

38. An antibody molecule having affinity for an interferon and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in a donor antibody having affinity for an interferon, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

39. An antibody molecule having affinity for an adhesion molecule and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in a donor antibody having affinity for an adhesion molecule, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

40. An antibody molecule having affinity for a hormone and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in a donor antibody having affinity for a hormone, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

41. An antibody molecule having affinity for a cancer marker and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in a donor antibody having affinity for a cancer marker, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

42. An antibody molecule having affinity for TNF-α and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in a donor antibody having affinity for TNF-α, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

43. An antibody molecule having affinity for mucin and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in a donor antibody having affinity for mucin, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

44. An antibody molecule having affinity for a receptor and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in a donor antibody having affinity for a receptor, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

45. An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in the donor murine antibody OKT3 having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

46. An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in the donor murine antibody OKT4A having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

47. An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in the donor murine antibody B72.3 having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor

residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

48. An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in the donor murine antibody 61E71 having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

49. An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in the donor murine antibody hTNF1 having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

50. An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework

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residues, the remaining heavy chain residues, corresponding to the equivalent residues in the donor murine antibody hTNF3 having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

51. An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in the donor murine antibody 101.4 having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

52. An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in the donor murine antibody A5B7 having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

53. A therapeutic composition comprising an antibody molecule according to

claim 24 or claim 25 in combination with a pharmaceutically acceptable carrier, diluent, or excipient.

54. A method of therapy comprising administering an effective amount of an antibody according to claim 24 or claim 25 to a human or animal subject.

55. A method of diagnosis comprising administering an effective amount of an antibody according to claim 24 or claim 25 to a human or animal subject.

REMARKS

The foregoing amendments are to remove improper multiple dependency and to otherwise advance prosecution. No new subject matter has been added. Support for the amendments can be found, *inter alia*, in claims 1-23 and Examples 1-5, of the application as originally filed. An early notification of allowance is earnestly requested.

Respectfully submitted,

Date: August 23, 1996

Doreen Yatko Trujillo

Doreen Yatko Trujillo
Registration No. **35,719**

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS
One Liberty Place - 46th Floor
Philadelphia, PA 19103
(215) 568-3100

5-1-97

DOCKET NO.: CARP-0057 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

3/B
D. Williams
9/11/97

In re application of:

Adair et al

Serial No.: Unassigned Group Art Unit: Unassigned

Filed: Herewith Examiner: Unassigned

For: HUMANIZED ANTIBODIES

I, Francis A. Paintin, Registration No. 19386 certify that this correspondence is being deposited with the U.S. Postal Service as First Class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

On May 1, 1997
Francis A. Paintin

Assistant Commissioner
for Patents
Washington, D.C. 20231

Dear Sir:

PRELIMINARY AMENDMENT AND REQUEST
FOR INTERFERENCE UNDER 37 CFR §1.607

Please amend the above-identified application as follows:

In the Specification:

At page 1, before line 1, insert the following:

--This application is a continuation of U.S. application Serial No.08/303,569, filed September 7, 1994, now U.S. Patent No. 5,859,205 which is a continuation of Serial No. 07/743,329, filed September 17, 1991, abandoned which is a U.S. national phase application stemming from PCT/GB90/02017, filed December 21, 1990, which PCT application claims priority benefit of GB national application Serial No. 89/28874.0, filed

December 21, 1989 in the United Kingdom, the entire content of each of said applications is incorporated by reference herein.--

Amend the above-identified specification in accordance with the enclosed copy of a preliminary amendment (dated 7/8/92) filed in applicants' Serial No. 07/743,329 application which enters the Sequence Listing as replacement pages 67-89, and renumbers original pages 67-70 as pages 90-93, respectively, and amends the specification to refer to said listing appropriately.

In the claims:

Cancel claim 1 without prejudice and enter the following claims 24-31 in this application:

--24. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglogulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1}, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions, wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of said donor amino acids is adjacent to a CDR in the donor immunoglobulin sequence.

25. A humanized immunoglobulin according to claim 24 which specifically binds to an antigen with an affinity in the range 10^8-10^{12} M^{-1}.

26. A humanized immunoglobulin according to claim 24, wherein the antigen is an IL-2 receptor.

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27. A humanized immunoglobulin according to claim 24, wherein the donor immunoglobulin is the anti-CD4 T-cell receptor antibody.

Sub H2

28. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglogulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an effective antigen binding affinity, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions, wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of said donor amino acids is adjacent to a CDR in the donor immunoglobulin sequence.

29. A humanized immunoglobulin according to claim 28 which specifically binds to an antigen with a binding affinity equivalent to that of a chimeric antibody formed from said donor immunoglobulin.

30. A humanized immunoglobulin according to claim 28, wherein the antigen is a human CD3 T-cell receptor.

31. A humanized immunoglobulin according to claim 28, wherein the donor immunoglobulin is the anti-CD3 T-cell receptor antibody.--

REMARKS

Applicants have entered claims 24-31 to request an interference in accordance with 37 CFR §1.607 as follows. It is noted that the Queen patent whose claims present the basis for an interference is classified in Class 424/133.1. MPEP §2306 suggests a transfer to the group where the patent is classified.

Compliance With 37 CFR §1.607(a)

(a) Identification of the Patent

Applicants request that an interference be declared between applicants' above-identified application and Queen et al., U.S. Patent No. 5,585,089 (hereinafter "the Queen patent"), issued December 17, 1996, a copy of which is enclosed herewith. Applicants have in claims 24-27 substantially copied claims 1, 5,

9 and 10 of the Queen patent. Applicants have fully complied with the requirements of 35 USC §135(b) in claiming substantially the same subject matter directed to the same invention as that claimed in the Queen patent prior to one year from the 12/17/96 date the Queen patent was granted.

(b) Presentation of a Proposed Count

Applicants present in Appendix A attached hereto the "Proposed Count." In compliance with 37 CFR §1.606, proposed Count 1 is broader than any of claims 1-4, the broadest claims in the Queen patent, and as broad as any one of claims 24-31 being entered into the instant application.

The proposed count contains disjunctive or alternative language to cover the claim terminology of the two parties. Such counts were expressly approved by the Board in *Hsing v. Myers*, 2 USPQ2d 1861 (Bd, Pat,. App. & Int. 1987). It is clear, however, that both alternatives are directed to the same invention as that claimed in the Queen patent.

For Queen's term, "Chothia CDRs", applicants' claims and the proposed count paragraph (b) use the alternative term "the structural loop CDRs of the variable regions." In the Queen patent (at col. 11, lines 38-44) it is stated that the light or heavy chain variable regions consist of a "framework" region interrupted by three "hypervariable regions, also called CDRs."

In Chothia et al., *J. Mol. Biol.*(1987) 197, pp.901-917,

the authors (at p. 904) define their "loops" as having "somewhat different" limits from those of the CDRs defined by Kabat et al.(1983). Chothia et al. (at page 904) describe six loops in the domains L1, L2, L3, H1, H2, and H3[1], and use the descriptive terms "hairpin loops" (Fig.1), "hairpin turns" (Table 2), and "hypervariable loops" (p.903) to describe their regions which Queen has chosen to call "Chothia CDRs". A copy of the Chothia et al. publication is enclosed.

(c) Identification of Claims Corresponding to the Count

Applicants identify all of the Queen patent claims 1-11 and applicant's claims 24-27 as corresponding to the Count and as being directed to the same patentable invention.

(d) Application of the Terms of Applicants' Disclosure to the Copied Claims

In attached Appendix B, applicants illustrate the representative support in their present application disclosure for the limitations of their claims 24-27, substantially copied from Queen claims 1, 5, 9 and 10. There is, of course, additional support in applicants' application omitted for the sake of brevity.

(e) Applicants' Effective Filing Date

Applicants' present application, being a Rule 60 continuation, has the identical specification and drawings as

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[1] In Fig. 1 of Chothia et al., these six domains are referred to as the "hypervariable regions."

that originally filed in U.S. application Serial No. 08/303.569, filed September 7, 1994, which is a U.S. national phase application stemming from PCT/GB-90/02017, filed December 21, 1990. The latter PCT application claimed priority benefit of GB national application Serial No. 89/28874.0, filed December 21, 1989. Enclosed is a copy of the GB application Serial No. 89/28874.0, a certified copy of which is on file in the aforesaid PCT/GB application.

In attached Appendix C is a diagram of support in applicants' 1989 GB application for each limitation of applicants' claims 28-31, which are also drawn to the same invention as proposed Count 1. Accordingly, applicants' effective filing date for their invention of Count 1 is 12/21/89, the filing date of their GB national application.

(f) Queen's Effective Filing Date

The Queen patent stems from U.S. Serial No. 08/477,728, filed June 7, 1995, which is a continuation of Serial No. 07/634,278, filed 12/19/90, which is a c-i-p of Serial No. 07/590,274, filed 9/28/90, and Serial No. 07/310,252, filed 2/13/89, which is a c-i-p of Serial No. 07/290,975, filed 12/28/88.

A careful study of Queen's 1988 and 1989 application disclosures reveals that that there is no disclosure therein for

certain critical limitations of the Queen patent claims and the proposed count, e.g., for the count limitation:

> ...said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework **outside: (a) the Kabat and Chothia CDRs, or (b) both the Kabat CDRs and the structural loop CDRs of the variable regions,....**

Neither of the Queen 1988 and 1989 applications contains any disclosure that teaches or suggests the requirement of amino acids from the donor Ig outside both the Kabat CDRs **and** structural loop (or Chothia) CDRs. This is a material limitation that was added to its claims by Queen to overcome the teachings of the prior art.[2]

The most that can be argued is that the 1988 and 1989 disclosures may be read to suggest that such amino acids are outside the Kabat CDRs; but nothing therein suggests that such amino acids also be outside the structural loop (or Chothia) CDRs. Thus, the absence of a disclosure of that presently claimed limitation in the earlier-filed Queen applications is clearly fatal to any attempt by Queen to claim priorty benefit thereof.

While applicants have been unable to locate a copy of the Queen application allegedly filed on 9/28/90 to evaluate its

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[2] In Queen's amendment of May, 31, 1996, at page 5, it was argued that the claims distinguish over the prior art because the immunoglobulins contain donor amino acids "outside the Kabat and Chothia CDRs."

disclosure, that application date is almost nine months later than the 12/21/89 filing date of applicants' GB application.

Queen should not be entitled to priority benefit of any application filed prior to 12/19/90, for the invention of Count 1 or its patent claims. Moreover, applicants do not concede that either the 12/19/90 application, or the application that matured into the Queen patent, contains an adequate disclosure of the invention of the proposed count. However, that issue need not be considered at this time.

Compliance With 37 CFR §1.608

Since applicants have the earlier effective filing date, there is no requirement for them to establish a prima facie case of earlier priority under §1.608.

The Requested Interference Should Be Declared

In applicants' parent application Serial No. 08/303,569, Queen's assignee, Protein Design Labs, Inc. ("PDL") has filed a Protest under 37 CFR §1.248. Therein, PDL specifically states (at page 2):

> [A]n interference analysis should be undertaken by the appropriate Examiner....

Thus, PDL acknowledges that there is interfering subject matter in the paties' respective applications. For that reason, applicants have filed the present application with claims specifically directed to the claimed ssubject matter of the Queen patent. This paper more accurately characterizes the effective

filing dates of the parties and shows that Queen would be the junior party of any interference declared hereon.

Applicants respectfully request that the proposed interference be promptly declared. MPEP §2307 states as follows:

> Examiners should note that 37 CFR 1.607 requires that examination of an application in which applicant seeks an interference with a patent "shall be conducted with special dispatch." See MPEP §708.01 (emphasis added herein).

Applicants wish to point out that in their efforts to provoke the interference, claims 1, 5, 9 and 10 of the Queen patent were substantially copied. Thus, most claim limitations are those that were examined and approved by the Examiner who allowed the Queen patent. Should the present examination involve rejections of applicants' claims that would have been equally applicable against the Queen claims, applicants respectfully note MPEP §2307.02, which requires the approval of the Group Director for such a rejection. Applicants are presumptively the prior inventors of the claimed subject matter and only desire an interference to prove that they are the actual prior inventors. Their opportunity to do so should not be unduly delayed.

Enclosed is a copy of an Information Disclosure Statement filed in applicants' parent Serial No. 08/303,569, filed 9/7/94, and Serial No. 07/743,929. Copies of the references are in said parent applications.

Please contact applicants' attorney, Francis A. Paintin, at 215-568 3100 if he can be of assistance in expediting this request.

Respectfully submitted,

Francis A. Paintin

Francis A. Paintin
Registration No. 19,386

Date: May 1, 1997

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS LLP
One Liberty Place - 46th Floor
Philadelphia, PA 19103
(215) 568-3100

APPENDIX A

PROPOSED COUNT FOR INTERFERENCE

Count 1:

A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglogulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with:

(i) an effective antigen binding activity, or

(ii) an affinity constant of at least 10^7 M^{-1} and no greater than about four-fold that of the donor immunoglobulin,

wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside:

(a) the Kabat and Chothia CDRs, or

(b) both the Kabat CDRs and the structural loop CDRs of the variable regions,

wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of said donor amino acids:

(I) is adjacent to a CDR in the donor immunoglobulin sequence, or

(II) contains an atom within a distance of 4 A^0 of a CDR in said humanized immunoglobulin .

APPENDIX B

Claim Limitation	Support in Adair Application
24. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglogulin heavy and light chains	See page 1, lines 5-16, and page 7, line 32, through page 8, line 21.
which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1},	See page 11, lines 27-30.
wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions,	See page 6, lines 14-23, page 8, lines 13-16, and page 19, line 16, to page 20, line 15.
wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks,	See page 6, line 12, to page 7, line 5.

and each of said donor amino acids is adjacent to a CDR in the donor immunoglobulin sequence.	See page 11, lines 16-20, showing that homology is maximized between donor and acceptor sequences adjacent CDRs within acceptor framework. At page 6, lines 25-35, it is indicated that the heavy chain "framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49...." In the heavy chain, Kabat CDR2 together with [Chothia] structural loop H2 extends from residues 50 to 65. Thus, residue 49 is immediately adjacent the beginning of this CDR2/H2 region. In Figs.3-4 residues marked with "N" to indicate near or adjacent a CDR (see p.38, l. 13.)
25. A humanized immunoglobulin according to claim 24 which specifically binds to an antigen with an affinity in the range 10^{8}-10^{12} M^{-1}.	Page 11, lines 27-30.
26. A humanized immunoglobulin according to claim 24, wherein the antigen is an IL-2 receptor.	Page 15, line 37, and page 16, line 2.
27. A humanized immunoglobulin according to claim 24, wherein the donor immunoglobulin is the anti-CD4 T-cell receptor antibody.	Page 53, Example 2.

APPENDIX C

Claim Limitation	Support in 1989 GB Application
28. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglogulin heavy and light chains	See page 1, lines 1-2 and 10-20; page 5, lines 8, to page 6, line 4; , and page 8.
which humanized immunoglobulin specifically binds to an antigen with an effective antigen binding affinity'	See page 5, lines 1-7; page 22, lines 27-35, page 23, lines 5-9, page 24, lines 1-4; page 25, lines 27-33; page 26 last paragraph.
wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions,	See page 5, lines 1-7; page 26, last paragraph, to page 27, top paragraph.
wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks,	See page 5, line 8, to page 6, line 4: page 7, lines 5-20.

and each of said donor amino acids is adjacent to a CDR in the donor immunoglobulin sequence.	See page 7, lines 11-14, showing that homology is maximized between donor and acceptor sequences adjacent CDRs within acceptor framework. At p.5, l. 9-16, reference is made to heavy chain "framework comprises donor at at least one of residues 6, 23 and/or 24, 48 and/or 49...." Residue 49 is immediately adjacent CDR2/H2 loop region. On Figs.20-21 residues marked "N" are near or adjacent a CDR.
29. A humanized immunoglobulin according to claim 28 which specifically binds to an antigen with a binding affinity as binding as a chimeric antibody formed from said donor immunoglobulin.	Page 23, lines 1-10; Fig. 29B.
30. A humanized immunoglobulin according to claim 28, wherein the antigen is a human CD3 T-cell receptor.	Page 11, lines 14-21. Page 17, lines 1-8; page 24, bottom paragraph.
31. A humanized immunoglobulin according to claim 28, wherein the donor immunoglobulin is the anti-CD3 T-cell receptor antibody.	Page 17, lines 1-8; page 24, bottom paragraph.

DOCKET NO.: CARP 0057 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

ADAIR ET AL.

For: HUMANIZED ANTIBODIES

Anticipated Classification of this application:

Class: 424, Subclass: 133.1

Prior Application
Examiner: D. ADAMS
Art Unit: 1816

"Express Mail" Label No. EM405876152US
Date of Deposit May 1, 1997

I hereby certify that this paper is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Typed Name: Bob Liforzato

BOX PATENT APPLICATION
Assistant Commissioner for Patents
Washington DC 20231

37 C.F.R. §1.60 TRANSMITTAL LETTER

Sir:

This is a request for filing a

(XX) Continuation ( ) Divisional

application under 37 CFR 1.60, of pending prior application Serial No.08/303,569, filed on September 7, 1994, which is a continuation of Serial No. 07/743,329, filed September 17, 1991.

1. (XX) Enclosed is a copy of prior application Serial No. 07/743,329, including the oath or declaration as originally filed.

I hereby verify that the attached papers comprise a true copy of the prior application Serial No. 07/743,329, as originally filed on September 17, 1991, and that no amendments referred to in the Oath or Declaration filed to complete the prior application introduced new matter therein.

The filing fee is calculated below on the basis of claims as filed in the prior application, less any claims cancelled or including any claims added by amendment listed below:

			SMALL ENTITY			OTHER THAN SMALL ENTITY	
For:	No. Filed	No. Extra	Rate	Fee	OR	Rate	Fee
BASIC FEE				$385	OR		$770
Total Claims	8 - 20 =		x $11=	$	OR	x $22=	$
Indep. Claims	2 - 3 =		x $40=	$	OR	x $80=	$
First Presentation Multiple Dependent Claims			+$130=	$	OR	+$260=	$
			TOTAL	$			$770

2. ( ) Verified Statement Claiming Small Entity Status is enclosed herewith.

3. ( ) Verified Statement Claiming Small Entity Status was filed in the parent case.

4. ( ) Please charge my Deposit Account No. 23-3050 in the amount of $_____. This sheet is attached in triplicate.

5. (XX) A check in the amount of $770.00 is attached. Please charge any deficiency or credit any overpayment to Deposit Account No. 23-3050.

6. (XX) The Commissioner is hereby authorized to charge payment of the following fees associated with this communication or credit any overpayment to Deposit Account No. 23-3050. This sheet is attached in triplicate.

   (XX) Any additional filing fees required under 37 CFR 1.16 including fees for presentation of extra claims.

   (XX) Any additional patent application processing fees under 37 CFR 1.17 and under 37 CFR 1.20(d).

7. (XX) The Commissioner is hereby authorized to charge payment of the following fees during the pendency of this application or credit any overpayment to Deposit Account No. 23-3050. This sheet is attached in triplicate.

(XX) Any patent application processing fees under 37 CFR 1.17 and under 37 CFR 1.20(d).

( ) The issue fee set in 37 CFR 1.18 at or before mailing of the Notice of Allowance, pursuant to 37 CFR 1.311(b).

(XX) Any filing fees under 37 CFR 1.16 including fees for presentation of extra claims.

8. (XX) Cancel in this application original claims 2-23 of the prior application before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)

9. (XX) Amend the specification as set forth in the accompanying preliminary amendment.

10. (XX) Formal drawings/photographs will be submitted when requested by the United States Patent and Trademark Office.

11. ( ) Please abandon said prior application as of the filing date accorded this application. A duplicate copy of this sheet is enclosed for filing in the prior application file. (May only be used if signed by person authorized by 37 CFR 1.138 and before payment of the base issue fee.)

12. (XX) Priority of GB application Serial No. 89/28874.0 filed on December 21, 1989 in United Kingdom (country) is claimed under 35 U.S.C. Section 119.

(XX) The certified copy has been filed in prior PCT application Serial No. PCT/GB90/02017, filed December 21, 1990.

13. (XX) The prior application is assigned of record to Celltech Limited.

14. ( ) Copy of the Assignment(s) of the invention and separate Form(s) 1595 for each Assignment will be submitted upon receipt of the Official Filing Receipt.

15. (XX) The power of attorney in the prior application is to Francis A. Paintin, Registration No. 19,386.

(XX) The power appears in the original papers in the prior application.

(XX) Since the associate power of attorney does not appear in the original papers, a copy of the associate power in the prior application is enclosed.

16. (XX) Address all future communications to:

Francis A. Paintin
WOODCOCK WASHBURN KURTZ MACKIEWICZ & NORRIS LLP
One Liberty Place - 46th Floor
Philadelphia, PA 19103

17. (XX) A preliminary amendment is enclosed. Claims added by this amendment have been properly numbered consecutively beginning with the number next following the highest numbered original claim in the prior application.

18. ( ) A Petition for Extension of Time has been filed in the parent application, Serial No. _____ filed _____. A copy of the Petition for Extension of Time is enclosed.

19. (XX) Enclosed is a Statement to Support Filing and Submission of DNA/Amino Acid Sequences in Accordance with 37 CFR §§ 1.821 through 1.825 as filed in Serial No. 08/303,569.

(XX) Enclosed is a copy of a Letter of Reference to Computer Readable Form filed in Serial No. 08/303,569.

Date: May 1, 1997

Francis A. Paintin
Signature
Francis A. Paintin
Attorney of Record
Registration No. **19,386**

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS LLP
One Liberty Place - 46th Floor
Philadelphia, PA 19103
(215) 568-3100

DOCKET NO.: CARP-0046 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF	:	John Robert Adair et al.
SERIAL NO.	:	08/485 686
FILED	:	JUNE 7, 1995
FOR	:	HUMANISED ANTIBODIES
GROUP ART UNIT	:	1816
EXAMINER	:	EVELYN RABIN Ph. D.

I, Doreen Yatko Trujillo, Registration No. 35,719 certify that this correspondence is being deposited with the U.S. Postal Service as First Class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

On August 20, 1997

Doreen Yatko Trujillo

Doreen Yatko Trujillo, Esquire Reg. No. 35,719

Assistant Commissioner for Patents
Washington, DC 20231.

RESPONSE AND AMENDMENT

Dear Sir:

Pursuant to 37 C.F.R. § 1.115, Applicants submit the following in response to the Office Action dated February 20, 1997.

In the Claims:

Please cancel claims 24 to 55, without prejudice.

Please add the following claims.

--56. An antibody molecule having affinity for a predetermined antigen and

comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarily determining regions (CDRS) , said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues corresponding to the equivalent residues in a donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain at least residues 31 to 35, 50 to 65 and 95 to 102 (the CDRS) and at least residues 23, 24, 49, 71, 73 and 78 (in the framework regions) correspond to the equivalent residues in said donor antibody.

57. The antibody molecule of claim 56, wherein additionally residues 26 to 30 in said composite heavy chain correspond to the equivalent residues in said donor antibody.

58. The antibody molecule of claim 56, wherein additionally at least one of residues 6, 37, 48 and 94 in said composite heavy chain corresponds, to the equivalent residue in said donor antibody.

59. The antibody molecule of claim 57, wherein additionally at least one of residues 6, 37, 48 and 94 in said composite heavy chain corresponds to the equivalent residue in said donor antibody.

60. The antibody molecule of claim 58, wherein additionally at least one of

residues 2, 4, 25, 36, 39, 47, 93, 103, 104, 106 and 107 in said composite heavy chain corresponds to the equivalent residue in said donor antibody.

61. The antibody molecule of claim 59, wherein additionally at least one of residues 2, 4, 25, 36, 39, 47, 93, 103, 104, 106 and 107 in said composite heavy chain corresponds to the equivalent residue in said donor antibody.

62. The antibody molecule of claim 56, wherein said complementary light chain is a composite light chain having a variable domain including CDRS, said variable domain comprising predominantly human acceptor antibody light chain framework residues, the remaining light chain residues corresponding to the equivalent residues in said donor antibody, wherein, according to the Kabat numbering system, in said composite light chain at least residues 24 to 34, 50 to 56 and 69 to 97 (the CDRs) and at least residues 46, 48, 58 and 71 (in the framework regions) correspond to the equivalent residues in said donor antibody.

63. The antibody molecule of claim 62, wherein additionally at least one of residues 2, 4, 6, 35, 38, 44, 47, 49, 62, 64 to 69, 85, 87, 98, 99, 101 and 102 in said composite light chain corresponds to the equivalent residue in said donor antibody.

64. The antibody molecule of any one of claims 56, 57 or 62 which ~~is~~ has specificity ~~specific~~ for a T-cell antigen.

65. The antibody molecule of any one of claims 56, 57 or 62 which ~~is~~ has specificity ~~specific~~ for a lymphokine.

66. The antibody molecule of any one of claims 56, 57 or 62 which ~~is~~ has specificity ~~specific~~ for a growth factor.

67. The antibody molecule of any one of claims 56, 57 or 62 which ~~is~~ has specificity ~~specific~~ for interferon.

68. The antibody molecule of any one of claims 56, 57 or 62 which ~~is~~ has specificity ~~specific~~ for an adhesion molecule.

69. The antibody molecule of any one of claims 56, 57 or 62 which ~~is~~ has specificity ~~specific~~ for a hormone.

70. The antibody molecule of any one of claims 56, 57 or 62 which ~~is~~ has specificity ~~specific~~ for a cancer marker.

71. The antibody molecule of any one of claims 56, 57 or 62 which ~~is~~ has specificity ~~specific~~ for TNF-α.

72. The antibody molecule of any one of claims 56, 57 or 62 which ~~is~~ has specificity ~~specific~~ for ~~mucin~~.

73. A ~~therapeutic~~ composition comprising ~~an~~ the antibody of any one of claims 56, 57 or 62 in combination with a pharmaceutically acceptable carrier, diluent or excipient. --

Remarks

This paper is being filed in response to the Office Action dated February 20, 1997. A petition for a three-month extension of time, and the appropriate fee, accompanies this response.

Claims 24 to 55 were pending. Claims 24 to 55 have been canceled herein without prejudice, and replaced by the new claims 56 to 73.

Support for present claim 56 can be found, *inter alia,* on page 19, lines 24 and 25, of the application as filed which defines the extent of the heavy chain CDRs, and page 7, lines 1 to 3, which defines the preferred basic set of framework residues which correspond between the donor and humanized chains.

Support for claim 57 can be found, *inter alia*, on page 17, lines 11 to 13 of the application as filed, which defines the preferred extent of the "CDRs". This extension, in fact, merely adds the Chothia loop residues to CDR1 in the heavy chain (see also page 8, lines 17 to 22).

Claims 58 and 59 are based on page 7, lines 7 to 9 of the application as filed. Claims 60 and 61 are based on page 7, lines 9 to 14 of the application as filed.

Support for claim 62 can be found, *inter alia*, is based on page 17, lines 1-4 to 16, of

the application as filed, which defines the extent of the light chain CDRs, and page 18, line 21, which defines the preferred basic set of light chain framework residues which correspond between the donor and humanized chains.

Basis for claim 63 is found on page 18, lines 23 to 28 of the application as filed.

Basis for claims 64 to 72 is found on page 15, line 24 through page 16, line 3 and in the Examples of the application as filed. Basis for claim 73 is found on page 16, lines 5 to 8 of the application as filed.

In view of the foregoing amendments, and arguments which follow, Applicants hereby request withdrawal of all rejections upon reconsideration. To the extent the rejections of claims 24 to 55 may be applied to the present claims, Applicants submit the following.

Rejections Under 35 USC § 112

In light of the amendments to the claims, it is submitted that the points raised by the Examiner in Sections 3, 4 and 5 (35 U.S.C. § 112, first paragraph) are rendered moot.

As regards the objection set forth in the last two paragraphs of the Examiner's Section 5, Applicants respectfully submit that such a rejection is unfounded. It is first to be noted that the Examiner has made a number of sweeping assertions which are totally unsupported by any evidence, reference to the statute, or reference to legal precedent. In particular, the Examiner has provided no evidence of a technical nature.

It is to be pointed out that, by the priority date of the present application, a large number of monoclonal antibodies had been successfully used in therapy. In particular, the OKT3

antibody, which is the subject of one of the examples, had been in use since the early 1980's in the treatment of acute rejection episodes in liver transplant patients. Thus, by the priority date, it was well known how to make a therapeutic antibody composition.

As is shown in the present description, the humanized antibodies of the present invention have affinities similar to those of the monoclonal antibodies from which they are derived. It would therefore have been clear to the skilled person that a composition containing a humanized antibody would be very similar to a composition containing the monoclonal antibody. It is therefore submitted that a skilled person would have been able readily to produce a therapeutic composition containing a humanized antibody of the present invention.

It may be that the Examiner has doubts as to the efficacy of the humanized antibodies of the present invention. Again, however, the Examiner has provided no evidence to support any such doubts. The Applicants have a number of humanized antibodies according to the present invention in clinical trials at various stages. These trials have shown that the humanized antibodies are effective. It is therefore submitted that there is no basis for the Examiner's, rejection which should therefore be withdrawn.

It is believed that the point raised in Section 6 by the Examiner is now moot. In any event, it is submitted that it is not necessary to use the particular monoclonal antibodies recited by the Examiner. The procedure described in the application is a general procedure which can be applied to any donor antibody and to any acceptor antibody. The Applicants have shown that this protocol can be applied generally, and have successfully produced a number of humanized antibodies according to the invention. It is therefore submitted that the rejection should be withdrawn.

Rejection Under 35 USC § 102(e)

In her Section 8, the Examiner refers to U.S. Patent No. 5,530,101 issued to Queen et al. The Examiner indicates, almost as a footnote, that the Queen Patent apparently has an effective filing date of December 28, 1988. While this may be true, the disclosures of the earliest Queen applications do not support this rejection. Following on from the initial application filed on December 28, 1988, there was a continuation-in-part application (CIP) filed February 13, 1989. There was then a separate new application filed September 28, 1990. A further continuation-in-part application was filed December 19, 1990, which claims priority to the prior continuation-in-part application and he separate new application. The '101 patent granted on the last continuation-in-part application.

The present application has a priority date of December 21, 1989. Only the initial application and the first continuation-in-part application of the '101 patent were filed before Applicants' priority date. Therefore, in order for the Examiner to be able to show that the present claims lack novelty, it must be shown that any subject matter which may be relevant was disclosed in these two earliest Queen applications.[1] Applicants respectfully submit that the Examiner has not shown this.

Applicants have been able to study the two early Queen applications in connection with the application from which the present application is a continuation. This study shows that there is

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[1] Similarly, the most recently issued patent from the same family, U.S. Patent No. 5,585,089 issued to Queen et al. on December 17, 1996 (the "'089 patent") is to no avail. The claims specify that the changes be made "outside the Kabat *and* Chothia CDRs." (emphasis added) There was no mention of Chothia. CDRs in the two earliest Queen applications. Thus, the '089 patent is not entitled to a filing date of December 28, 1988.

considerably more subject matter in the granted '101 patent than in either of the two early Queen applications. As far as Applicants can tell, the only working example provided in the two early Queen applications is the anti-tac antibody which is referred to in Figures 1A and 1B of the '101 patent.

The Examiner will see from a study of Figure lB of the Queen Patent that residue 74 (according to the numbering in the Figure) is different between the mouse (top line) and human (bottom line) sequences. It is indicated in the Figure that the places where changes were made are indicated by double underlining. At residue 74, no change was made and so there is a clear difference between the humanized and mouse sequences. In Figure 1B, the sequences are numbered linearly and not according to the Kabat system. If the sequences are numbered according to the Kabat system, as specified in the present claim, linear residue 74 becomes Kabat residue 73. Since it is specified in present claim 56 that Kabat residue 73 should be identical between donor and acceptor sequences, it can be seen that the antibody of Figure 1 of the '101 Patent does not destroy the novelty of claim 56.

It is therefore submitted that there is no specific disclosure in the '101 patent entitled to the date of the two early Queen applications which destroys the novelty of present claim 56.

The general disclosure is equally unavailing. Looking first at the initial Queen application, it can be readily seen that it is not directed to a general process for humanizing antibodies. As is made clear from the introduction on pages 1 to 4 and the Summary of the Invention, the initial application is very specifically directed to the production of a humanized anti-tac antibody. Thus, there is absolutely no disclosure or suggestion of a claim to humanized antibodies in

general; nor is there any disclosure or suggestion to make the specific residues recited in the present claims donor.

Even if (which is denied) such a general teaching for humanizing anitbodies could be derived from the initial Queen application, it must be borne in mind that the teaching in the initial application is not enabling. It will be seen from pages 21 and 22 that there are disclosed three criteria for selecting framework residues outside the Kabat CDRs which can be considered for changing. One of these criteria is that the framework residues should be physically close to the antigen binding region. However, this criterion is so vague as to be meaningless. There is no description as to what "physically close" means. How close is close? How does, a skilled person build a model to determine whether a residue is close? What is the antigen binding region? It can thus be seen that it would be impossible for a skilled person to put this part of the teaching of the initial Queen application into effect.

The second Queen application has a slightly more general teaching. However, the general teaching is still not enabling for the invention recited therein, much less the present claims. It requires the skilled person to put into practice various criteria set out therein. One of these criteria is that residues a certain distance away from the antigen binding region should be identified. In order to identify these residues, it is necessary to build a molecular model of an antibody. Given the lack of detail in the Queen application, it is submitted that this would not have been possible.

By the date of filing of the continuation-in-part application, it was possible to build rough models of antibodies. However, these models could only be built using details which are not provided in this application. Even when such details were available, the models which were built

were not very accurate. The best accuracy was obtained for the main chain conformation of the framework regions, but even this was not very good. The modeled main chain conformations for the CDRs was poor. The results obtained for side chains, whether on framework or CDR residues, were not very accurate at all.

In the Queen applications, it is a requirement that a determination be made either of the physical closeness or of the distance between framework side chain atoms and CDR atoms. These are the two least accurate areas in any model. This clearly shows that it would not have been possible to put the teaching in the early Queen applications into effect in a reproducible manner, even using modern modeling programmes.

It is therefore submitted that there is no disclosure in either of the two early Queen applications, much less an enabling disclosure, of Applicants' invention. Thus, there can be no novelty-destroying disclosure therein.

Even if (which is denied) there were any enabling disclosure in the two Queen applications, it would still not be possible to obtain the subject matter of the present claims by following the teachings therein. Applicants have carried out their own modeling procedures using a current available modeling program. These results have shown that none of residues 23, 24, 71 and 73 in the heavy chain meet the criteria set out in the first Queen continuation-in-part application. A study of 39 solved X-ray crystal structures of antibodies homologous to the OKT3 antibody has shown that in NONE of these real (as opposed to modeled) structures does either residue 23 or residue 24 meet the distance criterion of the first Queen CIP application. Thus, as these residues would never be identified, there is no novelty-destroying disclosure in the two early Queen

applications.

If the Examiner requires, details of any of the studies the Applicants have made, can be submitted.

Accordingly, Applicants respectfully submit that the rejection under 35 U.S.C. §102(e) should be withdrawn.

Rejections Under 35 USC § 103

Regarding the Examiner's Section 10, it is hereby confirmed that the subject matter of the various claims was commonly owned at the time that the inventions covered by these claims were made.

In the remaining sections of the Office Action, the Examiner raises a number of obviousness rejections. These are all based on the Queen '101 patent which was discussed above in connection with novelty, discussion incorporated herein. It is submitted that the secondary references do not overcome the deficiencies of the '101 patent. The secondary references are relied upon for the specific antigen recitations in the dependent claims.

As stated above, even by the priority date of the present application, it was not possible to build accurate models of antibodies. The identification of residues which might be changed in the Queen applications is based on the use of molecular modeling. Since no accurate models could have been built, it would not have been possible to apply the teaching of the two early Queen applications to any antibody except the specific one referred to in the Queen applications. It has been shown above that this does not destroy the novelty of the present claims. Further, applying any teaching

derived from the Queen applications would still not produce anything falling within the present claims.

It should again be stressed that, in the CIP application, it is specified that residues within a certain distance of the CDR atoms should be selected as candidates for changing. Using modern molecular modeling techniques, it is possible to show that residues 23, 24, 71 and 73 in the heavy chain can never meet this distance criterion. As discussed previously, this is confirmed by studies of resolved structures for residues 23 and 24. Thus, even if it had been possible to build a molecular model at the priority date of the present application, doing so would not have identified the above four residues. It would not have been possible to produce anything falling within the present claims based on the teaching in these two early Queen applications.

None of the remaining references, or combination thereof, cited by the Examiner overcomes these deficiencies. Applicants respectfully submit that the rejections under 35 U.S.C. § 103(a) should be withdrawn.

Summary

It is submitted that the new claims submitted for the Examiner's attention are supported by the description as originally filed, are novel over any disclosure in the two early Queen applications, and are nonobvious over the '101 patent, whether taken alone or in combination with any other documents.

It is therefore submitted that the application is in condition for allowance, notice of which is hereby respectfully requested.

Respectfully requested

Date: August 20, 1997

Signature
Doreen Yatko Trujillo
Registration No. **35,719**

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS LLP
One Liberty Place - 46th Floor
Philadelphia, PA 19103
(215) 568-3100

93542

LAW OFFICES

WOODCOCK WASHBURN KURTZ MACKIEWICZ & NORRIS

A PARTNERSHIP INCLUDING PROFESSIONAL CORPORATIONS

ONE LIBERTY PLACE - 46TH FLOOR
PHILADELPHIA, PA 19103-7301

(215) 568-3100

Facsimile: (215) 568-3439
Cable: WOODCOCK

DATE: January 28, 1998

Please deliver this and the following pages to:

Name:	**Lila Feisee, Supervisory Patent Examiner**
Company/Firm:	**U.S. Patent and Trademark Office, Group 1806**
Telecopier No.:	**(703) 305-7230**
Client/Matter No.:	**CARP-0032; Serial No. 08/303,569**
SENDER'S NAME:	**Doreen Y. Trujillo**
PAGES TO FOLLOW:	2

If transmission is not complete, please call (215) 568-3100.

COVER MESSAGE: A proposed amendment to the previously allowed claims consistent with our discussions is attached. I look forward to speaking with you.

PLEASE DELIVER UPON RECEIPT. HARD COPY WILL FOLLOW.

PROPOSED CLAIMS

120. An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in a donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 6, 23, 24, and 49 at least are donor residues, provided that said antibody is not the Queen et al. humanized anti-tac antibody.

121. The antibody molecule of claim 120, wherein amino acid residues 26 to 30 and 59 to 65 in said composite heavy chain are additionally donor residues.

122. The antibody molecule of claim 120, wherein amino acid residues 71, 73, and 78 in said composite heavy chain are additionally donor residues.

123. The antibody molecule of claim 120, wherein at least one of amino acid residues 1, 3, and 76 in said composite heavy chain are additionally donor residues.

124. The antibody molecule of claim 120, wherein at least one of amino acid residues 36, 94, 104, 106, and 107 in said composite heavy chain are additionally donor residues.

125. The antibody molecule of claim 124, wherein at least one of amino acid residues 2, 4, [6,] 38, 46, 67, and 69 in said composite heavy chain are additionally donor residues.

126. The antibody molecule of claim 120, wherein said complementary light chain is a composite light chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody light chain framework residues, the remaining light chain residues corresponding to the equivalent residues in a donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite light chain; said CDRs comprise donor residues at least at residues 24 to 34, 50 to 56, and 89 to 97; and amino acid residues 46, 48, 58, and 71 at least are donor residues.

127. The antibody molecule of claim 126, wherein amino acid residues 1, 3, 60 (if this residue can form a salt bridge with residue 54), and 70 (if this residue can form a salt bridge with residue 24) in said composite light chain are additionally donor residues.

DOCKET NO.: CARP-0032 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: **John Robert Adair, Diljeet Singh Athwal, and John Spencer Emtage**

Serial No.: **08/303,569** Group Art Unit: **1816**

Filed: **9/07/94** Examiner: **L. Feisee**

For: **HUMANISED ANTIBODIES**

Certificate of Facsimile Transmission

I Doreen Yatko Trujillo, Registration No.: 35,719 hereby certify that this paper is being facsimile transmitted to Examiner Feisee at (703)305-7230 of the U.S. Patents and Trademark Office, Washington, D.C. 20231, on the date shown below

On February 23 1998

Doreen Yatko Trujillo, Registration Reg. No. 35,719

U.S. Patent and Trademark Office
Office of Publications
Query and Correspondence Branch
Crystal Plaza 2 Room-6C30
Washington DC 20231

Dear Sir:

AMENDMENT PURSUANT TO 37 C.F.R. §1.312

Pursuant to 37 C.F.R. §1.312 (b), please amend the above-identified application as follows. A petition and appropriate fee accompanies this Amendment.

In the claims:

120. (Twice Amended) An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues corresponding to the equivalent residues in a donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50

to 58, and 95 to 102; and amino acid residues 6, 23, 24, and 49 at least are donor residues, provided that said antibody is not the humanized anti-tac antibody described in WO 90/07861.

125. (Amended) The antibody molecule of claim 124, wherein at least one of amino acid residues 2, 4, [6,] 38, 46, 67, and 69 in said composite heavy chain are additionally donor residues.

REMARKS

The foregoing amendments are being made to advance the present case to issuance. Although the issue fee had already been paid on December 4, 1996, issuance was delayed following the filing and subsequent entry of a Protest. These amendments are being submitted following the Examiner's consideration of the Protest, Applicants' response thereto, and a personal interview conducted with the Examiner on October 16, 1997. Thus, these amendments do not require additional search or examination, nor could they been submitted ealier. Support for the amendments can be found, *inter alia*, on page 5, line 10 through page 6, line 37, of the application as originally filed. The humanized anti-tac antibody of WO 90/07861 is therein described and distinguished from the present invention. The inclusion of residue 6 as a donor residue is also disclosed therein. As is clear from the foregoing, no new matter is added by these amendments. Applicants respectfully request that they be entered.

Respectfully submitted,

Date: February 23, 1998

Doreen Yatko Trujillo
Registration No. **35,719**

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS
One Liberty Place - 46th Floor
Philadelphia, PA 19103
(215) 568-3100

1642 1647

DOCKET NO.: CARP-0032 **PATENT**

Issue Batch No.: D80
Date of Notice
of Allowance : September 4, 1996
Serial No. : 08/303,569

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

John Robert Adair, Diljeet Singh Athwal, and John Spencer Emtage

Serial No.: **08/303,569** Group Art Unit: **1642**

Filed: **9/07/94** Examiner: **L. Feisee**

For: **HUMANISED ANTIBODIES**

Assistant Commissioner of
Patents and Trademarks
Washington, D.C. 20231

JUL 14 1998

MAIL ROOM & RECORDS
SERVICE CENTER

Dear Sir:

AMENDMENT PURSUANT TO 37 C.F.R. §1.312

Pursuant to 37 C.F.R. §1.312 (b), please amend the above-identified application as follows. A petition and the appropriate fee accompanies this Amendment.

In the specification:

Page 6, line 9, after "heavy chain", please insert -- SEQ ID NO:31 --.

Please replace pages 67-92 of the Sequence Listing with the attached substitute Sequence Listing, pages 67-93. Please renumber the pages thereafter accordingly.

07/17/1998 CGRIFFIN 00000035 08303569
01 FC:122 130.00 OP

In the claims:

120. (Twice Amended) An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues corresponding to the

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Carter v. Adair
Interference No. 105,744

equivalent residues in a donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 6, 23, 24, and 49 at least are donor residues, provided that said composite heavy chain does not comprise the amino acid sequence of SEQ ID NO: 31.

125. (Amended) The antibody molecule of claim 124, wherein at least one of amino acid residues 2, 4, [6,] 38, 46, 67, and 69 in said composite heavy chain are additionally donor residues.

REMARKS

The foregoing amendments are being made to advance the present case to issuance. Although the issue fee had already been paid on December 4, 1996, issuance was delayed following the filing and subsequent entry of a Protest in the above-identified application. In the Protest, U.S. Patent No. 5,585,089 issued to Queen et al. on December 17, 1996 was cited as relevant. These amendments are being submitted following the Examiner's consideration of the Protest, Applicants' response thereto, a personal interview conducted with the Examiner on October 16, 1997, and telephonic discussions and communications with the Examiner, as well as telephonic communications with Examiner Schwartz. These amendments do not require additional search or examination.

Support for the proviso in claim 120 can be found, *inter alia*, on page 5, line 10 through page 6, line 37, of the application as originally filed. The heavy chain variable region of the humanized anti-tac antibody of WO 90/07861 is therein described and distinguished from the present invention. The heavy chain variable region of the humanized anti-tac antibody of WO 90/07861 was, thus, incorporated by reference. The inherent amino acid sequence of the heavy chain variable region of the humanized anti-tac antibody of WO 90/07861 was, thus, also incorporated by reference. In view of its recitation in claim 120, the amino acid sequence of the heavy chain variable region of the humanized anti-tac antibody of WO 90/07861 is presumed essential. Accordingly, Applicants have amended the specification to include the sequence of

the heavy chain variable region of the humanized anti-tac antibody described in WO 90/07861. The sequence is included as SEQ ID NO: 31 of the substitute Sequence Listing. A paper and computer-readable copy of the substitute Sequence Listing, and accompanying papers, are included herein. A Declaration by the undersigned that the amendatory material consists of the same material incorporated by reference is included pursuant to M.P.E.P. 608.01(p). Applicants have also corrected typographical errors in the sequence listing inadvertently introduced in the substitute Sequence Listing previously submitted on December 4, 1996.

Support for the recitation of residue 6 as a donor residue can be found, *inter alia*, on page 6, line 35, of the application as filed.

No new matter is added by any of the foregoing.

Applicants request that the foregoing amendments be entered and that, upon their entry, the application be allowed to issue. Pursuant to M.P.E.P. § 1309, Applicants request that the application be tagged to ensure appropriate printing priority in the publishing division. This application is at least entitled to category (2) priority as listed in M.P.E.P. § 1309.

If anything remains outstanding, the Examiner is requested to contact the undersigned at (215) 564-8352.

Respectfully submitted,

Doreen Yatko Trujillo

Date: July 13, 1998

Doreen Yatko Trujillo
Registration No. **35,719**

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS LLP
One Liberty Place - 46th Floor
Philadelphia, PA 19103
[(215) 568-3100

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ALLOWED CLAIMS

120. An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues corresponding to the equivalent residues in a donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 6, 23, 24, and 49 at least are donor residues, provided that said composite heavy chain does not comprise the amino acid sequence of SEQ ID NO: 31.

121. The antibody molecule of claim 120, wherein amino acid residues 26 to 30 and 59 to 65 in said composite heavy chain are additionally donor residues.

122. The antibody molecule of claim 120, wherein amino acid residues 71, 73, and 78 in said composite heavy chain are additionally donor residues.

123. The antibody molecule of claim 120, wherein at least one of amino acid residues 1, 3, and 76 in said composite heavy chain are additionally donor residues.

124. The antibody molecule of claim 120, wherein at least one of amino acid residues 36, 94, 104, 106, and 107 in said composite heavy chain are additionally donor residues.

125. The antibody molecule of claim 124, wherein at least one of amino acid residues 2, 4, 38, 46, 67, and 69 in said composite heavy chain are additionally donor residues.

126. The antibody molecule of claim 120, wherein said complementary light chain is a composite light chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody light chain framework residues, the remaining light chain residues corresponding to the equivalent residues in a donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite light chain; said CDRs comprise donor residues at least at residues 24 to 34, 50 to 56, and 89 to 97; and amino acid residues 46, 48, 58, and 71 at least are donor residues.

127. The antibody molecule of claim 126, wherein amino acid residues 1, 3, 60 (if this residue can form a salt bridge with residue 54), and 70 (if this residue can form a salt bridge with residue 24) in said composite light chain are additionally donor residues.

DOCKET NO.: CARP-0057 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Adair et al

Serial No.: 08/846,658 Group Art Unit: 1642

Filed: May 1, 1997 Examiner: J. Reeves

For: HUMANIZED ANTIBODIES

I, Francis A. Paintin, Registration No. 19386 certify that this correspondence is being deposited with the U.S. Postal Service as First Class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

On Nov. 5, 1998

Francis A. Paintin

Assistant Commissioner
for Patents
Washington, D.C. 20231

Dear Sir:

FOURTH PRELIMINARY AMENDMENT

Please amend the above-identified application as follows:

In the claims:

Please enter the following claims 32-48 in this application:

32. First and second polynucleotides respectively encoding heavy and light chain variable regions of a humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chain frameworks, which humanized immunoglobulin

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02 FC:102 902.00 CH

specifically binds to an antigen with an affinity constant of at least about $10^8\ M^{-1}$ and no greater than about four-fold that of the donor immunoglobulin, wherein the sequence of the humanized immunoglobulin heavy chain variable region framework is at least 65% identical to the sequence of the donor immunoglobulin heavy chain variable region framework and comprises at least 70 amino acid residues identical to those in the acceptor human immunoglobulin heavy chain variable region framework.

33. A vector comprising first and second polynucleotides according to claim 32.

34. First and second polynucleotides respectively encoding heavy and light chain variable regions of a humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from acceptor immunoglobulin heavy and light chain frameworks, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least about $10^8\ M^{-1}$ and no greater than about four-fold that of the donor immunoglobulin, wherein the sequence of the acceptor immunoglobulin heavy chain variable region framework is a

consensus sequence of human immunoglobulin heavy chain variable region frameworks.

35. First and second polynucleotides respectively encoding heavy and light chain variable regions of a humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least about $10^8\ M^{-1}$ and no greater than about four-fold that of the donor immunoglobulin, wherein said humanized immunoglobulin heavy chain comprises one or more amino acids from the donor immunoglobulin heavy chain framework outside the Kabat and Chothia CDRs, wherein the donor amino acids substitute for corresponding amino acids in the acceptor immunoglobulin heavy chain framework, and each of these said donor amino acids:

(I) is adjacent to a CDR in the donor immunoglobulin sequence, or

(II) contains an atom within a distance of 6 ANGSTROM of a CDR in said humanized immunoglobulin.

36. First and second polynucleotides respectively encoding heavy and light chain variable regions of a humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of a least about $10^8\ M^{-1}$ and no greater than about four-fold that of the donor immunoglobulin, wherein said humanized immunoglobulin heavy chain comprises one or more amino acids from the donor immunoglobulin heavy chain framework outside the Kabat and Chothia CDRs that substitute for the corresponding amino acids in the acceptor immunoglobulin heavy chain framework, wherein each of these said donor amino acids:

(I) is adjacent to a CDR in the donor immunoglobulin sequence, or

(II) is capable of interacting with amino acids in the CDRs, or

(III) is typical at its position for human immunoglobulin sequences, and the substituted amino acid in the acceptor is rare at its position for human immunoglobulin sequences.

37. A cell line transfected with a vector according to claim 33.

38. First and second polynucleotides respectively encoding heavy and light chain variable regions of a humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chain frameworks, which humanized immunoglobulin specifically binds to an antigen with an affinity constant within about four-fold of that of the donor immunoglobulin, wherein the sequence of the humanized immunoglobulin heavy chain variable region framework is at least 65% identical to the sequence of the donor immunoglobulin heavy chain variable region framework and comprises at least 70 amino acid residues identical to those in the acceptor human immunoglobulin heavy chain variable region framework.

39. First and second polynucleotides respectively encoding heavy and light chain variable regions of a humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from acceptor immunoglobulin heavy and light chain frameworks, which humanized immunoglobulin specifically binds to an antigen with an affinity constant within about four-fold of that of the donor

immunoglobulin, wherein the sequence of the acceptor immunoglobulin heavy chain variable region framework is a consensus sequence of human immunoglobulin heavy chain variable region frameworks.

40. First and second polynucleotides respectively encoding heavy and light chain variable regions of a humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant within about four-fold of the donor immunoglobulin, wherein said humanized immunoglobulin heavy chain comprises one or more amino acids from the donor immunoglobulin heavy chain framework outside the Kabat and Chothia CDRs, wherein the donor amino acids substitute for corresponding amino acids in the acceptor immunoglobulin heavy chain framework, and each of these said donor amino acids:

(I) is adjacent to a CDR in the donor immunoglobulin sequence, or

(II) contains an atom within a distance of 6 ANGSTROM of a CDR in said humanized immunoglobulin.

41. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variableregion frameworks from human acceptor immunoglobulin heavy and light chain frameworks, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^7 M^{-1} and no greater than about four-fold that of the donor immunoglobulin, wherein the sequence of the humanized immunoglobulin heavy chain variable region framework is at least 65% identical to the sequence of the donor immunoglobulin heavy chain variable region framework and comprises at least 70 amino acid residues identical to an acceptor human immunoglobulin heavy chain variable region amino acid sequence.

42. A humanized immunoglobulin according to claim 41 which is an antibody comprising two light chain/heavy chain dimers.

43. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from acceptor immunoglobulin heavy and light chain frameworks, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least about 10^8 M^{-1} and no

greater than about four-fold that of the donor immunoglobulin, wherein the sequence of the acceptor immunoglobulin heavy chain variable region framework is a consensus sequence of human immunoglobulin heavy chain variable region frameworks.

44. A pharmaceutical composition comprising a humanized immunoglobulin of claim 41 in a pharmaceutically acceptable carrier.

45. A method of producing the humanized immunoglobulin of claim 41 comprising:

introducing DNA segments encoding the humanized immunoglobulin heavy and light chains into a cell; and

expressing the DNA segments in the cell to produce the humanized immunoglobulin.

46. A method of producing a humanized immunoglobulin, comprising the steps of:

(1) comparing the sequence of a donor immunoglobulin heavy chain variable region against a collection of sequences of human heavy chain variable regions;

(2) selecting a human heavy chain variable region from the collection of human heavy chain variable regions to provide an acceptor heavy chain variable region, wherein the selected

variable region framework is at least 65% identical to the donor immunoglobulin heavy chain variable region framework;

(3) synthesizing a DNA segment encoding a humanized heavy chain variable region, comprising CDRs from the donor immunoglobulin heavy chain variable region and a variable region framework from the selected acceptor heavy chain variable region;

(4) introducing the DNA segment encoding the humanized immunoglobulin heavy chain variable region and a DNA segment encoding a humanized immunoglobulin light chain variable region into a cell; and

(5) expressing the DNA segments in the cell to produce the humanized immunoglobulin.

47. A method of producing a humanized immunoglobulin, comprising the steps of:

(1) comparing the sequence of a donor immunoglobulin light chain variable region against a collection of sequences of human light chain variable regions;

(2) selecting a human light chain variable region from the collection of human light chain variable regions to provide an acceptor light chain variable region, wherein the selected

variable region framework is at least 65% identical to the donor immunoglobulin light chain variable region framework;

(3) synthesizing a DNA segment encoding a humanized light chain variable region, comprising CDRs from the donor immunoglobulin light chain variable region and a variable region framework from the selected acceptor light chain variable region;

(4) introducing the DNA segment encoding the humanized immunoglobulin light chain variable region and a DNA segment encoding a humanized immunoglobulin heavy chain variable region into a cell; and

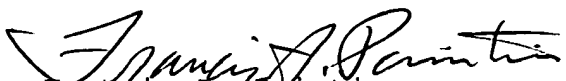
(5) expressing the DNA segments in the cell to produce the humanized immunoglobulin.

48. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from acceptor immunoglobulin heavy and light chain frameworks, which humanized immunoglobulin specifically binds to an antigen with an affinity constant within about four-fold of that of the donor immunoglobulin, wherein the sequence of the acceptor immunoglobulin heavy chain variable region framework is a consensus sequence of human immunoglobulin heavy chain variable region frameworks.

REMARKS

Newly added claims 32-40 have been copied from claims in Queen et al., U.S. Patent No. 5,693,761. Claims 41-48 have been copied from claims in Queen et al., U.S. Patent No. 5,693,762. Copies of both patents are enclosed. Applicants are in compliance with 35 USC §135(b) since both Queen patents were issued on December 2, 1997.

Respectfully submitted,



Francis A. Paintin
Registration No. 19,386

Date: November 5, 1998

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS LLP
One Liberty Place - 46th Floor
Philadelphia, PA 19103
(215) 568-3100

Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 10029–10033, December 1989
Immunology

A humanized antibody that binds to the interleukin 2 receptor

(chimeric antibody/antibody affinity/autoimmune disease)

CARY QUEEN*, WILLIAM P. SCHNEIDER*, HAROLD E. SELICK*†, PHILIP W. PAYNE*, NICHOLAS F. LANDOLFI*, JAMES F. DUNCAN*‡, NEVENKA M. AVDALOVIC*, MICHAEL LEVITT§, RICHARD P. JUNGHANS¶, AND THOMAS A. WALDMANN¶

*Protein Design Labs, 3181 Porter Drive, Palo Alto, CA 94304; §Department of Cell Biology, Stanford University, Stanford, CA 94305; and ¶Metabolism Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

Contributed by Thomas A. Waldmann, August 30, 1989

ABSTRACT **The anti-Tac monoclonal antibody is known to bind to the p55 chain of the human interleukin 2 receptor and to inhibit proliferation of T cells by blocking interleukin 2 binding. However, use of anti-Tac as an immunosuppressant drug would be impaired by the human immune response against this murine antibody. We have therefore constructed a "humanized" antibody by combining the complementarity-determining regions (CDRs) of the anti-Tac antibody with human framework and constant regions. The human framework regions were chosen to maximize homology with the anti-Tac antibody sequence. In addition, a computer model of murine anti-Tac was used to identify several amino acids which, while outside the CDRs, are likely to interact with the CDRs or antigen. These mouse amino acids were also retained in the humanized antibody. The humanized anti-Tac antibody has an affinity for p55 of 3×10^9 M^{-1}, about 1/3 that of murine anti-Tac.**

The cellular receptor for the lymphokine interleukin 2 (IL-2) plays an important role in regulation of the immune response (reviewed in ref. 1). The complete IL-2 receptor (IL-2R) consists of at least two IL-2-binding peptide chains: the p55 or Tac peptide (2, 3), and the recently discovered p75 peptide (4, 5). Identification and characterization of the p55 peptide were facilitated by the development of a monoclonal antibody, anti-Tac, which binds to human p55 (2). The p55 peptide was found to be expressed on the surface of T cells activated by an antigen or mitogen but not on resting T cells. Treatment of human T cells with anti-Tac antibody strongly inhibits their proliferative response to antigen or to IL-2 by preventing IL-2 binding (3, 6).

These results suggested that anti-IL-2R antibodies would be immunosuppressive when administered *in vivo*. Indeed, injection of an anti-IL-2R antibody into mice and rats greatly prolonged survival of heart allografts (7, 8). Anti-IL-2R was also effective in rats against experimental graft-versus-host disease (9). In animal models of autoimmune disease, an anti-IL-2R antibody alleviated insulitis in nonobese diabetic mice and lupus nephritis in NZB × NZW mice (10). Anti-Tac itself was highly effective in prolonging survival of kidney allografts in cynomolgus monkeys (11).

In human patients, the specificity of anti-Tac for activated T cells might give it an advantage as an immunosuppressive agent over OKT3 (monoclonal anti-CD3), which is effective in treating kidney transplant rejection (12), but which suppresses the entire peripheral T-cell population. In fact, in phase I clinical trials for kidney transplantation, prophylactic administration of anti-Tac significantly reduced the incidence of early rejection episodes, without associated toxicity (13). Furthermore, treatment with anti-Tac induced temporary partial or complete remission in three of nine patients with Tac-expressing adult T-cell leukemia (14). However, as a murine monoclonal antibody, anti-Tac elicits a strong human antibody response against itself, as does OKT3 (15). This response would prevent its long-term use in treating autoimmune conditions or suppressing organ transplant rejection.

The immune response against a murine monoclonal antibody may potentially be reduced by transforming it into a chimeric antibody. Such antibodies, produced by methods of genetic engineering, combine the variable (V) region binding domain of a mouse (or rat) antibody with human antibody constant (C) regions (16–18). Hence, a chimeric antibody retains the binding specificity of the original mouse antibody but contains less amino acid sequence foreign to the human immune system. Chimeric antibodies have been produced against a number of tumor-associated antigens (19–21). In some but not all cases, the chimeric antibodies have mediated human complement-dependent cytotoxicity (CDC) or antibody-dependent cellular cytotoxicity (ADCC) more efficiently than the mouse antibodies (21).

When the murine antibody OKT3 is used in human patients, much of the resulting antibody response is directed against the V region of OKT3 rather than the C region (15). Hence, chimeric antibodies in which the V region is still nonhuman may not have sufficient therapeutic advantages over mouse antibodies. To further reduce the immunogenicity of murine antibodies, Winter and colleagues constructed "humanized" antibodies in which only the minimum necessary parts of the mouse antibody, the complementarity-determining regions (CDRs), were combined with human V region frameworks and human C regions (22–25). We report here the construction of chimeric and humanized anti-Tac antibodies.‖ For the humanized antibody, sequence homology and molecular modeling were used to select a combination of mouse and human sequence elements that would reduce immunogenicity while retaining high binding affinity.

MATERIALS AND METHODS

Construction of Plasmids. cDNA cloning was by the method of Gubler and Hoffman (26), and sequencing was by the dideoxy method (27). The plasmid pVκ1 (Fig. 1*A*) was constructed from the following fragments: an approximately 4550-base-pair (bp) *Bam*HI–*Eco*RI fragment from the plas-

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Abbreviations: IL-2R, interleukin 2 receptor; CDR, complementarity-determining region; CDC, complement-dependent cytotoxicity; ADCC, antibody-dependent cellular cytotoxicity; V, variable; J, joining; C, constant.

†Present address: Biospan, 440 Chesapeake Drive, Redwood City, CA 94063.

‡Present address: Beckman Instruments, 1050 Page Mill Road, Palo Alto, CA 94304.

‖The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M28250 and M28251).

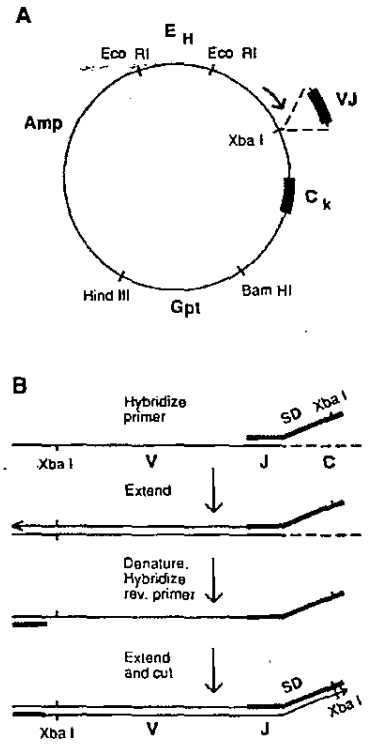


FIG. 1. (*A*) Schematic diagram of the plasmids pVκ1 and pLTac. Light chain exons are shown as boxes. An arrow indicates the direction of transcription from the κ promoter. E_H, heavy chain enhancer. Not drawn to scale. (*B*) Schematic diagram of the method used to excise the *V–J* region. SD, splice donor sequence; rev. primer, reverse primer.

mid pSV2gpt (28) containing the *amp* and *gpt* genes; an 1800-bp *Eco*RI–*Bgl* II fragment from pKcatH (29) containing the heavy chain enhancer and κ promoter; and a 1500-bp *Eco*RI–*Xba* I fragment containing the human C_κ region (30). Similarly, pVγ1 was constructed starting from a 4850-bp *Bam*HI–*Eco*RI fragment of the plasmid pSV2hph (a gift of A. Smith, A. Miyajima, and D. Strehlow, Stanford University), which is analogous to pSV2gpt except that the *gpt* gene is replaced by the *hyg* gene (31). This fragment was combined with the *Eco*RI–*Bgl* II fragment from pKcatH and a 2800-bp *Hin*dIII–*Pvu* II fragment containing the human γ1 constant region, isolated from a phage kindly provided by L. Hood (32). In each case, the fragments were combined by standard methods (ref. 33, pp. 390–401), with an *Xba* I linker inserted between the κ promoter fragment and the 5′ end of the C region fragment.

Construction of Chimeric Genes. *Eco*RI fragments containing the anti-Tac light and heavy chain cDNAs were separately inserted into the *Eco*RI site of the phage M13mp11D, a variant of M13mp11 (34) in which the *Eco*RI and *Xba* I sites of the polylinker were filled in and joined. The resulting phage, in which the 5′ ends of the cDNAs abutted the *Xba* I site, were respectively denoted M13L and M13H. The V–J (J, joining) segments of the cDNAs, followed by splice donor signals, were precisely excised from these phage, using a double-priming scheme (Fig. 1*B*). For the light chain, the following primer was synthesized (Applied Biosystems model 380B DNA synthesizer) and purified by gel electrophoresis: 5′-CCAGAATTCTAGAAAAGTGTACTTAC-GTTTCAGCTCCAGCTTGGTCCC-3′. From the 3′ end, the first 22 residues of the primer are the same as the last 22 residues of the $J_\kappa 5$ segment (noncoding strand). The next 16 nucleotides are the same as the sequence that follows $J_\kappa 5$ in mouse genomic DNA and therefore includes a splice donor signal. The final 10 nucleotides of the oligonucleotide include an *Xba* I site.

We hybridized this oligonucleotide to M13L and extended it with the Klenow fragment of DNA polymerase. The DNA was heat-denatured, hybridized with an excess of the "reverse primer" 5′-AACAGCTATGACCATG-3′, again extended with Klenow DNA polymerase, and digested with *Xba* I. The digested DNA was run on a gel, and an approximately 400-bp fragment was excised and inserted into the *Xba* I site of pVκ1. Sequencing showed that the fragment consisted of the V–J region of the light chain cDNA followed by the splice donor "tail," as expected (Fig. 1*B*), and pLTac, a clone with the appropriate orientation, was chosen. In an analogous fashion, the heavy chain V–J segment, followed by the mouse $J_H 2$ splice donor sequence, was excised from M13H and inserted into the *Xba* I site of pVγ1 to yield pGTac.

Computer Analysis. Sequences were manipulated and homology searches were performed with the MicroGenie Sequence Analysis Software (Beckman). The molecular model of the anti-Tac V region was constructed with the ENCAD program (35) and examined with the MIDAS program (36) on an IRIS 4D-120 graphics workstation (Silicon Graphics).

Construction of Genes for Humanized Antibody. Nucleotide sequences were selected that encoded the protein sequences of the humanized light and heavy chain V regions including signal peptides (*Results*), generally utilizing codons found in the mouse anti-Tac sequence. These nucleotide sequences also included the same splice donor signals used in the chimeric genes and an *Xba* I site at each end. For the heavy chain V region, four overlapping 120- to 130-nucleotide-long oligonucleotides were synthesized that encompassed the entire sequence on alternating strands. The oligonucleotides were phosphorylated with polynucleotide kinase, annealed, extended with T4 DNA polymerase, cut with *Xba* I, and ligated into the *Xba* I site of pUC19 (34), using standard reaction conditions. An insert with the correct sequence was recloned in pVγ1. The humanized light chain V region was constructed similarly.

Transfections. For each antibody constructed, the light chain plasmid was first transfected into Sp2/0 mouse myeloma cells (ATTC CRL 1581) by electroporation (Bio-Rad Gene Pulser) and cells were selected for *gpt* expression (28). Clones secreting a maximal amount of light chain, as determined by ELISA, were transfected with the heavy chain plasmid and cells were selected for hygromycin B resistance (31). Clones secreting a maximal amount of complete antibody were detected by ELISA. The clones were used for preparation of chimeric and humanized antibodies.

Antibody Purification. Medium from confluent cells was passed over a column of staphylococcal protein A-Sepharose CL-4B (Pharmacia), and antibody was eluted with 3 M $MgCl_2$. Antibody was further purified by ion-exchange chromatography on BakerBond ABx (J. T. Baker). Final antibody concentration was determined, assuming that 1 mg/ml has an A_{280} of 1.4. Anti-Tac antibody itself was purified as described (2).

Affinity Measurements. Affinities were determined by competition binding. HuT-102 human T-lymphoma cells (ATTC TIB 162) were used as source of p55 Tac antigen. Increasing amounts of competitor antibody (anti-Tac, chimeric, or humanized) were added to 1.5 ng of radioiodinated (Pierce Iodo-Beads) tracer anti-Tac antibody (2 μCi/μg; 1 Ci = 37 GBq) and incubated with 4×10^5 HuT cells in 0.2 ml of binding buffer (RPMI 1040 medium with 10% fetal calf serum, human IgG at 100 μg/ml, 0.1% sodium azide) for 3 hr at room temperature. Cells were washed and pelleted, and their radioactivities were measured, and the concentrations of bound and free tracer antibody were calculated. The affinity of mouse anti-Tac was determined by Scatchard plot analy-

sis, using anti-Tac itself as the competitor. Then the affinities of the chimeric and humanized antibodies were each calculated according to the formula $[X] - [\text{anti-Tac}] = (1/K_x) - (1/K_a)$, where K_a is the affinity of anti-Tac ($9 \times 10^9\ M^{-1}$), K_x is the affinity of the competitor X, [ ] indicates the concentration of competitor antibody at which bound/free tracer binding is $R_0/2$, and R_0 is maximal bound/free tracer binding (37).

RESULTS

Cloning of Light and Heavy Chain cDNA. A cDNA library in λgt10 was prepared from anti-Tac hybridoma cells and screened with oligonucleotide probes for the mouse κ and γ2a constant regions. The cDNA inserts from four κ-positive and four γ2a-positive phage were subcloned in M13mp19. Partial sequencing showed that two of the κ isolates had one sequence, and the other two had another sequence. In one pair, a V_κ gene segment was joined to the $J_\kappa 2$ segment out of its reading frame. In addition, the conserved cysteine at position 23 was absent from this V segment, and the sequences of the two isolates differed slightly. Presumably, these clones were the result of an aberrant joining event in one κ allele, which continued to undergo somatic mutation after the formation of the hybridoma.

The V–J segments of the other pair of κ clones were sequenced completely and were identical. This light chain uses the $J_\kappa 5$ segment. Partial sequencing of the four γ2a clones showed they were all from the same gene. The V–J segments of two were sequenced completely and were identical. This heavy chain uses the $J_H 2$ segment and is of subgroup II (38). The DNA sequences have been deposited with GenBank;[‖] the deduced protein sequences are shown in Fig. 2. As both alleles of the κ light chain were accounted for and only one heavy chain sequence was detected, we tentatively assigned these sequences to the anti-Tac antibody genes.

Construction of Chimeric Genes. Plasmid vectors were prepared for the construction and expression of chimeric light and heavy chain genes. The plasmid pVκ1 (Fig. 1*A*) contains the human genomic C_κ segment, including 336 bp of the preceding intron and the poly(A) signal. It also contains the promoter sequence from the MOPC 41 κ gene and the heavy chain enhancer sequence, which synergize to form a very strong transcriptional unit (29). There is a unique *Xba* I site between the promoter and the intron. A similar plasmid, pVγ1, was prepared by using the human $C_\gamma 1$ region in place of the C_κ region. In that case, the region inserted between the *Xba* I and *Bam*HI sites extended from about 210 bp 5′ of the $C_H 1$ exon to beyond the $C_H 3$ exon.

Our strategy was to insert the V–J region from the anti-Tac κ cDNA, followed by a splice donor signal, at the *Xba* I site of pVκ1 to construct the plasmid pLTac. Doing so created a chimeric κ gene with a short synthetic intron between the mouse V–J and human C_κ segments (Fig. 1*A*). For this purpose, we used a form of double primer-directed mutagenesis (*Materials and Methods*; Fig. 1*B*). Similarly, the V–J region from the anti-Tac γ2a heavy chain cDNA, followed by a splice donor signal, was inserted into the *Xba* I site of pVγ1. The resulting plasmid, pGTac, contained a chimeric heavy chain gene, with a synthetic intron between the mouse V–J and human $C_\gamma 1$ segments.

Construction of a Humanized Anti-Tac Antibody. In selecting a human antibody to provide the variable region framework for the humanized anti-Tac antibody, we reasoned that the more homologous the human antibody was to the original anti-Tac antibody, the less likely would combining the anti-Tac CDRs with the human framework be to introduce distortions into the CDRs. The anti-Tac heavy chain sequence was therefore compared by computer with all the human heavy chain sequences in the National Biomedical Research Foundation Protein Identification Resource (release 15). The heavy chain V region of the Eu antibody (of human heavy chain subgroup I; ref. 38) was 57% identical to the anti-Tac heavy chain V region (Fig. 2*B*); all other complete V_H regions in the data bank were 30–52% identical. However, no one human light chain V region was especially homologous to the anti-Tac light chain. We therefore chose to use the Eu light chain (of human light chain subgroup I; ref. 38) together with the Eu heavy chain to supply the framework sequences for the humanized antibody. The CDRs in the humanized antibody were of course chosen to be identical to the anti-Tac CDRs (Fig. 2).

A computer program was used to construct a plausible molecular model of the anti-Tac V domain (Fig. 3), based on homology to other antibody V domains with known crystal structure and on energy minimization. Graphic manipulation shows that a number of amino acid residues outside of the CDRs are in fact close enough to them to either influence their conformation or interact directly with antigen. When these residues differ between the anti-Tac and Eu antibodies, the residue in the humanized antibody was chosen to be the anti-Tac residue rather than the Eu residue. This choice was made for residues 27, 30, 48, 67, 68, 98, and 106 in the humanized heavy chain, and for 47 and 59 in the humanized light chain (Figs. 2 and 3; amino acids shown in blue in Fig. 3), although we now consider the light chain residue 59, which was chosen on the basis of an earlier model, to be doubtful. In this way, we hoped to better preserve the precise structure of the CDRs at the cost of possibly making the humanized antibody slightly less "human."

Different human light or heavy chain V regions exhibit strong amino acid homology outside of the CDRs, within the framework regions. However, a given V region will usually

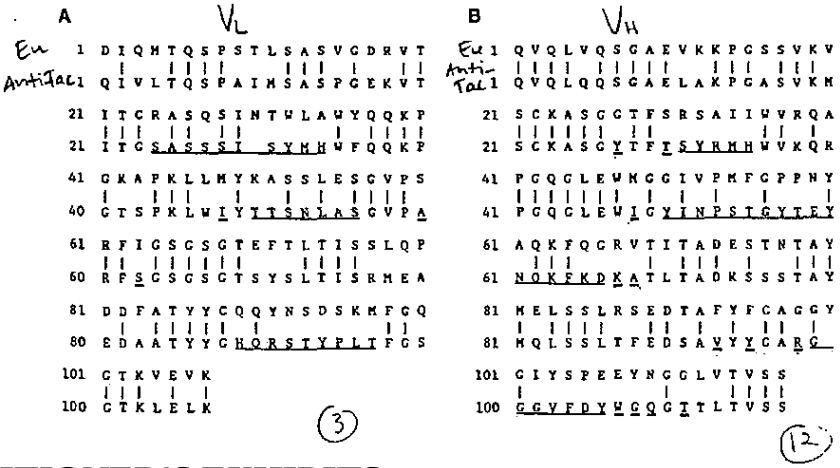


FIG. 2. Amino acid sequences of the humanized anti-Tac light (*A*) and heavy (*B*) chains. The sequences of the Eu antibody light and heavy chains (upper lines) are shown aligned above the mouse anti-Tac light and heavy chain sequences (lower lines), with a | indicating identity of amino acids. The three CDRs in each chain are underlined, and the other mouse amino acids used in the humanized antibody are double underlined. Hence, the humanized sequences are the same as the upper (Eu) sequences, except where the amino acid is underlined or double underlined.

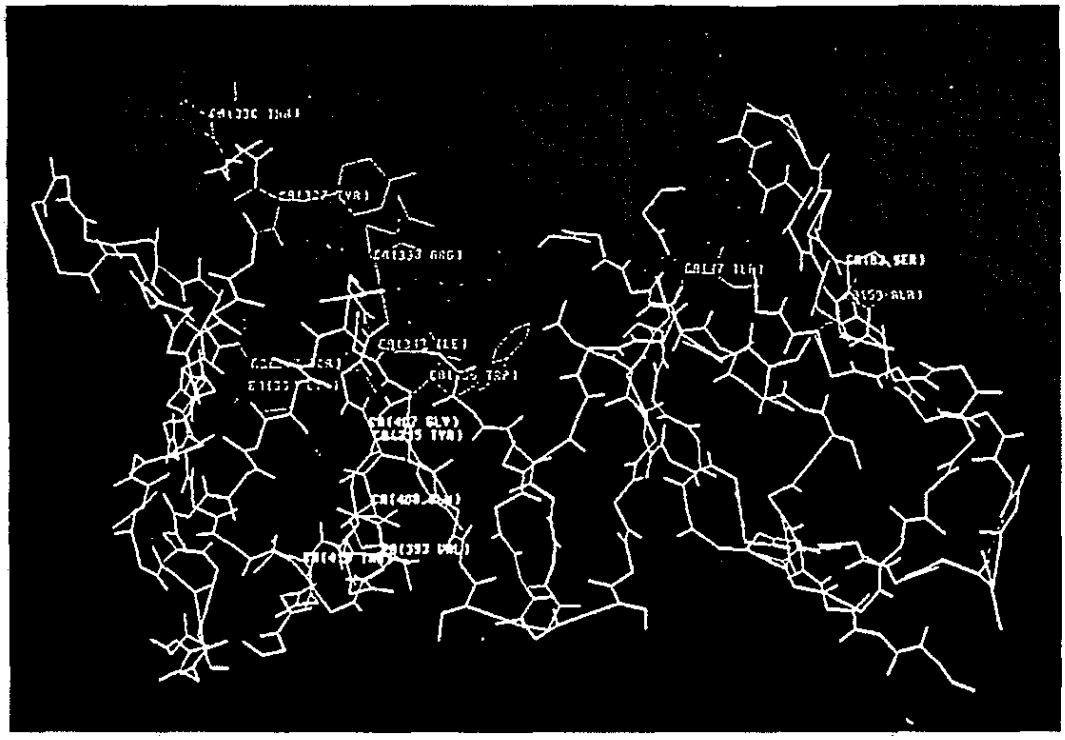


FIG. 3. Model of the mouse anti-Tac antibody V region, generated with the ENCAD program and displayed with the MIDAS program. Amino acids in the CDRs are shown in red; amino acids potentially interacting with the CDRs are shown in blue; other mouse amino acids used in the humanized antibody are shown in yellow, as described in the text. Thus, all amino acids transferred from the anti-Tac sequence to the humanized antibody are shown in red, blue, or yellow. Residue 1 is the first amino acid of V_κ; residue 301 is the first amino acid of V_H.

contain exceptional amino acids, atypical of other human V regions, at several framework positions. The Eu antibody contains such unusual residues at positions corresponding to 93, 95, 98, 106, 107, 108, and 110 of the humanized heavy chain and 47 and 62 of the light chain (Fig. 2), as determined by visual comparison of the Eu heavy and light chain V regions with other human V regions of subgroup I (38). The Eu antibody contains several other unusual residues, but at the listed positions, the murine anti-Tac antibody actually has a residue much more typical of human sequences than does Eu. At these positions, we therefore chose to use the anti-Tac residue rather than the Eu residue in the humanized antibody, to make the antibody more generically human. Some of these residues had already been selected because of their proximity to the CDRs, as described above (the remaining ones are shown in yellow in Fig. 3).

These criteria allowed the selection of all amino acids in the humanized antibody V regions as coming from either anti-Tac or Eu (Fig. 2). DNA segments encoding the desired heavy and light chain amino acid sequences were synthesized. These DNA segments also encoded typical immunoglobulin signal sequences for processing and secretion, and they contained splice donor signals at their 3′ end. The light and heavy chain segments were cloned, respectively, in pVκ1 and pVγ1 to form the plasmids pHuLTac and pHuGTac.

Properties of Chimeric and Humanized Antibodies. Sp2/0 cells, a nonproducing mouse myeloma line, were transfected sequentially with pLTac and pGTac (chimeric genes) or with pHuLTac and pHuGTac (humanized genes). Cell clones were selected first for antibiotic resistance and then for maximal antibody secretion, which reached 3 μg/10^6 cells per 24 hr. S1 nuclease mapping of RNA extracted from the cells transfected with pLTac and pGTac showed that the synthetic introns between the V and C regions (Fig. 1*A*) were correctly spliced (data not shown). Antibody was purified from the culture medium of cells producing the chimeric or humanized antibody. When analyzed by reducing SDS/polyacrylamide gel electrophoresis, the antibodies showed only two bands, having the expected molecular weights 50,000 and 25,000.

Flow cytometry showed that the chimeric and humanized antibodies bound to Hut-102 and CRII.2 cells, two human T-cell lines that express the p55 chain of the IL-2R, but not to CEM and other cell lines that do not express the IL-2R. To determine the binding affinity of the chimeric and humanized antibodies, their ability to compete with labeled mouse anti-Tac for binding to Hut-102 cells was determined. The affinity of chimeric anti-Tac was indistinguishable from that of anti-Tac (data not shown), as expected from the fact that their entire V regions are identical. The affinity of humanized anti-Tac for membrane-bound p55 was 3×10^9 M^{-1}, about 1/3 the measured affinity of 9×10^9 M^{-1} of anti-Tac itself (Fig. 4).

DISCUSSION

Because monoclonal antibodies can be produced that are highly specific for a wide variety of cellular targets, antibody therapy holds great promise for the treatment of cancer, autoimmune conditions, and other diseases. However, this promise has not been widely realized, largely because most monoclonal antibodies, which are of mouse origin, are immunogenic when used in human patients and are ineffective at recruiting human immune effector functions such as CDC and ADCC. A partial solution to this problem is the use of chimeric antibodies (16), which combine the V region binding domains of mouse antibodies with human antibody C regions. Initially, chimeric antibodies were constructed by combining genomic clones of the V and C region genes. However, this method is very time consuming because of the difficulty of genomic cloning, especially from tetraploid hybridomas.

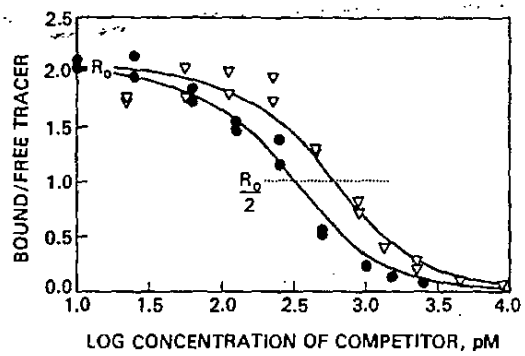


FIG. 4. Competitive binding of labeled anti-Tac tracer to Hut-102 cells. Duplicate samples are shown. ●, Mouse anti-Tac competitor; ▽, humanized anti-Tac competitor.

More recently, cDNA clones of the V and C regions have been combined, but this method is also tedious because of the need to join the V and C regions precisely (20, 21). Here we show that the V region from a readily obtainable cDNA clone can be easily joined to a human genomic C region, which need only be cloned once, by leaving a synthetic intron between the V and C regions. When linked to suitable transcriptional regulatory elements and transfected into an appropriate host cell, such chimeric genes produce antibody at a high level.

Chimeric antibodies represent an improvement over mouse antibodies for use in human patients, because they are presumably less immunogenic and sometimes mediate CDC or ADCC more effectively (21). For example, chimeric anti-Tac mediates ADCC with activated human effector cells, whereas murine anti-Tac does not (unpublished data). However, the mouse V region can itself be highly immunogenic (15). Winter and colleagues therefore took the further, innovative, step of combining the CDRs from a mouse (or rat) antibody with the framework region from a human antibody (22–25), thus reducing the xenogeneic elements in the humanized antibody to a minimum. Unfortunately, in some cases the humanized antibody had significantly less binding affinity for antigen than did the original mouse antibody. This is not surprising, because transferring the mouse CDRs from the mouse framework to the human framework could easily deform them.

In humanizing the anti-Tac antibody, which binds to the p55 chain of the human IL-2R, we have introduced two ideas that may have wider applicability. First, the human framework was chosen to be as homologous as possible to the original mouse antibody to reduce any deformation of the mouse CDRs. Second, computer modeling was used to identify several framework amino acids in the mouse antibody that might interact with the CDRs or directly with antigen, and these amino acids were transferred to the human framework along with the CDRs. The resulting humanized antibody has a high affinity, $3 \times 10^9\ M^{-1}$, for its antigen. Further work is needed to determine to what extent the choice of human framework and the preservation of particular mouse amino acids in fact contributed to the affinity of the humanized antibody. The extent to which humanization eliminates immunogenicity will need to be addressed in clinical trials, where humanized anti-Tac will be administered to patients with Tac-expressing lymphomas or selected autoimmune diseases or to patients receiving organ transplants.

1. Waldmann, T. A. (1989) *Annu. Rev. Biochem.* **58**, 875–911.
2. Uchiyama, T., Broder, S. & Waldmann, T. A. (1981) *J. Immunol.* **126**, 1393–1397.
3. Leonard, W. J., Depper, J. M., Uchiyama, T., Smith, K. A., Waldmann, T. A. & Greene, W. C. (1982) *Nature (London)* **300**, 267–269.
4. Tsudo, M., Kozak, R. W., Goldman, C. K. & Waldmann, T. A. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 9694–9698.
5. Sharon, M., Klausner, R. D., Cullen, B. R., Chizzonite, R. & Leonard, W. J. (1986) *Science* **234**, 859–863.
6. Depper, J. M., Leonard, W. J., Robb, R. J., Waldmann, T. A. & Greene, W. C. (1983) *J. Immunol.* **131**, 690–696.
7. Kirkman, R. L., Barrett, L. V., Gaulton, G. N., Kelley, V. E., Ythier, A. & Strom, T. B. (1985) *J. Exp. Med.* **162**, 358–362.
8. Kupiec-Weglinski, J. W., Diamantstein, T., Tilney, N. L. & Strom, T. B. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 2624–2627.
9. Volk, H.-D., Brocke, S., Osawa, H. & Diamantstein, T. (1986) *Clin. Exp. Immunol.* **66**, 126–131.
10. Kelley, V. E., Gaulton, G. N., Hattori, M., Ikegami, H., Eisenbarth, G. & Strom, T. B. (1988) *J. Immunol.* **140**, 59–61.
11. Reed, M. H., Shapiro, M. E., Strom, T. B., Milford, E. L., Carpenter, C. B., Weinberg, D. S., Reimann, K. A., Letvin, N. L., Waldmann, T. A. & Kirkman, R. L. (1989) *Transplantation* **47**, 55–59.
12. Ortho Multicenter Transplant Study Group (1985) *N. Engl. J. Med.* **313**, 337–342.
13. Kirkman, R. L., Shapiro, M. E., Carpenter, C. B., Milford, E. L., Ramos, E. L., Tilney, N. L., Waldmann, T. A., Zimmerman, C. E. & Strom, T. B. (1989) *Transplant. Proc.* **21**, 1766–1768.
14. Waldmann, T. A., Goldman, C. K., Bongiovanni, K. F., Sharrow, S. O., Davey, M. P., Cease, K. B., Greenberg, S. J. & Longo, D. L. (1988) *Blood* **72**, 1805–1816.
15. Jaffers, G. J., Fuller, T. C., Cosimi, A. B., Russell, P. S., Winn, H. J. & Colvin, R. B. (1986) *Transplantation* **41**, 572–578.
16. Morrison, S. L., Johnson, M. J., Herzenberg, L. A. & Oi, V. T. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 6851–6855.
17. Boulianne, G. L., Hozumi, N. & Shulman, M. J. (1984) *Nature (London)* **312**, 643–646.
18. Neuberger, M. S., Williams, G. T., Mitchell, E. B., Jouhal, S. S., Flanagan, J. G. & Rabbitts, T. H. (1985) *Nature (London)* **314**, 268–270.
19. Sun, L. K., Curtis, P., Rakowicz-Szulczynska, E., Ghrayeb, J., Chang, N., Morrison, S. L. & Koprowski, H. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 214–218.
20. Whittle, N., Adair, J., Lloyd, C., Jenkins, L., Devine, J., Schlom, J., Raubitschek, A., Colcher, D. & Bodmer, M. (1987) *Protein Eng.* **1**, 499–505.
21. Liu, A. Y., Robinson, R. R., Hellstrom, K. E., Murray, E. D., Jr., Chang, C. P. & Hellstrom, I. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 3439–3443.
22. Jones, P. T., Dear, P. H., Foote, J., Neuberger, M. S. & Winter, G. (1986) *Nature (London)* **321**, 522–525.
23. Verhoeyen, M., Milstein, C. & Winter, G. (1988) *Science* **239**, 1534–1536.
24. Reichmann, L., Clark, M., Waldmann, H. & Winter, G. (1988) *Nature (London)* **332**, 323–327.
25. Hale, G., Dyer, M. J. S., Clark, M. R., Phillips, J. M., Marcus, R., Riechmann, L., Winter, G. & Waldmann, H. (1988) *Lancet* **i**, 1394–1399.
26. Gubler, U. & Hoffman, B. J. (1983) *Gene* **25**, 263–269.
27. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
28. Mulligan, R. C. & Berg, P. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 2072–2076.
29. Garcia, J. V., Bich-Thuy, L. T., Stafford, J. & Queen, C. (1986) *Nature (London)* **322**, 383–385.
30. Hieter, P. A., Max, E. E., Seidman, J. G., Maizel, J. V., Jr., & Leder, P. (1980) *Cell* **22**, 197–207.
31. Sugden, B., Marsh, K. & Yates, J. (1985) *Mol. Cell. Biol.* **5**, 410–413.
32. Ellison, J. W., Berson, B. J. & Hood, L. E. (1982) *Nucleic Acids Res.* **10**, 4071–4079.
33. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
34. Yanisch-Perron, C., Vieira, J. & Messing, J. (1985) *Gene* **33**, 103–119.
35. Levitt, M. (1983) *J. Mol. Biol.* **168**, 595–617.
36. Ferrin, T. E., Huang, C. C., Jarvis, L. E. & Langridge, R. (1988) *J. Mol. Graphics* **6**, 13–27.
37. Berzofsky, J. A. & Berkower, I. J. (1984) in *Fundamental Immunology*, ed. Paul W. E. (Raven, New York), pp. 595–644.
38. Kabat, E. A., Wu, T. T., Reid-Miller, M., Perry, H. M. & Gottesman, K. S. (1987) *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD).

US005859205A

United States Patent [19]

Adair et al.

[11] **Patent Number: 5,859,205**

[45] **Date of Patent: Jan. 12, 1999**

[54] **HUMANISED ANTIBODIES**

[75] Inventors: **John Robert Adair**, High Wycombe; **Diljeet Singh Athwal**, London; **John Spencer Emtage**, Marlow, all of United Kingdom

[73] Assignee: **Celltech Limited**, Berkshire, United Kingdom

[21] Appl. No.: **303,569**

[22] Filed: **Sep. 7, 1994**

Related U.S. Application Data

[63] Continuation of Ser. No. 743,329, Sep. 17, 1991, abandoned.

[30] **Foreign Application Priority Data**

Dec. 21, 1989 [GB] United Kingdom 8928874

[51] **Int. Cl.**[6] **A61K 39/395**

[52] **U.S. Cl.** **530/387.3**; 530/387.1

[58] **Field of Search** 530/387.1, 387.3, 530/388.22, 867, 864

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,348,376	9/1982	Goldenberg .

FOREIGN PATENT DOCUMENTS

0239400 A2	3/1987	Enropean Pat. Off. .
A1 0323806	7/1989	European Pat. Off. .
0 328 404 A1	8/1989	European Pat. Off. .
0 365 209 A2	4/1990	European Pat. Off. .
0 403 156 A1	12/1990	European Pat. Off. .
WO 89/07452	8/1989	WIPO .
WO 90/07861	7/1990	WIPO .
WO 92/04381	3/1992	WIPO .
WO 92/11018	7/1992	WIPO .
WO 92/15683	9/1992	WIPO .
WO 92/16553	10/1992	WIPO .

OTHER PUBLICATIONS

Chothia, Cyrus et al (Dec. 1989) *Nature*, "Conformations of Immunoglobulin Hypervariable Regions", vol. 342, pp. 877–883.

Queen, C. et al (Dec. 1989) Proceedings of the National Academy of Sciences, "A Humanized Antibody That Binds to Interleukin 2 Receptor" vol. 86, pp. 10029–10033.

Riechmann et al (Mar. 1988) Nature, "Reshaping Human Antibodies for Therapy," vol. 332, pp. 323–327.

Roberts et al, "Generation of Antibody with Enhanced Affinity and Specificity for its Antigen by Protein Engineering" Nature, 328(20):731–734, Aug., 1987.

Verhoeyen et al, "Reshaping Human Antibodies: Grafting an Antilysozyme Activity", Science, 239:1534–36 Mar. 25, 1988.

Jones et al., "Replacing the complementarity–Determining Regions in a Human Antibody with those from a Mouse", Nature, 321:522–525, 1986.

Ward et al., "Binding activities of a Repertoire of Single Immunoglobulin Variable Domains Secreted from *Escherichia Coli*", Nature, 341:544–546, 1989.

Primary Examiner—Donald E. Adams

Attorney, Agent, or Firm—Woodcock Washburn Kurtz Mackiewicz & Norris

[57] **ABSTRACT**

CDR-grafted antibody heavy and light chains comprise acceptor framework and donor antigen binding regions, the heavy chains comprising donor residues at at least one of positions (**6**, **23**) and/or (**24**, **48**) and/or (**49**, **71**) and/or (**73**, **75**) and/or (**76**) and/or (**78**) and (**88**) and/or (**91**). The CDR-grafted light chains comprise donor residues at at least one of positions (**1**) and/or (**3**) and (**46**) and/or (**47**) or at at least one of positions (**46**, **48**, **58**) and (**71**). The CDR-grafted antibodies are preferably humanised antibodies, having non human, e.g. rodent, donor and human acceptor frameworks, and may be used for in vivo therapy and diagnosis. A generally applicable protocol is disclosed for obtaining CDR-grafted antibodies.

8 Claims, 18 Drawing Sheets

Carter Exhibit 2024
Carter v. Adair
Interference No. 105,744

```
  1  GAATTCCCAA AGACAAAatg gattttcaag tgcagatttt cagcttcctg
 51  ctaatcagtg cctcagtcat aatatccaga ggacaaattg ttctcaccca
101  gtctccagca atcatgtctg catctccagg ggagaaggtc accatgacct
151  gcagtgccag ctcaagtgta agttacatga actggtacca gcagaagtca
201  ggcacctccc ccaaaagatg gatttatgac acatccaaac tggcttctgg
251  agtccctgct cacttcaggg gcagtgggtc tgggacctct tactctctca
301  caatcagcgg catggaggct gaagatgctg ccacttatta ctgccagcag
351  tggagtagta  accattcac gttcggctcg gggacaaagt tggaaataaa
401  ccgggctgat actgcaccaa ctgtatccat cttcccacca tccagtgagc
451  agttaacatc tggaggtgcc tcagtcgtgt gcttcttgaa caacttctac
501  cccaaagaca tcaatgtcaa gtggaagatt gatggcagtg aacgacaaaa
551  tggcgtcctg aacagttgga ctgatcagga cagcaaagac agcacctaca
601  gcatgagcag caccctcacg ttgaccaagg acgagtatga acgacataac
651  agctatacct gtgaggccac tcacaagaca tcaacttcac ccattgtcaa
701  gagcttcaac aggaatgagt gtTAGAGACA AAGGTCCTGA GACGCCACCA
751  CCAGCTCCCA GCTCCATCCT ATCTTCCCTT CTAAGGTCTT GGAGGCTTCC
801  CCACAAGCGC tTACCACTGT TGCGGTGCTC TAAACCTCCT CCCACCTCCT
851  TCTCCTCCTC CTCCCTTTCC TTGGCTTTTA TCATGCTAAT ATTTGCAGAA
901  AATATTCAAT AAAGTGAGTC TTTGCCTTGA AAAAAAAAAA AAA
(SEQ ID NO:4)
```

FIG. 1a

```
  1  MDFQVQIFSF LLISASVIIS RG□QIVLTQSP AIMSASPGEK VTMTCSASSS
 51  VSYMNWYQQK SGTSPKRWIY DTSKLASGVP AHFRGSGSGT  SYSLTISGME
101  AEDAATYYCQ QWSSNPFTFG SGTKLEINRA DTAPTVSIFP  PSSEQLTSGG
151  ASVVCFLNNF YPKDINVKWK IDGSERQNGV LNSWTDQDSK  DSTYSMSSTL
201  TLTKDEYERH NSYTCEATHK TSTSPIVKSF NRNEC* (SEQ ID NO:5)
```

FIG. 1b

```
   1  GAATTCCCCT CTCCACAGAC ACTGAAAACT CTGACTCAAC ATGGAAAGGC
  51  ACTGGATCTT TCTACTCCTG TTGTCAGTAA CTGCAGGTGT CCACTCCCAG
 101  GTCCAGCTGC AGCAGTCTGG GGCTGAACTG GCAAGACCTG GGGCCTCAGT
 151  GAAGATGTCC TGCAAGGCTT CTGGCTACAC CTTTACTAGG TACACGATGC
 201  ACTGGGTAAA ACAGAGGCCT GGACAGGGTC TGGAATGGAT TGGATACATT
 251  ATTCCTAGCC GTGGTTATAC TAATTACAAT CAGAAGTTCA AGGACAAGGC
 301  CACATTGACT ACAGACAAAT CCTCCAGCAC AGCCTACATG CAACTGAGCA
 351  GCCTGACATC TGAGGACTCT GCAGTCTATT ACTGTGCAAG ATATTATGAT
 401  GATCATTACT GCCTTGACTA CTGGGGCCAA GGCACCACTC TCACAGTCTC
 451  CTCAGCCAAA ACAACAGCCC CATCGGTCTA TCCACTGGCC CCTGTGTGTG
 501  GAGATACAAC TGGCTCCTCG GTGACTCTAG GATGCCTGGT CAAGGGTTAT
 551  TTCCCTGAGC CAGTGACCTT GACCTGGAAC TCTGGATCCC TGTCCAGTGG
 601  TGTGCACACC TTCCCAGCTG TCCTGCAGTC TGACCTCTAC ACCCTCAGCA
 651  GCTCAGTGAC TGTAACCTCG AGCACCTGGC CCAGCCAGTC CATCACCTGC
 701  AATGTGGCCC ACCCGGCAAG CAGCACCAAG GTGGACAAGA AAATTGAGCC
 801  ACCTCTTGGG TGGACCATCC GTCTTCATCT TCCCTCCAAA GATCAAGGAT
 851  GTACTCATGA TCTCCCTGAG CCCCATAGTC ACATGTGTGG TGGTGGATGT
 901  GAGCGAGGAT GACCCAGATG TCCAGATCAG CTGGTTTGTG AACAACGTGG
 951  AAGTACACAC AGCTCAGACA CAAACCCATA GAGAGGATTA CAACAGTACT
1001  CTCCGGGTGG TCAGTGCCCT CCCCATCCAG CACCAGGACT GGATGAGTGG
1051  CAAGGAGTTC AAATGCAAGG TCAACAACAA AGACCTCCCA GCGCCCATCG
1101  AGAGAACCAT CTCAAAACCC AAAGGGTCAG TAAGAGCTCC ACAGGTATAT
1151  GTCTTGCCTC CACCAGAAGA AGAGATGACT AAGAAACAGG TCACTCTGAC
1201  CTGCATGGTC ACAGACTTCA TGCCTGAAGA CATTTACGTG GAGTGGACCA
1251  ACAACGGGAA AACAGAGCTA AACTACAAGA ACACTGAACC AGTCCTGGAC
1301  TCTGATGGTT CTTACTTCAT GTACAGCAAG CTGAGAGTGG AAAAGAAGAA
1351  CTGGGTGGAA AGAAATAGCT ACTCCTGTTC AGTGGTCCAC GAGGGTCTGC
1401  ACAATCACCA CACGACTAAG AGCTTCTCCC GGACTCCGGG TAAATGAGCT
1451  CAGCACCCAC AAAACTCTCA GGTCCAAAGA GAGACCCACA CTCATCTCCA
1501  TGCTTCCCTT GTATAAATAA AGCACCCAGC AATGCCTGGG ACCATGTAAA
1551  AAAAAAAAAA AAAGGAATTC  (SEQ ID NO:6)
```

FIG. 2a

OKT 3 HEAVY CHAIN PROTEIN SEQUENCE DEDUCED FROM DNA SEQUENCE

```
  1  MERHWIFLLL LSVTAGVHSQ VQLQQSGAEL ARPGASVKMS CKASGYTFTR
 51  YTMHWVKQRP GQGLEWIGYI NPSRGYTNYN QKFKDKATLT TDKSSSTAYM
101  QLSSLTSEDS AVYYCARYYD DHYCLDYWGQ GTTLTVSSAK TTAPSVYPLA
151  PVCGDTTGSS VTLGCLVKGY FPEPVTLTWN SGSLSSGVHT FPAVLQSDLY
201  TLSSSVTVTS STWPSQSITC NVAHPASSTK VDKKIEPRGP TIKPCPPCKC
251  PAPNLLGGPS VFIFPPKIKD VLMISLSPIV TCVVVDVSED DPDVQISWFV
301  NNVEVHTAQT QTHREDYNST LRVVSALPIQ HQDWMSGKEF KCKVNNKDLP
351  APIERTISKP KGSVRAPQVY VLPPPEEEMT KKQVTLTCMV TDFMPEDIYV
401  EWTNNGKTEL NYKNTEPVLD SDGSYFMYSK LRVEKKNWVE RNSYSCSVVH
451  EGLHNHHTTK SFSRTPGK* (SEQ ID NO:7)
```

FIG. 2b

```
           1                       23                      42
           NN      N                 N         N    N
RES TYPE   SBspSPESssBSbSsSssPSPSPsPSsse*s*p*Pi^ISsSe
Okt3vl     QIVLTQSPAIMSASPGEKVTMTCSASS.SVSYMNWYQQKSGT
REI        DIQMTQSPSSLSASVGDRVTITCQASQDIIKYLNWYQQIPGK
           ? ?
             CDR1   (LOOP)           *******
             CDR1   (KABAT)        **********


                      56                                85
           N  NN
RES TYPE   *IsiPpIeesesssSBEsePsPSBSSEsPspsPsseesSPePb
Okt3vl     SPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAAT
REI        APKLLIYEASNLQAGVPSRFSGSGSGTDYTFTISSLQPEDIAT (SEQ
ID NO:8)
           ?  ??                          ?  ?
                 *******  CDR2 (LOOP/KABAT)

                        102   108
RES TYPE   PiPIPies**iPIIsPPSPSPSS
Okt3vl     YYCQQWSSNPFTFGSGTKLEINR (SEQ ID NO:29)
REIvl      YYCQQYQSLPYTFGQGTKLQITR (SEQ ID NO:9)
                        ?    ?
              ******        CDR3 (LOOP)
            *********       CRD3(KABAT)
```

FIG. 3

```
          NN N                  23 26    32 35  N39  43
RES TYPE  SESPs^SBssS^sSSsSpSpSPsPSEbSBssBePi^PIpiesss
□kt3h     QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMNHWVKQRPGQ
K□L       QVQLVESGGGVVQPGRSLRLSCSSSGFIFSSYAMYWVRQAPGK
               ?                  ??
                                      ******     CDR1 (L□□P)
                                          *****  CDR1 (KABAT)

                    52a   60     65    N N  N   82abc     89
RES TYPE  IIeIppp^sssssssss^ps^pSSsbSpseSsSseSp^pSpsSBssS^ePb
□kt3vh    GLEWIGYINPSRGYTNTNQKFKRKATLTTDKSSSTAYMQLSSLTSEDSAV
K□L       GLEWVAIIWDDGSDQHYADSVKGRFTISRDNSKNTLFLQMDSLPPEDTGV
              ??                    ? ?  ?  ?            ?
                 ***********         CDR2   (L□□P)
                 ******************  CDR2   (KABAT)


           92 N                  107    113
RES TYPE  PiPIEisssssiiisssbibi*EIPIP*spSBSS
□kt3vh    YYCARYYDDHY.......CLDYWGQGTTLTVSS   (SEQ ID N□:30)
K□L       YFCARDGGHGFCSSASCFGPDYWGQGTPVTVSS   (SEQ ID N□:10)
               ****************  CRD4 (KABAT/L□□P)
```

FIG. 4

□KT 3 HEAVY CHAIN CDR GRAFTS

1. gh341 and derivatives

```
          1                          26        35  39  43
□kt3vh    QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQ
gH341     QVQLVESGGGVVQ□GRSLRLSCSSSGYTFTRYTMHWVRQAPGK   JA178
gH341A    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA185

gH341E    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA198
gH341*    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA2□7
gH341*    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA209
gH341D    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA197
gH341*    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA199
gH341C    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA184

gH341*    QVQLVQSGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA203
gH341*    QVQLVESGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA205
gH341B    QVQLVESGGGVVQPGRSLRLSCSSSGYTFTRYTMHWVRQAPGK   JA183
gH341*    QVQLVQSGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA204
gH341*    QVQLVESGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA206
gH341*    QVQLVQSGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA208
K□L       QVQLVESGGGVVQPGRSLRLSCSSSGFIFSSYAMYWVRQAPGK
```

FIG. 5a

```
        44    50              65                  83
□kt3vh  GLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLT
gH341   GLEWVAYINPSRGYTNYNQKFKDRFTISRDNSKNTLFLQMDSLR  JA178
gH341A  GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR  JA185

gH341E  GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR  JA198
gH341*  GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKNTAFLQMDSLR  JA207
gH341*  GLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTAFLQMDSLR  JA209
gH341D  GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKNTLFLQMDSLR  JA197
gH341*  GLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTLFLQMDSLR  JA199
gH341C  GLEWVAYINPSRGYTNYNQKFKDRFTISRDNSKNTLFLQMDSLR  JA184

gH341*  GLEWIGYINPSRGYTNYN□KVKDRFTISTDKSKSTAFLQMDSLR  JA207
gH341*  GLEWIGYINPSRGYTNYN□KVKDRFTISTDKSKSTAFLQMDSLR  JA205
gH341B  GLEWIGYINPSRGYTNYN□KVKDRFTISTDKSKSTAFLQMDSLR  JA183
gH341*  GLEWIGYINPSRGYTNYN□KVKDRFTISTDKSKSTAFLQMDSLR  JA204
gH341*  GLEWIGYINPSRGYTNYN□KVKDRFTISTDKSKSTAFLQMDSLR  JA206
gH341*  GLEWIGYINPSRGYTNYN□KVKDRFTISTDKSKNTAFLQMDSLR  JA208
K□L     GLEWVAIIWDDGSDQHYADSVKGRFTISRDNSKNTLFLQMDSLR
```

FIG. 5b

	84 95 102 113		SEQ ID NO:
□kt3vh	SEDSAVYYCARYYDDHY.....CLDYWGQGTTLTVSS		
gH341	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA178	30
gH341A	PEDTAVYYCARYYDDHY.....CLDYWGQGTTLTVSS	JA185	12
gH341E	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA198	13
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA207	14
gH341D	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA197	15
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA209	16
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA199	17
gH341C	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA184	18
gH341*	PEDTAVYYCARYYDDHY.....CLDYWGQGTTLTVSS	JA203	19
gH341*	PEDTAVYYCARYYDDHY.....CLDYWGQGTTLTVSS	JA205	20
gH341B	PEDTAVYYCARYYDDHY.....CLDYWGQGTTLTVSS	JA183	21
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA204	22
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA206	23
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA208	24
KOL	PEDTGVYFCARDGGHGFCSSASCFGPDYWGQGTPVTVSS		10

FIG. 5c

OKT3 LIGHT CHAIN CDR GRAFTING

1. gL221 and derivatives

```
          1                          24        34      42
Okt3vl    QIVLTQSPAOMSASPGEKVTMTCSASS.SVSYMNWYQQKSGT
gL221     DIQMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
gL221A    QIVMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
gL221B    QIVMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
gL221C    DIQMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
REI       DIQMTQSPSSLSASVGDRVTITCQASQDIIKYLNWYQQTPGK

          43     50    56                            85
Okt3vl    SPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAAT
gL221     APKLLIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
gL221A    APKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
gL221B    APKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
gL221C    APKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
REI       APKLLIYEASNLQAGVPSRFSGSGSGTDYTFTISSLQPEDIAT (SEQ ID NO:8)

          86   91   96          108
Okt3vl    YYCQQWSSNPFTFGSGTKLEINR       (SEQ ID NO:29)
gL221     YYCQQWSSNPETFGQGTKLQITR       (SEQ ID NO:25)
gL221A    YYCQQWSSNPETFGQGTKLQITR       (SEQ ID NO:26)
gL221B    YYCQQWSSNPETFGQGTKLQITR       (SEQ ID NO:27)
gL221C    YYCQQWSSNPETFGQGTKLQITR       (SEQ ID NO:28)
REI       YYCQQYQSLPYTFGQGTKLQITR       (SEQ ID NO:9)
```

CDR'S ARE UNDERLINED

FRAMEWORK RESIDUES INCLUDED IN THE GENE ARE DOUBLE UNDERLINED

FIG. 6

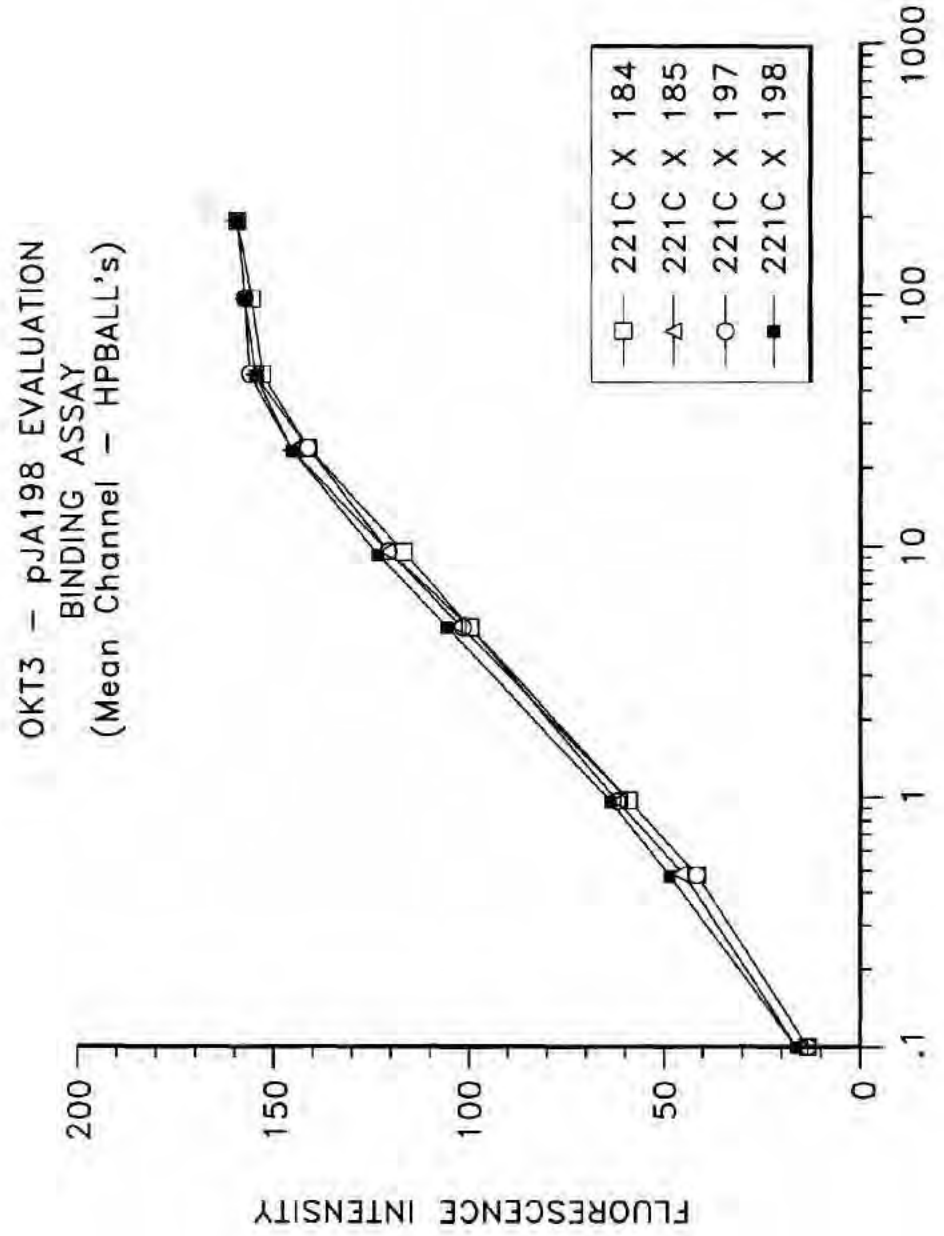


FIG. 7

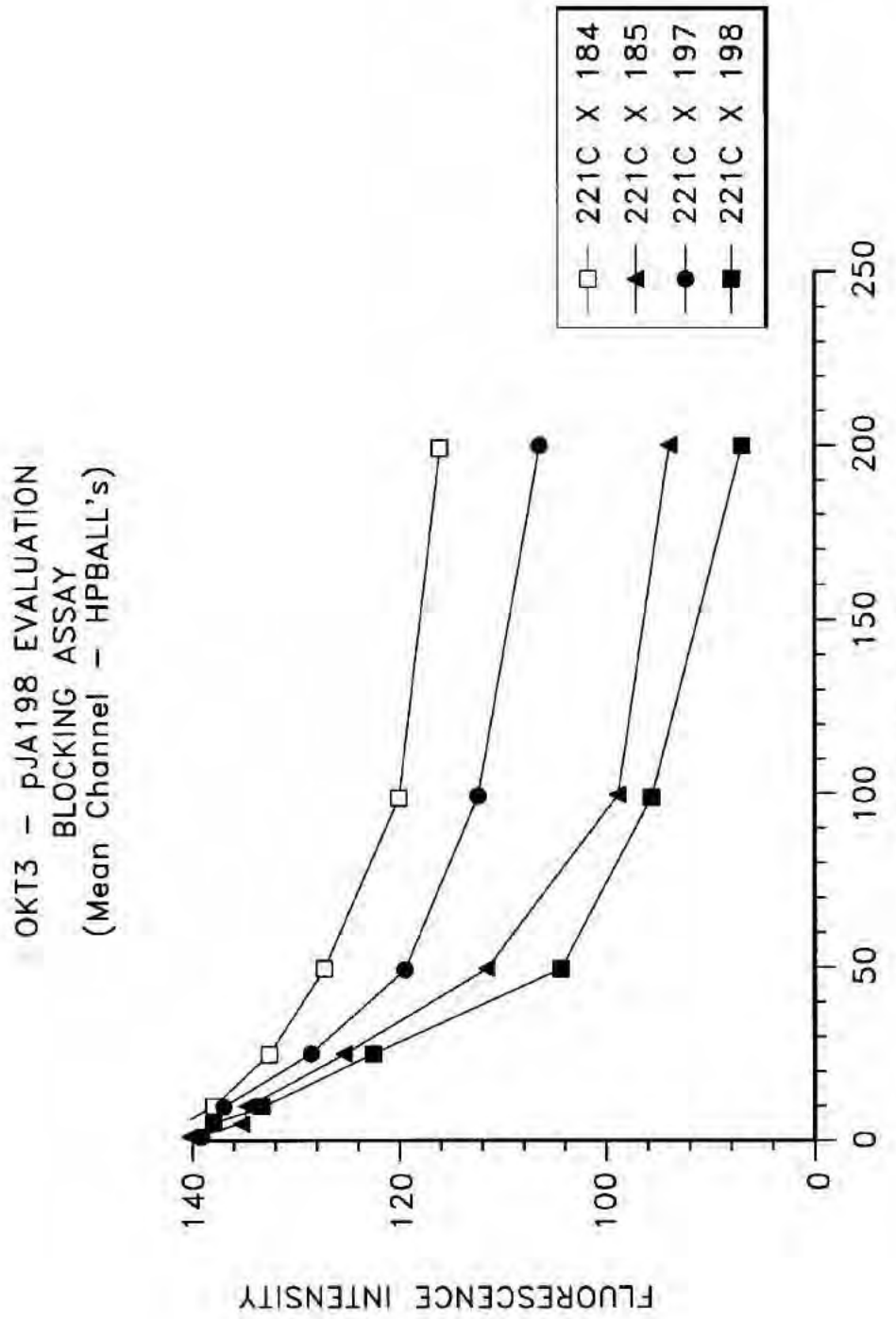


FIG. 8

Board Assigned Page #995

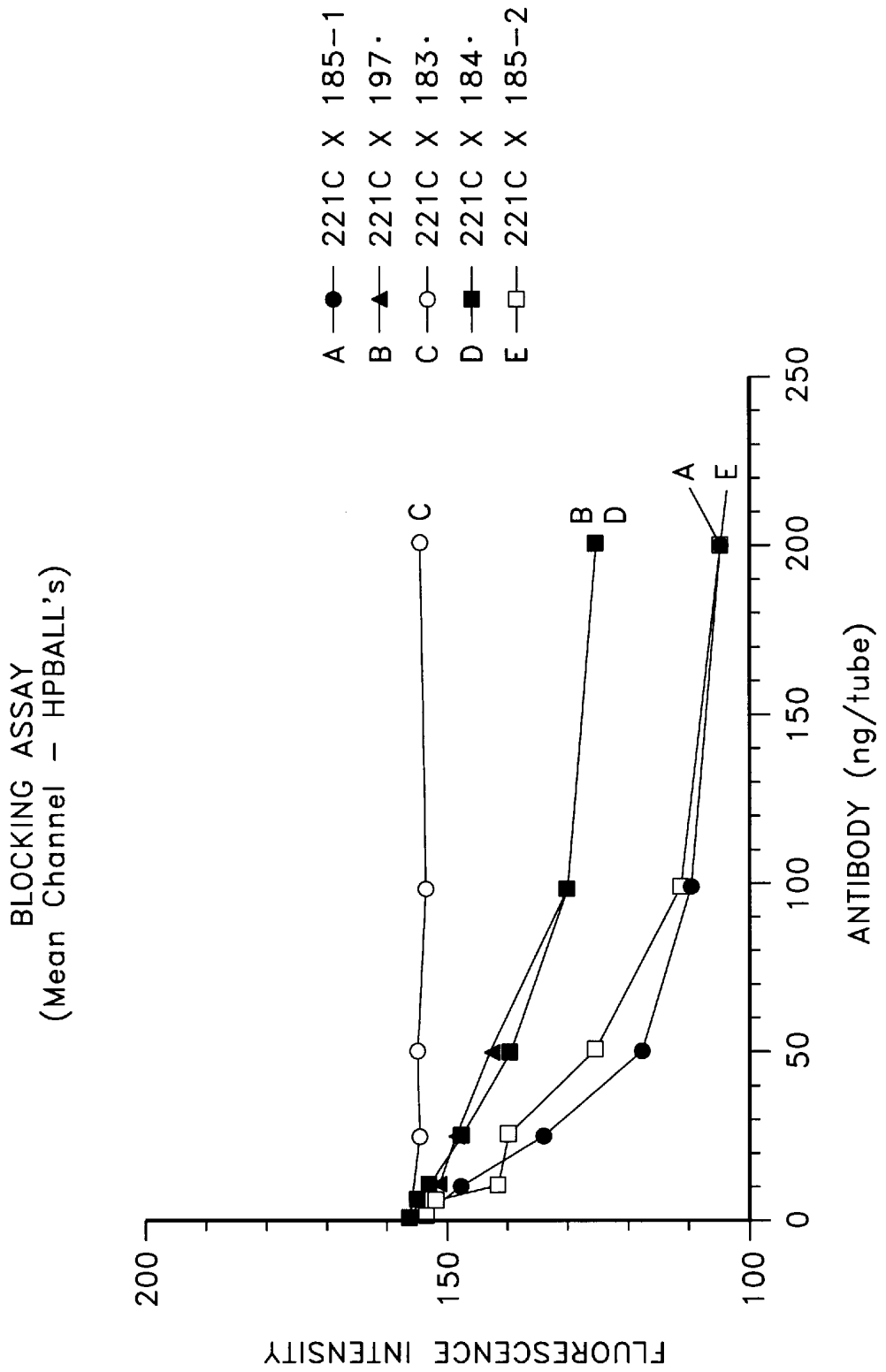

BLOCKING ASSAY
(Mean Channel – HPBALL's)
FLUORESCENCE INTENSITY
200
150
100
0
50
100
150
200
250
ANTIBODY (ng/tube)
A
B
C
D
E
A — 221C X 185–1
B — 221C X 197·
C — 221C X 183·
D — 221C X 184·
E — 221C X 185–2

FIG. 9

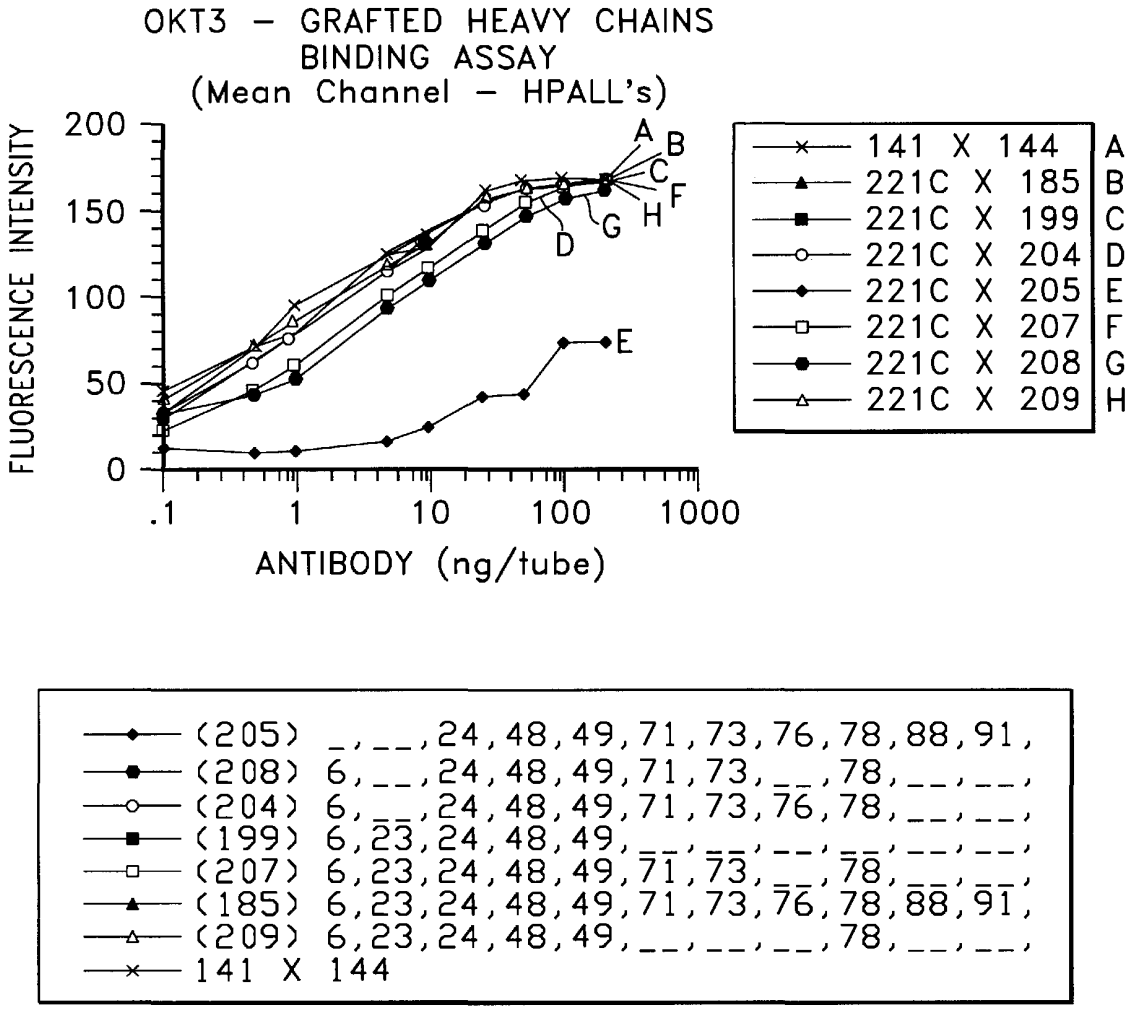

OKT3 – GRAFTED HEAVY CHAINS
BINDING ASSAY
(Mean Channel – HPALL's)
FLUORESCENCE INTENSITY
200
150
100
50
0
.1
1
10
100
1000
ANTIBODY (ng/tube)
A
B
C
F
H
G
D
E
141 X 144 A
221C X 185 B
221C X 199 C
221C X 204 D
221C X 205 E
221C X 207 F
221C X 208 G
221C X 209 H
(205) _,__,24,48,49,71,73,76,78,88,91,
(208) 6,__,24,48,49,71,73,__,78,__,__,
(204) 6,__,24,48,49,71,73,76,78,__,__,
(199) 6,23,24,48,49,__,__,__,__,__,__,
(207) 6,23,24,48,49,71,73,__,78,__,__,
(185) 6,23,24,48,49,71,73,76,78,88,91,
(209) 6,23,24,48,49,__,__,__,78,__,__,
141 X 144

FIG. 10a

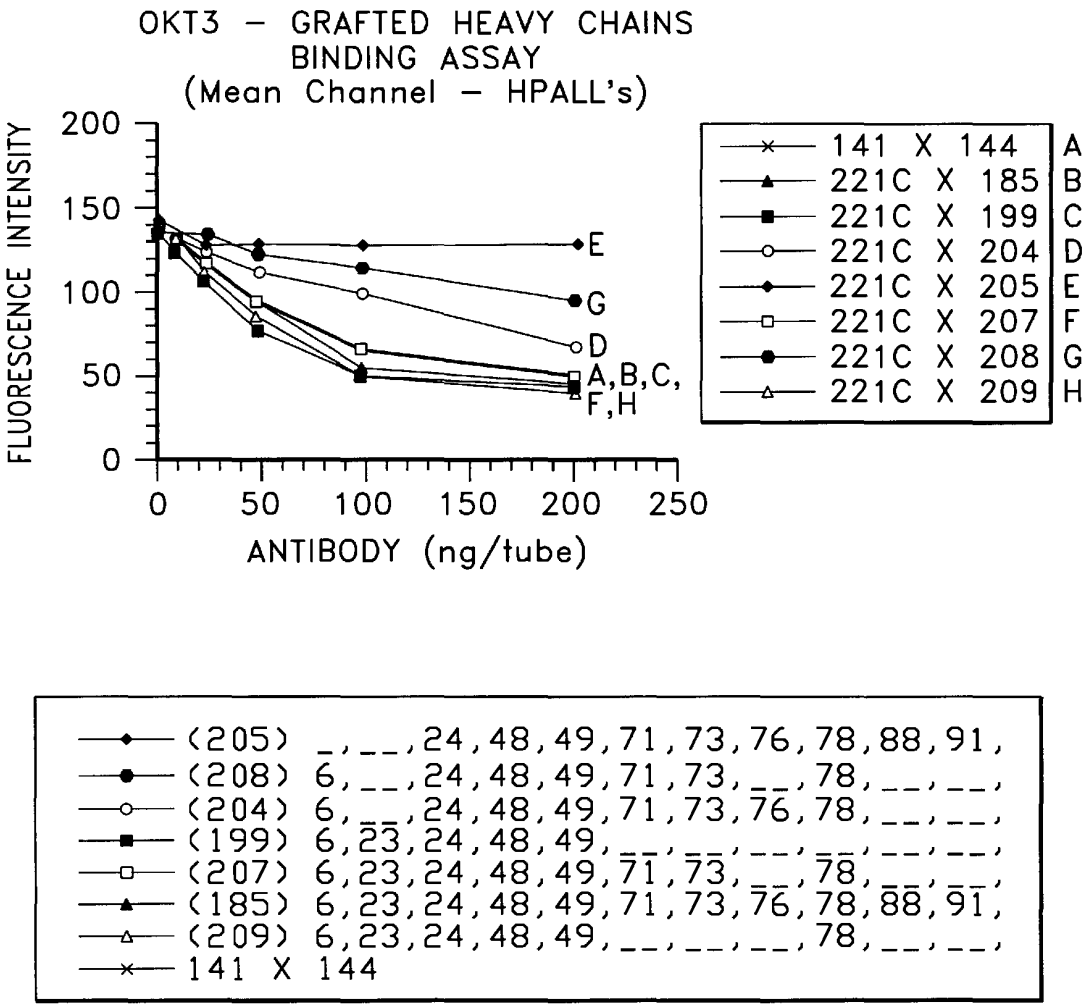

OKT3 – GRAFTED HEAVY CHAINS
BINDING ASSAY
(Mean Channel – HPALL's)
FLUORESCENCE INTENSITY
200
150
100
50
0
0
50
100
150
200
250
ANTIBODY (ng/tube)
E
G
D
A,B,C,
F,H
141 X 144 A
221C X 185 B
221C X 199 C
221C X 204 D
221C X 205 E
221C X 207 F
221C X 208 G
221C X 209 H
(205) _,__,24,48,49,71,73,76,78,88,91,
(208) 6,__,24,48,49,71,73,__,78,__,__,
(204) 6,__,24,48,49,71,73,76,78,__,__,
(199) 6,23,24,48,49,__,__,__,__,__,__,
(207) 6,23,24,48,49,71,73,__,78,__,__,
(185) 6,23,24,48,49,71,73,76,78,88,91,
(209) 6,23,24,48,49,__,__,__,78,__,__,
141 X 144

FIG. 10b

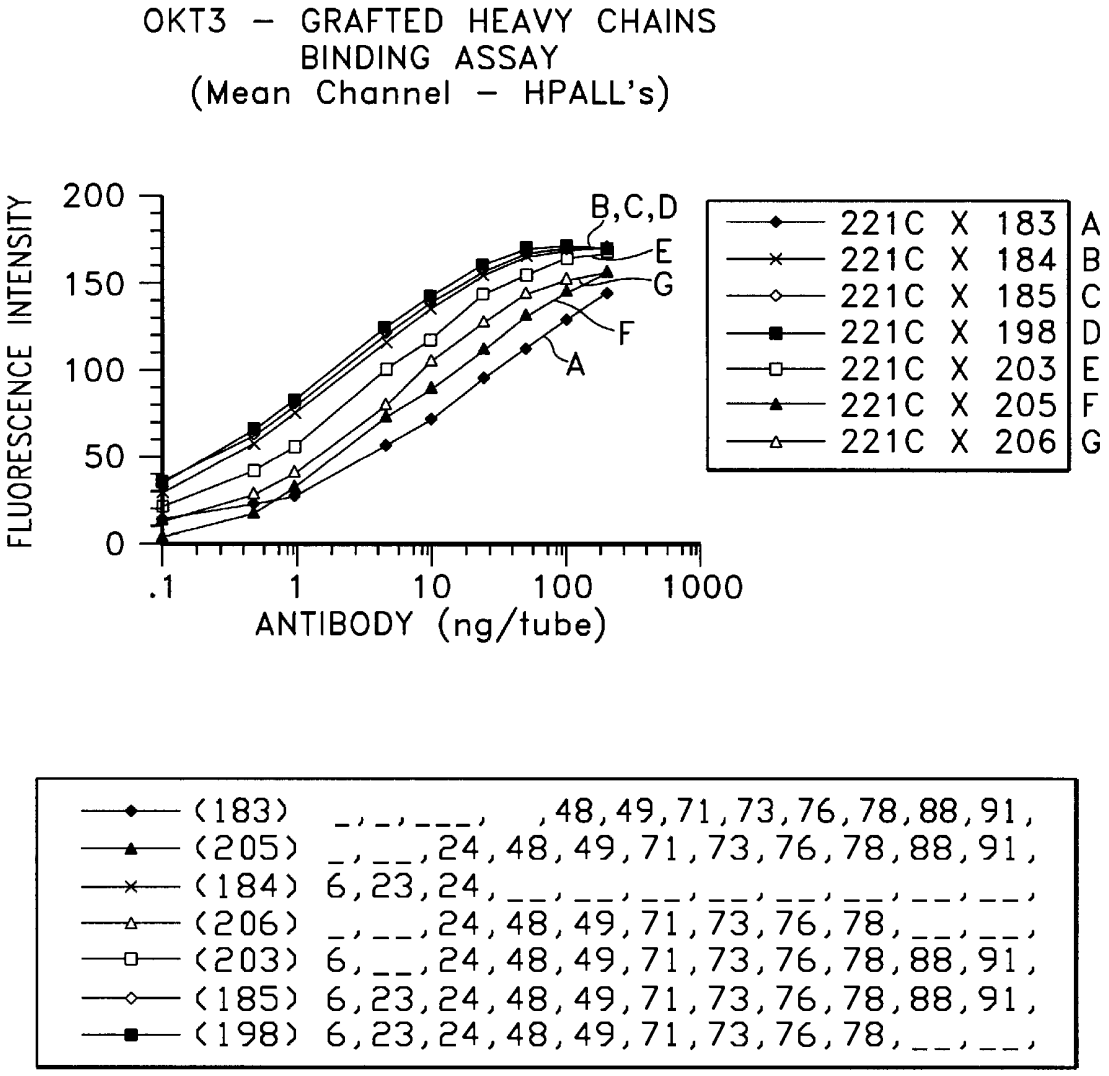
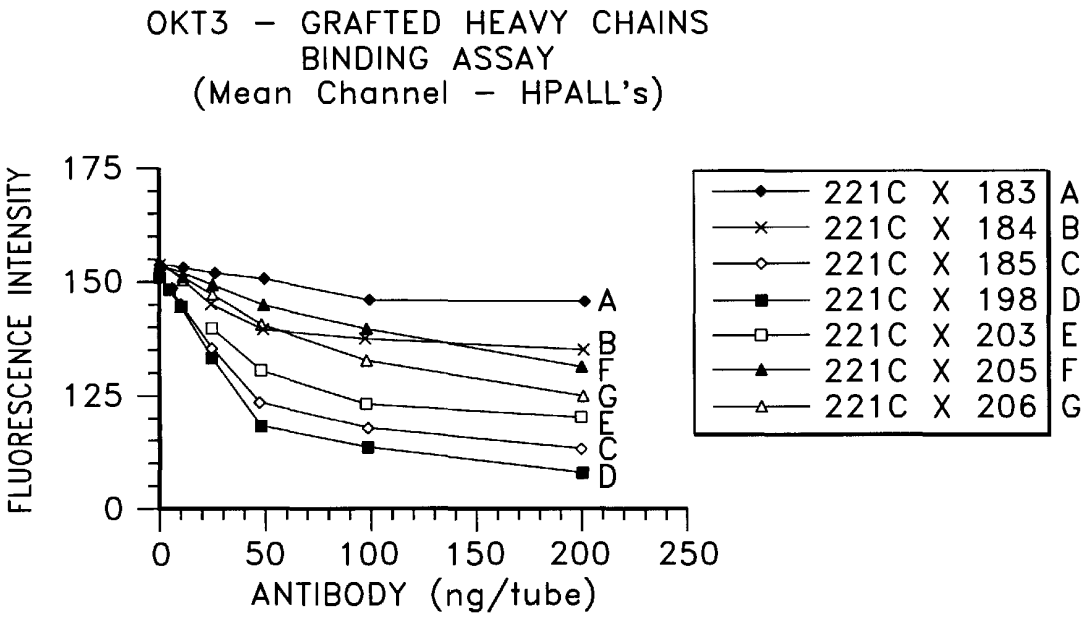

OKT3 – GRAFTED HEAVY CHAINS
BINDING ASSAY
(Mean Channel – HPALL's)
FLUORESCENCE INTENSITY
200
150
100
50
0
.1
1
10
100
1000
ANTIBODY (ng/tube)
B,C,D
E
G
F
A
221C X 183 A
221C X 184 B
221C X 185 C
221C X 198 D
221C X 203 E
221C X 205 F
221C X 206 G
(183) _,_,___, ,48,49,71,73,76,78,88,91,
(205) _,__,24,48,49,71,73,76,78,88,91,
(184) 6,23,24,__,__,__,__,__,__,__,__,
(206) _,__,24,48,49,71,73,76,78,__,__,
(203) 6,__,24,48,49,71,73,76,78,88,91,
(185) 6,23,24,48,49,71,73,76,78,88,91,
(198) 6,23,24,48,49,71,73,76,78,__,__,

FIG. 11a



(183) _,_,___, ,48,49,71,73,76,78,88,91,
(205) _,__,24,48,49,71,73,76,78,88,91,
(184) 6,23,24,__,__,__,__,__,__,__,__,
(206) _,__,24,48,49,71,73,76,78,__,__,
(203) 6,__,24,48,49,71,73,76,78,88,91,
(185) 6,23,24,48,49,71,73,76,78,88,91,
(198) 6,23,24,48,49,71,73,76,78,__,__,

FIG. 11b

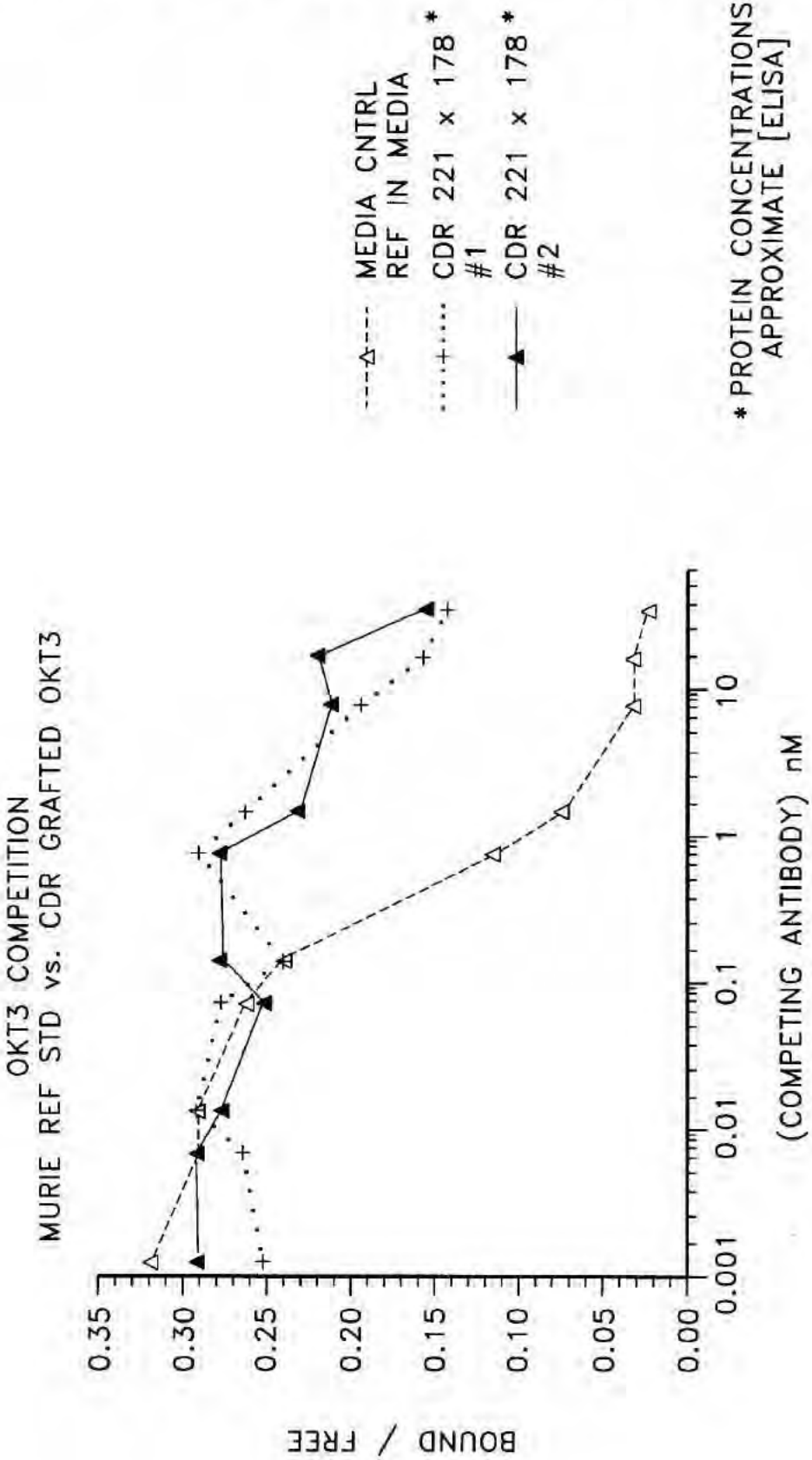


FIG. 12

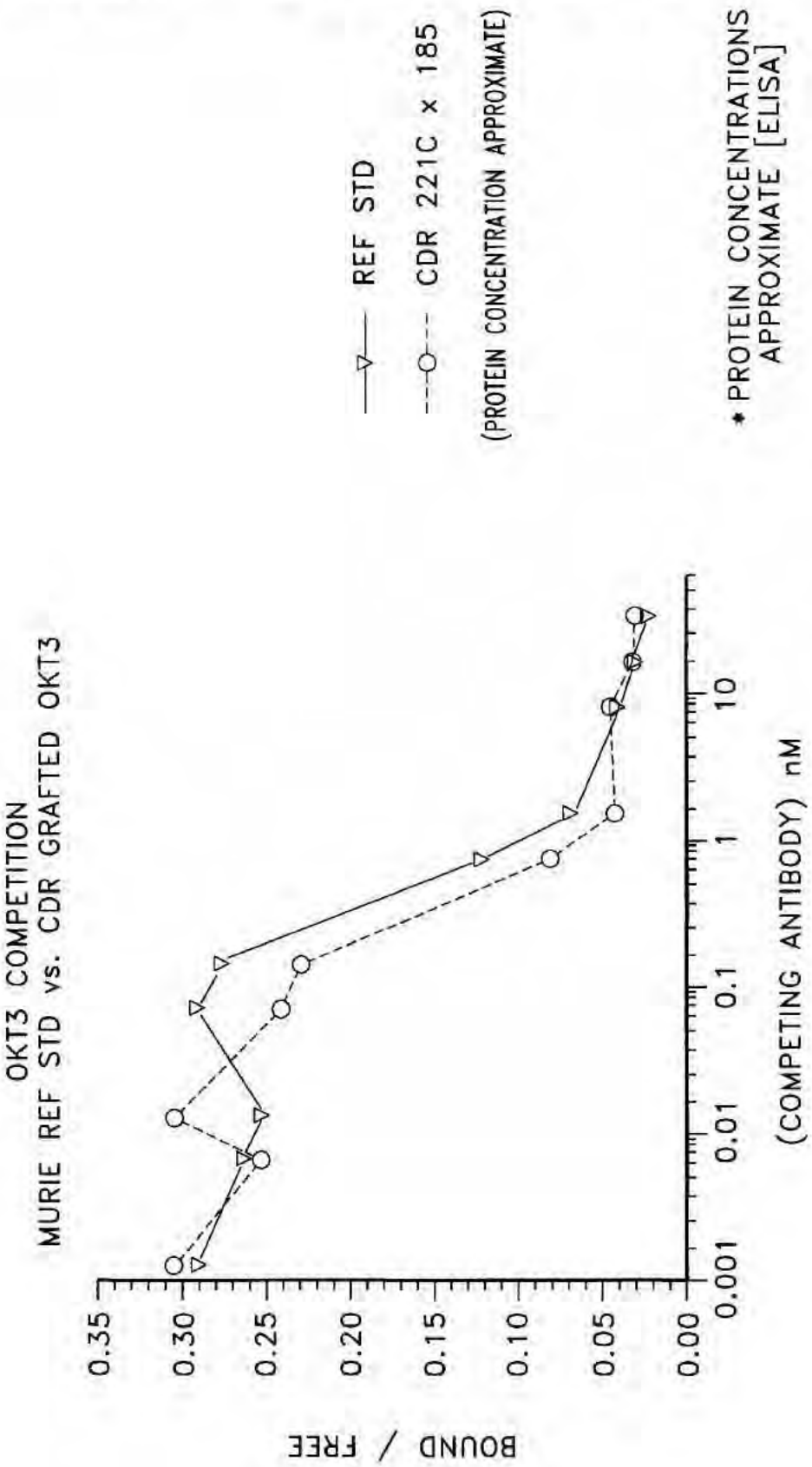


FIG. 13

Board Assigned Page #1002

HUMANISED ANTIBODIES

This is a continuation of application Ser. No. 07/743,329, filed Sep. 17, 1991, now abandoned.

FIELD OF THE INVENTION

The present invention relates to humanised antibody molecules, to processes for their production using recombinant DNA technology, and to their therapeutic uses.

The term "humanised antibody molecule" is used to describe a molecule having an antigen binding site derived from an immunoglobulin from a non-human species, and remaining immunoglobulin-derived parts of the molecule being derived from a human immunoglobulin. The antigen binding site typically comprises complementarity determining regions (CDRS) which determine the binding specificity of the antibody molecule and which are carried on appropriate framework regions in the variable domains. There are 3 CDRs (CDR1, CDR2 and CDR3) in each of the heavy and light chain variable domains.

In the description, reference is made to a number of publications by number. The publications are listed in numerical order at the end of the description.

BACKGROUND OF THE INVENTION

Natural immunoglobulins have been known for many years, as have the various fragments thereof, such as the Fab, $(Fab')_2$ and Fc fragments, which can be derived by enzymatic cleavage. Natural immunoglobulins comprise a generally Y-shaped molecule having an antigen-binding site towards the end of each upper arm. The remainder of the structure, and particularly the stem of the Y, mediates the effector functions associated with immunoglobulins.

Natural immunoglobulins have been used in assay, diagnosis and, to a more limited extent, therapy. However, such uses, especially in therapy, were hindered until recently by the polyclonal nature of natural immunoglobulins. A significant step towards the realisation of the potential of immunoglobulins as therapeutic agents was the discovery of procedures for the production of monoclonal antibodies (MAbs) of defined specificity (1).

However, most MAbs are produced by hybridomas which are fusions of rodent spleen cells with rodent myeloma cells. They are therefore essentially rodent proteins. There are very few reports of the production of human MAbs.

Since most available MAbs are of rodent origin, they are naturally antigenic in humans and thus can give rise to an undesirable immune response termed the HAMA (Human Anti-Mouse Antibody) response. Therefore, the use of rodent MAbs as therapeutic agents in humans is inherently limited by the fact that the human subject will mount an immunological response to the MAb and will either remove it entirely or at least reduce its effectiveness. In practice, MAbs of rodent origin may not be used in patients for more than one or a few treatments as a HAMA response soon develops rendering the MAb ineffective as well as giving rise to undesirable reactions. For instance, OKT3 a mouse IgG2a/k MAb which recognises an antigen in the T-cell receptor-CD3 complex has been approved for use in many countries throughout the world as an immunosuppressant in the treatment of acute allograft rejection [Chatenoud et al (2) and Jeffers et al (3)]. However, in view of the rodent nature of this and other such MAbs, a significant HAMA response which may include a major anti-idiotype component, may build up on use. Clearly, it would be highly desirable to

diminish or abolish this undesirable HAMA response and thus enlarge the areas of use of these very useful antibodies.

Proposals have therefore been made to render non-human MAbs less antigenic in humans. Such techniques can be generically termed "humanisation" techniques. These techniques typically involve the use of recombinant DNA technology to manipulate DNA sequences encoding the polypeptide chains of the antibody molecule.

Early methods for humanising MAbs involved production of chimeric antibodies in which an antigen binding site comprising the complete variable domains of one antibody is linked to constant domains derived from another antibody. Methods for carrying out such chimerisation procedures are described in EP0120694 (Celltech Limited), EP0125023 (Genentech Inc. and City of Hope), EP-A-0 171496 (Res. Dev. Corp. Japan), EP-A-0 173 494 (Stanford University), and WO 86/01533 (Celltech Limited). This latter Celltech application (WO 86/01533) discloses a process for preparing an antibody molecule having the variable domains from a mouse MAb and the constant domains from a human immunoglobulin. Such humanised chimeric antibodies, however, still contain a significant proportion of non-human amino acid sequence, i.e. the complete non-human variable domains, and thus may still elicit some HAMA response, particularly if administered over a prolonged period [Begent et al (ref. 4)].

In an alternative approach, described in EP-A-0239400 (Winter), the complementarity determining regions (CDRs) of a mouse MAb have been grafted onto the framework regions of the variable domains of a human immunoglobulin by site directed mutagenesis using long oligonucleotides. The present invention relates to humanised antibody molecules prepared according to this alternative approach, i.e. CDR-grafted humanised antibody molecules. Such CDR-grafted humanised antibodies are much less likely to give rise to a HAMA response than humanised chimeric antibodies in view of the much lower proportion of non-human amino acid sequence which they contain. The earliest work on humanising MAbs by CDR-grafting was carried out on MAbs recognising synthetic antigens, such as the NP or NIP antigens. However, examples in which a mouse MAb recognising lysozyme and a rat MAb recognising an antigen on human T-cells were humanised by CDR-grafting have been described by Verhoeyen et al (5) and Riechmann et al (6) respectively. The preparation of CDR-grafted antibody to the antigen on human T cells is also described in WO 89/07452 (Medical Research Council).

In Riechmann et al/Medical Research Council it was found that transfer of the CDR regions alone [as defined by Kabat refs. (7) and (8)] was not sufficient to provide satisfactory antigen binding activity in the CDR-grafted product. Riechmann et al found that it was necessary to convert a serine residue at position 27 of the human sequence to the corresponding rat phenylalanine residue to obtain a CDR-grafted product having improved antigen binding activity. This residue at position 27 of the heavy chain is within the structural loop adjacent to CDR1. A further construct which additionally contained a human serine to rat tyrosine change at position 30 of the heavy chain did not have a significantly altered binding activity over the humanised antibody with the serine to phenylalanine change at position 27 alone. These results indicate that changes to residues of the human sequence outside the CDR regions, in particular in the structural loop adjacent to CDR1, may be necessary to obtain effective antigen binding activity for CDR-grafted antibodies which recognise more complete antigens. Even so the binding affinity of the best CDR-grafted antibodies obtained was still significantly less than the original MAb.

Very recently Queen et al (9) have described the preparation of a humanised antibody that binds to the interleukin 2 receptor, by combining the CDRs of a murine MAb (anti-Tac) with human immunoglobulin framework and constant regions. The human framework regions were chosen to maximise homology with the anti-Tac MAb sequence. In addition computer modelling was used to identify framework amino acid residues which were likely to interact with the CDRs or antigen, and mouse amino acids were used at these positions in the humanised antibody.

In WO 90/07861 Queen et al propose four criteria for designing humanised immunoglobulins. The first criterion is to use as the human acceptor the framework from a particular human immunoglobulin that is unusually homologous to the non-human donor immunoglobulin to be humanised, or to use a consensus framework from many human antibodies. The second criterion is to use the donor amino acid rather than the acceptor if the human acceptor residue is unusual and the donor residue is typical for human sequences at a specific residue of the framework. The third criterion is to use the donor framework amino acid residue rather than the acceptor at positions immediately adjacent to the CDRs. The fourth criterion is to use the donor amino acid residue at framework positions at which the amino acid is predicted to have a side chain atom within about 3 A of the CDRs in a three-dimensional immunoglobulin model and to be capable of interacting with the antigen or with the CDRs of the humanised immunoglobulin. It is proposed that criteria two, three or four may be applied in addition or alternatively to criterion one, and may be applied singly or in any combination.

WO 90/07861 describes in detail the preparation of a single CDR-grafted humanised antibody, a humanised antibody having specificity for the p55 Tac protein of the IL-2 receptor. The combination of all four criteria, as above, were employed in designing this humanised antibody, the variable region frameworks of the human antibody Eu (7) being used as acceptor. In the resultant humanised antibody the donor CDRs were as defined by Kabat et al (7 and 8) and in addition the mouse donor residues were used in place of the human acceptor residues, at positions 27, 30, 48, 66, 67, 89, 91, 94, 103, 104, 105 and 107 in the heavy chain SEQ ID NO:31 and at positions 48, 60 and 63 in the light chain, of the variable region frameworks. The humanised anti-Tac antibody obtained is reported to have an affinity for p55 of $3\times10^{9}M^{-1}$, about one-third of that of the murine MAb.

We have further investigated the preparation of CDR-grafted humanised antibody molecules and have identified a hierarchy of positions within the framework of the variable regions (i.e. outside both the Kabat CDRs and structural loops of the variable regions) at which the amino acid identities of the residues are important for obtaining CDR-grafted products with satisfactory binding affinity. This has enabled us to establish a protocol for obtaining satisfactory CDR-grafted products which may be applied very widely irrespective of the level of homology between the donor immunoglobulin and acceptor framework. The set of residues which we have identified as being of critical importance does not: coincide with the residues identified by Queen et al (9).

SUMMARY OF THE INVENTION

Accordingly, in a first aspect the invention provides a CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

In preferred embodiments, the heavy chain framework comprises donor residues at positions 23, 24, 49, 71, 73 and 78 or at positions 23, 24 and 49. The residues at positions 71, 73 and 78 of the heavy chain framework are preferably either all acceptor or all donor residues.

In particularly preferred embodiments the heavy chain framework additionally comprises donor residues at one, some or all of positions 6, 37, 48 and 94. Also it is particularly preferred that residues at positions of the heavy chain framework which are commonly conserved across species, i.e. positions 2, 4, 25, 36, 39, 47, 93, 103, 104, 106 and 107, if not conserved between donor and acceptor, additionally comprise donor residues. Most preferably the heavy chain framework additionally comprises donor residues at positions 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

In addition the heavy chain framework optionally comprises donor residues at one, some or all of positions:

1 and 3,

72 and 76,

69 (if 48 is different between donor and acceptor),

38 and 46 (if 48 is the donor residue),

80 and 20 (if 69 is the donor residue),

67,

82 and 18 (if 67 is the donor residue),

91,

88, and

any one or more of 9, 11, 41, 87, 108, 110 and 112.

In the first and other aspects of the present invention reference is made to CDR-grafted antibody products comprising acceptor framework and donor antigen binding regions. It will be appreciated that the invention is widely applicable to the CDR-grafting of antibodies in general. Thus, the donor and acceptor antibodies may be derived from animals of the same species and even same antibody class or sub-class. More usually, however, the donor and acceptor antibodies are derived from animals of different species. Typically the donor antibody is a non-human antibody, such as a rodent MAb, and the acceptor antibody is a human antibody.

In the first and other aspects of the present invention, the donor antigen binding region typically comprises at least one CDR from the donor antibody. Usually the donor antigen binding region comprises at least two and preferably all three CDRs of each of the heavy chain and/or light chain variable regions. The CDRs may comprise the Kabat CDRs, the structural loop CDRs or a composite of the Kabat and structural loop CDRs and any combination of any of these. Preferably, the antigen binding regions of the CDR-grafted hearty chain variable domain comprise CDRs corresponding to the Kabat CDRs at CDR2 (residues 50–65) and CDR3 (residues 95–100) and a composite of the Kabat and structural loop CDRs at CDR1 (residues 26–35).

The residue designations given above and elsewhere in the present application are numbered according to the Kabat numbering [refs. (7) and (8)]. Thus the residue designations do not always correspond directly with the linear numbering of the amino acid residues. The actual linear amino acid sequence may contain fewer or additional amino acids than in the strict Kabat numbering corresponding to a shortening of, or insertion into, a structural component, whether framework or CDR, of the basic variable domain structure. For example, the heavy chain variable region of the anti-Tac

antibody described by Queen et al (9) contains a single amino acid insert (residue 52a) after residue 52 of CDR2 and a three amino acid insert (residues 82a, 82b and 82c) after framework residue 82, in the Kabat numbering. The correct Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

The invention also provides in a second aspect a CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 1 and/or 3 and 46 and/or 47. Preferably the CDR grafted light chain of the second aspect comprises donor residues at positions 46 and/or 47.

The invention also provides in a third aspect a CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, 58 and 71.

In a preferred embodiment of the third aspect, the framework comprises donor residues at all of positions 46, 48, 58 and 71.

In particularly preferred embodiments of the second and third aspects, the framework additionally comprises donor residues at positions 36, 44, 47, 85 and 87. Similarly positions of the light chain framework which are commonly conserved across species, i.e. positions 2, 4, 6, 35, 49, 62, 64–69, 98, 99, 101 and 102, if not conserved between donor and acceptor, additionally comprise donor residues. Most preferably the light chain framework additionally comprises donor residues at positions 2, 4, 6, 35, 36, 38, 44, 47, 49, 62, 64–69, 85, 87, 98, 99, 101 and 102.

In addition the framework of the second or third aspects optionally comprises donor residues at one, some or all of positions:

1 and 3,

60 (if 60 and 54 are able to form at potential saltbridge),

70 (if 70 and 24 are able to form a potential saltbridge),

73 and 21 (if 47 is different between donor and acceptor),

37 and 45 (if 47 is different between donor and acceptor), and

any one or more of 10, 12, 40, 80, 103 and 105.

Preferably, the antigen binding regions; of the CDR-grafted light chain variable domain comprise CDRs corresponding to the Kabat CDRs at CDR1 (residue 24–34), CDR2 (residues 50–56) and CDR3 (residues 89–97).

The invention further provides in a fourth aspect a CDR-grafted antibody molecule comprising at least one CDR-grafted heavy chain and at least one CDR-grafted light chain according to the first and second or first and third aspects of the invention.

The humanised antibody molecules and chains of the present invention may comprise: a complete antibody molecule, having full length heavy and light chains; a fragment thereof, such as a Fab, $(Fab')_2$ or FV fragment; a light chain or heavy chain monomer or dimer; or a single chain antibody, e.g. a single chain FV in which heavy and light chain variable regions are joined by a peptide linker; or any other CDR-grafted molecule with the same specificity as the original donor antibody. Similarly the CDR-grafted heavy and light chain variable region may be combined with other antibody domains as appropriate.

Also the heavy or light chains or humanised antibody molecules of the present invention may have attached to them an effector or reporter molecule. For instance, it may have a macrocycle, for chelating a heavy metal atom, or a toxin, such as ricin, attached to it by a covalent bridging structure. Alternatively, the procedures of recombinant DNA technology may be used to produce an immunoglobulin molecule in which the Fc fragment or CH3 domain of a complete immunoglobulin molecule has been replaced by, or has attached thereto by peptide linkage, a functional non-immunoglobulin protein, such as an enzyme or toxin molecule.

Any appropriate acceptor variable region framework sequences may be used having regard to class/type of the donor antibody from which the antigen binding regions are derived. Preferably, the type of acceptor framework used is of the same/similar class/type as the donor antibody. Conveniently, the framework may be chosen to maximise/optimise homology with the donor antibody sequence particularly at positions close or adjacent to the CDRs. However, a high level of homology between donor and acceptor sequences is not important for application of the present invention. The present invention identifies a hierarchy of framework residue positions at which donor residues may be important or desirable for obtaining a CDR-grafted antibody product having satisfactory binding properties. The CDR-grafted products usually have binding affinities of at least 10^5 M^{-1}, preferably at least about 10^8 M^{-1}, or especially in the range 10^8–10^{12} M^{-1}. In principle, the present invention is applicable to any combination of donor and acceptor antibodies irrespective of the level of homology between their sequences. A protocol for applying the invention to any particular donor-acceptor antibody pair is given hereinafter. Examples of human frameworks which may be used are KOL, NEWM, REI, EU, LAY and POM (refs. 4 and 5) and the like; for instance KOL and NEWM for the heavy chain and REI for the light chain and EU, LAY and POM for both the heavy chain and the light chain.

Also the constant region domains of the products of the invention may be selected having regard to the proposed function of the antibody in particular the effector functions which may be required. For example, the constant region domains may be human IgA, IgE, IgG or IgM domains. In particular, IgG human constant region domains may be used, especially of the IgGI and IgG3 isotypes, when the humanised antibody molecule is intended for therapeutic uses, and antibody effector functions are required. Alternatively, IgG2 and IgG4 isotypes may be used when the humanised antibody molecule is intended for therapeutic purposes and antibody effector functions are not required, e.g. for simple blocking of lymphokine activity.

However, the remainder of the antibody molecules need not comprise only protein sequences from immunoglobulins. For instance, a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain is fused to a DNA sequence encoding the amino acid sequence of a functional polypeptide such as an effector or reporter molecule.

Preferably the CDR-grafted antibody heavy and light chain and antibody molecule products are produced by recombinant DNA technology.

Thus in further aspects the invention also includes DNA sequences coding for the CDR-grafted heavy and light chains, cloning and expression vectors containing the DNA sequences, host cells transformed with the DNA sequences and processes for producing the CDR-grafted chains and antibody molecules comprising expressing the DNA sequences in the transformed host cells.

The general methods by which the vectors may be constructed, transfection methods and culture methods are

well known per se and form no part of the invention. Such methods are shown, for instance, in references 10 and 11.

The DNA sequences which encode the donor amino acid sequence may be obtained by methods well known in the art. For example the donor coding sequences may be obtained by genomic cloning, or cDNA cloning from suitable hybridoma cell lines. Positive clones may be screened using appropriate probes for the heavy and light chain genes in question. Also PCR cloning may be used.

DNA coding for acceptor, e.g. human acceptor, sequences may be obtained in any appropriate way. For example DNA sequences coding for preferred human acceptor frameworks such as KOL, REI, EU and NEWM, are widely available to workers in the art.

The standard techniques of molecular biology may be used to prepare DNA sequences coding for the CDR-grafted products. Desired DNA sequences may be synthesised completely or in part using oligonucleotide synthesis techniques. Site-directed mutagenesis and polymerase chain reaction (PCR) techniques may be used as appropriate. For example oligonucleotide directed synthesis as described by Jones et al (ref. 20) may be used. Also oligonucleotide directed mutagenesis of a pre-exising variable region as, for example, described by Verhoeyen et al (ref. 5) or Riechmann et al (ref. 6) may be used. Also enzymatic filling in of gapped oligonucleotides using T_4 DNA polymerase as, for example, described by Queen et al (ref. 9) may be used.

Any suitable host cell/vector system may be used for expression of the DNA sequences coding for the CDR-grafted heavy and light chains. Bacterial e.g. *E. coli,* and other microbial systems may be used, in particular for expression of antibody fragments such as FAb and $(Fab')_2$ fragments, and especially FV fragments; and single chain antibody fragments e.g. single chain FVs. Eucaryotic e.g. mammalian host cell expression systems may be used for production of larger CDR-grafted antibody products, including complete antibody molecules. Suitable mammalian host cells include CHO cells and myeloma or hybridoma cell lines.

Thus, in a further aspect the present invention provides a process for producing a CDR-grafted antibody product comprising:

(a) producing in an expression vector an operon having a DNA sequence which encodes an antibody heavy chain according to the first aspect of the invention;

and/or

(b) producing in an expression vector an operon having a DNA sequence which encodes a complementary antibody light chain according to the second or third aspect of the invention;

(c) transfecting a host cell with the or each vector; and

(d) culturing the transfected cell line to produce the CDR-grafted antibody product.

The CDR-grafted product may comprise only heavy or light chain derived polypeptide, in which case only a heavy chain or light chain polypeptide coding sequence is used to transfect the host cells.

For production of products comprising both heavy and light chains, the cell line may be transfected with two vectors, the first vector may contain an operon encoding a light chain-derived polypeptide and the second vector containing an operon encoding a heavy chain-derived polypeptide. Preferably, the vectors are identical, except in so far as the coding sequences and selectable markers are concerned, so as to ensure as far as possible that each polypeptide chain is equally expressed. Alternatively, a single vector may be used, the vector including the sequences encoding both light chain- and heavy chain-derived polypeptides.

The DNA in the coding sequences for the light and heavy chains may comprise cDNA or genomic DNA or both. However, it is preferred that the DNA sequence encoding the heavy or light chain comprises at least partially, genomic DNA, preferably a fusion of cDNA and genomic DNA.

The present invention is applicable to antibodies of any appropriate specificity. Advantageously, however, the invention may be applied to the humanisation of non-human antibodies which are used for in vivo therapy or diagnosis. Thus the antibodies may be site-specific antibodies such as tumour-specific or call surface-specific antibodies, suitable for use in in vivo therapy or diagnosis, e.g. tumour imaging. Examples of cell surface-specific antibodies are anti-T cell antibodies, such as anti-CD3, and CD4 and adhesion molecules, such as CR3, ICAM and ELAM. The antibodies may have specificity for interleukins (including lymphokines, growth factors and stimulating factors), hormones and other biologically active compounds, and receptors for any of these. For example, the antibodies may have specificity for any of the following: Interferons α, β, Γ or δ IL1, IL2, IL3, or IL4, etc., TNF, GCSF, GMCSF, EPO, hGH, or insulin, etc.

The the present invention also includes therapeutic and diagnostic compositions comprising the CDR-grafted products of the invention and uses of such compositions in therapy and diagnosis.

Accordingly in a further aspect the invention provides a therapeutic or diagnostic composition comprising a CDR-grafted antibody heavy or light chain or molecule according to previous aspects of the invention in combination with a pharmaceutically acceptable carrier, diluent or excipient.

Accordingly also the invention provides a method of therapy or diagnosis comprising administering an effective amount of a CDR-grafted antibody heavy or light chain or molecule according to previous aspects of the invention to a human or animal subject.

A preferred protocol for obtaining CDR-grafted antibody heavy and light chains in accordance with the present invention is set out below together with the rationale by which we have derived this protocol. This protocol and rationale are given without prejudice to the generality of the invention as hereinbefore described and defined.

Protocol

It is first of all necessary to sequence the DNA coding for the heavy and light chain variable regions of the donor antibody, to determine their amino acid sequences. It is also necessary to choose appropriate acceptor heavy and light chain variable regions, of known amino acid sequence. The CDR-grafted chain is then designed starting from the basis of the acceptor sequence. It will be appreciated that in some cases the donor and acceptor amino acid residues may be identical at a particular position and thus no change of acceptor framework residue is required.

1. As a first step donor residues are substituted for acceptor residues in the CDRs. For this purpose the CDRs are preferably defined as follows:

Heavy chain-CDR1: residues 26–35
-CDR2: residues 50–65
-CDR3: residues 95–102
Light chain-CDR1: residues 24–34
-CDR2: residues 50–56
-CDR3: residues 89–97

The positions at which donor residues are to be substituted for acceptor in the framework are then chosen as follows, first of all with respect to the heavy chain and subsequently with respect to the light chain.

2. Heavy Chain

2.1 Choose donor residues at all of positions 23, 24, 49, 71, 73 and 78 of the heavy chain or all of positions 23, 24 and 49 (71, 73 and 78 are always either all donor or all acceptor).

2.2 Check that the following have the same amino acid in donor and acceptor sequences, and if not preferably choose the donor: 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

2.3 To further optimise affinity consider choosing donor residues at one, some or any of:

i. 1, 3

ii. 72, 76

iii. If 48 is different between donor and acceptor sequences, consider 69

iv. If at 48 the donor residue is chosen, consider 38 and 46

v. If at 69 the donor residue is chosen, consider 80 and then 20

vi. 67

vii. If at 67 the donor residue is chosen, consider 82 and then 18

viii. 91

ix. 88

x. 9, 11, 41, 87, 108, 110, 112

3. Light Chain

3.1 Choose donor at 46, 48, 58 and 71

3.2 Check that the following have the same amino acid in donor and acceptor sequences, if not preferably choose donor:

2, 4, 6, 35, 38, 44, 47, 49, 62, 64–69 inclusive, 85, 87, 98, 99, 101 and 102

3.3 To further optimise affinity consider choosing donor residues at one, some or any of:

i. 1, 3

ii. 63

iii. 60, if 60 and 54 are able to form potential saltbridge

iv. 70, if 70 and 24 are able to form potential saltbridge

v. 73, and 21 if 47 is different between donor and acceptor

vi. 37, and 45 if 47 is different between donor and acceptor

vii. 10, 12, 40, 80, 103, 105

Rationale

In order to transfer the binding site of an antibody into a different acceptor framework, a number of factors need to be considered.

1. The extent of the CDRs

The CDRs (Complementary Determining Regions) were defined by Wu and Kabat (refs,. 4 and 5) on the basis of an analysis of the variability of different regions of antibody variable regions. Three regions per domain were recognised. In the light chain the sequences are 24–34, 50–56, 89–97 (numbering according to Kabat (ref. 4), Eu Index) inclusive and in the heavy chain the sequences are 31–35, 50–65 and 95–102 inclusive.

When antibody structures became available it became apparent that these CDR regions corresponded in the main to loop regions which extended from the β barrel framework of the light and heavy variable domains. For H1 there was a discrepancy in that the loop was from 26 to 32 inclusive and for H2 the loop was 52 to 56 and for L2 from 50 to 53. However, with the exception of H1 the CDR regions encompassed the loop regions and extended into the β strand frameworks. In H1 residue 26 tends to be a serine and 27 a phenylalanine or tyrosine, residue 29 is a phenylalanine in most cases. Residues 28 and 30 which are surface residues exposed to solvent might be involved in antigen-binding. A prudent definition of the H1 CDR therefore would include residues 26–35 to include both the loop region and the hypervariable residues 33–35.

It is of interest to note the example of Riechmann et al (ref. 3), who used the residue 31–35 choice for CDR-H1. In order to produce efficient antigen binding, residue 27 also needed to be recruited from the donor (rat) antibody.

2. Non-CDR residues which contribute to antigen binding

By examination of available X-ray structures we have identified a number of residues which may have an effect on net antigen binding and which can be demonstrated by experiment. These residues can be sub-divided into a number of groups.

2.1 Surface residues near CDR [all numbering as in Kabat et al (ref. 7)].

2.1.1. Heavy Chain—Key residues are 23, 71 and 73.

Other residues which may contribute to a lesser extent are 1, 3 and 76. Finally 25 is usually conserved but the murine residue should be used if there is a difference. 2.1.2 Light Chain—Many residues close to the CDRs, e.g. 63, 65, 67 and 69 are conserved. If conserved none of the surface residues in the light chain are likely to have a major effect. However, if the murine residue at these positions is unusual, then it would be of benefit to analyse the likely contribution more closely. Other residues which may also contribute to binding are 1 and 3, and also 60 and 70 if the residues at these positions and at 54 and 24 respectively are potentially able to form a salt bridge i.e. 60+54; 70+24.

2.2 Packing residues near the CDRs.

2.2.1. Heavy Chain—Key residues are 24, 49 and 78. Other key residues would be 36 if not a tryptophan, 94 if not an arginine, 104 and 106 if not glycines and 107 if not a threonine. Residues which may make a further contribution to stable packing of the heavy chain and hence improved affinity are 2, 4, 6, 38, 46, 67 and 69. 67 packs against the CDR residue 63 and this pair could be either both mouse or both human. Finally, residues which contribute to packing in this region but from a longer range are 18, 20, 80, 82 and 86. 82 packs against 67 and in turn 18 packs against 82. 80 packs against 69 and in turn 20 packs against 80. 86 forms an H bond network with 33 and 46. Many of the mouse-human differences appear minor e.g. Leu-Ile, but could have an minor impact on correct packing which could translate into altered positioning of the CDRs.

2.2.2. Light Chain—Key residues are 48, 58 and 71. Other key residues would be 6 if not glutamine, 35 if not tryptophan, 62 if not phenylalanine or tryosine, 64, 66, 68, 99 and 101 if not glycines and 102 if not a threonine. Residues which make a further contribution are 2, 4, 37, 45 and 47. Finally residues 73 and 21 and 19 may make long distance packing contributions of a minor nature.

2.3. Residues at the variable domain interface between heavy and light chains—In both the light and heavy chains most of the non-CDR interface residues are conserved. If a conserved residue is replaced by a residue of different character, e.g. size or charge, it should be considered for retention as the murine residue.

2.3.1. Heavy Chain—Residues which need to be considered are 37 if the residue is not a valine but is of larger side chain volume or has a charge or polarity. Other residues are 39 if not a glutamine, 45 if not a leucine, 47 if not a tryptophan, 91 if not a phenylalanine or tyrosine, 93 if not an alanine and 103 if not a tryptophan. Residue 89 is also at the interface but is not in a position where the side chain could be of great impact.

2.3.2. Light Chain—Residues which need to be considered are 36, if not a tyrosine, 38 if not a glutamine, 44 if not a proline, 46, 49 if not a tyrosine, residue 85, residue 87 if not a tyrosine and 98 if not a phenylalanine.

2.4. Variable-Constant region interface—The elbow angle between variable and constant regions may be affected by alterations in packing of key residues in the variable region against the constant region which may affect the position of V_L and V_H with respect to one another. Therefore it is worth noting the residues likely to be in contact with the constant region. In the heavy chain the surface residues potentially in contact with the variable region are conserved between mouse and human antibodies therefore the variable region contact residues may influence the V-C interaction. In the light chain the amino acids found at a number of the constant region contact points vary, and the V & C regions are not in such close proximity as the heavy chain. Therefore the influences of the light chain V-C interface may be minor.

2.4.1. Heavy Chain—Contact residues are 7, 11, 41, 87, 108, 110, 112.

2.4.2. Light Chain—In the light chain potentially contacting residues are 10, 12, 40, 80, 83, 103 and 105.

The above analysis coupled with our considerable practical experimental experience in the CDR-grafting of a number of different antibodies have lead us to the protocol given above.

The present invention is now described, by way of example only, with reference to the accompanying FIGS. **1–13**.

BRIEF DESCRIPTION OF THE FIGURES

FIGS. **1***a* and **1***b* show DNA and amino acid sequences of the OKT3 light chain (SEQ ID NO:4 and 5);

FIGS. **2***a* and **2***b* shows DNA and amino acid sequences of the OKT3 heavy chain;

FIG. **3** shows the alignment of the OKT3 light variable region amino acid sequence with that of the light variable region of the human antibody REI(SEQ ID NO:29, 8 and 9);

FIG. **4** shows the alignment of the OKT3 heavy variable region amino acid sequence with that of the heavy variable region of the human antibody KOL(SEQ ID NO:30 and 10);

FIGS. **5***a–c* show the heavy variable region amino acid sequences of OKT3, KOL and various corresponding CDR grafts(SEQ ID NO:30 and 10–24);

FIG. **6** shows the light variable region amino acid sequences of OKT3, REI and various corresponding CDR grafts(SEQ ID NO:29, 9 and 25);

FIG. **7** shows a graph of binding assay results for various grafted OKT3 antibodies'

FIG. **8** shows a graph of blocking assay results for various grafted OKT3 antibodies;

FIG. **9** shows a similar graph of blocking assay results;

FIGS. **10***a* and *b* show similar graphs for both binding assay and blocking assay results;

FIGS. **11***a* and *b* show further similar graphs for both binding assay and blocking assay results;

FIG. **12** shows a graph of competition assay results for a minimally grafted OKT3 antibody compared with the OKT3 murine reference standard, and

FIG. **13** shows a similar graph of competition assay results comparing a fully grafted OKT3 antibody with the murine reference standard.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

Example 1

CDR-grafting of OKT3

Material and Methods

1. Incoming Cells

Hybridoma cells producing antibody OKT3 were provided by Ortho (seedlot 4882.1) and were grown up in antibiotic free Dulbecco's Modified Eagles Medium (DMEM) supplemented with glutamine and 5% foetal calf serum, and divided to provide both an overgrown supernatant for evaluation and cells for extraction of RNA. The overgrown supernatant was shown to contain 250 ug/mL murine IgG2a/kappa antibody. The supernatant was negative for murine lambda light chain and IgG1, IgG2b, IgG3, IgA and IgM heavy chain. 20 mL of supernatant was assayed to confirm that the antibody present was OKT3.

2. Molecular Biology Procedures

Basic molecular biology procedures were as described in Maniatis et al (ref. 9) with, in some cases, minor modifications. DNA sequencing was performed as described in Sanger et al (ref. 11) and the Amersham International Plc sequencing handbook. Site directed mutagenesis was as described in Kramer et al (ref. 12) and the Anglian Biotechnology Ltd. handbook. COS cell expression and metabolic labelling studies were as described in Whittle et al (ref. 13)

3. Research Assays

3.1. Assembly Assays

Assembly assays were performed on supernatants from transfected COS cells to determine the amount of intact IgG present.

3.1.1. COS Cells Transfected With Mouse OKT3 Genes

The assembly assay for intact mouse IgG in COS cell supernatants was an ELISA with the following format:

96 well microtitre plates were coated with $F(ab')_2$ goat anti-mouse IgG Fc. The plates were washed in water and samples added for 1 hour at room temperature. The plates were washed and $F(ab')_2$ goat anti-mouse IgG $F(ab')_2$ (HRPO conjugated) was then added. Substrate was added to reveal the reaction. UPC10, a mouse IgG2a myeloma, was used as a standard.

3.1.2. COS and CHO Cells Transfected With Chimeric or CDR-grafted OKT3 Genes

The assembly assay for chimeric: or CDR-grafted antibody in COS cell supernatants was an ELISA with the following format:

96 well microtitre plates were coated with $F(ab')_2$ goat anti-human IgG Fc. The plates were washed and samples added and incubated for 1 hour at room temperature. The plates were washed and monoclonal mouse anti-human kappa chain was added for 1 hour at room temperature. The plates were washed and $F(ab')_2$ goat anti-mouse IgG Fc (HRPO conjugated) was added. Enzyme substrate was added to reveal the reaction. Chimeric B72.3 (IgG4) (ref. 13) was used as a standard. The use of a monoclonal anti-kappa chain in this assay allows grafted antibodies to be read from the chimeric standard.

3.2. Assay for Antigen Binding Activity

Material from COS cell supernatants was assayed for OKT3 antigen binding activity onto CD3 positive cells in a direct assay. The procedure was as follows:

HUT 78 cells (human T cell line, CD3 positive) were maintained in culture. Monolayers of HUT 78 cells were prepared onto 96 well ELISA plates using poly-L-lysine and glutaraldehyde. Samples were added to the monolayers for 1 hour at room temperature.

The plates were washed gently using PBS. $F(ab')_2$ goat anti-human IgG Fc (HRPO conjugated) or $F(ab')_2$ goat anti-mouse IgG Fc (HRPO conjugated) was added as appropriate for humanised or mouse samples. Substrate was added to reveal the reaction. The negative control for the cell-based assay was chimeric B72.3. The positive control was mouse

Orthomune OKT3 or chimeric OKT3, when available. This cell-based assay was difficult to perform, and an alternative assay was developed for CDR-grafted OKT3 which was more sensitive and easier to carry out. In this system CDR-grafted OKT3 produced by COS cells was tested for its ability to bind to the CD3-positive HPB-ALL (human peripheral blood acute lymphocytic leukemia) cell line. It was also tested for its ability to block the binding of murine OKT3 to these cells. Binding was measured by the following procedure: HPB-ALL cells were harvested from tissue culture. Cells were incubated at 4° C. for 1 hour with various dilutions of test antibody, positive control antibody, or negative control antibody. The cells were washed once and incubated at 4° C. for 1 hour with an FITC-labelled goat anti-human IgG (Fc-specific, mouse absorbed). The cells were washed twice and analysed by cytofluorography. Chimeric OKT3 was used as a positive control for direct binding. Cells incubated with mock-transfected COS cell supernatant, followed by the FITC-labelled goat anti-human IgG, provided the negative control. To test the ability of CDR-grafted OKT3 to block murine OKT3 binding, the HPB-ALL cells were incubated at 4° C. for 1 hour with various dilutions of test antibody or control antibody. A fixed saturating amount of FITC OKT3 was added. The samples were incubated for 1 hour at 4° C., washed twice and analysed by cytofluorography.

FITC-labelled OKT3 was used as a positive control to determine maximum binding. Unlabelled murine OKT3 served as a reference standard for blocking. Negative controls were unstained cells with or without mock-transfected cell supernatant. The ability of the CDR-grafted OKT3 light chain to bind CD3-positive cells and block the binding of murine OKT3 was initially tested in combination with the chimeric OKT3 heavy chain. The chimeric OKT3 heavy chain is composed of the murine OKT3 variable region and the human IgG4 constant region. The chimeric heavy chain gene is expressed in the same expression vector used for the CDR-grafted genes. The CDR-grafted light chain expression vector and the chimeric heavy chain expression vector were co-transfected into COS cells. The fully chimeric OKT3 antibody (chimeric light chain and chimeric heavy chain) was found to be fully capable of binding to CD3 positive cells and blocking the binding of murine OKT3 to these cells.

3.3 Determination of Relative Binding Affinity

The relative binding affinities of CDR-grafted anti-CD3 monoclonal antibodies were determined by competition binding (ref. 6) using the HPB-ALL human T cell line as a source of CD3 antigen, and fluorescein-conjugated murine OKT3 (Fl-OKT3) of known binding affinity as a tracer antibody. The binding affinity of Fl-OKT3 tracer antibody was determined by a direct binding assay in which increasing amounts of Fl-OKT3 were incubated with HPB-ALL (5×10^5) in PBS with 5% foetal calf serum for 60 min. at 4° C. Cells were washed, and the fluorescence intensity was determined on a FACScan flow cytometer calibrated with quantitative microbead standards (Flow Cytometry Standards, Research Triangle Park, N.C.). Fluorescence intensity per antibody molecule (F/P ratio) was determined by using microbeads which have a predetermined number of mouse IgG antibody binding sites (Simply Cellular beads, Flow Cytometry Standards). F/P equals the fluorescence intensity of beads saturated with Fl-OKT3 divided by the number of binding sites per bead. The amount of bound and free Fl-OKT3 was calculated from the mean fluorescence intensity per cell, and the ratio of bound/free was plotted against the number of moles of antibody bound. A linear fit was used to determine the affinity of binding (absolute value of the slope).

For competitive binding, increasing amounts of competitor antibody were added to a sub-saturating dose of Fl-OKT3 and incubated with 5×10^5 HPB-ALL in 200 ml of PBS with 5% foetal calf serum, for 60 min at 4° C. The fluorescence intensities of the cells were measured on a FACScan flow cytometer calibrated with quantitative microbead standards. The concentrations of bound and free Fl-OKT3 were calculated. The affinities of competing antibodies were calculated from the equation $[X]-[OKT3]=(1/Kx)-(1/Ka)$, where Ka is the affinity of murine OKT3, Kx is the affinity of competitor X, [ ] is the concentration of competitor antibody at which bound/free binding is R/2, and R is the maximal bound/free binding.

4. cDNA Library Construction

4.1. mRNA Preparation and cDNA Synthesis

OKT3 producing cells were grown as described above and 1.2×10^9 cells harvested and MRNA extracted using the guanidinium/LiCl extraction procedure. cDNA was prepared by priming from Oligo-dT to generate full length cDNA. The cDNA was methylated and EcoR1 linkers added for cloning.

4.2. Library Construction

The cDNA library was ligated to pSP65 vector DNA which had been EcoR1 cut and the 5' phosphate groups removed by calf intestinal phosphatase (EcoR1/CIP). The ligation was used to transform high transformation efficiency *Escherichia coli* (*E.coli*) HB101. A cDNA library was prepared. 3600 colonies were screened for the light chain and 10000 colonies were screened for the heavy chain.

5. Screening

E.coli colonies positive for either heavy or light chain probes were identified by oligonucleotide screening using the oligonucleotides:

5' TCCAGATGTTAACTGCTCAC (SEQ ID NO:1) for the light chain, which is complementary to a sequence in the mouse kappa constant region, and 5' CAGGGGCCAGTGGATGGATAGAC (SEQ ID NO: 2) for the heavy chain which is complementary to a sequence in the mouse IgG2a constant CH1 domain region. 12 light chain and 9 heavy chain clones were identified and taken for second round screening. Positive clones from the second round of screening were grown up and DNA prepared. The sizes of the gene inserts were estimated by gel electrophoresis and inserts of a size capable of containing a full length cDNA were subcloned into M13 for DNA sequencing.

6. DNA Sequencing

Clones representing four size classes for both heavy and light chains were obtained in M13. DNA sequence for the 5' untranslated regions, signal sequences, variable regions and 3' untranslated regions of full length cDNAs [FIGS. **1**(*a*) and **2**(*a*)(SEQ ID NO:6)] were obtained and the corresponding amino acid sequences predicted [(FIGS. **1**(*b*) and **2**(*b*)(SEQ ID NO:7]. In FIG. **1**(*a*) the untranslated DNA regions are shown in uppercase, and in both FIGS. **1** (SEQ ID NO:4 and 5) and **2** (SEQ ID NO:6 and 7) the signal sequences are underlined.

7. Construction of cDNA Expression Vectors

Celltech expression vectors are based on the plasmid pEE6hCMV (ref. 14). A polylinker for the insertion of genes to be expressed has been introduced after the major immediate early promoter/enhancer of the human Cytomegalovirus (hCMV). Marker genes for selection of the plasmid in transfected eukaryotic cells can be inserted as BamH1 cassettes in the unique BamH1 site of pEE6 hCMV; for instance, the neo marker to provide pEE6 hCMV neo. It is usual practice to insert the neo and gpt markers prior to

insertion of the gene of interest, whereas the GS marker is inserted last because of the presence of internal EcoR1 sites in the cassette.

The selectable markers are expressed from the SV40 late promoter which also provides an origin of replication so that the vectors can be used for expression in the COS cell transient expression system.

The mouse sequences were excised from the M13 based vectors described above as EcoR1 fragments and cloned into either pEE6-hCMV-neo for the heavy chain and into EE6-hCMV-gpt for the light chain to yield vectors pJA136 and pJA135 respectively.

8. Expression of cDNAS in COS Cells

Plasmids pJA135 and pJA136 were co-transfected into COS cells and supernatant from the transient expression experiment was shown to contain assembled antibody which bound to T-cell enriched lymphocytes. Metabolic labelling experiments using ^{35}S methionine showed expression and assembly of heavy and light chains.

9. Construction of Chimeric Genes

Construction of chimeric genes followed a previously described strategy [Whittle et al (ref. 13)]. A restriction site near the 3' end of the variable domain sequence is identified and used to attach an oligonucleotide adapter coding for the remainder of the mouse variable region and a suitable restriction site for attachment to the constant region of choice.

9.1. Light Chain Gene Construction

The mouse light chain cDNA sequence contains an Aval site near the 3' end of the variable region [FIG. **1**(*a*)(SEQ ID NO:4)]. The majority of the sequence of the variable region was isolated as a 396 bp. EcoR1-Aval fragment. An oligonucleotide adapter was designed to replace the remainder of the 3' region of the variable region from the Aval site and to include the 5' residues of the human constant region up to and including a unique Nar1 site which had been previously engineered into the constant region.

A Hind111 site was introduced to act as a marker for insertion of the linker.

The linker was ligated to the V_L fragment and the 413 bp EcoR1-Nar1 adapted fragment was purified from the ligation mixture.

The constant region was isolated as an Nar1-BamH1 fragment from an M13 clone NW361 and was ligated with the variable region DNA into an EcoR1/BamH1/C1P pSP65 treated vector in a three way reaction to yield plasmid JA143. Clones were isolated after transformation into *E.coli* and the linker and junction sequences were confirmed by the presence of the Hind111 site and by DNA sequencing.

9.2 Light Chain Gene Construction—Version 2

The construction of the first chimeric light chain gene produces a fusion of mouse and human amino acid sequences at the variable-constant region junction. In the case of the OKT3 light chain the amino acids at the chimera junction are:

. . .Leu—Glu—Ile—Asn—Arg / — / Thr—Val—Ala —Ala (SEQ ID NO: 3)

VARIABLE CONSTANT

This arrangement of sequence introduces a potential site for Asparagine (Asn) linked (N-linked) glycosylation at the V-C junction. Therefore, a second version of the chimeric light chain oligonucleotide adapter was designed in which the threonine (Thr), the first amino acid of the human constant region, was replaced with the equivalent amino acid from the mouse constant region, Alanine (Ala).

An internal Hind111 site was not included in this adapter, to differentiate the two chimeric light chain genes.

The variable region fragment was isolated as a 376 bp EcoR1-Aval fragment. The oligonucleotide linker was ligated to Nar1 cut pNW361 and then the adapted 396bp constant region was isolated after recutting the modified pNW361 with EcoR1. The variable region fragment and the modified constant region fragment were ligated directly into EcoR1/C1P treated pEE6hCMVneo to yield pJA137. Initially all clones examined had the insert in the incorrect orientation. Therefore, the insert was re-isolated and recloned to turn the insert round and yield plasmid pJA141. Several clones with the insert in the correct orientation were obtained and the adapter sequence of one was confirmed by DNA sequencing

9.3. Heavy Chain Gene Construction

9.3.1. Choice of Heavy Chain Gene Isotype

The constant region isotype chosen for the heavy chain was human IgG4.

9.3.2. Gene Construction

The heavy chain cDNA sequence showed a Ban1 site near the 3' end of the variable region [FIG. **2**(*a*)(SEQ ID NO:6)]. The majority of the sequence of the variable region was isolated as a 426bp. EcoR1/C1P/Ban1 fragment. An oligonucleotide adapter was designated to replace the remainder of the 3' region of the variable region from the Ban1 site up to and including a unique HindIII site which had been previously engineered into the first two amino acids of the constant region.

The linker was ligated to the V_H fragment and the EcoR1-Hind111 adapted fragment was purified from the ligation mixture.

The variable region was ligated to the constant region by cutting pJA91 with EcoR1 and Hind111 removing the intron fragment and replacing it with the V_H to yield pJA142. Clones were isolated after transformation into *E.coli* JM101 and the linker and junction sequences were confirmed by DNA sequencing. (N.B. The Hind111 site is lost on cloning).

10. Construction of Chimeric Expression Vectors

10.1. neo and gpt Vectors

The chimeric light chain (version 1) was removed from pJA143 as an EcoR1 fragment and cloned into EcoR1/C1P treated pEE6hCMVneo expression vector to yield pJA145. Clones with the insert in the correct orientation were identified by restriction mapping.

The chimeric light chain (version 2) was constructed as described above.

The chimeric heavy chain gene was isolated from pJA142 as a 2.5Kbp EcoR1/BamH1 fragment and cloned into the EcoR1/Bcl1/C1P treated vector fragment of a derivative of pEE6hCMVgpt to yield plasmid pJA144.

10.2. GS Separate Vectors

GS versions of pJA141 and pJA144 were constructed by replacing the neo and gpt cassettes by a BamH1/Sa11/C1P treatment of the plasmids, isolation of the vector fragment and ligation to a GS-containing fragment from the plasmid pRO49 to yield the light chain vector pJA179 and the heavy chain vector pJA180.

10.3. GS Single Vector Construction

Single vector constructions containing the cL (chimeric light), cH (chimeric heavy) and GS genes on one plasmid in the order cL-cH-GS, or cH-cL-GS and with transcription of the genes being head to tail e.g. cL>cH>GS were constructed. These plasmids were made by treating pJA179 or pJA180 with BamH1/C1P and ligating in a Bgl11/Hind111 hCMV promoter cassette along with either the Hind111/BamH1 fragment from pJA141 into pJA180 to give the cH-cL-GS plasmid pJA182 or the Hind111/BamH1 fragment from pJA144 into pJA179 to give the cL-cH-GS plasmid pJA181.

11. Expression of Chimeric Genes

11.1. Expression in COS Cells

The chimeric antibody plasmid pJA145 (cL) and pJA144 (cH) were co-transfected into COS cells and supernatant from the transient expression experiment was shown to contain assembled antibody which bound to the HUT 78 human T-cell line. Metabolic labelling experiments using ^{35}S methionine showed expression and assembly of heavy and light chains. However the light chain mobility seen on reduced gels suggested that the potential glycosylation site was being glycosylated. Expression in COS cells in the presence of tunicamycin showed a reduction in size of the light chain to that shown for control chimeric antibodies and the OKT3 mouse light chain. Therefore JA141 was constructed and expressed. In this case the light chain did not show an aberrant mobility or a size shift in the presence or absence of tunicamycin. This second version of the chimeric light chain, when expressed in association with chimeric heavy (cH) chain, produced antibody which showed good binding to HUT 78 cells. In both cases antigen binding was equivalent to that of the mouse antibody.

11.2 Expression in Chinese Hamster Ovary (CHO) Cells

Stable cell lines have been prepared from plasmids PJA141/pJA144 and from pJA179/pJA180, pJA181 and pJA182 by transfection into CHO cells.

12. CDR-grafting

The approach taken was to try to introduce sufficient mouse residues into a human variable region framework to generate antigen binding activity comparable to the mouse and chimeric antibodies.

12.1. Variable Region Analysis

From an examination of a small database of structures of antibodies and antigen-antibody complexes it is clear that only a small number of antibody residues make direct contact with antigen. Other residues may contribute to antigen binding by positioning the contact residues in favourable configurations and also by inducing a stable packing of the individual variable domains and stable interaction of the light and heavy chain variable domains. The residues chosen for transfer can be identified in a number of ways:

(a) By examination of antibody X-ray crystal structures the antigen binding surface can be predominantly located on a series of loops, three per domain, which extend from the B-barrel framework.

(b) By analysis of antibody variable domain sequences regions of hypervariability [termed the Complementarity Determining Regions (CDRs) by Wu and Kabat (ref. 5)]can be identified. In the most but not all cases these CDRs correspond to, but extend a short way beyond, the loop regions noted above.

(c) Residues not identified by (a) and (b) may contribute to antigen binding directly or indirectly by affecting antigen binding site topology, or by inducing a stable packing of the individual variable domains and stabilising the inter-variable domain interaction. These residues may be identified either by superimposing the sequences for a given antibody on a known structure and looking at key residues for their contribution, or by sequence alignment analysis and noting "idiosyncratic" residues followed by examination of their structural location and likely effects.

12.1.1. Light Chain

FIG. **3** (SEQ ID NO:29, 8 and 9) shows an alignment of sequences for the human framework region RE1 (SEQ ID NO:8and 9) and the OKT3 light variable region (SEQ ID NO:29). The structural loops (LOOP) and CDRs (KABAT) believed to correspond to the antigen binding region are marked. Also marked are a number of other residues which may also contribute to antigen binding as described in 13.1(c). Above the sequence in FIG. **3** (SEQ ID NO:29, 8 and 9) the residue type indicates the spatial location of each residue side chain, derived by examination of resolved structures from X-ray crystallography analysis. The key to this residue type designation is as follows:

N — near to CDR (From X-ray Structures)	
P — Packing	B — Buried Non-Packing
S — Surface	E — Exposed
I — Interface	* — Interface
— Packing/Part Exposed	
? — Non-CDR Residues which may reguire to be left as Mouse sequence.	

Residues underlined in FIG. **3** are amino acids. RE1 (SEQ ID NO:8 and 9) was chosen as the human framework because the light chain is a kappa chain and the kappa variable regions show higher homology with the mouse sequences than a lambda light variable region, e.g. KOL (SEQ ID NO:10)(see below). RE1 (SEQ ID NO:8 and 9) was chosen in preference to another kappa light chain because the X-ray structure of the light chain has been determined so that a structural examination of individual residues could be made.

12.1.2. Heavy Chain

Similarly FIG. **4** shows an alignment of sequences for the human framework region KOL (SEQ ID NO:10) and the OKT3 (SEQ ID NO:30) heavy variable region. The structural loops and CDRs believed to correspond to the antigen binding region are marked. Also marked are a number of other residues which may also contribute to antigen binding as described in 12.1(c). The residue type key and other indicators used in FIG. **4** are the same as those used in FIG. **3**. KOL (SEQ ID NO:10) was chosen as the heavy chain framework because the X-ray structure has been determined to a better resolution than, for example, NEWM and also the sequence alignment of OKT3 heavy variable region (SEQ ID NO:7) showed a slightly better homology to KOL (SEQ ID NO:10) than to NEWM.

12.2. Design of Variable Genes

The variable region domains were designed with mouse variable region optimal codon usage [Grantham and Perrin (ref. 15)] and used the B72.3 signal sequences [Whittle et al (ref. 13)]. The sequences were designed to be attached to the constant region in the same way as for the chimeric genes described above. Some constructs contained the "Kozak consensus sequence" [Kozak (ref. 16)] directly linked to the 5' of the signal sequence in the gene. This sequence motif is believed to have a beneficial role in translation initiation in eukaryotes.

12.3. Gene Construction

To build the variable regions, various strategies are available. The sequence may be assembled by using oligonucleotides in a manner similar to Jones et al (ref. 17) or by

simultaneously replacing all of the CDRs or loop regions by oligonucleotide directed site specific mutagenesis in a manner similar to Verhoeyen et al (ref. 2). Both strategies were used and a list of constructions is set out in Tables 1 and 2 and FIGS. **4** and **5***a–c*. It was noted in several cases that the mutagenesis approach led to deletions and rearrangements in the gene being remodelled, while the success of the assembly approach was very sensitive to the quality of the oligonucleotides.

13. Construction of Expression Vectors

Genes were isolated from M13 or SP65 based intermediate vectors and cloned into pEE6hCMVneo for the light chains and pEE6hCMVgpt for the heavy chains in a manner similar to that for the chimeric genes as described above.

TABLE 1

CDR-GRAFTED GENE CONSTRUCTS

CODE	MOUSE SEQUENCE CONTENT	METHOD OF CONSTRUCTION	KOZAK SEQUENCE –	KOZAK SEQUENCE +
LIGHT CHAIN	ALL HUMAN FRAMEWORK RE1			
121	26–32, 50–56, 91–96 inclusive	SDM and gene assembly	+	n.d.
121A	26–32, 50–56, 91–96 inclusive +1, 3, 46, 47	Partial gene assembly	n.d.	+
121B	26–32, 50–56, 91–96 inclusive +46, 47	Partial gene assembly	n.d.	+
221	24–24, 50–56, 91–96 inclusive	Partial gene assembly	+	+
221A	24–34, 50–56, 91–96 inclusive +1, 3, 46, 47	Partial gene assembly	+	+
221B	24–34, 50–56, 91–96 inclusive +1, 3	Partial gene assembly	+	+
221C	24–34, 50–56, 91–96 inclusive	Partial gene assembly	+	+
HEAVY CHAIN	ALL HUMAN FREMEWORK KOL			
121	26–32, 50–56, 95–100B inclusive	Gene assembly	n.d.	+
131	26–32, 50–58, 95–100B inclusive	Gene assembly	n.d.	+
141	26–32, 50–65, 95–100B inclusive	Partial gene assembly	+	n.d.
321	26–35, 50–56, 95–100B inclusive	Partial gene assembly	+	n.d.
331	26–35, 50–58, 95–100B inclusive	Partial gene assembly Gene assembly	+ 	 +
341	26–35, 50–65, 95–100B inclusive	SDM Partial gene assembly	+ 	 +
341A	26–35, 50–65, 95–100B inclusive +6, 23, 24, 48, 49, 71, 73, 76, 78, 88, 91 (+63 = human) (SEQ ID NO: 28)	Gene assembly	n.d.	+
34B	26–35, 50–65, 95–100B inclusive +48, 49, 71, 73, 76, 78, 88, 91 (+63 + human)	Gene assembly	n.d.	+

KEY
n.d. not done
SDM Site directed mutagenesis
Gene assembly Variable region assembled entirely from oligonucleotides
Partial gene assembly Variable region assembled by combination of restriction fragments either from other genes originally created by SDM and gene assembly or by oligonucleotide assembly of part of the variable region and reconstruction with restriction fragments from other genes originally created by SDM and gene assembly

14. Expression of CDR-grafted Genes

14.1. Production of Antibody Consisting of Grafted Light (gL) Chains With Mouse Heavy (mH) or Chimeric Heavy (cH) Chains

All gL chains, in association with mH or cH produced reasonable amounts of antibody. Insertion of the Kozak consensus sequence at a position 5' to the ATG (kgL constructs) however, led to a 2–5 fold improvement in net expression. Over an extended series of experiments expression levels were raised from approximately 200 ng/ml to approximately 500 ng/ml for kgL/cH or kgL/mH combinations.

When direct binding to antigen on HUT 78 cells was measured, a construct designed to include mouse sequence based on loop length (gL121) did not lead to active antibody in association with mH or cH. A construct designed to include mouse sequence based on Kabat CDRs (gL221) (SEQ ID NO:25) demonstrated some weak binding in association with mH or cH. However, when framework residues 1, 3, 46, 47 were changed from the human to the murine OKT3 equivalents based on the arguments outlined in Section 12.1 antigen binding was demonstrated when both of the new constructs, which were termed 121A and 221A were co-expressed with cH. When the effects of these residues were examined in more detail, it appears that residues 1 and 3 are not major contributing residues as the product of the gL221B gene (SEQ ID NO:27) shows little detectable binding activity in association with cH. The light chain product of gL221C(SEQ ID NO:28), in which mouse sequences are present at 46 and 47, shows good binding activity in association with cH.

14.2 Production of Antibody Consisting of Grafted Heavy (gH) Chains With Mouse Light (mL) or Chimeric Light (cL) Chains

Expression of the gH genes proved to be more difficult to achieve than for gL. First, inclusion of the Kozak sequence appeared to have no marked effect on expression of gH genes. Expression appears to be slightly improved but not to the same degree as seen for the grafted light chain.

Also, it proved difficult to demonstrate production of expected quantities of material when the loop choice (amino acid 26–32) for CDR1 is used, e.g. gH121, 131, 141 and no conclusions can be drawn about these constructs.

Moreover, co-expression of the gH341 gene (SEQ ID NO:11) with cL or mL has been variable and has tended to produce lower amounts of antibody than the cH/cL or mH/mL combinations. The alterations to gH341 (SEQ ID NO:11) to produce gH341(SEQ ID NO:12) and gH341B (SEQ ID NO:21) lead to improved levels of expression.

This may be due either to a general increase in the fraction of mouse sequence in the variable region, or to the alteration at position 63 where the residue is returned to the human amino acid Valine (Val) from Phenylalanine (Phe) to avoid possible internal packing problems with the rest of the human framework. This arrangement also occurs in gH331 and gH321.

When gH321 or gH331 were expressed in association with cL, antibody was produced but antibody binding activity was not detected.

When the more conservative gH341 gene (SEQ ID NO:11) was used antigen binding could be detected in association with cL or mL, but the activity was only marginally above the background level.

When further mouse residues were substituted based on the arguments in 12.1, antigen binding could be clearly demonstrated for the antibody produced when kgH341A and kgH341B were expressed in association with cL.

14.3 Production of Fully CDR-grafted Antibody

The kgL221A gene was co-expressed with kgH341, kgH341A or kgH341B. For the combination kgH221A/kgH341 very little material was produced in a normal COS cell expression.

For the combinations kgL221A/kgH341A or kgH221A/kgH341B amounts of antibody similar to gL/cH was produced.

In several experiments no antigen binding activity could be detected with kgH221A/gH341 or kgH221A/kgH341 combinations, although expression levels were very low.

Antigen binding was detected when kgL221A/kgH341A or kgH221A/kgH341B combinations were expressed. In the case of the antibody produced from the kgL221A/kgH341A combination the antigen binding was very similar to that of the chimeric antibody.

An analysis of the above results is given below.

15. Discussion of CDR-grafting Results

In the design of the fully humanised antibody the aim was to transfer the minimum number of mouse amino acids that would confer antigen binding onto a human antibody framework.

15.1. Light Chian

15.1.1. Extent of the CDRs

For the light chain the regions defining the loops known from structural studies of other antibodies to contain the antigen contacting residues, and those hypervariable sequences defined by Kabat et al (refs. 4 and 5) as Complementarity Determining Regions (CDRs) are equivalent for CDR2. For CDRI the hypervariable region extends from residues 24–34 inclusive while the structural loop extends from 26–32 inclusive. In the case of OKT3 (SEQ ID NO:5) there is only one amino acid difference between the two options, at amino acid 24, where the mouse sequence is a serine and the human framework RE1 has glutamine. For CDR3 the loop extends from residues 91–96 inclusive while the Kabat hypervariability extends from residues 89–97 inclusive. For OKT3 amino acids 89, 90 and 97 are the same between OKT3 and RE1 (FIG. **3**)(SEQ ID NO:29, 8 and 9). When constructs based on the loop choice for CDR1 (gL121) and the Kabat choice (gL221) were made and co-expressed with mH or cH no evidence for antigen binding activity could be found for gL121, but trace activity could be detected for the gL221, suggesting that a single extra mouse residue in the grafted variable region could have some detectable effect. Both gene constructs were reasonably well expressed in the transient expression system.

15.1.2. Framework Resides

The remaining framework residues were then further examined, in particular amino acids known from X-ray analysis of other antibodies to be close to the CDRs and also those amino acids which in OKT3 showed differences from the consensus framework for the mouse subgroup (subgroup VI) to which OKT3 shows most homology. Four positions 1, 3, 46 and 47 were identified and their possible contribution was examined by substituting the mouse amino acid for the human amino acid at each position. Therefore gL221A (gL221+D1Q, Q3V, L46R, L47W, see FIG. **3** and Table 1) was made, cloned in EE6hCMVneo and co-expressed with cH (pJA144). The resultant antibody was well expressed and showed good binding activity. When the related genes gL221B (SEQ ID NO:28)(gL221+D1Q, Q3V) and gL221C (gL221+L46R, L47W) were made and similarly tested, while both genes produced antibody when co-expressed with cH, only the gL221C/cH combination showed good antigen binding. When the gL121A (gL124+D1Q, Q3V, L46R, L47W) gene was made and co-expressed with cH, antibody was produced which also bound to antigen.

15.2. Heavy Chain

15.2.1. Extent of the CDRs

For the heavy chain the loop and hypervariability analyses agree only in CDR3. For CDR1 the loop region extends from residues 26–32 inclusive whereas the Kabat CDR extends from residues 31–35 inclusive. For CDR2 the loop region is from 50–58 inclusive while the hypervariable region covers amino acids 50–65 inclusive. Therefore humanised heavy chains were constructed using the framework from antibody KOL and with various combinations of these CDR choices, including a shorter choice for CDR2 of 50–56 inclusive as there was some uncertainty as to the definition of the end point for the CDR2 loop around residues 56 to 58. The genes were co-expressed with mL or cL initially. In the case of the gH genes with loop choices for CDR1 e.g. gH121, gH131, gH141 very little antibody was produced in the culture supernatants. As no free light chain was detected it was presumed that the antibody was being made and assembled inside the cell but that the heavy chain was aberrant in some way, possibly incorrectly folded, and therefore the antibody was being degraded internally. In some experiments trace amounts of antibody could be detected in ^{35}S labelling studies.

As no net antibody was produced, analysis of these constructs was not pursued further.

When, however, a combination of the loop choice and the Kabat choice for CDR1 was tested (mouse amino acids 26–35 inclusive) and in which residues 31 (Ser to Arg), 33 (Ala to Thr), and 35 (Tyr to His) were changed from the human residues to the mouse residue and compared to the first series, antibody was produced for gH321, kgH331 and kgH341 when co-expressed with cL. Expression was gen-

erally low and could not be markedly improved by the insertion of the Kozak consensus sequence 5' to the ATG of the signal sequence of the gene, as distinct from the case of the gL genes where such insertion led to a 2–5 fold increase in net antibody production. However, only in the case of gH341/mL or kgH341/cL could marginal antigen binding activity be demonstrated. When the kgH341 gene was co-expressed with kgL221A(SEQ ID NO:26), the net yield of antibody was too low to give a signal above the background level in the antigen binding assay.

15.2.2. Framework Residues

As in the case of the light chain the heavy chain frameworks were re-examined. Possibly because of the lower initial homology between the mouse and human heavy variable domains compared to the light chains, more amino acid positions proved to be of interest. Two genes kgH341A and kgH341B were constructed, with 11 or 8 human residues respectively substituted by mouse residues compared to gH341, and with the CDR2 residue 63 returned to the human amino acid potentially to improve domain packing. Both showed antigen binding when combined with cL or kgL221A, the kgH341A gene with all 11 changes appearing to be the superior choice.

15.3 Interim Conclusions

It has been demonstrated, therefore, for OKT3 that to transfer antigen binding ability to the humanised antibody, mouse residues outside the CDR regions defined by the Kabat hypervariability or structural loop choices are required for both the light and heavy chains. Fewer extra residues are needed for the light chain, possibly due to the higher initial homology between the mouse and human kappa variable regions.

Of the changes seven (1 and 3 from the light chain and 6, 23, 71, 73 and 76 from the heavy chain) are predicted from a knowledge of other antibody structures to be either partly exposed or on the antibody surface. It has been shown here that residues 1 and 3 in the light chain are not absolutely required to be the mouse sequence; and for the heavy chain the gH341B heavy chain in combination with the 221A light chain generated only weak binding activity. Therefore the presence of the 6, 23 and 24 changes are important to maintain a binding affinity similar to that of the murine antibody. It was important, therefore, to further study the individual contribution of othe other 8 mouse residues of the kgH341A gene compared to kgH341.

16. Further CDR-grafting Experiments

Additional CDR-grafted heavy chain genes were prepared substantially as described above. With reference to Table 2 the further heavy chain genes were based upon the gh341 (plasmid pJA178) and gH341A (plasmid pJA185)(SEQ ID NO:12) with either mouse OKT3 or human KOL residues at 6, 23, 24, 48, 49, 63, 71, 73, 76, 78, 88 and 91, as indicated. The CDR-grafted light chain genes used in these further experiments were gL221 (SEQ ID NO:25), gL221A(SEQ ID NO:26), gL221B (SEQ ID NO:27) and gL221C (SEQ ID NO:28) as described above.

TABLE 2

OKT3 HEAVY CHAIN CDR GRAFTS

1. gH341 and derivatives

RES NUM	6	23	24	48	49	63	71	73	76	78	88	91	
OKT3vh	Q	K	A	I	G	F	T	K	S	A	A	Y	
gH341	E	S	S	V	A	F	R	N	N	L	G	F	JA178
gH341A	Q	K	A	I	G	V	T	K	S	A	A	Y	JA185
gH341E	Q	K	A	I	G	V	T	K	S	A	G	G	JA198
gH341*	Q	K	A	I	G	V	T	K	N	A	G	F	JA207
gH341*	Q	K	A	I	G	V	R	N	N	A	G	F	JA209
gH341D	Q	K	A	I	G	V	T	K	N	L	G	F	JA197
gH341*	Q	K	A	I	G	V	R	N	N	L	G	F	JA199
gH341C	Q	K	A	V	A	F	R	N	N	L	G	F	JA184
gH341*	Q	S	A	I	G	V	T	K	S	A	A	Y	JA203
gH341*	E	S	A	I	G	V	T	K	S	A	A	Y	JA205
gH341B	E	S	S		G	V	T	K	S	A	A	Y	JA183
gH341*	Q	S	A	I	G	V	T	K	S	A	G	F	JA204
gH341*	E	S	A	I	G	V	T	K	S	A	G	F	JA206
gH341*	Q	S	A	I	G	V	T	K	N	A	G	F	JA208
KOL (SEQ ID NO:30, 10 AND 11–24)	E	S	S	V	A		R	N	N	L	G	F	

TABLE 2-continued

OKT3 LIGHT CHAIN CDR GRAFTS

2. gL221 and derivatives

RES NUM	1	3	46	47	
OKT3v1	Q	V	R	W	
GL221	D	Q	L	L	DA221
gL221A	Q	V	R	W	DA221A
gL221B	Q	V	L	L	DA221B
GL221C	D	Q	R	W	DA221C
RE1	D	Q	L	L	

(SEQ ID NO:29, 8, 9 and 25–28)

MURINE RESIDUES ARE UNDERLINED

The CDR-grafted heavy and light chain genes were co-expressed in COS cells either with one another in various combinations but also with the corresponding murine and chimeric heavy and light chain genes substantially as described above. The resultant antibody products were then assayed in binding and blocking assays with HPB-ALL cells as described above.

The results of the assays for various grafted heavy chains co-expressed with the gL221C light chain (SEQ ID NO:28) are given in FIGS. **7** and **8** (for the JA184, JA185, JA197 and JA198 constructs—see Table 2), in FIG. **9** (for the JA183, JA184, JA185 and JA197 constructs) in FIG. **10***a* and *b* (for the chimeric, JA185, JA199, JA204, JA205, JA207, JA208 and JA209 constructs) and in FIG. **11***a* and *b* (for the JA183, JA184, JA185, JA198, JA203, JA205 and JA206 constructs).

The basic grafted product without any human to murine changes in the variable frameworks, i.e. gL221 (SEQ ID NO:25) co-expressed with gh341 (JA178)(SEQ ID NO:11), and also the "fully grafted" product, having most human to murine changes in the grafted heavy chain framework, i.e. gL221C (SEQ ID NO:28) co-expressed with gh341A (JA185)(SEQ ID NO:12), were assayed for relative binding affinity in a competition assay against murine OKT3 reference standard, using HPB-ALL cells. The assay used was as described above in section 3.3. The results obtained are given in FIG. **12** for the basic grafted product and in FIG. **13** for the fully grafted product. These results indicate that the basic grafted product has neglibible binding ability aLs compared with the OKT3 murine reference standard; whereas the "fully grafted" product has a binding ability very similar to that of the OKT3 murine reference standard.

The binding and blocking assay results indicate the following:

The JA198 and JA207 constructs appear to have the best binding characteristics and similar binding abilities, both substantially the same as the chimeric and fully grafted gH341A products. This indicates that positions 88 and 91 and position 76 are not highly critical for maintaining the OKT3 binding ability; whereas at least some of positions 6, 23, 24, 48, 49, 71, 73 and 78 are more important.

This is borne out by the finding that the JA209 and JA199, although of similar binding ability to one another, are of lower binding ability than the JA198 and JA207 constructs. This indicates the importance of having mouse residues at positions 71, 73 and 78, which are either completely or partially human in the JA199 and JA209 constructs respectively.

Moreover, on comparing the results obtained for the JA205 and JA183 constructs it is seen that there is a decrease in binding going from the JA205 to the JA183 constructs. This indicates the importance of retaining a mouse residue at position 23, the only position changed between JA205 and JA183.

These and other results lead us to the conclusion that of the 11 mouse framework residues used in the gH341A (JA185) construct, it is important to retain mouse residues at all of positions 6, 23, 24, 48 and 49, and possibly for maximum binding affinity at 71, 73 and 78.

Similar Experiments were carried out to CDR-graft a number of the rodent antibodies including antibodies having specificity for CD4 (OKT4), ICAM-1 (R6-5), TAG72 (B72.3), and TNFα(61E71, 101.4, hTNF1, hTNF2 and hTNF3).

Example 2

CDR-grafting of a Murine Anti-CD4 T Cell Receptor Antibody, OKT4A

Anti OKT4A CDR-grafted heavy and light chain genes were prepared, expressed and tested substantially as described above in Example 1 for CDR-grafted OKT3. The CDR grafting of OKT4A is described in detail in Ortho patent application PCT/GB 90 . . . of even date herewith entitled "Humanised Antibodies". The disclosure of this Ortho patent application PCT/GB 90 . . . is incorporated herein by reference. A number of CDR-grafted OKT4 antibodies have been prepared. Presently the CDR-grafted OKT4A of choice is the combination of the grafted light chain LCDR2 and the grafted heavy chain HCDR10.

The Light Chain

The human acceptor framework used for the grafted light chains was RE1 (SEQ ID NO:8 and 9) The preferred LCDR2 light chain has human to mouse changes at positions 33, 34, 38, 49 and 89 in addition to the structural loop CDRs. Of these changed positions, positions 33, 34 and 89 fall within the preferred extended CDRs of the present invention (positions 33 and 34 in CDR1 and position 89 in CDR3). The human to murine changes at positions 38 and 49 corresponds to positions at which the amino acid residues are preferably donor murine amino acid residues in accordance with the present invention.

A comparison of the amino acid sequences of the donor murine light chain variable domain and the RE1 human

acceptor light chain variable further reveals that the murine and human residues are identical at all of positions 46, 48 and 71 and at all of positions 2, 4, 6, 35, 36, 44, 47, 62, 64–69, 85, 87, 98, 99 and 101 and 102. However the amino acid residue at position 58 in LCDR2 is the human RE1 framework residue not the mouse OKT4 residue as would be preferred in accordance with the present invention.

The Heavy Chain

The human acceptor framework used for the grafted heavy chains was KOL(SEQ ID NO:10).

The preferred CDR graft HCDR10 heavy chain has human to mouse changes at positions 24, 35, 57, 58, 60, 88 and 91 in addition to the structural loop CDRs.

Of these positions, positions 35 (CDR1) and positions 57, 58 and 60 (CDR2) fall within the preferred extended CDRs of the present invention. Also the human to mouse change at position 24 corresponds to a position at which the amino acid residue is a donor murine residue in accordance with the present invention. Moreover, the human to mouse changes at positions 88 and 91 correspond to positions at which the amino acid residues are optionally donor murine residues.

Moreover, a comparison of the murine OKT4A and human KOL heavy chain variable amino acid sequences reveals that the murine and human residues are identical at all of positions 23, 49, 71, 73 and 78 and at all of positions 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

Thus the OKT4A CDR-grafted heavy chain HCDR10 corresponds to a particularly preferred embodiment according to the present invention.

Example 3

CDR-grafting of an Anti-mucin Specific Murine Antibody, B72.3

The cloning of the genes coding for the anti-mucin specific murine monoclonal antibody B72.3 and the preparation of B72.3 mouse-human chimeric antibodies has been described previously (ref. 13 and WO 89/01783). CDR-grafted versions of B72.3 were prepared as follows.

(a) B72.3 Light Chain

CDR-grafting of this light chain was accomplished by direct transfer of the murine CDRs into the framework of the human light chain RE1. The regions transferred were:

CDR Number	Residues
1	24–34
2	50–56
3	90–96

The activity of the resulting grafted light chain was assessed by co-expression in COS cells, of genes for the combinations:

B72.3 cH/B72.3 cL

and B72.3 cH/B72.3 gL

Supernatants were assayed for antibody concentration and for the ability to bind to microtitre plates coated with mucin. The results obtained indicated that., in combination with the B72.3 cH chain, B72.3 cL and B72.3 gL had similar binding properties.

Comparison of the murine B72.3 and REI (SEQ ID NO:8 and 9) light chain amino acid sequences reveals that the residues are identical at positions 46, 58 and 71 but are different at position 48. Thus changing the human residue to the donor mouse residue at position 48 may further improve the binding characteristics of the CDR-grafted light chain, (B72.3 gL) in accordance with the present invention.

(b) B72.3 heavy chain

i. Choice of framework

At the outset it was necessary to make a choice of human framework. Simply put, the question was as follows: Was it necessary to use the framework regions from an antibody whose crystal structure was known or could the choice be made on some other criteria?

For B72.3 heavy chain, it was reasoned that, while knowledge of structure was important, transfer of the CDRs from mouse to human frameworks might be facilitated if the overall homology between the donor and receptor frameworks was maximised. Comparison of the B72.3 heavy chain sequence with those in Kabat (ref. 4) for human heavy chains showed clearly that B72.3 had poor homology for KOL (SEQ ID NO:10) and NEWM (for which crystal structures are available) but was very homologous to the heavy chain for EU.

On this basis, EU was chosen for the CDR-grafting and the following residues transferred as CDRs.

CDR Number	Residues
1	27–36
2	50–63
3	93–102

Also it was noticed that the FR4 region of EU was unlike that of any other human (or mouse) antibody. Consequently, in the grafted heavy chain genes this was also changed to produce a "consensus" human sequence. (Preliminary experiments showed that grafted heavy chain genes containing the EU FR4 sequence expressed very poorly in transient expression systems.)

ii. Results with grafted heavy chain genes

Expression of grafted heavy chain genes containing all human framework regions with either gL or cL genes produced a grafted antibody with little ability to bind to mucin. The grafted antibody had about 1% the activity of the chimeric antibody. In these experiments, however, it was noted that the activity of the grafted antibody could be increased to ~10% of B72.3 by exposure to pHs of 2–3.5.

This observation provided a clue as to how the activity of the grafted antibody could be improved without acid treatment. It was postulated that acid exposure brought about the protonation of an acidic residue (pKa of aspartic acid=3.86 and of glutamine acid=4.25) which in turn caused a change in structure of the CDR loops, or allowed better access of antigen.

From comparison of the sequences of B72.3 (ref. 13) and EU (refs. 4 and 5), it was clear that, in going from the mouse to human frameworks, only two positions had been changed in such a way that acidic residues had been introduced. These positions are at residues 73 and 81, where K to E and Q to E changes had been made, respectively.

Which of these positions might be important was determined by examining the crystal structure of the KOL antibody. In KOL heavy chain (SEQ ID NO:10), position 831 is far removed from either of the CDR loops.

Position 73, however, is close to both CDRs 1 and 3 of the heavy chain and, in this position it was possible to envisage that a K to E change in this region could have a detrimental effect on antigen binding.

iii. Framework changes in B72.3 gH gene

On the basis of the above analysis, E73 was mutated to a lysine (K). It was found that this change had a dramatic effect on the ability of the grafted Ab to bind to mucin. Further the ability of the grafted B72.3 produced by the mutated gH/gL combination to bind to mucin was similar to that of the B72.3 chimeric antibody.

iv. Other framework changes

In the course of the above experiments, other changes were made in the heavy chain framework regions. Within the accuracy of the assays used, none of the changes, either alone or together, appeared beneficial.

v. Other

All assays used measured the ability of the grafted Ab to bind to mucin and, as a whole, indicated that the single framework change at position 73 is sufficient to generate an antibody with similar binding properties to B72.3.

Comparison of the B72.3 murine and EU heavy chain sequence reveals that the mouse and human residues are identical at positions 23, 24, 71 and 78.

Thus the mutated CDR-grafted B72.3 heavy chain corresponds to a preferred embodiment of the present invention.

Example 4

CDR-graftin of a Murine Anti-ICAM-1 Monoclonal Antibody

A murine antibody, R6-5-D6 (EP 0314863) having specificity for Intercellular Adhesion Molecule 1 (ICAM-1) was CDR-grafted substantially as described above in previous examples. This work is described in greater detail in co-pending application, British Patent Application No. 9009549.8, the disclosure of which is incorporated herein by reference.

The human EU framework was used as the acceptor framework for both heavy and light chains. The CDR-grafted antibody currently of choice is provided by co-expression of grafted light chain gL221A (SEQ ID NO:26) and grafted heavy chain gH341D (SEQ ID NO:16) which has a binding affinity for ICAM 1 of about 75% of that of the corresponding mouse-human chimeric antibody.

Light Chain

gL221A has murine CDRs at positions 24–34 (CDR1), 50–56 (CDR2) and 89–97 (CDR3). In addition several framework residues are also the murine amino acid. These residues were chosen after consideration of the possible contribution of these residues to domain packing and stability of the conformation of the antigen binding region. The residues which have been retained as mouse are at positions 2, 3, 48 (?), 60, 84, 85 and 87.

Comparison of the murine anti-ICAM 1 and human EU light chain amino acid sequences reveals that the murine and human residues are identical at positions 46, 58 and 71.

Heavy Chain

gH341D has murine CDRs at positions 26–35 (CDR1), 50–56 (CDR2) and94-100B (CDR3). In addition murine residues were used in gH341D at positions 24, 48, 69, 71, 73, 80, 88 and 91. Comparison of the murine anti-ICAM 1 and human EU heavy chain amino acid sequences are identical at positions 23, 49 and 78.

Example 5

CDR-Grafting of Murine Anti-TNFa Antibodies

A number of murine anti-TNFa monoclonal antibodies were CDR-grafted substantially as described above in previous examples. These antibodies include the murine monoclonal antibodies designated 61 E71, hTNF1, hTNF3 and 101.4 A brief summary of the CDR-grafting of each of these antibodies is given below.

61E71

A similar analysis as described above (Example 1, Section 12.1.) was done for 61E71 and for the heavy chain 10 residues were identified at 23, 24, 48, 49, 68, 69, 71, 73, 75 and 88 as residues to potentially retain as murine. The human frameworks chosen for CDR-grafting of this antibody, and the hTNF3 and 101.4 antibodies were RE1 for the light chain and KOL for the heavy chain. Three genes were built, the first of which contained 23, 24, 48, 49, 71 and 73 [gH341(6)] as murine residues. The second gene also had 75 and 88 as murine residues [gH341(8)] while the third gene additionally had 68, 69, 75 and 88 as murine residues [gH341(10)]. Each was co-expressed with gL221, the minimum grafted light chain (CDRs only). The gL221/gH341(6) and gL221/gH341(8) antibodies both bound as well to TNF as murine 61E71. The gL221/gH341(10) antibody did not express and this combination was not taken further.

Subsequently the gL221/gH341(6) antibody was assessed in an L929 cell competition assay in which the antibody competes against the TNF receptor on L929 cells for binding to TNF in solution. In this assay the gL221/gH341(6) antibody was approximately 10% as active as murine 61E71.

hTNF1

hTNF1 is a monoclonal antibody which recognises an epitope on human TNF-. The EU human framework was used for CDR-grafting of both the heavy and light variable domains.

Heavy Chain

In the CDR-grafted heavy chain (ghTNF1) mouse CDRs were used at positions 26–35 (CDR1), 50–65 (CDR2) and 95–102 (CDR3). Mouse residues were also used in the frameworks at positions 48, 67, 69, 71, 73, 76, 89, 91, 94 and 108. Comparison of the TNF1 mouse and EU human heavy chain residues reveals that these are identical at positions 23, 24, 29 and 78.

Light Chain

In the CDR-grafted light chain (gLhTNF1) mouse CDRs wre used at positions 24–34 (CDR1), 50–56 (CDR2) and 89–97 (CDR3). In addition mouse residues were used in the frameworks at positions 3, 42, 48, 49, 83, 106 and 108. Comparison of the hTNF1 mouse and EU human light chain residues reveals that these are identical at positions 46, 58 and 71.

The grafted hTNF1 heavy chain was co-expressed with the chimeric light chain and the binding ability of the product compared with that of the chimeric light chain/chimeric heavy chain product in a TNF binding assay. The grafted heavy chain product appeared to have binding ability for TNF slightly better than the fully chimeric product.

Similarly, a grafted heavy chain/grafted light chain product was co-expressed and compared with the fully chimeric product and found to have closely similar binding properties to the latter product.

hTNF3

hTNF3 recognises an epitope on human TNF-α. The sequence of hTNF3 shows only 21 differences compared to 61E71 in the light and heavy chain variable regions, 10 in the light chain (2 in the CDRs at positions 50, 96 and 8 in the framework at 1, 19, 40, 45, 46, 76, 103 and 106) and 11 in the heavy chain (3 in the CDR regions at positions 52, 60 and 95 and 8 in the framework at 1, 10, 38, 40, 67, 73, 87 and 105). The light and heavy chains of the 61E71 and hTNF3 chimeric antibodies can be exchanged without loss of activity in the direct binding assay. However 61E71 is an order of magnitude less able to compete with the TNF receptor on L929 cells for TNF-a compared to hTNF3.

Based on the 61E71 CDR grafting data gL221 and gH341 (+23, 24, 48, 49 71 and 73 as mouse) genes have been built for hTNF3 and tested and the resultant grafted antibody binds well to TNF-a, but competes very poorly in the L929 assay. It is possible that in this case also the framework residues identified for OKT3 programme may improve the competitive binding ability of this antibody.

101.4

101.4 is a further murine monoclonal antibody able to recognise human TNF-a. The heavy chain of this antibody shows good homology to KOL and so the CDR-grafting has been based on RE1 for the light chain and KOL for the heavy chain. Several grafted heavy chain genes have been constructed with conservative choices for the CDR's (gH341) (SEQ ID NO:11) and which have one or a small number of non-CDR residues at positions 73, 78 or 77–79 inclusive, as the mouse amino acids. These have been co-expressed with cL or gL221. In all cases binding to TNF equivalent to the chimeric antibody is seen and when co-expressed with cL the resultant antibodies are able to compete well in the L929 assay. However, with gL221 the resultant antibodies are at least an order of magnitude less able to compete for TNF against the TNF receptor on 1,929 cells. Mouse residues at other positions in the heavy chain, for example, at 23 and 24 together or at 76 have been demonstrated to provide no improvement to the competitive ability of the grafted antibody in the L929 assay.

A number of other antibodies including antibodies having specificity for interleukins e.g. IL1 and cancer markers such as carcinoembryonic antigen (CEA) e.g. the monoclonal antibody A5B7 (ref. 21), have been successfully CDR-grafted according to the present invention.

It will be appreciated that the foregoing examples are given by way of illustration only and are not intended to limit the scope of the claimed invention. Changes and modifications may be made to the methods described whilst still falling within the spirit and scope of the invention.

REFERENCES

1. Kohler & Milstein, Nature, 265, 295–497, 1975.
2. Chatenoud et al, (1986), J. Immunol. 137, 830–838.
3. Jeffers et al, (1986), Transplantation, 41, 572–578.
4. Begent et al, Br. J. Cancer 62: 487 (1990).
5. Verhoeyen et al, Science, 239, 1534–1536, 1988.
6. Riechmann et al, Nature, 332, 323–324, 1988.
7. Kabat, E. A., Wu, T. T., Reid-Miller, M., Perry, H. M., Gottesman, K. S., 1987, in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA.
8. Wu, T. T., and Kabat, E. A., 1970, J. Exp. Med. 132 211–250.
9. Queen et al, (1989), Proc. Natl. Acad. Sci. USA, 86, 10029–10033 and WO 90/07861
10. Maniatis et al, Molecular Cloning, Cold Spring Harbor, N.Y., 1989.
11. Primrose and Old, Principles of Gene Manipulation, Blackwell, Oxford, 1980.
12. Sanger, F., Nicklen, S., Coulson, A. R., 1977, Proc. Natl. Acad. Sci. USA, 74 5463
13. Kramer, W., Drutsa, V., Jansen, H. -W., Kramer, B., Plugfelder, M., Fritz, H. -J., 1934, Nucl. Acids Res. 12, 9441
14. Whittle, N., Adair, J., Lloyd, J.C., Jenkins, E., Devine, J., Schlom, J., Raubitshek, A., Colcher, D., Bodmer, M., 1987, Protein Engineering 1, 499.
15. Sikder, S. S., Akolkar, P. N., Kaledas, P. M., Morrison, S. L., Kabat, E. A., 1985, J. Immunol. 135, 4215.
16. Wallick, S. C., Kabat, E. A., Morrison, S. L., 1988, J. Exp. Med. 168, 1099
17. Bebbington, C. R., Published International Patent Application WO 89/01036.
18. Granthan and Perrin 1986, Immunology Today 7, 160.
19. Kozak, M., 1987, J. Mol. Biol. 196, 947.
20. Jones, T. P., Dear, P. H., Foote, J., Neuberger, M. S., Winter, G., 1986, Nature, 321, 522
21. Harwood et al, Br. J. Cancer, 54, 75–82 (1986).

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i i i) NUMBER OF SEQUENCES: 31

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TCCAGATGTT AACTGCTCAC 20

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

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(x i) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 943 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i x) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 18..722

(i x) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 84..722

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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                   Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu
                   -22 -20                 -15

CTA ATC AGT GCC TCA GTC ATA ATA TCC AGA GGA CAA ATT GTT CTC ACC          98
Leu Ile Ser Ala Ser Val Ile Ile Ser Arg Gly Gln Ile Val Leu Thr
-10                 -5                   1                   5

CAG TCT CCA GCA ATC ATG TCT GCA TCT CCA GGG GAG AAG GTC ACC ATG         146
Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met
            10                  15                  20

ACC TGC AGT GCC AGC TCA AGT GTA AGT TAC ATG AAC TGG TAC CAG CAG         194
Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln
        25                  30                  35

AAG TCA GGC ACC TCC CCC AAA AGA TGG ATT TAT GAC ACA TCC AAA CTG         242
Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu
    40                  45                  50

GCT TCT GGA GTC CCT GCT CAC TTC AGG GGC AGT GGG TCT GGG ACC TCT         290
Ala Ser Gly Val Pro Ala His Phe Arg Gly Ser Gly Ser Gly Thr Ser
55                  60                  65

TAC TCT CTC ACA ATC AGC GGC ATG GAG GCT GAA GAT GCT GCC ACT TAT         338
Tyr Ser Leu Thr Ile Ser Gly Met Glu Ala Glu Asp Ala Ala Thr Tyr
70                  75                  80                  85

TAC TGC CAG CAG TGG AGT AGT AAC CCA TTC ACG TTC GGC TCG GGG ACA         386
Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr Phe Gly Ser Gly Thr
                90                  95                 100

AAG TTG GAA ATA AAC CGG GCT GAT ACT GCA CCA ACT GTA TCC ATC TTC         434
Lys Leu Glu Ile Asn Arg Ala Asp Thr Ala Pro Thr Val Ser Ile Phe
            105                 110                 115

CCA CCA TCC AGT GAG CAG TTA ACA TCT GGA GGT GCC TCA GTC GTG TGC         482
Pro Pro Ser Ser Glu Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys
        120                 125                 130
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TTC TTG AAC AAC TTC TAC CCC AAA GAC ATC AAT GTC AAG TGG AAG ATT          530
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    135                 140                 145

GAT GGC AGT GAA CGA CAA AAT GGC GTC CTG AAC AGT TGG ACT GAT CAG          578
Asp Gly Ser Glu Arg Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln
150                 155                 160                 165

GAC AGC AAA GAC AGC ACC TAC AGC ATG AGC AGC ACC CTC ACG TTG ACC          626
Asp Ser Lys Asp Ser Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr
                170                 175                 180

AAG GAC GAG TAT GAA CGA CAT AAC AGC TAT ACC TGT GAG GCC ACT CAC          674
Lys Asp Glu Tyr Glu Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His
            185                 190                 195

AAG ACA TCA ACT TCA CCC ATT GTC AAG AGC TTC AAC AGG AAT GAG TGT          722
Lys Thr Ser Thr Ser Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
        200                 205                 210

TAGAGACAAA GGTCCTGAGA CGCCACCACC AGCTCCCAGC TCCATCCTAT CTTCCCTTCT        782

AAGGTCTTGG AGGCTTCCCC ACAAGCGCTT ACCACTGTTG CGGTGCTCTA AACCTCCTCC        842

CACCTCCTTC TCCTCCTCCT CCCTTTCCTT GGCTTTTATC ATGCTAATAT TTGCAGAAAA        902

TATTCAATAA AGTGAGTCTT TGCCTTGAAA AAAAAAAAAA A                            943
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 235 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser
-22  -20                 -15                 -10

Val Ile Ile Ser Arg Gly Gln Ile Val Leu Thr Gln Ser Pro Ala Ile
 -5                  1                   5                  10

Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser Ala Ser
                15                  20                  25

Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser
            30                  35                  40

Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro
        45                  50                  55

Ala His Phe Arg Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile
    60                  65                  70

Ser Gly Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp
75                  80                  85                  90

Ser Ser Asn Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Asn
                95                  100                 105

Arg Ala Asp Thr Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu
            110                 115                 120

Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe
        125                 130                 135

Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg
    140                 145                 150

Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser
155                 160                 165                 170

Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu
                175                 180                 185
```

-continued

```
Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser
                190                 195                 200

Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
            205                 210
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1570 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i x) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 41..1444

(i x) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 98..1444

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
GAATTCCCCT CTCCACAGAC ACTGAAAACT CTGACTCAAC ATG GAA AGG CAC TGG      55
                                             Met Glu Arg His Trp
                                             -19                 -15

ATC TTT CTA CTC CTG TTG TCA GTA ACT GCA GGT GTC CAC TCC CAG GTC     103
Ile Phe Leu Leu Leu Leu Ser Val Thr Ala Gly Val His Ser Gln Val
            -10                 -5                  1

CAG CTG CAG CAG TCT GGG GCT GAA CTG GCA AGA CCT GGG GCC TCA GTG     151
Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala Ser Val
    5                   10                  15

AAG ATG TCC TGC AAG GCT TCT GGC TAC ACC TTT ACT AGG TAC ACG ATG     199
Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr Thr Met
20                  25                  30

CAC TGG GTA AAA CAG AGG CCT GGA CAG GGT CTG GAA TGG ATT GGA TAC     247
His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Tyr
35                  40                  45                  50

ATT AAT CCT AGC CGT GGT TAT ACT AAT TAC ATT CAG AAG TTC AAG GAC     295
Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe Lys Asp
                55                  60                  65

AAG GCC ACA TTG ACT ACA GAC AAA TCC TCC AGC ACA GCC TAC ATG CAA     343
Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln
            70                  75                  80

CTG AGC AGC CTG ACA TCT GAG GAC TCT GCA GTC TAT TAC TGT GCA AGA     391
Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg
        85                  90                  95

TAT TAT GAT GAT CAT TAC TGC CTT GAC TAC TGG GGC CAA GGC ACC ACT     439
Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly Thr Thr
    100                 105                 110

CTC ACA GTC TCC TCA GCC AAA ACA ACA GCC CCA TCG GTC TAT CCA CTG     487
Leu Thr Val Ser Ser Ala Lys Thr Thr Ala Pro Ser Val Tyr Pro Leu
115                 120                 125                 130

GCC CCT GTG TGT GGA GAT ACA ACT GGC TCC TCG GTG ACT CTA GGA TGC     535
Ala Pro Val Cys Gly Asp Thr Thr Gly Ser Ser Val Thr Leu Gly Cys
                135                 140                 145

CTG GTC AAG GGT TAT TTC CCT GAG CCA GTG ACC TTG ACC TGG AAC TCT     583
Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Leu Thr Trp Asn Ser
            150                 155                 160

GGA TCC CTG TCC AGT GGT GTG CAC ACC TTC CCA GCT GTC CTG CAG TCT     631
Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
        165                 170                 175

GAC CTC TAC ACC CTC AGC AGC TCA GTG ACT GTA ACC TCG AGC ACC TGG     679
```

-continued

```
Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Thr Ser Ser Thr Trp
    180                 185                 190

CCC AGC CAG TCC ATC ACC TGC AAT GTG GCC CAC CCG GCA AGC AGC ACC      727
Pro Ser Gln Ser Ile Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr
195                 200                 205                 210

AAG GTG GAC AAG AAA ATT GAG CCC AGA GGG CCC ACA ATC AAG CCC TGT      775
Lys Val Asp Lys Lys Ile Glu Pro Arg Gly Pro Thr Ile Lys Pro Cys
                215                 220                 225

CCT CCA TGC AAA TGC CCA GCA CCT AAC CTC TTG GGT GGA CCA TCC GTC      823
Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Leu Gly Gly Pro Ser Val
            230                 235                 240

TTC ATC TTC CCT CCA AAG ATC AAG GAT GTA CTC ATG ATC TCC CTG AGC      871
Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu Met Ile Ser Leu Ser
        245                 250                 255

CCC ATA GTC ACA TGT GTG GTG GTG GAT GTG AGC GAG GAT GAC CCA GAT      919
Pro Ile Val Thr Cys Val Val Val Asp Val Ser Glu Asp Asp Pro Asp
    260                 265                 270

GTC CAG ATC AGC TGG TTT GTG AAC AAC GTG GAA GTA CAC ACA GCT CAG      967
Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val His Thr Ala Gln
275                 280                 285                 290

ACA CAA ACC CAT AGA GAG GAT TAC AAC AGT ACT CTC CGG GTG GTC AGT     1015
Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Leu Arg Val Val Ser
                295                 300                 305

GCC CTC CCC ATC CAG CAC CAG GAC TGG ATG AGT GGC AAG GAG TTC AAA     1063
Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys Glu Phe Lys
            310                 315                 320

TGC AAG GTC AAC AAC AAA GAC CTC CCA GCG CCC ATC GAG AGA ACC ATC     1111
Cys Lys Val Asn Asn Lys Asp Leu Pro Ala Pro Ile Glu Arg Thr Ile
        325                 330                 335

TCA AAA CCC AAA GGG TCA GTA AGA GCT CCA CAG GTA TAT GTC TTG CCT     1159
Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln Val Tyr Val Leu Pro
    340                 345                 350

CCA CCA GAA GAA GAG ATG ACT AAG AAA CAG GTC ACT CTG ACC TGC ATG     1207
Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val Thr Leu Thr Cys Met
355                 360                 365                 370

GTC ACA GAC TTC ATG CCT GAA GAC ATT TAC GTG GAG TGG ACC AAC AAC     1255
Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val Glu Trp Thr Asn Asn
                375                 380                 385

GGG AAA ACA GAG CTA AAC TAC AAG AAC ACT GAA CCA GTC CTG GAC TCT     1303
Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu Pro Val Leu Asp Ser
            390                 395                 400

GAT GGT TCT TAC TTC ATG TAC AGC AAG CTG AGA GTG GAA AAG AAG AAC     1351
Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg Val Glu Lys Lys Asn
        405                 410                 415

TGG GTG GAA AGA AAT AGC TAC TCC TGT TCA GTG GTC CAC GAG GGT CTG     1399
Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val Val His Glu Gly Leu
    420                 425                 430

CAC AAT CAC CAC ACG ACT AAG AGC TTC TCC CGG ACT CCG GGT AAA         1444
His Asn His His Thr Thr Lys Ser Phe Ser Arg Thr Pro Gly Lys
435                 440                 445

TGAGCTCAGC ACCCACAAAA CTCTCAGGTC CAAAGAGACA CCCACACTCA TCTCCATGCT   1504

TCCCTTGTAT AAATAAAGCA CCCAGCAATG CCTGGGACCA TGTAAAAAAA AAAAAAAAAG   1564

GAATTC                                                              1570
```

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 468 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

-continued

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Met Glu Arg His Trp Ile Phe Leu Leu Leu Leu Ser Val Thr Ala Gly
-19                 -15                 -10                  -5

Val His Ser Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg
  1                   5                  10

Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe
 15                  20                  25

Thr Arg Tyr Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu
 30                  35                  40                  45

Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn
                 50                  55                  60

Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser
 65                  70                  75

Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val
 80                  85                  90

Tyr Tyr Cys Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp
 95                 100                 105

Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Ala Pro
110                 115                 120                 125

Ser Val Tyr Pro Leu Ala Pro Val Cys Gly Asp Thr Thr Gly Ser Ser
                130                 135                 140

Val Thr Leu Gly Cys Leu Val Lys Gly Thr Phe Pro Glu Pro Val Thr
145                 150                 155

Leu Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro
160                 165                 170

Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val
175                 180                 185

Thr Ser Ser Thr Trp Pro Ser Gln Ser Ile Thr Cys Asn Val Ala His
190                 195                 200                 205

Pro Ala Ser Ser Thr Lys Val Asp Lys Lys Ile Glu Pro Arg Gly Pro
                210                 215                 220

Thr Ile Lys Pro Cys Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Leu
225                 230                 235

Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu
240                 245                 250

Met Ile Ser Leu Ser Pro Ile Val Thr Cys Val Val Val Asp Val Ser
255                 260                 265

Glu Asp Asp Pro Asp Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu
270                 275                 280                 285

Val His Thr Ala Gln Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr
                290                 295                 300

Leu Arg Val Val Ser Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser
305                 310                 315

Gly Lys Glu Phe Lys Cys Lys Val Asn Asn Lys Asp Leu Pro Ala Pro
320                 325                 330

Ile Glu Arg Thr Ile Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln
335                 340                 345

Val Tyr Val Leu Pro Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val
350                 355                 360                 365

Thr Leu Thr Cys Met Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val
                370                 375                 380
```

-continued

```
Glu Trp Thr Asn Asn Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu
385                 390                 395

Pro Val Leu Asp Ser Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg
400                 405                 410

Val Glu Lys Lys Asn Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val
415                 420                 425

Val His Glu Gly Leu His Asn His His Thr Thr Lys Ser Phe Ser Arg
430                 435                 440                 445

Thr Pro Gly Lys
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                  10                  15

Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Ile Lys Tyr
            20                  25                  30

Leu Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Thr Glu Ala Ser Asn Leu Gln Ala Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Ile Ala Thr
                85
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```
Tyr Tyr Cys Gln Gln Tyr Gln Ser Leu Pro Tyr Thr Phe Gly Gln Gly
1               5                  10                  15

Thr Lys Leu Gln Ile Thr Arg
            20
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 126 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Ser Ser Ser Gly Phe Ile Phe Ser Ser Tyr
            20                  25                  30
```

-continued

```
Ala Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
            35                  40                  45

Ala Ile Ile Trp Asp Asp Gly Ser Asp Gln His Tyr Ala Asp Ser Val
        50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Asp Gly Gly His Gly Phe Cys Ser Ser Ala Ser Cys Phe Gly
            100                 105                 110

Pro Asp Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser
        115                 120                 125
```

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

```
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1                  5                  10                  15

Ser Leu Arg Leu Ser Cys Ser Ser Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
            35                  40                  45

Ala Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe
        50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1                  5                  10                  15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
            35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
        50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65                  70                  75                  80
```

-continued

```
Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1               5                  10                  15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1               5                  10                  15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Asn Thr Ala Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
```

-continued

115

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
50 55 60

Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Ala Phe
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
50 55 60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Asn Thr Leu Phe
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

-continued

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

```
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1               5                  10                  15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1               5                  10                  15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1               5                  10                  15
```

-continued

```
Ser Leu Arg Leu Ser Cys Ser Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ser Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ser Ser Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60
```

-continued

```
Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
 65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1               5                  10                  15

Ser Leu Arg Leu Ser Cys Ser Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
 65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1               5                  10                  15

Ser Leu Arg Leu Ser Cys Ser Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
 65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
```

-continued

100 105 110

Thr Thr Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ser Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
50 55 60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
20 25 30

Asn Trp Tyr Gly Gln Thr Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu
65 70 75 80

Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr Arg
100 105

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid

(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:26:

```
Gln Ile Val Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1               5                  10                  15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
            20                  25                  30

Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr
        35                  40                  45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu
65                  70                  75                  80

Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr
                85                  90                  95

Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr Arg
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```
Gln Ile Val Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1               5                  10                  15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
            20                  25                  30

Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr
        35                  40                  45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu
65                  70                  75                  80

Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr
                85                  90                  95

Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr Arg
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:28:

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1               5                  10                  15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
            20                  25                  30

Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr
        35                  40                  45
```

-continued

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu
65 70 75 80

Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr Arg
100 105

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Gln Ile Val Leu Thr Gln Ser Pro Ala Ile
1 5 10

Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser Ala Ser
15 20 25

Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser
30 35 40

Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro
45 50 55

Ala His Phe Arg Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile
60 65 70

Ser Gly Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp
75 80 85

Ser Ser Asn Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Asn
90 95 100 105

Arg

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg
1 5 10

Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe
15 20 25

Thr Arg Tyr Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu
30 35 40 45

Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn
50 55 60

Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser
65 70 75

Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val
80 85 90

Tyr Tyr Cys Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp
95 100 105

-continued

```
Gly Gln Gly Thr Thr Leu Thr Val Ser Ser
110                 115
```

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 135 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:31:

```
Met Gly Trp Ser Trp Ile Phe Leu Phe Leu Leu Ser Gly Thr Ala Gly
 1               5                  10                  15

Val His Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys
            20                  25                  30

Pro Gly Ser Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
        35                  40                  45

Thr Ser Tyr Arg Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
    50                  55                  60

Glu Trp Ile Gly Tyr Ile Asn Pro Ser Thr Gly Tyr Thr Glu Tyr Asn
65                  70                  75                  80

Gln Lys Phe Lys Asp Lys Ala Thr Ile Thr Ala Asp Glu Ser Thr Asn
                85                  90                  95

Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
            100                 105                 110

Tyr Tyr Cys Ala Arg Gly Gly Gly Val Phe Asp Tyr Trp Gly Gln Gly
        115                 120                 125

Thr Leu Val Thr Val Ser Ser
    130                 135
```

We claim:

1. An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues corresponding to the equivalent residues in a donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 6 23, 24, and 49 at least are donor residues, provided that said composite heavy chain does not comprise the amino acid sequence of SEQ ID NO:31.

2. The antibody molecule of claim **1**, wherein amino acid residues 26 to 30 and 59 to 65 in said composite heavy chain are additionally donor residues.

3. The antibody molecule of claim **1**, wherein amino acid residues 71, 73, and 78 in said composite heavy chain are additionally donor residues.

4. The antibody molecule of claim **1**, wherein at least one of amino acid residues 1, 3, and 76 in said composite heavy chain are additionally donor residues.

5. The antibody molecule of claim **1**, wherein at least one of amino acid residues 36, 94, 104, 106, and 107 in said composite heavy chain are additionally donor residues.

6. The antibody molecule of claim **5**, wherein at least one of amino acid residues 2, 4, 38, 46, 67, and 69 in said composite heavy chain are additionally donor residues.

7. The antibody molecule of claim **1**, wherein said complementary light chain is a composite light chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody light chain framework residues, the remaining light chain residues corresponding to the equivalent residues in a donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite light chain; said CDRs comprise donor residues at least at residues 24 to 34, 50 to 56, and 89 to 97; and amino acid residues 46, 48, 58, and 71 at least are donor residues.

8. The antibody molecule of claim **7**, wherein amino acid residues 1, 3, 60 (if this residue can form a salt bridge with residue 54), and 70 (if this residue can form a salt bridge with residue 24) in said composite light chain are additionally donor residues.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,859,205
DATED : January 12, 1999
INVENTOR(S) : Adair et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title page,
Item [30], **Foreign Application Priority Data** section thereof:
Please insert -- PCT/GB90/02017, International Filing Date: December 21, 1990 --
after "Dec. 21, 1989, [GB], United Kingdom, 8928874".

Item [56], **References Cited**, U.S. PATENT DOCUMENTS section after
4,348,376, 9/1982, Goldberg., please insert -- 5,225,539, 7/1993, Winter . --
After 5,225,539, 7/1993, Winter.,
Please insert -- 5,585,089, 12/1996, Queen et al. . --

FOREIGN PATENT DOCUMENTS section at 0239400 A2, 3/1987, European Pat. Off. .
Please delete "0239400 A2" and insert -- 0 239 400 A2 --
At A1 0323806, 7/1989, European Pat. Off. .
Please delete "Al 0323806" and insert -- 0 323 806 A1 --

OTHER PUBLICATIONS section at Chothia, Cyrus et al (Dec. 1989) *Nature,* "Conformations of Immunoglobulin Hypervariable Regions", vol. 342, pp. 877-883., it should read:

-- Chothia et al., "Conformations of Immunoglobulin Hypervariable Regions", *Nature,* 342:877-883, Dec., 1989. --

At Queen, C. et al (Dec. 1989) Proceedings of the National Academy of Sciences, "A Humanized Antibody That Binds to Interleukin 2 Receptor" vol. 86, pp. 10029-10033., it should read:

-- Queen et al., "A Humanized Antibody that Binds to the Interleukin 2 Receptor," *Proceedings of the National Academy of Sciences, USA,* 86:10029-10033, Dec., 1989. --

At Reichmann et al (Mar. 1988) Nature, "Reshaping Human Antibodies for Therapy," vol. 332, pp. 323-327., it should read:

-- Reichmann et al., "Reshaping Human Antibodies for Therapy," *Nature,* 332:323-327, Mar. 1988. --

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,859,205
DATED : January 12, 1999
INVENTOR(S) : Adair et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Item [56], **References Cited**, OTHER PUBLICATIONS section at Roberts et al. "Generation of Antibody with Enhanced Affinity and Specificity for its Antigen by Protein Engineering" Nature, 328(20):731-734, Aug., 1987., it should read:

-- Roberts et al., "Generation of Antibody with Enhanced Affinity and Specificity for its Antigen by Protein Engineering," *Nature,* 328(20):731-734, Aug., 1987. --

At Verhoeyen et al. "Reshaping Human Antibodies: Grafting an Antilysozyme Activity", Science, 239:1534-36 Mar. 25, 1988., it should read:

-- Verhoeyen et al., "Reshaping Human Antibodies: Grafting an Antilysozyme Activity", *Science,* 239:1534-36, Mar., 1988. --

At Jones et al. "Replacing the complementarity-Determining Regions in a Human Antibody with those from a Mouse", Nature, 321:522-525, 1986., it should read:

-- Jones et al., "Replacing the complementarity-Determining Regions in a Human Antibody with those from a Mouse," *Nature,* 321:522-525, May, 1986. --

At Ward et al. "Binding activities of a Repertoire of Single Immunoglobulin Variable Domains Secreted from *Escherichia coli*", *Nature*, 341:544-546, 1989., it should read:

-- Ward et al., "Binding activities of a Repertoire of Single Immunoglobulin Variable Domains Secreted from *Escherichia coli*," *Nature*, 341:544-546 Oct., 1989. --

Drawings,
Please replace Sheet 8 of 18, FIG. 5c with new Sheet 8 of 18 FIG. 5c attached.
Please replace Sheet 9 of 18, FIG. 6 with new Sheet 9 of 18 FIG. 6 attached. .

Column 2,
Line 65, "complete antigens" should read -- complex antigens --.

Column 3,
Line 59, "not: coincide" should read -- not coincide --.

Column 5,
Between lines 37 and 38, insert -- 63, --.
Line 45, "regions; of " should read -- regions of --.

Column 7,
Line 32, "FV fragments; and" should read -- FV fragments and --.

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 8,
Line 23, "The the present" should read -- The present --.

Column 10,
Line 20, please make "2.1.2 Light Chain...70+24." a new paragraph.
Line 40, "with 33 and 46" should read -- with 38 and 46 --.

Column 11,
Line 29, "FIGS. **2*a*** and **2*b*** shows" should read -- FIGS. **2*a*** and **2*b*** show --.
Line 30, "heavy chain;" should read -- heavy chain (SEQ ID NO:6 and 7); --.
Line 43, "(SEQ ID NO:29, 9 and 25)" should read -- (SEQ ID NO:29, 8, 9 and 25-28) --.
Line 45, "antibodies' " should read -- antibodies; --.

Column 12,
Line 39, "chimeric: or CDR-grafted" should read -- chimeric or CDR-grafted --.

Column 13,
Line 4, please make "In this system...cytofluorography." a new paragraph.

Column 14,
Line 51, "[FIGS. **1**(***a***) and" should read -- [FIGS. **1**(***a***)(SEQ ID NO:4) and --.
Line 53, "[FIGS. **1**(***b***) and" should read -- [FIGS. **1**(***b***)(SEQ ID NO:5) and --.

Column 18,
Line 28, "Residues underlined in FIG. **3**" should read -- Residues underlined in FIG. **3** (SEQ ID NO:29, 8 and 9) --.
Line 51, "ID NO:7" should read -- ID NO:30 --.

Column 21,
Line 56, "15.1. Light Chian" should read -- 15.1. Light Chain --.

Column 22,
Line 15, "15.1.2. Framework Resides" should read -- 15.1.2. Framework Residues --.
Line 29, "gL221B (SEQ ID NO:28)(gL221+D1Q, Q3V) and gL221C" should read -- gL221B (gL221 +D1Q, Q3V) and gL221 C (SEQ ID N0:28) --.
Line 33, "When the gL121 A (gL124+D1Q, Q3V" should read -- When the gL121A (gL121+D1Q, Q3V --.

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 24,
Line 16, "individual contribution of othe other 8 mouse residues of the" should read -- individual contribution of other 8 mouse residues of the --.
Table 2, on the same line as the second gH341*, "R N N A G F" should read --R N N A G F --.
Table 2, on the same line as the first gH341B, "E S S G V" should read -- E S S I G V --.
Table 2, on the same line as the sixth gH341*, "Q S A I G V" should read -- Q S A I G V --.
Table 2, on the same line as the eighth gH341*, "Q S A I G V" should read -- Q S A I G V --.

Column 25,
Line 47, "basic grafted product has neglibible binding ability aLs" should read -- basic grafted product has neglibible binding ability as --.

Column 28,
Line 55, "body. In KOL heavy chain (SEQ ID NO:10), position 831 is" should read -- body. In KOL heavy chain (SEQ ID NO:10), position 81 is --.

Column 29,
Line 17, "CDR-graftin of a Murine Anti-ICAM-1 Monoclonal" should read -- CDR-grafting of a Murine Anti-ICAM-1 Monoclonal --.
Line 49, "50-56 (CDR2) and94-100B (CDR3). In addition murine" should read -- 50-56 (CDR2) and 94-100B (CDR3). In addition murine --.
Line 57, "CDR-Grafting of Murine Anti-TNFa Antibodies" should read -- CDR-Grafting of Murine Anti-TNFα Antibodies --.
Line 58, "A number of murine anti-TNFa monoclonal antibodies" should read -- A number of murine anti-TNFα monoclonal antibodies --.

Column 30,
Line 38, "wre used at positions 24-34 (CDR1), 50-56 (CDR2) and" should read -- were used at positions 24-34 (CDR1), 50-56 (CDR2) and --.
Line 67, "receptor on L929 ells for TNF-a compared to hTNF3" should read -- receptor on L929 ells for TNF-α compared to hTNF3 --.

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,859,205
DATED : January 12, 1999
INVENTOR(S) : Adair et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 31,
Line 2, "(+23, 24, 48, 49 71 and 73 as mouse) genes have been built" should read -- (+23, 24, 48, 49, 71 and 73 as mouse) genes have been built --.
Line 4, "binds well to TNF-a, but competes very poorly in the L929" should read -- binds well to TNF-α, but competes very poorly in the L929 --.
Line 11, "recognise human TNF-a. The heavy chain of this antibody" should read -- recognise human TNF-α. The heavy chain of this antibody --.
Line 23, please make "Mouse residues at other positions...assay." a new paragraph.

Column 32,
Line 22, in the REFERENCES section "13. Kramer, W., Drutsa, V., Jansen, H.-W., Kramer, B., Plugfelder, M., Fritz, H.-J., 1934, Nucl. Acids. Res. 12, 9441" should read -- 13. Kramer, W., Drutsa, V., Jansen, H.-W., Kramer, B., Plugfelder, M., Fritz, H.-J., 1984, Nucl. Acids. Res. 12, 9441 --

IN THE SEQUENCE LISTING:
Please replace the Sequence Listing with the attached Sequence Listing.

Column 63,
Line 52, "residues 6 23, 24, and 49 at least are donor residues." should read -- residues 6, 23, 24, and 49 at least are donor residues. --.

Signed and Sealed this

Twelfth Day of November, 2002

Attest:

Attesting Officer

JAMES E. ROGAN
Director of the United States Patent and Trademark Office

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Adair, John R.
Athwal, Diljeet S.
Emtage, John S.

(ii) TITLE OF INVENTION: Humanised Antibodies

(iii) NUMBER OF SEQUENCES: 30

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Woodcock Washburn Kurtz Mackiewicz & Norris
(B) STREET: One Liberty Place - 46th Floor
(C) CITY: Philadelphia
(D) STATE: PA
(E) COUNTRY: USA
(F) ZIP: 19103

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/303,569
(B) FILING DATE: 07-SEP-1994
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Trujillo, Doreen Yatko
(B) REGISTRATION NUMBER: 35,719
(C) REFERENCE/DOCKET NUMBER: CARP-0032

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (215) 568-3100
(B) TELEFAX: (215) 568-3439

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TCCAGATGTT AACTGCTCAC 20

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CAGGGGCCAG TGGATGGATA GAC 23

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Leu Glu Ile Asn Arg Thr Val Ala Ala
1 5

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 943 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 18..722

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 84..722

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
GAATTCCCAA AGACAAA ATG GAT TTT CAA GTG CAG ATT TTC AGC TTC CTG            50
                   Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu
                   -22     -20                 -15

CTA ATC AGT GCC TCA GTC ATA ATA TCC AGA GGA CAA ATT GTT CTC ACC           98
Leu Ile Ser Ala Ser Val Ile Ile Ser Arg Gly Gln Ile Val Leu Thr
    -10                 -5                  1               5

CAG TCT CCA GCA ATC ATG TCT GCA TCT CCA GGG GAG AAG GTC ACC ATG          146
Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met
                10                  15                  20

ACC TGC AGT GCC AGC TCA AGT GTA AGT TAC ATG AAC TGG TAC CAG CAG          194
Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln
            25                  30                  35

AAG TCA GGC ACC TCC CCC AAA AGA TGG ATT TAT GAC ACA TCC AAA CTG          242
Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu
        40                  45                  50

GCT TCT GGA GTC CCT GCT CAC TTC AGG GGC AGT GGG TCT GGG ACC TCT          290
Ala Ser Gly Val Pro Ala His Phe Arg Gly Ser Gly Ser Gly Thr Ser
    55                  60                  65

TAC TCT CTC ACA ATC AGC GGC ATG GAG GCT GAA GAT GCT GCC ACT TAT          338
Tyr Ser Leu Thr Ile Ser Gly Met Glu Ala Glu Asp Ala Ala Thr Tyr
70                  75                  80                  85

TAC TGC CAG CAG TGG AGT AGT AAC CCA TTC ACG TTC GGC TCG GGG ACA          386
Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr Phe Gly Ser Gly Thr
                90                  95                  100

AAG TTG GAA ATA AAC CGG GCT GAT ACT GCA CCA ACT GTA TCC ATC TTC          434
Lys Leu Glu Ile Asn Arg Ala Asp Thr Ala Pro Thr Val Ser Ile Phe
            105                 110                 115

CCA CCA TCC AGT GAG CAG TTA ACA TCT GGA GGT GCC TCA GTC GTG TGC          482
Pro Pro Ser Ser Glu Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys
        120                 125                 130
```

```
TTC TTG AAC AAC TTC TAC CCC AAA GAC ATC AAT GTC AAG TGG AAG ATT          530
Phe Leu Asn Asn Phe Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile
    135                 140                 145

GAT GGC AGT GAA CGA CAA AAT GGC GTC CTG AAC AGT TGG ACT GAT CAG          578
Asp Gly Ser Glu Arg Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln
150                 155                 160                 165

GAC AGC AAA GAC AGC ACC TAC AGC ATG AGC AGC ACC CTC ACG TTG ACC          626
Asp Ser Lys Asp Ser Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr
                170                 175                 180

AAG GAC GAG TAT GAA CGA CAT AAC AGC TAT ACC TGT GAG GCC ACT CAC          674
Lys Asp Glu Tyr Glu Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His
            185                 190                 195

AAG ACA TCA ACT TCA CCC ATT GTC AAG AGC TTC AAC AGG AAT GAG TGT          722
Lys Thr Ser Thr Ser Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
        200                 205                 210

TAGAGACAAA GGTCCTGAGA CGCCACCACC AGCTCCCAGC TCCATCCTAT CTTCCCTTCT        782

AAGGTCTTGG AGGCTTCCCC ACAAGCGCTT ACCACTGTTG CGGTGCTCTA AACCTCCTCC        842

CACCTCCTTC TCCTCCTCCT CCCTTTCCTT GGCTTTTATC ATGCTAATAT TTGCAGAAAA        902

TATTCAATAA AGTGAGTCTT TGCCTTGAAA AAAAAAAAAA A                            943
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 235 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser
-22      -20                 -15                 -10

Val Ile Ile Ser Arg Gly Gln Ile Val Leu Thr Gln Ser Pro Ala Ile
     -5                  1                   5                  10

Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser Ala Ser
                15                  20                  25
```

```
Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser
          30                  35                  40

Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro
      45                  50                  55

Ala His Phe Arg Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile
  60                  65                  70

Ser Gly Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp
75                  80                  85                  90


Ser Ser Asn Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Asn
              95                  100                 105

Arg Ala Asp Thr Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu
          110                 115                 120

Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe
      125                 130                 135

Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg
  140                 145                 150

Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser
155                 160                 165                 170

Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu
              175                 180                 185

Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser
          190                 195                 200

Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
      205                 210
```

(2) INFORMATION FOR SEQ ID NO:6:

SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1570 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 41..1444

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 98..1444

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
GAATTCCCCT CTCCACAGAC ACTGAAAACT CTGACTCAAC ATG GAA AGG CAC TGG          55
                                            Met Glu Arg His Trp
                                            -19             -15

ATC TTT CTA CTC CTG TTG TCA GTA ACT GCA GGT GTC CAC TCC CAG GTC          103
Ile Phe Leu Leu Leu Leu Ser Val Thr Ala Gly Val His Ser Gln Val
               -10                 -5                   1

CAG CTG CAG CAG TCT GGG GCT GAA CTG GCA AGA CCT GGG GCC TCA GTG          151
Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala Ser Val
          5                  10                  15

AAG ATG TCC TGC AAG GCT TCT GGC TAC ACC TTT ACT AGG TAC ACG ATG          199
Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr Thr Met
     20                  25                  30

CAC TGG GTA AAA CAG AGG CCT GGA CAG GGT CTG GAA TGG ATT GGA TAC          247
His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Tyr
 35                  40                  45                  50

ATT AAT CCT AGC CGT GGT TAT ACT AAT TAC ATT CAG AAG TTC AAG GAC          295
Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe Lys Asp
                55                  60                  65

AAG GCC ACA TTG ACT ACA GAC AAA TCC TCC AGC ACA GCC TAC ATG CAA          343
Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln
           70                  75                  80

CTG AGC AGC CTG ACA TCT GAG GAC TCT GCA GTC TAT TAC TGT GCA AGA          391
Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg
      85                  90                  95

TAT TAT GAT GAT CAT TAC TGC CTT GAC TAC TGG GGC CAA GGC ACC ACT          439
Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly Thr Thr
 100                 105                 110

CTC ACA GTC TCC TCA GCC AAA ACA ACA GCC CCA TCG GTC TAT CCA CTG          487
Leu Thr Val Ser Ser Ala Lys Thr Thr Ala Pro Ser Val Tyr Pro Leu
115                 120                 125                 130
```

```
GCC CCT GTG TGT GGA GAT ACA ACT GGC TCC TCG GTG ACT CTA GGA TGC          535
Ala Pro Val Cys Gly Asp Thr Thr Gly Ser Ser Val Thr Leu Gly Cys
                135                 140                 145

CTG GTC AAG GGT TAT TTC CCT GAG CCA GTG ACC TTG ACC TGG AAC TCT          583
Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Leu Thr Trp Asn Ser
            150                 155                 160

GGA TCC CTG TCC AGT GGT GTG CAC ACC TTC CCA GCT GTC CTG CAG TCT          631
Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
        165                 170                 175

GAC CTC TAC ACC CTC AGC AGC TCA GTG ACT GTA ACC TCG AGC ACC TGG          679
Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Thr Ser Ser Thr Trp
    180                 185                 190

CCC AGC CAG TCC ATC ACC TGC AAT GTG GCC CAC CCG GCA AGC AGC ACC          727
Pro Ser Gln Ser Ile Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr
195                 200                 205                 210

AAG GTG GAC AAG AAA ATT GAG CCC AGA GGG CCC ACA ATC AAG CCC TGT          775
Lys Val Asp Lys Lys Ile Glu Pro Arg Gly Pro Thr Ile Lys Pro Cys
                215                 220                 225

CCT CCA TGC AAA TGC CCA GCA CCT AAC CTC TTG GGT GGA CCA TCC GTC          823
Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Leu Gly Gly Pro Ser Val
            230                 235                 240

TTC ATC TTC CCT CCA AAG ATC AAG GAT GTA CTC ATG ATC TCC CTG AGC          871
Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu Met Ile Ser Leu Ser
        245                 250                 255

CCC ATA GTC ACA TGT GTG GTG GTG GAT GTG AGC GAG GAT GAC CCA GAT          919
Pro Ile Val Thr Cys Val Val Val Asp Val Ser Glu Asp Asp Pro Asp
    260                 265                 270

GTC CAG ATC AGC TGG TTT GTG AAC AAC GTG GAA GTA CAC ACA GCT CAG          967
Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val His Thr Ala Gln
275                 280                 285                 290

ACA CAA ACC CAT AGA GAG GAT TAC AAC AGT ACT CTC CGG GTG GTC AGT         1015
Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Leu Arg Val Val Ser
                295                 300                 305

GCC CTC CCC ATC CAG CAC CAG GAC TGG ATG AGT GGC AAG GAG TTC AAA         1063
Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys Glu Phe Lys
            310                 315                 320

TGC AAG GTC AAC AAC AAA GAC CTC CCA GCG CCC ATC GAG AGA ACC ATC         1111
Cys Lys Val Asn Asn Lys Asp Leu Pro Ala Pro Ile Glu Arg Thr Ile
        325                 330                 335
```

```
TCA AAA CCC AAA GGG TCA GTA AGA GCT CCA CAG GTA TAT GTC TTG CCT        1159
Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln Val Tyr Val Leu Pro
    340                 345                 350

CCA CCA GAA GAA GAG ATG ACT AAG AAA CAG GTC ACT CTG ACC TGC ATG        1207
Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val Thr Leu Thr Cys Met
355                 360                 365                 370

GTC ACA GAC TTC ATG CCT GAA GAC ATT TAC GTG GAG TGG ACC AAC AAC        1255
Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val Glu Trp Thr Asn Asn
                375                 380                 385

GGG AAA ACA GAG CTA AAC TAC AAG AAC ACT GAA CCA GTC CTG GAC TCT        1303
Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu Pro Val Leu Asp Ser
            390                 395                 400

GAT GGT TCT TAC TTC ATG TAC AGC AAG CTG AGA GTG GAA AAG AAG AAC        1351
Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg Val Glu Lys Lys Asn
        405                 410                 415


TGG GTG GAA AGA AAT AGC TAC TCC TGT TCA GTG GTC CAC GAG GGT CTG        1399
Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val Val His Glu Gly Leu
    420                 425                 430


CAC AAT CAC CAC ACG ACT AAG AGC TTC TCC CGG ACT CCG GGT AAA            1444
His Asn His His Thr Thr Lys Ser Phe Ser Arg Thr Pro Gly Lys
435                 440                 445

TGAGCTCAGC ACCCACAAAA CTCTCAGGTC CAAAGAGACA CCCACACTCA TCTCCATGCT      1504

TCCCTTGTAT AAATAAAGCA CCCAGCAATG CCTGGGACCA TGTAAAAAAA AAAAAAAAAG      1564

GAATTC                                                                 1570
```

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 468 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Met Glu Arg His Trp Ile Phe Leu Leu Leu Leu Ser Val Thr Ala Gly
-19             -15                 -10                 -5
```

```
Val His Ser Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg
             1               5                  10

Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe
    15                  20                  25

Thr Arg Tyr Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu
 30                  35                  40                  45

Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn
                50                  55                  60

Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser
            65                  70                  75

Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val
         80                  85                  90

Tyr Tyr Cys Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp
     95                  100                 105

Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Ala Pro
110                 115                 120                 125

Ser Val Tyr Pro Leu Ala Pro Val Cys Gly Asp Thr Thr Gly Ser Ser
                130                 135                 140

Val Thr Leu Gly Cys Leu Val Lys Gly Thr Phe Pro Glu Pro Val Thr
            145                 150                 155

Leu Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro
        160                 165                 170

Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val
    175                 180                 185

Thr Ser Ser Thr Trp Pro Ser Gln Ser Ile Thr Cys Asn Val Ala His
190                 195                 200                 205

Pro Ala Ser Ser Thr Lys Val Asp Lys Lys Ile Glu Pro Arg Gly Pro
                210                 215                 220

Thr Ile Lys Pro Cys Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Leu
            225                 230                 235

Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu
        240                 245                 250

Met Ile Ser Leu Ser Pro Ile Val Thr Cys Val Val Val Asp Val Ser
    255                 260                 265
```

```
Glu Asp Asp Pro Asp Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu
270                 275                 280                 285

Val His Thr Ala Gln Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr
                290                 295                 300

Leu Arg Val Val Ser Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser
            305                 310                 315

Gly Lys Glu Phe Lys Cys Lys Val Asn Asn Lys Asp Leu Pro Ala Pro
        320                 325                 310

Ile Glu Arg Thr Ile Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln
    335                 340                 345

Val Tyr Val Leu Pro Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val
350                 355                 360                 365

Thr Leu Thr Cys Met Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val
                370                 375                 380

Glu Trp Thr Asn Asn Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu
            385                 390                 395

Pro Val Leu Asp Ser Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg
        400                 405                 410

Val Glu Lys Lys Asn Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val
    415                 420                 425

Val His Glu Gly Leu His Asn His His Thr Thr Lys Ser Phe Ser Arg
430                 435                 440                 445

Thr Pro Gly Lys
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15
```

```
Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Ile Lys Tyr
            20                  25                  30

Leu Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Thr Glu Ala Ser Asn Leu Gln Ala Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Ile Ala Thr
                85
```

2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```
Tyr Tyr Cys Gln Gln Tyr Gln Ser Leu Pro Tyr Thr Phe Gly Gln Gly
1               5                   10                  15

Thr Lys Leu Gln Ile Thr Arg
            20
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 126 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15
```

```
Ser Leu Arg Leu Ser Cys Ser Ser Ser Gly Phe Ile Phe Ser Ser Tyr
            20                  25                  30

Ala Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Ile Ile Trp Asp Asp Gly Ser Asp Gln His Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Thy Phe Cys
                85                  90                  95

Ala Arg Asp Gly Gly His Gly Phe Cys Ser Ser Ala Ser Cys Phe Gly
            100                 105                 110

Pro Asp Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser
        115                 120                 125
```

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

```
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1                   5                  10                  15

Ser Leu Arg Leu Ser Cys Ser Ser Ser Gly Try Thr Phe Thr Arg Tyr
            20                  25                  30

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95
```

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
50 55 60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60
```

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Asn Thr Ala Phe
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
50 55 60

Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Ala Phe
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Asn Thr Leu Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

```
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                  10                  15
```

```
Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95
```

```
Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ser Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Ser Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Ser Ser Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60
```

```
Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ser Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Ash Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110
```

Thr Thr Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ser Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
50 55 60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ser Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CIARACTERISTICS:
(A) LENGTI: 107 amino acids
(B) TYPE: amino acid
(D) TOPOLDGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
            20                  25                  30

Asn Trp Tyr Gly Gln Thr Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
        35                  40                  45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
    50                  55                  60
```

```
Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu
65                  70                  75                  80

Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr
                85                  90                  95

Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr Arg
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTI: 107 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

```
Gln Ile Val Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
            20                  25                  30

Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr
        35                  40                  45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu
65                  70                  75                  80

Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr
                85                  90                  95

Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr Arg
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```
Gln Ile Val Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                  10                  15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
            20                  25                  30

Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
        35                  40                  45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu
65                  70                  75                  80

Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr
                85                  90                  95

Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr Arg
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTI: 107 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                  10                  15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
            20                  25                  30

Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr
        35                  40                  45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu
65                  70                  75                  80
```

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30

```
            Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg
            1               5                   10

Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe
     15                  20                  25

Thr Arg Tyr Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu
 30                  35                  40                  45

Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn
                50                  55                  60

Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser
             65                  70                  75

Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val
          80                  85                  90

Tyr Tyr Cys Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp
     95                  100                 105

Gly Gln Gly Thr Thr Leu Thr Val Ser Ser
110                 115
```

4-19-99
Amdt
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DOCKET NO.: CARP-0057 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of: **Adair et al.**

Serial No.: **08/846,658** Group No.: **1642**

Filed: **May 1, 1997** Examiner: **J. Reeves**

For: **Humanised Antibodies**

I, Doreen Yatko Trujillo, Registration No. 35,179 certify that this correspondence is being deposited with the U.S. Postal Service as First Class Mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

On April 9, 1999
Doreen Yatko Trujillo
Doreen Yatko Trujillo Reg. No. 35,179

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

AMENDMENT AND REQUEST FOR RECONSIDERATION

Pursuant to 37 C.F.R. § 1.115, please amend the above-identified application as a follows.

In the specification:

Page 1, after "September 7, 1994," please insert -- now U.S. Patent No. 5.859,205, --.

Page 1, after "September 17, 1991," please insert -- abandoned, --.

In the claims:

24. (Amended) A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least

H1

10^8 M^{-1}, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions, wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of said donor amino acids is adjacent to a CDR in the donor immunoglobulin sequence or contributes to antigen binding as determined by X-ray crystallography.

28. (Amended) A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an effective antigen binding affinity, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions, wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of said donor amino acids is adjacent to a CDR in the donor immunoglobulin sequence or contributes to antigen binding as determined by X-ray crystallography.

29. (Amended) A humanized immunoglobulin according to claim 28 which specifically binds to an antigen with a binding affinity [equivalent to that of a chimeric antibody formed from] similar to that of said donor immunoglobulin.

REMARKS

This paper is being filed in response to the Office Action dated November 16, 1998. A petition for a two-month extension of time and the appropriate fee accompany this response.

Claims 24-31 are pending. In the Office Action, all pending claims were rejected. In view of the foregoing amendments and the arguments that follow, Applicants

respectfully submit that allowable subject matter has been identified and request that the interference be declared.

Preliminarily, as requested by the Examiner, the specification has been amended to update the status of parent applications.

Additionally, the Examiner stated that the Information Disclosure Statements filed in the parent cases will not be considered unless they are filed with the present case and the references have been submitted. This appears to be contrary to MPEP § 609, page 600-103, specifically. As stated therein, information that has been considered by the Office in a parent application of a FWC filed prior to December 1, 1997 will be part of the file and need not be resubmitted to have the information considered. Likewise, an Examiner will consider information that has been considered by the Office in a parent application when examining a continuation under 36 C.F.R. § 1.60. The present application is a continuation under 37 C.F.R. § 1.60 of prior Application Serial No. 08/303,569, filed September 7, 1994, which is a continuation under 37 C.F.R.§ 1.62 (i.e., FWC) of Application Serial No. 07/7443,329, filed September 17, 1991. According to MPEP § 609, then, information considered in both parent applications is to be considered by the Examiner.

Rejections Under 35 U.S.C. § 112, first paragraph

Claims 24-31 were rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors had possession of the claimed invention at the time the application was filed. Applicants respectfully traverse this rejection for the reasons that follow. For the Examiner's convenience, the paragraphs are designated to correspond to the Examiner's paragraphs under this section.

a. The Examiner rejected claims 24 and 28 alleging that the specification does not provide support for the concept that only substitutions adjacent CDRs are envisaged. Claims 24 and 28 have been amended herein to recite that each of the donor amino acids to be replaced is adjacent a CDR or contributes to antigen binding as determined by X-ray crystallography. Support for these amendments can be found, *inter alia*, on page 38, lines 1-

12, and lines 23 through 38, of the application as filed. As is clear therefrom, the contribution to antigen binding can be indirect, e.g., by affecting antigen binding site topology or inducing stable packing, i.e., the residues are spatially near a CDR. On page 17, lines 9-11, of the application as filed, the extents of the heavy chain CDRs are taught. On page 6, lines 25-35, it is indicated that the heavy chain "framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49...." Residue 49 is clearly adjacent a CDR. As evident from Figure 4, residues 6, 23, 24, and 48 contribute to antigen binding, as determined by X-ray crystallography. Applicants respectfully request that this rejection be withdrawn.

b. The Examiner rejected claims 27, 30, and 31, seeking evidence that CD3 is the same as "OKT3" and that CD4 is the same as "OKT4." Actually, one term refers to the antibody, while the other refers to the antigen bound. Specifically, OKT3 refers to a monoclonal antibody that recognizes the CD3 antigen and OKT4 refers to a monoclonal antibody that recognizes the CD4 antigen. Consistent therewith, on page 28, lines 19-22, of the application as filed, the testing of the ability of CDR-grafted OKT3 light chain to bind to CD3 positive cells is disclosed, and on page 52, line 29, of the application as filed, the reference "CD4 (OKT4)" is made. Applicants respectfully request that this rejection be withdrawn.

Claim 29 was rejected under 35 U.S.C. § 112, first paragraph, in view of the phrase "which specifically binds to an antigen with a binding affinity equivalent to that of a chimeric antibody formed from said donor immunoglobulin." The Examiner requested that Applicants point to support in the specification for the phrase. Claim 29 has been amended herein to recite that the binding affinity is "similar to that of" the donor. Support for this amendment can be found, *inter alia*, on page 48, lines 24-27 and page 51, lines 27-31 of the application as filed. Applicants respectfully request that this rejection be withdrawn.

Rejection Under 35 U.S.C. § 102(e)

Claims 24-31 were rejected under 35 U.S.C. § 102(e) in view of U.S. Patent No. 5,585,089 ("Queen et al."). Applicants respectfully traverse this rejection.

The Examiner observed that Queen et al. is entitled to priority back to "at least

12/28/90." (It is assumed that the Examiner meant 12/19/90, the filing date of the latest application designated as a continuation-in-part in the series of Queen et al. applications.) Although seeming to recognize that Queen et al. may not be entitled to a priority date earlier than 12/19/90, the Examiner, nonetheless, proceeded to argue that limitations recited in claims 24-31 are found in the earlier Queen et al. applications. The relevant inquiry for Queen et al. to be an appropriate reference under 102(e) is whether there is support for the claims *as allowed* in the priority applications, see MPEP 2136.03, p. 2100-85, citing *In re Wertheim*, 209 USPQ 554 (CCPA 1981), not simply whether the limitations can be found in the priority document. Regardless, Applicants maintain that the limitation "outside the Kabat and Chothia CDRs" is not found in, nor supported by, the priority documents.

This limitation requires that the framework residues to be replaced be outside both the Kabat and Chothia CDRs. As submitted in the Preliminary Amendment filed concurrently with the present application, however, the earliest Queen et al. applications do not teach, either explicitly or implicitly, that the framework residues to be replaced by donor **must be** outside **both** the Kabat and Chothia CDRs. Indeed, in the only example found in these early applications, and even in the specification of the Queen et al. patent as issued, changes were made to residues inside what Queen et al. denotes as CDRH1 of Chothia, i.e., inside a Chothia CDR. Considering that this limitation was required for patentability, Queen et al. cannot be entitled to a priority date earlier than the filing date of the application in which this limitation was first introduced, i.e., 12/19/90. Queen et al., thus, fails as a reference under 102(e) because, as also submitted in the Preliminary Amendment filed concurrently with the present application, Applicants are entitled to their GB priority date of 12/21/89.

Applicants respectfully request that this rejection be withdrawn.

Presentation of a Revised Proposed Count

Applicants present in Appendix A attached hereto a revised "Proposed Count." In compliance with 37 CFR §1.606, the revised proposed Count 1 is broader than any of claims 1-4, the broadest claims in the Queen patent, and as broad as any one of claims 24-31 being entered into the instant application.

The proposed count contains disjunctive or alternative language to cover the claim terminology of the two parties. Such counts were expressly approved by the Board in *Hsing v. Myers*, 2 USPQ2d 1861 (Bd, Pat,. App. & Int. 1987). It is clear, however, that both alternatives are directed to the same invention as that claimed in the Queen patent.

(c) Identification of Claims Corresponding to the Count

Applicants identify all of the Queen patent claims 1-11 and applicant's claims 24-31 as corresponding to the Count and as being directed to the same patentable invention.

(d) Application of the Terms of Applicants' Disclosure to the Copied Claims

In attached Appendix B, applicants illustrate the representative support in their present application disclosure for the limitations of their amended claim 24 substantially copied from Queen claims 1, 5, 9 and 10. There is, of course, additional support in applicants' application omitted for the sake of brevity.

(e) Applicants' Effective Filing Date

Applicants' present application, being a Rule 60 continuation, has the identical specification and drawings as that originally filed in U.S. application Serial No. 08/303.569, filed September 7, 1994, which is a U.S. national phase application stemming from PCT/GB-90/02017, filed December 21, 1990. The latter PCT application claimed priority benefit of GB national application Serial No. 89/28874.0, filed December 21, 1989.

In attached Appendix C is a diagram of support in applicants' 1989 GB application for each limitation of applicants' amended claims 28 and 29 which are also drawn to the same invention as proposed Count 1. Accordingly, applicants' effective filing date for their invention of Count 1 is 12/21/89, the filing date of their GB national application.

In view of the foregoing, Applicants respectfully submit that allowable subject matter has been identified and request that the Examiner advise them as soon as possible whether the Examiner intends to declare an interference between the present application and

Queen et al. Specifically, the Examiner is requested to contact the undersigned at (215) 564-8352.

Respectfully submitted,

Doreen Yatko Trujillo
Registration No. 35,719

Date: April 9, 1999

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS LLP
One Liberty Place - 46th Floor
Philadelphia, PA 19103
(215) 568-3100

APPENDIX A

PROPOSED COUNT FOR INTERFERENCE

Count 1:

A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with:

(i) an effective antigen binding activity, or

(ii) an affinity constant of at least 10^7 M^{-1} and no greater than about four-fold that of the donor immunoglobulin,

wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside:

(a) the Kabat and Chothia CDRs, or

(b) both the Kabat CDRs and the structural loop CDRs of the variable regions,

wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of said donor amino acids:

(I) is adjacent to a CDR in the donor immunoglobulin sequence, or

(II) (a) contains an atom within a distance of 4 of or (b) is spatially close to a CDR in said humanized immunoglobulin .

APPENDIX B

Claim Limitation	Support in Adair Application
24. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains	See page 1, lines 5-16, and page 7, line 32, through page 8, line 21.
which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1},	See page 11, lines 27-30.
wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions,	See page 6, lines 14-23, page 8, lines 13-16, and page 19, line 16, to page 20, line 15.
wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks,	See page 6, line 12, to page 7, line 5.
and each of said donor amino acids is adjacent to a CDR in the donor immunoglobulin sequence	See page 11, lines 16-20, showing that homology is maximized between donor and acceptor sequences adjacent CDRs within acceptor framework. At page 6, lines 25-35, it is indicated that the heavy chain "framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49...." In the heavy chain, Kabat CDR2 together with [Chothia] structural loop H2 extends from residues 50 to 65. Thus, residue 49 is immediately adjacent the beginning of this CDR2/H2 region.

or contributes to antigen binding as determined by X-ray crystallography.	Page 38, lines 1-12, and lines 23-38, and Figs. 3-4 of the application as filed reference residues that may "contribute to antigen binding" as determined using X-ray crystallography. Residues 6, 23,24, and 48 are identified in Figure 4.

APPENDIX C

Claim Limitation	Support in 1989 GB Application
28. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains	See page 1, lines 1-2 and 10-20; page 5, lines 8, to page 6, line 4; , and page 8.
which humanized immunoglobulin specifically binds to an antigen with an effective antigen binding affinity	See page 5, lines 1-7; page 22, lines 27-35, page 23, lines 5-9, page 24, lines 1-4; page 25, lines 27-33; page 26 last paragraph.
wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions,	See page 5, lines 1-7; page 26, last paragraph, to page 27, top paragraph.
wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks,	See page 5, line 8, to page 6, line 4: page 7, lines 5-20.
and each of said donor amino acids is adjacent to a CDR in the donor immunoglobulin sequence	See page 7, lines 11-14, showing that homology is maximized between donor and acceptor sequences adjacent CDRs within acceptor framework. At p.5, l. 9-16, reference is made to heavy chain "framework comprises donor at at least one of residues 6, 23 and/or 24, 48 and/or 49...." Residue 49 is immediately adjacent CDR2/H2 loop region.
or contributes to antigen binding as determined by X-ray crystallography.	Page 18, lines 11-17, and lines 33-37, and Figs. 20-21 of the application as filed reference residues that may "contribute to antigen binding" as determined using X-ray crystallography. Residues 6, 23,24, and 48 are identified in Figure 21.

29. A humanized immunoglobulin according to claim 28 which specifically binds to an antigen with a binding affinity similar to that of said donor immunoglobulin.	Page 23, lines 1-10.

AF

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WOODCOCK WASHBURN KURTZ MACKIEWICZ & NORRIS
A PARTNERSHIP INCLUDING PROFESSIONAL CORPORATIONS
ONE LIBERTY PLACE - 46TH FLOOR
PHILADELPHIA, PA 19103-7301
(215) 568-3100
Facsimile: (215) 568-3439
Cable: WOODCOCK

75962

DATE: November 4, 1999

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PATENT

~~RESPONSE UNDER 37 CFR 1.116~~
~~EXPEDITED PROCEDURE~~
~~EXAMINING GROUP NO. 1642~~

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(NE)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of: **Adair et al.**

Serial No.: **08/846,658** Group No.: **1642**

Filed: **May 1, 1997** Examiner: **J. Burke**

For: **Humanised Antibodies**

I, Doreen Yatko Trujillo, Registration No. 35,719 certify that this correspondence is being transmitted by facsimile to Examiner Burke of the U.S. Patent and Trademark Office, Washington, D.C. 20231.

On November 3, 1999

Doreen Yatko Trujillo

Doreen Yatko Trujillo Reg. No. 35,719

Do not enter 98 17Nov99

BOX AF
Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

AMENDMENT AND REQUEST FOR RECONSIDERATION

11/09/1999 LPENDER 00000003 23[illegible]
01 FC:117 [illegible]

Pursuant to 37 C.F.R. § 1.116, please amend the above-identified application as a follows.

In the claims:

24. (Twice Amended) A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1}, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the

variable regions, wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of said donor amino acids [is adjacent to a CDR in the donor immunoglobulin sequence or] contributes to antigen binding as determined by X-ray crystallography.

28. (Twice Amended) A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an effective antigen binding affinity, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions, wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of said donor amino acids [is adjacent to a CDR in the donor immunoglobulin sequence or] contributes to antigen binding as determined by X-ray crystallography.

Please add the following claim:

32. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1}, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions, wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks at residues 48, 49, 71, 73, 76, 78, 88, and 91.

REMARKS

This paper is being filed in response to the Final Rejection dated May 28, 1999. No extension of time is believed necessary for responding to the Final Rejection. To the extent this belief is in error, Applicants hereby request the necessary extension and the undersigned authorizes charging any such fee to Deposit Account 23-3050.

Claims 24-31 were pending. In the Final Rejection, all pending claims were rejected. Claim 32 has been added herein. In view of the foregoing amendments and the arguments that follow, Applicants respectfully submit that allowable subject matter has been identified and request that the interference be declared.

The Examiner stated that the Information Disclosure Statements filed in the parent cases will be considered once the references are submitted. To the extent the Examiner is requiring that Applicants resubmit references already submitted, this appears to be contrary to MPEP § 609, page 600-103. Applicants are not required to resubmit references to get them considered by the Examiner.

Rejections Under 35 U.S.C. § 112, first paragraph

Claims 24-31 were again rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors had possession of the claimed invention at the time the application was filed. Applicants respectfully traverse this rejection for the reasons that follow. For the Examiner's convenience, the paragraphs are designated to correspond to the Examiner's paragraphs for the rejection remaining under this section.

a. and b. The Examiner again rejected claims 24 and 28 alleging that the specification does not provide support for the concept that only substitutions adjacent CDRs are envisaged. Claims 24 and 28 were previously amended to recite that each of the donor amino acids to be replaced is adjacent a CDR or contributes to antigen binding as determined by X-ray crystallography. During a telephone conference between the Examiner and the undersigned, the Examiner indicated that removal of the "adjacent to a CDR" language would

obviate this rejection. Although Applicants disagree with the Examiner's reasoning, the claims have been amended herein to remove the recitation "adjacent to a CDR in the donor immunoglobulin sequence." As Applicants made clear in the previous response, the contribution to antigen binding need not be direct and, indeed, can be indirect, e.g., by affecting antigen binding site topology or inducing stable packing. Naturally, even for an indirect effect, the residues must be spatially near the CDR.

Applicants respectfully request that this rejection be withdrawn.

As the Examiner is aware, Applicants desire to provoke an interference between the present application and the Queen patent (U.S. Patent No. 5,585,089). Although Applicants are confident that the present claims are directed to the same invention as the Queen patent, new claim 32 is submitted herewith. New claim 32 recites the residues changed in example g341B disclosed in Applicants' specification as filed and, indeed, in GB8928874. Of the residues recited, all are either adjacent a CDR (49), or contribute to antigen binding as determined by X-ray crystallography (48, 71, 73, 76, 78, 88, and 91). Claim 32 is clearly allowable and clearly directed to the same invention as claim 1 of the Queen patent.

> If at least one of the presented claims is not rejectable on any [ ] ground and is claiming the same invention as at least one claim of the patent, the examiner should proceed to initiate an interference.

MPEP 2307.02.

Applicants respectfully request that an interference between the present application and the Queen patent be declared.

Rejection Under 35 U.S.C. § 102(e)

Claims 24-31 were again rejected under 35 U.S.C. § 102(e) in view of the Queen patent. Applicants respectfully traverse this rejection. Again, the relevant inquiry as to whether the Queen patent is an appropriate reference under 102(e) is whether there is support for the claims *as allowed* in the priority applications, see MPEP 2136.03, p. 2100-85, citing *In re Wertheim*, 209 USPQ 554 (CCPA 1981), not simply whether the limitations can be found in the priority document. Regardless, Applicants maintain that the limitation "outside the

Kabat and Chothia CDRs" is not found in, nor supported by, the priority documents.

This limitation requires that framework residues be changed outside both the Kabat and Chothia CDRs. This limitation is significant because the "CDRs" as defined by Kabat and Chothia differ.[1] Kabat defines CDR1 of the heavy chain as amino acids 31-35. Chothia defines the first hypervariable loop of the heavy chain as residues 26-32. As submitted in the Preliminary Amendment filed concurrently with the present application, the earliest Queen patent applications do not teach, either explicitly or implicitly, that the framework residues to be replaced by donor **must** be outside **both** the Kabat and Chothia CDRs. Indeed, in the specification of the Queen patent as issued, changes were made to residues inside what the Queen patent denotes as CDRH1 of Chothia, i.e., inside a Chothia CDR. Considering that the "outside the Kabat and Chothia CDRs" limitation was required for patentability, the Queen patent cannot be entitled to a priority date earlier than the filing date of the application in which this limitation was first introduced, i.e., 12/19/90.

The Examiner argued in the Final Rejection that the limitation is taught, for example, on page 9, lines 1-5 of Queen priority Application Serial No. 07/290,975 ("Queen '975") and page 13, lines 1-8 of Queen priority Application Serial No. 08/310,252 ("Queen '252"). The passages cited by the Examiner, however, do not support the Examiner's position.

The passage on page 9, lines 1-5, of Queen '975, contains a background discussion of the hypervariable regions, which it is therein stated are also called the CDRs. References by Kabat and Chothia are cited, and incorporated by reference. This is the only in passage in Queen '975 linking the Chothia reference to the term "CDRs." Other passages specifically referring to the CDRs as encompassed by the invention of Queen '975 make it clear that the CDRs are as defined by Kabat. For example, on page 10, line 2, the framework regions are defined in terms of Kabat. If the framework regions are defined in terms of Kabat,

---

[1]Notably, the Chothia reference refers to loops and carefully distinguishes these loops from the Kabat CDRs.

the CDRs must be as well. On page 21, the protocol for selecting which residues in the heavy chain are to be donor is set out. In lines 19-22, residues which fall in positions within a CDR "as defined by Kabat, [i.e.,] amino acids 31-35, 50-66, and 99-106" are to be donor. In lines 28-30, amino acid 30 is listed as a position immediately adjacent to a CDR to be changed to donor. Amino acid 30 is adjacent the heavy chain Kabat CDR, but within the heavy chain Chothia "CDR" as that term is used in Queen '975. The description of Figure 1 of Queen '975 indicates that it refers to the heavy chains and that the three CDRs are underlined (page 6, lines 1-6). In Figure 1, amino acids 31-35 are underlined for CDR1. Clearly, all specific references to CDRs were to Kabat CDRs only.

Further, in Figure 1, framework amino acids changed to donor are indicated by asterisks. Amino acids 27 and 30 are so designated. These residues are clearly within the Chothia "CDR." Neither the specification nor the claims require that more than one amino acid be changed to donor. Thus, Queen '975 teaches changing only one or two amino acids, and that both can be within the Chothia CDR. There is no support in Queen '975 for the limitation that the residues changed to donor must be outside both the Kabat and Chothia "CDRs."

Neither is there support for the limitation in Queen '252. In this instance, the passage relied upon by the Examiner for referring to Chothia is in the context of computer programs for computer models. There is no reference to CDRs. Contrastingly, the specific references to CDRs make it clear that the CDRs are as defined by Kabat. On page 8, lines 22-26, Queen '252 reports that the extents of the framework region and CDRs have been "precisely defined" by Kabat. On page 21, the protocol for selecting which residues in the heavy chain are to be donor is set out. In lines 20-22, residues which fall in positions within a CDR "as defined by Kabat, [i.e.,] amino acids 31-35, 50-66, and 99-106" are to be donor. In lines 27-29, amino acid 30 is listed as a position immediately adjacent to a CDR to be changed to donor. Amino acid 30 is adjacent the heavy chain Kabat CDR, but within the heavy chain Chothia "CDR" as that term is used in Queen '975.

Again, in Figure 1, framework amino acids changed to donor are indicated by

asterisks. Amino acids 27 and 30 are so designated. These residues are clearly within the Chothia "CDR." Neither the specification nor the claims require that more than one amino acid be changed to donor. Thus, Queen '252 teaches changing only one or two amino acids to donor, and that both can be within the Chothia CDR. There is no support in Queen '252 for the limitation that the residues changed to donor must be outside both the Kabat and Chothia "CDRs."

Applicants respectfully request that this rejection be withdrawn.

The Proposed Count is the same as that submitted with the Amendment filed April 9, 1999. Applicants again identify all of the Queen patent claims 1-11 and Applicants' claims 24-32 as corresponding to the Proposed Count.

In attached Appendix A, applicants illustrate the representative support in their present application disclosure for the limitations of their amended claim 24 and new claim 32. There is, of course, additional support in applicants' application omitted for the sake of brevity.

In attached Appendix B is a diagram of support in applicants' 1989 GB application for each limitation of applicants' amended claim 28 and new claim 38 which are also drawn to the same invention as proposed Count 1. Accordingly, applicants' effective filing date for their invention of Count 1 is 12/21/89, the filing date of their GB national application.

In view of the foregoing, Applicants respectfully submit that allowable subject matter has been identified and request that the Examiner declare an interference between the

present application and the Queen patent. Specifically, the Examiner is requested to contact the undersigned at (215) 564-8352.

Respectfully submitted,

Doreen Yatko Trujillo
Registration No. 35,719

Date: November 3, 1999

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS LLP
One Liberty Place - 46th Floor
Philadelphia, PA 19103
(215) 568-3100

APPENDIX A

Claim Limitation	Support in Adair Application
24. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains	See page 1, lines 5-16, and page 7, line 32, through page 8, line 21.
which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1}.	See page 11, lines 27-30.
wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions,	See page 6, lines 14-23, page 8, lines 13-16, and page 19, line 16, to page 20, line 15.
wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks,	See page 6, line 12, to page 7, line 5.
and each of said donor amino acids contributes to antigen binding as determined by X-ray crystallography.	Page 38, lines 1-12, and lines 23-38, and Figs. 3-4 of the application as filed reference residues that may "contribute to antigen binding" as determined using X-ray crystallography. Residues 48, 49, 71, 73, 76, 78, 88, and 91 are so identified in Figure 4.
32. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains	See page 1, lines 5-16, and page 7, line 32, through page 8, line 21.

which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1}.	See page 11, lines 27-30.
wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions,	See page 6, lines 14-23, page 8, lines 13-16, and page 19, line 16, to page 20, line 15.
wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks	See page 6, line 12, to page 7, line 5.
at residues 48, 49, 71, 73, 76, 78, 88, and 91.	See Light chain 341B of Table 1, page 41.

APPENDIX B

Claim Limitation	Support in 1989 GB Application
28. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains	See page 1, lines 1-2 and 10-20; page 5, lines 8, to page 6, line 4; , and page 8.
which humanized immunoglobulin specifically binds to an antigen with an effective antigen binding affinity	See page 5, lines 1-7; page 22, lines 27-35, page 23, lines 5-9, page 24, lines 1-4; page 25, lines 27-33; page 26 last paragraph.
wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions,	See page 5, lines 1-7; page 26, last paragraph, to page 27, top paragraph.
wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks,	See page 5, line 8, to page 6, line 4: page 7, lines 5-20.
and each of said donor amino acids contributes to antigen binding as determined by X-ray crystallography.	Page 18, lines 11-17, and lines 33-37, and Figs. 20-21 of the application as filed reference residues that may "contribute to antigen binding" as determined using X-ray crystallography. Residues 48, 49, 71, 73, 76, 78, 88, and 91 are identified in Figure 21.
32. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains	See page 1, lines 5-16, and page 7, line 32, through page 8, line 21.

which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1}.	See page 11, lines 27-30.
wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions,	See page 6, lines 14-23, page 8, lines 13-16, and page 19, line 16, to page 20, line 15.
wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks	See page 6, line 12, to page 7, line 5.
at residues 48, 49, 71, 73, 76, 78, 88, and 91.	See Light chain 341B of Table 1, page 20.

DOCKET NO.: CARP-0057 **PATENT**

~~RESPONSE UNDER 37 CFR 1.116~~
~~EXPEDITED PROCEDURE~~
~~EXAMINING GROUP NO. 1642~~

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of: **Adair et al.**

Serial No.: **08/846,658** Group No.: **1642**

Filed: **May 1, 1997** Examiner: **J. Burke**

For: **Humanised Antibodies**

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I, Doreen Yatko Trujillo, Registration No. 35,719 certify that this correspondence is being transmitted by facsimile to Examiner Burke of the U.S. Patent and Trademark Office, Washington, D.C. 20231.

On January 19, 2000
Doreen Yatko Trujillo
Doreen Yatko Trujillo Reg No. 35,719

BOX AF
Assistant Commissioner for Patents
Washington, D.C. 20231

Please do not enter 2/3/00 QE

Dear Sir:

AMENDMENT AND REQUEST FOR RECONSIDERATION

Pursuant to 37 C.F.R. § 1.116, please amend the above-identified application as a follows.

In the claims:

24. (Twice Amended) A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1}, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the

variable regions, wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of said donor amino acids [is adjacent to a CDR in the donor immunoglobulin sequence or] contributes to antigen binding as determined by X-ray crystallography.

28. (Twice Amended) A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an effective antigen binding affinity, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions, wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of said donor amino acids [is adjacent to a CDR in the donor immunoglobulin sequence or] contributes to antigen binding as determined by X-ray crystallography.

Please add the following claim:

49. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1}, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions, wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy chain framework at residues 48, 49, 71, 73, 76, 78, 88, and 91.

DOCKET NO.: CARP-0057 **PATENT**

REMARKS

This paper is being filed following the Advisory Action dated December 2, 1999. A Notice of Appeal was filed November 29, 1999. Accordingly, it is Applicants' belief that no extension of time or accompanying fee is required. If Applicants' belief is erroneous, this serves to request the requisite extension of time and authorizes the charging of any fee to Deposit Account 23-3050.

Claims 24-31 were pending. In the Final Rejection, all pending claims were rejected. An Amendment and Request for Reconsideration ("Amendment") was filed November 3, 1999 in response to the Final Rejection. The Amendment was not entered in view of what the Examiner considered new matter in a newly submitted claim. The Advisory Action, however, indicated that the Amendment would have overcome the then outstanding rejections under 112 and for new matter of claims 24 and 28. The previous amendments to claims 24 and 28 are resubmitted herein. Their entry is earnestly requested.

New claim 49 has been added herein. New claim 49 refers to specific replacements in the heavy chain. In that regard, the Examiner is directed to Table 1 of the application as filed, specifically to the "Heavy Chain" designated as 341 b. Applicants respectfully submit that new claim 49 does not contain new matter and does not raise new 35 U.S.C. § 112, first and second paragraph issues, nor does it raise new 102/103 issues. Claim 49 is submitted herein in an abundance of caution in view of the removal of the phrase "adjacent to a CDR in the donor immunoglobulin sequence" from claims 24 and 28 as suggested by the Examiner in the Final Rejection. Claim 49 recites a specific residue that is adjacent a CDR, i.e., residue 49. If, however, Applicants' submission of claim 49 is all that stands between the application being in condition for interference , Applicants respectfully request that the Examiner so advise the undersigned at (215) 564-8352.

In view of the foregoing amendments and the arguments that follow, Applicants respectfully submit that allowable subject matter has been identified and request that the interference be declared.

The Examiner stated that the Information Disclosure Statements filed in the

parent cases will be considered once the references are submitted. To the extent the Examiner is requiring that Applicants resubmit references already submitted, this appears to be contrary to MPEP § 609, page 600-103. Applicants are not required to resubmit references to get them considered by the Examiner.

Rejections Under 35 U.S.C. § 112, first paragraph

Claims 24-31 were again rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors had possession of the claimed invention at the time the application was filed. Applicants respectfully traverse this rejection for the reasons that follow. For the Examiner's convenience, the paragraphs are designated to correspond to the Examiner's paragraphs for the rejection remaining under this section.

a. and b. The Examiner again rejected claims 24 and 28 alleging that the specification does not provide support for the concept that only substitutions adjacent CDRs are envisaged. Claims 24 and 28 were previously amended to recite that each of the donor amino acids to be replaced is adjacent a CDR or contributes to antigen binding as determined by X-ray crystallography. During a telephone conference between the Examiner and the undersigned, the Examiner indicated that removal of the "adjacent to a CDR" language would obviate this rejection. Although Applicants disagree with the Examiner's reasoning, the claims have been amended herein to remove the recitation "adjacent to a CDR in the donor immunoglobulin sequence." As Applicants made clear in the previous response, the contribution to antigen binding need not be direct and, indeed, can be indirect, e.g., by affecting antigen binding site topology or inducing stable packing. Naturally, even for an indirect effect, the residues must be spatially near the CDR.

Applicants respectfully request that this rejection be withdrawn.

As the Examiner is aware, Applicants desire to provoke an interference between the present application and the Queen patent (U.S. Patent No. 5,585,089). Although Applicants are confident that the present claims are directed to the same invention as the

Queen patent, new claim 49 is submitted herewith. New claim 49 recites the residues changed in example g341B disclosed in Applicants' specification as filed and, indeed, in GB8928874. Of the residues recited, all are either adjacent a CDR (49), or contribute to antigen binding as determined by X-ray crystallography (48, 71, 73, 76, 78, 88, and 91). Claim 49 is clearly allowable and clearly directed to the same invention as claim 1 of the Queen patent.

> If at least one of the presented claims is not rejectable on any [ ] ground and is claiming the same invention as at least one claim of the patent, the examiner should proceed to initiate an interference.

MPEP 2307.02.

Applicants respectfully request that an interference between the present application and the Queen patent be declared.

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Claims 24-31 were again rejected under 35 U.S.C. § 102(e) in view of the Queen patent. Applicants respectfully traverse this rejection. Again, the relevant inquiry as to whether the Queen patent is an appropriate reference under 102(e) is whether there is support for the claims *as allowed* in the priority applications, see MPEP 2136.03, p. 2100-85, citing *In re Wertheim*, 209 USPQ 554 (CCPA 1981), not simply whether the limitations can be found in the priority document. Regardless, Applicants maintain that the limitation "outside the Kabat and Chothia CDRs" is not found in, nor supported by, the priority documents.

This limitation requires that framework residues be changed outside both the Kabat and Chothia CDRs. This limitation is significant because the "CDRs" as defined by Kabat and Chothia differ.[1] Kabat defines CDR1 of the heavy chain as amino acids 31-35. Chothia defines the first hypervariable loop of the heavy chain as residues 26-32. As submitted in the Preliminary Amendment filed concurrently with the present application, the earliest Queen patent applications do not teach, either explicitly or implicitly, that the

[1]Notably, the Chothia reference refers to loops and carefully distinguishes these loops from the Kabat CDRs.

framework residues to be replaced by donor **must be outside both** the Kabat and Chothia CDRs. Indeed, in the specification of the Queen patent as issued, changes were made to residues inside what the Queen patent denotes as CDRH1 of Chothia, i.e., inside a Chothia CDR. Considering that the "outside the Kabat and Chothia CDRs" limitation was required for patentability, the Queen patent cannot be entitled to a priority date earlier than the filing date of the application in which this limitation was first introduced, i.e., 12/19/90.

The Examiner argued in the Final Rejection that the limitation is taught, for example, on page 9, lines 1-5 of Queen priority Application Serial No. 07/290,975 ("Queen '975") and page 13, lines 1-8 of Queen priority Application Serial No. 08/310,252 ("Queen '252"). The passages cited by the Examiner, however, do not support the Examiner's position.

The passage on page 9, lines 1-5, of Queen '975, contains a background discussion of the hypervariable regions, which it is therein stated are also called the CDRs. References by Kabat and Chothia are cited, and incorporated by reference. This is the only in passage in Queen '975 linking the Chothia reference to the term "CDRs." Other passages specifically referring to the CDRs as encompassed by the invention of Queen '975 make it clear that the CDRs are as defined by Kabat. For example, on page 10, line 2, the framework regions are defined in terms of Kabat. If the framework regions are defined in terms of Kabat, the CDRs must be as well. On page 21, the protocol for selecting which residues in the heavy chain are to be donor is set out. In lines 19-22, residues which fall in positions within a CDR "**as defined by Kabat, [i.e.,] amino acids 31-35, 50-66, and 99-106**" are to be donor. In lines 28-30, amino acid 30 is listed as a position **immediately adjacent to a CDR** to be changed to donor. Amino acid 30 is adjacent the heavy chain Kabat CDR, but **within** the heavy chain Chothia "CDR" as that term is used in Queen '975. The description of Figure 1 of Queen '975 indicates that it refers to the heavy chains and that the three CDRs are underlined (page 6, lines 1-6). In Figure 1, amino acids 31-35 are underlined for CDR1. Clearly, all specific references to CDRs were to Kabat CDRs only.

Further, in Figure 1, framework amino acids changed to donor are indicated by

asterisks. Amino acids 27 and 30 are so designated. These residues are clearly within the Chothia "CDR." Neither the specification nor the claims require that more than one amino acid be changed to donor. Thus, Queen '975 teaches changing only one or two amino acids, and that both can be within the Chothia CDR. There is no support in Queen '975 for the limitation that the residues changed to donor must be outside both the Kabat and Chothia "CDRs."

Neither is there support for the limitation in Queen '252. In this instance, the passage relied upon by the Examiner for referring to Chothia is in the context of computer programs for computer models. There is no reference to CDRs. Contrastingly, the specific references to CDRs make it clear that the CDRs are as defined by Kabat. On page 8, lines 22-26, Queen '252 reports that the extents of the framework region and CDRs have been "precisely defined" by Kabat. On page 21, the protocol for selecting which residues in the heavy chain are to be donor is set out. In lines 20-22, residues which fall in positions within a CDR "**as defined by Kabat, [i.e.,] amino acids 31-35, 50-66, and 99-106**" are to be donor. In lines 27-29, amino acid 30 is listed as a position **immediately adjacent to a CDR** to be changed to donor. Amino acid 30 is adjacent the heavy chain Kabat CDR, but **within** the heavy chain Chothia "CDR" as that term is used in Queen '975.

Again, in Figure 1, framework amino acids changed to donor are indicated by asterisks. Amino acids 27 and 30 are so designated. These residues are clearly within the Chothia "CDR." Neither the specification nor the claims require that more than one amino acid be changed to donor. Thus, Queen '252 teaches changing only one or two amino acids to donor, and that both can be within the Chothia CDR. There is no support in Queen '252 for the limitation that the residues changed to donor must be outside both the Kabat and Chothia "CDRs."

Applicants respectfully request that this rejection be withdrawn.

The Proposed Count is the same as that submitted with the Amendment filed April 9, 1999. Applicants again identify all of the Queen patent claims 1-11 and Applicants' claims 24-31 and 49 as corresponding to the Proposed Count.

DOCKET NO.: CARP-0057 **PATENT**

In attached Appendix A, applicants illustrate the representative support in their present application disclosure for the limitations of their amended claim 24 and new claim 49. There is, of course, additional support in applicants' application omitted for the sake of brevity.

In attached Appendix B is a diagram of support in applicants' 1989 GB application for each limitation of applicants' amended claim 28 and new claim 49 which are also drawn to the same invention as proposed Count 1. Accordingly, applicants' effective filing date for their invention of Count 1 is 12/21/89, the filing date of their GB national application.

In view of the foregoing, Applicants respectfully submit that allowable subject matter has been identified and request that the Examiner declare an interference between the present application and the Queen patent. Specifically, the Examiner is requested to contact the undersigned at (215) 564-8352.

Respectfully submitted,

Doreen Yatko Trujillo

Doreen Yatko Trujillo
Registration No. 35,719

Date: January 19, 2000

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS LLP
One Liberty Place - 46th Floor
Philadelphia, PA 19103
(215) 568-3100

DOCKET NO.: CARP-0057 **PATENT**

APPENDIX A

Claim Limitation	Support in Adair Application
24. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains	See page 1, lines 5-16, and page 7, line 32, through page 8, line 21.
which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1}.	See page 11, lines 27-30.
wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions,	See page 6, lines 14-23, page 8, lines 13-16, and page 19, line 16, to page 20, line 15.
wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks,	See page 6, line 12, to page 7, line 5.
and each of said donor amino acids contributes to antigen binding as determined by X-ray crystallography.	Page 38, lines 1-12, and lines 23-38, and Figs. 3-4 of the application as filed reference residues that may "contribute to antigen binding" as determined using X-ray crystallography. Residues 48, 49, 71, 73, 76, 78, 88, and 91 are so identified in Figure 4.
49. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains	See page 1, lines 5-16, and page 7, line 32, through page 8, line 21.

which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1}.	See page 11, lines 27-30.
wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions,	See page 6, lines 14-23, page 8, lines 13-16, and page 19, line 16, to page 20, line 15.
wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy chain framework	See page 6, line 12, to page 7, line 5.
at residues 48, 49, 71, 73, 76, 78, 88, and 91.	See Light chain 341B of Table 1, page 41.

DOCKET NO.: CARP-0057 **PATENT**

APPENDIX B

Claim Limitation	Support in 1989 GB Application
28. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains	See page 1, lines 1-2 and 10-20; page 5, lines 8, to page 6, line 4; , and page 8.
which humanized immunoglobulin specifically binds to an antigen with an effective antigen binding affinity	See page 5, lines 1-7; page 22, lines 27-35, page 23, lines 5-9, page 24, lines 1-4; page 25, lines 27-33; page 26 last paragraph.
wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions,	See page 5, lines 1-7; page 26, last paragraph, to page 27, top paragraph.
wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks,	See page 5, line 8, to page 6, line 4: page 7, lines 5-20.
and each of said donor amino acids contributes to antigen binding as determined by X-ray crystallography.	Page 18, lines 11-17, and lines 33-37, and Figs. 20-21 of the application as filed reference residues that may "contribute to antigen binding" as determined using X-ray crystallography. Residues 48, 49, 71, 73, 76, 78, 88, and 91 are identified in Figure 21.
49. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains	See page 1, lines 5-16, and page 7, line 32, through page 8, line 21.

DOCKET NO.: CARP-0057 **PATENT**

which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1},	See page 11, lines 27-30.
wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions,	See page 6, lines 14-23, page 8, lines 13-16, and page 19, line 16, to page 20, line 15.
wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy chain framework	See page 6, line 12, to page 7, line 5.
at residues 48, 49, 71, 73, 76, 78, 88, and 91.	See Light chain 341B of Table 1, page 20.

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SERIAL NUMBER	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
07/743,329	09/17/91	ADAIR	J CARP-0009

EXAMINER: BENNETT, L

ART UNIT: 1807 | PAPER NUMBER: 19

18N1/0907

FRANCIS A. PAINTIN
WOODCOCK, WASHBURN, KURTZ, MACKIEWICZ & NORRIS
ONE LIBERTY PLACE-46TH FLOOR
PHILADELPHIA, PA 19103

DATE MAILED: 09/07/93

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

☑ This application has been examined ☑ Responsive to communication filed on April 9, 1993 ☑ This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s), ___ days from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☑ Notice of References Cited by Examiner, PTO-892, 4 pages
2. ☐ Notice re Patent Drawing, PTO-948.
3. ☑ Notice of Art Cited by Applicant, PTO-1449, 4 pages
4. ☐ Notice of Informal Patent Application, Form PTO-152.
5. ☐ Information on How to Effect Drawing Changes, PTO-1474.
6. ☐ _____

Part II SUMMARY OF ACTION

1. ☑ Claims 67-119 are pending in the application.
   Of the above, claims Ø are withdrawn from consideration.
2. ☑ Claims 1-66 have been cancelled.
3. ☐ Claims _____ are allowed.
4. ☑ Claims 67-119 are rejected.
5. ☐ Claims _____ are objected to.
6. ☐ Claims _____ are subject to restriction or election requirement.
7. ☑ This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.
8. ☐ Formal drawings are required in response to this Office action.
9. ☐ The corrected or substitute drawings have been received on _____. Under 37 C.F.R. 1.84 these drawings are ☐ acceptable. ☐ not acceptable (see explanation or Notice re Patent Drawing, PTO-948).
10. ☐ The proposed additional or substitute sheet(s) of drawings, filed on _____ has (have) been ☐ approved by the examiner, ☐ disapproved by the examiner (see explanation).
11. ☐ The proposed drawing correction, filed on _____, has been ☐ approved. ☐ disapproved (see explanation).
12. ☐ Acknowledgment is made of the claim for priority under U.S.C. 119. The certified copy has ☐ been received ☐ not been received ☐ been filed in parent application, serial no. _____; filed on _____.
13. ☐ Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.
14. ☐ Other

EXAMINER'S ACTION

PTOL-326 (Rev. 9-89)

Carter Exhibit 2028
Carter v. Adair
Interference No. 105,744

15. This Action is in response to the paper filed April 21, 1993. Claims 1-66 have been cancelled, and claims 67-119 have been newly added. All of Applicant's arguments have been thoroughly reviewed but are deemed non-persuasive for the reasons which follow. This Action is made FINAL.

The current status of the pending claims is as follows:

Claims 67-119 are rejected under 35 U.S.C. 112, first paragraph, for introducing new matter.

Claims 67-119 stand rejected under 35 U.S.C. 112, first paragraph scope.

Claims 67-117 stand rejected under 35 U.S.C. 103.

16. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> "The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention."

The specification is objected to under 35 U.S.C. 112, first paragraph, as the specification, as originally filed, does not provide support for the invention as is now claimed. Claims 67-119 have been amended to include the limitation that "in said composite heavy chain, amino acid residues 5,8,10,12-17,19,21,22,40,42-44,66,68,70,74,77,79,81,83-85,90,92, 105,109,111-113 at least are acceptor residues". However, nowhere in the specification is the invention described as containing these particular acceptor amino acid residues. Applicant points to the specification as teaching a number of residues which can be considered for changing from acceptor to donor residues and alleges that this teaching is support for the amendment on the grounds that "it follows that if a residue has not been considered for changing, that it must remain as in the acceptor chain". This

argument is not convincing because it does not necessarily follow that the unmentioned residues were originally contemplated as only being acceptor residues. That is, by not specifically describing whether particular residues are to acceptor or donor, can be interpreted to mean that the source of these residues was irrelevant, i.,e. they could be whether acceptor or donor residues. Therefore, this amendment introduces new matter into the specification which is not supported by the original specification.

Claims 71, 78, 85, 92, ,99,106,118 have been further amended to include a limitation which is not supported by the original specification. These claims have been amended to recite that the amino acid residues 2,4,6,38,48, 67 and 69 as being donor residues is supported by the passage on page 21, lines 13-16 of the specification. However, these pages teach that amino acid residues 2,4,6,38,46, 67 and 69 can be additionally changed to donor residues but does not teach that amino acid 48 is changed to a donor residue. Therefore, this amendment introduces new matter which is not supported by the original specification.

Claims 72, 79, 86, 93, 100,107 have also been further amended to include a limitation which is not supported by the original specification. These claims recite that amino acid residues 7,9,11,18,20,25,37,37,41, 45, 47,48,72,75,80,82,86-89,91,93,103,108,110 and 112 are additionally donor residues. However, the specification does not teach the concept that these particular amino acid residues are limited to being only acceptor amino acids. Applicant argues that this limitation was derived by taking all the donor residues mentioned in claims 67 to 71 and specifying that all other residues are acceptor residues. This rationale is not convincing because the original specification does not describe the invention as encompassing antibodies in which the amino acid residues which remain acceptor residues are specifically identified as these particular amino acid residues recited in claims 72, 79, 86, 93, 100,107. Therefore, this amendment introduces new matter into the specification which is not supported by the original specification.

Claims 108-113 have been further amended to specifically recite particular light chain amino acid residues which are limited to being only acceptor amino acids residues, i.e. residues 5,7-9,11, 13-18,20,22,23,39,41-43,57,59,61,72,74-79,81,82,84,86,88, 100,104,106 and 107. The specification does not teach that these particular positions in the disclosed antibodies are limited to being only acceptor residues. The specification does not discuss these amino acid position and therefore the original specification appears to teach that the source of these amino acids, i.e. from acceptor or donor, is not important to the invention. Therefore, this amendment introduces new matter which is not supported but the original specification.

Claims 118 and 119, drawn to a method for producing recombinant antigen binding molecule, are not supported by the original specification. Applicant points to now cancelled claims 66 and 67 submitted in the amendment filed February 9, 1993, has providing support for claims 118 and 199 respectively. However, the February 9, 1993 amendment does not appear to point to a passage in the originally filed specification which supports the particulars of the claimed method. The specification does not appear to disclose a method having steps in the specific order as claimed. The specification also does not describe the list of amino acid positions which are at least maintained as the acceptor amino acids as previously discussed. The specification appears to discuss amino acid positions which may be important in the structural and functional integrity of the humanized antibodies. The specification does not describe the particular order of making amino acid changes as is now claimed in the steps of claims 118 and 119. Specifically the specification does not appear to teach that the affinity of a generated humanized antibody is measured in order to determine if additional amino acid substitutions to the acceptor sequence are to be made. Therefore the amendment of these claims introduces new matter which is not supported by the original specification.

17. Claims 67-119 are rejected under 35 U.S.C. 112, first paragraph, for the reasons set forth in the objection to the specification.

18. The objection to the disclosure because of the use of terms such as "humanised" and "humanisation" is withdrawn in light of Applicant's convincing arguments.

19. The objection of claims 5,11-16,22 and 23 made under 37 CFR 1.75(c) as being in improper form has been obviated by the cancellation of these claims.

20. The objection of claims 1-23 made over the recitation of "CDR-grafted" has been obviated by the cancellation of these claims.

21. The rejection of claims 1-12 made under 35 U.S.C. 101 because the claimed invention is inoperative and therefore lacks patentable utility has been obviated by the cancellation of these claims.

22. The rejection of claims 17 made under 35 U.S.C. 101 because the claimed invention is drawn to non-statutory subject matter has been obviated by the cancellation of claim 17.

23. The rejection of claims 22-23 made under 35 U.S.C. 101 because the invention was inoperative and therefore lacked patentable utility, has been obviated by the cancellation of these claims drawn to therapeutic compositions.

24. The objection to the specification and the rejection of claims 1-12 made under 35 U.S.C. 112, first paragraph, as failing to adequately teach how to use the isolated heavy and light chains antibodies fragments for the disclosed utility, has been obviated by the cancellation of claims 1-12.

25. The objection to the specification and the rejection of claims 22-23 made under 35 U.S.C. 112, first paragraph, as failing to adequately teach how to use the claimed compositions as therapeutic or diagnostic agents, has been obviated by the cancellation of claims 22-23.

26. The rejection of claims 13-16 made under 35 U.S.C. 112, first paragraph, as the disclosure is enabling only for claims limited to specific

CDR-grafted antibodies disclosed in the specification as having effective binding affinities for their specific antigen, has been obviated by the cancellation of claims 13-16. **However**, the rejection now applies to newly added claims 67-117. The claims are not commensurate in scope with the present disclosure. Insufficient guidance and working examples are provided in the specification to support the broad claims drawn to any CDR-grafted antibodies which contain donor residues at the recited framework amino acid positions for the heavy and light chains. The specification does not sufficiently develop the concept that there are certain framework amino acids which when changed in the acceptor sequence to be the same as in the donor sequence result in an increase in antigen binding affinity. The specification does describe several examples where particular framework amino acid changes result in increased antigen binding affinity, such as an for OKT-3, OKT-4, and anti-ICAM. However, the specification does not clearly establish that every time the recited amino acid positions are the same between the donor and the acceptor. "good" binding to antigen is observed. The specification does not provide actual biding values for most of the examples, but instead qualitatively describes the binding of the humanized antibody to antigen, Furthermore, in light of the prior art (for instance, Reichmann et al., Queen et al., and Chothia et al.) such a universal property appears to be unpredictable since different antibodies will have different amino acids in the framework which are important for antigen binding and stability. The prior art does not teach that a standardized principle of which amino acids must always be changed is possible, but instead appears to teach that three dimensional structures of the antibodies and an understanding of protein folding properties, is necessary to be able to reasonably predict which amino acids will always be effective in increasing or retaining antigen binding ability. Therefore, this analysis shows that undue experimentation would be required of the skilled artisan in order to practice the invention as claimed.

Applicant traverses the rejection on the following grounds. First, Applicant states that Queen et al. provides little guidance for making recombinant antibodies but acknowledges that Queen et al. does teach to first select a human chain which is as closely comparable to the murine chain

as possible, followed by computer modelling to determine which residues outside of the CDRs are important for antigen binding. Applicant states that Queen et al. does not provide guidance as to which residues are critical for improving affinity. Applicant argues that the teachings in the present application in contrast to the teachings of Queen et al. can be applied to any antibody. Applicant asserts that computer modelling is not necessary in the present method. Applicant argues that the specification refers to nine different antibodies which have been successfully humanized, and therefore Applicant that the skilled artisan would readily predict that the concept is applicable to other antibodies. Applicant points to Figures 7-13 as showing data and page 60 as teaching binding affinities of the humanized anti-ICAM.

Applicant's arguments have been thoroughly reviewed but are deemed non-persuasive for the following reasons. First, as amended, the claims are broadly drawn to all antibodies having the specified amino acid donor and acceptor amino acids. However, the specification does not teach an antibody which possesses all of the recited amino acids as claimed. The specification teaches antibodies which have been altered at some of these positions, but does not teach antibodies in general which retain binding affinity for antigen every the acceptor residues are changed or the same as the recited donor residues in the claims. Therefore, although the specification does describe nine different CDR grafted antibodies, the specification does not teach variants of these antibodies which have been additionally modified as recited in the claims. Since the specification does not teach a representative number of the antibodies which are encompassed by the broadly written claims, the specification does not appear to have established the generality of the recited amino acid positions being important for antigen binding and stability. Because no standard and reproducible rules are available for predicting protein folding, the ability to predict that all the recited amino acid positions will always produce functional antibodies regardless of antigen binding specificity and source of antibody acceptor and donor is not reliable. Therefore this rejection is maintained and made FINAL.

27. The rejection of claims 1-23 made under 35 U.S.C. 112, second paragraph, as being indefinite has been obviated by the cancellation of claims 1-23.

28. The rejection of claims 1,5,6-8,12-22 made under 35 U.S.C. 102(b) as being anticipated by Reichmann et al. has been obviated by the cancellation of these claims.

29. The rejection of claims 1-6 and 12-22 made under 35 U.S.C. 102(b) as being anticipated by Queen et al. has been obviated by the cancellation of these claims.

30. The rejection of claims 1-21 made under 35 U.S.C. 103 as being unpatentable over Reichmann et al. and Queen et al. has been obviated by the cancellation of claims 1-21. **However**, this rejection now applies to newly added claims 67-117.

Both Reichmann et al. and Queen et al. teach how to make humanized antibodies using a human antibody variable domain framework as an acceptor and a rat antibody (in the case of Reichmann et al.) or a murine antibody (in the case of Queen et al.) as the complementarity determining region donor. Both of these references also teach how to identify framework amino acids which are important for retaining the binding effective conformation of the CDRs. Specifically, Queen et al. teach that the more homologous the human antibody is to the murine antibody reduces the likelihood of producing distortions in the CDRs. Furthermore, Queen et al. teach making a database comparison of all known human antibodies with the donor antibody to determine the most similar human antibody to use as the framework (page 10031, col. 2, paragraph 2). Queen et al. also teach making a molecular model of the donor variable domain (in this case the anti-Tac V domain) based upon homology to other antibody V domains whose crystal structure is known. By doing so, Queen et al. teach that amino acids outside of the CDRs which are close enough to the CDRs to influence the CDR

conformation or to directly interact with the antigen. When the residues were different between the human and the donor murine antibodies, the human framework amino acid was changed to the corresponding murine amino acid (page 10031, col. 2 paragraph 3). Finally, when the human acceptor antibody contains unusual amino acids with respect to consensus sequences in homologous antibodies, Queen et al. recommends changing these amino acids to the consensus amino acid (page 10032, col. 1) Reichmann et al. and Queen et al. further teach that different changes will be necessary depending on the specific donor and acceptor antibodies which are used. Both references each the cDNA encoding the heavy and light antibody chains which are the templates for making the specific changes in the sequences of CDR-grafted antibodies. The references also both teach the insertion of the cDNAs into vectors, transfection of host cells and co-expression of the heavy and light chains to result in the expression of a complete CDR-grafted antibody molecule.

Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to use the guidelines taught by Reichmann et al. and Queen et al. to reshape any given antibody to "humanize" that antibody by making the changes in the framework regions of the human acceptor sequence to the donor residue when those residues are close to the CDR's and when the amino acids would be expected to affect the conformation of the CDRs. One of ordinary skill would have been motivated to make the changes in the framework regions from the human amino acid to the donor amino acid in order to achieve the expected benefit of increasing the binding affinity of the humanized antibody for the specific antigen over the binding affinity observed in the humanized antibodies which do not contain the framework changes as taught by Queen et al. (page 10032, col. 1, para. 3 through col. 2) and Reichmann et al. (Figure 4).

Applicants traverse the rejection on the following grounds. First, applicants argue that Reichmann et al. does not go beyond the original idea of Winter et al. WO-A 89/07452 which teaches transferring only the CDRs to a human framework. Applicant further argues that Reichmann et al. only changed residues 27 and 30 because the at donor sequence was found to be

unusual. Also, Applicant points out that Reichmann et al. did not make any framework residue changes to the light chain of the antibody outside of the CDRs. Applicant argues that Reichmann et al. do not teach that these changes are generally applicable to other antibodies. Also, Applicant states that Reichmann et al. do not suggest that altering residues remote from the CDRs might be effective in improving affinity nor that there might by a hierarchy of residues which should be considered.

Second Applicant argues that Queen et al. teach the amino acid sequence of the donor antibody chain should be determined and then compared to that of known acceptor chains and an acceptor chain chosen which is as homologous as possible to the donor chain. Applicant further states that the next step in Queen et al. is to carry out a computer modelling exercise to determine the residues which may be involved in antigen binding. Applicant alleges that this step may not always lead to the same results. Applicant also alleges that the fact that the donor sequence is compared to a number of possible acceptor sequences and that a computer model of the donor must be made, shows that the procedure is specific to one antibody at a time. Applicant asserts that Queen et al. does not suggest that the changes taught for reshaping the anti-TAC antibody could be expected to be the same necessary in another recombinant antibody. Applicant also states that Queen et al. doe snot teach an antibody containing all the donor residues recited in the claims.

Applicant's arguments have been thoroughly reviewed but are deemed non-persuasive for the following reasons. First, the claims have not been rejected as obvious over Reichmann et al. alone nor over Queen et al. alone. Instead, the claims have been rejected over the combined teachings of both Reichmann et al. and Queen et al. Consequently, Applicant's arguments do not address the rejection made. Second, Applicant's arguments are directed to a procedure of making recombinant antibodies but claims 67-117 are drawn to recombinant antibodies not to a method of making those antibodies. Therefore, when the prior art teaches an antibody which is encompassed by the broadly written claims which is made by a different method than the procedure disclosed in the specification, the prior

art still reads on the claims. Therefore, while Reichmann et al. and Queen et al. do not specifically teach that certain non-CDR framework amino acids must always be either acceptor or donor residues, these references do teach that best antigen binding affinities would be expected when the overall sequence the donor is most similar to the acceptor and that amino acids which come into contact with the CDRs should be donor residues. How these residues are identified is irrelevant when the claims are drawn to the antibodies themselves. Furthermore, the claims as written are not limited to antibodies in which the donor is non-human or that all the "donor residues are from the same donor. Many of the specific residues recited in the claims as being donor residues, are identical in the acceptor and the donor. Consequently, Th references teach many of the specific amino acid limitations without teaching that these amino acids need to be changed. Therefore, for all of these reasons, this rejection is maintained and made FINAL.

31. No claims are allowable.

32. **THIS ACTION IS MADE FINAL**. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a). The practice of automatically extending the shortened statutory period an additional month upon the filing of a timely first response to a final rejection has been discontinued by the Office. See 1021 TMOG 35.

A SHORTENED STATUTORY PERIOD FOR RESPONSE TO THIS FINAL ACTION IS SET TO EXPIRE THREE MONTHS FROM THE DATE OF THIS ACTION. IN THE EVENT A FIRST RESPONSE IS FILED WITHIN TWO MONTHS OF THE MAILING DATE OF THIS FINAL ACTION AND THE ADVISORY ACTION IS NOT MAILED UNTIL AFTER THE END OF THE THREE-MONTH SHORTENED STATUTORY PERIOD, THEN THE SHORTENED STATUTORY PERIOD WILL EXPIRE ON THE DATE THE ADVISORY ACTION IS MAILED, AND ANY EXTENSION FEE

PURSUANT TO 37 CFR 1.136(a) WILL BE CALCULATED FROM THE MAILING DATE OF THE ADVISORY ACTION. IN NO EVENT WILL THE STATUTORY PERIOD FOR RESPONSE EXPIRE LATER THAN SIX MONTHS FROM THE DATE OF THIS FINAL ACTION.

33. Papers relating to this application may be submitted to Group 180 by facsimile transmission. Papers should be faxed to Group 180 via the P.T.O. Fax Center located in Crystal Mall 1. The CM1 Fax Center number is (703) 308-2730. Papers may be submitted Monday-Friday between 8:00 am and 4:45 pm (EST). Please note that the faxing of such papers must conform with the Notice to Comply in the Official Gazette, 1096 OG 30 (November 15, 1989).

34. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Lisa Bennett Arthur (nee Lisa T. Bennett) whose telephone number is (703) 308-3988. Any inquiry of a general nature or relating to the status of an application should be directed to the Group 180 receptionist whose telephone number is (703) 308-0196.

LBA

Lisa Bennett Arthur
September 2, 1993

M. Parr

MARGARET PARR
SUPERVISORY PATENT EXAMINER
GROUP 1800

DOCKET NO.: CARP-0046 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of: **John R. Adair, Diljeet S. Athwal and John S. Emtage**

Serial No.: **08/485,686** Group No.: **1642**

Filed: **June 7, 1995** Examiner: **J. Burke Reeves**

For: **Humanized Antibodies**

I, Doreen Yatko Trujillo, Registration No.35,719 certify that this correspondence is being deposited with the U.S. Postal Service as First Class mail in an envelope addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231.

On August 29, 2000

Doreen Yatko Trujillo, Reg. No. 35,719

#23H 09

Assistant Commissioner for Patents
Washington, D.C. 20231.

Dear Sir,

REQUEST FOR RECONSIDERATION

This responds to the Office Action dated February 29, 2000. A petition for a three-month extension of time and the appropriate fee accompanies this response.

Claims 56-73 were pending. All pending claims were rejected in the Office Action. In view of the arguments and amendments that follow, Applicants respectfully request withdrawal of all rejections upon reconsideration.

In the specification:

Please amend the specification as follows:

Page 1, line 1, after "9/07/94", replace "copending" with -- issued as

U.S. 5,859,205 --.

Page 23, line 20, please delete "Figure 1 shows" and insert -- Figures 1 a and b show --.

At page 23, line 21, insert --(SEQ ID NO: 4 and 5)--between "chain" and ";".

Page 23, line 22, please delete "Figure 2 shows" and insert -- Figures 2 a and b show --.

At page 23, line 23, insert --(SEQ ID NO: 6 and 7)--between "chain" and ";".

At page 23, line 26, insert --(SEQ ID NO: 5, 8, and 9)--between "REI" and ";".

At page 23, line 29, insert --(SEQ ID NO: 7 and 10) – between "KOL" and ";".

At page 23, line 30, please delete "Figure 5 shows" and insert -- Figures 5 a - c show --.

At page 23, line 32, insert --(SEQ ID NO: 7 and 11-24) – between "grafts" and ";".

At page 23, line 35, insert --(SEQ ID NO: 5, 8, 9, and 25-28) -- between "grafts" and ";".

At page 24, line 6, please delete "Figure 10 shows" and insert -- Figures 10 a and b show --.

At page 24, line 8, please delete "Figure 11 shows" and insert -- Figures 11 a and b show --.

At page 30, line 31, insert --(SEQ ID NO: 1) -- between "TCCAGATGTTAACTGCTCAC" and "for".

At page 30, line 33, insert --(SEQ ID NO: 2) -- after "CAGGGGCCAGTGGATGGATAGAC".

At page 33, line 26, insert --(SEQ ID NO: 3) -- after

"Leu-Glu-Ile-Asn-Arg/ -/Thr-Val-Ala -Ala".

At page 40, line 14, after "5", please insert -- a - c --.

At page 41, between lines 29 and 30, insert -- (SEQ ID NO: 8-28) -- .

At page 50, line 24, please insert – (SEQ ID NO: 7, 10, and 11-24) –.

At page 50, line 36, please insert – (SEQ ID NO: 5, 8, 9, and 25-28) –.

At page 51, line 13, after "10", please insert -- a and b --.

At page 51, line 15, after "11", please insert -- a and b --.

In the claims:

Please amend the claims as follows:

56. (Twice Amended) An antibody molecule having affinity for [a predetermined] an antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions ([CDRS] CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues corresponding to the equivalent residues using the Kabat numbering system in a donor antibody having binding affinity for said [predetermined] antigen, wherein, according to the Kabat numbering system, in said composite heavy chain at least residues 31 to 35, 50 to 65 and 95 to 102 in [(] the [CDRS] CDRs [)] and at least residues 23, 24, 49, 71, and 73 [(] in the framework regions [)] correspond to the equivalent residues in said donor antibody.

62. (Twice Amended) The antibody molecule of claim 56, wherein said complementary light chain is a composite light chain having a variable domain including [CDRS] CDRs, said variable

domain comprising predominantly human acceptor antibody light chain framework residues, the remaining light chain residues corresponding to the equivalent residues using the Kabat numbering system in said donor antibody, wherein, according to the Kabat numbering system, in said composite light chain at least residues 24 to 34, 50 to 56 and 89 to 97 in [(] the CDRs [)] and at least residues 46, 48, 58 and 71 [(] in the framework regions [)] correspond to the equivalent residues in said donor antibody.

63. The antibody molecule of claim 62, wherein additionally at least one of residues 2, 4, 6, 35, 38, 44, 47, 49, 62, [64 to 69] 64, 65, 66, 67, 68, 69, 85, 87, 98, 99, 101 and 102 in said composite light chain corresponds to the equivalent residue in said donor antibody.

In claim 64, line 1, please delete "is specific" and insert – has specificity –.

In claim 65, line 1, please delete "is specific" and insert – has specificity –.

In claim 66, line 1, please delete "is specific" and insert – has specificity –.

In claim 67, line 1, please delete "is specific" and insert – has specificity --.

In claim 68, line 2, please delete "is specific" and insert – has specificity –.

In claim 69, line 1, please delete "is specific" and insert – has specificity –.

In claim 70, line 1, please delete "is specific" and insert – has specificity –.

In claim 71, line 1, please delete "is specific" and insert – has specificity –.

In claim 72, line 1, please delete "is specific" and insert – has specificity –.

In claim 73, line 1, delete "therapeutic"; and

replace "an antibody" with -- the antibody molecule --.

Remarks

Preliminarily, Applicants note with appreciation the Examiner's observation that the claims are free of the prior art.

The specification has been objected to because, *inter alia*, the first line of the specification needs to be updated to reflect the status of any parent applications, and to reflect the parent international application. Applicants direct the Examiner to the transmittal letter of the present application in which the latter amendment was effected; the former amendment has been effected herein.

The Brief Description of the Drawings was objected to as not conforming with the labelling of the figures. The specification has been amended herein to place the Brief Description of the Drawings in conformity with the figures. No new matter was added thereby.

The specification was objected to as not complying with the Sequence Rules and Regulations. Specifically, the Examiner suggested that the specification be checked for missing sequence identifiers. The specification has been amended herein to add sequence identifiers. No new matter is added thereby.

The specification was further objected to for missing text on pages 53 and 62. A substitute specification with the complete information is enclosed. Because the missing text was simply a copying error, Applicants have not submitted a marked-up copy showing the addition. If the Examiner so requires, one will be forwarded upon request.

I. Rejections under 35 USC § 112, Second Paragraph

Claims 56-73 have been rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite in the recitation of "CDRS." Claims 56 and 62 have been amended to replace "CDRS" with "CDRs."

Claims 56-73 have been rejected as allegedly indefinite in reciting parentheses around the phrases "in the framework regions" and "the CDRs." The parentheses have been removed from claims 56 and 62 and an appropriate preposition added.

Claims 56-73 have been rejected as allegedly indefinite for reciting "predetermined antigen." Claim 56 has been amended to remove "predetermined." Applicants respectfully submit that the claim as amended covers predetermined antigens.

Claims 56-73 have been rejected as allegedly indefinite for reciting "an antibody molecule having affinity." Claim 56 has been amended to recite "binding affinity." Support for this amendment can be found, *inter alia*, on page 6, lines 21-22, of the application as filed.

Claims 56-73 have been rejected as allegedly indefinite for reciting "the remaining heavy chain residues corresponding to the equivalent residues in a donor antibody." In claim 56 and 62, "using the Kabat numbering system" has been inserted after "equivalent residues." Support for this amendment can be found, *inter alia*, on page 8, lines 24-26, of the application as filed.

Claim 58 has been rejected as allegedly indefinite for the inclusion of a comma after "corresponds." In claim 58, the comma after "corresponds" as been deleted.

Claim 63 has been rejected as allegedly indefinite for reciting "at least one of residues 2, 4. . . 64 to 69. . ." Claim 63 has been amended to recite the residues individually.

Claims 64-72 have be rejected as allegedly indefinite for reciting "which is specific for." The Examiner alleged that is unclear whether the antibody molecule binds to the specific antigen or is otherwise "specific." Applicants respectfully disagree. Nonetheless, claims 64-72 have been amended

to recite "which has specificity for" in replace of "which is specific for." Support for this amendment can be found, *inter alia*, in the paragraph bridging pages 15-16 of the application as originally filed. It is clear from the discussion therein that the reference to "specificity" means that the antibody molecule binds the particular antigen.

Claim 73 has been rejected as allegedly indefinite for recitation of "a therapeutic composition." The Examiner suggests that deleting the term "therapeutic" would obviate the rejection. Accordingly, the term "therapeutic" has been deleted from claim 73. Compositions with therapeutic applications are included in the scope of claim 73.

Claim 73 has been rejected as allegedly indefinite in the recitation of "an antibody." Claim 73 has been amended to recite "the antibody molecule." Support for this amendment can be found, *inter alia*, in claim 13 as originally filed. (This rejection was apparently levied twice – see sub-paragraphs j and n.)

Claims 56-73 have been rejected as allegedly indefinite in the recitation of "said variable domain comprising predominantly human acceptor..." Applicants respectfully disagree and note that this term is present in the claims of issued U.S. Patent No. 5,859,205, the parent of the present application. The term is used to distinguish the claims from chimeric antibodies in which the entire variable domain is from the donor antibody. Clearly, since the claims recite that the variable domain comprises predominantly human acceptor framework residues, the Examiner's query whether only framework residues are counted is correct. Further, the Applicants respectfully submit that it is clear to one skilled in the art that, if the donor and acceptor residues are identical for a particular position, they are counted

as acceptor. Applicants respectfully submit this term is definite and request that this rejection should be withdrawn.

Claims 56-73 have been rejected as allegedly indefinite in the recitation of "the remaining heavy chain residues corresponding to the equivalent residues in a donor antibody having affinity for said predetermined antigen." The Examiner alleges that the CDRs appear to be outside the scope of the phrase "remaining heavy chain residues." This allegation is based upon a clear misreading of the claims. Clearly, a correct reading of the claims reveals that the CDRs correspond to the equivalent residues in the donor antibody. See, for example, claim 56.

Claims 58-61 and 63 have been rejected as allegedly indefinite. The Examiner improper alleges that it is unclear whether the claims intend to recite the residues in the alternative or in Markush grouping. Both means of claiming, however, accomplish the same end -- alternative claiming. The Examiner is directed to MPEP 2173.05(h). Applicants respectfully request that this rejection be withdrawn.

In view of the foregoing, Applicants respectfully request that the rejections under 35 U.S.C. § 112, second paragraph be withdrawn.

II. Rejections under 35 USC § 112, First Paragraph

Claims 56-73 have been rejected under 35, U.S.C. §112, first paragraph, as containing subject matter which allegedly was not described in the specification in such a way as to allow one skilled in the art to make and/or use the invention. The Examiner alleges that, in particular, the specification is lacking in guidance in choosing the donor-acceptor antibody pair (Office Action pp. 8-9). The Examiner

indicates that it could not have been expected that antibody molecules of the present invention would be functional because a number of criteria, such as homology between the donor and acceptor antibodies and the identity of packing residues near the CDRs, do not form part of the claims.

Applicants respectfully submit that the Examiner misapprehends Applicants' invention and, indeed, is inappropriately reading disclosure from the specification into the claims. Applicants' invention is based upon the identity of a hierarchy of residues that are of universal import without the need to compare antibodies or identify packing residues. The invention enables one skilled in the art to make an antibody molecule having a composite heavy chain wherein the heavy chain CDRs are from a donor antibody and at least framework residues 23, 24, 49, 71 and 73 are from the same donor antibody. Based on this information, one skilled on the art can obtain functional antibody molecules.

In order to demonstrate that antibody molecules having the claimed features are functional, Applicants enclose the Declaration of Geoffrey T. Yarranton, which was forwarded in the parent application, 08/303,569, on September 18, 1995. Dr. Yarranton's Declaration contains three tables relating to a number of antibodies having the claimed features, i.e. wherein at least residues 31 to 35, 50 to 65, 95 to 102, 23, 24, 49, 71 and 73 of the heavy chain variable domain correspond to residues from a donor antibody. The first table relates to the heavy chain and the second table relates to the light chain. The third table sets out the degree of affinity recovered as a percentage of the affinity of the donor antibody. A comparison with antibodies which have been produced by other methods is also provided. B1.8, D1.3, CAMPATH, and anti-TAC are such antibodies. As is evident therefrom, the subject matter of the present application enables one skilled in the art to obtain functional antibody molecules.

The Examiner further alleges that the claims recite CDR residues 31-35, 50-65, and 95-102 as numbered by the Kabat system, while the specification teaches different boundaries of the CDRs (Office Action p. 9). Furthermore, the Examiner points to constructs 121-141 of the specification to show that resides 26-35 are required for binding activity of the hybrid antibody (Office Action, p. 10). The Examiner also indicates that the range of the CDRs needs to be determined by structural analysis. Applicants respectfully disagree. The specification is clearly in agreement with the claims. See, for example, the disclosure bridging pages 19-20 of the application as filed, under the heading "The extent of the CDRs." As is clear therefrom, the CDRs are as defined by Kabat; the structural loops corresponded to the CDRs and, indeed, are completely encompassed within the CDRs, with the exception of CDR1 of the heavy chain. In the case of CDR1 of the heavy chain, the structural loop corresponds to residues 26-32; residues 26-30, thus, are part of the structural loop not contained within the Kabat CDR. Regarding the constructs referred to in Table 1, Applicants note that the table reports the changes made and remind the Examiner that no change is necessary if the donor and acceptor residues are the same at a particular position.

The Examiner alleges that while the claims recite the limitation of heavy chain residues 23, 24, 49, 71 and 73, other required residues are taught by the specification. In particular, the Examiner alleges that the specification specifically teaches that residues 71, 73 and 78 will always be all donor or all acceptor (Office Action, p. 10). This allegation is not correct. The specification clearly indicates that residue 78 is optional (see specification page 6, line 34: "residues at at least one of positions . . ."). Further, it is stated that

> "The residues at positions 71, 73 and 78 of the heavy chain framework are preferably either all acceptor or all donor residues.

(Specification page 7, lines 3-5, emphasis added.). Page 17, section 2.1 of the specification is cited by the Examiner. This section is part of a protocol. On page 16, fifth paragraph, it is stated that:

> "This protocol and rationale are give without prejudice to the generality of the invention as hereinbefore described...." (Emphasis added)

The present application clearly indicates that it is merely preferred that residues 71, 73 and 78 are either all donor or all acceptor residues, not that it is required. Claim 56 therefore does not have to specify that residue 78 is a donor residue. Furthermore, in Example 5 of the application, antibody molecules 61E71 and hTNF1 are described. Neither of these antibodies have a donor residue at position 78. This can clearly be seen from the enclosed tables. Furthermore, both antibodies were found to have 100% of the potency of the parent antibody.

The Examiner also alleges that the specification teaches that residue 6 is necessary to retain binding functions. The previous argument also applies to residue 6, which is referred to as being important to binding affinity in the protocol given in the specification. As indicated above, the protocol is without prejudice to the generality of the invention. Again, in Example 5 of the application, antibody molecules 61E71 and hTNF1 are described. Neither of these antibodies have a donor residue at position 6. This can clearly be seen from the enclosed tables. Furthermore, both antibodies were found to have 100% of the potency of the parent antibody.

The Examiner alleges that the unpredictability in the art is high and undue experimentation would be required to make the invention. Rudikoff et al., Panka et al., and Amit et al. are cited as

examples in which one amino acid change in a CDR or framework region dramatically affected antigen binding (Office Action, p 11). None of these documents suggest that the antibody molecules as defined in the present claims do not function to bind antigen.

Rudikoff et al. describes amino acid changes to CDRs. The finding that by changing the sequence of a CDR, which is known to determine the binding affinity of the antibody, actually results in a decreased binding affinity is not relevant to the presently claimed subject matter. The presently claimed subject matter recites that the residues in the CDRs (defined by Kabat numbering) entirely correspond to residues in the CDRs of the donor antibody. There are no alterations in the sequence of the CDRs of the donor antibody molecule and therefore Rudikoff et al. is irrelevant.

Panka et al. describes a single amino acid substitution at position 94. Although this amino acid substitution alters the binding affinity of the antibody, the antibody still binds the antigens digoxin and digitoxin (see Abstract). Panka et al. thus reports that amino acid changes made in the framework region can alter the binding affinity of antibodies. The same is clearly taught in the present application. See, for example, pages 20 to 21 wherein non-CDR, i.e. framework, residues which contribute to antigen binding are discussed. In particular, on page 21, lines 10-12, it is dislcosed that residue 94 should be changed if it is not arginine. The subject matter of the present application enables one skilled in the art to produce antibody molecules having affinity for a predetermined antigen. The fact that the antibody molecules may not have the optimum binding affinity for the antigen is not relevant to the claimed subject matter.

Amit et al. is said to indicate that at least one amino acid in the framework region of an antibody is involved in antigen binding. We assume that the Examiner is referring to residue 30 in the heavy chain of the antibody. All that is said concerning this residue is that it contacts the antigen. There is no indication that this residue is required for affinity binding of an antigen. There is no disclosure of substituting residue 30 for another amino acid and therefore no evidence to suggest that the binding affinity will change. The disclosure of Amit et al. is therefore not relevant to the presently claimed subject matter.

In view of the foregoing, Applicants respectfully request that the rejections under 35 U.S.C. § 112, first paragraph be withdrawn.

For the foregoing reasons, Applicants submit that the present claims meet all the requirements for patentability. The Examiner is respectfully requested to allow all the present claims. If the Examiner is of a contrary view, it is requested that she contact the undersigned at (215) 557-5948.

Respectfully submitted,

Doreen Yatko Trujillo
Registration No. **35,719**

Date: 29 August 2000

Woodcock Washburn Kurtz
Mackiewicz and Norris LLP
One Liberty Place - 46th Floor,
Philadelphia, PA 19103,
(215) 568-3100

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WOODCOCK WASHBURN KURTZ MACKIEWICZ & NORRIS
A PARTNERSHIP INCLUDING PROFESSIONAL CORPORATIONS
ONE LIBERTY PLACE - 46TH FLOOR
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(215) 568-3100 FAX
Facsimile: (215) 568-3439
Cable: WOODCOCK

00035

DATE: September 14, 2000

Please deliver this and the following pages to:

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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of: **Adair et al.**

Serial No.: **08/846,658** Group No.: **1642**

Filed: **May 1, 1997** Examiner: **J. Burke**

For: **Humanised Antibodies**

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

SUPPLEMENTAL AMENDMENT AND REQUEST FOR RECONSIDERATION

Pursuant to 37 C.F.R. § 1.111, please amend the above-identified application as a follows.

In the claims:

24. (Twice Amended) A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1}, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the

variable regions, wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of said donor amino acids [is adjacent to a CDR in the donor immunoglobulin sequence or] contributes to antigen binding as determined by X-ray crystallography.

28. (Twice Amended) A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an effective antigen binding affinity, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions, wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of said donor amino acids [is adjacent to a CDR in the donor immunoglobulin sequence or] contributes to antigen binding as determined by X-ray crystallography.

REMARKS

This paper is being filed to supplement the amendment referred to in the Request for Continued Examination filed June 1, 2000 ("the RCE"). No extension of time is believed to be necessary. To the extent this belief is in error, Applicants hereby request the necessary extension and the undersigned authorizes charging any such fee to Deposit Account 23-3050.

The amendment referred to in the RCE referenced certain claim amendments that had, inadvertently, not been included in the amendment. Specifically, the foregoing amendments to claims 24 and 28 were referenced but not effected in the amendment referred to in the RCE. Applicants respectfully request entry of the foregoing amendments and respectfully submit that, upon entry of these amendments, Applicants will have allowable subject matter.

In view of the foregoing, Applicants respectfully request that the Examiner declare an interference between the present application and the Queen patent. The Examiner is

requested to contact the undersigned at (215) 564-8352 if she feels a telephonic discussion will be helpful.

Respectfully submitted,

Doreen Yatko Trujillo
Registration No. 35,719

Date: September 14, 2000

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS LLP
One Liberty Place - 46th Floor
Philadelphia, PA 19103
(215) 568-3100

DOCKET NO.: CARR0046 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Adair et al.

Serial No.: **08/485,686**

Group Art Unit: **1642**

Filed: **June 7, 1995**

Examiner: **M. Davis**

For: **Humanised Antibodies**

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2-6-02

I, **Paul K. Legaard**, Registration No. **38,534** certify that this correspondence is being deposited with the U.S. Postal Service as First Class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

On November 12, 2001

Paul K Legaard

Paul K. Legaard Reg. No. 38,534

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

AMENDMENT AND REQUEST FOR RECONSIDERATION

In response to the Office Action mailed August 10, 2001 in connection with the above-identified patent application, Applicants respectfully request that the application be amended as follows.

In the Application:

Please delete pages 67-89 of the application as filed containing the Sequence Listing and insert substitute pages 1-22 enclosed herewith, which contain the amended Sequence Listing formatted under the new rules for the Sequence Listing. In addition, please renumber the remaining pages of the application, containing the claims and Abstract, accordingly.

In the Claims:

Please amend claims 56, 58 and 62 to read as follows:

56. (Amended four times) An antibody molecule having affinity for an antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs) and framework regions, wherein said framework regions of said variable domain comprise predominantly human acceptor antibody heavy chain framework region residues, the remaining heavy chain framework region residues corresponding to the equivalent residues using the Kabat numbering system in a donor antibody, said donor antibody having binding affinity for said antigen, wherein, according to the Kabat numbering system, in said composite heavy chain at least residues 31 to 35, 50 to 65 and 95 to 102 in the CDRs and at least residues 23, 24, 49, 71, and 73 in the framework regions correspond to the equivalent residues in said donor antibody.

58. (Amended three times) The antibody molecule of claim 56, wherein additionally at least one of the residues selected from the group consisting of residues 6, 37, 48 and 94 in said composite heavy chain corresponds to the equivalent residue in said donor antibody.

62. (Amended four times) The antibody molecule of claim 56, wherein said complementary light chain is a composite light chain having a variable domain including CDRs and framework regions, wherein said framework regions of said light chain variable domain comprise predominantly human acceptor antibody light chain framework region residues, the remaining light chain framework region residues corresponding to the equivalent residues using the Kabat numbering system in said donor antibody, wherein, according to the Kabat numbering system, in said composite light chain at least residues 24 to 34, 50 to 56 and 89 to 97 in the CDRs and at least residues 46, 48, 58 and 71 in the framework regions correspond to the equivalent residues in said donor antibody.

REMARKS

Claims 56-73 are pending in the present application. Claims 56, 58 and 62 have been amended herein. No new matter has been added. Upon entry of the present amendment, claims 56-73 will remain pending.

As a preliminary matter, claim 58 has been amended as suggested in the Office Action to delete the comma inside brackets.

Applicants acknowledge receipt of the "Attachment for PTO-948" outlining changes for prosecution of applications containing drawings. In addition, Applicants enclose herewith a Drawing Change Authorization Request in which changes to Figures 5c and 6 are proposed. In particular, the changes in regard to Figure 5c are directed to reciting the correct sequence identifier. No new matter is added. In addition, the changes to Figure 6 are directed to replacing the "RW" amino acids with "LL" amino acids, support for which can be found, for example, in Table 2 at page 50 of the specification where positions 46 and 47 are both indicated to be "L" amino acids. Thus, no new matter is being added. The drawings have also been amended to incorporate sequence identifiers. Formal drawings have been filed on date even herewith under separate cover to the Draftsperson, including formal drawings of Figures 5c and 6, in order to be completely responsive to the Office Action.

Applicants have amended the Sequence Listing to correct the typographical error in SEQ ID NO:27 set forth above (e.g., replacement of "RW" with "LL"). New pages are provided to comply with the Sequence Rules set forth in 37 CFR §§ 1.821-1.825. In addition, enclosed herewith is a Statement to Support Filing and Submission of DNA/Amino Acid Sequences in Accordance with 37 CFR §§ 1.821-1.825, and a computer readable form (CRF). No new matter has been added. In addition, the contents of the paper copy of the Sequence Listing and computer readable copy of the Sequence Listing, submitted in accordance with 37 CFR §§ 1.821(c) and (e), are the same.

I. There Is No Obviousness-Type Double Patenting

Claims 56 and 62 remain rejected under the doctrine of obviousness-type double patenting as allegedly being unpatentable over claims 3 and 7 of U.S. Patent No. 5,859,205 (the "'205 patent").

Applicants traverse the rejection and request reconsideration thereof because a proper *prima facie* case of obviousness has not been made.

The only reasoning provided in the Office Action for the rejection is that claims 56 and 62 of the present application and claims 3 and 7 of the '205 patent relate to the same inventive concept and that claims 56 and 62 are **generic** to claims 3 and 7 because they have all of the characteristics of the claimed humanised antibody. These assertions, however, are insufficient to support a proper *prima facie* case of obviousness-type double patenting. Rather, an obviousness-type double patenting rejection is analogous to a failure to meet the non-obviousness requirement of 35 U.S.C. § 103. *In re Braithwaite*, 154 U.S.P.Q. 29, 34 (C.C.P.A. 1967) and *In re Longi*, 225 U.S.P.Q. 645, 648 n.4 (Fed. Cir. 1985). Thus, under the law, the pivotal question in an obviousness-type double patenting analysis is: Does any claim in the application define merely an obvious variation of an invention disclosed and claimed in the patent? *In re Vogel*, 164 U.S.P.Q. 619 (C.C.P.A. 1970). If the answer to this question is no, there can be no double patenting.

In making the obviousness-type double patenting analysis, then, the proper inquiry is as taught in *Graham v. John Deere Co.*, 383 U.S. 1 (1966). See, M.P.E.P. § 804. A determination whether one patent is generic to another patent is not the appropriate inquiry. The following quotation from *In re Kaplan*, 229 U.S.P.Q. 678, 681 (Fed. Cir. 1986) is instructive:

> By domination we refer ... to that phenomenon ... whereunder one patent has a broad or "generic" claim which "reads on" an invention defined by another narrower or more specific claim in another patent, the former "dominating" the latter because the more narrowly claimed invention cannot be practiced without infringing the broader claim ... In possibly simpler terms, one patent dominates another if a claim of the first patent reads on a device built or process practiced according to the second patent disclosure. This commonplace situation is not, *per se*, double patenting as the board seems to think. (citations omitted).

Thus, that some of Applicants' antibodies claimed in the present patent application may also meet limitations of claims in the '205 patent is not, alone, grounds for an obviousness-type double patenting rejection. It may simply be a case of one patent application dominating another patent application. Domination by itself cannot support a double patenting rejection. The obviousness-type

double patenting rejection is, therefore, misplaced. Further, the law requires more than a mere overlap in claim scope when concluding that particular compounds are obvious variants. Indeed, as stated by the Federal Circuit:

> The fact that a claimed compound may be encompassed by a disclosed generic formula does not by itself render that compound obvious. (citation omitted)

In re Baird, 29 U.S.P.Q.2d 1550, 1552 (Fed. Cir. 1994). As stated in § 804 of the M.P.E.P., the analysis employed in an obviousness-type double patenting determination parallels the guidelines for analysis of a 35 U.S.C. § 103 rejection, which requires analysis of the factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1 (1966). No such factual inquiries have, in fact, been set forth in the Office Action. In view of the foregoing, Applicants request that the obviousness-type double patenting rejection be withdrawn.

II. The Claims Are Clear And Definite

Claims 56-73 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as their invention. Applicants traverse the rejection and request reconsideration thereof because the claims are clear and definite.

The Office Action acknowledges that the terms "predominantly" and "remaining" are terms commonly used in English and that the term "predominantly" is defined in dictionaries. The Office Action, quite remarkably, asserts that the "metes and bounds" of these terms are not defined in the specification or the dictionary. As set forth in the previous response, "predominantly" means "having numerical superiority or advantage" (as defined in *Random House Webster's Dictionary*, 2nd ed., Random House, New York, 1997, p.1026). Thus, for a particular antibody having human acceptor antibody heavy chain framework residues (acceptor residues) and also having residues corresponding to residues in a donor antibody (donor residues), such antibody has "predominantly" acceptor residues if there is a greater number of acceptor residues than donor residues. For example, if a heavy chain has 51 acceptor residues and 50 donor residues, then it has "predominantly" acceptor

residues. Regarding the term "remaining," Applicants respectfully submit that this term must not be viewed in a vacuum. Claim 56 recites that the variable domain comprises predominantly human acceptor antibody heavy chain framework residues and that the "remaining" heavy chain residues correspond to equivalent residues in a donor antibody. Thus, the term "remaining" refers to those residues that are not the "human acceptor antibody heavy chain framework region residues." To be even more clear, Applicants have amended claims 56 and 62 to recite "remaining heavy chain framework region residues" and "remaining light chain framework region residues," respectively. Claims 56 and 62 have also been amended to provide antecedent basis for these recitations. Persons of ordinary skill would have no difficulty in determining whether a particular antibody meets these criteria. Thus, the claims are definite within the meaning of § 112. *In re Mercier*, 185 U.S.P.Q. 774 (C.C.P.A. 1975) (claims sufficiently define an invention so long as one skilled in the art can determine what subject matter is or is not within the scope of the claims). Because claims 56-73 are clear and definite, Applicants request that the rejection under 35 U.S.C. § 112, second paragraph be withdrawn.

III. The Claimed Invention Is Sufficiently Described

Claims 56-73 are rejected under 35 U.S.C. § 112, first paragraph as allegedly failing to provide a sufficient written description. The Office Action mistakenly asserts that Applicants' specification fails to adequately describe the heavy chain CDR ranges. Applicants traverse the rejection and request reconsideration because Applicants' specification permits a person skilled in the art to clearly recognize that Applicants had possession of the claimed invention.

As stated in the "Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, ¶1, 'Written Description' Requirement,":

> A description as filed is presumed to be adequate, unless or until sufficient evidence or reasoning to the contrary has been presented by the examiner to rebut the presumption. The Examiner, therefore, must have a reasonable basis to challenge the adequacy of the written description. The examiner has the initial burden of presenting by a preponderance of evidence why a person skilled in the art would not recognize in an applicant's disclosure a description of the invention defined by the claims. In rejecting a claim, the

> examiner must set forth express findings of fact regarding the above analysis which support the lack of written description conclusion. These findings should: (1) Identify the claim limitation at issue; and (2) Establish a *prima facie* case by providing reasons why a person skilled in the art at the time the application was filed would not have recognized that the inventor was in possession of the invention as claimed in view of the disclosure of the application as filed.

In accordance with these standards, Applicants have indeed, provided a sufficient written description of the claimed inventions. The Office Action fails to establish a *prima facie* case, let alone show sufficient evidence to maintain this rejection.

In stark contrast to the mistaken assertion in the Office Action that Applicants did not contemplate nor disclose use of the CDRs defined by Wu and Kabat for humanising antibodies, Applicants provide ample written description regarding the heavy chain CDR ranges for not only the Kabat CDRs, but also for all claimed inventions. Applicants teach, for example, at page 8, lines 8-16 of the specification, that the antibody molecules of the present invention can comprise three donor CDRs that can be: 1) the Kabat CDRs; 2) the structural loop CDRs; 3) a composite of the Kabat and structural loop CDRs; and 4) any combination of any of these. Applicants teach, for example, at page 19, lines 19-23 of the specification, that the Kabat CDRs comprise residues 31-35, 50-65, and 95-102 of the heavy chain. Thus, Applicants ***clearly*** teach the ranges of heavy chain CDRs recited in claim 56 (*i.e.*, 31 to 35, 50 to 65 and 95 to 102). At page 19, lines 24-31 of the specification, Applicants teach that the structural loop CDRs comprise residues 26-32 of the heavy chain. Residues 26 to 35, thus, represent a composite of the Kabat CDR H1 and the structural loop CDR H1. To make this CDR composite, residues 26-30, in addition to residues 31-35, are donor in the heavy chain. Indeed, page 17, lines 6-11 of the specification, ***expressly teaches*** that donor residues that are substituted for acceptor residues in the CDRs include regions defined as residues 26-35, 50-65 and 95-102. Claim 57 recites that, in addition to residues 31 to 35 (see claim 56 from which claim 57 depends), residues 26 to 30 also come from the donor antibody. Thus, in effect, claim 57 encompasses antibodies that comprise the composite CDR (*i.e.*, residues 26 to 35). The effective range of the particular heavy chain CDR recited in claim 57 (*i.e.*, 26 to 35) is clearly supported by

Applicants' specification. Thus, Applicants' specification ***clearly*** provides written description of heavy chain CDRs having the recited residues, which are ***clearly*** taught as regions that can be substituted. Accordingly, Applicants request that the written description rejection under 35 U.S.C. § 112, first paragraph be withdrawn.

IV. The Claimed Invention Is Enabled

Claims 56-73 are rejected under 35 U.S.C. § 112, first paragraph as allegedly failing to provide an enabling disclosure. Applicants traverse the rejection and request reconsideration because one skilled in the art would be able to practise the claimed invention without being required to perform undue experimentation.

A. Residues 23, 24, 49, 71 and 73

The Office Action mistakenly asserts that it would require undue experimentation for one skilled in the art to retain antigen binding in an antibody wherein at least residues 23, 24, 49, 71 and 73 in the framework region correspond to the equivalent residues in the donor antibody. In particular, the Examiner doubts whether substitution of residues 23, 24, 49, 71 and 73, without also substituting position 48, would result in an antibody that retained antigen binding. As will be recognized, however, the enablement requirement of §112 is satisfied so long as a disclosure contains sufficient information that persons of ordinary skill in the art having the disclosure before them would be able to make and use the invention. *In re Wands*, 8 U.S.P.Q.2d 1400 (Fed. Cir. 1988) (the legal standard for enablement under §112 is whether one skilled in the art would be able to practice the invention without undue experimentation). In this respect, the following statement from *In re Marzocchi*, 169 U.S.P.Q. 367, 369-370 (C.C.P.A. 1971), is noteworthy:

> The only relevant concern of the Patent Office under these circumstances should be over the truth of any such assertion. The first paragraph of §112 requires nothing more than objective enablement. How such a teaching is set forth, either by the use of illustrative examples or by broad terminology, is of no importance.

> As a matter of Patent Office practice, then, a specification disclosure which contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented **must** be taken as in compliance with the enabling requirements of the first paragraph of §112 unless there is reason to doubt the objective truth of the statements contained therein which must be relied upon for enabling support. (emphasis added)

Any assertion by the Patent Office that an enabling disclosure is not commensurate in scope with the protection sought must be supported by evidence or reasoning substantiating the doubts so expressed. *In re Dinh-Nguyen*, 181 U.S.P.Q. 46 (C.C.P.A. 1974); *In re Bowen*, 181 U.S.P.Q. 48 (C.C.P.A. 1974).

The reasoning provided in the Office Action in support of the enablement rejection is three-fold: 1) humanised antibodies 61E71 and hTNF3 in Applicants' specification require substitution at position 48; 2) U.S. Patent No. 5,530,101 shows that different humanised antibodies require different combination of mouse residues, and 3) Applicants' specification is alleged to teach that residues 71, 73 and 78 will "always" be all donor or all acceptor residues. Each of these reasons will be addressed separately below.

First, what may be required for a particular antibody is not necessarily required for all antibodies. Applicants teach a hierarchy of residues which can, if necessary, be changed in sequence. Depending on the antibody, different residues may need to be changed. Applicants teach, at page 20, line 25 of the specification, that particular key residues near the CDR contribute to antigen binding, *i.e.*, residues 23, 71 and 73. Each of these residues are recited in claim 56. Applicants also teach, at page 21, line 9 of the specification, that particular key packing residues near the CDR contribute to antigen binding, *i.e.*, residues 24, 49 and 78. Residues 24 and 49 are recited in claim 56. Thus, five of the six residues identified as being key residues are recite in claim 56. Residue 48, identified in the Office Action and alleged to be necessary, is not among these. If the Examiner maintains that residue 48 is required to be a donor residue, Applicants request that the Examiner provide an affidavit containing evidence substantiating this position. 37 C.F.R. § 1.104(d)(2).

U.S. Patent No. 5,530,101 (the "'101 patent") is alleged in the Office Action to show that different humanised antibodies require different combination of mouse residues for antigen binding. Applicants respectfully submit that the '101 patent is irrelevant. Regardless, Applicants cannot find, nor did the Office Action point out, any portion of the '101 patent that teaches that an antibody molecule that comprises donor residues at positions 23, 24, 49, 71 and 73 will be unable to bind an antigen. Rather, the Office Action supports such an erroneous conclusion by attempting to show that a change in one amino acid in the OKT3 antibody disclosed in Applicants' specification "could drastically change the antibody affinity," referring to antibody constructs JA207 and JA197. Applicants cannot find, however, where their specification shows a "drastic" change in affinity between JA207 and JA197. Indeed, when one skilled in the art examines Figures 7 and 10a, it is quite clear that JA197 and JA207 have binding affinities that are very close to the binding affinity of JA185, which is the "fully grafted" product that has a binding affinity very similar to that of the OKT3 murine reference antibody (see, page 51, lines 29-31 of the specification). In the absence of any specific teaching that an antibody molecule that comprises donor residues at positions 23, 24, 49, 71 and 73 will be unable to bind an antigen, Applicants' statements that such antibodies have affinity for an antigen must be taken as in compliance with the enabling requirements.

Finally, the section of the specification referred to in the Office Action (page 17, section 2.1) for supporting the allegation that residues 71, 73 and 78 are either all donor or all acceptor merely points to a "preferred protocol" for practicing the invention. Indeed, the Summary of the Invention states that these residues are "preferably either all donor or all acceptor" (page 7, lines 3-5 of the specification). Further, Applicants teach, for example, at page 6, lines 28-35 of the specification, that the framework comprises donor residues at at least **one** of positions "71 and/or 73, 75 and/or 76 and/or 78..." Thus, Applicants' specification clearly teaches that residues at positions 71, 73 and 78 can independently be substituted by donor residues.

Thus, in view of the foregoing, there is no reason to believe that one skilled in the art would be required to perform any amount of experimentation, let alone an undue amount, in order to make and use the claimed invention wherein the antibody molecules comprise donor residues at positions 23, 24, 49, 71 and 73. Accordingly, Applicants respectfully request that the rejection under 35

U.S.C. § 112, first paragraph, in regard to donor residue positions 23, 24, 49, 71 and 73 be withdrawn.

B. Framework Regions

The Office Action mistakenly asserts that it would require undue experimentation for one skilled in the art to use a humanised antibody "wherein its framework region is from any human framework region." In particular, the Examiner asserts that "one necessary criteria for choosing these frameworks [EU, REI, KOL, LAY, HIL, SGI, and SGIII] is that they are substantially analogous to the donor framework." This assertion is, however, wholly unsupported by any evidence and is, in fact, explicitly contrary to the teachings in Applicants' specification.

Applicants teach at, for example page 11, lines 21-33 of the specification:

> However, a high level of homology between donor and acceptor sequences is not important for application of the present invention. The present invention identifies a hierarchy of residue positions at which donor residues may be important or desirable for obtaining a CDR-grafted antibody product having satisfactory binding properties. The CDR-grafted products usually have binding affinities of at least 10^5 M^{-1}, preferably at least about 10^8 M^{-1}, or especially in the range 10^8-10^{12} M^{-1}. In principle, the present invention is applicable to any combination of donor and acceptor antibodies irrespective of the level of homology between their sequences.

Thus, contrary to the erroneous assertions in the Office Action, substantial homology between the acceptor and donor framework is not a necessary criteria. If the Examiner maintains that a particular level of homology is a necessary criteria, Applicants request that the Examiner provide an affidavit containing evidence substantiating this position. 37 C.F.R. § 1.104(d)(2).

The Office Action also asserts that Applicants' specification teaches that human KOL and NEWM heavy chain frameworks "could not be used for humanizing antibody B72.3" because of poor homology as allegedly disclosed on page 56 of the specification. Applicants' specification, however, does **not** teach that KOL and NEWM "could not be used for humanizing antibody B72.3." Rather, Applicants teach that the EU heavy chain was chosen for B72.3 to determine **whether** "transfer of the CDRs from mouse to human frameworks might be facilitated if the overall homology

between the donor and the receptor frameworks was maximised." There is **no** discussion or suggestion that KOL and NEWM would not work. Indeed, the contrary is suggested for B72.3; Applicants were trying to see whether the human framework selection had to be of a known crystal structure, or could be based on another criteria. That some experimentation may be required (and Applicants maintain that no further experimentation is required) does not preclude enablement so long as the amount of experimentation is not undue. *W. L. Gore & Associates, Inc. v. Garlock, Inc.*, 220 U.S.P.Q. 303, 316 (Fed. Cir. 1983). Further, the Office Action fails to establish that if any experimentation is required, it is anything other than routine experimentation. Indeed, routine experimentation does not constitute undue experimentation.

> The test [for undue experimentation] is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed to enable the determination of how to practice a desired embodiment of the claimed invention.

PPG Indus., Inc. v. Guardian Indus. Corp., 75 F.3d 1558, 1564, 37 U.S.P.Q.2d 1618, 1623 (Fed. Cir. 1996) (quotation and citation omitted). Thus, the Office Action fails to establish that any experimentation, let alone undue experimentation, is required to practice the claimed invention.

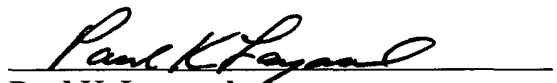
The Office Action asserts that the binding data in Table 2 of the Yarranton Declaration is confusing, and appears to allege that the data conflicts. Applicants submit there is no conflict between the data provided therein. The Examiner continues to misread the data. For 61E71, the first data point for 61E71, *i.e.*, 100, falls under the heading "POTENCY RELATIVE TO RODENT ANTIBODY" and represents the potency relative to the rodent antibody as measured by the relative ability to compete with the murine antibody for binding to the antigen. Thus, 61E71 is as potent in antigen binding as the murine antibody. The second data point for 61E71, *i.e.*, <1, falls under the heading "ANTI-CYTOKINE" and represents the score using a cytokine neutralization assay in which antibody binds to the cytokine and the resultant complex is tested for the ability to affect growth of L929 cells, which are dependent on TNFα. Thus, 61E71 is a potent inhibitor of L929 cell growth, demonstrating that the antibody not only binds TNFα but also has biological effectiveness.

Thus, in view of the foregoing, there is no reason to believe that one skilled in the art would be required to perform any amount of undue experimentation in order to make and use the claimed invention wherein the antibody framework region is from any human framework region. Accordingly, Applicants respectfully request that the rejection under 35 U.S.C. § 112, first paragraph, in regard to framework regions be withdrawn.

V. Conclusion

In view of the foregoing, Applicants respectfully submit that the claims are in condition for allowance. An early notice of the same is earnestly solicited. The Examiner is invited to contact Applicants' undersigned representative at (215) 564-8906 if there are any questions regarding Applicants' claimed invention. Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned **"Version with markings to show changes made."**

Respectfully submitted,



Paul K. Legaard
Registration No. **38,534**

Date: **November 12, 2001**

WOODCOCK WASHBURN LLP
One Liberty Place - 46th Floor
Philadelphia, PA 19103
Telephone: (215) 568-3100
Facsimile: (215) 568-3439

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Claims:

Claims 56, 58 and 62 have been amended as follows:

56. (Amended four times) An antibody molecule having affinity for an antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs) and framework regions, [said] wherein said framework regions of said variable domain comprise [comprising] predominantly human acceptor antibody heavy chain framework region residues, the remaining heavy chain framework region residues corresponding to the equivalent residues using the Kabat numbering system in a donor antibody, said donor antibody having binding affinity for said antigen, wherein, according to the Kabat numbering system, in said composite heavy chain at least residues 31 to 35, 50 to 65 and 95 to 102 in the CDRs and at least residues 23, 24, 49, 71, and 73 in the framework regions correspond to the equivalent residues in said donor antibody.

58. (Amended three times) The antibody molecule of claim 56, wherein additionally at least one of the residues selected from the group consisting of residues 6, 37, 48 and 94 in said composite heavy chain corresponds [,] to the equivalent residue in said donor antibody.

62. (Amended four times) The antibody molecule of claim 56, wherein said complementary light chain is a composite light chain having a variable domain including CDRs and framework regions, [said] wherein said framework regions of said light chain variable domain [comprising] comprise predominantly human acceptor antibody light chain framework region residues, the remaining light chain framework region residues corresponding to the equivalent residues using the Kabat numbering system in said donor antibody, wherein, according to the Kabat numbering system, in said composite light chain at least residues 24 to 34, 50 to 56 and 89 to 97 in the CDRs and at least residues 46, 48, 58 and 71 in the framework regions correspond to the equivalent residues in said donor antibody.

WOODCOCK WASHBURN
INTELLECTUAL PROPERTY LAW

PHILADELPHIA
One Liberty Place, 46th floor
Philadelphia, PA 19103
Phone: 215-568-3100
Fax: 215-568-3439

SEATTLE
999 Third Avenue, Suite 1606
Seattle, WA 98104
Phone: 206-332-1380
Fax: 206-624-7317

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DATE: March 18, 2002 JOB CODE: 37136.6

Please deliver this and the following pages to:

Name:	Examiner M. Davis
Company/Firm:	U.S.P.T.O.
Telecopier No.:	(703) 746-7145
Client/Matter No.:	U.S. Serial No. 08/485,686; Our Docket No. CARP-0046
Sender's Name:	Paul K. Legaard
Pages to Follow:	3

If transmission is not complete, please call (215) 568-3100
COVER MESSAGE:

Examiner Davis, attached is a copy of proposed amendments to the claims which corresponds with our discussion last week. Please call me to discuss them. If you concur with these amendments, I will prepare a formal amendment to be faxed to you later this afternoon. Best regards, Paul.

Serial No.: 08/485,686
Filed: June 7, 1995

CARP-0046 PENDING CLAIMS

56. (AMEND) An antibody molecule having affinity for an antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs) and framework regions, wherein said framework regions of said variable domain comprise predominantly human acceptor antibody heavy chain framework region residues, the remaining heavy chain framework region residues corresponding to the equivalent residues using the Kabat numbering system ~~in a donor antibody~~, said donor antibody having binding affinity for said antigen, wherein, according to the Kabat numbering system, [in] said composite heavy chain [at least] comprises residues 31 to 35, 50 to 65 and 95 to 102 in the CDRs and [at least] comprises residues 23, 24, 49, 71, [and] 73, and 78 in the framework regions that correspond to the equivalent residues in said donor antibody.

are donor residues.

Key ? f. to mean residues near CDR

57. (AMEND) The antibody molecule of claim 56, wherein additionally residues 26 to 30 [and 78] in said composite heavy chain correspond to the equivalent residues in said donor antibody.

58. (AMEND) The antibody molecule of claim 56, wherein additionally [at least one of the residues] a residue selected from the group consisting of residues 6, 37, 48 and 94 in said composite heavy chain corresponds to the equivalent residue in said donor antibody.

59. (AMEND) The antibody molecule of claim 57, wherein additionally [at least one of residues] a residue selected from the group consisting of 6, 37, 48 and 94 in said composite heavy chain corresponds to the equivalent residue in said donor antibody.

60. (AMEND) The antibody molecule of claim 58, wherein additionally [at least one of residues] a residue selected from the group consisting of 2, 4, 25, 36, 39, 47, 93, 103, 104, 106 and 107 in said composite heavy chain corresponds to the equivalent residue in said donor antibody.

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CARP-0046 PENDING CLAIMS

61. (AMEND) The antibody molecule of claim 59, wherein additionally [at least one of residues] a residue selected from the group consisting of 2, 4, 25, 36, 39, 47, 93, 103, 104, 106 and 107 in said composite heavy chain corresponds to the equivalent residue in said donor antibody.

62. (AMEND) The antibody molecule of claim 56, wherein said complementary light chain is a composite light chain having a variable domain including CDRs and framework regions, wherein said framework regions of said light chain variable domain comprise predominantly human acceptor antibody light chain framework region residues, the remaining light chain framework region residues corresponding to the equivalent residues using the Kabat numbering system in said donor antibody, wherein, according to the Kabat numbering system, [in] said composite light chain [at least] comprises residues 24 to 34, 50 to 56 and 89 to 97 in the CDRs and [at least] comprises residues [46], 48, 58 and 71 in the framework regions correspond to the equivalent residues in said donor antibody.

63. (AMEND) The antibody molecule of claim 62, wherein additionally [at least one of residues] a residue selected from the group consisting of 2, 4, 6, 35, 38, 44, 47, 49, 62, 64, 65, 66, 67, 68, 69, 85, 87, 98, 99, 101 and 102 in said composite light chain corresponds to the equivalent residue in said donor antibody.

64. The antibody molecule of any one of claims 56, 57 or 62 which has specificity for a T-cell antigen.

65. The antibody molecule of any one of claims 56, 57 or 62 which has specificity for a lymphokine.

66. The antibody molecule of any one of claims 56, 57 or 62 which has specificity for a growth factor.

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67. The antibody molecule of any one of claims 56, 57 or 62 which has specificity for interferon.

68. The antibody molecule of any one of claims 56, 57 or 62 which has specificity for an adhesion molecule.

69. The antibody molecule of any one of claims 56, 57 or 62 which has specificity for a hormone.

70. The antibody molecule of any one of claims 56, 57 or 62 which has specificity for a cancer marker.

71. The antibody molecule of any one of claims 56, 57 or 62 which has specificity for a TNF-α.

72. The antibody molecule of any one of claims 56, 57 or 62 which has specificity for mucin.

73. (AMEND) A [therapeutic] composition comprising the antibody molecule of any one of claims 56, 57 or 62 in combination with a pharmaceutically acceptable carrier, diluent or excipient.

DOCKET NO.: CARP-0046 **PATENT-DRAFT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Adair et al.

Serial No.: **08/485,686**

Group Art Unit: **1642**

Filed: **June 7, 1995**

Examiner: **M. Davis**

For: **Humanised Antibodies**

I, Doreen Yatko Trujillo, Registration No. 35,719 certify that this correspondence is being deposited with the U.S. Postal Service as First Class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

On August 9, 2002

Doreen Yatko Trujillo

Doreen Yatko Trujillo, Reg. No. 35,719

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

AMENDMENT AND REQUEST FOR RECONSIDERATION

In response to the Office Action mailed April 9, 2002 in connection with the above-identified patent application, Applicants respectfully request that the application be amended as follows. The period for responding to the Office Action has been extended, by enclosure of a petition and fee, to and through August 9, 2002.

In the Claims:

Please amend claims 56-63 and 73 to read as follows:

56. (Amended five times) An antibody molecule having affinity for an antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs) and framework regions, wherein said framework regions of said variable domain comprise predominantly human acceptor

antibody heavy chain framework region residues, the remaining heavy chain framework region residues corresponding to the equivalent residues using the Kabat numbering system in a donor antibody, said donor antibody having binding affinity for said antigen, wherein, according to the Kabat numbering system, said composite heavy chain comprises residues 31 to 35, 50 to 65 and 95 to 102 in the CDRs and comprises residues 23, 24, 49, 71, 73, and 78 in the framework regions that correspond to the equivalent residues in said donor antibody.

57. (Amended twice) The antibody molecule of claim 56, wherein additionally residues 26 to 30 in said composite heavy chain correspond to the equivalent residues in said donor antibody.

58. (Amended four times) The antibody molecule of claim 56, wherein additionally a residue selected from the group consisting of residues 6, 37, 48 and 94 in said composite heavy chain corresponds to the equivalent residue in said donor antibody.

59. (Amended) The antibody molecule of claim 57, wherein additionally a residue selected from the group consisting of 6, 37, 48 and 94 in said composite heavy chain corresponds to the equivalent residue in said donor antibody.

60. (Amended) The antibody molecule of claim 58, wherein additionally a residue selected from the group consisting of 2, 4, 25, 36, 39, 47, 93, 103, 104, 106 and 107 in said composite heavy chain corresponds to the equivalent residue in said donor antibody.

61. (Amended) The antibody molecule of claim 59, wherein additionally a residue selected from the group consisting of 2, 4, 25, 36, 39, 47, 93, 103, 104, 106 and 107 in said composite heavy chain corresponds to the equivalent residue in said donor antibody.

62. (Amended five times) The antibody molecule of claim 56, wherein said complementary light chain is a composite light chain having a variable domain including CDRs and framework

regions, wherein said framework regions of said light chain variable domain comprise predominantly human acceptor antibody light chain framework region residues, the remaining light chain framework region residues corresponding to the equivalent residues using the Kabat numbering system in said donor antibody, wherein, according to the Kabat numbering system, said composite light chain comprises residues 24 to 34, 50 to 56 and 89 to 97 in the CDRs and comprises residues 48, 58 and 71 in the framework regions correspond to the equivalent residues in said donor antibody.

63. (Amended twice) The antibody molecule of claim 62, wherein additionally a residue selected from the group consisting of 2, 4, 6, 35, 38, 44, 47, 49, 62, 64, 65, 66, 67, 68, 69, 85, 87, 98, 99, 101 and 102 in said composite light chain corresponds to the equivalent residue in said donor antibody.

73. (Amended twice) A composition comprising the antibody molecule of any one of claims 56, 57 or 62 in combination with a pharmaceutically acceptable carrier, diluent or excipient.

REMARKS

Claims 56-73 are pending in the present application. Claims 56-63 and 73 have been amended herein. No new matter has been added. Upon entry of the present amendment, claims 56-73 will remain pending.

As a preliminary matter, Applicants thank the Examiner for taking the time and effort to engage Applicants' representative in an interview on April 3, 2002, as well as subsequent discussions thereafter. The following remarks are based upon the substance of the interview.

In addition, the Interview Summary provided along with the present Office Action requests that Applicants provide a "comparison between the sequences having substituted residues of Queen et al as shown for example in Table I and the claimed sequences after adjusting for differences in numbering systems." During the interview, the Examiner indicated a desire to see a comparison similar to the one filed in connection with application Serial No. 08/116,247. Although Applicants contend that such a showing is not required, Applicants submit herewith a comparison similar to the

comparison submitted in application Serial No. 08/116,247.

All four sequences presented in the attachments were reported in Queen *et al.*, *PNAS-USA*, *86*:10029-10033, **1989**. More specifically, the sequences are found at the bottom of page 10031 of the reference. Applicants duplicated the sequences in the attachments to facilitate comparison of linear numbering with Kabat numbering. All sequences in the attachments are presented in single letter amino acid code.

The first sequence on the first page of the attachments is the top sequence in Panel A on page 10031 and represents the amino acid sequence of the light chain variable domain of the human Eu antibody. The second sequence on the first page is the bottom sequence in Panel A and represents the amino acid sequence of the light chain variable domain of an anti-Tac antibody. The first sequence on the second page of the attachments is the top sequence in Panel B on page 10031 and represents the amino acid sequence of the heavy chain variable domain of the human Eu antibody. The second sequence on the second page is the bottom sequence in Panel B and represents the amino acid sequence of the heavy chain variable domain of an anti-Tac antibody.

The amino acids of the sequences presented in the attachments are numbered using two different numbering systems. The numbers above each sequence are according to the numbering system used in Queen *et al.*, which represents the linear numbering system. The numbers below each sequence are according to the Kabat numbering system. As is evident from the attachments, the two numbering systems result in the assignment of the same residue number to a particular amino acid in some instances - *i.e.*, the first sequence on the first page of the attachments. However, in other instances - *i.e.*, the remaining sequences of the attachments - the two numbering systems do not result in the assignment of the same residue number to a particular amino acid.

The differences between the two approaches is clearly evident from the comparison. The residues which are specified to be donor residues are indicated by horizonal bars. The blue bars above the sequences depict the residues which are specified as donor in Queen *et al.* The red bars below the sequences depict the ***minimum*** number of residues specified as donor in Applicants' invention (as set forth in, for example, claims 56 and 62).

I. No New Matter Has Been Introduced Into The Claims

Claims 56-73 were rejected under 35 U.S.C. § 112, first paragraph as allegedly containing new matter. The Office Action objects to the phrase "at least" in claims 56 and 62. Applicants request reconsideration in view of the amended claims.

Although Applicants provide ample written description of an antibody molecule having, *inter alia*, a composite heavy chain in which at least residues 23, 24, 49, 71, and 73 in the framework regions correspond to the equivalent residues in a donor antibody (referring to claim 56) and an antibody molecule having, *inter alia*, a composite light chain in which at least residues 46, 48, 58 and 71 in the framework regions correspond to the equivalent residues in a donor antibody (referring to claim 62), to advance prosecution of the present application Applicants have amended the claims as suggested during the teleconference with the Examiner on April 3, 2002. In particular, the Examiner suggested deleting "at least" and replacing it with "comprises." In addition, the Examiner suggested that Applicants insert "78" in claim 56 and delete "46" in claim 62. Applicants have also amended claims 57-61, 63 and 73 to be consistent with the language of the claims from which they depend. In addition, claim 73 has been amended, as suggested by the Examiner, to provide additional clarity.

In view of the forgoing, Applicants respectfully request that the new matter rejection under 35 U.S.C. § 112, first paragraph be withdrawn.

II. There Is No Obviousness-Type Double Patenting

Claims 56 and 62 remain rejected under the doctrine of obviousness-type double patenting as allegedly being unpatentable over claims 3 and 7 of U.S. Patent No. 5,859,205 (the "'205 patent"). Applicants, again, traverse the rejection and request reconsideration thereof because **a proper *prima facie* case of obviousness has not been made**.

The only reason of record provided by the Examiner (see, the Office Action mailed August 10, 2001) for the rejection is that claims 56 and 62 of the present application and claims 3 and 7 of the '205 patent relate to the same inventive concept and that claims 56 and 62 are **generic** to claims 3 and 7 because they have all of the characteristics of the claimed humanised antibody. **These assertions, however, are insufficient to support a proper *prima facie* case of obviousness-type**

double patenting. Rather, an obviousness-type double patenting rejection is analogous to a failure to meet the non-obviousness requirement of 35 U.S.C. § 103. *In re Braithwaite*, 154 U.S.P.Q. 29, 34 (C.C.P.A. 1967) and *In re Longi*, 225 U.S.P.Q. 645, 648 n.4 (Fed. Cir. 1985). Thus, under the law, the pivotal question in an obviousness-type double patenting analysis is: Does any claim in the application define merely an obvious variation of an invention disclosed and claimed in the patent? *In re Vogel*, 164 U.S.P.Q. 619 (C.C.P.A. 1970). If the answer to this question is no, there can be no double patenting.

In making the obviousness-type double patenting analysis, then, the proper inquiry is as taught in *Graham v. John Deere Co.*, 383 U.S. 1 (1966). See, M.P.E.P. § 804. **A determination whether one patent is generic to another patent is not the appropriate inquiry.** The following quotation from *In re Kaplan*, 229 U.S.P.Q. 678, 681 (Fed. Cir. 1986) is instructive:

> By domination we refer ... to that phenomenon ... whereunder one patent has a broad or "generic" claim which "reads on" an invention defined by another narrower or more specific claim in another patent, the former "dominating" the latter because the more narrowly claimed invention cannot be practiced without infringing the broader claim ... In possibly simpler terms, one patent dominates another if a claim of the first patent reads on a device built or process practiced according to the second patent disclosure. This commonplace situation is not, *per se*, double patenting as the board seems to think. (citations omitted).

Thus, that some of Applicants' antibodies claimed in the present patent application may also meet limitations of claims in the '205 patent is not, alone, grounds for an obviousness-type double patenting rejection. It may simply be a case of one patent application dominating another patent application. **Domination by itself cannot support a double patenting rejection.** The obviousness-type double patenting rejection is, therefore, misplaced. Further, the law requires more than a mere overlap in claim scope when concluding that particular compounds are obvious variants. Indeed, as stated by the Federal Circuit:

> The fact that a claimed compound may be encompassed by a disclosed generic formula does not by itself render that compound obvious. (citation omitted)

In re Baird, 29 U.S.P.Q.2d 1550, 1552 (Fed. Cir. 1994). As stated in § 804 of the M.P.E.P., the analysis employed in an obviousness-type double patenting determination parallels the guidelines for analysis of a 35 U.S.C. § 103 rejection, which requires analysis of the factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1 (1966). No such factual inquiries have, in fact, been set forth in the Office Action.

The only rebuttal offered by the Examiner in response to the above arguments in Applicants previous response is that because "the instant claims 56 and 62 are narrower than claims 3 and 7 of PN=5,859,205" claims 56 and 62 "would be subjected to obviousness-type double patenting." Applicants understand that the Examiner may want to "subject" the claims of the present application to an obviousness-type double patenting "analysis." The Examiner, however, fails to carry out such an analysis, let alone provide any reasoning or evidence supporting the obviousness of the 56 and 62. **Thus, the Examiner has not established a *prima facie* case of obviousness.** Again, merely because some of Applicants' antibodies claimed in the present patent application may also meet limitations of claims in the '205 patent is not, alone, grounds for an obviousness-type double patenting rejection. If the present rejection is not withdrawn, Applicants respectfully request that the Examiner call Applicants' undersigned representative so that an interview can be scheduled with the Examiner and the Examiner's supervisor.

In view of the foregoing, Applicants request that the obviousness-type double patenting rejection be withdrawn.

III. The Amendments to the Drawings are Supported by the Specification

The Office Action objects to the proposed changes in Figure 6 and instructs Applicants to correct the same. In particular, the Office Action asserts that the drawing changes have not been granted because "it seems that changing the amino acid residues RW to LL of the sequence gL221B would be new matter." Applicants respectfully request that this objection be withdrawn and the formal drawings accepted because the specification provides ample written description supporting the changes to the drawings.

As pointed out in the previously filed response, **the proposed change in Figure 6, in**

which the "RW" amino acids are replaced with "LL" amino acids, find support, for example, in Table 2 at page 50 of the specification. In particular, Table 2 provides explicit written description showing that amino acids at positions 46 and 47 of gL221B are both "L" (*i.e.*, leucine). Thus, the specification provides explicit written description support for the proposed changes to Figure 6. No new matter is being added to Figure 6.

In view of the foregoing, Applicants request that the objections to the proposed changes to the drawings be withdrawn and that the formal drawings be accepted.

IV. Conclusion

In view of the foregoing, Applicants respectfully submit that the claims are in condition for allowance. An early notice of the same is earnestly solicited. The Examiner is respectfully requested to contact Applicants' undersigned representative at (215) 564-8352 if a Notice of Allowance is not forthcoming so that an interview can be scheduled. Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned **"Version with markings to show changes made."**

Respectfully submitted,

Doreen Yatko Trujillo

Doreen Yatko Trujillo
Registration No. **35,719**

Date: August 9, 2002

WOODCOCK WASHBURN LLP
One Liberty Place - 46th Floor
Philadelphia, PA 19103
Telephone: (215) 568-3100
Facsimile: (215) 568-3439

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Claims:

Claims 56-63 and 73 have been amended as follows:

56. (Amended five times) An antibody molecule having affinity for an antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs) and framework regions, wherein said framework regions of said variable domain comprise predominantly human acceptor antibody heavy chain framework region residues, the remaining heavy chain framework region residues corresponding to the equivalent residues using the Kabat numbering system in a donor antibody, said donor antibody having binding affinity for said antigen, wherein, according to the Kabat numbering system, [in] said composite heavy chain [at least] comprises residues 31 to 35, 50 to 65 and 95 to 102 in the CDRs and [at least] comprises residues 23, 24, 49, 71, [and] 73, and 78 in the framework regions that correspond to the equivalent residues in said donor antibody.

57. (Amended twice) The antibody molecule of claim 56, wherein additionally residues 26 to 30 [and 78] in said composite heavy chain correspond to the equivalent residues in said donor antibody.

58. (Amended four times) The antibody molecule of claim 56, wherein additionally [at least one of the residues] a residue selected from the group consisting of residues 6, 37, 48 and 94 in said composite heavy chain corresponds to the equivalent residue in said donor antibody.

59. (Amended) The antibody molecule of claim 57, wherein additionally [at least one of residues] a residue selected from the group consisting of 6, 37, 48 and 94 in said composite heavy chain corresponds to the equivalent residue in said donor antibody.

60. (Amended) The antibody molecule of claim 58, wherein additionally [at least one of residues] a residue selected from the group consisting of 2, 4, 25, 36, 39, 47, 93, 103, 104, 106 and 107 in said composite heavy chain corresponds to the equivalent residue in said donor antibody.

61. (Amended) The antibody molecule of claim 59, wherein additionally [at least one of residues] a residue selected from the group consisting of 2, 4, 25, 36, 39, 47, 93, 103, 104, 106 and 107 in said composite heavy chain corresponds to the equivalent residue in said donor antibody.

62. (Amended five times) The antibody molecule of claim 56, wherein said complementary light chain is a composite light chain having a variable domain including CDRs and framework regions, wherein said framework regions of said light chain variable domain comprise predominantly human acceptor antibody light chain framework region residues, the remaining light chain framework region residues corresponding to the equivalent residues using the Kabat numbering system in said donor antibody, wherein, according to the Kabat numbering system, [in] said composite light chain [at least] comprises residues 24 to 34, 50 to 56 and 89 to 97 in the CDRs and [at least] comprises residues [46], 48, 58 and 71 in the framework regions correspond to the equivalent residues in said donor antibody.

63. (Amended twice) The antibody molecule of claim 62, wherein additionally [at least one of residues] a residue selected from the group consisting of 2, 4, 6, 35, 38, 44, 47, 49, 62, 64, 65, 66, 67, 68, 69, 85, 87, 98, 99, 101 and 102 in said composite light chain corresponds to the equivalent residue in said donor antibody.

73. (Amended twice) A [therapeutic] composition comprising the antibody molecule of any one of claims 56, 57 or 62 in combination with a pharmaceutically acceptable carrier, diluent or excipient.

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RECEIPT FOR DOCUMENTS FOR A PATENT APPLICATION

Documents for a patent application have been received and recorded under the provisions of the Patents Act 1977 in the name(s) shown below.

The documents bear this number which should be used on all correspondence concerning them: 8928874 · O

The filing date provisionally given to the application is: 21 DEC 1989

Applicants:

Celltech Limited

RECEIVED
10 JAN 1990

THE DOCUMENTS RECEIVED PURPORT TO BE:

REQUEST FOR GRANT OF A PATENT	✓
DESCRIPTION	✓
CLAIMS	X
DRAWINGS (No of Sheets)	35+35
ABSTRACT	X
STATEMENT OF INVENTORSHIP (Form 7/77)	X
REQUEST FOR SEARCH (Form 9/77)	X
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Address for service.

P. E. Crawley
Celltech Limited
216 Bath Road,
Slough,
SL1 4EN Berks.

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FILED 21 DEC 1989

HUMANISED ANTIBODIES

The present invention relates to humanised antibody molecules (HAMs), to processes for their production using recombinant DNA technology, and to their therapeutic uses.

In the present application, the term "recombinant antibody molecule" (RAM) is used to describe an antibody produced by an process involving the use of recombinant DNA technology, including any analogues of natural immunoglobulins or their fragments. The term "humanised antibody molecule" (HAM) is used to describe a molecule having an antigen binding site derived from an immunoglobulin from a non-human species, remaining immunoglobulin-derived parts of the molecule being derived from a human immunoglobulin. The antigen binding site may comprise either complete variable domains fused onto constant domains or complementarity determining regions grafted onto appropriate framework regions in the variable domains. The abbreviation "MAb" is used to indicate a monoclonal antibody.

In the description, reference is made to a number of publications by number. The publications are listed in numerical order at the end of the description.

Natural immunoglobulins have been known for many years, as have the various fragments thereof, such as the Fab, $(Fab')_2$ and Fc fragments, which can be derived by enzymatic cleavage. Natural immunoglobulins comprise a generally Y-shaped molecule having an antigen-binding site towards the end of each upper arm. The remainder of the structure, and particularly the stem of the Y, mediates the effector functions associated with immunoglobulins.

Natural immunoglobulins have been used in assay, diagnosis and, to a more limited extent, therapy. However, such uses, especially in therapy, have been hindered by the polyclonal nature of natural immunoglobulins. A significant step towards the realisation of the potential of immunoglobulins as therapeutic agents was the discovery of techniques for the preparation of monoclonal antibodies of defined specificity (1). However, most MAbs are produced by fusions of rodent spleen cells with rodent myeloma cells. They are therefore essentially rodent proteins. There are very few reports of the production of human MAbs.

Since most available MAbs are of rodent origin, they are naturally antigenic in humans and thus can give rise to an undesirable immune response termed the HAMA (Human Anti-Mouse Antibody) response. Therefore, the use of rodent MAbs as therapeutic agents in humans is inherently limited by the fact that the human subject will mount an immunological response to the MAb and will either remove it entirely or at least reduce its effectiveness. Thus, in practice, MAbs of rodent origin may not be used in patients for more than one or a few treatments as a HAMA response soon develops rendering the MAb ineffective as well as giving rise to undesirable reactions.

Proposals have therefore been made to render non-human MAbs less antigenic in humans. Such techniques can be generically termed "humanisation" techniques. These techniques generally involve the use of recombinant DNA technology to manipulate DNA sequences encoding the polypeptide chains of the antibody molecule.

Some early methods for carrying out such a procedure are described in EP-A-0 171 496 (Res. Dev. Corp. Japan), EP-A-0 173 494 (Stanford University), EP-A-0 194 276 (Celltech Limited) and WO-A-8 702 671 (Int. Gen. Eng. Inc.). The Celltech application discloses a process for preparing an antibody molecule having the variable domains from a mouse MAb and the constant domains from a human immunoglobulin. It also shows the production of an antibody molecule comprising the variable domains of a mouse MAb, the CH1 and CL domains of a human immunoglobulin, and a non-immunoglobulin-derived protein in place of the Fc portion of the human immunoglobulin.

In an alternative approach, described in EP-A-0239400 (Winter), the complementarity determining regions (CDRs) of a mouse MAb have been grafted onto the framework regions of the variable domains of a human immunoglobulin by site directed mutagenesis using long oligonucleotides. There are 3 CDRs (CDR1, CDR2 and CDR3) in each of the heavy and light chain variable regions. The present invention relates to HAMs prepared according to this alternative approach, i.e. CDR-grafted HAMs.

The earliest work on humanising MAbs by CDR-grafting was carried out on MAbs recognising synthetic antigens, such as the NP or NIP antigens. However, examples in which a mouse MAb recognising lysozyme and a rat MAb recognising an antigen on human T-cells respectively were humanised by CDR-grafting are shown by Verhoeyen et al (2) and Riechmann et al (3).

In the latter case (Riechmann et al) it was found that transfer of the CDR regions alone (as defined by Kabat refs. 4 and 5) was not sufficient to provide

satisfactory antigen binding activity in the CDR-grafted product. Riechmann et al found that it was necessary to convert a serine residue at position 27 of the human sequence to the corresponding rat phenylalanine residue to obtain a CDR-grafted product having satisfactory antigen binding activity. This residue at position 27 of the heavy chain is within the structural loop adjacent to CDR1. A further construct which additionally contained a human serine to rat tyrosine change at position 30 of the heavy chain did not have a significantly altered binding activity over the humanised antibody with the serine to phenylalanine change at position 27 alone. These results indicate that changes to residues of the human sequence outside the CDR regions, in particular in the loop adjacent to CDR1, may be necessary to obtain effective antigen binding activity for CDR-grafted antibodies which recognise more complex antigens.

In recent years a number of rodent MAbs have been developed for therapeutic applications. For instance, OKT3 a mouse IgG2a/k MAb which recognises an antigen in the T-cell receptor-CD3 complex has been approved for use in the USA as an immunosuppressant in the treatment of acute allograft rejection (Chatenond et al (1986) J. Immunol., 137, 830-838, and Jeffers et al (1986) Transplantation, 41, 572-578). However, in view of the rodent nature of this and other such MAbs, a significant HAMA response which may include a major anti-idiotype component, builds up on use. Clearly, it would be highly desirable to diminish or abolish this undesirable HAMA response by suitable humanisation or other recombinant DNA manipulation of these very useful antibody and thus enlarge their areas of use.

We have further investgated the preparation of CDR-grafted HAMs and have identified residues within the framework of the variable region (i.e. outside both the Kabat CDRs and structural loops of the variable regions) the amino acid identities of which are important for obtaining CDR-grafted products with satisfactory binding affinity.

Accordingly, in a first aspect the invention provides a CDR-grafted antibody heavy chain having a variable region domain comprising human framework and non-human (rodent) antigen binding regions wherein the human framework comprises non-human (rodent) residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

Preferably, the antigen binding regions of the CDR-grafted heavy chain variable domain comprise CDRs corresponding to the Kabat CDR at CDR2 (residues 50-65), the structural loop residues at CDR3 (residues 95-100) and a composite of the Kabat and structural loop CDRs at CDR1 (residues 26-35).

The invention also provides in a second aspect a CDR-grafted antibody light chain having a variable region domain comprising human framework and non-human (rodent) antigen binding regions wherein the human framework comprises non-human (rodent) residues at at least one of positions 1 and/or 3 and 46 and/or 47.

Preferably, the antigen binding regions of the CDR-grafted light chain variable domain comprise CDRs corresponding to the Kabat CDRs at CDR1 (residue 24-34) and CDR2 (residues 50-56) and the structural loop residues at CDR3 (residues 91-96).

The invention further provides a CDR-grafted HAM comprising at least one CDR-grafted heavy chain and at least one CDR-grafted light chain according to the first and second aspects of the invention.

The residue designations given above and elsewhere in the present specification are numbered according to the Kabat numbering (refs. 4 and 5).

Preferably the CDR-grafted heavy chain comprises non-human (rodent) residues at positions 23 and/or 24, 48 and/or 49 and 71 and/or 73. Preferably, the CDR-grafted light chain comprises non-human (rodent) residues at positions 46 and/or 47.

Preferably the CDR-grafted antibody heavy and light chains and HAM are produced by recombinant DNA technology.

The HAM of the present invention may comprise: a complete antibody molecule, having full length heavy and light chains; a fragment thereof, such as the Fab or $(Fab')_2$ fragment; a light chain or heavy chain monomer or dimer; or any other molecule with the same specificity as the original non-human (rodent) antibody. Similarly the CDR-grafted heavy and light chain variable region may be combined with other antibody domains as appropriate.

Alternatively, the heavy or light chains or HAM of the present invention may have attached to them an effector or reporter molecule. For instance, they may have a macrocycle, for chelating a heavy metal atom, or a toxin, such as ricin, attached to it by a covalent bridging structure. Alternatively, the procedures of recombinant DNA technology may be used

to produce an immunoglobulin molecule in which the Fc fragment or CH3 domain of a complete immunoglobulin molecule has been replaced by an enzyme or toxin molecule.

For CDR-grafted products of the invention, appropriate variable region framework sequences may be used having regard to class/type of the donor antibody from which the antigen binding regions are derived. Preferably, the type of human framework used is of the same/similar class/type as the donor antibody. Advantageously, the framework is chosen to maximise/optimise homology with the donor antibody sequence particularly at positions close or adjacent to the CDRs. It will be appreciated that in some cases that the non-human and human amino acid residues, identified above in connection with the first and second aspects of the invention, may be the same and thus no change of the human framework to the corresponding non-human framework residue is required.

Also human constant region domains of the products of the invention may be selected having regard to the proposed function of the antibody in particular the effector functions which may be required. For example, the constant region domains may be human IgA, IgE, IgG or IgM domain. In particular, IgG human constant region domains may be used especially of the IgG1 and IgG3 isotypes, when the HAM is intended for therapeutic uses.

However, the remainder of the HAM need not comprise only protein sequences from the human immunoglobulin. For instance, a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain is fused to a DNA sequence

encoding the amino acid sequence of a polypeptide effector or reporter molecule.

Thus, according to a further aspect the present invention provides a process for producing an anti-CD3 HAM which process comprises:

(a) producing in an expression vector an operon having a DNA sequence which encodes an antibody heavy or light chain according to the first or second aspect of the invention;

(b) producing in an expression vector an operon having a DNA sequence which encodes a complementary antibody light or heavy chain according to the second or first aspect of the invention;

(c) transfecting a host cell with the or each vector; and

(d) culturing the transfected cell line to produce the HAM.

The cell line may be transfected with two vectors, the first vector containing an operon encoding a light chain-derived polypeptide and the second vector containing an operon encoding a heavy chain-derived polypeptide. Preferably, the vectors are identical except in so far as the coding sequences and selectable markers are concerned so as to ensure as far as possible that each polypeptide chain is equally expressed.

Alternatively, a single vector may be used, the vector including the sequences encoding both light chain- and heavy chain-derived polypeptides.

The DNA in the coding sequences for the light and heavy chains may comprise cDNA or genomic DNA or both.

The present invention also includes cloning and expression vectors and transfected cell lines used in the process of the invention, therapeutic and diagnostic compositions comprising the variable domains or the HAM of the invention and uses of such compositions in therapy and diagnosis.

The general methods by which the vectors may be constructed, transfection methods and culture methods are well known per se and form no part of the invention. Such methods are shown, for instance, in references 6 and 7.

The present invention is now described, by way of example only, with reference to the accompanying Figures 1 - 29.

MATERIAL AND METHODS

1. INCOMING CELLS

Hybridoma cells producing antibody OKT3 were provided by Ortho (seedlot 4882-1) and were grown up in antibiotic free Dulbecco's Modified Eagles Medium (DMEM) supplemented with glutamine and 5% foetal calf serum, and divided to provide both an overgrown supernatant for evaluation and cells for extraction of RNA. The overgrown supernatant was shown to contain 250 ug/mL murine IgG2a/kappa antibody. The supernatant was negative for murine lambda light chain and IgG1, IgG2b, IgG3, IgA and IgM heavy chain. 20mL. of supernatant was sent to Ortho to confirm that the antibody present was OKT3.

2. MOLECULAR BIOLOGY PROCEDURES

Basic molecular biology procedures were as Maniatis et al.(ref. 6) with, in some cases minor modifications. DNA sequencing was performed as described in Sanger et al.(ref. 7) and the Amersham International Plc sequencing handbook. Site directed mutagenesis was as described in Kramer et al.(ref.8)and the Anglian Biotechnology Ltd handbook. COS cell expression and metabolic labelling studies were as described in Whittle et al. (ref. 9)

3. RESEARCH ASSAYS

3.1 ASSEMBLY ASSAYS

Assembly assays were performed on supernatants from transfected COS cells to determine the amount of intact IgG present.

3.1.1 COS CELLS TRANSFECTED WITH MOUSE OKT3 GENES

The assembly assay for intact mouse IgG in COS cell supernatants was an ELISA with the following format:-

96 well microtitre plates were coated with $F(ab')_2$ goat anti mouse IgG Fc. The plates were washed in water and samples added for 1 hour at room temperature. The plates were washed and $F(ab')_2$ goat anti mouse IgG $F(ab')_2$ (HRPO conjugated) was then added. Substrate was added to reveal the reaction.

UPC10, a mouse IgG2a myeloma, was used as a standard.

3.1.2 COS CELLS TRANSFECTED WITH CHIMAERIC OR CDR GRAFTED OKT3 GENES

The assembly assay for intact humanised OKT3 in COS cell supernatants was an ELISA with the following format:-

96 well microtitre plates were coated with $F(ab')_2$ goat anti-human IgG Fc. The plates were washed and samples added for 1 hour at room temperature. The plates were washed and monoclonal mouse anti-human kappa chain was added for 1 hour at room temperature.

The plates were washed and $F(ab')_2$ goat anti mouse IgG Fc (HRPO conjugated) was added. Substrate was added to reveal the reaction.

Chimaeric B72.3 (IgG4) was used as a standard. The use of a monoclonal anti-kappa chain in this assay allows grafted antibodies to be read from the chimaeric standard.

3.2 ASSAY FOR OKT3 ANTIGEN BINDING ACTIVITY

Material from COS cell supernatants was assayed for OKT3 antigen binding activity onto CD3 positive cells in a direct assay. The procedure was as follows:-

HUT 78 cells (human T cell line, CD3 positive) were maintained in culture. Monolayers of HUT 78 cells were prepared onto 96 well ELISA plates using poly-L-lysine and glutaraldehyde. Samples were added to the monolayers for 1 hour at room temperature.

The plates were washed gently using PBS. $F(ab')_2$ goat anti-human IgG Fc (HRPO conjugated) or $F(ab')_2$ goat anti mouse IgG Fc (HRPO conjugated) was added as appropriate for humanised or mouse samples. Substrate was added to reveal the reaction.

The negative control for the cell-based assay was chimaeric B72.3. The positive control was mouse Orthomune OKT3 or chimaeric OKT3, when available. This cell-based assay was difficult to perform and gave poorly reproducible results with a high background.

4. cDNA LIBRARY CONSTRUCTION

4.1 mRNA PREPARATION AND cDNA SYNTHESIS

Cells were grown as described above (OKT3 producing) and 1.2 x 10^9 cells harvested and mRNA extracted using the guanidinium/LiCl extraction procedure. cDNA was prepared by priming from Oligo-dT to generate full length cDNA. The cDNA was methylated and EcoR1 linkers added for cloning.

4.2 LIBRARY CONSTRUCTION

The cDNA library was ligated to pSP65 vector DNA which had been EcoR1 cut and the 5' phosphate groups removed by calf intestinal phosphatase (EcoRI/CIP). The ligation was used to transform high transformation efficiency *Escherichia coli (E.coli)* HB101. A cDNA library was prepared. 3600 colonies were screened for the light chain and 10000 colonies were screened for the heavy chain.

5. SCREENING

E.coli colonies positive for either heavy or light chain probes were identified by oligonucleotide screening using the oligonucleotides :

5' TCCAGATGTTAACTGCTCAC for the light chain, which is complementary to a sequence in the mouse kappa constant region, and 5' CAGGGGCCAGTGGATGGATAGAC for the heavy chain which is complementary to a sequence in the mouse IgG2a constant CH1 domain region. 12 light chain and 9 heavy chain clones were identified and taken for second round screening. Positive clones from the second round of screening were grown up and DNA prepared. The sizes of the gene inserts were estimated by gel electrophoresis and inserts of a size capable of containing a full length cDNA were subcloned into M13 for DNA sequencing.

6. DNA SEQUENCING

Clones representing four size classes for both heavy and light chains were obtained in M13. DNA sequence for the 5' untranslated regions, signal sequences, variable regions and 3' untranslated regions of full length cDNA s were obtained (Figs 1 and 2).

ANALYSIS OF SEQUENCES

DNA sequences from cDNA's were compared with RNA sequences

provided by Ortho. The cDNA sequences included 5' untranslated region sequence as well as signal peptide sequence. The 3' untranslated region was also sequenced. A single coding difference was observed at position 9 in the heavy chain where the mRNA suggested a Proline but the cDNA sequence read as an Alanine. The cDNA sequence was used for further analysis.

The light chain is a member of the mouse V_L subgroup VI and uses a J_K4 minigene. The heavy chain is probably a member of the mouse V_H subgroup II, most probably IIb, although it also has significant homology to the consensus for group Va which itself is very homologous to subgroup II. The D region is currently unclassified and the JH region is J_H2 (Figs 3 and 4).

The light chain shows a high degree of homology to the Ox-1 germline gene and to the published antibodies 45.21.1, 14.6b.1 and 26.4.1. The heavy chain shows reasonable homology to a subgroup of the J558 family including 14.6b.1. These combinations of light and heavy chain genes have previously resulted in antibodies with affinity for alpha-1-6 dextran (Sikder et al.(ref.10)Wallick et al. (ref.11)).

The heavy chain has the sequence Asparagine (Asn)- Proline (Pro)- Serine (Ser) in CDR2. Normally Asn-X-Ser would be a potential glycosylation site, but when X is Pro these sites tend not to be glycosylated.

8. CONSTRUCTION OF cDNA EXPRESSION VECTORS

Celltech expression vectors are based on the plasmid pEE6hCMV (Fig. 5) (ref. 12)A polylinker for the insertion of genes to be expressed has been introduced after the major immediate early promoter/enhancer of the human Cytomegalovirus (hCMV). Marker genes for selection of the plasmid in transfected eukaryotic cells can be inserted as BamHI cassettes in the unique BamHI site of pEE6 hCMV. It is usual practice to insert the *neo* and *gpt* markers prior to insertion of the gene of interest, whereas the GS marker is inserted last because of the presence of internal EcoRI sites in the cassette.

The selectable markers are expressed from the SV40 late promoter which also provides an origin of replication so that the vectors can be used for expression in the COS cell transient expression system.

The mouse sequences were excised as EcoRI fragments and cloned into either EE6-hCMV-neo for the heavy chain (Fig 6) and into EE6-hCMV-gpt

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for the light chain (Fig 7).

9. EXPRESSION OF cDNA'S IN COS CELLS

Plasmids pJA135 (Fig 7) and pJA136 (fig 6) were co-transfected into COS cells and supernatant from the transient expression experiment was shown to contain assembled antibody which bound to T-cell enriched peripheral blood lymphocytes. Metabolic labelling experiments using ^{35}S methionine showed expression and assembly of heavy and light chains.

10. CONSTRUCTION OF CHIMAERIC GENES

Construction of chimaeric genes followed a previously described strategy (Whittle et al (ref. 9)) A restriction site near the 3' end of the variable domain sequence is identified and used to attach an oligonucleotide adapter coding for the remainder of the mouse variable region and a suitable restriction site for attachment to the constant region of choice.

10.1 LIGHT CHAIN GENE CONSTRUCTION- VERSION 1

The mouse light chain cDNA sequence showed an AvaI site near the 3' end of the variable region (Fig 8). The majority of the sequence of the variable region was isolated as a 376 bp. EcoRI-AvaI fragment. An oligonucleotide adapter was designed to replace the remainder of the 3' region of the variable region from the AvaI site and to include the 5' residues of the human constant region up to and including a unique NarI site which had been previously engineered into the constant region.

TOP STRAND 5' TCGGGGACAAAGCTTGAAATAAACAGAACTGTGGCGG 3'

BOTTOM STRAND 3' CCTGTTTCGAACTTTATTTGTCTTGACACCGCCGC 5'

A Hin_dIII site, shown in bold type within the oligonucleotide sequence above, was introduced to act as a marker for insertion of the linker.

The linker was ligated to the V_L fragment and the 413 bp EcoRI-NarI adapted fragment was purified from the ligation mixture.

The constant region was isolated as an NarI-BamHI fragment from an M13 clone NW361 and was ligated with the variable region DNA into an EcoRI/BamHI/CIP pSP65 treated vector in a three way reaction. Clones were isolated after transformation into *E.coli* and the linker and junction sequences were confirmed by the presence of the HinDIII site and by DNA sequencing (Fig 9).

10.2 LIGHT CHAIN GENE CONSTRUCTION- VERSION 2

The construction of the first chimaeric light chain gene produces a fusion of mouse and human amino acid sequences at the variable -constant region junction. In the case of the OKT3 light chain the amino acids at the chimaera junction are:

.....Leu-Glu-Ile-Asn-Arg/ -/Thr-Val-Ala -Ala

VARIABLE CONSTANT

This arrangement of sequence introduces a potential site for Asparagine (Asn) linked (N-linked) glycosylation at the V-C junction. As will be seen later, this sequence can be glycosylated. Therefore, a second version of the chimaeric light chain oligonucleotide adapter was designed in which the threonine (Thr), the first amino acid of the human constant region, was replaced with the equivalent amino acid from the mouse constant region, Alanine (Ala).

TOP STRAND 5' TCGGGGACAAAGTTGGAAATAAACAGAGCTGTGGCGG 3'

BOTTOM STRAND 3' CCTGTTTCAACCTTTATTTGTCTCGACACCGCCGC 5'

The internal HinDIII site present in the version 1 adapter was not included to differentiate the two chimaeric light chain genes.

The variable region fragment was isolated as a 376 bp. EcoRI-AvaI fragment. The oligonucleotide linker was ligated to NarI cut pNW361 and then the adapted 396bp constant region was isolated after recutting the modified pNW361 with EcoRI. The variable region fragment and the modified constant region fragment were ligated directly into EcoRI/CIP treated pEE6hCMVneo.

Initially all clones examined had the insert in the incorrect orientation. Therefore, the insert was re-isolated and recloned to turn the insert round. Several clones with the insert in the correct orientation were obtained and the adapter sequence of one was confirmed by DNA sequencing (Fig10).

10.3 HEAVY CHAIN GENE CONSTRUCTION

10.3.1 CHOICE OF HEAVY CHAIN GENE ISOTYPE

The constant region isotype chosen for the heavy chain was human IgG4.

10.3.2 GENE CONSTRUCTION

The heavy chain cDNA sequence showed a BanI site near the 3' end of the variable region (Fig 11). The majority of the sequence of the variable region was isolated as a 426bp. EcoRI/CIP/BanI fragment. An oligonucleotide adapter was designed to replace the remainder of the

3' region of the variable region from the BanI site up to and including a unique HinDIII site which had been previously engineered into the first two amino acids of the constant region.

TOP STRAND 5' GCACCACTCTCACCGTGAGCTC3'

BOTTOM STRAND 3'GTGAGAGTGGCACTCGAGTCGA 5'

The linker was ligated to the V_H fragment and the EcoRI-HinDIII adapted fragment was purified from the ligation mixture.

The variable region was ligated to the constant region by cutting mJA91with EcoRI and HindIII removing the intron fragment and replacing it with the V_H (Fig 12). Clones were isolated after transformation into *E.coli* JM101 and the linker and junction sequences were confirmed by DNA sequencing. (NB. The HindIII site is lost on cloning).

11. CONSTRUCTION OF CHIMAERIC EXPRESSION VECTORS

11.1 neo AND gpt VECTORS

The chimaeric light chain (version 1) was removed from pJA143 (Fig 9) as an EcoRI fragment and cloned into EcoRI/CIP treated pEE6hCMVneo expression vector. Clones with the insert in the correct orientation were identified by restriction mapping (Fig 13).

The chimaeric light chain (version 2) was constructed as described above (see Fig 10).

The chimaeric heavy chain gene was isolated as a 2.5Kbp EcoRI/BamHI fragment and cloned into the EcoRI/BclI/CIP treated vector fragment of pJA97, a derivative of pEE6hCMVgpt (Fig 14).

11.2 GS SEPARATE VECTORS

GS versions of pJA141 (Fig 10) and pJA144 (Fig 14) were constructed by replacing the neo and gpt cassettes by BamHI/SalI/CIP treatment of the plasmids, isolation of the vector fragment and ligation to a GS-containing fragment from the plasmid pRO49 (Figs 15 and 16)

11.3 GS SINGLE VECTOR CONSTRUCTION

Single vector constructions containing the cL, cH and GS genes on one plasmid in the order cL-cH-GS, or cH-cL-GS and with transcription of the genes being head to tail eg. cL>cH>GS were constructed. These plasmids were made by treating pJA179 (Fig 15) or pJA180 (Fig 16) with BamHI/CIP and ligating in a BglII/HinDIII hCMV cassette from pJA146 along with either the HinDIII/BamHI from pJA141 into pJA180 to give the cH-cL-GS plasmid pJA182 (Fig17), or the HinDIII/BamHI from pJA144 into pJA179 to give the cL-cH-GS plasmid pJA181 (Fig 18).

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12. EXPRESSION OF CHIMAERIC GENES

12.1 EXPRESSION IN COS CELLS

The chimaeric antibody plasmids pJA145 (cL) and pJA144 (cH) were cotransfected into COS cells and supernatant from the transient expression experiment was shown to contain assembled antibody which bound to the HUT 78 human T-cell line. Metabolic labelling experiments using ^{35}S methionine showed expression and assembly of heavy and light chains. However the light chain mobility seen on reduced gels (Fig 19) suggested that the potential glycosylation site was being glycosylated. Expression in COS cells in the presence of tunicamycin showed a reduction in size of the light chain to that shown for control chimaeric antibodies and the OKT3 mouse light chain. Therefore JA141 was constructed and expressed. In this case the light chain did not show an aberrant mobility or a size shift in the presence or absence of tunicamycin (Fig 19). This second version of the chimaeric light chain, when expressed in association with chimaeric heavy (cH) chain, produced antibody which showed good binding to HUT 78 cells. In both cases antigen binding was equivalent to that of the mouse antibody.

12.2 EXPRESSION IN CHINESE HAMSTER OVARY (CHO) CELLS

Stable cell lines are being prepared from plasmids pJA141/pJA144 and from pJA179/pJA180, pJA181, and pJA182 by transfection into CHO cells.

13. CDR GRAFTING

The approach taken was to try to introduce sufficient mouse residues into a human variable region framework to generate antigen binding activity comparable to the mouse and chimaeric antibodies.

13.1 VARIABLE REGION ANALYSIS

From an examination of a small database of structures of antibodies and antigen-antibody complexes it is clear that only a small number of antibody residues make direct contact with antigen. Other residues may contribute to antigen binding by positioning the contact residues in favourable configurations and also by inducing a stable packing of the individual variable domains and stable interaction of the light and

heavy chain variable domains.

The residues chosen for transfer can be identified in a number of ways:

A. By examination of antibody X-ray crystal structures the antigen binding surface can be predominantly located on a series of loops, three per domain, which extend from the B-barrel framework.

B. By analysis of antibody variable domain sequences,regions of hypervariability (termed the Complementarity Determining Regions (CDRs) by Wu and Kabat (ref. 5))can be identified. In the most but not all cases these CDRs correspond to, but extend a short way beyond, the loop regions noted above.

C. Residues not identified by A and B above may contribute to antigen binding directly or indirectly by affecting antigen binding site topology, or by inducing a stable packing of the individual variable domains and stabilising the inter-variable domain interaction. These residues may be identified either by superimposing the sequences for a given antibody on a known structure and looking at key residues for their contribution, or by sequence alignment analysis and noting "idiosyncratic" residues followed by examination of their structural location and likely effects.

13.1.1 LIGHT CHAIN

Figure 20 shows an alignment of sequences for the human framework region REI and the OKT3 light variable region. The structural loops and CDRs believed to correspond to the antigen binding region are marked. Also marked are a number of other residues which may also contribute to antigen binding as described in 13.1C. REI was chosen as the human framework because the light chain is a Kappa chain and the kappa variable regions show higher homology with the mouse sequences than a lambda light variable region eg KOL (see below). REI was chosen in preference to another kappa light chain because the X-ray structure of the light chain has been determined so that a structural examination of individual residues could be made.

13.1.2 HEAVY CHAIN

Figure 21 shows an alignment of sequences for the human framework region KOL and the OKT3 heavy variable region. The structural loops and CDRs believed to correspond to the antigen binding region are marked. Also marked are a number of other residues which may also contribute to antigen binding as described in 13.1C. KOL was chosen as the heavy chain framework because the X-ray structure has been determined to a better resolution than, for example, NEWM and also

the sequence alignment of OKT3 heavy variable region showed a slightly better homology to KOL than to NEWM.

13.2 DESIGN OF VARIABLE GENES

The variable region domains were designed with mouse variable region optimal codon usage (Grantham and Perrin(ref.13))and used the B72.3 signal sequences (Whittle et al.(ref.9))The sequences were designed to be attached to the constant region in the same way as for the chimaeric genes described above. Some constructs contained the "Kozak consensus sequence" (Kozak,(ref.14))directly linked to the 5' of the signal sequence in the gene. This sequence motif is believed to have a beneficial role in translation initiation in eukaryotes.

13.3 GENE CONSTRUCTION

To build the variable regions two strategies are available. Either to assemble the sequence using oligonucleotides in a manner similar to Jones et al. (ref. 15)or to simultaneously replace all of the CDRs or loop regions by oligonucleotide directed site specific mutagenesis in a manner similar to Verhoeyen et al. (ref. 2) Both strategies were used and a list of constructions is set out in Table 1. It was noted in several cases that the mutagenesis approach led to deletions and rearrangements in the gene being remodelled, while the success of the assembly approach was very sensitive to the quality of the oligonucleotides. Figs 22a and b and 23a and b show by way of example the nucleotide sequences and procedures required to construct gH341 by site directed mutagenesis and kgH341A by oligonucleotide assembly.

14 CONSTRUCTION OF EXPRESSION VECTORS

Genes were isolated from M13 or SP65 based intermediate vectors and cloned into pEE6hCMVneo for the light chains and pEE6hCMVgpt for the heavy chains in a manner similar to that for the chimaeric genes as described above.

15 EXPRESSION OF CDR GRAFTED GENES

A number of points should be noted.

1. There is no standard for the antigen binding assay when chimaeric or CDR grafted antibody are being measured, except when the heavy chain of the antibody is murine when murine OKT3 can be used as standard with an anti-murine Fc antibody as revealing antibody. Therefore all comparisons of antigen binding assays with chimaeric (c) or CDR grafted (g) genes can only be made within an individual

TABLE 1 CDR GRAFTED GENE CONSTRUCTIONS

CODE	MOUSE SEQUENCE CONTENT	METHOD OF CONSTRUCTION	KOZAK SEQUENCE -	KOZAK SEQUENCE +
LIGHT CHAIN	ALL HUMAN FRAMEWORK REI			
121	26-32, 50-56, 91-96 inclusive	SDM and gene assembly	+	n.d.
121A	26-32, 50-56, 91-96 inclusive +1, 3, 46, 47	Partial gene assembly	n.d.	+
121B	26-32, 50-56, 91-96 inclusive +46, 47	Partial gene assembly	n.d.	+
221	24-24, 50-56, 91-96 inclusive	Partial gene assembly	+	+
221A	24-34, 50-56, 91-96 inclusive +1, 3, 46, 47	Partial gene assembly	+	+
221B	24-34, 50-56, 91-96 inclusive +1, 3	Partial gene assembly	+	+
221C	24-34, 50-56, 91-96 inclusive +46, 47	Partial gene assembly	+	+
HEAVY CHAIN	ALL HUMAN FRAMEWORK KOL			
121	26-32, 50-56, 95-100B inclusive	Gene assembly	n.d.	+
131	26-32, 50-58, 95-100B inclusive	Gene assembly	n.d.	+
141	26-32, 50-65, 95-100B inclusive	Partial gene assembly	+	n.d.
321	26-35, 50-56, 95-100B inclusive	Partial gene assembly	+	n.d.
331	26-35, 50-58, 95-100B inclusive	Partial gene assembly Gene assembly	+ 	 +
341	26-35, 50-65, 95-100B inclusive	SDM Partial gene assembly	+ 	 +
341A	26-35, 50-65, 95-100B inclusive +6, 23, 24, 48, 49, 71, 73, 76, 78, 88, 91 (+63=human)	Gene assembly	n.d.	+
341B	26-35, 50-65, 95-100B inclusive +48, 49, 71, 73, 76, 78, 88, 91(+63=human)	Gene assembly	n.d.	+

KEY

n.d.	not done
SDM	Site directed mutagenesis
Gene assembly	Variable region assembled entirely from oligonucleotides
Partial gene assembly	Variable region assembled by combination of restriction fragments either from other genes originally created by SDM and gene assembly or by oligonucleotide assembly of part of the variable region and reconstruction with restriction fragments from other genes originally created by SDM and gene assembly.

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experiment.

2. The cell-based antigen binding assay is not robust and resulting data varies depending on cell binding to the plates and the amount of antibody used. Therefore several experiments are needed to confirm marginal results.

3. The COS cell expression system can give batch to batch variation in antibody yield which has a direct bearing on the results obtained in the antigen binding assay.

Bearing these factors in mind the data can be divided into three groups. Table 2 shows a summary of data for the various constructs.

15.1 PRODUCTION OF ANTIBODY CONSISTING OF GRAFTED LIGHT (gL) CHAINS WITH MOUSE HEAVY (mH) OR CHIMAERIC HEAVY (cH) CHAINS.

All gL chains, in association with mH or cH produced reasonable amounts of antibody. Insertion of the Kozak consensus sequence at a position 5' to the ATG (kgL constructs) however, led to a 2-5 fold improvement in net expression (Fig 24a and b). Over an extended series of experiments expression levels were raised from approx 200ng/mL to approx. 500 ng/mL for kgL/cH or kgL/mH combinations.

When direct binding to antigen on HUT 78 cells was measured, a construct designed to include mouse sequence based on loop length (gL121) did not lead to active antibody in association with mH or cH. A construct designed to include mouse sequence based on Kabat CDRs (gL221) demonstrated some weak binding in association with mH or cH (Fig 25B). However when framework residues 1, 3, 46, 47 were changed from the human to the murine OKT3 equivalents based on the arguments outlined in Section 13.1 antigen binding can be demonstrated when both of the new constructs, which were termed 121A and 221A ,are coexpressed with cH (Fig 25A and B). When the effects of these residues are examined in more detail it appears that residues 1 and 3 are not major contributing residues as the product of the gL221B gene shows little detectable binding activity in association with cH (Fig 25B). The light chain product of gL221C, in which mouse sequences are present at 46 and 47, shows good binding activity in association with cH (Fig 25 B).

15.2 PRODUCTION OF ANTIBODY CONSISTING OF GRAFTED HEAVY (gH) CHAINS WITH MOUSE LIGHT (mL) OR CHIMAERIC LIGHT(cL) CHAINS.

Expression of the gH genes has proven to be more difficult to achieve than for gL. First, inclusion of the Kozak sequence appears to have

had no marked effect on expression of gH genes (Fig 26). Expression may be slightly improved but not to the same degree as seen for the grafted light chain.

Second, it has proven difficult to demonstrate production of expected quantities of material when the loop choice (amino acid 26-32) for CDR1 is used eg. gH121, 131, 141 (Fig 27) and no conclusions can be drawn about these constructs. Further, in experiments where low antibody production was seen it has not been possible to detect free light chain expression and secretion which would be expected if heavy chain expression was not occurring at all. Therefore the data suggests, but does not confirm, that in these cases the heavy chain is being expressed but the processing of the chain once it has become associated with light chain is aberrant leading to degradation of assembled or partially assembled antibody inside the cell. Experiments to determine gH mRNA levels, or to attempt to demonstrate the presence of antibody within the cells have not been done.

Third, coexpression of the gH341 gene with cL or mL has been variable and has tended to produce lower amounts of antibody than the cH/cL or mH/mL combinations. The alterations to gH341 to produce gH341A and gH341B appear to lead to improved levels of expression (Fig 27 lanes h-k). This may partly be due either to a general increase in the fraction of mouse sequence in the variable region, or to the alteration at position 63 where the residue is returned to the human amino acid Valine (Val) from Phenylalanine (Phe) to avoid possible internal packing problems with the rest of the human framework. This arrangement also occurs in gH331 and gH321.

When gH321 or gH331 are expressed in association with cL, antibody is produced but antibody binding activity has not been detected (Table 2). When the more conservative gH341 gene is used antigen binding can be detected in association with cL or mL, but the activity is only marginally above the background level (Fig 28). When further mouse residues are substituted based on the arguments in 13.1 antigen binding can be clearly demonstrated for the antibody produced when kgH341A and kgH341B are expressed in association with cL (Fig 29).

15.3 PRODUCTION OF FULLY CDR GRAFTED ANTIBODY

The kgL221A gene was co-expressed with kgH341, kgH341A, or kgH341B. For the combination kgH221A/kgH341 very little material was produced in a normal COS cell expression experiment (Fig 29A ancd C). For the

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combinations kgL221A/kgH341A or kgH221A/kgH341B amounts of antibody similar to gL/cH or cL/cH was produced (Fig 29A and C).

In several experiments no antigen binding activity could be detected with kgH221A/gH341 or kgH221A/kgH341 combinations (see for example Fig 29), although expression levels were very low.

Antigen binding was detected when kgL221A/kgH341A or kgH221A/kgH341B combinations were expressed (Fig 29B). In the case of the antibody produced from the kgL221A/kgH341A combination the antigen binding was very similar to that of the chimaeric antibody (Fig 29B).

The objectives of the programme were to produce both a chimaeric mouse variable-human constant IgG4/K antibody and a fully humanised antibody retaining the antigen binding activity of the murine monoclonal antibody OKT3.

Cells were obtained from Ortho and mRNA prepared. A cDNA library was screened for heavy and light chain cDNAs using oligonucleotide probes. Full length cDNAs were obtained and the variable regions were sequenced (Figs 1 and 2). The cDNAs showed a high level of homology with sequences of antibodies which have specificity for alpha-1-6-dextrans. It would be of interest to test OKT3 to determine whether it recognises and binds to dextran antigens.

The cDNAs were transferred to expression vectors (Figs 6 and 7) and expressed in COS cells. Antibody was produced which bound to an enriched T-cell population from peripheral blood cells.

Two versions of the chimaeric antibody were produced, differing in the light chain at the first amino acid of the constant region. In version 1 (Figs 9 and 13) the amino acid sequence which resulted at the V-C junction when the chimaeric light chain was constructed generates a potential N-linked glycosylation site at the "elbow' region. This region is an extended sequence of peptide between the V and C domains and is potentially accessible to the enzymes of the glycosylation process. Fig 19 shows that the version 1 chimaeric light chain is glycosylated demonstrating that the secondary structure generated at the elbow is sufficient for the Asn-Arg-Thr motif to be used for glycosylation.

A second version of the chimaeric light chain was constructed (Fig 10) in which the first amino acid of the human constant region (Thr) was returned to the mouse amino acid (Ala), so removing the glycosylation site. Antibody was produced by coexpression with chimaeric heavy chain (Fig 13) and in both versions the chimaeric material was equivalent in binding to the mouse OKT3 (see Fig 19). These observations have been confirmed by Ortho staff (L Jolliffe pers. comm.).

Vectors for the expression of chimaeric OKT3 using neo/gpt or glutamine synthetase (GS) selection were prepared, including vectors in which both genes were on the same plasmid (Figs 15 to18).

In the design of the fully humanised antibody the aim was to transfer the minimum number of mouse amino acids that would confer antigen binding onto a human antibody framework.

For the light chain the regions defining the loops known from

structural studies of other antibodies to contain the antigen contacting residues, and those hypervariable sequences defined by Kabat et al. as Complementarity Determining Regions (CDRs) are equivalent for CDR 2. For CDR1 the hypervariable region extends from residues 24-34 inclusive while the structural loop extends from 26-32 inclusive. In the case of OKT3 there is only one amino acid difference between the two options, at amino acid 24, where the mouse sequence is a serine and the human framework REI has glutamine. For CDR 3 the loop extends from residues 91-96 inclusive while the Kabat hypervariability extends from 89-97 inclusive. For OKT3 amino acids 89, 90 and 97 are the same between OKT3 and REI (Fig 20). When constructs based on the loop choice for CDR1 (gL121) and the Kabat choice (gL221) were made and coexpressed with mH or cH no evidence for antigen binding activity could be found for gL121, but trace activity could be detected for the gL221, suggesting that a single extra mouse residue in the grafted variable region could have some detectable effect. Both gene constructs were reasonably well expressed in the transient expression system.

The remaining framework residues were then further examined, in particular amino acids known from X-ray analysis of other antibodies to be close to the CDRs and also those amino acids which in OKT3 showed differences from the consensus framework for the mouse subgroup (subgroup VI) to which OKT3 shows most homology. Four positions, 1, 3, 46 and 47 were identified and their possible contribution was examined by substituting the mouse amino acid for the human amino acid at each position. Therefore gL221A (gL221 + D1Q, Q3V, L46R, L47W, see Fig 20 and Table 1) was made, cloned in EE6hCMVneo and coexpressed with cH (pJA144). The resultant antibody was well expressed and showed good binding activity(Fig 25 and Table 2). When the related genes gL221B (gL221 + D1Q, Q3V) and gL221C (gL221 + L46R, L47W) were made and similarly tested, while both genes produced antibody when coexpressed with cH, only the gL221C/cH combination showed good antigen binding (Fig 25). When the gL121A (gL121 + D1Q, Q3V, L46R, L47W) gene was made and coexpressed with cH antibody was produced which also bound to antigen (Fig 25).

For the heavy chain the loop and hypervariability analyses agree only in CDR3. For CDR1 the loop region extends from residues 26-32 inclusive whereas the Kabat CDR extends from residues 31-35 inclusive. For CDR2 the loop region is from 50-58 inclusive while the hypervariable region covers amino acids 50-65 incusive. Therefore humanised heavy chains were constructed using the framework from antibody KOL and with various

combinations of these CDR choices, including a shorter choice for CDR2 of 50-56 inclusive as there was some uncertainty as to the definition of the end point for the CDR2 loop around residues 56 to 58. The genes were coexpressed with mL or cL initially. In the case of the gH genes with loop choices for CDR1 eg. gH121, gH131, gH141 very little antibody was produced in the culture supernatants (see Fig 27). As no free light chain was detected it was presumed that the antibody was being made and assembled inside the cell but that the heavy chain was aberrant in some way, possibly incorrectly folded, and therefore the antibody was being degraded internally. In some experiments trace amounts of antibody could be detected in ^{35}S labelling studies (see Fig 27). As no net antibody was produced, analysis of these constructs was not pursued further.

When, however, a combination of the loop choice and the Kabat choice for CDR1 was tested (mouse amino acids 26-35 inclusive) and in which residues 31 (Ser to Arg), 33 (Ala to Thr), and 35 (Tyr to His) were changed from the human residue to the mouse residue and compared to the first series, antibody was produced for gH321, kgH331 and kgH341 when coexpressed with cL (Fig 27). Expression was generally low and could not be markedly improved by the insertion of the Kozak consensus sequence 5' to the ATG of the signal sequence of the gene, as distinct from the case of the gL genes where such insertion led to a 2-5 fold increase in net antibody production (compare Figs 24 and 26). However, only in the case of gH341/mL or kgH341/cL could marginal antigen binding activity be demonstrated (see Fig 28 and Table 2). When the kgH341 gene was coexpressed with kgL221A, the net yield of antibody was too low (see Figs 29A column 6 and 29C laneE) to give a signal above the background level in the antigen binding assay (see Fig 29A column 5).

As in the case of the light chain the heavy chain frameworks were reexamined. Possibly because of the lower initial homology between the mouse and human heavy variable domains compared to the light chains, more amino acid positions proved to be of interest. Two genes, kgH341A and kgH341B were constructed, with 11 or 8 human residues respectively substituted by mouse residues compared to gH341, and with the CDR2 residue 63 returned to the human amino acid potentially to improve domain packing. Both showed good levels of expression with cL or kgL221A (Fig 29A) and both showed antigen binding with cL or kgL221A, the kgH341A gene with all 11 changes appearing to be the superior choice (Fig 29B).

It has been demonstrated here for OKT3 that to transfer antigen binding ability to the humanised antibody mouse residues outside the

CDR regions defined by the Kabat hypervariability or structural loop choices are required for both the light and heavy chains. Fewer extra residues are needed for the light chain, possibly due to the higher initial homology between the mouse and human Kappa variable regions.

Of the changes seven (1 and 3 from the light chain and 6, 23, 71, 73 and 76 from the heavy chain) are predicted from a knowledge of other antibody structures to be either partly exposed or on the antibody surface. It has already been shown here that residues 1 and 3 in the light chain are not absolutely required to be the mouse sequence and for the heavy chain the gH341B heavy chain in combination with the 221A light chain generates activity without the presence of the 6 and 23 changes. It would be of interest to determine by further study the individual contribution of the other 8 mouse residues of the kgH341A gene compared to kgH341 and to determine whether the 7 extra mouse surface residues in the antibody produced by the kgH341A/kgL221A combination contribute to idiotypic epitopes which can be detected by sera from patients treated with murine OKT3.

References

1. Kohler & Milstein, Nature, 265, 295-497, 1975.

2. Verhoeyen et al, Science, 239, 1534-1536, 1988.

3. Riechmann et al, Nature, 332, 323-324, 1988.

4. Kabat, E.A., Wu, T.T., Reid-Miller, M., Perry, H.M., Gottesman, K.S., 1987, in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA.

5. Wu, T.T., and Kabat, E.A., 1970, J. Exp. Med. 132 211-250.

6. Maniatis et al, Molecular Cloning, Cold Spring Harbor, New York, 1982.

7. Sanger, F., Nicklen, S., Coulson, A.R., 1977, Proc. Natl. Acad. Sci. USA, 74 5463

8. Kramer, W., Drutsa, V., Jansen, H.-W., Kramer, B., Plugfelder, M., Fritz, H.-J., 1984, Nucl. Acids Res. 12 9441

9. Whittle, N., Adair, J., Lloyd, J.C., Jenkins, E., Devine, J., Schlom, J., Raubitshek, A., Colcher, D., Bodmer, M., 1987, Protein Engineering 1, 499.

10. Sikder, S.S., Akolkar, P.N., Kaledas, P.M., Morrison, S.L., Kabat, E.A., 1985, J. Immunol. 135, 4215.

11. Wallick, S.C., Kabat, E.A., Morrison, S.L., 1988, J. Exp. Med. 168, 1099

12. Bebbington, C.R., Published International Patent Application WO 89/01036.

13. Granthan and Perrin 1986, Immunology Today 7, 160.

14. Kozak, M., 1987, J. Mol. Biol. 196, 947.

15. Jones, T.P., Dear, P.H., Foote, J., Neuberger, M.S., Winter, G., 1986, Nature, 321, 522

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   1 GAATTCCCAA AGACAAAatg gattttcaag tgcagatttt cagcttcctg
  51 ctaatcagtg cctcagtcat aatatccaga ggacaaattg tctcacccca
 101 gtctccagca atcatgtctg catctccagg ggagaaggtc accatgacct
 151 gcagtgccag ctcaagtgta agttacatga actggtacca gcagaagtca
 201 ggcacctccc ccaaaagatg gatttatgac acatccaaac tggcttctgg
 251 agtccctgct cacttcaggg gcagtgggtc tgggacctct tactctctca
 301 caatcagcgg catggaggct gaagatgctg ccacttatta ctgccagcag
 351 tggagtagta acccattcac gttcggctcg gggacaaagt tggaaataaa
 401 ccgggctgat actgcaccaa ctgtatccat cttcccacca tccagtgagc
 451 agttaacatc tggaggtgcc tcagtcgtgt gcttcttgaa caacttctac
 501 cccaaagaca tcaatgtcaa gtggaagatt gatggcagtg aacgacaaaa
 551 tggcgtcctg aacagttgga ctgatcagga cagcaaagac agcacctaca
 601 gcatgagcag caccctcacg ttgaccaagg acgagtatga acgacataac
 651 agctatacct gtgaggccac tcacaagaca tcaacttcac ccattgtcaa
 701 gagcttcaac aggaatgagt gtTAGAGACA AAGGTCCTGA GACGCCACCA
 751 CCAGCTCCCA GCTCCATCCT ATCTTCCCTT CTAAGGTCTT GGAGGCTTCC
 801 CCACAAGCGC tTACCACTGT TGCGGTGCTC tAAACCTCCT CCCACCTCCT
 851 TCTCCTCCTC CTCCCTTTCC TTGGCTTTTA TCATGCTAAT ATTTGCAGAA
 901 AATATTCAAT AAAGTGAGTC TTTGCCTTGA AAAAAAAAAA AAA
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SEQUENCE LENGTH 943 RESIDUES
INITIATOR MET AT 18
MATURE SEQUENCE BEGINS AT 84
CODING SEQUENCE 639 RESIDUES

NB. KAPPA CHAIN SEQUENCE OBTAINED FROM PUBLISHED SEQUENCE. ONLY THE JUNCTION WITH VARIABLE REGION AND 3' UNTRANSLATED REGION HAS BEEN CHECKED.

OKT3 LIGHT CHAIN PROTEIN SEQUENCE DEDUCED FROM DNA SEQUENCE

```
   1 MDFQVQIFSF LLISASVIIS RGQIVLTQSP AIMSASPGEK VTMTCSASSS
  51 VSYMNWYQQK SGTSPKRWIY DTSKLASGVP AHFRGSGSGT SYSLTISGME
 101 AEDAATYYCQ QWSSNPFTFG SGTKLEINRA DTAPTVSIFP PSSEQLTSGG
 151 ASVVCFLNNF YPKDINVKWK IDGSERQNGV LNSWTDQDSK DSTYSMSSTL
 201 TLTKDEYERH NSYTCEATHK TSTSPIVKSF NRNEC*
```

FIGURE 1

The DNA sequence of the OKT3 light chain as deduced from DNA sequencing of cDNA's and, for the Kappa constant region, from known sequence.

Untranslated regions are shown in uppercase type and the signal sequence is underlined. Also shown is the protein sequence translated from the major open reading frame.

```
   1  GAATTCCCCT CTCCACAGAC ACTGAAAACT CTGACTCAAC ATGGAAGGGC
  51  ACTGGATCTT TCTACTCCTG TTGTCAGTAA CTGCAGGTGT CCACTCCCAG
 101  GTCCAGCTGC AGCAGTCTGG GGCTGAACTG GCAAGACCTG GGGCCTCAGT
 151  GAAGATGTCC TGCAAGGCTT CTGGCTACAC CTTTACTAGG TACACGATGC
 201  ACTGGGTAAA ACAGAGGCCT GGACAGGGTC TGGAATGGAT TGGATACATT
 251  AATCCTAGCC GTGGTTATAC TAATTACAAT CAGAAGTTCA AGGACAAGGC
 301  CACATTGACT ACAGACAAAT CCTCCAGCAC AGCCTACATG CAACTGAGCA
 351  GCCTGACATC TGAGGACTCT GCAGTCTATT ACTGTGCAAG ATATTATGAT
 401  GATCATTACT GCCTTGACTA CTGGGGCCAA GGCACCACTC TCACAGTCTC
 451  CTCAGCCAAA ACAACAGCCC CATCGGTCTA TCCACTGGCC CCTGTGTGTG
 501  GAGATACAAC TGGCTCCTCG GTGACTCTAG GATGCCTGGT CAAGGGTTAT
 551  TTCCCTGAGC CAGTGACCTT GACCTGGAAC TCTGGATCCC TGTCCAGTGG
 601  TGTGCACACC TTCCCAGCTG TCCTGCAGTC TGACCTCTAC ACCCTCAGCA
 651  GCTCAGTGAC TGTAACCTCG AGCACCTGGC CCAGCCAGTC CATCACCTGC
 701  AATGTGGCCC ACCCGGCAAG CAGCACCAAG GTGGACAAGA AAATTGAGCC
 751  CAGAGGGCCC ACAATCAAGC CCTGTCCTCC ATGCAAATGC CCAGCACCTA
 801  ACCTCTTGGG TGGACCATCC GTCTTCATCT TCCCTCCAAA GATCAAGGAT
 851  GTACTCATGA TCTCCCTGAG CCCCATAGTC ACATGTGTGG TGGTGGATGT
 901  GAGCGAGGAT GACCCAGATG TCCAGATCAG CTGGTTTGTG AACAACGTGG
 951  AAGTACACAC AGCTCAGACA CAAACCCATA GAGAGGATTA CAACAGTACT
1001  CTCCGGGTGG TCAGTGCCCT CCCCATCCAG CACCAGGACT GGATGAGTGG
1051  CAAGGAGTTC AAATGCAAGG TCAACAACAA AGACCTCCCA GCGCCCATCG
1101  AGAGAACCAT CTCAAAACCC AAAGGGTCAG TAAGAGCTCC ACAGGTATAT
1151  GTCTTGCCTC CACCAGAAGA AGAGATGACT AAGAAACAGG TCACTCTGAC
1201  CTGCATGGTC ACAGACTTCA TGCCTGAAGA CATTTACGTG GAGTGGACCA
1251  ACAACGGGAA AACAGAGCTA AACTACAAGA ACACTGAACC AGTCCTGGAC
1301  TCTGATGGTT CTTACTTCAT GTACAGCAAG CTGAGAGTGG AAAAGAAGAA
1351  CTGGGTGGAA AGAAATAGCT ACTCCTGTTC AGTGGTCCAC GAGGGTCTGC
1401  ACAATCACCA CACGACTAAG AGCTTCTCCC GGACTCCGGG TAAATGAGCT
1451  CAGCACCCAC AAAACTCTCA GGTCCAAAGA GACACCCACA CTCATCTCCA
1501  TGCTTCCCTT GTATAAATAA AGCACCCAGC AATGCCTGGG ACCATGTAAA
1551  AAAAAAAAAA AAAGGAATTC
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SEQUENCE LENGTH 1570 RESIDUES
INITIATOR MET AT 41
SIGNAL SEQUENCE UNDERLINED
MATURE SEQUENCE BEGINS AT 98
CODING SEQUENCE 1407 RESIDUES
5' UNTRANSLATED REGION 40 RESIDUES 3' UNTRANSLATED REGION 123 RESIDUES

OKT3 HEAVY CHAIN PROTEIN SEQUENCE DEDUCED FROM DNA SEQUENCE

8928874.0

```
  1  MERHWIFLLL LSVTAGVHSQ VQLQQSGAEL ARPGASVKMS CKASGYTFTR
 51  YTMHWVKQRP GQGLEWIGYI NPSRGYTNYN QKFKDKATLT TDKSSSTAYM
101  QLSSLTSEDS AVYYCARYYD DHYCLDYWGQ GTTLTVSSAK TTAPSVYPLA
151  PVCGDTTGSS VTLGCLVKGY FPEPVTLTWN SGSLSSGVHT FPAVLQSDLY
201  TLSSSVTVTS STWPSQSITC NVAHPASSTK VDKKIEPRGP TIKPCPPCKC
251  PAPNLLGGPS VFIFPPKIKD VLMISLSPIV TCVVVDVSED DPDVQISWFV
301  NNVEVHTAQT QTHREDYNST LRVVSALPIQ HQDWMSGKEF KCKVNNKDLP
351  APIERTISKP KGSVRAPQVY VLPPPEEEMT KKQVTLTCMV TDFMPEDIYV
401  EWTNNGKTEL NYKNTEPVLD SDGSYFMYSK LRVEKKNWVE RNSYSCSVVH
451  EGLHNHHTTK SFSRTPGK*
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FIGURE 2

The DNA sequence of the OKT3 heavy chain chain as deduced from DNA sequencing of cDNA's and, for the constant regions, from known sequence.

The signal sequence is underlined.

Also shown is the protein sequence translated from the major open reading frame.

```
  1 QIVLTQSPAIMSASPGEKVTMTCSASSSVSYMNWYQQKSGTSPKRWIYDT 50
    |||||||||||||||||||||||||||||||| ||||||||||||||||
  1 QIVLTQSPAIMSASPGEKVTMTCSASSSVSYMHWYQQKSGTSPKRWIYDT 50

 51 SKLASGVPAHFRGSGSGTSYSLTISGMEAEDAATYYCQQWSSNP..FTFG 98
    ||||||||| | |||||||||||| ||||||||||||||||||   |||
 51 SKLASGVPARFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPPMLTFG 100

 99 SGTKLEINR 107
    |||||  |
101 AGTKLELKR 109
```

HOMOLOGY 92.5%

UPPER LINE OKT3 V_L

LOWER LINE MOUSE V_L SUB GROUP 6

FIGURE 3

The protein sequence comparison of the OKT3 light chain variable region with the Kabat mouse sub group 6 consensus sequence (Kabat et al. 1987).

```
   OKT3 QVQLQQSGaELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQGLEWIGY 50
        ||||||| ||  |||||||||||||||||| |   |||| |  |||||
2A CONS evqlqqsgpelvkpgasvkmsckasgytftdyymkwvkqshgks1ewigd 50

     51 INPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCAR.. 98
        |||  | | |||||| ||||| |||||||||||| ||||||||||||||
     51 inpnnggtsynqkfkgkatltvdkssstaymqlnsltsedsavyycards 100

     99 .YYDDHYCLDYWGQGTTLTVss 119
                |||||||| ||||
    101 ywyfxyywfdywgqgttvtvss 122     Percent Similarity: 78.151


   OKT3 QVQLQQSGaELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQGLEWIGY 50
        |||||| ||||  |||||| |||||||||| | ||||||||||||||||
2B CONS qvqlqqpgaelvkpgasvklsckasgytftsywmhwvkqrpgqglewigr 50

     51 INPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARY. 99
        | |  | | || ||| ||||| ||||||||||||||||||||||||||
     51 idpnsggtkynekfkskatltvdkssstaymqlssltsedsavyycaryx 100

    100 ......YDDHYCLDYWGQGTTLTVss 119
                  ||||||||||||
    101 yygsssxgyyxyfdywgqgttltvss 126 Percent Similarity: 81.513


   OKT3 QVQLQQSGaELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQGLEWIGY 50
        ||||||||| ||  |||||| || |||       |||||||| |||||||
2C CONS qvqlqqsgaelvkpgasvklsctasgfnikdtymhwvkqrpeqglewigr 50

     51 INPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYY 100
        | |  | | |  ||  ||| | | || ||| ||||||||| ||||||| |
     51 idpangntkydpkfqgkatitadtssntaylqlssltsedtavyycargy 100

    101 D..DHYCLDYWGQGTTLTVss 117
           | |  |||||||  ||||
    101 xxydyyamdywgqgtsvtvss 121      Percent Similarity: 69.231


   OKT3 QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQGLEWIGY 50
         |||||||||| | | |||||||||||||| |   ||||||||||||||
5A CONS evqlqqsgaelvragssvkmsckasgytftsyginwvkqrpgqglewigy 50

     51 INPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCAR.. 98
        |||  ||| || ||| | ||| |||||||||| |||||||||| |||
     51 inpgngytkynekfkgkttltvdkssstaymqlrsltsedsavyfcarsn 100

     99 YYDDHYC..LDYWGQGTTLTVSS 119
        ||   |    ||||||||||||
    101 yyggsyyfffdywgqgttltvss 123    Percent Similarity: 81.513
```

FIGURE 4

The protein sequence comparison of the OKT3 heavy chain variable region with the Kabat mouse sub groups 2A, 2B, 2C and 5A (Kabat et al. 1987)

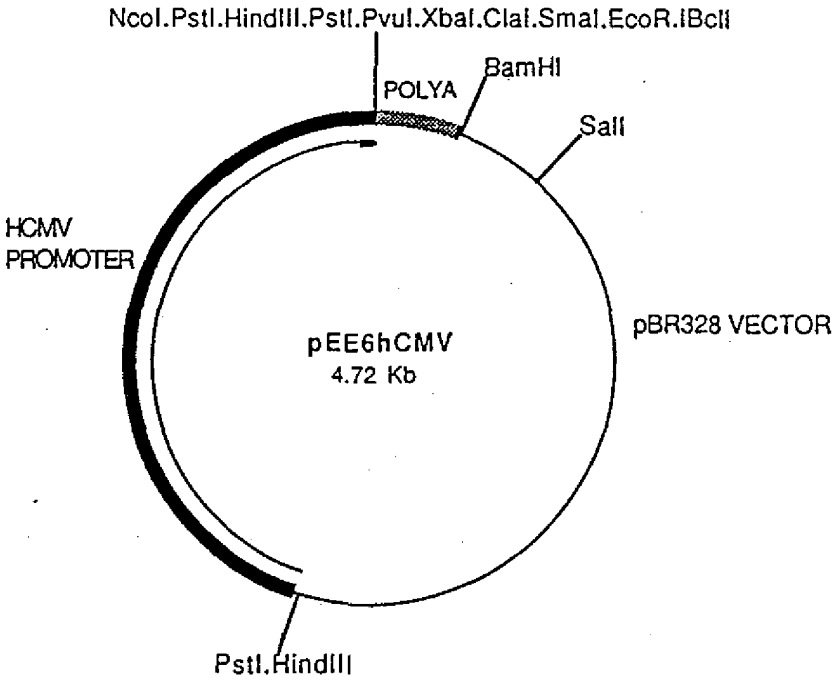


FIGURE 5

A map for the EE6hCMV expression vector used in this study
Only necessary sites are shown.

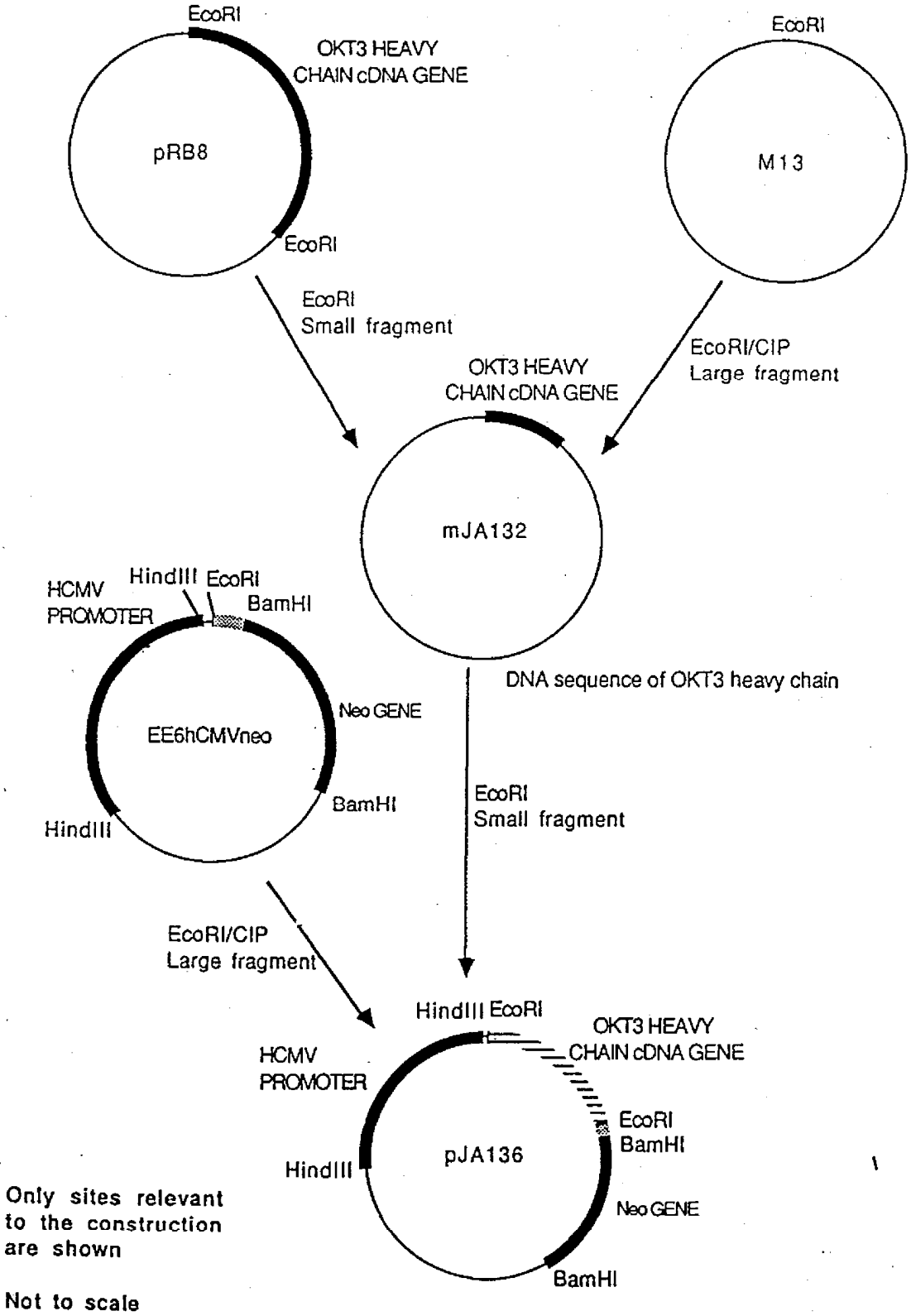


FIGURE 6
An outline schematic of the procedures involved in the construction of pJA136, a vector for the expression in eukaryotic cells of the OKT3 heavy chain cDNA gene.

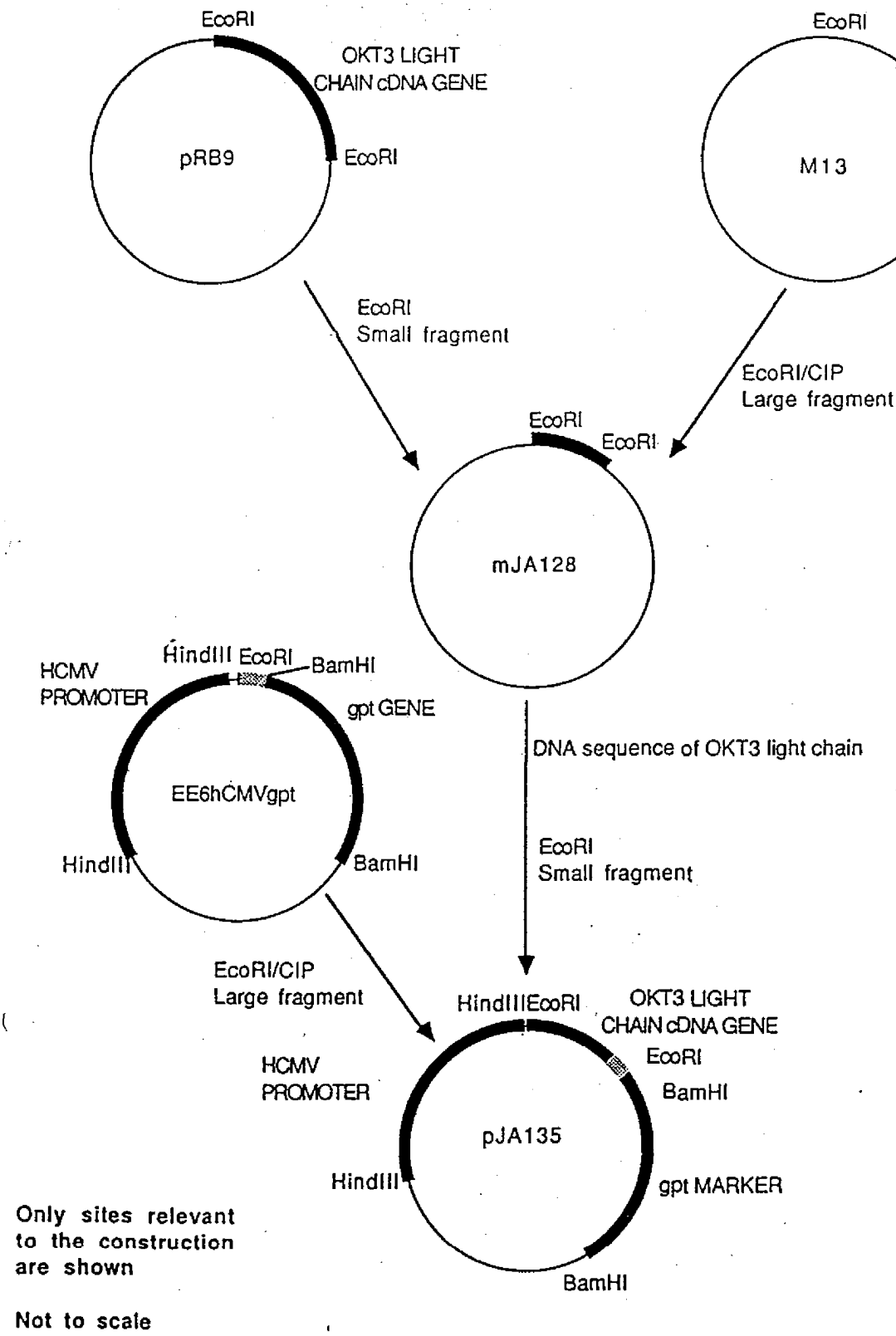


FIGURE 7
An outline schematic of the procedures involved in the construction of pJA135, a vector for the expression in eukaryotic cells of the OKT3 light chain cDNA gene.

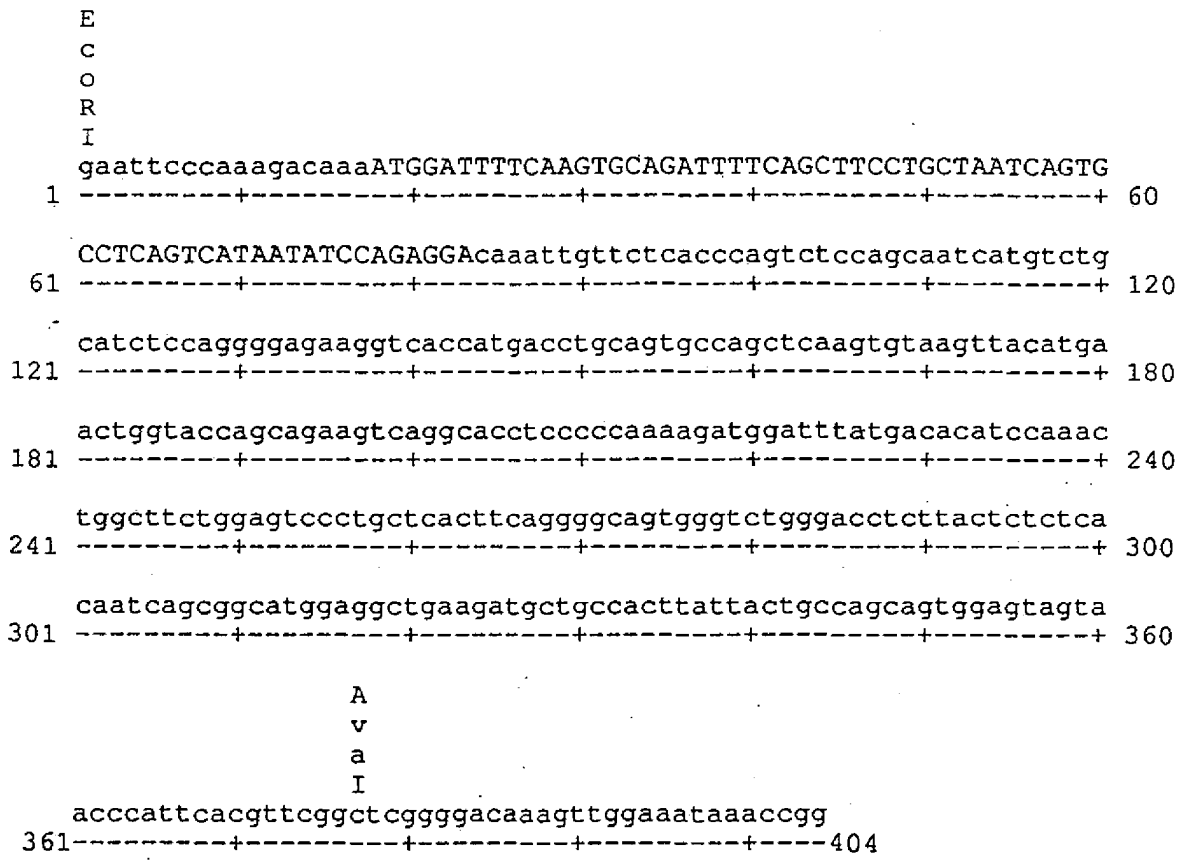
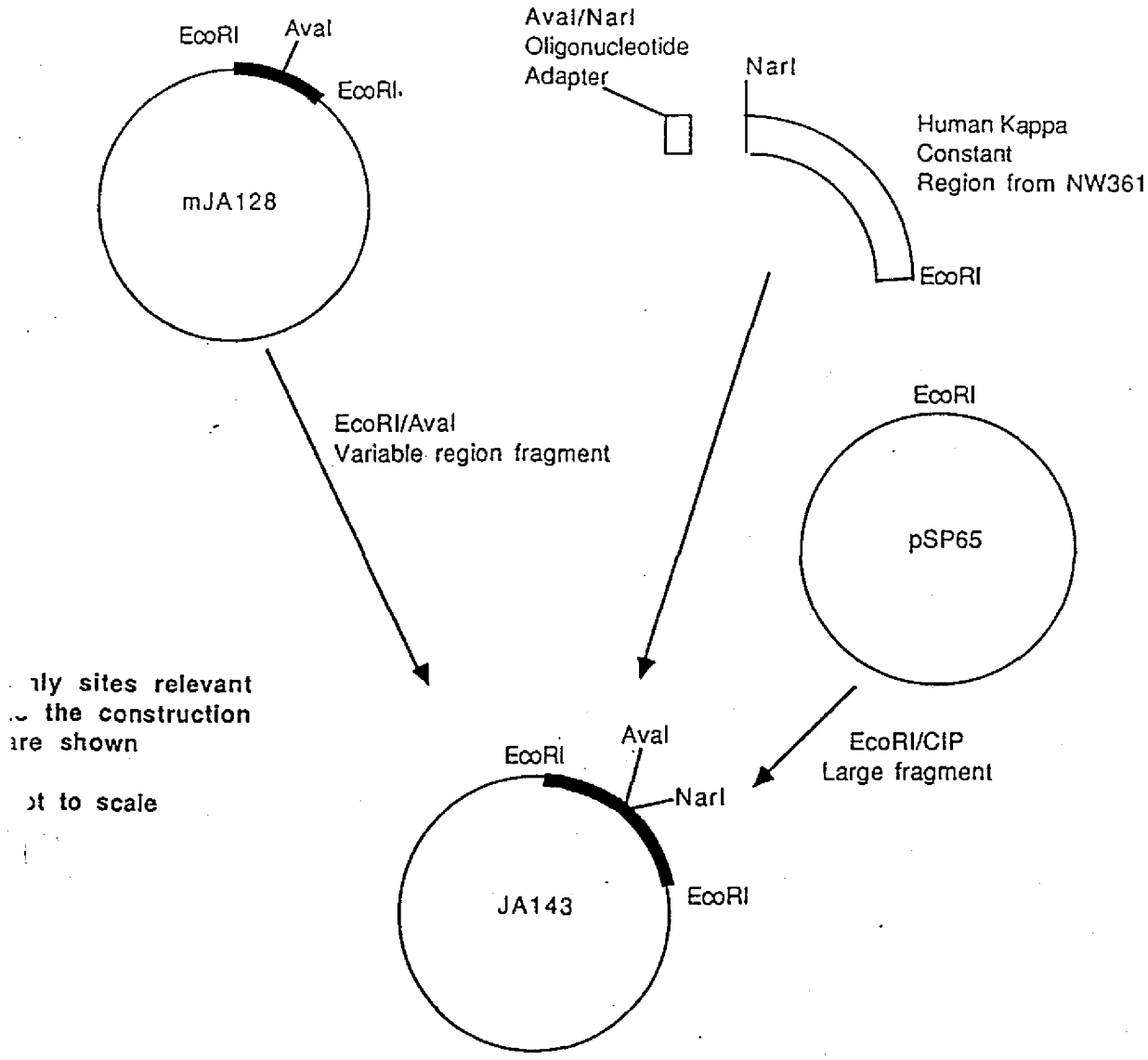


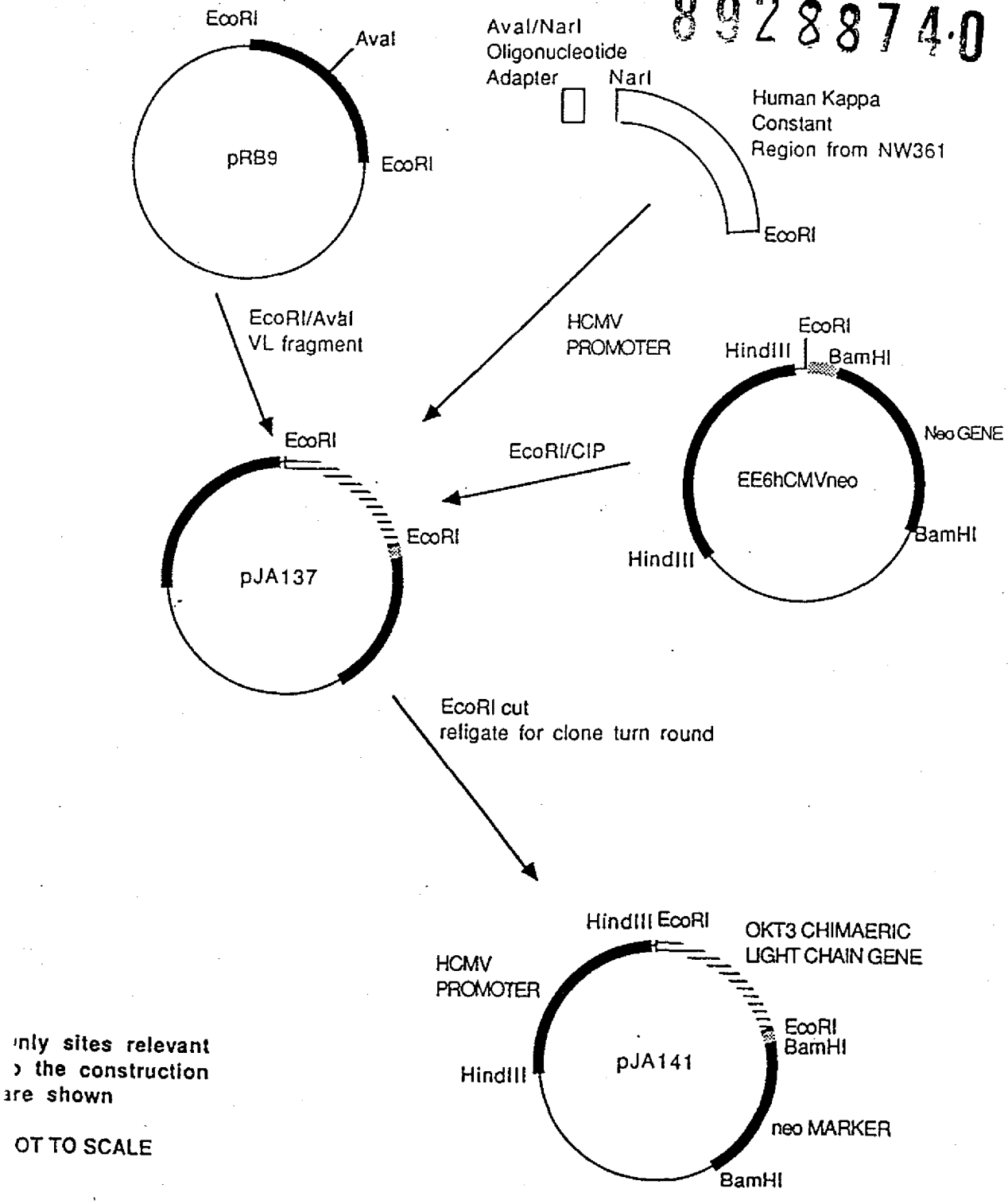
FIGURE 8
The nucleotide sequence for the OKT3 light chain variable region and the location of the EcoRI and AvaI sites used in the construction of the chimaeric OKT3 light chain gene.



ıly sites relevant
.e the construction
ıre shown

ɔt to scale

FIGURE 9
An outline schematic of the procedures involved in the construction of pJA143, an MI3 vector, including the OKT3 chimaeric light chain gene (Version1).



nly sites relevant
the construction
are shown

OT TO SCALE

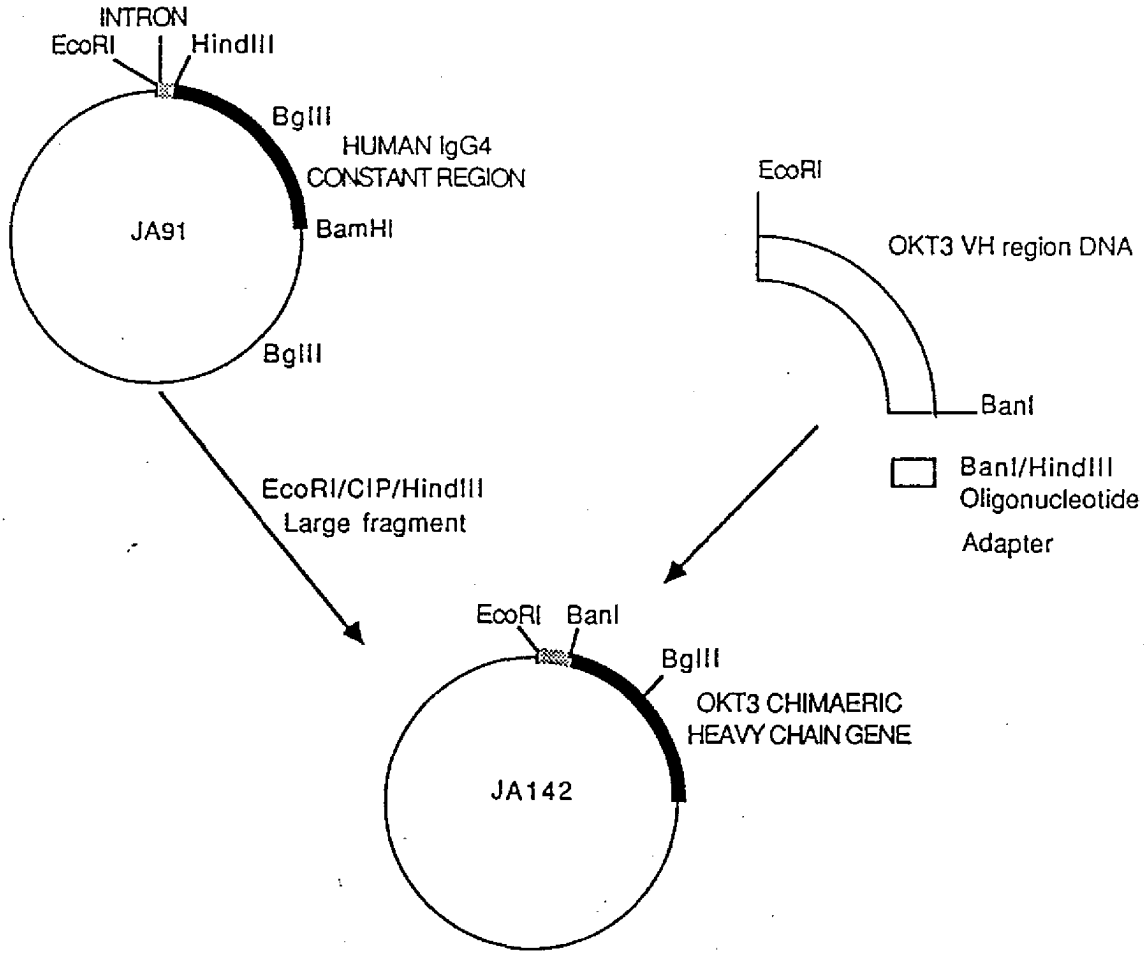
FIGURE 10

An outline schematic of the procedures involved in the construction of pJA141, a vector for the expression in eukaryotic cells of the OKT3 chimaeric light chain gene (Version 2).

```
    E
    c
    o
    R
    I
    GAATTCCCCTCTCCACAGACACTGAAAACTCTGACTCAACATGGAAAGGCACTGGATCTT
1   ---------+---------+---------+---------+---------+---------+ 60

    TCTACTCCTGTTGTCAGTAACTGCAGGTGTCCACTCCCAGGTCCAGCTGCAGCAGTCTGG
61  ---------+---------+---------+---------+---------+---------+ 120

    GGCTGAACTGGCAAGACCTGGGGCCTCAGTGAAGATGTCCTGCAAGgCTTCTGGCTACAC
121 ---------+---------+---------+---------+---------+---------+ 180

    CTTTACTAGGTACACGATGCACTGGGTAAAACAGAGGCCTGGACAGGGTCTGGAATGGAT
181 ---------+---------+---------+---------+---------+---------+ 240

    TGGATACATTAATCCTAGCCGTGGTTATACTAATTACAATCAGAAGTTCAAGGACAAGGC
241 ---------+---------+---------+---------+---------+---------+ 300

    CACATTGACTACAGACAAATCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATC
301 ---------+---------+---------+---------+---------+---------+ 360

    TGAGGACTCTGCAGTCTATTACTGTGCAAGATATTATGATGATCATTACTGCCTTGACTA
361 ---------+---------+---------+---------+---------+---------+ 420

         B
         a
         n
         I
    CTGGGGCCAAGGCACCACTCTCACAGTCTCCTCA
421 ---------+---------+---------+---- 454
```

FIGURE 11

The nucleotide sequence for the OKT3 heavy chain variable region and the location of the EcoRI and BanI sites used in the construction of the chimaeric OKT3 heavy chain gene.



Only sites relevant to the construction are shown

FIGURE 12

An outline schematic of the procedures involved in the construction of pJA142, an MI3 vector including the OKT3 chimaeric heavy chain gene.

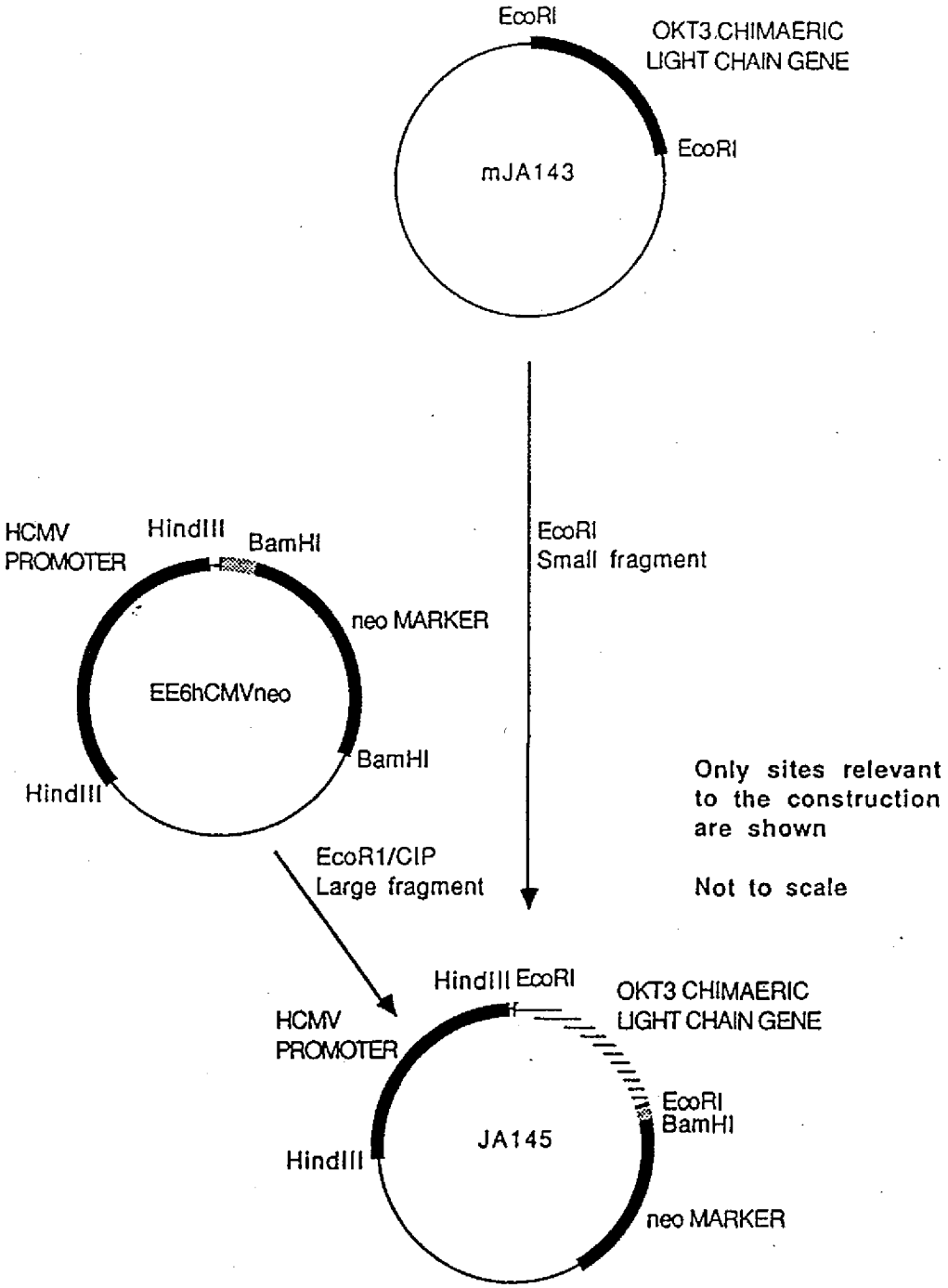


FIGURE 13
An outline schematic of the procedures involved in theconstruction of pJA145, a vector for the expression in eukaryotic cells of the OKT3 chimaeric light chain gene (Version 1).

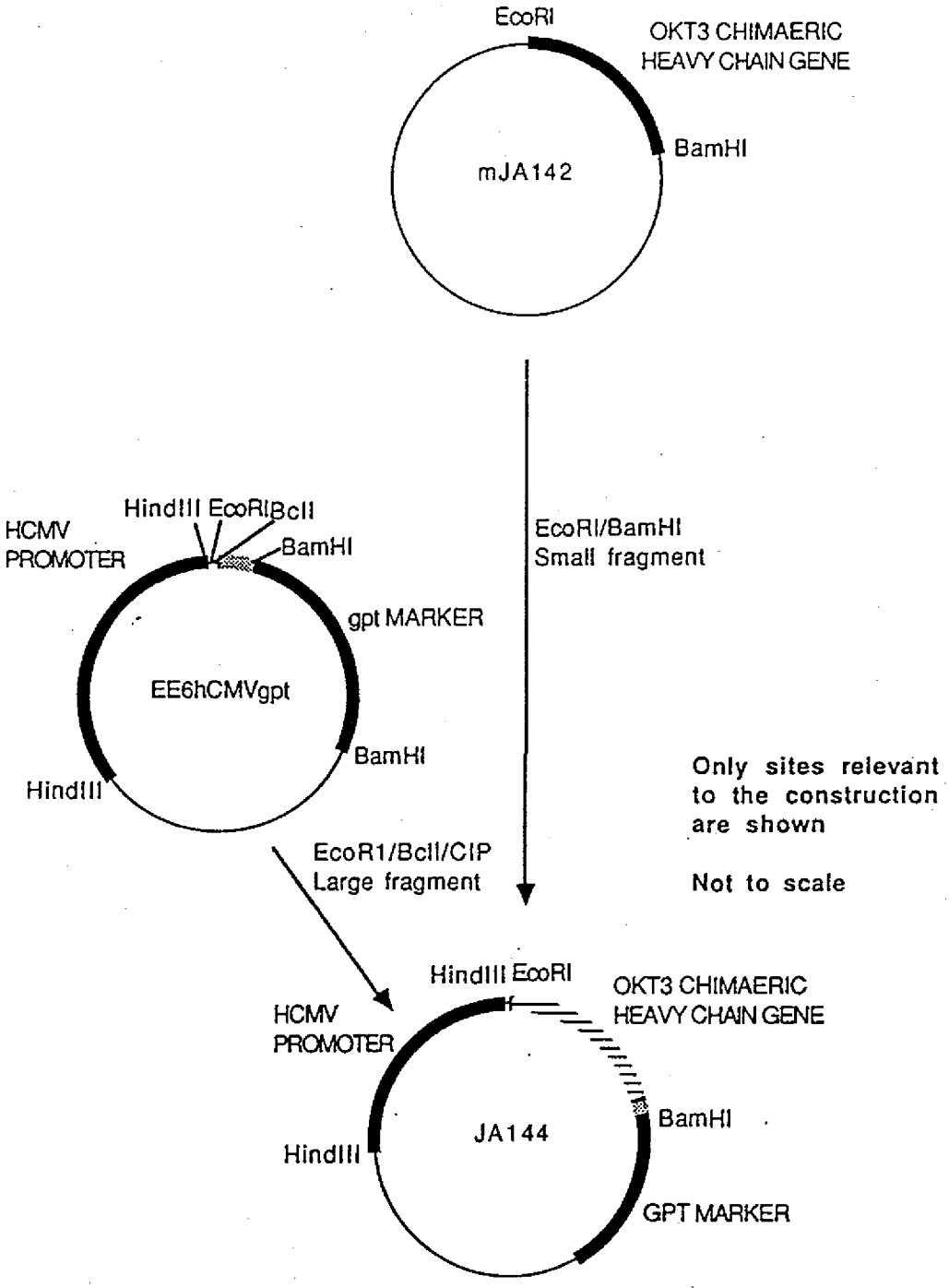


FIGURE 14
An outline schematic of the procedures involved in the construction of pJA144, a vector for the expression in eukaryotic cells of the OKT3 chimaeric heavy chain gene.

FILED 21 DEC 1989

8923874.0

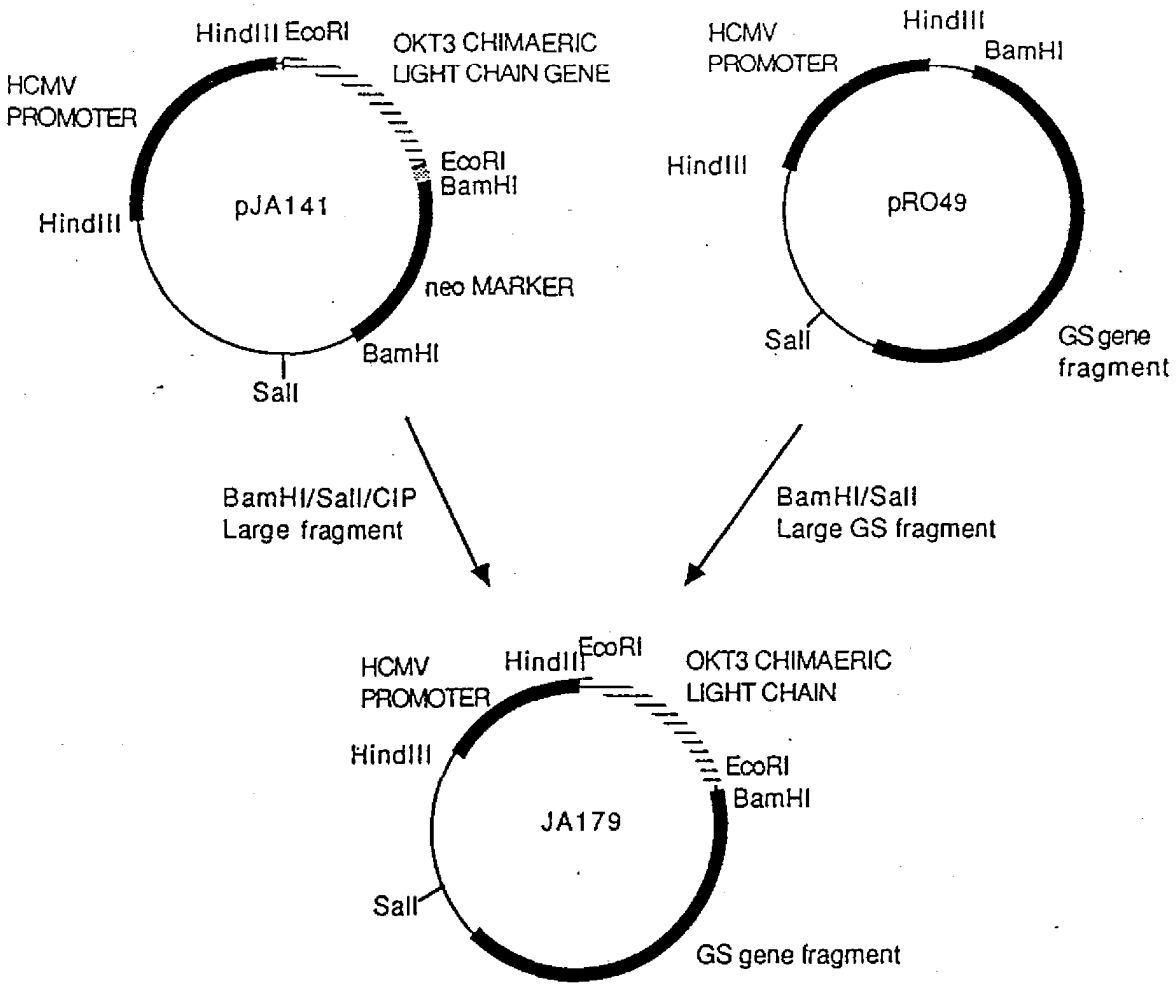


FIGURE 15
An outline schematic of the procedures involved in the construction of pJA179, a vector for the expression in eukaryotic cells using the GS amplification system of the OKT3 chimaeric light chain gene (Version 2).

FILED 21 DEC 1989 8928874-0

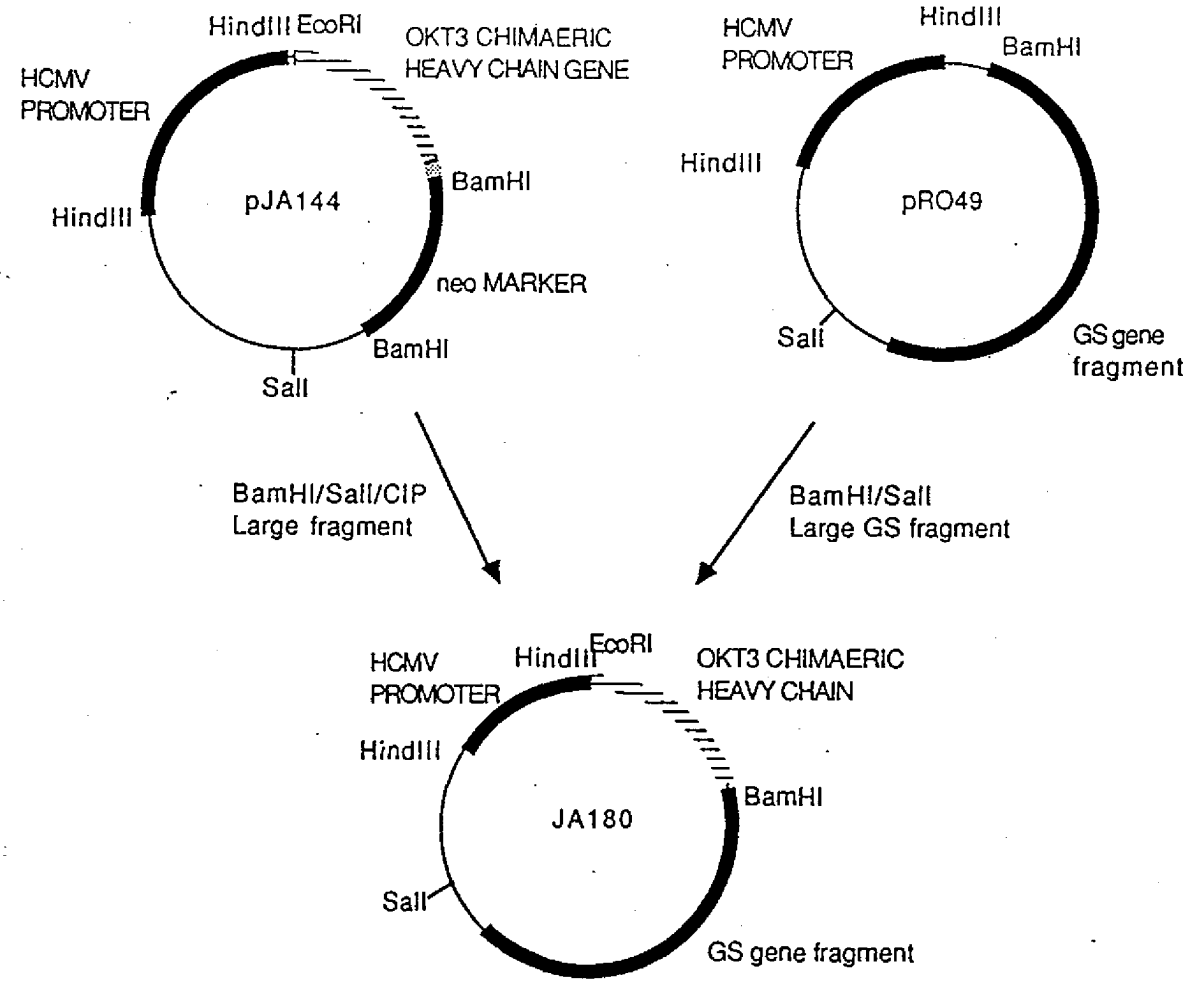


FIGURE 16
An outline schematic of the procedures involved in the construction of pJA179, a vector for the expression in eukaryotic cells using the GS amplification system of the OKT3 chimaeric heavy chain gene.

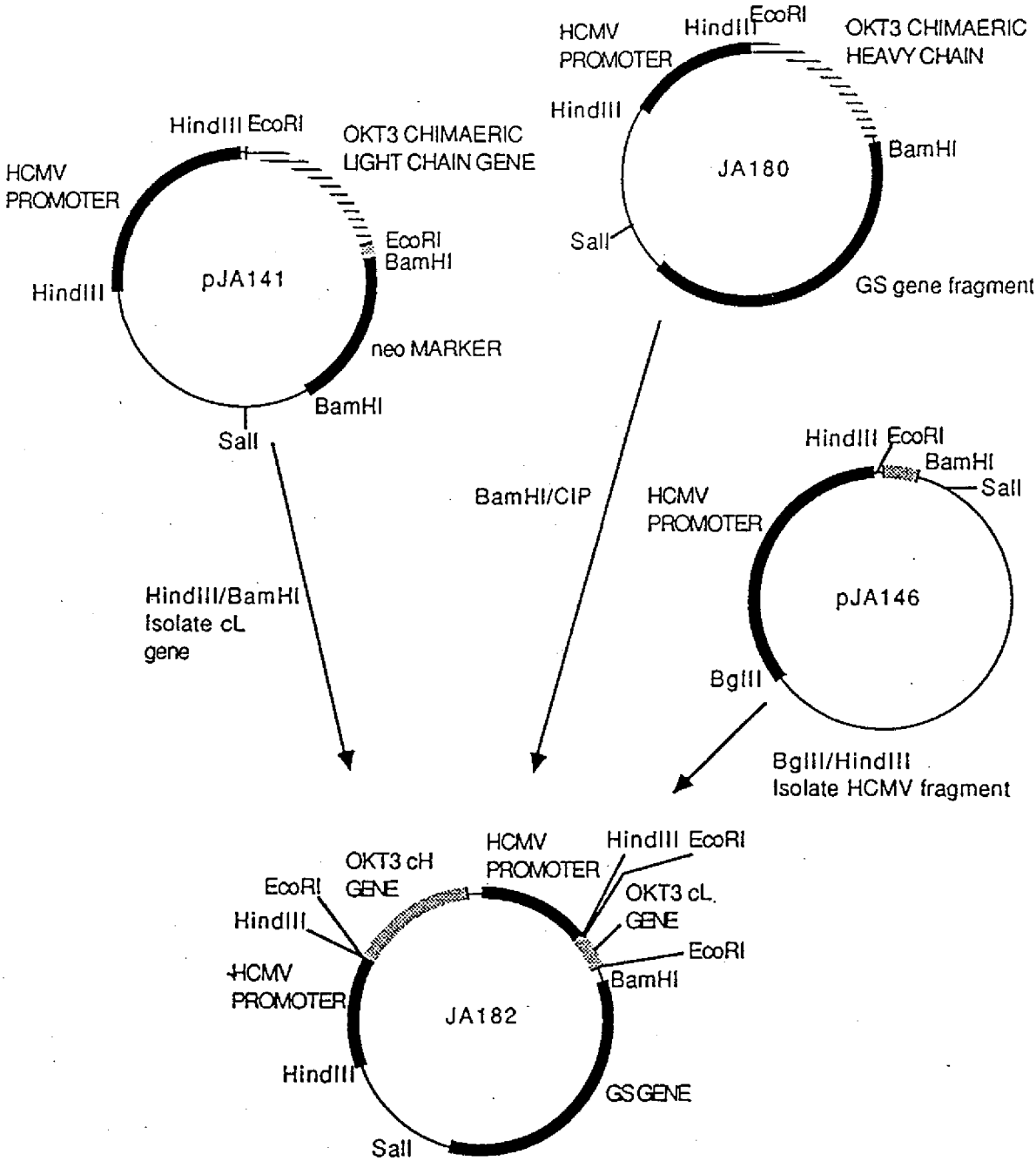


FIGURE 17
An outline schematic of the procedures involved in the construction of pJA182, a vector for the expression in eukaryotic cells using the GS amplification system of the OKT3 chimaeric heavy chain gene and chimaeric light chain gene (Version 2) in the transcription order cH>cL>GS.

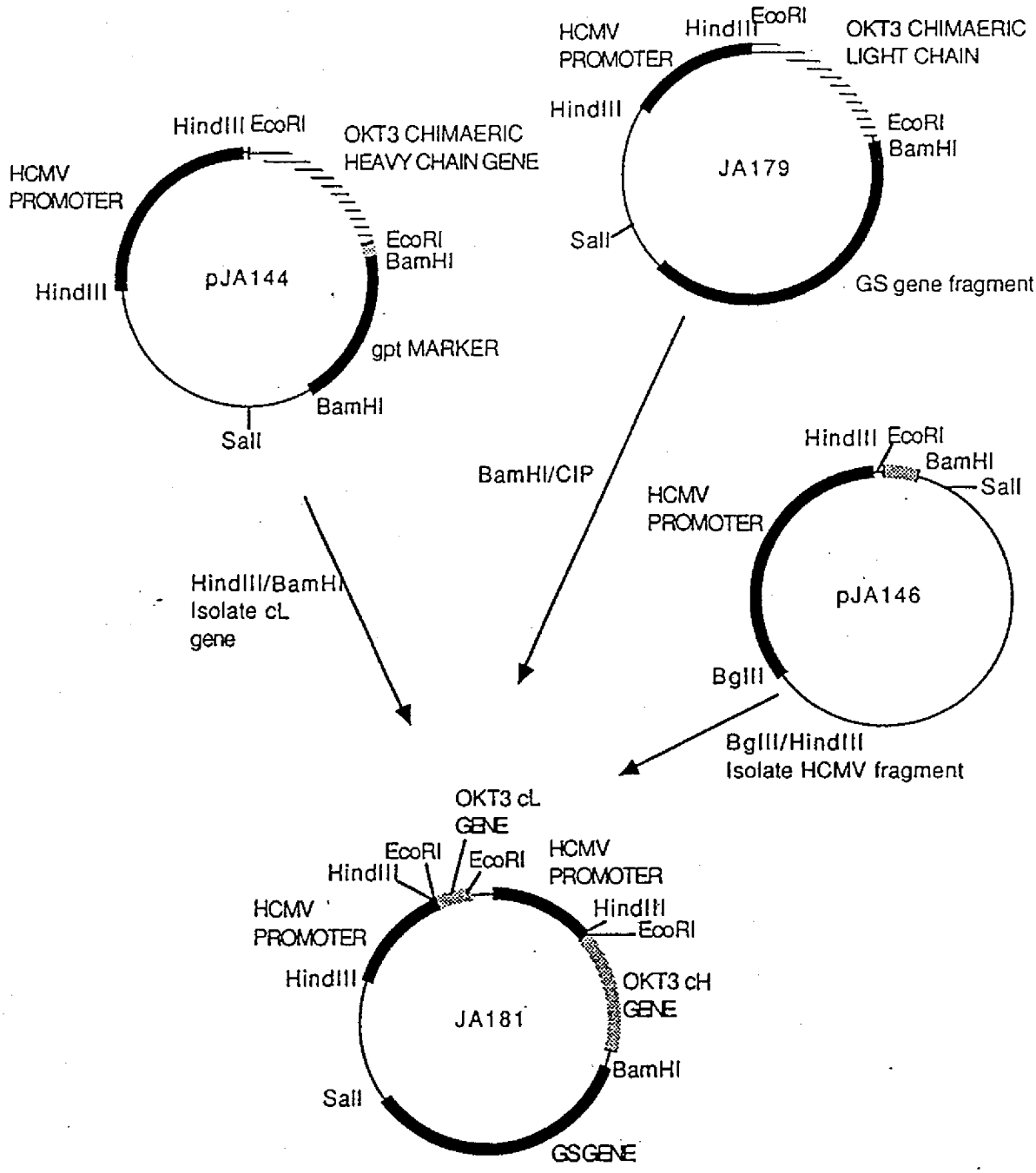


FIGURE 18
An outline schematic of the procedures involved in the construction of pJA181, a vector for the expression in eukaryotic cells using the GS amplification system, of the OKT3 chimaeric light chain gene (Version 2) and chimaeric heavy chain gene in the transcription order cL>cH>GS.

FILED 21 DEC 1987

89288740

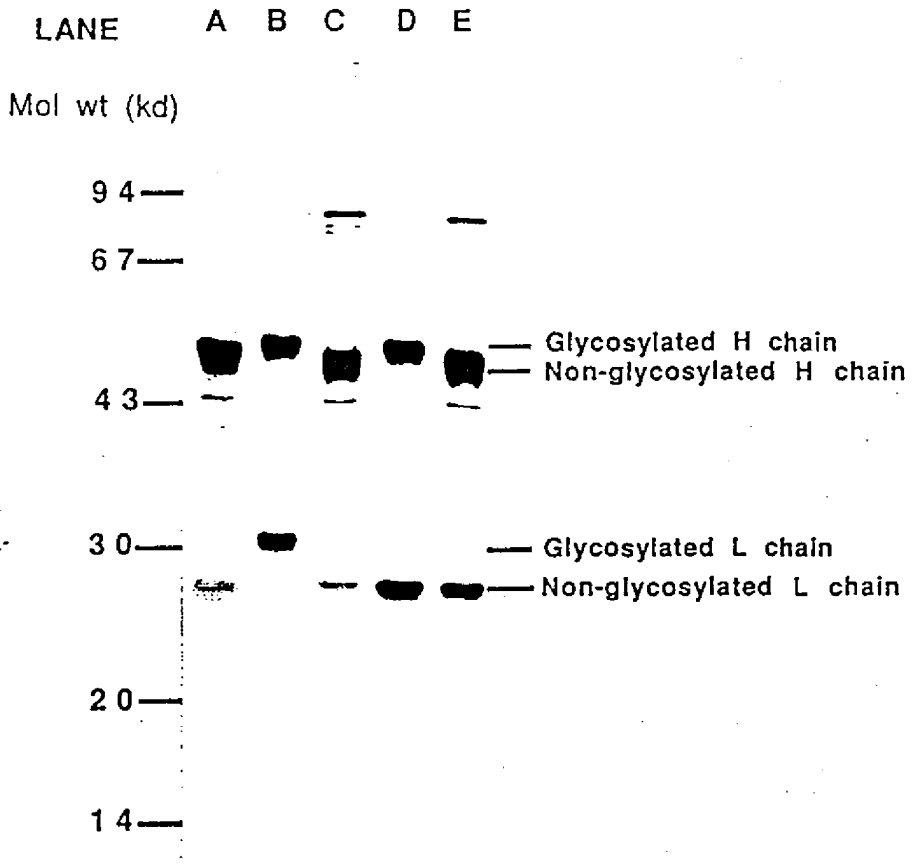


Fig 19. Effect on glycosylation of the presence of tunicamycin during cell growth

Reducing SDS-PAGE of ^{35}S labelled antibody produced in the absence (lanes A ,B, D) or presence (lanes C & E) of tunicamycin. COS cells were transfected and medium replaced after 24hrs by medium with or without tunicamycin. Antibody was recovered from culture supernatants by protein-A Sepharose precipitation. after 48hrs further incubation.

Key:

A. - cLcH B72.3 control
B. - cL*cH - Tunicamycin
C. - cL*cH + Tunicamycin
D. - cLcH - Tunicamycin
E. - cLcH + Tunicamycin

NB: cL* - chimaeric light chain version 1
cL - chimaeric light chain version 2

```
                1                   23                  42
                NN      N               N       N       N
 RES TYPE   SBspSPESssBSbSsSssPSPSPsPSsse*s*p*Pi^ISsSe
Okt3v1      QIVLTQSPAIMSASPGEKVTMTCSASS.SVSYMNWYQQKSGT
REI         DIQMTQSPSSLSASVGDRVTITCQASQDIIKYLNWYQQTPGK
            ? ?
               CDR1   (LOOP)            *******
               CDR1   (KABAT)         ***********

                          56                              85
            N  NN
 RES TYPE   *IsiPpIeesesssSBEsePsPSBSSEsPspsPsseesSPePb
Okt3v1      SPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAAT
REI         APKLLIYEASNLQAGVPSRFSGSGSGTDYTFTISSLQPEDIAT
            ?  ??                           ?   ?
                    ******* CDR2   (LOOP/KABAT)

                          102   108
RES TYPE    PiPIPies**iPIIsPPSPSPSS
Okt3v1      YYCQQWSSNPFTFGSGTKLEINR
REIv1       YYCQQYQSLPYTFGQGTKLQITR
                         ?     ?
                  ******        CDR3   (LOOP)
                 *********      CDR3   (KABAT)
```

KEY TO RES TYPE

N NEAR TO CDR (FROM X RAY STRUCTURES)

P PACKING — B BURIED NON PACKING

S SURFACE — E EXPOSED

I INTERFACE — * INTERFACE/PART EXPOSED

^ PACKING/PART EXPOSED

? NON-CDR RESIDUES WHICH MAY REQUIRE TO BE LEFT AS MOUSE SEQUENCE

FIGURE 20

The alignment of the OKT3 light variable region amino acid sequence with that of the light variable region of the human antibody REI. Above the sequence the residue type (defined in the key) notes the spatial location of each residue side chain (derived by examination of resolved structures from Xray crystallography analysis). Residues in bold type refer to amino acids which differ from the residue found at that position in the consensus sequence for the species subgroup to which the antibody belongs ie. mouse sub group 6 for the OKT3 sequence and human sub group 1 for the REI sequence.

```
          NN N                  23 26    32 35 N39  43
RES TYPE  SESPs^SBssS^sSSsSpSpSPsPSEbSBssBePiPIpiesss
Okt3vh    QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQ
KOL       QVQLVESGGGVVQPGRSLRLSCSSSGFIFSSYAMYWVRQAPGK
               ?                ??
                                   ******     CDR1 (LOOP)
                                       *****  CDR1 (KABAT)

                  52a      60  65     N N  N    82abc     89
RES TYPE  IIeIppp^ssssssss^ps^pSSsbSpseSsSseSp^pSpsSBssS^ePb
Okt3vh    GLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAV
KOL       GLEWVAIIWDDGSDQHYADSVKGRFTISRDNSKNTLFLQMDSLRPEDTGV
              ??                      ? ?  ?  ?            ?
                 ************        CDR2  (LOOP)
                 ******************** CDR2  (KABAT)

           92 N                  107    113
RES TYPE  PiPIEissssiiisssbibi*EIPIP*spSBSS
Okt3vh    YYCARYYDDHY.......CLDYWGQGTTLTVSS
KOL       YFCARDGGHGFCSSASCFGPDYWGQGTPVTVSS
               ***************** CDR3 (KABAT/LOOP)
```

KEY TO RES TYPE

N NEAR TO CDR (FROM X RAY STRUCTURES)

P PACKING — B BURIED NON PACKING

S SURFACE — E EXPOSED

I INTERFACE — * INTERFACE/PART EXPOSED

^ PACKING/PART EXPOSED

? AMINO ACIDS WHICH MAY NEED TO REMAIN AS MOUSE IN CDR GRAFT

FIGURE 21

The alignment of the OKT3 heavy variable region amino acid sequence with that of the heavy variable region of the human antibody KOL. Above the sequence the residue type (defined in the key) notes the spatial location of each residue side chain (derived by examination of resolved structures from Xray crystallography analysis). Residues in bold type refer to amino acids which differ from the residue found at that position in the consensus sequence for the species subgroup to which the antibody belongs ie. mouse sub group 2B for the OKT3 sequence and human sub group 3 for the KOL sequence.

```
  1  AATTCATGGA ATGGAGCTGG GTCTTTCTCT TCTTCCTGTC AGTAACTACA
 51  GGTGTCCACT CCCAGGTTCA GCTGGTGGAG TCTGGAGGAG GAGTCGTCCA

                                                26  27 28 29 30
                                                G   Y  T  F  T
101  GCCTGGAAGG TCCCTGAGAC TGTCTTGTTC TTCTTCTGGA TACACATTCA
                                  oligo JA88-44 cct atgtgtaagt...
                                                 PROBE JA88-45
                                                 G   Y  T  F

      31 32 33  34 35
       D  H  A   M  Y  W
151  CAGACCACGC TATGTACTGG GTCAGACAGG CTCCTGGAAA GGGACTGGAG
       ****  *      *                          oligo JA88-40    c...
  ...gttctatgtg atacgtgacc cagtctgtcc 5' R1198
     gttctatgtg atacgt 5' R1197
     T  R  Y  T   M  H  W

              50  51 51 52a 53 54 55  56 57 58  59 60 61 62
               Y   I  S  P   G  N  D   D  I  K   Y  N  E  K
201  TGGGTCGCTT ACATCTCTCC TGGAAATGAC GACATCAAGT ACAATGAGAA
                   ***     * * ** ** *   **  *
  ...acccagcgaa tgtaattagg atcgtctcct atgtgtttaa tgttagtctt...
        PROBE JA88-41  gg atcgtctcct atgtgttta 5' R1153
             Y   I  N  P   S  R  G   Y  T  N  Y   N  Q  K

      63 64 65  66
      F  K  G   R
251  GTTCAAGGGA AGATTCACAA TTTCTAGAGA CAATTCTAAG AATACACTGT
             **
  ...caagttcctg tctaagtgtt aaagatc 5' R1152
      F  K  D   R

301  TCCTGCAGAT GGACTCACTC AGACCTGAGG ACACAGGAGT CTACTTCTGT
                                  oligo JA88-42 tgaagaca

           95 96  97 98 99  100  a  b 101102
           S  Y   Y  G  H            D  Y
351  GCTAGATCCT ACTACGGCCA C......... GACTACTGGG GCCAAGGTAC
             *     *   *     *********
     cgatctatga tgctgctggt gatgacagac ctgatgaccc cggtt 5' R1154
PROBE JA88-43 a tgctgctggt gatg 5' R1155
           Y  Y   D  D  H   Y  C  L   D  Y

401  CCCGGTCACC GTGAGCTC
```

KEY

LINE 1 AMINO ACID SEQUENCE NUMBERS (KABAT NOMENCLATURE)
LINE 2 AMINO ACID SEQUENCE OF B72.3 GH341V_H REGION (PARENT)
LINE 3 NUCLEOTIDE SEQUENCE OF JA148 (B72.3 GH341 PARENT SEQ)
LINE 4 * LOCATION OF POINT MUTATIONS
LINE 5 NUCLEOTIDE SEQUENCE OF MUTAGENIC OLIUGONUCLEOTIDES
LINE 6 NUCLEOTIDE SEQUENCE OF PROBE OLIGONUCLEOTIDES
LINE 7 AMINO ACID SEQUENCE OF MUTATED SEQUENCE (GH341 OKT3)

FIGURE 22A

The DNA sequence of the B72.3 grafted heavy (gH341) sequence (J Adair and A Docherty unpublished) and the sequences of oligonucleotides necessary to replace the CDR regions with OKT3 CDRs and to act as specific probes for the desired alterations.

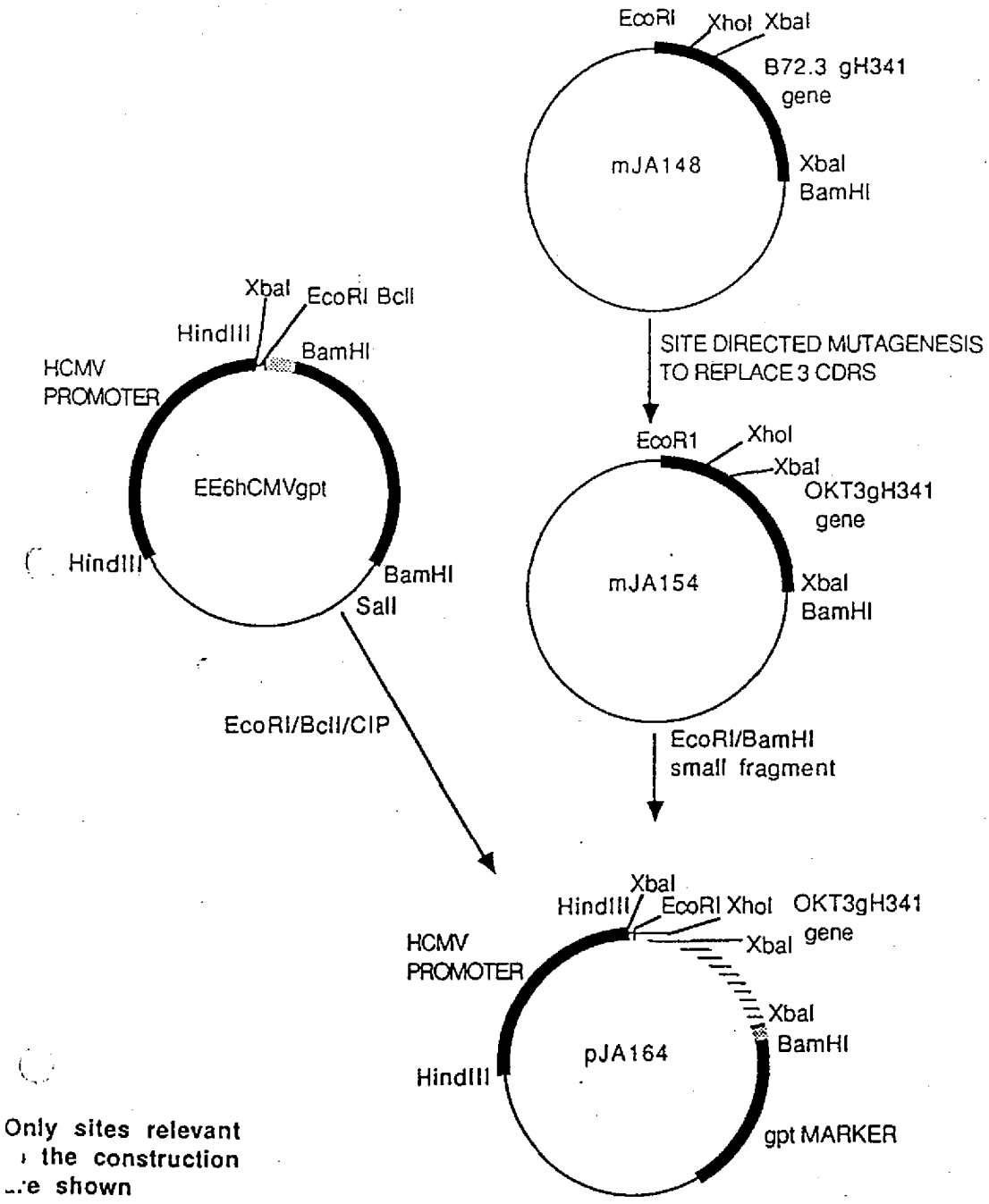


FIGURE 22B
An outline schematic of the procedures involved in the construction of pJA164, a vector for the expression in eukaryotic cells of the OKT3 gH341 CDR grafted heavy chain gene.

```
                    KOZAK SEQ   SIGNAL SEQ
                                M  E  W  S  W  V  F  L  F  F  L  S  V
R1387   1  AATTCGCCGCCACCATGGAATGGAGCTGGGTCTTTCTCTTCTTCCTGTCAGTA   51
R1388   2     GCGGCGGTGGTACCTTACCTCGACCCAGAAAGAGAAGAAGGAC         43

                                MATURE V_H
                    T  T  G  V  H  S  Q  V  Q  L  V
R1089   3        ACTACAGGTGTCCACTCCCAGGTTCAGCTGGTG          33
R1091   4  AGTCATTGATGTCCACAGGTGAGGGTCCAAGTC                33

                    q  S  G  G  G  V  V  Q  P  G
R1594   5        cAGTCTGGAGGAGGAGTCGTCCAGCCTGGA             30
R1595   6  GACCACgTCAGACCTCCTCCTCAGCAGGTC                   30

                    R  S  L  R  L  S  C  k  a
R1540   7        AGGTCCCTGAGACTGTCTTGTaaggct                27
R1095   8  GGACCTTCCAGGGACTCTGACAGAACA                      27

                    S  G  Y  T  F  T  R  Y  T  M  H          CDR1
R1385   9        TCTGGATACACCTTCACTAGATACACAATGCAC           33
R1590  10  ttccgaAGACCTATGTGGAAGTGATCTATGTGTTACGTGACCCAG     45

                 W  V  R  Q  A  P  G  K  G  L  E  W  i
R1591  11  TGGGTCAGACAGGCTCCTGGAAAGGGACTCGAGTGGatt          39
R1258  12        TCTGTCCGAGGACCTTTCCCTGAGCTC                27
                                        -XhoI-

                    g  Y  I  N  P  S  R  G  Y  T  N  Y
R1586  13        ggaTACATTAATCCTAGCAGAGGTTATACTAACTAC
R1587  14  ACCtaacctATGTAATTAGGATCGTCTCCAATATGATTGATG

                 N  Q  K  v  K  D  R                      CDR2
                 AATCAGAAGgtgAAGGACAGA                    57
                 TTAGTCTTCcacTTCCTGTCTAAGTGT              69

                  F  T  I  S  t  D  k  S  K  s  T  a
R1599  15  TTCACAATTTCTactGACaaaTCTAAGagcACAgcc           36
R1600  16        TAAAGAtgaCTGtttAGATTCtcg                  24

                    F  L  Q  M  D  S  L  R  P
R1106  17        TTCCTGCAGATGGACTCACTCAGACCT               27
R1601  18  TGTcggAAGGACGTCTACCTGAGTGAG                     27

                    E  D  T  a  V  Y  y  C  A
R1680  19        GAGGATACCGccGTCTAtTatTGTGCT               27
R1681  20  TCTGGACTCCTATGGCggCAGATaAta                     27

                    R  Y  Y  D  D  H  Y  C  L  D  Y  W          CDR3
R1426  21        AGATATTACGATGACCACTACTGTCTGGACTACTGG        36
R1427  22  ACACGATCTATAATGCTACTGGTGATGACAGACCTGATGACCCCGGTT  48

                 G  Q  G  T  P  V  T  V  S  S  a
R1114  23  GGCCAAGGTACCCCGGTCACCGTGAGCTC                   29
R1115  24        CCATGGGGCCAGTGGCACTCGAGTCGA               27
                                        >CH1 DOMAIN
```

FIGURE 23A

The sequences of oligonucleotides necessary to construct, by oligonucleotide assembly procedures, the OKT3 CDR grafted kgH341A gene. Above the nucleotide sequences are shown the peptide sequences coded by the oligonucleotides. Lower case nucleotide and amino acid residues show differences compared to the gH341 sequence (see Fig 22A).

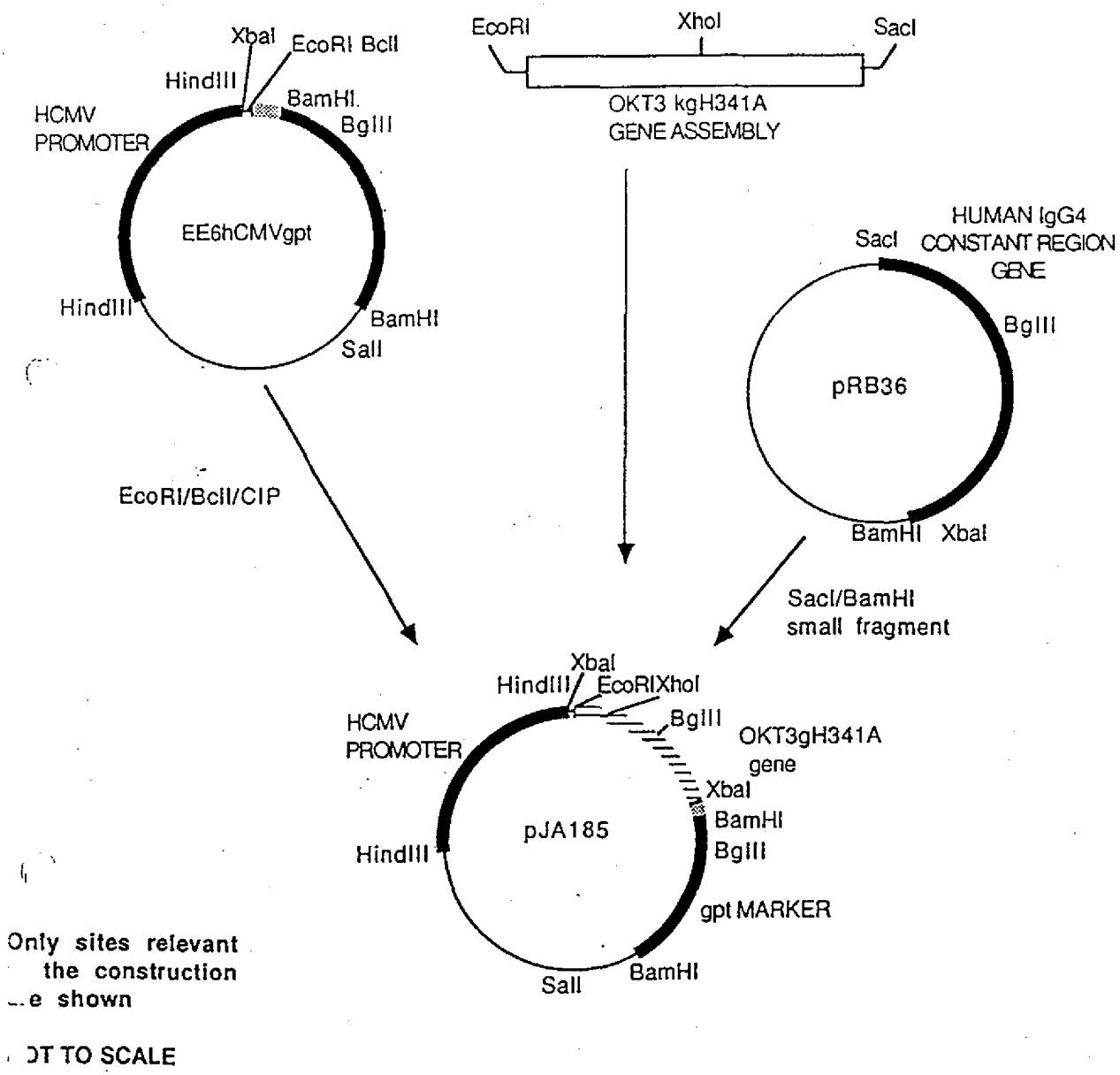


FIGURE 23B
An outline schematic of the procedures involved in theconstruction of pJA185, a vector for the expression in eukaryotic cells of the OKT3 gH341 CDR grafted heavy chain gene.

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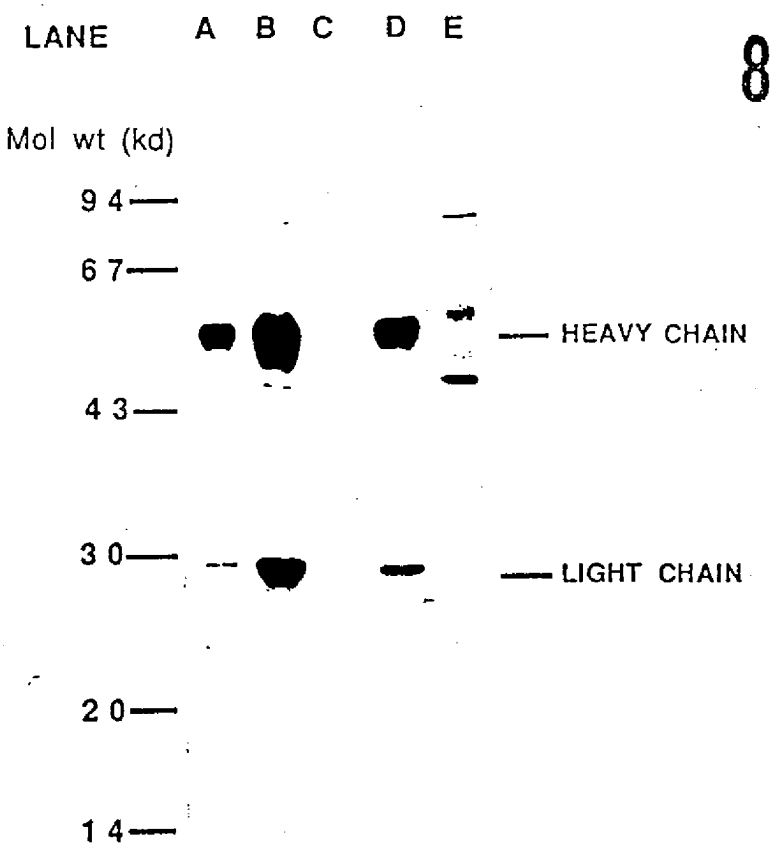

Fig 24a. Effect on antibody expression of the presence of a "Kozak consensus sequence" immediately preceding the gL gene.

Reducing SDS-PAGE of ^{35}S labelled antibody purified from a COS cell transient expression experiment by Protein A-Sepharose precipitation.

KEY:
A. - gL221 cH
B. - gLK221 cH
C. - gL221A cH
D. - gLK221A cH
E. - Mock transfection

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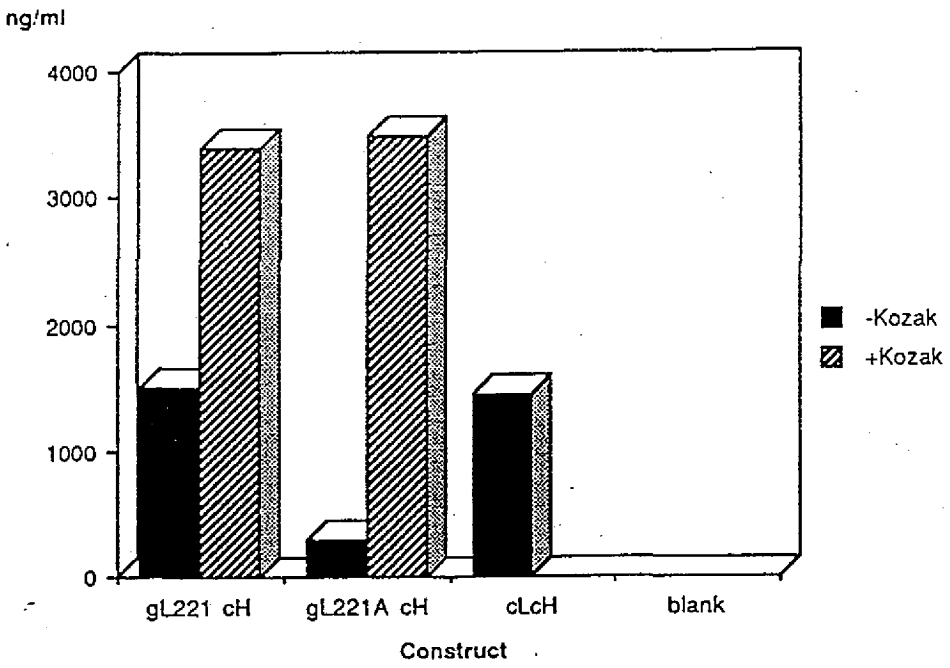

Fig 24b. Effect on antibody expression in the presence of the Kozak consensus sequence immediately preceding the gL gene.

Yield of antibody (ng/ml) from COS cell transient expression experiment.

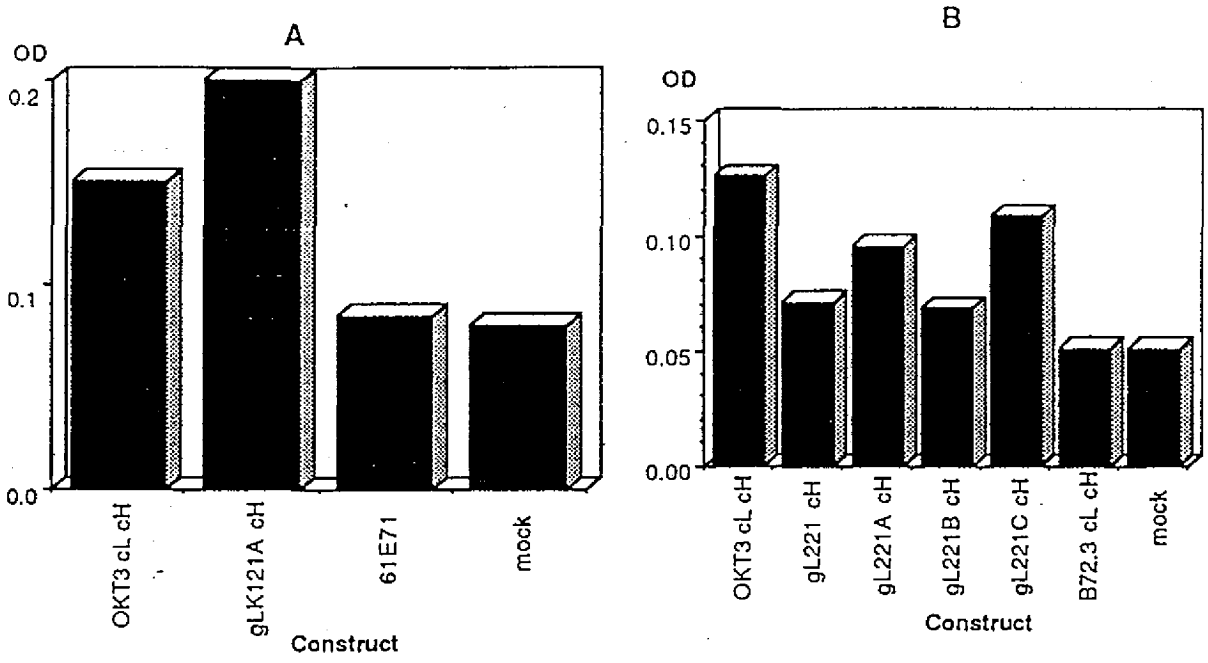

Fig 25. Antigen binding data for gL series genes

Culture supernatants from COS cell transient expression experiments. Various combinations of gL and cH genes were tested for binding to Hut 78 cells.

Chimaeric B72.3 or chimaeric 61E71 was used as a negative control

For codes to genes see table 1.

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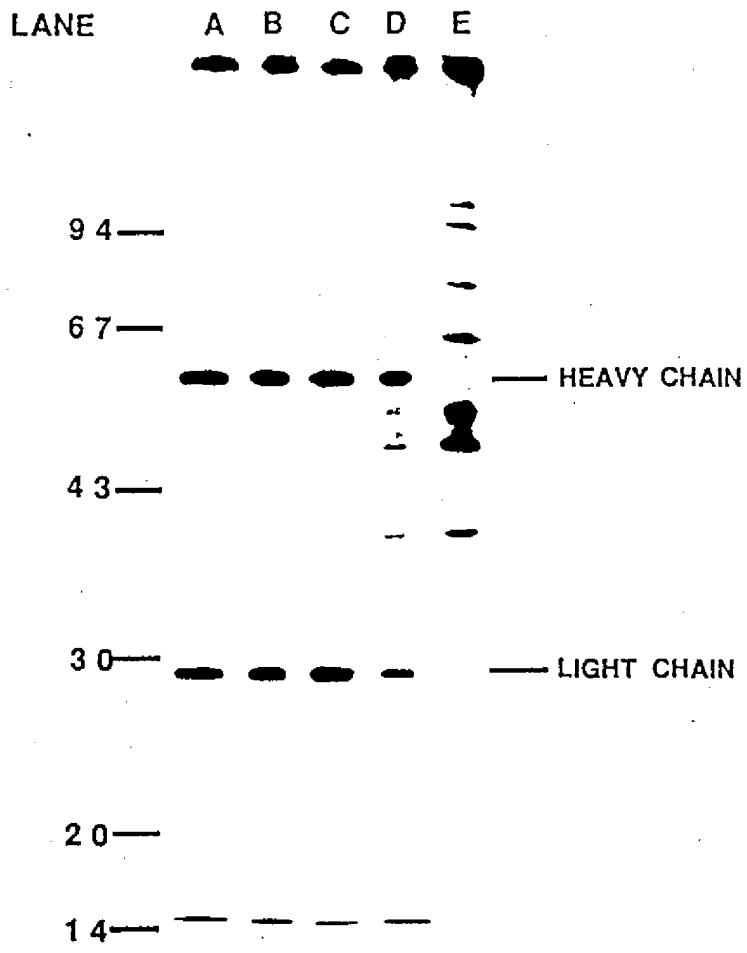

Fig 26. Effect on antibody expression of the presence of a "Kozak consensus sequence" immediatly preceding the gH gene.

Reducing SDS-PAGE of ^{35}S labelled antibody purified from a COS cell transient expression experiment by Protein A-Sepharose precipitation.

KEY:
A. - gH331 cL
B. - gHK331 cL
C. - gH341 cL
D. - gHK341 cL
E. - Mock transfection

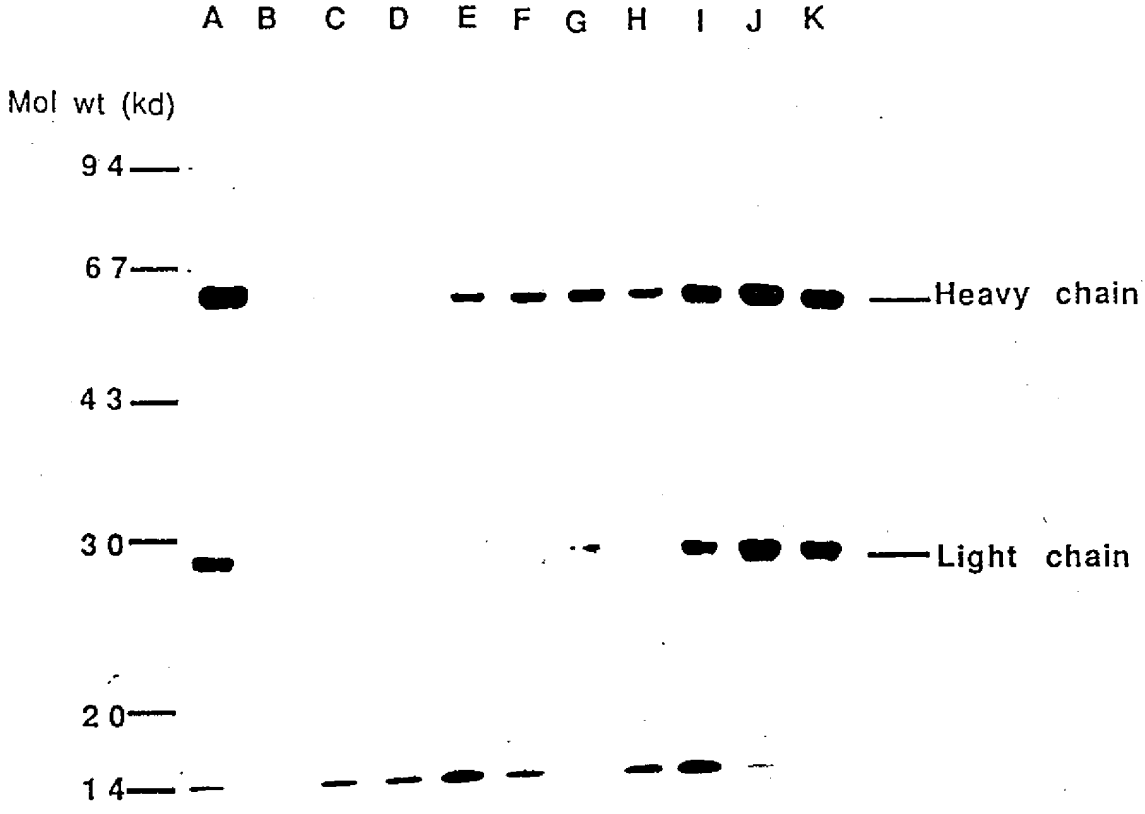

Fig 27. Expression of gH chain genes with cL chain

Reducing SDS-PAGE of ^{35}S labelled antibody produced from COS cell transient expression experiment. Antibody was recovered from culture supernatant by binding to polyclonal anti-human $F(ab')_2$ and then by precipitation with Protein A-Sepharose.

KEY:

A. cLcH OKT3
B. gHK121 cL
C. gHK131 cL
D. gH141 cL
E. gH321 cL
F. gH331 cL
G. gHK331 cL
H. gH341 cL
I. gHK341 cL
J. gHK341B cL
K. gHK341A cL

1. CDR GRAFTED LIGHT (gL) WITH MOUSE (mH) OR CHIMAERIC (cH) HEAVY CHAIN GENES

GENE COMBINATIONS		ANTIGEN BINDING	EXPRESSION
gL121	cH	-	+
KgL121A	cH	+	+
gL221	cH	+/-	+
KgL221	cH	-	++
gL221A	cH	+	+
KgL221A	cH	+	++
gL221B	cH	-	+
KgL221B	cH	-	++
gL221C	cH	+	+
KgL221C	cH	+	++

2. CDR GRAFTED HEAVY (gH) WITH MOUSE (mL) OR CHIMAERIC (cL) LIGHT CHAIN GENES

GENE COMBINATIONS		ANTIGEN BINDING	EXPRESSION
KgH121	mL	not det.	-
KgH121	cL	not det.	-
KgH131	mL	not det.	-
KgH131	cL	not det.	-
gH141	mL	-	+/-
gH141	cL	-	+/-
gH321	cL	-	+
gH331	cL	-	+
KgH331	cL	-	+
gH341	mL	+	+
gH341	cL	+/-	+
KgH341	cL	+/-	+
KgH341A	cL	+	+
KgH341B	cL	+	+

3. FULLY CDR GRAFTED ANTIBODY

GENE COMBINATIONS		ANTIGEN BINDING	EXPRESSION
KgL221A	KgH121	not det.	-
KgL221A	KgH131	not det.	-
KgL221A	gH141	not det.	-
KgL221A	KgH331	not det.	-
KgL221A	gH341	not det.	-
KgL221A	KgH341	not det.	-
KgL221A	KgH341A	+	+
KgL221A	KgH341B	+	+

KEY		
	L	LIGHT CHAIN GENE (SEE TABLE 1 FOR NUMBER CODE)
	H	HEAVY CHAIN GENE
	m	MOUSE
	c	CHIMAERIC
	g	CDR GRAFTED
	K	PRESENCE OF KOZAK CONSENSUS SEQUENCE
	not det.	NOT DETERMINED (EXPRESSION LEVELS TOO LOW)

TABLE 2
A summary of the expression and antigen binding data for the CDR grafted genes constructed in this study

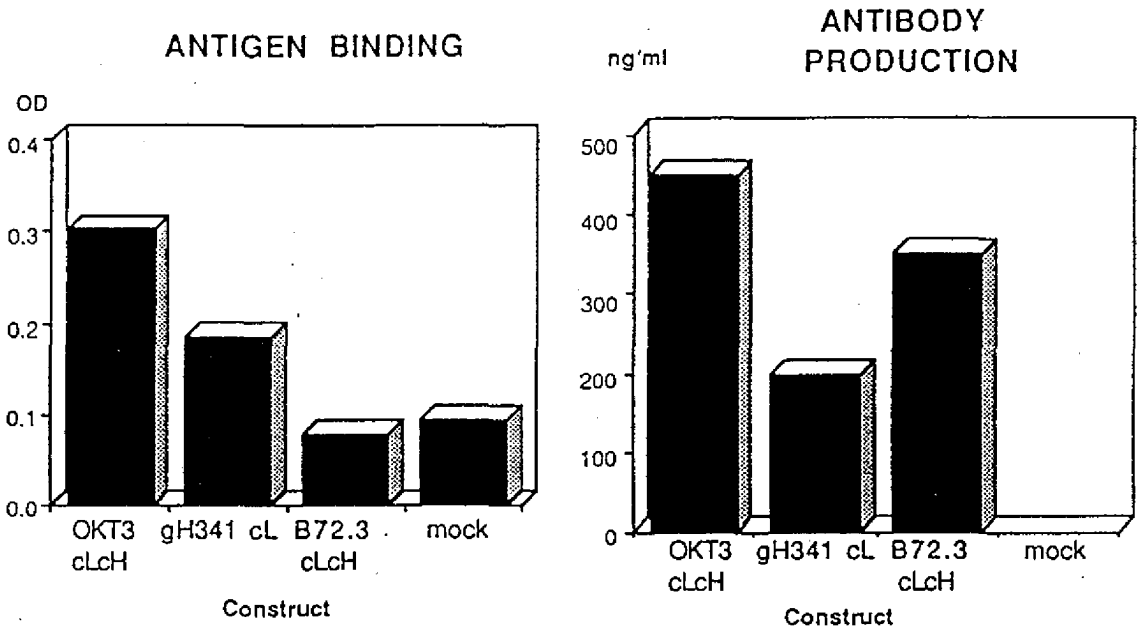

Fig 28 Antigen binding assay for gH341 cL gene combination.

Culture supernatants from COS cell transient expression experiments were tested for binding to Hut-78 cells (panel A) and for yield of assembled antibody (panel B). Chimaeric B72.3 was included as a negative control.

Note. Poor expression of gH341 cL gene combination.

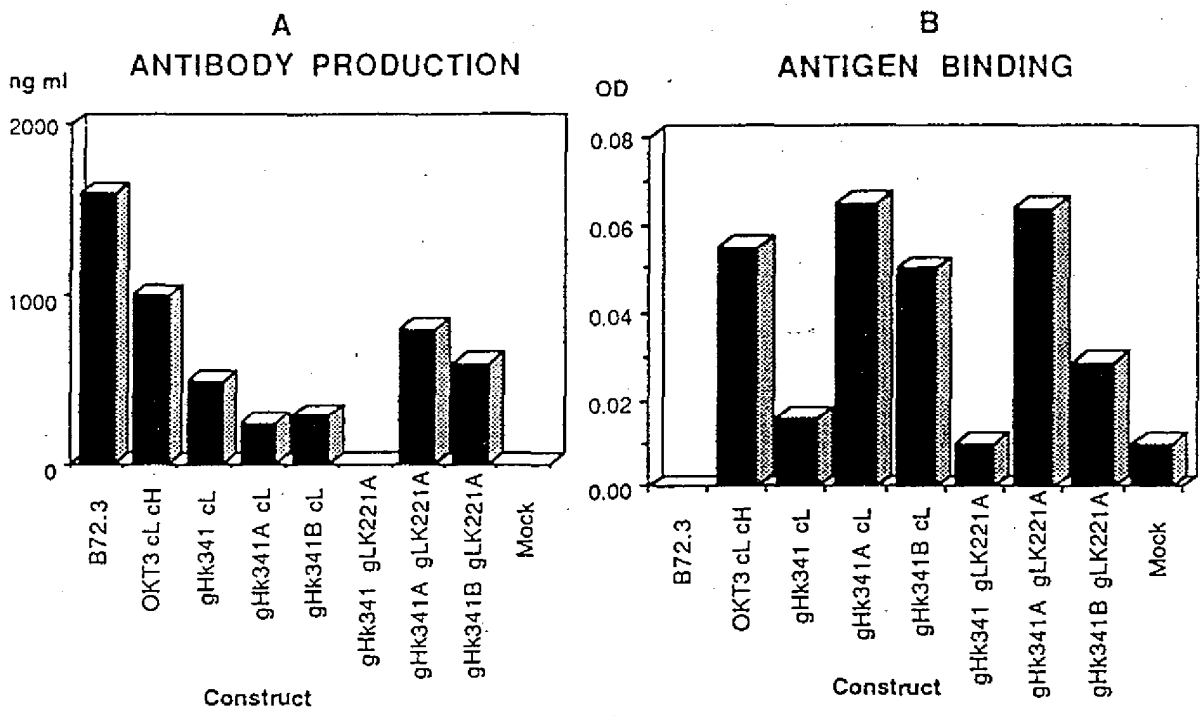

Fig 29 Antigen Binding assay for grafted OKT3 combinations

Culture supernatant from COS cell transient expression experiments were tested for yield of assembled antibody (Panel A) and for binding to Hut 78 cells (Panel B). Chimaeric B72.3 was included as a negative control. Panels show level of antibody produced and resultant antigen binding for various combinations of heavy and light chain genes cotransfected into COS cells.
NB: In panel B binding data has been normalised so that the level of binding B72.3 is set to zero.

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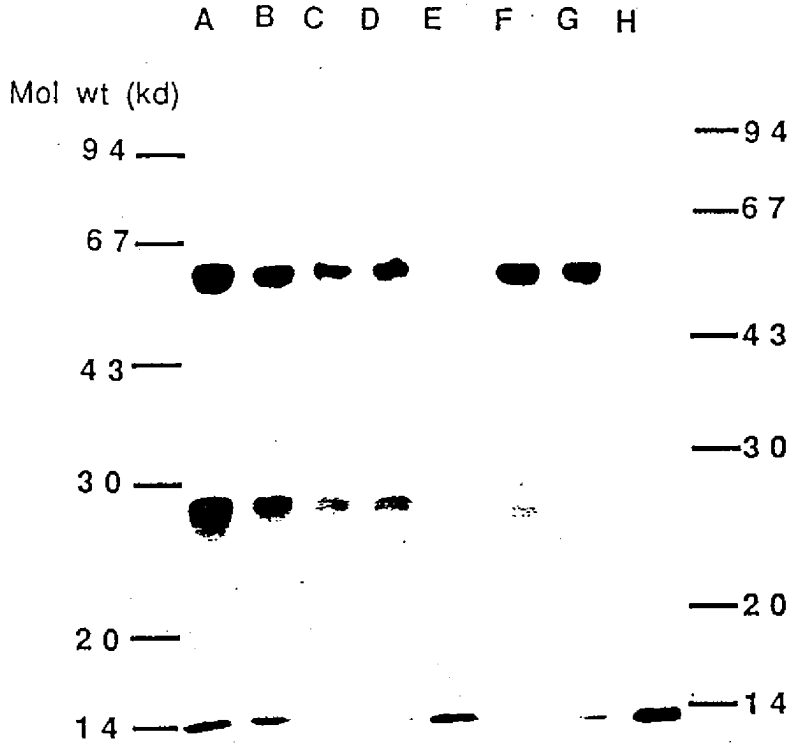

Fig 29c. gH341 series cotransfected with cL and gLK221A

Key:
A. cL cH OKT3
B. gHK341 cL
C. gHK341A cL
D. gHK341B cL
E. gHK341 gLK221A
F. gHK341A gLK221A
G. gHK341B gLK221A
H. Mock transfection

HUMANISED ANTIBODIES

Field of the Invention

The present invention relates to humanised antibody molecules ~~(HAMs)~~, to processes for their production using recombinant DNA technology, and to their therapeutic uses.

~~In the present application, the term "recombinant antibody molecule" (RAM) is used to describe an antibody produced by an process involving the use of recombinant DNA technology, including any analogues of natural immunoglobulins or their fragments.~~ The term "humanised antibody molecule" ~~(HAM)~~ is used to describe a molecule having an antigen binding site derived from an immunoglobulin from a non-human species, and remaining immunoglobulin-derived parts of the molecule being derived from a human immunoglobulin. The antigen binding site ~~may comprise either complete variable domains fused onto constant domains or~~typically comprises complementarity determining regions ~~grafted onto~~(CDRs) which determine the binding specificity of the antibody molecule and which are carried on appropriate framework regions in the variable domains. ~~The abbreviation "MAb" is used to indicate a monoclonal antibody~~ There are 3 CDRs (CDR1, CDR2 and CDR3) in each of the heavy and light chain variable domains.

In the description, reference is made to a number of publications by number. The publications are listed in numerical order at the end of the description.

Background of the Invention

Natural immunoglobulins have been known for many years, as have the various fragments thereof, such as the Fab, $(Fab')_2$ and Fc fragments, which can be derived by enzymatic cleavage. Natural immunoglobulins comprise a generally Y-shaped molecule having an antigen-binding site towards the end of each upper arm. The remainder of the structure, and particularly the stem of the Y, mediates the effector functions associated with immunoglobulins.

Natural immunoglobulins have been used in assay, diagnosis and, to a more limited extent, therapy. However, such uses, especially in therapy, ~~have been~~were hindered until recently by the polyclonal nature of natural immunoglobulins. A significant step towards the realisation of the potential of immunoglobulins as therapeutic agents was the discovery of ~~techniques~~procedures for the ~~preparation~~production of monoclonal antibodies (MAbs) of defined specificity (1).

However, most MAbs are produced by hybridomas which are fusions of rodent spleen cells with rodent myeloma cells. They are therefore essentially rodent proteins. There are very few reports of the production of human MAbs.

Since most available MAbs are of rodent origin, they are naturally antigenic in humans and thus can give rise to an undesirable immune response termed the HAMA (Human Anti-Mouse Antibody) response. Therefore, the use of rodent MAbs as therapeutic agents in humans is inherently limited by the fact that the human subject will mount an immunological response to the MAb and will either remove it entirely or at least reduce its effectiveness. ~~Thus, in~~In practice,

MAbs of rodent origin may not be used in patients for more than one or a few treatments as a HAMA response soon develops rendering the MAb ineffective as well as giving rise to undesirable reactions. For instance, OKT3 a mouse IgG2a/k MAb which recognises an antigen in the T-cell receptor-CD3 complex has been approved for use in many countries throughout the world as an immunosuppressant in the treatment of acute allograft rejection [Chatenoud et al (2) and Jeffers et al (3)]. However, in view of the rodent nature of this and other such MAbs, a significant HAMA response which may include a major anti-idiotype component, may build up on use. Clearly, it would be highly desirable to diminish or abolish this undesirable KAMA response and thus enlarge the areas of use of these very useful antibodies.

Proposals have therefore been made to render non-human MAbs less antigenic in humans. Such techniques can be generically termed "humanisation" techniques. These techniques ~~generally~~typically involve the use of recombinant DNA technology to manipulate DNA sequences encoding the polypeptide chains of the antibody molecule.

~~Some early methods for carrying out such a procedure~~Early methods for humanising MAbs involved production of chimeric antibodies in which an antigen binding site comprising the complete variable domains of one antibody is linked to constant domains derived from another antibody. Methods for carrying out such chimerisation procedures are described in EP~~-A-0 171 496~~0120694 (Celltech Limited), EP0125023 (Genentech Inc. and City of Hope), EP-A-0 171496 (Res. Dev. Corp. Japan), EP-A-0 173 494 (Stanford University), ~~EP-A-0 194 276~~and WO 86/01533 (Celltech Limited)~~ and WO-A-8 702 671 (Int. Gen. Eng. Inc.). The~~. This latter Celltech application (WO 86/01533) discloses a process for preparing an antibody molecule having the variable domains from a mouse MAb and the constant domains from a human immunoglobulin. ~~It also shows the production of an antibody molecule comprising the variable domains of a mouse MAb, the CH1 and CL domains of a human immunoglobulin, and a non-immunoglobulin-derived protein in place of the Fc portion of the human immunoglobulin~~Such humanised chimeric antibodies, however, still contain a significant proportion of non-human amino acid sequence, i.e. the complete non-human variable domains, and thus may still elicit some HAMA response, particularly if administered over a prolonged period [Begent et al (ref. 4)].

In an alternative approach, described in EP-A-0239400 (Winter), the complementarity determining regions (CDRs) of a mouse MAb have been grafted onto the framework regions of the variable domains of a human immunoglobulin by site directed mutagenesis using long oligonucleotides~~. There are 3 CDRs (CDR1, CDR2 and CDR3) in each of the heavy and light chain variable regions~~. The present invention relates to ~~HAMs~~humanised antibody molecules prepared according to this alternative approach, i.e. CDR-grafted ~~HAMs~~humanised antibody molecules. Such
CDR-grafted humanised antibodies are much less likely to give rise to a HAMA response than humanised chimeric antibodies in view of the much lower proportion of non-human amino acid sequence which they contain.

The earliest work on humanising MAbs by CDR-grafting was carried out on MAbs recognising synthetic antigens, such as the NP or NIP antigens. However, examples in which a mouse MAb recognising lysozyme and a rat MAb recognising an antigen on human T-cells ~~respectively~~ were humanised by CDR-grafting ~~are shown~~have been described by Verhoeyen et al (~~2~~5) and

Riechmann et al (~~3~~6) respectively. The preparation of CDR-grafted antibody to the antigen on human T cells is also described in WO 89/07452 (Medical Research Council).

In ~~the latter case (~~Riechmann et al~~)~~/Medical Research Council it was found that transfer of the CDR regions alone ~~(~~[as defined by Kabat refs. ~~4~~(7) and ~~5~~(8)] was not sufficient to provide satisfactory antigen binding activity in the CDR-grafted product. Riechmann et al found that it was necessary to convert a serine residue at position 27 of the human sequence to the corresponding rat phenylalanine residue to obtain a CDR-grafted product having ~~satisfactory~~improved antigen binding activity. This residue at position 27 of the heavy chain is within the structural loop adjacent to
CDR1. A further construct which additionally contained a human serine to rat tyrosine change at position 30 of the heavy chain did not have a significantly altered binding activity over the humanised antibody with the serine to phenylalanine change at position 27 alone. These results indicate that changes to residues of the human sequence outside the CDR regions, in particular in the structural loop adjacent to CDR1, may be necessary to obtain effective antigen binding activity for CDR-grafted antibodies which recognise more complex antigens. Even so the binding affinity of the best CDR-grafted antibodies obtained was still significantly less than the original MAb.

Very recently Queen et al (9) have described the preparation of a humanised antibody that binds to the interleukin 2 receptor, by combining the CDRs of a murine MAb (anti-Tac) with human immunoglobulin framework and constant regions. The human framework regions were chosen to maximise homology with the anti-Tac MAb sequence. In addition computer modelling was used to identify framework amino acid residues which were likely to interact with the CDRs or antigen, and mouse amino acids were used at these positions in the humanized antibody.

In WO 90/07861 Queen et al propose four criteria for designing humanised immunoglobulins. The first criterion is to use as the human acceptor the framework from a particular human immunoglobulin that is unusually homologous to the non-human donor immunoglobulin to be humanised, or to use a consensus framework from many human antibodies. The second criterion is to use the donor amino acid rather than the acceptor if the human acceptor residue is unusual and the donor residue is typical for human sequences at a specific residue of the framework. The third criterion is to use the donor framework amino acid residue rather than the acceptor at positions immediately adjacent to the CDRs. The fourth criterion is to use the donor amino acid residue at framework positions at which the amino acid is predicted to have a side chain atom within about 3 Å of the CDRs in a three-dimensional immunoglobulin model and to be capable of interacting with the antigen or with the CDRs of the humanised immunoglobulin. It is proposed that criteria two, three or four may be applied in addition or alternatively to criterion one, and may be applied singly or in any combination.

~~In recent years a number of rodent MAbs have been developed for therapeutic applications. For instance, OKT3 a mouse IgG2a/k MAb which recognizes an antigen in the T cell receptor CD3 complex has been approved for use in the USA as an immunosuppressant in the treatment of acute allograft rejection (Chatenond et al (1986) J. Immunol., 137, 830-838, and Jeffers et al (1986) Transplantation, 41, 572-578). However, in view of the rodent nature of this and other such MAbs, a significant HAMA response which may include a major anti-idiotype component, builds up on use. Clearly, it would be highly desirable to diminish or abolish this undesirable HAMA~~

~~response by suitable humanisation or other recombinant DNA manipulation of these very useful antibody and thus enlarge their areas of use.~~
WO 90/07861 describes in detail the preparation of a single CDR-grafted humanised antibody, a humanized antibody having specificity for the p55 Tac protein of the IL-2 receptor. The combination of all four criteria, as above, were employed in designing this humanised antibody, the variable region frameworks of the human antibody Eu (7) being used as acceptor. In the resultant humanized antibody the donor CDRs were as defined by Kabat et al (7 and 8) and in addition the mouse donor residues were used in place of the human acceptor residues, at positions 27, 30, 48, 66, 67, 89, 91, 94, 103, 104, 105 and 107 in the heavy chain and at positions 48, 60 and 63 in the light chain, of the variable region frameworks. The humanized anti-Tac antibody obtained is reported to have an affinity for p55 of 3×10^9 M^{-1}, about one-third of that of the murine MAb.

We have further ~~investgated~~investigated the preparation of CDR-grafted ~~HAMs~~humanised antibody molecules and have identified ~~residues~~a hierarchy of positions within the framework of the variable ~~region~~regions (i.e. outside both the Kabat CDRs and structural loops of the variable regions) at which the amino acid identities of ~~which~~the residues are important for obtaining CDR-grafted products with satisfactory binding affinity. This has enabled us to establish a protocol for obtaining satisfactory CDR-grafted products which may be applied very widely irrespective of the level of homology between the donor immunoglobulin and acceptor framework. The set of residues which we have identified as being of critical importance does not coincide with the residues identified by Queen et al (9).

Summary of the Invention

Accordingly, in a first aspect the invention provides a CDR-grafted antibody heavy chain having a variable region domain comprising ~~human~~acceptor framework and ~~non-human (rodent)~~donor antigen binding regions wherein the ~~human~~ framework comprises ~~non-human (rodent)~~donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

In preferred embodiments, the heavy chain framework comprises donor residues at positions 23, 24, 49, 71, 73 and 78 or at positions 23, 24 and 49. The residues at positions 71, 73 and 78 of the heavy chain framework are preferably either all acceptor or all donor residues.

In particularly preferred embodiments the heavy chain framework additionally comprises donor residues at one, some or all of positions 6, 37, 48 and 94. Also it is particularly preferred that residues at positions of the heavy chain framework which are commonly conserved across species, i.e. positions 2, 4, 25, 36, 39, 47, 93, 103, 104, 106 and 107, if not conserved between donor and acceptor, additionally comprise donor residues. Most preferably the heavy chain framework additionally comprises donor residues at positions 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

In addition the heavy chain framework optionally comprises donor residues at one, some or all of positions:
1 and 3,

72 and 76,
69 (if 48 is different between donor and acceptor),
38 and 46 (if 48 is the donor residue),
80 and 20 (if 69 is the donor residue),
67,
82 and 18 (if 67 is the donor residue),
91,
88, and
any one or more of 9, 11, 41, 87, 108, 110 and 112.

In the first and other aspects of the present invention reference is made to CDR-grafted antibody products comprising acceptor framework and donor antigen binding regions. It will be appreciated that the invention is widely applicable to the CDR-grafting of antibodies in general. Thus, the donor and acceptor antibodies may be derived from animals of the same species and even same antibody class or sub-class. More usually, however, the donor and acceptor antibodies are derived from animals of different species. Typically the donor antibody is a non-human antibody, such as a rodent MAb, and the acceptor antibody is a human antibody.

In the first and other aspects of the present invention, the donor antigen binding region typically comprises at least one CDR from the donor antibody. Usually the donor antigen binding region comprises at least two and preferably all three CDRs of each of the heavy chain and/or light chain variable regions. The CDRs may comprise the Kabat CDRs, the structural loop CDRs or a composite of the Kabat and structural loop CDRs and any combination of any of these.

Preferably, the antigen binding regions of the CDR-grafted heavy chain variable domain comprise CDRs corresponding to the Kabat CDRs at CDR2 (residues 50-65) and CDR3 (residues 95-100) and a composite of the Kabat and structural loop CDRs at CDR1 (residues 26-35).

~~Preferably, the antigen binding regions of the CDR-grafted heavy chain variable domain comprise CDRs corresponding to the Kabat CDR at CDR2 (residues 50-65), the structural loop residues at CDR3 (residues 95-100) and a composite of the Kabat and structural loop CDRs at CDR1 (residues 26-35).~~
The residue designations given above and elsewhere in the present application are numbered according to the Kabat numbering [refs. (7) and (8)]. Thus the residue designations do not always correspond directly with the linear numbering of the amino acid residues. The actual linear amino acid sequence may contain fewer or additional amino acids than in the strict Kabat numbering corresponding to a shortening of, or insertion into, a structural component, whether framework or CDR, of the basic variable domain structure. For example, the heavy chain variable region of the anti-Tac antibody described by Queen et al (9) contains a single amino acid insert (residue 52a) after residue 52 of CDR2 and a three amino acid insert (residues 82a, 82b and 82c) after framework residue 82, in the Kabat numbering. The correct Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

The invention also provides in a second aspect a CDR-grafted antibody light chain having a variable region domain comprising ~~human~~acceptor framework and ~~non-human (rodent)~~donor

antigen binding regions wherein the ~~human~~ framework comprises ~~non-human (rodent)~~donor residues at at least one of positions 1 and/or 3 and 46 and/or 47. Preferably the CDR grafted light chain of the second aspect comprises donor residues at positions 46 and/or 47.

The invention also provides in a third aspect a CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, 58 and 71.

In a preferred embodiment of the third aspect, the framework comprises donor residues at all of positions 46, 48, 58 and 71.

In particularly preferred embodiments of the second and third aspects, the framework additionally comprises donor residues at positions 36, 44, 47, 85 and 87. Similarly positions of the light chain framework which are commonly conserved across species, i.e. positions 2, 4, 6, 35, 49, 62, 64-69, 98, 99, 101 and 102, if not conserved between donor and acceptor, additionally comprise donor residues. Most preferably the light chain framework additionally comprises donor residues at positions 2, 4, 6, 35, 36, 38, 44, 47, 49, 62, 64-69, 85, 87, 98, 99, 101 and 102.

In addition the framework of the second or third aspects optionally comprises donor residues at one, some or all of positions:
1 and 3,
63,
60 (if 60 and 54 are able to form at potential saltbridge),
70 (if 70 and 24 are able to form a potential saltbridge),
73 and 21 (if 47 is different between donor and acceptor),
37 and 45 (if 47 is different between donor and acceptor),
and
any one or more of 10, 12, 40, 80, 103 and 105.

Preferably, the antigen binding regions of the CDR-grafted light chain variable domain comprise CDRs corresponding to the Kabat CDRs at CDR1 (residue 24-34) ~~and~~, CDR2 (residues 50-56) and ~~the structural loop residues at~~ CDR3 (residues ~~91~~89-~~96~~97).

The invention further provides in a fourth aspect a CDR-grafted ~~HAM~~antibody molecule comprising at least one CDR-grafted heavy chain and at least one CDR-grafted light chain according to the first and second or first and third aspects of the invention.

The ~~residue designations given above and elsewhere in the present specification are numbered according to the Kabat numbering (refs. 4 and 5).~~

~~Preferably the CDR-grafted heavy chain comprises non-human (rodent) residues at positions 23 and/or 24, 48 and/or 49 and 71 and/or 73. Preferably, the CDR-grafted light chain comprises non-human (rodent) residues at positions 46 and/or 47.~~

~~Preferably the CDR-grafted~~humanised antibody ~~heavy~~molecules and ~~light~~ chains ~~and HAM are produced by recombinant DNA technology.The HAM~~ of the present invention may comprise: a

complete antibody molecule, having full length heavy and light chains; a fragment thereof, such as ~~the~~a Fab ~~or~~, $(Fab')_2$ or FV fragment; a light chain or heavy chain monomer or dimer; or ~~any other~~a single chain antibody, e.g. a single chain FV in which heavy and light chain variable regions are joined by a peptide linker; or any other CDR-grafted molecule with the same specificity as the original ~~non-human (rodent)~~donor antibody. Similarly the CDR-grafted heavy and light chain variable region may be combined with other antibody domains as appropriate.

~~Alternatively,~~Also the heavy or light chains or ~~HAM~~humanised antibody molecules of the present invention may have attached to them an effector or reporter molecule. For instance, ~~they~~it may have a macrocycle, for chelating a heavy metal atom, or a toxin, such as ricin, attached to it by a covalent bridging structure. Alternatively, the procedures of recombinant DNA technology may be used to produce an immunoglobulin molecule in which the Fc fragment or CH3 domain of a complete immunoglobulin molecule has been replaced by, or has attached thereto by peptide linkage, a functional non-immunoglobulin protein, such as an enzyme or toxin molecule.

~~For CDR-grafted products of the invention,~~Any appropriate acceptor variable region framework sequences may be used having regard to class/type of the donor antibody from which the antigen binding regions are derived. Preferably, the type of ~~human~~acceptor framework used is of the same/similar class/type as the donor antibody. ~~Advantageously~~Conveniently, the framework ~~is~~may be chosen to maximise/optimise homology with the donor antibody sequence particularly at positions close or adjacent to the CDRs. ~~It will be appreciated that in some cases that the non-human and human amino acid residues, identified above in connection with the first and second aspects of the invention, may be the same and thus no change of the human framework to the corresponding non-human framework residue is required~~However, a high level of homology between donor and acceptor sequences is not important for application of the present invention. The present invention identifies a hierarchy of framework residue positions at which donor residues may be important or desirable for obtaining a CDR-grafted antibody product having satisfactory binding properties. The CDR-grafted products usually have binding affinities of at least 10^5 M^{-1}, preferably at least about 10^8 M^{-1}, or especially in the range 10^8-10^{12} M^{-1}. In principle, the present invention is applicable to any combination of donor and acceptor antibodies irrespective of the level of homology between their sequences. A protocol for applying the invention to any particular donor-acceptor antibody pair is given hereinafter. Examples of human frameworks which may be used are KOL, NEWM, RE1, EU, LAY and POM (refs. 4 and 5) and the like; for instance KOL and NEWM for the heavy chain and RE1 for the light chain and EU, LAY and POM for both the heavy chain and the light chain.

Also ~~human~~the constant region domains of the products of the invention may be selected having regard to the proposed function of the antibody in particular the effector functions which may be required. For example, the constant region domains may be human IgA, IgE, IgG or IgM ~~domain~~domains. In particular, IgG human constant region domains may be used, especially of the IgG1 and IgG3 isotypes, when the ~~HAM~~humanised antibody molecule is intended for therapeutic uses, and antibody effector functions are required. Alternatively, IgG2 and IgG4 isotypes may be used when the humanised antibody molecule is intended for therapeutic purposes and antibody effector functions are not required, e.g. for simple blocking of lymphokine activity.

However, the remainder of the ~~HAM~~antibody molecules need not comprise only protein sequences from ~~the human immunoglobulin~~immunoglobulins. For instance, a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain is fused to a DNA sequence encoding the amino acid sequence of a functional polypeptide such as an effector or reporter molecule.

Preferably the CDR-grafted antibody heavy and light chain and antibody molecule products are produced by recombinant DNA technology.

Thus in further aspects the invention also includes DNA sequences coding for the CDR-grafted heavy and light chains, cloning and expression vectors containing the DNA sequences, host cells transformed with the DNA sequences and processes for producing the CDR-grafted chains and antibody molecules comprising expressing the DNA sequences in the transformed host cells.

The general methods by which the vectors may be constructed, transfection methods and culture methods are well known per se and form no part of the invention. Such methods are shown, for instance, in references 10 and 11.

The DNA sequences which encode the donor amino acid sequence may be obtained by methods well known in the art. For example the donor coding sequences may be obtained by genomic cloning, or cDNA cloning from suitable hybridoma cell lines. Positive clones may be screened using appropriate probes for the heavy and light chain genes in question. Also PCR cloning may be used.

DNA coding for acceptor, e.g. human acceptor, sequences may be obtained in any appropriate way. For example DNA sequences coding for preferred human acceptor frameworks such as KOL, RE1, EU and NEWM, are widely available to workers in the art.

The standard techniques of molecular biology may be used to prepare DNA sequences coding for the CDR-grafted products. Desired DNA sequences may be synthesized completely or in part using oligonucleotide synthesis techniques. Site-directed mutagenesis and polymerase chain reaction (PCR) techniques may be used as appropriate. For example oligonucleotide directed synthesis as described by Jones et al (ref. 20) may be used. Also oligonucleotide directed mutagenesis of a pre-exising variable region as, for example, described by Verhoeyen et al (ref. 5) or Riechmann et al (ref. 6) may be used. Also enzymatic filling in of gapped oligonucleotides using T_4 DNA polymerase as, for example, described by Queen et al (ref. 9) may be used.

Any suitable host cell/vector system may be used for expression of the DNA sequences coding for the CDR-grafted heavy and light chains. Bacterial e.g. E. coli, and other microbial systems may be used, in particular for expression of antibody fragments such as FAb and (Fab')2 fragments, and especially FV fragments and single chain antibody fragments e.g. single chain FVs. Eucaryotic e.g. mammalian host cell expression systems may be used for production of larger CDR-grafted antibody products, including complete antibody molecules. Suitable mammalian host cells include CHO cells and myeloma or hybridoma cell lines.

Thus, ~~according to~~in a further aspect the present invention provides a process for producing ~~an anti-CD3 HAM which process comprises~~a CDR-grafted antibody product comprising:

(a) producing in an expression vector an operon having a DNA sequence which encodes an antibody heavy ~~or light~~ chain according to the first ~~or second~~ aspect of the invention; and/or

(b) producing in an expression vector an operon having a DNA sequence which encodes a complementary antibody light ~~or heavy~~ chain according to the second or ~~first~~third aspect of the invention;

(c) transfecting a host cell with the or each vector; and

(d) culturing the transfected cell line to produce the ~~HAM~~CDR-grafted antibody product.

The CDR-grafted product may comprise only heavy or light chain derived polypeptide, in which case only a heavy chain or light chain polypeptide coding sequence is used to transfect the host cells.

For production of products comprising both heavy and light chains, the cell line may be transfected with two vectors, the first vector ~~containing~~may contain an operon encoding a light chain-derived polypeptide and the second vector containing an operon encoding a heavy chain-derived polypeptide. Preferably, the vectors are identical, except in so far as the coding sequences and selectable markers are concerned, so as to ensure as far as possible that each polypeptide chain is equally expressed. Alternatively, a single vector may be used, the vector including the sequences encoding both light chain- and heavy chain-derived polypeptides.

The DNA in the coding sequences for the light and heavy chains may comprise cDNA or genomic DNA or both. However, it is preferred that the DNA sequence encoding the heavy or light chain comprises at least partially, genomic DNA, preferably a fusion of cDNA and genomic DNA.

The present invention ~~also includes cloning and expression vectors and transfected cell lines used in the process of the invention, therapeutic and diagnostic compositions comprising the variable domains or the HAM of the invention and uses of such compositions in therapy and diagnosis.~~is applicable to antibodies of any appropriate specificity. Advantageously, however, the invention may be applied to the humanisation of non-human antibodies which are used for in vivo therapy or diagnosis. Thus the antibodies may be site-specific antibodies such as tumour-specific or cell surface-specific antibodies, suitable for use in in vivo therapy or diagnosis, e.g. tumour imaging. Examples of cell surface-specific antibodies are anti-T cell antibodies, such as anti-CD3, and CD4 and adhesion molecules, such as CR3, ICAM and ELAM. The antibodies may have specificity for interleukins (including lymphokines, growth factors and stimulating factors), hormones and other biologically active compounds, and receptors for any of these. For example, the antibodies may have specificity for any of the following: Interferons α, β, γ, or δ, IL1, IL2, IL3, or IL4, etc., TNF, GCSF, GMCSF, EPO, hGH, or insulin, etc.

The the present invention also includes therapeutic and diagnostic compositions comprising the CDR-grafted products of the invention and uses of such compositions in therapy and diagnosis.

Accordingly in a further aspect the invention provides a therapeutic or diagnostic composition comprising a CDR-grafted antibody heavy or light chain or molecule according to previous aspects of the invention in combination with a pharmaceutically acceptable carrier, diluent or excipient.

Accordingly also the invention provides a method of therapy or diagnosis comprising administering an effective amount of a CDR-grafted antibody heavy or light chain or molecule according to previous aspects of the invention to a human or animal subject.

A preferred protocol for obtaining CDR-grafted antibody heavy and light chains in accordance with the present invention is set out below together with the rationale by which we have derived this protocol. This protocol and rationale are given without prejudice to the generality of the invention as hereinbefore described and defined.

Protocol

It is first of all necessary to sequence the DNA coding for the heavy and light chain variable regions of the donor antibody, to determine their amino acid sequences. It is also necessary to choose appropriate acceptor heavy and light chain variable regions, of known amino acid sequence. The CDR-grafted chain is then designed starting from the basis of the acceptor sequence. It will be appreciated that in some cases the donor and acceptor amino acid residues may be identical at a particular position and thus no change of acceptor framework residue is required.

1. As a first step donor residues acceptor residues in the CDRs. For this purpose the CDRs are preferably defined as follows:

Heavy chain	- CDR1:	residues 26-35
	- CDR2:	residues 50-65
	- CDR3:	residues 95-102
Light chain	- CDR1:	residues 24-34
	- CDR2:	residues 50-56
	- CDR3:	residues 89-97

The positions at which donor residues are to be substituted for acceptor in the framework are then chosen as follows, first of all with respect to the heavy chain and subsequently with respect to the light chain.

2. Heavy Chain

2.1 Choose donor residues at all of positions 23, 24, 49, 71, 73 and 78 of the heavy chain or all of positions 23, 24 and 49 (71, 73 and 78 are always either all donor or all acceptor).

2.2 Check that the following have the same amino acid in donor and acceptor sequences, and if not preferably choose the donor: 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

2.3 To further optimise affinity consider choosing donor residues at one, some or any of:

i. 1, 3
ii. 72, 76
iii. If 48 is different between donor and acceptor sequences, consider 69
iv. If at 48 the donor residue is chosen, consider 38 and 46
v. If at 69 the donor residue is chosen, consider 80 and then 20
vi. 67
vii. If at 67 the donor residue is chosen, consider 82 and then 18
viii. 91
ix. 88
x. 9, 11, 41, 87, 108, 110, 112

3. Light Chain

3.1 Choose donor at 46, 48, 58 and 71

3.2 Check that the following have the same amino acid in donor and acceptor sequences, if not preferably choose donor:

2, 4, 6, 35, 38, 44, 47, 49, 62, 64-69 inclusive, 85, 87, 98, 99, 101 and 102

3.3 To further optimise affinity consider choosing donor residues at one, some or any of:

i. 1, 3
ii. 63
iii. 60, if 60 and 54 are able to form potential saltbridge
iv. 70, if 70 and 24 are able to form potential saltbridge
v. 73, and 21 if 47 is different between donor and acceptor
vi. 37, and 45 if 47 is different between donor and acceptor
vii. 10, 12, 40, 80, 103, 105

Rationale

In order to transfer the binding site of an antibody into a different acceptor framework, a number of factors need to be considered.

1. The extent of the CDRs
The CDRs (Complementary Determining Regions) were defined by Wu and Kabat (refs. 4 and 5) on the basis of an analysis of the variability of different regions of antibody variable regions. Three regions per domain were recognised. In the light chain the sequences are 24-34, 50-56, 89-97 (numbering according to Kabat (ref. 4), Eu Index) inclusive and in the heavy chain the sequences are 31-35, 50-65 and 95-102 inclusive.

When antibody structures became available it became apparent that these CDR regions corresponded in the main to loop regions which extended from the β barrel framework of the light and heavy variable domains. For H1 there was a discrepancy in that the loop was from 26 to 32 inclusive and for H2 the loop was 52 to 56 and for L2 from 50 to 53. However, with the exception of H1 the CDR regions encompassed the loop regions and extended into the β strand frameworks. In H1 residue 26 tends to be a serine and 27 a phenylalanine or tyrosine, residue 29 is a phenylalanine in most cases. Residues 28 and 30 which are surface residues exposed to solvent might be involved in antigen-binding. A prudent definition of the H1 CDR therefore would include residues 26-35 to include both the loop region and the hypervariable residues 33-35.

It is of interest to note the example of Riechmann et al (ref. 3), who used the residue 31-35 choice for CDR-H1. In order to produce efficient antigen binding, residue 27 also needed to be recruited from the donor (rat) antibody.

2. Non-CDR residues which contribute to antigen binding

By examination of available X-ray structures we have identified a number of residues which may have an effect on net antigen binding and which can be demonstrated by experiment. These residues can be sub-divided into a number of groups.

2.1 Surface residues near CDR [all numbering as in Kabat et al (ref. 7)].

2.1.1. Heavy Chain - Key residues are 23, 71 and 73. Other residues which may contribute to a lesser extent are 1, 3 and 76. Finally 25 is usually conserved but the murine residue should be used if there is a difference.

2.1.2 Light Chain - Many residues close to the CDRs, e.g. 63, 65, 67 and 69 are conserved. If conserved none of the surface residues in the light chain are likely to have a major effect. However, if the murine residue at these positions is unusual, then it would be of benefit to analyse the likely contribution more closely. Other residues which may also contribute to binding are 1 and 3, and also 60 and 70 if the residues at these positions and at 54 and 24 respectively are potentially able to form a salt bridge i.e. 60 + 54; 70 + 24.

2.2 Packing residues near the CDRs.

2.2.1 Heavy Chain - Key residues are 24, 49 and 78. Other key residues would be 36 if not a tryptophan, 94 if not an arginine, 104 and 106 if not glycines and 107 if not a threonine. Residues which may make a further contribution to stable packing of the heavy chain and hence improved affinity are 2, 4, 6, 38, 46, 67 and 69. 67 packs against the COR residue 63 and this pair could be either both mouse or both human. Finally, residues which contribute to packing in this region but from a longer range are 18, 20, 80, 82 and 86. 82 packs against 67 and in turn 18 packs against 82. 80 packs against 69 and in turn 20 packs against 80. 86 forms an H bond network with 38 and 46. Many of the mouse-human differences appear minor e.g. Leu-Ile, but could have an minor impact on correct packing which could translate into altered positioning of the CDRs.

2.2.2 Light Chain - Key residues are 48, 58 and 71. Other key residues would be 6 if not glutamine, 35 if not tryptophan, 62 if not phenylalanine or tryosine, 64, 66, 68, 99 and 101 if not glycines and 102 if not a threonine. Residues which make a further contribution are 2, 4, 37, 45 and- 47. Finally residues 73 and 21 and 19 may make long distance packing contributions of a minor nature.

2.3 Residues at the variable domain interface between heavy and light chains - In both the light and heavy chains most of the non-CDR interface residues are conserved. If a conserved residue is replaced by a residue of different character, e.g. size or charge, it should be considered for retention as the murine residue.

2.3.1 Heavy Chain - Residues which need to be considered are 37 if the residue is not a valine but is of larger side chain volume or has a charge or polarity. Other residues are 39 if not a glutamine, 45 if not a leucine, 47 if not a tryptophan, 91 if not a phenylalanine or tyrosine, 93 if not an alanine and 103 if not a tryptophan. Residue 89 is also at the interface but is not in a position where the side chain could be of great impact.

2.3.2 Light Chain - Residues which need to be considered are 36, if not a tyrosine, 38 if not a glutamine, 44 if not a proline, 46, 49 if not a tyrosine, residue 85, residue 87 if not a tyrosine and 98 if not a phenylalanine.

2.4 Variable-Constant region interface - The elbow angle between variable and constant regions may be affected by alterations in packing of key residues in the variable region against the constant region which may affect the position of V_L and V_H with respect to one another. Therefore it is worth noting the residues likely to be in contact with the constant region. In the heavy chain the surface residues potentially in contact with the variable region are conserved between mouse and human antibodies therefore the variable region contact residues may influence the V-C interaction. In the light chain the amino acids found at a number of the constant region contact points vary, and the V & C regions are not in such close proximity as the heavy chain. Therefore the influences of the light chain V-C interface may be minor.

2.4.1 Heavy Chain - Contact residues are 7, 11, 41, 87, 108, 110, 112.

2.4.2 Light Chain - In the light chain potentially contacting residues are 10, 12, 40, 80, 83, 103 and 105.

~~The general methods by which the vectors may be constructed, transfection methods and culture methods are well known per se and form no part of the invention. Such methods are shown, for instance, in references 6 and 7.~~
The above analysis coupled with our considerable practical experimental experience in the CDR-grafting of a number of different antibodies have lead us to the protocol given above.

The present invention is now described, by way of example only, with reference to the accompanying Figures 1 - ~~29.~~13.

Brief Description of the Figures

Figure 1 shows DNA and amino acid sequences of the OKT3 light chain;
Figure 2 shows DNA and amino acid sequences of the OKT3 heavy chain;
Figure 3 shows the alignment of the OKT3 light variable region amino acid sequence with that of the light variable region of the human antibody RE1;
Figure 4 shows the alignment of the OKT3 heavy variable region amino acid sequence with that of the heavy variable region of the human antibody KOL;
Figure 5 shows the heavy variable region amino acid sequences of OKT3, KOL and various corresponding CDR grafts;
Figure 6 shows the light variable region amino acid sequences of OKT3, RE1 and various corresponding CDR grafts;
Figure 7 shows a graph of binding assay results for various grafted OKT3 antibodies
Figure 8 shows a graph of blocking assay results for various grafted OKT3 antibodies;
Figure 9 shows a similar graph of blocking assay results;
Figure 10 shows similar graphs for both binding assay and blocking assay results;
Figure 11 shows further similar graphs for both binding assay and blocking assay results;
Figure 12 shows a graph of competition assay results for a minimally grafted OKT3 antibody compared with the OKT3 murine reference standard, and
Figure 13 shows a similar graph of competition assay results comparing a fully grafted OKT3 antibody with the murine reference standard.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

EXAMPLE 1

CDR-GRAFTING OF OKT3

MATERIAL AND METHODS

1. INCOMING CELLS

Hybridoma cells producing antibody OKT3 were provided by Ortho (seedlot ~~4882 1~~4882.1) and were grown up in antibiotic free Dulbecco's Modified Eagles Medium (DMEM) supplemented with glutamine and 5% foetal calf serum, and divided to provide both an overgrown supernatant for evaluation and cells for extraction of RNA. The overgrown supernatant was shown to contain 250 ug/mL murine IgG2a/kappa antibody. The supernatant was negative for murine lambda light chain and IgG1, IgG2b, IgG3, IgA and IgM heavy chain. 20mL~~.~~ of supernatant was ~~sent to Ortho~~assayed to confirm that the antibody present was OKT3.

2. MOLECULAR BIOLOGY PROCEDURES

Basic molecular biology procedures were as described in Maniatis et al~~.~~ (ref. ~~6~~9) with, in some cases, minor modifications. DNA sequencing was performed as described in Sanger et al~~.~~ (ref. ~~7~~11) and the Amersham International Plc sequencing handbook. Site directed mutagenesis was as described in Kramer et al~~.~~ (ref. ~~8~~12) and the Anglian Biotechnology Ltd, handbook. COS cell expression and metabolic labelling studies were as described in Whittle et al~~.~~ (ref. ~~9~~13)

3. RESEARCH ASSAYS

~~3.1~~3.1. ASSEMBLY ASSAYS

Assembly assays were performed on supernatants from transfected COS cells to determine the amount of intact IgG present.

~~3.1 .1~~3.1.1. COS CELLS TRANSFECTED WITH MOUSE OKT3 GENES

The assembly assay for intact mouse IgG in COS cell supernatants was an ELISA with the following format:-

96 well microtitre plates were coated with F(ab')2 goat anti -mouse IgG Fc. The plates were washed in water and samples added for 1 hour at room temperature. The plates were washed and F(ab')2 goat anti -mouse IgG F(ab')2 (HRPO conjugated) was then added. Substrate was added to reveal the reaction. UPC10, a mouse IgG2a myeloma, was used as a standard.

~~3.1.2~~3.1.2. COS AND CHO CELLS TRANSFECTED WITH ~~CHIMAERIC~~CHIMERIC OR CDR -GRAFTED OKT3 GENES

The assembly assay for ~~intact humanised OKT3~~chimeric or CDR-grafted antibody in COS cell supernatants was an ELISA with the following format:-

96 well microtitre plates were coated with F(ab')2 goat anti-human IgG Fc. The plates were washed and samples added and incubated for 1 hour at room temperature. The plates were washed and monoclonal mouse anti-human kappa chain was added for 1 hour at room temperature.

The plates were washed and F(ab')2 goat anti -mouse IgG Fc (HRPO conjugated) was added. ~~Substrate~~Enzyme substrate was added to reveal the reaction.~~Chimaeric~~ Chimeric B72.3 (IgG4) (ref. 13) was used as a standard. The use of a monoclonal anti-kappa chain in this assay allows grafted antibodies to be read from the ~~chimaeric~~chimeric standard.

~~3,2~~3.2. ASSAY FOR ~~OKT3~~ ANTIGEN BINDING ACTIVITY

Material from COS cell supernatants was assayed for OKT3 antigen binding activity onto CD3 positive cells in a direct assay. The procedure was as follows:-

HUT 78 cells (human T cell line, CD3 positive) were maintained in culture. Monolayers of HUT 78 cells were prepared onto 96 well ELISA plates using poly-L-lysine and glutaraldehyde. Samples were added to the monolayers for 1 hour at room temperature.

The plates were washed gently using PBS. F(ab')2 goat anti-human IgG Fc (HRPO conjugated) or F(ab')2 goat anti -mouse IgG Fc (HRPO conjugated) was added as appropriate for humanised or mouse samples. Substrate was added to reveal the reaction. The negative control for the cell-based assay was chimeric B72.3. The positive control was mouse Orthomune OKT3 or

chimeric OKT3, when available. This cell-based assay was difficult to perform, and an alternative assay was developed for CDR-grafted OKT3 which was more sensitive and easier to carry out.

In this system CDR-grafted OKT3 produced by COS cells was tested for its ability to bind to the CD3-positive HPB-ALL (human peripheral blood acute lymphocytic leukemia) cell line. It was also tested for its ability to block the binding of murine OKT3 to these cells. Binding was measured by the following procedure: HPB-ALL cells were harvested from tissue culture. Cells were incubated at 4°C for 1 hour with various dilutions of test antibody, positive control antibody; or negative control antibody. The cells were washed once and incubated at 4°C for 1 hour with an FITC-labelled goat anti-human IgG (Fc-specific, mouse absorbed). The cells were washed twice and analysed by cytofluorography. Chimeric OKT3 was used as a positive control for direct binding. Cells incubated with mock- transfected COS cell supernatant, followed by the FITC-labelled goat anti-human IgG, provided the negative control. To test the ability of CDR-grafted OKT3 to block murine OKT3 binding, the HPB-ALL cells were incubated at 4°C for 1 hour with various dilutions of test antibody or control antibody. A fixed saturating amount of FITC OKT3 was added. The samples were incubated for 1 hour at 4°C, washed twice and analysed by cytofluorography. FITC-labelled OKT3 was used as a positive control to determine maximum binding. Unlabelled murine OKT3 served as a reference standard for blocking. Negative controls were unstained cells with or without mock-transfected cell supernatant. The ability of the CDR-grafted OKT3 light chain to bind CD3-positive cells and block the binding of murine OKT3 was initially tested in combination with the chimeric OKT3 heavy chain. The chimeric OKT3 heavy chain is composed of the murine OKT3 variable region and the human IgG4 constant region. The chimeric heavy chain gene is expressed in the same expression vector used for the CDR-grafted genes. The CDR-grafted light chain expression vector and the chimeric heavy chain expression vector were co-transfected into COS cells. The fully chimeric OKT3 antibody (chimeric light chain and chimeric heavy chain) was found to be fully capable of binding to CD3 positive cells and blocking the binding of murine OKT3 to these cells.

3.3 DETERMINATION OF RELATIVE BINDING AFFINITY

The relative binding affinities of CDR-grafted anti-CD3 monoclonal antibodies were determined by competition binding (ref. 6) using the HPB-ALL human T cell line as a source of CD3 antigen, and fluorescein-conjugated murine OKT3 (FI-OKT3) of known binding affinity as a tracer antibody. The binding affinity of FI-OKT3 tracer antibody was determined by a direct binding assay in which increasing amounts of FI-OKT3 were incubated with HPB-ALL ($5x10^5$) in PBS with 5% foetal calf serum for 60 min. at 4°C. Cells were washed, and the fluorescence intensity was determined on a FACScan flow cytometer calibrated with quantitative microbead standards (Flow Cytometry Standards, Research Triangle Park, NC). Fluorescence intensity per antibody molecule (F/P ratio) was determined by using microbeads which have a predetermined number of mouse IgG antibody binding sites (Simply Cellular beads, Flow Cytometry Standards). F/P equals the fluorescence intensity of beads saturated with FI-OKT3 divided by the number of binding sites per bead. The amount of bound and free FI-OKT3 was calculated from the mean fluorescence intensity per cell, and the ratio of bound/free was plotted against the number of moles of antibody bound. A linear fit was used to determine the affinity of binding (absolute value of the slope).

~~The negative control for the cell based assay was chimaeric B72.3. The positive control was mouse Orthomune OKT3 or chimaeric OKT3, when available. This cell based assay was difficult to perform and gave poorly reproducible results with a high background.~~For competitive binding, increasing amounts of competitor antibody were added to a sub-saturating dose of FI-OKT3 and incubated with $5xl0^5$ HPB-ALL in 200 ml of PBS with 5% foetal calf serum, for 60 min at 4^oC. The fluorescence intensities of the cells were measured on a FACScan flow cytometer calibrated with quantitative microbead standards. The concentrations of bound and free FI-OKT3 were calculated. The affinities of competing antibodies were calculated from the equation [X] - [OKT3] = (l/Kx) - (l/Ka), where Ka is the affinity of murine OKT3, Kx is the affinity of competitor X, [ ] is the concentration of competitor antibody at which bound/free binding is R/2, and R is the maximum bound/free binding.

4. cDNA LIBRARY ~~CONSTRUTION~~CONSTRUCTION

~~4.1~~4.1. mRNA PREPARATION AND cDNA SYNTHESIS

OKT3 producing cells were grown as described above and 1.2 x 10^9 cells harvested and mRNA extracted using the guanidinium/LiCl extraction procedure. cDNA was prepared by priming from Oligo-dT to generate full length cDNA. The cDNA was methylated and EcoR1 linkers added for cloning.

~~4.2~~4.2. LIBRARY CONSTRUCTION

The cDNA library was ligated to pSP65 vector DNA which had been EcoR1 cut and the 5' phosphate groups removed by calf intestinal phosphatase (EcoR1/CIP). The ligation was used to transform high transformation efficiency Escherichia coli (E.coli) HB101. A cDNA library was prepared. 3600 colonies were screened for the light chain and 10000 colonies were screened for the heavy chain.

5. SCREENING

E.coli colonies positive for either heavy or light chain probes were identified by oligonucleotide screening using the oligonucleotides:
5' TCCAGATGTTAACTGCTCAC for the light chain, which is complementary to a sequence in the mouse kappa constant region, and 5' CAGGGGCCAGTGGATGGATAGAC for the heavy chain which is complementary to a sequence in the mouse IgG2a constant CH1 domain region. 12 light chain and 9 heavy chain clones were identified and taken for second round screening. Positive clones from the second round of screening were grown up and DNA prepared. The sizes of the gene inserts were estimated by gel electrophoresis and inserts of a size capable of containing a full length cDNA were subcloned into M13 for DNA sequencing.

6. DNA SEQUENCING

Clones representing four size classes for both heavy and light chains were obtained in M13. DNA sequence for the 5' untranslated regions, signal sequences, variable regions and 3' untranslated regions of full length cDNAs [Figures l(a) and 2(a)] were obtained ~~(Figs 1 and 2).~~and the

corresponding amino acid sequences predicted [(Figures l(b) and 2(b)]. In Figure l(a) the untranslated DNA regions are shown in uppercase, and in both Figures 1 and 2 the signal sequences are underlined.

~~ANALYSIS OF SEQUENCES~~

~~DNA sequences from cDNA's were compared with RNA sequences provided by Ortho. The cDNA sequences included 5' untranslated region sequence as well as signal peptide sequence. The 3' untranslated region was also sequenced. A single coding difference was observed at position 9 in the heavy chain where the mRNA suggested a Proline but the cDNA sequence read as an Alanine. The cDNA sequence was used for further analysis.~~

~~The light chain is a member of the mouse V_L subgroup VI and uses a J_K4 minigene. The heavy chain is probably a member of the mouse V_H subgroup II, most probably IIb, although it also has significant homology to the consensus for group Va which itself is very homologous to subgroup II. The D region is currently unclassified and the JH region is J_H2 (Figs 3 and 4).~~

~~The light chain shows a high degree of homology to the Ox-1 germline gene and to the published antibodies 45.21.1, 14.6b.1 and 26.4.1. The heavy chain shows reasonable homology to a subgroup of the J558 family including l4.6b.1. These combinations of light and heavy chain genes have previously resulted in antibodies with affinity for alpha-1-6 dextran (Sikder et al. (ref. l0) Wallick et al. (ref. 11)).~~

~~The heavy chain has the sequence Asparagine (Asn)-Proline (Pro)-Serine (Ser) in CDR2. Normally Asn-X-Ser would be a potential glycosylation site, but when X is Pro these sites tend not to be glycosylated.~~

~~8.~~7. CONSTRUCTION OF cDNA EXPRESSION VECTORS

Celltech expression vectors are based on the plasmid pEE6hCMV ~~(Fig. 5)~~ (ref. ~~12~~14) . A polylinker for the insertion of genes to be expressed has been introduced after the major immediate early promoter/enhancer of the human Cytomegalovirus (hCMV). Marker genes for selection of the plasmid in transfected eukaryotic cells can be inserted as ~~BamHI~~BamH1 cassettes in the unique ~~BamHI~~BamH1 site of pEE6 hCMV; for instance, the neo marker to provide pEE6 hCMV neo. It is usual practice to insert the neo and gpt markers prior to insertion of the gene of interest, whereas the GS marker is inserted last because of the presence of internal ~~EcoRI~~EcoR1 sites in the cassette.

The selectable markers are expressed from the SV40 late promoter which also provides an origin of replication so that the vectors can be used for expression in the COS cell transient expression system.

The mouse sequences were excised ~~as EcoRI~~from the M13 based vectors described above as EcoR1 fragments and cloned into either ~~EE~~pEE6-hCMV-neo for the heavy chain ~~(Fig 6)~~ and into EE6-hCMV-gpt for the light chain ~~(Fig. 7)~~to yield vectors pJA136 and pJA135 respectively.

~~9.~~8. EXPRESSION OF ~~cDNA'S~~cDNAS IN COS CELLS

Plasmids pJA135 ~~(Fig 7)~~ and pJA136 ~~(fig 6)~~ were co-transfected into COS cells and supernatant from the transient expression experiment was shown to contain assembled antibody which bound to T-cell enriched ~~peripheral blood~~ lymphocytes. Metabolic labelling experiments using ^{35}S methionine showed expression and assembly of heavy and light chains.

~~10.~~9. CONSTRUCTION OF ~~CHIMAERIC~~CHIMERIC GENES

Construction of ~~chimaeric~~chimeric genes followed a previously described strategy ~~([~~[Whittle et al (ref. ~~9~~ 13)~~)~~]. A restriction site near the 3' end of the variable domain sequence is identified and used to attach an oligonucleotide adapter coding for the remainder of the mouse variable region and a suitable restriction site for attachment to the constant region of choice.

~~10.1~~9.1. LIGHT CHAIN GENE CONSTRUCTION ~~– VERSION 1~~

The mouse light chain cDNA sequence ~~showed~~contains an Aval site near the 3' end of the variable region ~~([~~[Fig ~~8. 1(a)~~]. The majority of the sequence of the variable region was isolated as a ~~376~~396 bp. ~~EcoRI~~EcoR1-Aval fragment. An oligonucleotide adapter was designed to replace the remainder of the 3' region of the variable region from the Aval site and to include the 5' residues of the human constant region up to and including a unique Narl site which had been previously engineered into the constant region.

~~TOP STRAND 5TCGGGGACAAAGCTTGAAATAAACAGAACTGTGGCGG 3'~~
~~BOTTOM STRAND 3' CCTGTTTCGAACTTTATTTGTCTTGACACCGCCGC 5'~~

A Hind ~~III site, shown in bold type within the oligonucleotide sequence above,~~111 site was introduced to act as a marker for insertion of the linker.

The linker was ligated to the V_L fragment and the 413 bp ~~EcoRI~~EcoR1-Narl adapted fragment was purified from the ligation mixture.

The constant region was isolated as an Narl-~~BamHI~~BamH1 fragment from an M13 clone NW361 and was ligated with the variable region DNA into an ~~EcoRI/BamHI/ClP~~EcoR1/BamH1/C1P pSP65 treated vector in a three way reaction ~~.~~ to yield plasmid JA143. Clones were isolated after transformation into E.coli and the linker and junction sequences were confirmed by the presence of the ~~HinDIII~~Hind111 site and by DNA sequencing ~~(Fig 9)~~.

~~10.2.~~9.2 LIGHT CHAIN GENE CONSTRUCTION - VERSION 2

The construction of the first ~~chimaeric~~chimeric light chain gene produces a fusion of mouse and human amino acid sequences at the variable-constant region junction. In the case of the OKT3 light chain the amino acids at the ~~chimaera~~chimera junction are:

……. Leu-Glu-~~Ile~~Ile-Asn-Arg/ -/Thr-Val-Ala -Ala
VARIABLE CONSTANT

This arrangement of sequence introduces a potential site for Asparagine (Asn) linked (N-linked) glycosylation at the V-C junction. ~~As will be seen later, this sequence can be glycosylated.~~ Therefore, a second version of the ~~chimaeric~~chimeric light chain oligonucleotide adapter was designed in which the threonine (Thr), the first amino acid of the human constant region, was replaced with the equivalent amino acid from the mouse constant region, Alanine (Ala).
~~TOP STRAND 5' TCGGGGACAMGTIGGAMTAMCAG IIGCTGTGGCGG 3'~~
~~BOTIOM STRAND 3' CCTGTTTCAACCTTTATTTGTCTCGACACCGCCGC 5'~~

~~The~~An internal ~~HinDIII~~Hind111 site ~~present in the version 1 adapter~~ was not included in this adapter, to differentiate the two ~~chimaeric~~chimeric light chain genes.

The variable region fragment was isolated as a 376 bp~~. EcoRI~~ EcoR1-AvaI fragment. The oligonucleotide linker was ligated to NarI cut pNW361 and then the adapted 396bp constant region was isolated after recutting the modified pNW361 with ~~EcoRI.~~EcoR1. The variable region fragment and the modified constant region fragment were ligated directly into ~~EcoRI/CIP~~EcoR1/C1P treated pEE6hCMVneo~~.~~ to yield pJA137. Initially all clones examined had the insert in the incorrect orientation. Therefore, the insert was re-isolated and recloned to turn the insert round~~.~~ and yield plasmid pJA141. Several clones with the insert in the correct orientation were obtained and the adapter sequence of one was confirmed by DNA sequencing ~~(Fig10).~~

~~10.3~~9.3. HEAVY CHAIN GENE CONSTRUCTION
~~10.3.1~~9.3.1. CHOICE OF HEAVY CHAIN GENE ISOTYPE

The constant region isotype chosen for the heavy chain was human IgG4.

~~10.3.2~~9.3.2. GENE CONSTRUCTION
The heavy chain cDNA sequence showed a ~~BanI~~Ban1 site near the 3' end of the variable region ~~(~~[Fig ~~11~~. 2(a)). The majority of the sequence of the variable region was isolated as a 426bp. ~~EcoRI/CIP/BanI~~EcoR1/C1P/Ban1 fragment. An oligonucleotide adapter was ~~designed~~designated to replace the remainder of the 3' region of the variable region from the ~~BanI~~Ban1 site up to and including a unique ~~HinDIII~~HindIII site which had been previously engineered into the first two amino acids of the constant region.
~~TOP STRAND 5' GCACCACTCTCACCGTGtlGCTC3'~~
~~BOTTOM STRAND 3'GTGAGAGTGGCACTCGAGTCGA 5'~~

The linker was ligated to the V_H fragment and the ~~EcoRI HinDIII~~EcoR1-Hind111 adapted fragment was purified from the ligation mixture.

The variable region was ligated to the constant region by cutting ~~mJA~~pJA91 with ~~EcoRI~~EcoR1 and Hind~~III~~111 removing the intron fragment and replacing it with the V_H ~~(Fig 12).~~to yield pJA142. Clones were isolated after transformation into E.coli JM101 and the linker and junction sequences were confirmed by DNA sequencing. (~~NB~~N. B. The ~~HindIII~~Hind111 site is lost on cloning).

~~11.~~10. CONSTRUCTION OF ~~CHIMAERIC~~CHIMERIC EXPRESSION VECTORS
~~11.1~~10.1. neo AND gpt VECTORS

The ~~chimaeric~~chimeric light chain (version 1) was removed from pJA143 ~~(Fig 9)~~ as an ~~EcoRI~~EcoR1 fragment and cloned into ~~EcoRI/CIP~~EcoR1/C1P treated pEE6hCMVneo expression vector~~.~~ to yield pJA145. Clones with the insert in the correct orientation were identified by restriction mapping ~~(Fig 13)~~.

The ~~chimaeric~~chimeric light chain (version 2) was constructed as described above ~~(see Fig 10)~~.

The ~~chimaeric~~chimeric heavy chain gene was isolated from pJAl42 as a 2.5Kbp ~~EcoRI/BamHI~~EcoR1/BamH1 fragment and cloned into the ~~EcoRI/BcII/CIP~~EcoR1/Bc11/C1P treated vector fragment of ~~pJA97,~~ a derivative of pEE6hCMVgpt ~~(Fig 14).~~to yield plasmid pJA144.

~~11.2~~10.2. GS SEPARATE VECTORS

GS versions of pJA141 ~~(Fig 10)~~ and pJA144 ~~(Fig 14)~~ were constructed by replacing the neo and gpt cassettes by ~~BamHI/SaII/CIP~~a BamH1/Sa11/C1P treatment of the plasmids, isolation of the vector fragment and ligation to a
GS-containing fragment from the plasmid pRO49 ~~(Figs 15 and 16)~~to yield the light chain vector pJA179 and the heavy chain vector pJA180.

~~11.3~~ 10.3. GS SINGLE VECTOR CONSTRUCTION

Single vector constructions containing the cL (chimeric light), cH (chimeric heavy) and GS genes on one plasmid in the order cL-cH-GS, or cH-cL-GS and with transcription of the genes being head to tail ~~ege~~. g. cL>cH>GS were constructed. These plasmids were made by treating pJA179 ~~(Fig 15)~~ or pJA180 ~~(Fig 16)~~ with ~~BamHI/CIP~~BamH1/C1P and ligating in a ~~BgIII/HinDIII~~Bg111/Hind111 hCMV promoter cassette ~~from pJA146~~ along with either the ~~HinDIII/BamHI~~Hind111/BamH1 fragment from pJA141 into pJA180 to give the cH-cL-GS plasmid pJA182 ~~(Fig17),~~ or the ~~HinDIII/BamHI~~Hind111/BamH1 fragment from pJA144 into pJA179 to give the cL-cH-GS plasmid pJA~~181 (Fig 18).~~181.

~~12.~~11. EXPRESSION OF ~~CHIMAERIC~~CHIMERIC GENES

~~12.1~~11.1. EXPRESSION IN COS CELLS

The ~~chimaeric~~chimeric antibody ~~plasmids~~plasmid pJA145 (cL) and pJA144 (cH) were ~~cotransfected~~co-transfected into COS cells and supernatant from the transient expression experiment was shown to contain assembled antibody which bound to the HUT 78 human T-cell line. Metabolic labelling experiments using ^{35}S methionine showed expression and assembly of heavy and light chains. However the light chain mobility seen on reduced gels ~~(Fig 19)~~ suggested that the potential glycosylation site was being glycosylated. Expression in COS cells in the presence of tunicamycin showed a reduction in size
of the light chain to that shown for control ~~chimaeric~~chimeric antibodies and the OKT3 mouse light chain. Therefore JA141 was constructed and expressed. In this case the light chain did not show an aberrant mobility or a size shift in the presence or absence of tunicamycin ~~(Fig 19)~~. This second version of the ~~chimaeric~~chimeric light chain, when expressed in association with

~~chimaeric~~chimeric heavy (cH) chain, produced antibody which showed good binding to HUT 78 cells. In both cases antigen binding was equivalent to that of the mouse antibody.

~~12.2~~11.2. EXPRESSION IN CHINESE HAMSTER OVARY (CHO) CELLS

Stable cell lines ~~are being~~have been prepared from plasmids ~~pJA~~PJA141/pJA144 and from pJA179/pJA180, pJA~~181,~~181 and pJA182 by transfection into CHO cells.

~~13.~~12. CDR ~~-~~GRAFTING

The approach taken was to try to introduce sufficient mouse residues into a human variable region framework to generate antigen binding activity comparable to the mouse and ~~chimaeric~~chimeric antibodies.

~~13.1~~12.1. VARIABLE REGION ANALYSIS

From an examination of a small database of structures of antibodies and antigen-antibody complexes it is clear that only a small number of antibody residues make direct contact with antigen. Other residues may contribute to antigen binding by positioning the contact residues in favourable configurations and also by inducing a stable packing of the individual variable domains and stable interaction of the light and heavy chain variable domains.

The residues chosen for transfer can be identified in a number of ways:

~~A.~~(a) By examination of antibody X-ray crystal structures the antigen binding surface can be predominantly located on a series of loops, three per domain, which extend from the B-barrel framework.

~~B.~~ (b) By analysis of antibody variable domain sequences~~,~~ regions of hypervariability ~~(~~[termed the Complementarity Determining Regions (CDRs) by Wu and Kabat (ref.~~-~~ 5)~~)~~] can be identified. In the most but not all cases these CDRs correspond to, but extend a short way beyond, the loop regions noted above.

~~C.~~ (c) Residues not identified by ~~A~~(a) and ~~B above~~(b) may contribute to antigen binding directly or indirectly by affecting antigen binding site topology, or by inducing a stable packing of the individual variable domains and stabilising the inter-variable domain interaction. These residues may be identified either by superimposing the sequences for a given antibody on a known structure and looking at key residues for their contribution, or by sequence alignment analysis and noting "idiosyncratic" residues followed by examination of their structural location and likely effects.

~~13.1.1~~12.1.1. LIGHT CHAIN

Figure ~~20~~3 shows an alignment of sequences for the human framework region ~~REI~~RE1 and the OKT3 light variable region. The structural loops (LOOP) and CDRs (KABAT) believed to correspond to the antigen binding region are marked. Also marked are a number of other residues which may also contribute to antigen binding as described in 13.1~~C. REI~~(c).

Above the sequence in Figure 3 the residue type indicates the spatial location of each residue side chain, derived by examination of resolved structures from X-ray crystallography analysis. The key to this residue type designation is as follows:

N - near to CDR (From X-ray Structures)

P - Packing	B - Buried Non-Packing
S - Surface	E - Exposed
I - Interface	* - Interface
- Packing/Part Exposed	

? - Non-CDR Residues which may require to be left as Mouse sequence.

Residues underlined in Figure 3 are amino acids. RE1 was chosen as the human framework because the light chain is a ~~Kappa~~kappa chain and the kappa variable regions show higher homology with the mouse sequences than a lambda light variable region ~~eg~~, e.g. KOL (see below). ~~REI~~RE1 was chosen in preference to another kappa light chain because the X-ray structure of the light chain has been determined so that a structural examination of individual residues could be made.

~~13.1.2~~12.1.2. HEAVY CHAIN

Similarly Figure ~~21~~4 shows an alignment of sequences for the human framework region KOL and the OKT3 heavy variable region. The structural loops and CDRs believed to correspond to the antigen binding region are marked. Also marked are a number of other residues which may also contribute to antigen binding as described in ~~13.1C.~~12.1(c). The residue type key and other indicators used in Figure 4 are the same as those used in Figure 3. KOL was chosen as the heavy chain framework because the X-ray structure has been determined to a better resolution than, for example, NEWM and also the sequence alignment of OKT3 heavy variable region showed a slightly better homology to KOL than to NEWM.

~~13.2~~12.2. DESIGN OF VARIABLE GENES

The variable region domains were designed with mouse variable region optimal codon usage ~~([~~[Grantham and Perrin~~,~~ (ref.~~13~~ 15))] and used the B72.3 signal sequences ~~([~~[Whittle et al~~.~~ (ref. ~~9~~13))]. The sequences were designed to be attached to the constant region in the same way as for the ~~chimaeric~~chimeric genes described above. Some constructs contained the "Kozak consensus sequence" ~~([~~[Kozak~~,~~ (ref. ~~14~~16))] directly linked to the 5' of the signal sequence in the gene. This sequence motif is believed to have a beneficial role in translation initiation in eukaryotes.

~~13.3~~ 12.3. GENE CONSTRUCTION

To build the variable regions ~~two~~, various strategies are available. ~~Either to assemble the~~The sequence may be assembled by using oligonucleotides in a manner similar to Jones et al~~.~~ (ref. ~~15~~17) or ~~to~~by simultaneously ~~replace~~replacing all of the CDRs or loop regions by oligonucleotide directed site specific mutagenesis in a manner similar to Verhoeyen et al~~.~~ (ref. 2) . Both strategies were used and a list of constructions is set out in ~~Table 1.~~Tables 1 and 2 and Figures 4 and 5. It was noted in several cases that the mutagenesis approach led to deletions and rearrangements in the gene being remodelled, while the success of the assembly approach was very sensitive to the quality of the oligonucleotides. ~~Figs 22a and b and 23a and b show by way of example the~~

~~nucleotide sequences and procedures required to construct gH341 by site directed mutagenesis and kgH341A by oligonucleotide assembly.~~

~~14~~13. CONSTRUCTION OF EXPRESSION VECTORS

Genes were isolated from M13 or SP65 based intermediate vectors and cloned into pEE6hCMVneo for the light chains and pEE6hCMVgpt for the heavy chains in a manner similar to that for the ~~chimaeric~~chimeric genes as described above.

~~15 EXPRESSION OF CDR GRAFTED GENES~~

~~A number of points should be noted.~~

~~1. There is no standard for the antigen binding assay when chimaeric or CDR grafted antibody are being measured, except when the heavy chain of the antibody is murine when murine OKT3 can be used as standard with an anti-murine Fc antibody as revealing antibody. Therefore all comparisons of antigen binding assays with chimaeric (c) or CDR grafted (g) genes can only be made within an individual experiment.~~

TABLE 1 CDR GRAFTED GENE CONSTRUCTIONS

CODE	MOUSE SEQUENCE CONTENT	METHOD OF CONSTRUCTION	KOZAK SEQUENCE +
LIGHT CHAIN	ALL HUMAN FRAMEWORK ~~REI~~RE1		
121	26-32, 50-56, 91-96 inclusive	SDM and gene assembly	+ n.d.
121A	26-32, 50-56, 91-96 inclusive +1, 3, 46, 47	Partial gene assembly	n.d. +
121B	26-32, 50-56, 91-96 inclusive +46, 47	Partial gene assembly	n.d. +
221	24-24, 50-56, 91-96 inclusive	Partial gene assembly	+ +
221A	24-34, 50-56, 91-96 inclusive +1, 3, 46, 47	Partial gene assembly	+ +
221B	24-34, 50-56, 91-96 inclusive +1, 3	Partial gene assembly	+ +
221C	24-34, 50-56, 91-96 inclusive +46, 47	Partial gene assembly	+ +
HEAVY CHAIN	ALL HUMAN FRAMEWORK KOL		
121	26-32, 50-56, 95-100B inclusive	Gene assembly	n.d. +
131	26-32, 50-58, 95-100B inclusive	Gene assembly	n.d. +
141	26-32, 50-65, 95-100B inclusive	Partial gene assembly	+ n.d.
321	26-35, 50-56, 95-100B inclusive	Partial gene assembly	+ n.d.
331	26-35, 50-58, 95-100B inclusive	Partial gene assembly Gene assembly	+ +
341	26-35, 50-65, 95-100B inclusive	SDM Partial gene assembly	+ +

341A	26-35, 50-65, 95-100B inclusive +6, 23, 24, 48, 49, 71, 73, 76, 78, 88, 91 (+ 63 = human)	Gene assembly	n.d. +
341B	26-35, 50-65, 95-100B inclusive +48, 49, 71, 73, 76, 78, 88, 91 (+63 =± human)	Gene assembly	n.d. +

KEY

n.d.	not done
SDM	Site directed mutagenesis
Gene assembly	Variable region assembled entirely from oligonucleotides
Partial gene assembly	Variable region assembled by combination of restriction fragments either from other genes originally created by SDM and gene assembly or by oligonucleotide assembly of part of the variable region and reconstruction with restriction fragments from other genes originally created by SDM and gene assembly.

~~2. The cell based antigen binding assay is not robust and resulting data varies depending on cell binding to the plates and the amount of antibody used. Therefore several experiments are needed to confirm marginal results.~~

~~3. The COS cell expression system can give batch to batch variation in antibody yield which has a direct bearing on the results obtained in the antigen binding assay.~~

~~Bearing these factors in mind the data can be divided into three groups. Table 2 shows a summary of data for the various constructs.~~

14. EXPRESSION OF CDR-GRAFTED GENES

~~15.1~~ 14.1. PRODUCTION OF ANTIBODY CONSISTING OF GRAFTED LIGHT (gL) CHAINS WITH MOUSE HEAVY (mH) OR ~~CHIMAERIC~~CHIMERIC HEAVY (cH) CHAINS~~.~~

All gL chains, in association with mH or cH produced reasonable amounts of antibody. Insertion of the Kozak consensus sequence at a position 5' to the ATG (kgL constructs) however, led to a 2-5 fold improvement in net expression ~~(Fig 24a and b)~~. Over an extended series of experiments expression levels were raised from ~~approx~~approximately 200ng/~~mL~~ml to ~~approx.~~approximately 500 ng/~~mL~~ml for kgL/cH or kgL/mH combinations.

When direct binding to antigen on HUT 78 cells was measured, a construct designed to include mouse sequence based on loop length (gL121) did not lead to active antibody in association with mH or cH. A construct designed to include mouse sequence based on Kabat CDRs (gL221) demonstrated some weak binding in association with mH or cH ~~(Fig 25B)~~. However, when framework residues 1, 3, 46, 47 were changed from the human to the murine OKT3 equivalents based on the arguments outlined in Section ~~13.1~~12.1 antigen binding ~~can be~~was demonstrated

when both of the new constructs, which were termed 121A and 221A~~, are coexpressed~~ were co-expressed with cH ~~(Fig 25A and B)~~. When the effects of these residues ~~are~~were examined in more detail, it appears that residues 1 and 3 are not major contributing residues as the product of the gL221B gene shows little detectable binding activity in association with cH ~~(Fig 25B)~~. The light chain product of gL221C, in which mouse sequences are present at 46 and 47, shows good binding activity in association with cH ~~(Fig 25 B)~~.

~~15.2~~ 14.2 PRODUCTION OF ANTIBODY CONSISTING OF GRAFTED HEAVY (gH) CHAINS WITH MOUSE LIGHT (mL) OR ~~CHIMAERIC~~CHIMERIC LIGHT (cL) CHAINS~~.~~

Expression of the gH genes ~~has proven~~proved to be more difficult to achieve than for gL. First, inclusion of the Kozak sequence ~~appears~~appeared to have ~~had~~ no marked effect on expression of gH genes ~~(Fig. 26)~~. Expression ~~may~~appears to be slightly improved but not to the same degree as seen for the grafted light chain.

~~Second~~Also, it ~~has proven~~proved difficult to demonstrate production of expected quantities of material when the loop choice (amino acid 26-32) for CDR1 is used ~~eg,~~ e.g. gH121, 131, 141 ~~(Fig 27)~~ and no conclusions can be drawn about these constructs. ~~Further, in experiments where low antibody production was seen it has not been possible to detect free light chain expression and secretion which would be expected if heavy chain expression was not occurring at all. Therefore the data suggests, but does not confirm, that in these cases the heavy chain is being expressed but the processing of the chain once it has become associated with light chain is aberrant leading to degradation of assembled or partially assembled antibody inside the cell. Experiments to determine gH mRNA levels, or to attempt to demonstrate the presence of antibody within the cells have not been done.~~

~~Third, coexpression~~Moreover, co-expression of the gH341 gene with cL or mL has been variable and has tended to produce lower amounts of antibody than the cH/cL or mH/mL combinations. The alterations to gH341 to produce gH341A and gH341B ~~appear to~~ lead to improved levels of expression ~~(Fig 27 lanes h-k).~~ .

This may ~~partly~~ be due either to a general increase in the fraction of mouse sequence in the variable region, or to the alteration at position 63 where the residue is returned to the human amino acid Valine (Val) from Phenylalanine (Phe) to avoid possible internal packing problems with the rest of the human framework. This arrangement also occurs in gH331 and gH321.

When gH321 or gH331 ~~are~~were expressed in association with cL, antibody ~~is~~was produced but antibody binding activity ~~has~~was not ~~been~~ detected ~~(Table 2)~~. When the more conservative gH341 gene ~~is~~was used antigen binding ~~can~~could be detected in association with cL or mL, but the activity ~~is~~was only marginally above the background level ~~(Fig 28)~~.

When further mouse residues ~~are~~were substituted based on the arguments in ~~13.1~~12.1, antigen binding ~~can~~could be clearly demonstrated for the antibody produced when kgH341A and kgH341B ~~are~~were expressed in association with cL ~~(Fig 29)~~.

~~15.3~~14.3 PRODUCTION OF FULLY CDR ~~-~~GRAFTED ANTIBODY

The kgL221A gene was co-expressed with kgH341, kgH341A~~,~~ or kgH341B. For the combination kgH221A/kgH341 very little material was produced in a normal COS cell expression ~~experiment (Fig 29A aned C)~~. For the combinations kgL221A/kgH341A or kgH221A/kgH341B amounts of antibody similar to gL/cH ~~or cL/cH~~ was produced ~~(Fig 29A and C)~~.

In several experiments no antigen binding activity could be detected with kgH221A/gH341 or kgH221A/kgH341 combinations ~~(see for example Fig 29)~~, although expression levels were very low.

Antigen binding was detected when kgL221A/kgH341A or kgH221A/kgH341B combinations were expressed ~~(Fig 29B)~~. In the case of the antibody produced from the kgL221A/kgH341A combination the antigen binding was very similar to that of the ~~chimaeric~~chimeric antibody ~~(Fig 29B)~~.

~~DISCUSSION~~

~~The objectives of the programme were to produce both a chimaeric mouse variable-human constant IgG4/K antibody and a fully humanised antibody retaining the antigen binding activity of the murine monoclonal antibody OKT3.~~

~~Cells were obtained from Ortho and mRNA prepared. A cDNA library was screened for heavy and light chain cDNAs using oligonucleotide probes. Full length cDNAs were obtained and the variable regions were sequenced (Figs 1 and 2). The cDNAs showed a high level of homology with sequences of antibodies which have specificity for alpha-1-6-dextrans. It would be of interest to test OKT3 to determine whether it recognises and binds to dextran antigens.~~

~~The cDNAs were transferred to expression vectors (Figs 6 and 7) and expressed in COS cells. Antibody was produced which bound to an enriched T-cell population from peripheral blood cells.~~

~~Two versions of the chimaeric antibody were produced differing in the light chain at the first amino acid of the constant region. In version 1 (Figs 9 and 13) the amino acid sequence which resulted at the V-C junction when the chimaeric light chain was constructed generates a potential N-linked glycosylation site at the "elbow' region. This region is an extended sequence of peptide between the V and C domains and is potentially accessible to the enzymes of the glycosylation process. Fig 19 shows that the version 1 chimaeric light chain is glycosylated demonstrating that the secondary structure generated at the elbow is sufficient for the Asn-Arg-Thr motif to be used for glycosylation.~~

~~A second version of the chimaeric light chain was constructed (Fig 10) in which the first amino acid of the human constant region (Thr) was returned to the mouse amino acid (Ala), so removing the glycosylation site. Antibody was produced by coexpression with chimaeric heavy chain (Fig 13) and in both versions the chimaerlc material was equivalent in binding to the mouse OKT3 (see Fig 19). These observations have been confirmed by Ortho staff (L Jolliffe pers. comm.).~~
An analysis of the above results is given below.

~~Vectors for the expression of chimaeric OKT3 using neo/gpt or glutamine synthetase (GS) selection were prepared. Including vectors in which both genes were on the same plasmid (Figs 15 to18).~~

15. DISCUSSION OF CDR-GRAFTING RESULTS

In the design of the fully humanised antibody the aim was to transfer the minimum number of mouse amino acids that would confer antigen binding onto a human antibody framework.

15.1. LIGHT CHAIN

15.1.1. EXTENT OF THE CDRs

For the light chain the regions defining the loops known from structural studies of other antibodies to contain the antigen contacting residues, and those hypervariable sequences defined by Kabat et al (refs. ~~as Cornplementarity~~4 and 5) as Complementarity Determining Regions (CDRs) are equivalent for CDR2. For CDR1 the hypervariable region extends from residues 24-34 inclusive while the structural loop extends from 26-32 inclusive. In the case of OKT3 there is only one amino acid difference between the two options, at amino acid 24, where the mouse sequence is a serine and the human framework ~~REI~~RE1 has glutamine. For CDR3 the loop extends from residues 91-96 inclusive while the Kabat hypervariability extends from residues 89-97 inclusive. For OKT3 amino acids 89, 90 and 97 are the same between OKT3 and ~~REI~~RE1 (Fig ~~20~~. 3). When constructs based on the loop choice for CDR1 (gL121) and the Kabat choice (gL221) were made and ~~coexpressed~~co-expressed with mH or cH no evidence for antigen binding activity could be found for gL121, but trace activity could be detected for the gL221, suggesting that a single extra mouse residue in the grafted variable region could have some detectable effect. Both gene constructs were reasonably well expressed in the transient expression system.

15.1.2. FRAMEWORK RESIDUES

The remaining framework residues were then further examined, in particular amino acids known from X-ray analysis of other antibodies to be close to the CDRs and also those amino acids which in OKT3 showed differences from the consensus framework for the mouse subgroup (subgroup VI) to which OKT3 shows most homology. Four positions~~,~~ 1, 3, 46 and 47 were identified and their possible contribution was examined by substituting the mouse amino acid for the human amino acid at each position. Therefore ~~gl~~gL221A (gL221 + D1Q, Q3V, L46R, L47W, see ~~Fig 20~~Figure 3 and Table 1) was made, cloned in EE6hCMVneo and ~~coexpressed~~co-expressed with cH (pJA144). The resultant antibody was well expressed and showed good binding activity ~~(Fig 25 and Table 2)~~. When the related genes gL221B (gL221 + D1Q, Q3V) and gL221C (gL221 + L46R, L47W) were made and similarly tested, while both genes produced antibody when ~~coexpressed~~co-expressed with cH, only the gL221C/cH combination showed good antigen binding ~~(Fig 25)~~. When the gL121A (gL121 + D1Q, Q3V, L46R, L47W) gene was made and ~~coexpressed~~co-expressed with cH, antibody was produced which also bound to antigen ~~(Fig 25)~~.

15.2. HEAVY CHAIN

15.2.1. EXTENT OF THE CDRs

For the heavy chain the loop and hypervariability analyses agree only in CDR3. For CDR1 the loop region extends from residues 26-32 inclusive whereas the Kabat CDR extends from residues 31-35 inclusive. For CDR2 the loop region is from 50-58 inclusive while the hypervariable region covers amino acids 50-65 ~~incusive~~inclusive. Therefore humanised heavy chains were constructed using the framework from antibody KOL and with various combinations of these CDR choices, including a shorter choice for CDR2 of 50-56 inclusive as there was some uncertainty as to the definition of the end point for the CDR2 loop around residues 56 to 58. The genes were ~~coexpressed~~co-expressed with mL or cL initially. In the case of the gH genes with loop choices for CDR1 ~~eg~~e.g. gH121, gH131, gH141 very little antibody was produced in the culture supernatants ~~(see Fig 27)~~. As no free light chain was detected it was presumed that the antibody was being made and assembled inside the cell but that the heavy chain was aberrant in some way, possibly incorrectly folded, and therefore the antibody was being degraded internally. In some experiments trace amounts of antibody could be detected in ^{35}S labelling studies ~~(see Fig 27).~~ .

As no net antibody was produced, analysis of these constructs was not pursued further.

When, however, a combination of the loop choice and the Kabat choice for CDR1 was tested (mouse amino acids 26-35 inclusive) and in which residues 31 (Ser to Arg) , 33 (Ala to Thr) , and 35 (Tyr to His) were changed from the human ~~residue~~residues to the mouse residue and compared to the first series, antibody was produced for gH321, kgH331 and kgH341 when ~~coexpressed~~co-expressed with cL ~~(Fig 27)~~. Expression was generally low and could not be markedly improved by the insertion of the Kozak consensus sequence 5' to the ATG of the signal sequence of the gene, as distinct from the case of the gL genes where such insertion led to a 2-5 fold increase in net antibody production ~~(compare Figs 24 and 26)~~. However, only in the case of gH341/mL or kgH341/cL could marginal antigen binding activity be demonstrated ~~(see Fig 28 and Table 2)~~. When the kgH341 gene was ~~coexpressed~~co-expressed with kgL221A, the net yield of antibody was too low ~~(see Figs 29A column 6 and 29C laneE)~~ to give a signal above the background level in the antigen binding assay ~~(see Fig 29A column 5)~~.

15.2.2. FRAMEWORK RESIDUES

As in the case of the light chain the heavy chain frameworks were ~~reexamined~~re-examined. Possibly because of the lower initial homology between the mouse and human heavy variable domains compared to the light chains, more amino acid positions proved to be of interest. Two genes~~,~~ kgH341A and kgH341B were constructed, with 11 or 8 human residues respectively substituted by mouse residues compared to gH341, and with the CDR2 residue 63 returned to the human amino acid potentially to improve domain packing. Both showed ~~good levels of expression with cL or kgL221A (Fig 29A) and both showed~~ antigen binding when combined with cL or kgL221A, the kgH341A gene with all 11 changes appearing to be the superior choice ~~(Fig 29B)~~.

15.3 INTERIM CONCLUSIONS

It has been demonstrated ~~here~~, therefore, for OKT3 that to transfer antigen binding ability to the humanised antibody, mouse residues outside the CDR regions defined by the Kabat hypervariability or structural loop choices are required for both the light and heavy chains. Fewer

extra residues are needed for the light chain, possibly due to the higher initial homology between the mouse and human ~~Kappa~~kappa variable regions.

Of the changes seven (1 and 3 from the light chain and 6, 23, 71, 73 and 76 from the heavy chain) are predicted from a knowledge of other antibody structures to be either partly exposed or on the antibody surface. It has ~~already~~ been shown here that residues 1 and 3 in the light chain are not absolutely required to be the mouse sequence; and for the heavy chain the gH341B heavy chain in combination with the 221A light chain ~~generates~~generated only weak binding activity ~~without~~. Therefore the presence of the ~~6 and 23 changes. It would be of interest to determine by~~6, 23 and 24 changes are important to maintain a binding affinity similar to that of the murine antibody. It was important, therefore, to further study the individual contribution of ~~the~~the other 8 mouse residues of the kgH341A gene compared to kgH~~341 and to determine whether the 7 extra mouse surface residues in the antibody produced by the kgH341A/kgL221A combination contribute to idiotypic epitopes which can be detected by sera from patients treated with murine OKT3.~~341.

16. FURTHER CDR-GRAFTING EXPERIMENTS

Additional CDR-grafted heavy chain genes were prepared substantially as described above. With reference to Table 2 the further heavy chain genes were based upon the gh341 (plasmid pJA178) and gH341A (plasmid pJA185) with either mouse OKT3 or human KOL residues at 6, 23, 24, 48, 49, 63, 71, 73, 76, 78, 88 and 91, as indicated. The CDR-grafted light -chain genes used in these further experiments were gL221, gL221A, gL221B and gL221C as described above.

TABLE 2

OKT3 HEAVY CHAIN CDR GRAFTS

1. gH341 and derivatives

RES NUM	6	23	24	48	49	63	71	73	76	78	88	91	
OKT3vh	Q	K	A	I	G	F	T	K	S	A	A	Y	
gH341	E	S	S	V	A	F	R	N	N	L	G	F	JA178
gH341A	Q	K	A	I	G	V	T	K	S	A	A	Y	JA185
gH341E	Q	K	A	I	G	V	T	K	S	A	G	G	JA198
gH341*	Q	K	A	I	G	V	T	K	N	A	G	F	JA207
gH341*	Q	K	A	I	G	V	R	N	N	A	G	F	JA209
gH341d	Q	K	A	I	G	V	T	K	N	L	G	F	JA197
gH341*	Q	K	A	I	G	V	R	N	N	L	G	F	JA199

gH341C	Q	K	A	V	A	F	R	N	N	L	G	F	JA184
gH341*	Q	S	A	I	G	V	T	K	S	A	A	Y	JA203
gH341*	E	S	A	I	G	V	T	K	S	A	A	Y	JA205
gH341B	E	S	S	I	G	V	T	K	S	A	A	Y	JA183
gH341*	Q	S	A	I	G	V	T	K	S	A	G	F	JA204
gH341*	E	S	A	I	G	V	T	K	S	A	G	F	JA206
gH341*	Q	S	A	I	G	V	T	K	N	A	G	F	JA208
KOL	E	S	S	V	A		R	N	N	L	G	F	

OKT3 LIGHT CHAIN CDR GRAFTS

2. gL221 and derivatives

RES NUM	1	3	46	47	
OKT3vl	Q	V	R	W	
GL221	D	Q	L	L	DA221
gL221A	Q	V	R	W	DA221A
gL221B	Q	V	L	L	DA221B
GL221C	D	Q	R	W	DA221C
RE1	D	Q	L	L	

MURINE RESIDUES ARE UNDERLINED

The CDR-grafted heavy and light chain genes were co-expressed in COS cells either with one another in various combinations but also with the corresponding murine and chimeric heavy and light chain genes substantially as described above. The resultant antibody products were then assayed in binding and blocking assays with BPS-ALL cells as described above.

The results of the assays for various grafted heavy chains co-expressed with the gL221C light chain are given in Figures 7 and 8 (for the JA184, JA185, JA197 and JA198 constructs - see Table 2), in Figure 9 (for the JA183, JA184 , JA185 and JA197 constructs) in Figure 10 (for the chimeric, JA185, JA199, JA204, JA205, JA207, JA208 and JA209 constructs) and in Figure 11 (for the JA183, JA184, JA185, JA198, JA203, JA205 and JA206 constructs).

The basic grafted product without any human to murine changes in the variable frameworks, i.e. gL221 co-expressed with gh341 (JAI78), and also the "fully grafted" product, having most human to murine changes in the grafted heavy chain framework, i.e. gL221C co-expressed with gh341A (JA185), were assayed for relative binding affinity in a competition assay against murine OKT3 reference standard, using HPB-ALL cells. The assay used was as described above in section 3.3. The results obtained are given in Figure 12 for the basic grafted product and in Figure 13 for the fully grafted product. These results indicate that the basic grafted product has neglibible binding ability as compared with the OKT3 murine reference standard; whereas the "fully grafted" product has a binding ability very similar to that of the OKT3 murine reference standard.

The binding and blocking assay results indicate the following:

The JA198 and JA207 constructs appear to have the best binding characteristics and similar binding abilities, both substantially the same as the chimeric and fully grafted gH341A products. This indicates that positions 88 and 91 and position 76 are not highly critical for maintaining the OKT3 binding ability; whereas at least some of positions 6, 23, 24, 48, 49, 71, 73 and 78 are more important.

This is borne out by the finding that the JA209 and JA199, although of similar binding ability to one another, are of lower binding ability than the JA198 and JA207 constructs. This indicates the importance of having mouse residues at positions 71, 73 and 78, which are either completely or partially human in the JA199 and JA209 constructs respectively.

Moreover, on comparing the results obtained for the JA205 and JA183 constructs it is seen that there is a decrease in binding going from the JA205 to the JA183 constructs. This indicates the importance of retaining a mouse residue at position 23, the only position changed between JA205 and JA183.

These and other results lead us to the conclusion that of the 11 mouse framework residues used in the gH341A (JA185) construct, it is important to retain mouse residues at all of positions 6, 23, 24, 48 and 49, and possibly for maximum binding affinity at 71, 73 and 78.

Similar Experiments were carried out to CDR-graft a number of the rodent antibodies including antibodies having specificity for CD4 (OKT4), ICAM-l (R6-5), TAG72 (B72.3), and TNF α(61E71, 101.4, hTNF1, hTNF2 and hTNF3).

EXAMPLE 2

CDR-GRAFTING OF A MURINE ANTI-CD4 T CELL RECEPTOR ANTIBODY, OKT4A

Anti OKT4A CDR-grafted heavy and light chain genes were prepared, expressed and tested substantially as described above in Example 1 for CDR-grafted OKT3. The CDR grafting of OKT4A is described in detail in Ortho patent application PCT/GB 90 ……. of even date herewith entitled "Humanised Antibodies". The disclosure of this Ortho patent application PCT/GB 90 ……. is incorporated herein by reference. A number of CDR-grafted OKT4

antibodies have been prepared. Presently the CDR-grafted OKT4A of choice is the combination of the grafted light chain LCDR2 and the grafted heavy chain HCDR10.

THE LIGHT CHAIN

The human acceptor framework used for the grafted light chains was RE1. The preferred LCDR2 light chain has human to mouse changes at positions 33, 34, 38, 49 and 89 in addition to the structural loop CDRs. Of these changed positions, positions 33, 34 and 89 fall within the preferred extended CDRs of the present invention (positions 33 and 34 in CDR1 and position 89 in CDR3). The human to murine changes at positions 38 and 49 corresponds to positions at which the amino acid residues are preferably donor murine amino acid residues in accordance with the present invention.

A comparison of the amino acid sequences of the donor murine light chain variable domain and the RE1 human acceptor light chain variable further reveals that the murine and human residues are identical at all of positions 46, 48 and 71 and at all of positions 2, 4, 6, 35, 36, 44, 47, 62, 64 - 69, 85, 87, 98, 99 and 101 and 102. However the amino acid residue at position 58 in LCDR2 is the human RE1 framework residue not the mouse OKT4 residue as would be preferred in accordance with the present invention.

THE HEAVY CHAIN

The human acceptor framework used for the grafted heavy chains was KOL.

The preferred CDR graft HCDR10 heavy chain has human to mouse changes at positions 24, 35, 57, 58, 60, 88 and 91 in addition to the structural loop CDRs.

Of these positions, positions 35 (CDR1) and positions 57, 58 and 60 (CDR2) fall within the preferred extended CDRs of the present invention. Also the human to mouse change at position 24 corresponds to a position at which the amino acid residue is a donor murine residue in accordance with the present invention. Moreover, the human to mouse changes at positions 88 and 91 correspond to positions at which the amino acid residues are optionally donor murine residues.

Moreover, a comparison of the murine OKT4A and human KOL heavy chain variable amino acid sequences reveals that the murine and human residues are identical at all of positions 23, 49, 71, 73 and 78 and at all of positions 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

Thus the OKT4A CDR-grafted heavy chain HCDR10 corresponds to a particularly preferred embodiment according to the present invention.

EXAMPLE 3

CDR-GRAFTING OF AN ANTI-MUCIN SPECIFIC MURINE ANTIBODY, B72.3

The cloning of the genes coding for the anti-mucin specific murine monoclonal antibody B72.3 and the preparation of B72.3 mouse-human chimeric antibodies has been described previously (ref. 13 and WO 89/01783). CDR-grafted versions of B72.3 were prepared as follows.

(a) B72.3 Light Chain

CDR-grafting of this light chain was accomplished by direct transfer of the murine CDRs into the framework of the human light chain RE1.

The regions transferred were:

CDR Number	Residues
1	24-34
2	50-56
3	90-96

The activity of the resulting grafted light chain was assessed by co-expression in COS cells, of genes for the combinations:

B72.3 cH/B72.3 cL
and B72.3 cH/B72.3 gL

Supernatants were assayed for antibody concentration and for the ability to bind to microtitre plates coated with mucin. The results obtained indicated that, in combination with the B72.3 cH chain, B72.3 cL and B72.3 gL had similar binding properties.

Comparison of the murine B72.3 and RE1 light chain amino acid sequences reveals that the residues are identical at positions 46, 58 and 71 but are different at position 48.

Thus changing the human residue to the donor mouse residue at position 48 may further improve the binding characteristics of the CDR-grafted light chain, (B72.3 gL) in accordance with the present invention.

(b) B72.3 heavy chain

i. Choice of framework

At the outset it was necessary to make a choice of human framework. Simply put, the question was as follows: Was it necessary to use the framework regions from an antibody whose crystal structure was known or could the choice be made on some other criteria?

For B72.3 heavy chain, it was reasoned that, while knowledge of structure was important, transfer of the CDRs from mouse to human frameworks might be facilitated if the overall homology between the donor and receptor frameworks was maximised. Comparison of the B72.3 heavy chain sequence with those in Kabat (ref. 4) for human heavy chains showed clearly that B72.3 had

poor homology for KOL and NEWM (for which crystal structures are available) but was very homologous to the heavy chain for EU.

On this basis, EU was chosen for the CDR-grafting and the following residues transferred as CDRs.

CDR Number	Residues
1	27-36
2	50-63
3	93-102

Also it was noticed that the FR4 region of EU was unlike that of any other human (or mouse) antibody. Consequently, in the grafted heavy chain genes this was also changed to produce a "consensus" human sequence. (Preliminary experiments showed that grafted heavy chain genes containing the EU FR4 sequence expressed very poorly in transient expression systems.)

ii. Results with grafted heavy chain genes

Expression of grafted heavy chain genes containing all human framework regions with either gL or cL genes produced a grafted antibody with little ability to bind to mucin. The grafted antibody had about 1% the activity of the chimeric antibody.

In these experiments, however, it was noted that the activity of the grafted antibody could be increased to ~10% of B72.3 by exposure to pHs of 2-3.5.

This observation provided a clue as to how the activity of the grafted antibody could be improved without acid treatment. It was postulated that acid exposure brought about the protonation of an acidic residue (pKa of aspartic acid = 3.86 and of glutamine acid = 4.25) which in turn caused a change in structure of the CDR loops, or allowed better access of antigen.

From comparison of the sequences of B72.3 (ref. 13) and EU (refs. 4 and 5), it was clear that, in going from the mouse to human frameworks, only two positions had been changed in such a way that acidic residues had been introduced. These positions are at residues 73 and 81, where K to E and Q to E changes had been made, respectively.

Which of these positions might be important was determined by examining the crystal structure of the KOL antibody. In KOL heavy chain, position 81 is far removed from either of the CDR loops.

Position 73, however, is close to both CDRs 1 and 3 of the heavy chain and, in this position it was possible to envisage that a K to E change in this region could have a detrimental effect on antigen binding.

iii. Framework changes in B72.3 gH gene

On the basis of the above analysis, E73 was mutated to a lysine (K). It was found that this change had a dramatic effect on the ability of the grafted Ab to bind to mucin. Further the ability of the

grafted B72.3 produced by the mutated gH/gL combination to bind to mucin was similar to that of the B72.3 chimeric antibody.

iv. Other framework changes

In the course of the above experiments, other changes were made in the heavy chain framework regions. Within the accuracy of the assays used, none of the changes, either alone or together, appeared beneficial.

v. Other

All assays used measured the ability of the grafted Ab to bind to mucin and, as a whole, indicated that the single framework change at position 73 is sufficient to generate an antibody with similar binding properties to B72.3.

Comparison of the B72.3 murine and EU heavy chain sequences reveals that the mouse and human residues are identical at positions 23, 24, 71 and 78.
Thus the mutated CDR-grafted B72.3 heavy chain corresponds to a preferred embodiment of the present invention.

CDR-GRAFTING OF A MURINE ANTI-ICAM-1 MONOCLONAL ANTIBODY

A murine antibody, R6-5-D6 (EP 0314863) having specificity for Intercellular Adhesion Molecule 1 (ICAM-l) was CDR-grafted substantially as described above in previous examples. This work is described in greater detail in co-pending application, British Patent Application No. 9009549.8, the disclosure of which is incorporated herein by reference.

The human EU framework was used as the acceptor framework for both heavy and light chains. The CDR-grafted antibody currently of choice is provided by co-expression of grafted light chain gL221A and grafted heavy chain gH341D which has a binding affinity for ICAM 1 of about 75% of that of the corresponding mouse-human chimeric antibody.

LIGHT CHAIN

gL221A has murine CDRs at positions 24-34 (CDR1), 50-56 (CDR2) and 89-97 (CDR3). In addition several framework residues are also the murine amino acid. These residues were chosen after consideration of the possible contribution of these residues to domain packing and stability of the conformation of the antigen binding region. The residues which have been retained as mouse are at positions 2, 3, 48 (?), 60, 84, 85 and 87. Comparison of the murine anti-ICAM 1 and human EU light chain amino acid sequences reveals that the murine and human residues are identical at positions 46, 58 and 71.

HEAVY CHAIN

gH341D has murine CDRs at positions 26-35 (CDR1), 50-56 (CDR2) and 94-100B (CDR3). In addition murine residues were used in gH341D at positions 24, 48, 69, 71, 73, 80, 88 and 91.

Comparison of the murine anti-ICAM 1 and human EU heavy chain amino acid sequences are identical at positions 23, 49 and 78.

EXAMPLE 5

CDR-Grafting of murine anti-TNFa antibodies

A number of murine anti-TNFa monoclonal antibodies were CDR-grafted substantially as described above in previous examples. These antibodies include the murine monoclonal antibodies designated 61 E71, hTNFl, hTNF3 and 101.4. A brief summary of the CDR-grafting of each of these antibodies is given below.

61E71

A similar analysis as described above (Example 1, Section 12.1.) was done for 61E71 and for the heavy chain 10 residues were identified at 23, 24, 48, 49, 68, 69, 71, 73, 75 and 88 as residues to potentially retain as murine. The human frameworks chosen for CDR-grafting of this antibody, and the hTNF3 and 101.4 antibodies were RE1 for the light chain and KOL for the heavy chain. Three genes were built, the first of which contained 23, 24, 48, 49, 71 and 73 [gH341(6)] as murine residues. The second gene also had 75 and 88 as murine residues [gH341(8)] while the third gene additionally had 68, 69, 75 and 88 as murine residues [gH341(10)]. Each was co-expressed with gL221, the minimum grafted light chain (CDRs only). The gL221/gH341(6) and gL221/gH341(8) antibodies both bound as well to TNF as murine 61E71. The gL221/gH341(10) antibody did not express and this combination was not taken further.

Subsequently the gL221/gH341(6) antibody was assessed in an L929 cell competition assay in which the antibody competes against the TNF receptor on L929 cells for binding to TNF in solution. In this assay the gL221/gH341(6) antibody was approximately 10% as active as murine 61E71.

hTNF1

hTNF1 is a monoclonal antibody which recognises an epitope on human TNF. The EU human framework was used for CDR-grafting of both the heavy and light variable domains.

Heavy Chain

In the CDR-grafted heavy chain (ghTNF1) mouse CDRs were used at positions 26-35 (CDR1), 50-65 (CDR2) and 95-102 (CDR3). Mouse residues were also used in the frameworks at positions 48, 67, 69, 71, 73, 76, 89, 91, 94 and 108. Comparison of the TNF1 mouse and EU human heavy chain residues reveals that these are identical at positions 23, 24, 29 and 78.

Light Chain

In the CDR-grafted light chain (gLhTNF1) mouse CDRs wre used at positions 24-34 (CDR1), 50-56 (CDR2) and 89-97 (CDR3). In addition mouse residues were used in the frameworks at

positions 3, 42, 48, 49, 83, 106 and 108. Comparison of the hTNF1 mouse and EU human light chain residues reveals that these are identical at positions 46, 58 and 71.

The grafted hTNF1 heavy chain was co-expressed with the chimeric light chain and the binding ability of the product compared with that of the chimeric light chain/chimeric heavy chain product in a TNF binding assay. The grafted heavy chain product appeared to have binding ability for TNF slightly better than the fully chimeric product.

Similarly, a grafted heavy chain/grafted light chain product was co-expressed and compared with the fully chimeric product and found to have closely similar binding properties to the latter product.

hTNF3

hTNF3 recognises an epitope on human TNF-α. The sequence of hTNF3 shows only 21 differences compared to 61E71 in the light and heavy chain variable regions, 10 in the light chain (2 in the CDRs at positions 50, 96 and 8 in the framework at 1, 19, 40, 45, 46, 76, 103 and 106) and 11 in the heavy chain (3 in the CDR regions at positions 52, 60 and 95 and 8 in the framework at 1, 10, 38, 40, 67, 73, 87 and 105). The light and heavy chains of the 61E71 and hTNF3 chimeric antibodies can be exchanged without loss of activity in the direct binding assay. However 61E71 is an order of magnitude less able to compete with the TNF receptor on L929 cells for TNF-a compared to hTNF3. Based on the 61E71 CDR grafting data gL221 and gH341(+23, 24, 48, 49 71 and 73 as mouse) genes have been built for hTNF3 and tested and the resultant grafted antibody binds well to TNF-a, but competes very poorly in the L929 assay. It is possible that in this case also the framework residues identified for OKT3 programme may improve the competitive binding ability of this antibody.

101. 4

101.4 is a further murine monoclonal antibody able to recognise human TNF-a. The heavy chain of this antibody shows good homology to KOL and so the CDR-grafting has been based on RE1 for the light chain and KOL for the heavy chain. Several grafted heavy chain genes have been constructed with conservative choices for the CDR's (gH341) and which have one or a small number of non-CDR residues at positions 73, 78 or 77-79 inclusive, as the mouse amino acids. These have been co-expressed with cL or gL221. In all cases binding to TNF equivalent to the chimeric antibody is seen and when co-expressed with cL the resultant antibodies are able to compete well in the L929 assay. However, with gL221 the resultant antibodies are at least an order of magnitude less able to compete for TNF against the TNF receptor on L929 cells.

Mouse residues at other positions in the heavy chain, for example, at 23 and 24 together or at 76 have been demonstrated to provide no improvement to the competitive ability of the grafted antibody in the L929 assay.

A number of other antibodies including antibodies having specificity for interleukins e.g. IL1 and cancer markers such as carcinoembryonic antigen (CEA) e.g. the monoclonal antibody A5B7 (ref. 21), have been successfully CDR-grafted according to the present invention.

It will be appreciated that the foregoing examples are given by way of illustration only and are not intended to limit the scope of the claimed invention. Changes and modifications may be made to the methods described whilst still falling within the spirit and scope of the invention.

ABSTRACT

CDR-grafted antibody heavy and light chains comprise acceptor framework and donor antigen binding regions, the heavy chains comprising donor residues at at least one of positions (6, 23) and/or (24, 48) and/or (49, 71) and/or (73, 75) and/or (76) and/or (78) and (88) and/or (91). The CDR-grafted light chains comprise donor residues at at least one of positions (1) and/or (3) and (46) and/or (47) or at at least one of positions (46, 48, 58) and (71).

The CDR-grafted antibodies are preferably humanised antibodies, having non human, e.g. rodent, donor and human acceptor frameworks, and may be used for in vivo therapy and diagnosis. A generally applicable protocol is disclosed for obtaining CDR-grafted antibodies.

References

1. Kohler & Milstein, Nature, 265, 295-497, 1975.

2. Chatenoud et al, (1986), J. Immunol. 137, 830-838.

3. Jeffers et al, (1986), Transplantation, 41, 572-578.

4. Begent et al, Br. J. Cancer 62: 487 (1990).

5. Verhoeyen et al, Science, 239, 1534-1536, 1988.

~~3.~~ 6. Riechmann et al, Nature, 332, 323-324, 1988.

~~4.~~ 7. Kabat, E.A., Wu, T.T., Reid-Miller, M., Perry, H.M., Gottesman, K.S., 1987, in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA.

~~5.~~ 8. Wu, T.T., and Kabat, E.A., 1970, J. Exp. Med. ~~132,~~132 211-250.

9. Queen et al, (1989), Proc. Natl. Acad. Sci. USA, 86, 10029-10033 and WO 90/07861

~~6.~~10. Maniatis et al, Molecular Cloning, Cold Spring Harbor, New York, ~~1982.~~1989.

11. Primrose and Old, principles of Gene Manipulation, Blackwell, Oxford, 1980.

~~7.~~12. Sanger, F., Nicklen, S., Coulson, A.R., 1977, Proc. Natl. Acad. Sci. USA, 74 ~~5463.~~5463

~~8.~~ 13. Kramer, W., Drutsa, V., Jansen, H.-W., Kramer, B., Plugfelder, M., Fritz, H.-J., 1984, Nucl. Acids Res. ~~12 9441.~~12, 9441

~~9.~~ 14. Whittle, N., Adair, J., Lloyd, J.C., Jenkins, E., Devine, J., Schlom, J., Raubitshek, A., Colcher, D., Bodmer, M., 1987, Protein Engineering 1, 499.

~~10.~~ 15. Sikder, S.S., Akolkar, P.N., Kaledas, P.M., Morrison, S.L., Kabat, E.A., 1985, J. Immunol. 135, 4215.

~~11.~~ 16. Wallick, S.C., Kabat, E.A., Morrison, S.L., 1988, J. Exp. Med. 168, ~~1099.~~1099

~~12.~~ 17. Bebbington, C.R., Published International Patent Application WO 89/01036.

~~13.~~ 18. Granthan and Perrin 1986, Immunology Today 7, 160.

~~14.~~19. Kozak, M., 1987, J. Mol. Biol. 196, 947.

~~15.~~ 20. Jones, T.P., Dear, P.H., Foote, J., Neuberger, M.S., Winter, G., 1986, Nature, 321, ~~522.~~522

21. Harwood et al, Br. J. Cancer, 54, 75-82 (1986).

CLAIMS

1. A CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

2. A CDR-grafted heavy chain according to Claim 1 comprising donor residues at positions 23, 24, 49, 71, 73 and 78, or at positions 23, 24 and 49.

3. A CDR-grafted heavy chain according to Claim 2 comprising donor residues at positions 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

4. A CDR-grafted heavy chain according to Claim 2 or 3, comprising donor residues at one, some or all of positions:
1 and 3,
69 (if 48 is different between donor and acceptor),
38 and 46 (if 48 is the donor residue),
67,
82 and 18 (if 67 is the donor residue),
91, and
any one or more of 9, 11, 41, 87, 108, 110 and 112.

5. A CDR-grafted heavy chain according to any of the preceding comprising donor CDRs at positions 26-35, 50-65 and 95-100.

6. A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 1 and/or 3 and 46 and/or 47.

7. A CDR-grafted light chain according to Claim 6 comprising donor residues at positions 46 and 47.

8. A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, 58 and 71.

9. A CDR-grafted light chain according to Claim 8 comprising donor residues at positions 46, 48, 58 and 71.

10. A CDR-grafted light chain according to Claim 8 or 9, comprising donor residues at positions 2, 4, 6, 35, 36, 38, 44, 47, 49, 62, 64-69, 85, 87, 98, 99, 101 and 102.

11. A CDR-grafted light chain according to Claim 9 or 10, comprising donor residues at one, some or all of positions:
1 and 3,
63,
60 (if 60 and 54 are able to form a potential saltbridge),
70 (if 70 and 24 are able to form a potential saltbridge),
73 and o 21 (if 47 is different between donor and acceptor) ,
37 and 45 (if 47 if different between donor and acceptor), and
any one or more of 10, 12, 40, 83, 103 and 105.

12. A CDR-grafted light chain according to anyone of Claims 6-11, comprising donor CDRs at positions 24-34, 50-56 and 89-97.

13. A CDR-grafted antibody molecule comprising at least one CDR-grafted heavy chain according to anyone of Claims 1-5 and at least one CDR-grafted light chain according to anyone of Claims 6-12.

14. A CDR-grafted antibody molecule according to Claim 13, which is a site-specific antibody molecule.

15. A CDR-grafted antibody molecule according to Claim 13 which has specificity for an interleukin, hormone or other biologically active compound or a receptor therefor.

16. A CDR-grafted antibody heavy or light chain or molecule according to anyone of the preceding claims comprising human acceptor residues and non-human donor residues.

17. A DNA sequence which codes for a CDR-grafted heavy chain according to Claim 1 or a CDR-grafted light chain according to Claim 6 or Claim 8.

18. A cloning or expression vector containing a DNA sequence according to Claim 17.

19. A host cell transformed with a DNA sequence according to Claim 17.

20. A process for the production of a CDR-grafted antibody sequence according to Claim 17 in a transformed host cell.

21. A process for producing a CDR-grafted antibody product comprising:

(a) producing in an expression vector an operon having a DNA sequence which encodes an antibody heavy chain according to Claim 1;

and/or

(b) producing in an expression vector an operon having a DNA sequence which encodes a complementary antibody light chain according to Claim 6 or Claim 8;

(c) transfecting a host cell with the or each vector;

and

(d) culturing the transfected cell line to produce the CDR-grafted antibody product.

22. A therapeutic or diagnostic composition comprising a CDR-grafted antibody heavy chain according to Claim 1, or a CDR-grafted light chain according to Claim 6 or Claim 8, or a CDR-grafted antibody molecule according to Claim 13 in combination with a pharmaceutically acceptable carrier, diluent or excipient.

23. A method of therapy or diagnosis comprising administering an effective amount of a CDR-grafted heavy chain according to Claim 1, or a CDR-grafted light chain according to Claim 6 or Claim 8, or a CDR-grafted antibody molecule according to Claim 13 to a human or animal subject.

Legend:	
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Statistics:	
	Count
Insertions	743
Deletions	424
Moved from	11
Moved to	11
Style change	0
Format changed	0
Total changes	1189

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Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
07/743,329	09/17/91	ADAIR	J CARP-0009

EXAMINER
BENNETT, L

ART UNIT	PAPER NUMBER
1807	11

DATE MAILED: 11/18/92

FRANCIS A. PAINTIN
WOODCOCK, WASHBURN, KURTZ, MACKIEWICZ & NORRIS
ONE LIBERTY PLACE-46TH FLOOR
PHILADELPHIA, PA 19103

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

☒ This application has been examined ☐ Responsive to communication filed on _____ ☐ This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s), 0 days from the date of this letter. Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☒ Notice of References Cited by Examiner, PTO-892.
2. ☒ Notice re Patent Drawing, PTO-948.
3. ☐ Notice of Art Cited by Applicant, PTO-1449.
4. ☐ Notice of Informal Patent Application, Form PTO-152.
5. ☐ Information on How to Effect Drawing Changes, PTO-1474.
6. ☐ _____

Part II SUMMARY OF ACTION

1. ☒ Claims 1-23 are pending in the application.
   Of the above, claims 0 are withdrawn from consideration.
2. ☐ Claims _____ have been cancelled.
3. ☐ Claims _____ are allowed.
4. ☒ Claims 1-23 are rejected.
5. ☒ Claims 1-23 are objected to.
6. ☒ Claims 0 are subject to restriction or election requirement.
7. ☒ This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.
8. ☐ Formal drawings are required in response to this Office action.
9. ☐ The corrected or substitute drawings have been received on _____. Under 37 C.F.R. 1.84 these drawings are ☐ acceptable. ☐ not acceptable (see explanation or Notice re Patent Drawing, PTO-948).
10. ☐ The proposed additional or substitute sheet(s) of drawings, filed on _____ has (have) been ☐ approved by the examiner. ☐ disapproved by the examiner (see explanation).
11. ☐ The proposed drawing correction, filed on _____, has been ☐ approved. ☐ disapproved (see explanation).
12. ☐ Acknowledgment is made of the claim for priority under U.S.C. 119. The certified copy has ☐ been received ☐ not been received ☐ been filed in parent application, serial no. _____; filed on _____.
13. ☐ Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.
14. ☐ Other

EXAMINER'S ACTION

PTOL-326 (Rev. 9-89)

15. The disclosure is objected to because the specification is replete with misspellings including "humanised" and "humanisation" instead of "humanized" and "humanization", respectively; "recognizes" on page 2, line 22, and page 4, lines 2 and 4 instead of "recognizes"; "chimerisation" on page 3, line 8 instead of "chimerization"; "maximise/optimise" on page 11, lines 18 and 19 instead of "maximize/optimize". The specification should be reviewed and amended to identify and correct other such misspellings. Appropriate correction is required.

16. Claims 5, 11-16, 22 and 23 are objected to under 37 CFR 1.75(c) as being in improper form because a multiple dependent claim cannot depend from any other multiple dependent claim. See MPEP 608.01(n).

17. Claims 1-23 are objected to over the recitation of "CDR-grafted" because abbreviations and acronyms are not appropriate in claim language since the same abbreviation can represent multiple terms. This objection by be overcome by amending the claims to recite instead "Complementarity determining region- grafted".

18. 35 U.S.C. 101 reads as follows:

> "Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter or any new and useful improvement thereof, may obtain a patent therefore, subject to the conditions and requirements of this title".

A) Claims 1-12 are rejected under 35 U.S.C. 101 because the claimed invention is inoperative and therefore lacks utility. Claims 1-5 are drawn to CDR-grafted antibody heavy chains having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises comprises donor residues at particular positions. Claims 6-12 are drawn to CDR-grafted antibody light chains having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at

particular positions. The specification discloses that a few particular CDR-grafted heavy chains when co-expressed with chimeric light chains, unmodified light chains or in one case a CDR-grafted light chain in COS cells retain similar binding affinity for the specific antigen as the chimeric antibody or the unmodified donor antibody. The specification also presents a few examples of co-expression of CDR-grafted light chains with chimeric or unmodified heavy chains where the resulting antibody possesses antigen binding affinity similar to the chimeric antibody. However, the specification does not show CDR-grafted antibody heavy or light chains alone which have the ability to bind antigen. Since antigen binding and therapeutic application are the only utility disclosed in the present application and since the prior art does not generally teach that isolated heavy and light antibody chains can bind antigen, a showing that isolated heavy and light chains have useful antigen binding utility would be required to support the operability. The single chains will not form a complete receptor structure for the antigen epitope for which the complete antibody is specific.

B) Claim 17 is rejected under 35 U.S.C. 101 because the claimed invention is drawn to non-statutory subject matter. Claim 17 is drawn to a DNA sequence coding for a CDR-grafted heavy or light chain. However, "DNA sequences" are not patentable because they are algorithms. M.P.E.P. 608.01 (P). This rejection may be overcome by amending the claims to recite "DNA molecule" instead of "DNA sequence".

C) Claims 22-23 are rejected under 35 U.S.C. 101 because the invention is inoperative and therefore lacks patentable utility. Claim 22 is drawn to a therapeutic or diagnostic composition comprising a CDR-grafted antibody heavy chain or a CDR grafted light chain or a CDR-grafted antibody molecule in combination with a pharmaceutical carrier. Claim 23 is drawn to a method of therapy or diagnosis comprising administering an effective amount of a CDR grafted heavy chain or a CDR grafted light chain or a CDR-grafted antibody to a human or animal subject. The specification fails to establish the utility of the claimed method and pharmaceutical composition using a CDR-grafted antibody in humans or any other animal. The specification does not present any **in vivo** or **in vitro** data to support the

utility of any CDR-grafted heavy chain, light chain or complete antibody molecule. Pharmaceutical therapy reshaped monoclonal antibodies is unpredictable in the absence of **in vivo** clinical data for the following reasons: (1) The antibody or the heavy and light chains may be inactivated before producing an effect such as by proteolytic degradation or due to an inherently short half-life of the modified antibody; (2) The humanized antibody or antibody chain may otherwise not reach the target area because the reshaped antibody may be absorbed by fluid cells or tissues where the antibody has no effect or the humanized antibody; (3) The reshaped antibody and antibody chains may have reduced affinity for the antigen, may be incapable of effector functions or both and as a consequence the antibody may not function as the original donor antibody; (4) The reshaped antibody may still be treated by the human or animal subject as a foreign antigen and result in a rejection reaction. See M.P.E.P. 608.01(P).

19. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> "The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention."

The specification is objected to under 35 U.S.C. 112, first paragraph, as failing to adequately teach how to make and use the claimed invention. Claims 1-5 are drawn to CDR-grafted antibody heavy chains having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises comprises donor residues at particular positions. Claims 6-12 are drawn to CDR-grafted antibody light chains having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at particular positions. The specification does not enable the skilled artisan to make and use the claimed CDR-grafted antibody heavy and light chains. The specification does not provide guidance and working examples

showing how to make and use antibody heavy and light chains to bind antigen. Since this is the basis of the utility disclosed in the present application for the claimed antibodies and antibody chains, the specification must demonstrate the operability of isolated antibody heavy and light chains for binding antigen. The specification only demonstrates the ability of CDR-grafted antibodies containing both a heavy and a light chain. The prior art also does not teach that isolated antibody heavy and light chains have the ability to generally bind antigen similar to the complete double chain antibody. Therefore, the isolated heavy and light chains appear to be inoperable for binding antigen as discussed above in the rejection made under 35 U.S.C. 101, and as a consequence undue experimentation would be required of the skilled artisan in order to practice the claimed invention.

Claims 1-12 are rejected under 35 U.S.C. 112, first paragraph, for the reasons set forth in the objection to the specification.

20. The specification is objected to under 35 U.S.C. 112, first paragraph, as failing to adequately teach how to make and use the claimed invention. Claim 22 is drawn to a therapeutic or diagnostic composition comprising a CDR-grafted antibody heavy chain or a CDR grafted light chain or a CDR-grafted antibody molecule in combination with a pharmaceutical carrier. Claim 23 is drawn to a method of therapy or diagnosis comprising administering an effective amount of a CDR grafted heavy chain or a CDR grafted light chain or a CDR-grafted antibody to a human or animal subject. The present specification does not enable the skilled artisan to make and use CDR-grafted antibody heavy, light antibody chains or complete antibodies as therapeutic or diagnostics. The specification provides no guidance or working examples to support the operability of the claimed therapeutic/diagnostic composition and method of treatment. The prior art also does not teach that CDR-grafted antibodies have general operability in treating disease in human or non-human subjects. The claimed invention appears to be inoperable without supporting **in vivo** data for the reasons discussed in the rejection made under 35 U.S.C. 101. Therefore undue

experimentation would be required of the skilled artisan in order to practice the claimed invention.

Claims 22 and 23 are rejected under 35 U.S.C. 112, first paragraph, for the reasons set forth in the objection to the specification.

21. Claims 13-16 are rejected under 35 U.S.C. 112, first paragraph, as the disclosure is enabling only for claims limited to specific CDR-grafted antibodies disclosed in the specification as having effective binding affinities for their specific antibody, i.e. which are similar to the non-humanized donor antibodies. The claims are not commensurate in scope with the present disclosure. Insufficient guidance and working examples are provided in the specification to support the broad claims drawn to any CDR-grafted antibodies which contain donor residues at the recited framework amino acid positions for the heavy and light chains. The specification does not sufficiently develop the concept that there are certain framework amino acids which when changed in the acceptor sequence to be the same in the donor sequence result in an increase in antigen binding affinity. The specification does describe several examples where particular framework amino acid changes result in increased antigen binding affinity, such as for OKT3, OKT4 and Anti-ICAM. However the specification does not clearly establish that every time the recited amino acid positions are the same between donor and acceptor, "good" binding to antigen is observed. The specification does not provide actual binding values for most of the examples but instead qualitatively describes the binding of the humanized antibody to antigen. Furthermore, in light of the prior art (for instance, Reichmann et al. and Queen et al. Chothia et al.) , such a universal property appears to be unpredictable since different antibodies will have different amino acids in the framework which are important in antigen binding and stability. The prior art does not teach that a standardized principle of which amino acids must always be changed is possible, but instead appears to teach that three dimensional structures of the antibodies and an understanding of protein folding properties, is necessary to be able to reasonable predict which amino acids will always be effect in increasing of retaining antigen binding ability. Therefore, this analysis shows that undue experimentation would be

required of the skilled artisan in order to practice the claimed invention. See MPEP 706.03(n) and 706.03(z).

22. Claims 1-23 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

A) Claims 1-5 are indefinite over the recitation of "at least one of positions 6,23 and/or 24,48 and/or 49,71 and/or 73,75 and/or 76 and/or 78 and 88 and/or 91". because the claims is unclear as to whether the antibody heavy chain has at least one of 6,23,24,48,49,71,73,75,76,78,88, or 91 or alternatively has at least one (6) or (23 and/or 24) or (48 and/or 49) or (71 and/or 73) or (75 and/or 76 and/or 78 and 88 and/or 91) or alternatively has at least one (6,23) and/or (24,48) and/or (49,71) and/or (73,75) and (76) and/or (78 and 88) and/or (91). The claims are further indefinite because these position numbers are arbitrary unless they are identified as positions in a Figure or relative to an identified' numbered sequence, for example. These position numbers are not identified as positions in a amino acid sequence or in a nucleotide sequence.

B) Claims 6-12 are indefinite over the recitation of "at least one of positions 1 and/or 3 and 46 and/or 47". The claims are unclear with regard to whether the light chain contains donor residues at at least one of positions 1,3, 46 and/or 47 or alternatively at at least one of (1 and/or 3) and (46 and/or 47) or alternatively (1) and/or (3 and 26) and/or (47). The claims are further indefinite because these position numbers are arbitrary unless they are identified as positions in a Figure or relative to an identified numbered sequence, for example. These position numbers are not identified as positions in a amino acid sequence or in a nucleotide sequence.

C) Claims 4 and 11 are indefinite and confusing because they are in an improper Markush listing. Correction is required.

D) Claim 21 is indefinite over the recitation of "a complementary antibody light chain" in step (b) because the method is unclear as to what "complementary" refers. Claim 21 is further indefinite over the recitation of "with the or each vector" in step (c) because the method is unclear as to which of the two expression vectors is "the vector" or if "the vector" refers to a different vector which lacks antecedent basis. Additionally step (c) is unclear with regard to whether the same host cell is transfected with both vectors or whether the vectors are transfected into different host cells. Finally, claim 21 is unclear because the recitation of "the transfected cell line" lacks antecedent basis.

23. The following is a quotation of the appropriate paragraphs of 35 U.S.C. § 102 that form the basis for the rejections under this section made in this Office action:

"A person shall be entitled to a patent unless -

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States."

A) Claims 1, 5, 6-8, 12-22 are rejected under 35 U.S.C. § 102 (b) as being anticipated by Riechmann et al. Claim 1 is drawn to a CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6,23, and/or 24,48 and/or 49,71 and/or 73,75 and/or 76 and/or 78 and 88 and/or 91. Claim 5 is further limited wherein the donor CDRs are at positions 26-35, 50-65 and 95-100. Claim 6 is drawn to a CDR-grafted antibody light chain having a variable domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 1 and/or 3 and 46 and/or 47. Claim 7 is further limited wherein the donor residues are at positions 46 and 47. Claim 8 is drawn to a CDR-

grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48,, 58 and 71. Claim 13 is drawn to a CDR-grafted antibody molecule comprising at least one CDR-grafted antibody heavy chain of claims 1-5 and at least one CDR-grafted antibody light chain of claims 6-12. Claims 14 and 15 are further limited wherein the antibody is a site-specific antibody molecule (claim 14) and wherein the antibody has specificity for an interleukin, hormone, other biologically active compound. Claim 16 is drawn to a CDR-grafted antibody heavy or light chain or molecule comprising human acceptor residues and non-human donor residues. Claim 17 is drawn to DNA encoding any of the above antibody chains. Claim 18 is drawn to an expression vector and claim 19 is drawn to a host cell transformed with the expression vector. Claim 20 is drawn to a method of making the antibody from the transformed cell and claim 21 is drawn to a process comprising producing an expression vector encoding the heavy chain and another expression vector encoding the light, transfecting a host cell with the vectors and culturing the transfected cell to make the CDR-grafted antibody product.

Riechmann et al. teach a CDR-grafted antibody heavy chains (Figure 1(a)), a CDR-grafted antibody light chain (Figure 1(b)) and a CDR-grafted antibody molecule containing a CDR-grafted heavy chain and a CDR grafted light chain (Figure 2) having a variable region domain comprising human acceptor framework (human NEW for the heavy chain and RE1 for the light chain) and rat donor antigen binding regions (heavy chain CDRs: CDR1 at 31-35, CDR2 at 50-65, and CDR3 at 95-102; light chain CDRs: CDR1 at 24-34, CDR2 at 50-56, CDR3 at 89-97), wherein the framework comprises rat donor residues at the following positions. For the heavy chain rat donor residues are present at amino acid positions 6, 49, 76, 88, 91, and for the light chain rat donor residues are at positions 1, 46 and 47, 48 and 71. Riechmann et al. also teach the DNA encoding these humanized antibodies in Figure 1, expression vectors containing the DNA encoding the reshaped antibodies (see description of Figure 2), a host cell transfected with these expression vectors, i.e lymphoid cells lines (see description of Figure 2) and a process for the production of the humanized antibodies comprising making an expression

vector for the reshaped heavy chain and another expression vector for the light chain, transfecting a host cell with both of these vectors and culturing the host cells so that the heavy and light chains are co-expressed. (description of Figure 2). Applicant should note that claim 1 and claim 6 are being interpreted to mean that the framework has donor residues at at least one of any of positions 6,23,24,48,49,71,73,75,76,78,88 or 91 in the heavy chain and (1,3,46 or 47) or (46,48,58 or 71) in the light chain. Therefore, the teachings of Riechmann et al. anticipate the invention as claimed.

B) Claims 1-6 and 12-22 are rejected under 35 U.S.C. § 102 (b) as being anticipated by Queen et al. Claim 1 is drawn to a CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6,23, and/or 24,48 and/or 49,71 and/or 73,75 and/or 76 and/or 78 and 88 and/or 91. Claims 2-5 are further limited wherein donor residues are at positions 23,24,49,71,73,78 or 23,24 and 49 (claim 2), wherein donor residues are additionally present at positions 2,4,6,25,36,37,39,47,48,93,94,103,104,106 and 107 (claim 3), wherein donor residues are additionally present at position at at least on of positions 1 and 3, 69, 38 and 46, 67 82 and 18, 91 and any of 9,11,41,87,108,110 and 112 (claim 4) and wherein the donor CDRs are at positions 26-35, 50-65 and 95-100. Claim 6 is drawn to a CDR-grafted antibody light chain having a variable domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 1 and/or 3 and 46 and/or 47. Claim 12 further limited wherein the donor CDRs are at positions 24-34, 50-56 and 89-97. Claim 13 is drawn to a CDR-grafted antibody molecule comprising at least one CDR-grafted antibody heavy chain of claims 1-5 and at least one CDR-grafted antibody light chain of claims 6-12. Claims 14 and 15 are further limited wherein the antibody is a site-specific antibody molecule (claim 14) and wherein the antibody has specificity for an interleukin, hormone, other biologically active compound. Claim 16 is drawn to a CDR-grafted antibody heavy or light chain or molecule comprising

human acceptor residues and non-human donor residues. Claim 17 is drawn to DNA encoding any of the above antibody chains. Claim 18 is drawn to an expression vector and claim 19 is drawn to a host cell transformed with the expression vector. Claim 20 is drawn to a method of making the antibody from the transformed cell and claim 21 is drawn to a process comprising producing an expression vector encoding the heavy chain and another expression vector encoding the light, transfecting a host cell with the vectors and culturing the transfected cell to make the CDR-grafted antibody product.

Queen et al. teach a CDR-grafted anti-Tac antibody using a human antibody framework (EU) as acceptor and murine anti-Tac antibody as donor. The antibody heavy chain of Queen et al. as murine amino acids at positions 2,4,6,23,24,25,36,37,39,47,48,49,71,73,78,93,94,103,104,106 and 107 and additionally at positions 1,3,69,67,9,41,108 and 110. (see Figure 2). The humanized antibody of Queen et al. contains murine CDRs at positions 26-35, 50-56 and 95-100. Queen et al. also teach a humanized antibody light chain having a donor amino acid at position 47 and CDRs at positions 24-34, 50-56 and 89-97. Queen et al. further teach the cDNA encoding the the humanized heavy and light chains (page 10030, col. 1 and 10031), the insertion of these cDNAs in expression vectors (10030 col 1-col 2), the transfection of host cells with these expression vectors(10031 col. 2) and the co-expression of the cDNAs to produce a CDR-grafted antibody molecule (10031, col. 2 and 10032). Therefore, since every recited claim limitation is taught by Queen et al., the invention, as claimed, is anticipated by Queen et al.

24. The following is a quotation of 35 U.S.C. 103 which forms the basis for all obviousness rejections set forth in this Office action:

> A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary

skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Subject matter developed by another person, which qualifies as prior art only under subsection (f) and (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103, the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of potential 35 U.S.C. 102(f) or (g) prior art under 35 U.S.C. 103.

Claims 1-21 are rejected under 35 U.S.C. 103 as being unpatentable over Riechmann et al. and Queen et al. Both Riechmann et al. and Queen et al. teach how to make humanized antibodies using a human antibody variable domain framework as an acceptor and a rat antibody (in the case of Riechmann et al.) or a murine antibody (in the case of Queen et al.) as the complementarity determining region donor. Both of these references also teach how to identify framework amino acids which are important for retaining the binding effective conformation of the CDRs. Specifically, Queen et al. teach that the more homologous the human antibody to the murine antibody the less likely would combining the anti-Tac CDRs with the human framework be to introduce distortions into the CDRs. Therefore, Queen et al. teach making a database comparison of all known human antibodies with the donor antibody to determine the most similar human antibody to use as the framework (page 10031, col. 2, paragraph 2). Queen et al. further teach making a molecular model of the donor variable domain (in this case the anti-Tac V domain) based on homology to other antibody V domains whose crystal structure is known. By doing so Queen et al. teach that amino acids

outside the CDRs which are close enough to the CDRs to influence the CDR conformation or to directly interact with the antigen. When the residues where different between the human and the donor murine antibodies, the human framework amino acid was changed to the corresponding murine amino acid. (page 10031, col. 2, paragraph 3). Finally, when the human acceptor antibody contains unusual amino acids with respect to consensus sequences in homologous antibodies, Queen recommends changing these amino acids to the consensus amino acid (page 10032, col. 1). Riechmann et al. and Queen et al. further teach that different changes will be necessary depending of the specific donor and acceptor antibodies which are used. Both references teach the cDNA encoding the heavy and light antibody chains which are the templates for making the specific changes in the sequences of CDR-grafted antibodies. The references also both teach the insertion of the cDNAs into vectors, transfection into host cells and co-expression of the heavy and light chains to result in the expression of a complete CDR-grafted antibody molecule.

Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to use the guidelines taught Riechmann et al. and Queen et al. to reshape any given antibody to "humanize" that antibody by making changes in the framework regions of the human acceptor to the donor residue when those residues are close to the CDRS and when those amino acids affect the conformation of the CDRS. One of ordinary skill would have been motivated to make the changes in the framework regions from the human amino acid to the donor amino acid in order to achieve the expected benefit of increasing the binding affinity of the humanized antibody for the specific antigen over the binding affinity observed in the humanized antibodies which do not contain the framework changes as taught by Queen et al. (page 10032, col. 1, paragraph 3 through col. 2) and Riechmann et al. (Figure 4).

25. No claims are allowable.

26. Papers relating to this application may be submitted to Group 180 by facsimile transmission. Papers should be faxed to Group 180 via the P.T.O. Fax Center located in Crystal Mall 1. The CM1 Fax Center number is (703) 308-4227. Papers may be submitted Monday-Friday between 8:00 am and 4:45 pm (EST). Please note that the faxing of such papers must conform with the Notice to comply in the Official Gazette, 1096 OG 30 (November 15, 1989).

27. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Lisa T. Bennett whose telephone number is (703) 308-3988. Any inquiry of a general nature or relating to the status of an application should be directed to the Group 180 receptionist whose telephone number is (703) 308-0196.

LTB
Lisa T. Bennett
November 14, 1992

MARGARET MOSKOWITZ
SUPERVISORY PATENT EXAMINER
GROUP 180

08/303569

FORM PTO-892 (REV. 3-78)	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	SERIAL NO.	GROUP ART UNIT	ATTACHMENT TO PAPER NUMBER	
NOTICE OF REFERENCES CITED		~~07/743,329~~	1807		11
		APPLICANT(S) ADAIR ET AL.			

U.S. PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE
	A						
	B						
	C						
	D						
	E						
	F						
	G						
	H						
	I						
	J						
	K						

FOREIGN PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	COUNTRY	NAME	CLASS	SUB-CLASS	PERTINENT SHTS. DWG.	PERTINENT PP. SPEC.
	L								
	M								
	N								
	O								
	P								
	Q								

OTHER REFERENCES (Including Author, Title, Date, Pertinent Pages, Etc.)

R CHOTHIA, CYRUS ET AL (Dec. 1989) NATURE, "CONFORMATIONS OF IMMUNOGLOBULIN HYPERVARIABLE REGIONS," VOLUME 342, pp. 877-883.

S QUEEN, C. ET AL (DEC. 1989) PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, "A HUMANIZED ANTIBODY THAT BINDS TO INTERLEUKIN 2 RECEPTOR"

T ~~RIECHM~~ VOLUME 86, pp. 10029-10033,

U RIECHMANN ET AL (MARCH 1988) NATURE, "RESHAPING HUMAN ANTIBODIES FOR THERAPY," VOLUME 332, pp. 323-327.

EXAMINER	DATE
Lisa L. Bennett	11-14-92

* A copy of this reference is not being furnished with this office action.
(See Manual of Patent Examining Procedure, section 707.05 (a).)

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
743,329	8/16/91	Adair et al.	CARP-0009

EXAMINER	
Lisa Bennett	
ART UNIT	PAPER NUMBER
1807	18

DATE MAILED:

EXAMINER INTERVIEW SUMMARY RECORD

All participants (applicant, applicant's representative, PTO personnel):

(1) Lisa Bennett (3) LIZA HOHENSCHUTZ

(2) Scott Chambers (4) FRANCIS A. PAINTIN
GALE MATTHEWS PHILLIP ANSELL
CHRIS MERCER
JOHN ADAIR

Date of interview 1/27/93

Type: ☐ Telephonic ☑ Personal (copy is given to ☐ applicant ☑ applicant's representative).

Exhibit shown or demonstration conducted: ☑ Yes ☐ No. If yes, brief description: Proposed amended claims.

Agreement ☐ was reached with respect to some or all of the claims in question. ☑ was not reached.

Claims discussed: All

Identification of prior art discussed: Queen et al and Reichmann et al

Description of the general nature of what was agreed to if an agreement was reached, or any other comments: proposed amend w/ overcome 112 2nd & spec obj: leaving 101, utility, enablement & art. Upon requested narrowing to use of human/mouse or human/rat: at present, specific residues for any antibodies were w/in genus of the claim. Thera claims will be put into a continuat. & applic will presently focus

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendments which would render the claims allowable is available, a summary thereof must be attached.)

Unless the paragraphs below have been checked to indicate to the contrary, A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW (e.g., items 1 – 7 on the reverse side of this form). If a response to the last Office action has already been filed, then applicant is given one month from this interview date to provide a statement of the substance of the interview.

☐ It is not necessary for applicant to provide a separate record of the substance of the interview.

☐ Since the examiner's interview summary above (including any attachments) reflects a complete response to each of the objections, rejections and requirements that may be present in the last Office action, and since the claims are now allowable, this completed form is considered to fulfill the response requirements of the last Office action.

Lisa L. Bennett
Examiner's Signature

PTOL-413 (REV. 1-84)

ORIGINAL FOR INSERTION IN RIGHT HAND FLAP OF FILE WRAPPER

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
743,329	8/16/91	Adair et al.	CARP-0009

EXAMINER	
Lisa Bennett	
ART UNIT	PAPER NUMBER
1807	#18

DATE MAILED:

EXAMINER INTERVIEW SUMMARY RECORD

All participants (applicant, applicant's representative, PTO personnel):

(1) Lisa Bennett, Scott Chambers, (3) _____

(2) Adair. (4) _____

Date of interview 1/27/93

Type: ☐ Telephonic ☑ Personal (copy is given to ☐ applicant ☐ applicant's representative).

Exhibit shown or demonstration conducted: ☑ Yes ☐ No. If yes, brief description: Proposed amended claims.

Agreement ☐ was reached with respect to some or all of the claims in question. ☑ was not reached.

Claims discussed: All

Identification of prior art discussed: Queen et al., Riechmann et al.

Description of the general nature of what was agreed to if an agreement was reached, or any other comments: on just binding regions, Can one of art make Ab from any source? Applicat indicates that great conservation of Ab would suggest that acceptor & donor can come from any species. Prior art rejections: Riechmann & Queen. Applic. have found specific residues which they find are important for the invent.

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendments which would render the claims allowable is available, a summary thereof must be attached.)

Unless the paragraphs below have been checked to indicate to the contrary, A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW (e.g., items 1–7 on the reverse side of this form). If a response to the last Office action has already been filed, then applicant is given one month from this interview date to provide a statement of the substance of the interview.

☐ It is not necessary for applicant to provide a separate record of the substance of the interview.

☐ Since the examiner's interview summary above (including any attachments) reflects a complete response to each of the objections, rejections and requirements that may be present in the last Office action, and since the claims are now allowable, this completed form is considered to fulfill the response requirements of the last Office action.

Lisa J. Bennett
Examiner's Signature

PTOL-413 (REV. 1-84)

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Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
743,329	8/16/91	Adair et al.	CARP—0009

EXAMINER	
Lisa Bennett	
ART UNIT	PAPER NUMBER
1807	#18

DATE MAILED:

EXAMINER INTERVIEW SUMMARY RECORD

All participants (applicant, applicant's representative, PTO personnel):

(1) Lisa Bennett, Scott Chambers (3) _____

(2) _____ (4) _____

Date of interview 1/27/93

Type: ☐ Telephonic ☑ Personal (copy is given to ☐ applicant ☑ applicant's representative).

Exhibit shown or demonstration conducted: ☑ Yes ☐ No. If yes, brief description: Proposed Claims.

Agreement ☐ was reached with respect to some or all of the claims in question. ☑ was not reached.

Claims discussed: All

Identification of prior art discussed: Queen et al., Riechmann et al.

Description of the general nature of what was agreed to if an agreement was reached, or any other comments: Queen starts from point of identifying areas of high homology & then uses computer modeling to determine. It is possible that Queen would come up w/ same sequence but Queen sometimes fails, applicant indicates they have not had a failure & average is 50-60%. Queen doesn't suggest some of the changes

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendments which would render the claims allowable is available, a summary thereof must be attached.)

Unless the paragraphs below have been checked to indicate to the contrary, A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW (e.g., items 1—7 on the reverse side of this form). If a response to the last Office action has already been filed, then applicant is given one month from this interview date to provide a statement of the substance of the interview.

☐ It is not necessary for applicant to provide a separate record of the substance of the interview.

☐ Since the examiner's interview summary above (including any attachments) reflects a complete response to each of the objections, rejections and requirements that may be present in the last Office action, and since the claims are now allowable, this completed form is considered to fulfill the response requirements of the last Office action.

Lisa L. Bennett
Examiner's Signature

PTOL-413 (REV. 1-84)

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4 of 4

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
743,529			

EXAMINER	
ART UNIT	PAPER NUMBER
	18

DATE MAILED:

EXAMINER INTERVIEW SUMMARY RECORD

All participants (applicant, applicant's representative, PTO personnel):

(1) Lisa Bennett, Scott Chambliss

(2) _____ (4) See page 1

Date of interview 1/27/92

Type: ☐ Telephonic ☒ Personal (copy is given to ☒ applicant ☐ applicant's representative).

Exhibit shown or demonstration conducted: ☐ Yes ☐ No. If yes, brief description: _____

Agreement ☐ was reached with respect to some or all of the claims in question. ☐ was not reached.

Claims discussed: _____

Identification of prior art discussed: _____

Description of the general nature of what was agreed to if an agreement was reached, or any other comments: the changes; applicant suggests that the "comprising" in eg clm 24 is not to be taken as "comprising" more residues than those in clm, i.e. claimed residues are not to be considered open-ended. Applic indicated they would clarify the latter issue. Queen does not teach charging residues: 73HC; 38HC; 71 on LC #1 on LC + #4 on LC, 36 on LC 46 on LC

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendments which would render the claims allowable is available, a summary thereof must be attached.)

Unless the paragraphs below have been checked to indicate to the contrary, A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW (e.g., items 1–7 on the reverse side of this form). If a response to the last Office action has already been filed, then applicant is given one month from this interview date to provide a statement of the substance of the interview.

☐ It is not necessary for applicant to provide a separate record of the substance of the interview.

☐ Since the examiner's interview summary above (including any attachments) reflects a complete response to each of the objections, rejections and requirements that may be present in the last Office action, and since the claims are now allowable, this completed form is considered to fulfill the response requirements of the last Office action.

Lisa Bennett
Examiner's Signature

PTOL-413 (REV. 1-84)

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Paper No. _____
Filed on behalf of: Party Carter
Filed: May 28, 2010

By: Oliver R. Ashe, Jr., Esq.
ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

Jeffrey P. Kushan, Esq.
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, DC 20005
Tel.: (202) 736-8914
Fax: (202) 736-8711
E-mail: jkushan@sidley.com

UNITED STATES PATENT AND TRADEMARK OFFICE

_____

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

_____

PAUL J. **CARTER** AND LEONARD G. PRESTA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR**, DILJEET SINGH ATHWAL,
AND JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

_____

Patent Interference 105,744 (SGL)
Technology Center 1600

_____

CARTER'S LIST OF EXHIBITS
(As of May 28, 2010)

CARTER'S LIST OF EXHIBITS

In accordance with ¶ 154.4 of the Standing Order (Paper No. 2) and Part E of the Order - Motion Times - Bd.R. 104(c) (Paper No. 23, p. 8), Carter herein provides a list of exhibits reflecting evidence filed and served in connection with Carter Substantive Motions 1 and 2 (*i.e.*, Ex. 2001-2029, 2031 and 2033-2039).

Designated Exhibit	Description of the Document
Ex. 2001	U.S. Patent No. 6,407,213 to Carter *et al.*, issued June 18, 2002.
Ex. 2002	U.S. Patent Application No. 11/284,261 to Adair *et al.*, filed November 21, 2005.
Ex. 2003	Preliminary Amendment and Request for Interference Under 37 C.F.R. § 42.202 [sic], filed November 21, 2005, in U.S. Patent Application No. 11/284,261 to Adair *et al.*
Ex. 2004	Request for Reconsideration, filed September 9, 2009, in U.S. Patent Application No. 11/284,261 to Adair *et al.*
Ex. 2005	PCT Application No. PCT/GB90/02017 to Adair *et al.*, filed December 21, 1990, published as WO 91/09967 on July 11, 1991 ("the PCT Application").
Ex. 2006	U.S. Patent Application No. 07/743,329 to Adair *et al.*, filed September 17, 1991.
Ex. 2007	Response to Office Action filed January 19, 1993, in U.S. Patent

	Application No. 07/743,329 to Adair *et al*.
Ex. 2008	Amendment filed April 7, 1993, in U.S. Patent Application No. 07/743,329 to Adair *et al*.
Ex. 2009	Letter regarding amendments filed September 9, 1993, in European Patent Application No. 91901433.2 to Adair *et al*.
Ex. 2010	Amendment filed February 7, 1994, in U.S. Patent Application No. 07/743,329 to Adair *et al*.
Ex. 2011	Riechmann *et al.*, *Nature*, Vol. 332, pp. 323-327 (March 1988).
Ex. 2012	Response to Advisory Action filed May 9, 1994, in U.S. Patent Application No. 07/743,329 to Adair *et al*.
Ex. 2013	Preliminary Amendment filed September 7, 1994, in U.S. Patent Application No. 08/303,569 to Adair *et al*.
Ex. 2014	Amendment filed September 18, 1995, in U.S. Patent Application No. 08/303,569 to Adair *et al*.
Ex. 2015	Preliminary Amendment filed August 23, 1996, in U.S. Patent Application No. 08/485,686 to Adair *et al*.
Ex. 2016	Preliminary Amendment and Request for Interference Under 37 C.F.R. § 1.607 filed May 1, 1997, in U.S. Patent Application No.

	08/846,658 to Adair *et al*.
Ex. 2017	Response and Amendment filed August 20, 1997, in U.S. Patent Application No. 08/485,686 to Adair *et al*.
Ex. 2018	Proposed claims transmitted January 28, 1998, in U.S. Patent Application No. 08/303,569 to Adair *et al*.
Ex. 2019	Amendment Pursuant to 37 C.F.R. § 1.312 filed February 23, 1998, in U.S. Patent Application No. 08/303,569 to Adair *et al*.
Ex. 2020	Amendment Pursuant to 37 C.F.R. § 1.312 filed July 13, 1998, in U.S. Patent Application No. 08/303,569 to Adair *et al*.
Ex. 2021	Allowed claims filed September 29, 1998, in U.S. Patent Application No. 08/846,658 to Adair *et al*.
Ex. 2022	Fourth Preliminary Amendment filed November 5, 1998, in U.S. Patent Application No. 08/846,658 to Adair *et al*.
Ex. 2023	Queen *et al.*, *Proc. Natl. Acad. Sci. USA*, Vol. 86, pp. 10029-10033 (December 1989).
Ex. 2024	U.S. Patent No. 5,859,205 to Adair *et al.*, issued January 12, 1999.
Ex. 2025	Amendment and Request for Reconsideration filed April 9, 1999, in U.S. Patent Application No. 08/846,658 to Adair *et al*.

Ex. 2026	Amendment and Request for Reconsideration filed November 3, 1999, in U.S. Patent Application No. 08/846,658 to Adair *et al*.
Ex. 2027	Amendment and Request for Reconsideration filed January 19, 2000, in U.S. Patent Application No. 08/846,658 to Adair *et al*.
Ex. 2028	Office Action mailed September 7, 1993, in U.S. Patent Application No. 07/743,329 to Adair *et al.*
Ex. 2029	Request for Reconsideration filed August 29, 2000, in U.S. Patent Application No. 08/485,686 to Adair *et al*.
Ex. 2030	Not used.
Ex. 2031	Supplemental Amendment and Request for Reconsideration filed September 14, 2000, in U.S. Patent Application No. 08/846,658 to Adair *et al*.
Ex. 2032	Not used.
Ex. 2033	Amendment and Request for Reconsideration filed November 12, 2001, in U.S. Patent Application No. 08/485,686 to Adair *et al*.
Ex. 2034	Proposed amendments to claims transmitted on March 18, 2002, in U.S. Patent Application No. 08/485,686 to Adair *et al*.
Ex. 2035	Amendment and Request for Reconsideration filed August 9, 2002,

	in U.S. Patent Application No. 08/485,686 to Adair *et al*.
Ex. 2036	Great Britain Application No. 8928874.0 to Adair *et al*., filed December 21, 1989 ("the UK Application").
Ex. 2037	Computer generated comparison (using Workshare™ Professional 5.2 SR2 software) of the typewritten text of the UK Application to the typewritten text of the PCT Application.
Ex. 2038	Office Action mailed November 18, 1992, in U.S. Patent Application No. 07/743,329 to Adair *et al*.
Ex. 2039	Examiner Interview Summary Record dated January 27, 1993, in U.S. Patent Application No. 07/743,329 to Adair *et al*.

Respectfully submitted,

May 28, 2010

/Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.
Registration No. 40,491
Counsel for Party Carter

ASHE, P.C.
11440 Isaac Newton Square North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

CERTIFICATE OF FILING

The undersigned certifies that a copy of the paper entitled **"CARTER SUBSTANTIVE MOTION 1"** and Exhibits 2001-2029, 2031, and 2033-2039 were filed this 28th day of May, 2010, via Interference Web Portal (https://acts.uspto.gov/ifiling/), with:

The Board of Patent Appeals and Interferences
Madison Building East, 9th Floor
600 Dulany Street
Alexandria, VA 22314
Tel.: 571-272-4683
Fax: 571-273-0042
E-mail: BoxInterferences@USPTO.GOV

May 28, 2010 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

CERTIFICATE OF SERVICE

The undersigned hereby certifies that a copy of the paper entitled **"CARTER SUBSTANTIVE MOTION 1"** and Exhibits 2001-2029, 2031, and 2033-2039 were served this 28th day of May, 2010, via Interference Web Portal (https://acts.uspto.gov/ifiling/), on the Attorney of Record for Adair:

Doreen Yatko Trujillo, Esq.
Cozen O'Connor P.C.
1900 Market Street, 7th Floor
Philadelphia, PA 19103
Tel.: 215-665-6593
Fax: 215-701-2005
E-mail: dtrujillo@cozen.com

May 28, 2010 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

Paper No. _____
Filed on behalf of: Party Carter
Filed: May 28, 2010

By: Oliver R. Ashe, Jr., Esq.
ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

Jeffrey P. Kushan, Esq.
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, DC 20005
Tel.: (202) 736-8914
Fax: (202) 736-8711
E-mail: jkushan@sidley.com

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

PAUL J. **CARTER** AND LEONARD G. PRESTA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR**, DILJEET SINGH ATHWAL,
AND JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

Patent Interference 105,744 (SGL)
Technology Center 1600

CARTER SUBSTANTIVE MOTION 1
(Adair Claim 24 Is Barred Under 35 U.S.C. § 135(b)(1))

Table of Contents

CARTER SUBSTANTIVE MOTION 1

I. PRECISE RELIEF REQUESTED

Carter moves under 37 C.F.R. § 41.121(a)(1)(iii) for judgment that Adair's involved claim 24 is not patentable to Adair under 35 U.S.C. § 135(b)(1).

II. THE EVIDENCE AND STATEMENT OF MATERIAL FACTS

A list of exhibits, papers, and appendices relied upon in support of this motion is set forth in Appendix 1. A statement of material facts relied upon in support of this motion is set forth in Appendix 2.

III. ARGUMENT

A. Introduction and Overview of Argument

Carter's involved U.S. Patent No. 6,407,213 ("the '213 patent") issued on June 18, 2002. (MF 36). The "critical date" for compliance with 35 U.S.C. § 135(b) is June 18, 2003. (MF 37). More than six years after the critical date, on September 9, 2009, Adair presented its involved claim 24. (MF 40).

To comply with the requirements of § 135(b), Adair must satisfy at least three conditions. First, Adair must have presented a "pre-critical date" claim that is patentable to Adair. Second, Adair must have presented a "pre-critical date" claim that defines the same or substantially the same subject matter as a Carter '213 patent claim.[1] Third, Adair's post-critical date claim (*i.e.*, Adair's involved claim 24) cannot differ in any "material limitation" from Adair's pre-critical date claim(s) that is patentable to Adair and defines substantially the same subject matter as a

[1] None of Adair's pre-critical date claims is identical to (*i.e.*, "the same as") a Carter '213 patent claim. (MF 41).

Carter '213 patent claim.[2] *In re Berger*, 279 F.3d 975, 981-82 (Fed. Cir. 2002), citing *Corbett v. Chisum*, 568 F.2d 759, 196 USPQ 337 (CCPA 1977). If Adair fails to satisfy any one of these three conditions, then Adair's claim 24 is not patentable to Adair and Adair loses its standing to participate in the interference. (37 C.F.R. § 41.201, defining "threshold issue.")

On November 21, 2005, Adair filed its involved application 11/284,261 ("the '261 application"), presented new claim 24, and filed a "Preliminary Amendment and Request for Interference under 37 C.F.R. § 42.202 [sic]."[3] (MF 38-39 and 43). In requesting an interference with the Carter '213 patent, Adair acknowledged the hurdle it faced to comply with the requirements of § 135(b). (MF 43-44). Specifically, Adair argued that its newly presented claim 24 was not barred by § 135(b) because claims 8 and 16 in its PCT application, when considered together, define subject matter that is the same or substantially the same as the subject matter defined by claim 1 of Carter's '213 patent.[4] (MF 44). Adair's position does not withstand scrutiny.

First, Adair's § 135(b) analysis entirely failed to address whether Adair's PCT claims are patentable to Adair, a prerequisite for compliance with § 135(b) of any post-critical date claim

[2] Adair claim 24 is not identical to (*i.e.*, "the same as") any pre-critical date Adair claim. MF 42.

[3] Adair thereafter amended claim 24. Adair's involved claim 24 was presented on September 9, 2009. A comparison of the 2005 version of claim 24 and the 2009 version of involved claim 24 is provided in Appendix 3. (MF 31).

[4] Carter '213 patent claim 1 is not designated as corresponding to Count 1, nor has Adair moved for such relief. (Declaration, Paper No. 1, p. 4; and Adair Motions List, Paper 20, p. 2, item 5).

seeking benefit of a pre-critical date claim.[5] As discussed below, Adair's original PCT claims were also presented as the original claims in Adair's '329 application and were immediately and soundly rejected under 35 U.S.C. §§ 101, 102, 103, and 112 first and second paragraph. (MF 5-6 and 11-20). In response, Adair promptly cancelled the original claims in its PCT and U.S. applications and presented new claims that Adair characterized as more clearly describing Adair's invention and distinguishing the claims over a number of prior art references. (MF 21-26).

Second, Adair's arguments regarding compliance with § 135(b) failed to address the critical issue of whether Adair claim 24 is materially different from Adair's pre-critical date claims. A comparison of Adair's PCT claim 16/8 (*i.e.*, the pre-critical claims identified by Adair for compliance with § 135(b) in its request for interference) and Adair's involved claim 24 reveals that the claims are directed to different subject matter. (MF 32 and Appendix 4). For example, the PCT claim 16/8 recites a "CDR-grafted light chain…" whereas involved claim 24 recites a "humanized antibody comprising a heavy chain…" (MF 32 and Appendix 4). The heavy and light chains of an antibody are different polypeptides and, therefore, a residue substitution in a light chain is physically different from a residue substitution in a heavy chain and *vice-versa*.

In summary, the § 135(b) argument that Adair advanced to provoke an interference with the Carter '213 patent 1) failed to address the unpatentability to Adair of the original PCT/U.S.

[5] As a matter of law, Adair cannot rely on a PCT application to satisfy the requirements of 35 U.S.C. § 135(b). However, Adair's original PCT and original U.S. claims are identical and, therefore, Adair's improper reliance on PCT claims rather than on U.S. claims does not appear to be of immediate significance to the relief requested in this motion.

claims upon which Adair relied for § 135(b) benefit; 2) failed to address the fact that Adair's pre-critical claims do not define the same or substantially the same subject matter as an involved Carter patent claim; and 3) failed to address the differences in material limitations between Adair's original PCT/U.S. claims and Adair post-critical date claim 24.

In this motion, Carter explains that Adair's involved claim 24 is statutorily barred under § 135(b) for any one of the following reasons:

1) none of Adair's original PCT/U.S. claims are patentable to Adair and, therefore, Adair involved claim 24 (or any other post-critical date claim) is not entitled to the benefit of these claims for compliance with § 135(b);

2) none of Adair's pre-critical date claims are directed to the same or substantially same subject matter as any one of Carter's involved '213 patent claims and, therefore, cannot be relied upon for compliance with § 135(b);

3) Adair involved claim 24 differs in material limitations from Adair's pre-critical date claims and, therefore, Adair is not entitled to the benefit of these claims for compliance with § 135(b).

In Carter Substantive Motion 2, Carter moves for judgment that Adair involved claim 24 is unpatentable to Adair for failure to comply with the written description requirement of 35 U.S.C. § 112, first paragraph. Regardless of whether the Board finds Adair claim 24 to satisfy the written description requirement, Adair claim 24 is barred under § 135(b) and adverse judgment should be entered against Adair.

B. <u>Adair Is Not Statutorily Entitled to Any of Its Original U.S. Claims</u>

On December 21, 1990, Adair filed PCT/GB90/02017 ("the PCT application"), containing claims 1-23 (*i.e.*, the "original PCT claims"). (MF 2). On September 17, 1991, Adair filed U.S. Patent Application No. 07/743,329 ("the '329 application"), which claims benefit of

the Adair PCT application. (MF 5). The original claims filed in the '329 application are identical to Adair's original PCT claims. (MF 6).

On November 18, 1992, the USPTO rejected the original Adair '329 claims 1-23 on a variety of statutory grounds (MF 11), including the following:

1. Claims 1-12, 17 and 22-23 were rejected under 35 U.S.C. § 101 as lacking utility (MF 12);

2. Claims 1-16 and 22-23 were rejected under 35 U.S.C. § 112, first paragraph, as failing to adequately teach how to make and use the claimed invention (MF 13);

3. Claims 1-23 were rejected under 35 U.S.C. § 112, second paragraph, as failing to particularly point out and distinctly claim the subject matter which Adair regarded as its invention (MF 14);

4. Claims 1, 5, 6-8, and 12-22 were rejected under 35 U.S.C. § 102(b) as being anticipated by Riechmann *et al.* (MF 15);

5. Claims 1-6 and 12-22 were rejected under 35 U.S.C. § 102(b) as being anticipated by Queen *et al.* (MF 16); and

6. Claims 1-21 were rejected under 35 U.S.C. § 103 as being obvious over Riechmann *et al.* and Queen *et al.* (MF 17).

In rejecting claims 1-16 and 22-23 for lack of enablement under § 112, first paragraph, the Examiner maintained, *inter alia*, that the Adair specification did not support making the range of residue changes recited in the claims and observed that the effects of the residue changes as described in Adair's original claims could not readily be predicted. (MF 18).

In rejecting claims 1-23 under 35 U.S.C. § 112, second paragraph, the Examiner maintained that the claims were indefinite in their recitation of "at least one of positions 6, 23

and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91" because it was unclear to the Examiner whether the heavy chain framework had donor residues at:

a. at least one of 6, 23, 24, 48, 49, 71, 73, 75, 76, 78, 88, or 91, or alternatively,

b. at least one of (6) or (23 and/or 24) or (48 and/or 49) or (71 and/or 73) or (75 and/or 76 and/or 78 and 88 and/or 91), or alternatively,

c. at least one of (6, 23) and/or (24, 48) and/or (49, 71) and/or (73, 75) and 76 and/or (78 and 88) and/or (91). (MF 19).

In rejecting the claims 1, 5, 6-8, and 12-22 under 102(b) as being anticipated by Riechmann *et al.*, the Examiner noted that the claims were being interpreted to mean that the framework has donor residues at at least one of any of positions 6, 23, 24, 48, 49, 71, 73, 75, 76, 78, 88, or 91 in the heavy chain. (MF 20).

On January 19, 1993, Adair responded to the November 1992 Office action by cancelling original claims 1-20, 22 and 23, amending original claim 21, and adding new claims 24-66. (MF 21).

Adair further responded to the § 112, first paragraph, rejections by relying on the disclosure added to its PCT application that described a "hierarchy of residues" protocol for preparing humanized antibodies according to Adair's invention. (MF 3-4). Adair argued that, in view of the specific rules set forth in Adair's "hierarchy of residues" protocol, it would not require undue experimentation to make and use the subject matter defined by Adair's newly added claims. (MF 3-4, 22).

In its January 1993 response, Adair did not contest the rejections under § 112, second paragraph but, rather, cancelled the rejected claims. (MF 21 and 24). Likewise, Adair did not contest the rejection of the claims under § 102(b) as being anticipated by Riechmann *et al.* and

Queen *et al.* but, rather, relied upon limitations in the newly presented claims to distinguish over these prior art references. (MF 25-26). Adair followed the filing of its response in the '329 application with an in-person Examiner interview on January 27, 1993, wherein Adair asserted that its claims were not open-ended with respect to unrecited residue substitutions. (MF 27).

On September 9, 1993, in the Adair PCT/EP Patent Application No. 91901433.2, Adair filed an amendment deleting original claims 1-23, replacing them with new claims 1-20, and acknowledging grounds of unpatentability of its original claims. (MF 28).

On February 7, 1994, Adair filed an amendment in the '329 application responding to the Office action mailed on September 7, 1993. Although substantial in length, the following quote captures several important points regarding Adair's own characterization of its invention, the scope of its claims, and the interpretation of its specification (MF 10 and 29):

> At a very helpful interview held at the beginning of 1993, there was some discussion of the word "comprising" as used in the claims under consideration at that time. In those claims, it was only specified that certain residues should be donor residues. It was considered that it was not clear whether these were the only residues which could be donor residues. The alternative view was that these were only the minimum number of residues which must be donor but that any of the other residues could also be donor. [Emphasis by Adair].
>
> If the second line of interpretation were taken, the claims could be read to cover a situation in which all except one of the residues in the variable domain were donor residues. In this case, the claims could then be interpreted to cover a structure similar to a "chimeric" antibody comprising a donor variable domain and a human constant region. Such chimeric antibodies were already well known at the priority date. [Emphasis by Adair].
>
> It plainly is not the intention of the Applicants to claim chimeric antibodies or any similar structures. As can be seen from the description, the superhumanised antibodies of the present invention are compared to the prior art chimeric antibodies. Moreover, the present invention was intended to deal with the problem of chimeric antibodies in that chimeric antibodies were believed to be too "foreign" because of the presence of the complete donor variable domain.
>
> For the above reasons, it is clear that the wording of the claims needed to be changed so that the Applicants' intention of excluding chimeric antibodies was

made effective. The language now present in the claims puts this intention clearly into effect.

As to support for this wording, the Examiner is referred firstly to page 16, under the heading "Protocol". It can be seen from this paragraph that the first step in the process involves the choice of an appropriate acceptor chain variable domain. This acceptor domain must be of known sequence. Thus, the protocol starts with a variable domain in which all the residues are acceptor residues. In the sentence bridging pages 16 and 17, it is stated that:

> "The CDR-grafted chain is then designed starting from the basis of the acceptor sequence".

On page 17, in the middle paragraph, it is stated that:

> "The positions at which donor residues are to be substituted for acceptor in the framework are then chosen as follows ...."

This again shows that, unless a residue is chosen for substitution, it will remain as in the acceptor sequence. [Emphasis by Adair].

It must also be borne in mind that the purpose of the invention is to obviate some of the disadvantages of prior art proposals. The proposal of using chimeric antibodies had the disadvantage that they were more "foreign" than desirable. The problem of making CDR-grafted antibodies was that they generally did not provide good recovery of affinity. Thus, the aim of the present invention was to minimise as far as possible the "foreign" nature of the antibody while maximising as far as possible its affinity.

Bearing the passages referred to above and the aim of the invention in mind, it would have been abundantly clear to the skilled person reading the application that as many residues as possible should remain as acceptor residues. If this were not the case, it could hardly be said that the composite chain is based on the acceptor sequence.

The skilled person reading the application can plainly see that certain residues have been considered for changing from acceptor to donor. These are clearly set out in the description. It would be plain to the skilled person that all other residues should not be considered for changing at all. It would therefore be obvious that any residue which is not specified as being under consideration for changing must remain as in the acceptor chain.

It may be that there is no explicit statement in the description that the specified residues should remain as in the acceptor chain. However, the disclosure in a specification is not limited to the explicit disclosure but also includes that which is implicit. It is implicit, in the recitation that the chain is based on the acceptor and that only certain residues are considered for changing, that all non-specified residues must remain as acceptor residues. Subject matter which might

be fairly deduced from the disclosure is not new matter. *Acme Highway Products Corp. v. D.S. Brown Co.*, 431 F.2d 1074, 1080, 167 U.S.P.Q. 129, 132-133(6th Cir. 1970), *cert denied*, 401 U.S. 956 (1971).

In summary, Adair has acknowledged that it is not statutorily entitled to its original PCT/U.S. claims, as these claims were ambiguous and could be reasonably interpreted as reading on the disclosures in Queen *et al.* and Reichmann *et al.* of humanized antibody polypeptides meeting the requirements of these original Adair claims, as well as on known chimeric antibody structures. The express teachings of the Adair specification and Adair's representations regarding the same provide compelling evidence that Adair is not statutorily entitled to its original PCT/U.S. claims and, therefore cannot rely upon those claims for purposes of satisfying the statutory requirements of § 135(b). *Adang v. Umbeck*, 2007 U.S. App. LEXIS 25198 (Fed. Cir. 2007); *Univ. of Rochester v. G.D. Searle & Co.*, 358 F.3d 916, 927 (Fed. Cir. 2004); *PIN/NIP, Inc. v. Platte Chem. Co.*, 304 F.3d 1235, 1247-48 (Fed. Cir. 2002); *In re Curtis*, 354 F.3d 1347, 1353-54 (Fed. Cir. 2004).

C. None of Adair's Pre-Critical Date Claims Defines the Same or Substantially the Same Subject Matter As Any One of Carter's Involved Patent Claims

None of Adair's pre-critical date claims define the same or substantially the same subject matter as an involved Carter '213 patent claim because each of Adair's pre-critical date claims differ in one or more material limitations relative to Carter's '213 patent claims. (MF 41). As a consequence, none of Adair's pre-critical date claims can serve as a basis for overcoming the barrier of § 135(b).

As discussed above, Adair's original PCT/U.S. claims were found not to comply with numerous statutory provisions for patentability, including that the claims were indefinite under 35 U.S.C. § 112, second paragraph. (MF 11-20). By contrast, Adair does not contest that Carter's involved patent claims are definite and thus satisfy 35 U.S.C. § 112, second paragraph.

Logically, Adair's original claims must differ in ways having patentable significance (*i.e.*, in material limitations) from Carter's '213 claims because the former were found to be indefinite by the Examiner, while the latter are conceded by Adair to not be indefinite. Adair's original PCT/U.S. claims are thus not directed to the same or substantially the same subject matter as any Carter's '213 patent claim.

After Adair cancelled its original PCT/U.S. claims in 1992, Adair presented a plethora of other claims prior to June 18, 2003 ("Adair's non-original pre-critical date claims"). (MF 30). None of Adair's non-original pre-critical date claims can be relied upon to satisfy the requirements of § 135(b).

First, many of Adair's non-original pre-critical date claims were determined to be not patentable to Adair and, therefore, cannot be relied upon to satisfy the requirements of § 135(b).

Second, Adair characterized these non-original pre-critical date claims as incorporating essential features of its "hierarchy of residues" protocol. Adair repeatedly emphasized that this protocol was the hallmark of its invention, and was the reason why its newly added claims were patentable over prior art. Adair also asserted that the newly presented claims, because they incorporated limitations adhering to its "protocol" of multiple required substitutions, cured a variety of maladies that plagued the patentability of Adair's original claims.

At page 6 of its involved specification, Adair stated (MF 7):

> We have further investigated the preparation of CDR-grafted humanized antibody molecules and have identified a hierarchy of positions within the framework of the variable regions (i.e., outside both the Kabat CDRs and structural loops of the variable regions) at which the amino acid identities of the residues are important for obtaining CDR-grafted products with satisfactory binding affinity. This has enabled us to establish a protocol for obtaining satisfactory CDR-grafted products which may be applied very widely irrespective of the level of homology between donor immunoglobulin and acceptor framework. The set of residues which we have identified as being of critical importance does not coincide with the residues identified by Queen et al (9).

In the amendment filed on January 19, 1993, responding to the enablement rejections, Adair again emphasized specific rules governing the "hierarchy of residues" set forth in the protocol in Adair's specification. Adair stated (MF 22):

> In contrast, the teaching in the present application can be applied without any undue experimentation to any antibody. All that is required is experimentation following a protocol which is clearly set out in the description, in particular at page 16, line 30 to page 19, line 9.
>
> …There is then no need to carryout computer modeling to determine which donor residues to substitute into the acceptor sequence. The protocol in the present application provides the teaching directly. It instructs the skilled person to compare the two sequences and change certain specified residues in the acceptor sequence to donor residues.
>
> …Thus, producing recombinant chains and testing them for affinity merely involves routine experimentation following a protocol which is clearly defined in the application. [Emphasis added].

After cancelling its original claims and presenting new claims in the January 1993 amendment, Adair made the following argument (MF 23):

> It is submitted that this identifies where the present invention makes a significant departure from the prior art. The prior art indicates that each antibody has to be treated individually. In contrast, the present invention teaches that, by following the protocol set forth in the present application, it is possible to reshape any antibody.

Adair also relied upon the "hierarchy of residues" to overcome prior art rejections. For example, in an amendment filed on April 7, 1993, Adair amended a claim reciting residues 71, 73 and 78 to distinguish over the anti-TAC antibody disclosed by Queen *et al.*. (MF 9 and 33):

> In claim 67, it has been specified that residues 71, 73 and 78 are all donor residues in order to ensure that claim 67 is novel over the anti-TAC antibody disclosed by Queen. This anti-TAC antibody has an acceptor residue at residue 73. However, as can be seen from page 7, lines 1 to 5, the Applicant considers that in general, residues 71, 73 and 78 can be either all donor or all acceptor.

In the April 1993 amendment, Adair again pointed to the substance of its protocol setting forth rules for substitutions to support its newly proposed claims. In particular, Adair explained

that, with respect to residues 23, 24, 49, 71, 73 and 78, there were only two alternatives of humanized antibodies being described in its specification; one involving changes at three residues in the heavy chain, and the other involving six residue changes (MF 9 and 34):

> It is stated on page 7, lines 1 to 5, that residues 71, 73 and 78 should all be either acceptor or donor. Claims 73, 80, 87, 94 and 101 cover the first alternative and claims 74, 81, 88, 95 and 102 cover the second alternative.

In other words Adair characterized the universe of choices for changes to residues 71, 73 and 78 to consist of two alternatives; all donor or all acceptor.

In responding to the Examiner's repeated enablement rejections, Adair made the following statements in an Amendment filed on February 7, 1994, in the '329 application (MF 35):

> It is specifically stated in the application that the present protocol represents a departure from the procedures of Reichmann [sic] and Queen, at least. Thus, the skilled person would not rely on Reichmann [sic] and Queen as teachings relevant to whether the present description is enabling.
>
> It is submitted that the skilled person would rely on the clear teaching given in the application and find that it is enabling. The specification plainly sets out what actions need to be taken. It is presumed that the Examiner agrees that the skilled person could have taken those actions. The application also sets out that, contrary to the teachings of Reichmann and Queen, the protocol is generally applicable. The application further shows that it had been successfully implemented. Thus, it is submitted that the skilled person would find that the present application is properly enabled the full extent of the claims.

Consistent with these arguments, Adair's non-original pre-critical date claims recite positions that must all be donor residues. In contrast, the Carter '213 patent claims do not require that each recited position have a donor residue but, rather, allow for combinations of donor and acceptor residues at the recited positions. The Adair pre-critical claims are not directed to the same or substantially the same subject matter as the Carter '213 patent claims because the claims differ in material limitations. Accordingly, Adair cannot rely upon any of Adair's non-original pre-critical date claims for purposes of complying with the requirements of 35 U.S.C. § 135(b).

D. Adair Involved Claim 24 Differs In Material Limitations From Adair's Non-Original Pre-Critical Date Claims

Adair involved claim 24 is not entitled to the benefit of any pre-critical date claims because there are differences in material limitations in the pre-critical date claims that are not present in Adair involved claim 24.

As noted above, Adair cannot rely on its original PCT/U.S. claims because the claims are not patentable to Adair. (MF 11-20). Also as explained above, Adair's non-original pre-critical date claims are firmly grounded in the specific rules governing the "hierarchy of residues" to which Adair attributed the patentability of its claims. (MF 30). These claims emphasize the criticality of each recited position having a donor residue. Adair's specification and prosecution history also emphasize the criticality of adhering to these rules to render the claims patentable over the prior art such as the Riechmann *et al.* and Queen *et al.* references.

Adair involved claim 24 does not contain material limitations present in Adair's non-original pre-critical date claims. Rather, claim 24 allows for any possible donor/acceptor residue combination at positions 23, 24, 49, 71, 73 and 78. Claim 24 also allows for donor residues at any unrecited position in the heavy chain variable region. In other words, the very concepts and claim limitations upon which Adair relied to distinguish over prior art and to justify the enablement of its claims are absent in Adair claim 24.

IV. CONCLUSION

For the above-stated reasons, Adair involved claim 24 is barred under § 135(b) and adverse judgment should be entered against Adair.

Respectfully submitted,

May 28, 2010

/Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.
Registration No. 40,491
Counsel for Party Carter

CERTIFICATE OF FILING

The undersigned certifies that a copy of the paper entitled **"CARTER SUBSTANTIVE MOTION 1"** was filed this 28th day of May, 2010, via Interference Web Portal (https://acts.uspto.gov/ifiling/), with:

The Board of Patent Appeals and Interferences
Madison Building East, 9th Floor
600 Dulany Street
Alexandria, VA 22314
Tel.: 571-272-4683
Fax: 571-273-0042
E-mail: BoxInterferences@USPTO.GOV

May 28, 2010 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

CERTIFICATE OF SERVICE

The undersigned hereby certifies that a copy of the paper entitled **"CARTER SUBSTANTIVE MOTION 1"** was served this 28th day of May, 2010, via Interference Web Portal (https://acts.uspto.gov/ifiling/), on the Attorney of Record for Adair:

Doreen Yatko Trujillo, Esq.
Cozen O'Connor P.C.
1900 Market Street, 7th Floor
Philadelphia, PA 19103
Tel.: 215-665-6593
Fax: 215-701-2005
E-mail: dtrujillo@cozen.com

May 28, 2010 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

Appendix 1

EVIDENCE

I. Exhibits Cited

The following exhibits are cited in support of this motion:

Ex. 2001 U.S. Patent No. 6,407,213 to Carter *et al.*, issued June 18, 2002.

Ex. 2002 U.S. Patent Application No. 11/284,261 to Adair *et al*., filed November 21, 2005.

Ex. 2003 Preliminary Amendment and Request for Interference Under 37 C.F.R. § 42.202 [sic], filed November 21, 2005, in U.S. Patent Application No. 11/284,261 to Adair *et al.*

Ex. 2004 Request for Reconsideration, filed September 9, 2009, in U.S. Patent Application No. 11/284,261 to Adair *et al*.

Ex. 2005 PCT Application No. PCT/GB90/02017 to Adair *et al*., filed December 21, 1990, published as WO 91/09967 on July 11, 1991 ("the PCT Application").

Ex. 2006 U.S. Patent Application No. 07/743,329 to Adair *et al*., filed September 17, 1991.

Ex. 2007 Response to Office Action filed January 19, 1993, in U.S. Patent Application No. 07/743,329 to Adair *et al*.

Ex. 2008 Amendment filed April 7, 1993, in U.S. Patent Application No. 07/743,329 to Adair *et al*.

Ex. 2009 Letter regarding amendments filed September 9, 1993, in European Patent Application No. 91901433.2 to Adair *et al*.

Ex. 2010 Amendment filed February 7, 1994, in U.S. Patent Application No. 07/743,329 to Adair *et al*.

Ex. 2011 Riechmann *et al.*, *Nature,* Vol. 332, pp. 323-327 (March 1988).

Ex. 2012 Response to Advisory Action filed May 9, 1994, in U.S. Patent Application No. 07/743,329 to Adair *et al*.

Ex. 2013 Preliminary Amendment filed September 7, 1994, in U.S. Patent Application No. 08/303,569 to Adair *et al*.

Ex. 2014 Amendment filed September 18, 1995, in U.S. Patent Application No. 08/303,569 to Adair *et al*.

Ex. 2015 Preliminary Amendment filed August 23, 1996, in U.S. Patent Application No. 08/485,686 to Adair *et al*.

Ex. 2016 Preliminary Amendment and Request for Interference Under 37 C.F.R. § 1.607 filed May 1, 1997, in U.S. Patent Application No. 08/846,658 to Adair *et al*.

Ex. 2017 Response and Amendment filed August 20, 1997, in U.S. Patent Application No. 08/485,686 to Adair *et al*.

Ex. 2018 Proposed claims filed January 28, 1998, in U.S. Patent Application No. 08/303,569 to Adair *et al*.

Ex. 2019 Amendment Pursuant to 37 C.F.R. § 1.312 filed February 23, 1998, in U.S. Patent Application No. 08/303,569 to Adair *et al*.

Ex. 2020 Amendment Pursuant to 37 C.F.R. § 1.312 filed July 13, 1998, in U.S. Patent Application No. 08/303,569 to Adair *et al*.

Ex. 2021 Allowed claims filed September 29, 1998, in U.S. Patent Application No. 08/846,658 to Adair *et al*.

Ex. 2022 Fourth Preliminary Amendment filed November 5, 1998, in U.S. Patent Application No. 08/846,658 to Adair *et al*.

Ex. 2023 Queen *et al.*, *Proc. Natl. Acad. Sci. USA*, Vol. 86, pp. 10029-10033 (December 1989).

Ex. 2024 U.S. Patent No. 5,859,205 to Adair *et al.*, issued January 12, 1999.

Ex. 2025 Amendment and Request for Reconsideration filed April 9, 1999, in U.S. Patent Application No. 08/846,658 to Adair *et al*.

Ex. 2026 Amendment and Request for Reconsideration filed November 3, 1999, in U.S. Patent Application No. 08/846,658 to Adair *et al*.

Ex. 2027 Amendment and Request for Reconsideration filed January 19, 2000, in U.S. Patent Application No. 08/846,658 to Adair *et al*.

Ex. 2028 Office Action mailed September 7, 1993, in U.S. Patent Application No. 07/743,329 to Adair *et al.*

Ex. 2029 Request for Reconsideration filed August 29, 2000, in U.S. Patent Application No. 08/485,686 to Adair *et al*.

Ex. 2031 Supplemental Amendment and Request for Reconsideration filed September 14, 2000, in U.S. Patent Application No. 08/846,658 to Adair *et al*.

Ex. 2033 Amendment and Request for Reconsideration filed November 12, 2001, in U.S. Patent Application No. 08/485,686 to Adair *et al*.

Ex. 2034 Pending claims filed March 18, 2002, in U.S. Patent Application No.

08/485,686 to Adair *et al*.

Ex. 2035 Amendment and Request for Reconsideration filed August 9, 2002, in U.S. Patent Application No. 08/485,686 to Adair *et al*.

Ex. 2036 Great Britain Application No. 8928874.0 to Adair *et al*., filed December 21, 1989 ("the UK Application").

Ex. 2037 Computer generated comparison (using Workshare™ Professional 5.2 SR2 software) of the typewritten text of the UK Application to the typewritten text of the PCT Application.

Ex. 2038 Office Action mailed November 18, 1992, in U.S. Patent Application No. 07/743,329 to Adair *et al*.

Ex. 2039 Examiner Interview Summary Record dated January 27, 1993, in U.S. Patent Application No. 07/743,329 to Adair *et al*.

II. Papers Cited

The following papers are cited in support of this motion:

Paper No. 1 Declaration entered February 2, 2010.

Paper No. 5 Adair Clean Copy of Claims filed February 16, 2010.

Paper No. 20 Adair Motions List filed April 9, 2010.

III. Appendices Cited

The following papers are cited in support of this motion:

Appendix 1 Evidence.

Appendix 2 Statement of Material Facts Relied Upon in Motion.

Appendix 3	Claim chart comparing Adair claim 24 presented in 2005 and Adair involved claim 24.
Appendix 4	Claim chart comparing Adair original PCT claims 8 and 16 with Adair involved claim 24.

Appendix 2

STATEMENT OF MATERIAL FACTS RELIED UPON IN MOTION

1. On December 21, 1989, Adair filed Great Britain Application No. 8928874.0 ("Adair UK Application"). (Ex. 2036).

2. On December 21, 1990, Adair filed PCT/GB90/02017("Adair's PCT application"), which contained claims 1-23. (Ex. 2005, pp. 67-70).

3. Exhibit 2037 is a computer generated comparison (using Workshare™ Professional 5.2 SR2 software) of the typewritten text of the UK Application to the typewritten text of the PCT Application. The last page of Exhibit 2037 contains a color-coded legend for identifying deletions, additions, and movement of text.

4. The PCT Application contains a section titled "Protocol" that is not contained in the UK Application. (Ex. 2005, pp. 16-19; Ex. 2036; and Ex. 2037, pp. 10-11).

5. On September 17, 1991, Adair entered the U.S. national stage by filing U.S. Patent Application No. 07/743,329 ("the '329 application"), claiming benefit to Adair's PCT application. (Ex. 2006).

6. Adair's U.S. '329 application contained claims 1-23, which are identical to claims 1-23 as originally filed with Adair's PCT application. (Ex. 2005, pp. 67-70 and Ex. 2006, pp. 67-70).

7. At page 6 of its involved specification, Adair stated:

> We have further investigated the preparation of CDR-grafted humanized antibody molecules and have identified a hierarchy of positions within the framework of the variable regions (i.e., outside both the Kabat CDRs and structural loops of the variable regions) at which the amino acid identities of the residues are important for obtaining CDR-grafted products with satisfactory binding affinity. This has enabled us to establish a protocol for obtaining

> satisfactory CDR-grafted products which may be applied very widely irrespective of the level of homology between donor immunoglobulin and acceptor framework. The set of residues which we have identified as being of critical importance does not coincide with the residues identified by Queen et al (9). [Ex. 2002, p. 6, lns. 15-28].

8. At page 6, lines 31-37, the Adair specification reads as follows:

> Accordingly, in a first aspect the invention provides a CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91. [Ex. 2002, p. 6, lns. 31-37].

9. At page 7, lines 1-5, the Adair specification reads as follows:

> In preferred embodiments, the heavy chain framework comprises donor residues at positions 23, 24, 49, 71, 73 and 78 or at positions 23, 24 and 49. The residues at positions 71, 73 and 78 of the heavy chain framework are preferably either all acceptor or all donor residues. [Ex. 2002, p. 7, lns. 1-5].

10. At page 17, lines 27-30, the involved Adair specification reads as follows under a section titled "Protocol":

> 2.1 Choose donor residues at all of positions 23, 24, 49, 71, 73 and 78 of the heavy chain or all of positions 23, 24 and 49 (71, 73 and 78 are always either all donor or all acceptor). [Ex. 2002, p. 17, lns. 27-30].

11. On November 18, 1992, the U.S. Patent and Trademark Office ("the USPTO") entered a non-final office action rejecting claims Adair's claims 1-23 on several statutory grounds. (Ex. 2038).

12. On November 18, 1992, the USPTO rejected the original Adair '329 claims 1-12, 17 and 22-23 under 35 U.S.C. § 101 for lack of utility. (Ex. 2038, pp. 1-3).

13. On November 18, 1992, the USPTO rejected the original Adair '329 claims 1-16 and 22-23 under 35 U.S.C. § 112, first paragraph, for failing to adequately teach how to make and use the claimed invention. (Ex. 2038, pp. 3-6).

14. On November 18, 1992, the USPTO rejected the original Adair '329 claims 1-23 were rejected under 35 U.S.C. § 112, second paragraph, for failing to particularly point out and distinctly claim the subject matter which Adair regarded as its invention. (Ex. 2038, pp. 6-7).

15. On November 18, 1992, the USPTO rejected the original Adair '329 claims 1, 5, 6-8, and 12-22 under 35 U.S.C. § 102(b) as being anticipated by Riechmann *et al.*, *Nature*, Vol. 332, pp. 323-327 (March 1988). (Ex. 2038, pp. 7-9 and Ex. 2011).

16. On November 18, 1992, the USPTO rejected the original Adair '329 claims 1-6 and 12-22 under 35 U.S.C. § 102(b) as being anticipated by Queen *et al.*, *Proc. Natl. Acad. Sci. USA*, Vol. 86, pp. 10029-10033 (December 1989). (Ex. 2038, pp. 9-10 and Ex. 2023).

17. On November 18, 1992, the USPTO rejected the original Adair '329 claims 1-21 under 35 U.S.C. § 103 as being obvious over Riechmann *et al.* and Queen *et al.*. (Ex. 2038, pp. 10-12).

18. At pages 3-6 of the November 1992 office action, the Examiner rejected claims 1-16 and 22-23 for lack of enablement under § 112, first paragraph, on the grounds that, *inter alia*, the specification did not support making the range of residue changes recited in the claims and that the effects of the residue changes as described in Adair's original claims could not readily be predicted. (Ex. 2038, pp. 3-6).

19. At page 6 of the November 1992 office action, the Examiner rejected claims 1-5 under 35 U.S.C. § 112, second paragraph, as being indefinite in their recitation of "at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91" because it was unclear to the Examiner whether the heavy chain,

a. had at least one of 6, 23, 24, 48, 49, 71, 73, 75, 76, 78, 88, or 91, or alternatively,

b. had at least one of (6) or (23 and/or 24) or (48 and/or 49) or (71 and/or 73) or (75 and/or 76 and/or 78 and 88 and/or 91), or alternatively,

c. had at least one (6, 23) and/or (24, 48) and/or (49, 71) and/or (73, 75) and 76 and/or (78 and 88) and/or (91). (Ex. 2038, p. 6).

20. At page 9 of the November 1992 office action, the Examiner rejected Adair's claims in view of Riechmann *et al.*, noting that the Examiner interpreted the claims to mean that the framework has donor residues at at least one of any of positions 6, 23, 24, 48, 49, 71, 73, 75, 76, 78, 88, or 91 in the heavy chain. (Ex. 2038, p. 9).

21. In a January 19, 1993 amendment, Adair responded to the November 1992 Office action by cancelling original claims 1-20, 22 and 23, amending original claim 21, and adding new claims 24-66. (Ex. 2007, pp. 1-13).

22. In the January 1993 amendment, Adair stated the following:

> In contrast, the teaching in the present application can be applied without any undue experimentation to any antibody. All that is required is experimentation following a protocol which is clearly set out in the description, in particular at page 16, line 30 to page 19, line 9. …
>
> There is then no need to carryout computer modeling to determine which donor residues to substitute in to the acceptor sequence. <u>The protocol in the present application provides the teaching directly</u>. It instructs the skilled person to compare the two sequences and change certain specified residues in the acceptor sequence to donor residues.
>
> …Thus, producing recombinant chains and testing them for affinity merely <u>involves routine experimentation following a protocol which is clearly defined in the application</u>. [Ex. 2007, pp. 26-27; Emphasis added].

23. In the January 1993 amendment, Adair stated the following:

> It is submitted that this identifies where the present invention makes a significant departure from the prior art. The prior art indicates that each antibody has to be treated individually. In contrast, the present invention teaches that, by following the protocol set forth in the present application, it is possible to reshape any antibody. [Ex. 2007, p. 28].

24. In the January 1993 amendment, Adair responded to the rejection of claims under 35 U.S.C. § 112, second paragraph, by cancelling claims 1-12. (Ex. 2007, pp. 29-32).

25. In the January 1993 amendment, Adair responded to the rejection of claims under 35 U.S.C. § 102(b) in view of Riechmann *et al.* as follows:

> In Part A of this rejection, claims 1, 5, 6-8, and 12-22 were rejected as anticipated by Riechmann et al. The Examiner stated that claim 1 and claim 6 were interpreted to mean that the framework has donor residues in at least one of any of positions 6, 23, 24, 48, 49, 71, 73, 75, 76, 78, 88, or 91 in the heavy chain and (1, 3, 46, or 47) or 46, 48, 58, or 71) in the light chain, and thus, the teachings of Riechmann et al. anticipate the invention as claimed.
>
> The Examiner contends that the original claims lacked novelty over Riechmann et al. Claims 1, 5, 6-8, 12 and 22 have been cancelled without prejudice and submitted as new claims that more distinctly point out certain aspects of the present invention.
>
> In present claims 24 and 25, it is specified that residues 23 and 24 in the heavy chain should be donor residues. However, as can be seen from Fig. 1, panel (a) in Riechmann et al., in the recombinant antibody shown there, residues 23 and 24 are acceptor residues. [Ex. 2007, p. 32-33].

26. In the January 1993 amendment, Adair responded to the rejection of claims under 35 U.S.C. § 102(b) in view of Queen *et al.* as follows:

> In Part B of the rejection, the Examiner rejected claims 1-6 and 12-22 as anticipated by Queen et al.
>
> Claims 1-6, 12-20 and 22 have been cancelled without prejudice and submitted as new claims that more distinctly point out certain aspects of the present invention.
>
> In present claims 24 and 25, it is specified that residues 48, 66, 67, 68, 93, 103 to 108 and 110 should all be acceptor residues. However, in Queen et al., as can be seen from Fig. 2B, in these positions Queen et al. uses donor, rather than acceptor, residues. It should again be borne in mind that Queen et al. does <u>not</u> use the Kabat numbering and it is therefore necessary to look carefully at the disclosure in Queen et al. before it is possible to come to any final conclusion. [Emphasis by Adair].

> In present claim 38, it is specified that residue 71 should be a donor residue. However, as can be seen from Fig. 2A of Queen et al., in that position Queen et al. uses an acceptor, rather than a donor residue.
>
> Applicants' claimed antigen-binding molecules are thus not anticipated by Queen et al. Withdrawal of this entire 35 USC § 102 (b) rejection is respectfully requested. [Ex. 2007, pp. 33-34].

27. An Examiner Interview Summary Record dated January 27, 1993, states "applicant suggests that the 'comprising' in eg clm 24 is not to be taken as 'comprising' more residues than those in clm, i.e. claimed residues are not to be considered open ended. Applicant indicated they would clarify the latter issue. Queen does not teach changing residues: 73HC; 38HC; 71 on LC # 1 on LC + #4 on LC, 36 on LC 46 on LC." (Ex. 2039, p. 4; Emphasis by Examiner).

28. On September 9, 1993, in the Adair PCT/EP Patent Application 91901433.2, Adair filed an amendment deleting original claims 1-23 and replacing them with new claims 1-20 and made the following statements:

> 2.10. In new claim 1, it has been specified that residues 71, 73 and 78 are all donor residues in order to ensure that new claim 1 is novel over the anti-TAC antibody disclosed in PNAS-USA, 86, 10029-10033, 1989 (Queen) (cited in the International Search Report). This anti-TAC antibody has an acceptor residue at residue 73. However, as can be seen from page 7, lines 1 to 5, the Applicant considers that in general, residues 71, 73 and 78 can be either all donor or all acceptor. [Ex. 2009, p. 3].

29. On February 7, 1994, Adair filed an amendment in the '329 application responding to an office action mailed on September 7, 1993 **(**Ex. 2028**)**, wherein Adair made the following statements:

> At a very helpful interview held at the beginning of 1993, there was some discussion of the word "comprising" as used in the claims under consideration at that time. In those claims, it was only specified that certain residues should be donor residues. [Emphasis by Adair]. It was considered that it was not clear whether these were the only residues which could be donor residues. The

alternative view was that these were only the minimum number of residues which must be donor but that any of the other residues could also be donor.

If the second line of interpretation were taken, the claims could be read to cover a situation in which all except one of the residues in the variable domain were donor residues. [Emphasis by Adair]. In this case, the claims could then be interpreted to cover a structure similar to a "chimeric" antibody comprising a donor variable domain and a human constant region. Such chimeric antibodies were already well known at the priority date.

It plainly is not the intention of the Applicants to claim chimeric antibodies or any similar structures. As can be seen from the description, the superhumanised antibodies of the present invention are compared to the prior art chimeric antibodies. Moreover, the present invention was intended to deal with the problem of chimeric antibodies in that chimeric antibodies were believed to be too "foreign" because of the presence of the complete donor variable domain.

For the above reasons, it is clear that the wording of the claims needed to be changed so that the Applicants' intention of excluding chimeric antibodies was made effective. The language now present in the claims puts this intention clearly into effect.

As to support for this wording, the Examiner is referred firstly to page 16, under the heading "Protocol". It can be seen from this paragraph that the first step in the process involves the choice of an appropriate acceptor chain variable domain. This acceptor domain must be of known sequence. Thus, the protocol starts with a variable domain in which all the residues are acceptor residues. In the sentence bridging pages 16 and 17, it is stated that:

> "The CDR-grafted chain is then designed starting from the basis of the acceptor sequence". [Emphasis by Adair].

On page 17, in the middle paragraph, it is stated that:

> "The positions at which donor residues are to be substituted for acceptor in the framework are then chosen as follows ...."

This again shows that, unless a residue is chosen for substitution, it will remain as in the acceptor sequence.

It must also be borne in mind that the purpose of the invention is to obviate some of the disadvantages of prior art proposals. The proposal of using chimeric antibodies had the disadvantage that they were more "foreign" than desirable. The problem of making CDR-grafted antibodies was that they generally did not provide good recovery of affinity. Thus, the aim of the present

invention was to minimise as far as possible the "foreign" nature of the antibody while maximising as far as possible its affinity.

Bearing the passages referred to above and the aim of the invention in mind, it would have been abundantly clear to the skilled person reading the application that as many residues as possible should remain as acceptor residues. If this were not the case, it could hardly be said that the composite chain is based on the acceptor sequence.

The skilled person reading the application can plainly see that certain residues have been considered for changing from acceptor to donor. These are clearly set out in the description. It would be plain to the skilled person that all other residues should not be considered for changing at all. It would therefore be obvious that any residue which is not specified as being under consideration for changing must remain as in the acceptor chain.

It may be that there is no explicit statement in the description that the specified residues should remain as in the acceptor chain. However, the disclosure in a specification is not limited to the explicit disclosure but also includes that which is implicit. It is implicit, in the recitation that the chain is based on the acceptor and that only certain residues are considered for changing, that all non-specified residues must remain as acceptor residues. Subject matter which might be fairly deduced from the disclosure is not new matter. *Acme Highway Products Corp. v. D.S. Brown Co.*, 431 F.2d 1074, 1080, 167 U.S.P.Q. 129, 132-133(6th Cir. 1970), *cert denied*, 401 U.S. 956 (1971).

Another way to look at it is to consider a different way in which the claim could be drafted. It could be specified that in the composite chain, at least a certain minimum number of residues are donor residues (as in the present claims) and at most a certain maximum number of residues are donor residues. The maximum number would be derived by listing all the residues which are considered for changing. Such an amendment would have clear explicit basis in the description because all those residues are mentioned as such. However, the effect of such an amendment would be to produce claims of exactly the same scope as the present claims. It can thus be seen that the present claims do not add subject matter but are plainly properly based on the disclosure in the description.

It is therefore submitted that the claims are fully supported by the description, are commensurate in scope with the disclosure in the description, and are properly delimited over the prior art. [Ex. 2010, pp. 3-7].

30. Adair's non-original pre-critical date claims are grounded in the specific rules governing the "hierarchy of residues" to which Adair attributed the patentability of its claims. (Ex. 2007-2010, 2012-2022, 2024-2027, 2029, 2031, and 2031-2035).

31. Appendix 3 is a claim chart comparing Adair claim 24 as originally filed in 2005 and Adair involved claim 24.

32. Appendix 4 is an accurate comparison of Adair original PCT claims 8 and 16 to Adair involved claim 24.

33. In an amendment filed on April 7, 1993, Adair amended a claim reciting residues 71, 73 and 78, stating the following:

> In claim 67, it has been specified that residues 71, 73 and 78 are all donor residues in order to ensure that claim 67 is novel over the anti-TAC antibody disclosed by Queen. This anti-TAC antibody has an acceptor residue at residue 73. However, as can be seen from page 7, lines 1 to 5, the Applicant considers that in general, residues 71, 73 and 78 can be either all donor or all acceptor. [Ex. 2008, p. 14].

34. In the April 1993 amendment, Adair stated the following:

> It is stated on page 7, lines 1 to 5, that residues 71, 73 and 78 should all be either acceptor or donor. Claims 73, 80, 87, 94 and 101 cover the first alternative and claims 74, 81, 88, 95 and 102 cover the second alternative. [Ex. 2008, p. 15].

35. In an Amendment filed on February 7, 1994, in the ‘329 application, Adair stated the following:

> It is specifically stated in the application that the present protocol represents a departure from the procedures of Reichmann [sic] and Queen, at least. Thus, the skilled person would not rely on Reichmann [sic] and Queen as teachings relevant to whether the present description is enabling.
>
> It is submitted that the skilled person would rely on the clear teaching given in the application and find that it is enabling. The specification plainly sets out what actions need to be taken. It is presumed that the Examiner agrees that the skilled person could have taken those actions. The application also sets out that, contrary to the teachings of Reichmann and Queen, the protocol is generally applicable. The application further shows that it had been successfully implemented. Thus, it is submitted that the skilled person would find that the present application is properly enabled the full extent of the claims. [Ex. 2010, pp. 11-12].

36. Carter's involved U.S. Patent No. 6,407,213 ("the '213 patent") issued on June 18, 2002. (Ex. 2001).

37. One year from the date on which the Carter '213 patent issued is June 18, 2003. (Ex. 2001).

38. On November 21, 2005, Adair filed its involved application 11/284,261 ("the 261 application"). (Ex. 2002).

39. On November 21, 2005, Adair presented new claim 24 as follows:

> Claim 24 (new) A humanised antibody heavy chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises an amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof, as numbered according to Kabat. [Ex. 2003, p. 3].

40. On September 9, 2009, Adair presented its involved claim 24 in the '261 application, which reads as follows:

> Claim 24 (currently amended): A humanised antibody comprising a heavy chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises a non-human amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof, as numbered according to Kabat. [Ex. 2004, p. 2; Adair Clean Copy of Claims, Paper No. 5, p. 4].

41. None of Adair's pre-critical date claims is identical to a Carter '213 patent claim. (Ex.2001, 2005-2010, 2012-2022, 2024-2027, 2029, 2031, and 2031-2035).

42. None of Adair's pre-critical date claims is identical to Adair's involved claim 24. (Ex. 2005-2010, 2012-2022, 2024-2027, 2029, 2031, and 2031-2035 and Adair Clean Copy Of Claims, Paper No. 5, p. 4).

43. Concurrent with the filing of the '261 application, Adair filed a "Preliminary Amendment and Request for Interference under 37 CFR § 42.202 [sic]." (Ex. 2003).

44. On page 4 of the 2005 amendment, Adair stated the following:

> **(b) Compliance with 35 USC § 135(b)**
> Although the present rules do not require a showing of compliance under 35 USC § 135(b), Applicants submit the following to advance the examination of the present application to allowability. […] Claims 1-23 as filed in the PCT application are attached as Appendix A.
>
> Under 35 USC § 135(b)(l), Applicants must show that they had a claim to the same, or substantially the same, subject matter as a claim of the 213 patent within one year of the issuance of the 213 patent, or June 18, 2003. The 213 patent issued on June 18, 2002. The PCT application was filed on December 21, 1990, over 10 years earlier than the 213 patent issued. The time limit of Section 135(b)(l) has been complied with fully. See *Corbett v. Chisholm*, 196 USPQ 337 (CCPA 1977).
>
> To meet the "same or substantially the same invention" requirement of Section 135(b)(l), Applicants must show that their claim contained all material limitations, i.e. limitations necessary to patentability, of the claim of the 213 patent alleged to be to the same, or substantially the same, invention. *Corbett v. Chisholm*, 196 USPQ 337 (C.C.P.A. 1977), *citing Wetmore v. Miller*, 477 F.2d 960, 177 USPQ 699 (C.C.P.A. 19730).
>
> As is evident from Appendix A, Applicants made a claim for the same, or substantially the same, subject matter as a claim of the 213 patent well before the issuance of the 213 patent. Claim 16 of the PCT application, as depending from claim 8, is to substantially the same subject matter as at least claim 1 of the 213 patent. For the Office's convenience, all three claims are duplicated below.
>
> > **Claim 8 of the PCT application**: A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, **58** and 71.
> >
> > **Claim 16 of the PCT application**: A CDR-grafted antibody heavy or **light chain** or molecule according to any one of the preceding claims comprising human acceptor residues and non-human donor residues.
> >
> > **Claim 1 of the 213 patent**: A humanized antibody variable domain comprising non-human Complementarity Determining

Region (CDR) amino acid residues which bind an antigen incorporated into a human antibody variable domain, and further comprising a Framework Region (FR) amino acid substitution at a site selected from the group consisting of: 4L, 38L, 43L, 44L, **58L**, 62L, 65L, 66L, 67L, 68L, 69L, 73L, 85L, 98L, 2H, 4H, 36H, 39H, 43H, 45H, 69H, 70H, 74H, and 92H, utilizing the numbering system set forth in Kabat. [Ex. 2003, pp. 4-6; Emphasis by Adair].

Appendix 3

CLAIM CHART COMPARING ADAIR CLAIM 24 PRESENTED IN 2005 AND ADAIR INVOLVED CLAIM 24

Adair Claim 24 Presented in 2005	Adair Involved Claim 24
A humanised antibody heavy chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises an amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof, as numbered according to Kabat.	A humanised antibody comprising a heavy chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises a non-human amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof, as numbered according to Kabat.

Appendix 4

CLAIM CHART COMPARING
ADAIR ORIGINAL PCT CLAIMS 8 AND 16 WITH ADAIR INVOLVED CLAIM 24

Claims 8 and 16 of Adair PCT/GB90/02017	Involved Claim 24 of Adair 11/284,261
8. A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, 58, and 71. 16. A CDR-grafted antibody heavy or light chain or molecule according to any one of the preceding claims comprising human acceptor residues and non-human donor residues.	A humanised antibody comprising a heavy chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises a non-human amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof, as numbered according to Kabat.

Paper No. _____
Filed on behalf of: Party Carter
Filed: May 28, 2010

By: Oliver R. Ashe, Jr., Esq.
ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

Jeffrey P. Kushan, Esq.
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, DC 20005
Tel.: (202) 736-8914
Fax: (202) 736-8711
E-mail: jkushan@sidley.com

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

PAUL J. **CARTER** AND LEONARD G. PRESTA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR**, DILJEET SINGH ATHWAL,
AND JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

Patent Interference 105,744 (SGL)
Technology Center 1600

CARTER SUBSTANTIVE MOTION 2
(Adair Claim 24 Lacks Written Description)

Table of Contents

CARTER SUBSTANTIVE MOTION 2

I. PRECISE RELIEF REQUESTED

Carter moves under 37 C.F.R. § 41.121(a)(1)(iii) for judgment that involved claim 24 of Adair's U.S. Patent Application No. 11/284,261 ("the '261 application") is not patentable to Adair under 35 U.S.C. § 112, first paragraph, for lack of written description.

II. THE EVIDENCE AND STATEMENT OF MATERIAL FACTS

A list of exhibits, papers, and appendices relied upon in support of this motion is set forth in Appendix 1. A statement of material facts relied upon in support of this motion is set forth in Appendix 2.

III. ARGUMENT

A. Overview

Adair involved claim 24 recites a human heavy chain framework region that "…comprises a non-human amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof…." (MF 37). Giving claim 24 its broadest reasonable construction, it encompasses a human heavy chain framework polypeptide having a single non-human amino acid residue at any of positions 23, 24, 49, 71, 73 or 78. Conversely, claim 24 encompasses human heavy chain framework region polypeptides having non-human residue substitutions within the human framework sequence at any number of locations, up to six, and in any combination. There is no description in Adair's specification of a human heavy chain framework region sequence as defined by claim 24.

The Adair specification recites a set of minimum requirements for its modified human heavy chain framework region polypeptides. These requirements are set forth at pages 16-19 of the specification in a detailed "Protocol" for producing humanized heavy chain framework region

polypeptides. (MF. 12; Ex. 2002). Step 1 of the protocol instructs one to insert all of the donor complementarity determining region ("CDR") sequences into the heavy and light human framework regions. See, *Id*. at 17, lines 7-22. Step 2 then sets forth rules for "the positions at which residues are to be substituted for acceptor in the framework." *Id*. at 17. The specification states (MF 13):

> 2. Heavy Chain
> 2.1 Choose donor residues at all of positions 23, 24, 49, 71, 73 and 78 of the heavy chain or all of positions 23, 24 and 49 (71, 73 and 78 are always either all donor or all acceptor). [Emphasis added].

That these substitutions are mandatory is demonstrated by the next rule in the Adair protocol, which uses different language to indicate optional substitutions. Specifically, Rule 2.2 states (MF 14):

> 2.2. Check that the following have the same amino acid in donor and acceptor sequences, and if not preferably choose the donor: 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107. [Emphasis added].

Thus, Adair expressly teaches that positions 23, 24 and 49 in the human heavy chain framework region must always be donor (*e.g.*, non-human) residues, and following the Adair rules yields only two possible sets of changes to the heavy chain framework region:

(i) replacement of residues 23, 24 and 49 of the human sequence with donor residues (*i.e.*, where residues 71, 73 and 78 are "all acceptor"), or

(ii) replacement of residues 23, 24, 49, 71, 73 and 78 of the human sequence with donor residues (*i.e.*, where 71, 73 and 78 are "all donor").

Therefore, the Adair specification does not provide written description for a claim encompassing acceptor/residues at any of positions 23, 24 and 49, as allowed by Adair claim 24.

Adair also does not describe human heavy chain framework substitutions that involve various combinations of substitutions at positions 23, 24, 49, 71, 73 and 78, as recited in Adair

claim 24. As noted above, the Adair specification requires all of residues 23, 24 and 49 to be donor residues and that positions 71, 73 and 78 must be either all donor (*i.e.*, non-human) residues or all acceptor (*i.e.*, human) residues. (MF 13). Therefore, the Adair specification does not provide written description for a claim encompassing acceptor/donor combinations at any of positions 23, 24, 49 71, 73 and 78, as recited in Adair claim 24.

Other portions of the Adair specification reinforce, rather than relax, these rules. For example, at pages 19-23, Adair offers a "rationale" for its protocol. (MF 15). For surface area residues near the CDR regions, the specification at page 20, line 27, states "Heavy Chain - Key residues are 23, 71 and 73." (MF 16). For the "packing residues near the CDRs," the specification at page 21, line 9, states "Heavy Chain - Key residues are 24, 49 and 78." (MF 17). In other words, the "key" residues to which Adair refers are residues 23, 24, 49, 71, 73 and 78 – the same set of residues governed by specific substitution rules set forth in Adair specification.

Adair's rules and rationale are further reinforced by Adair's observations in its working examples. For example, the Adair specification states: "the presence of the 6, 23 and 24 changes are important to maintain a binding affinity similar to that of the murine antibody." (MF 18). Then, at page 52, lines 25-29, the specification states (MF 19):

> These and other results lead us to the conclusion that of the 11 mouse framework residues used in the gH341A (JA185) construct, it is important to retain mouse residues at all of positions 6, 23, 24, 48 and 49, and possibly for maximum binding affinity at 71, 73 and 78.

The fact that some of the express rules governing substitution of residues 23, 24, 49, 71, 73 and 78 are characterized as "preferred" embodiments does not alter the conclusion that involved claim 24 lacks adequate written description under 35 U.S.C. § 112. First, there is no other disclosure in the Adair specification that describes changes to the specific group of residues recited in claim 24 other than the passages that set forth and reinforce the Adair protocol.

Second, Adair has repeatedly emphasized the necessity of following the express rules governing residues 23, 24, 49, 71, 73 and 78 in distinguishing over prior art references and in response to enablement rejections. (MF 20-33). Moreover, each of these express rules was newly added to the Adair disclosure when Adair filed its PCT benefit application PCT/GB90/02017 ("the PCT application"), at which time Adair also added disclosure to the PCT application acknowledging "recent" developments in the prior art. (MF 3 and 7).

Thus, viewing the involved Adair specification as a whole, claim 24 is unpatentable to Adair because it lacks adequate written description under 35 U.S.C. § 112.

In Carter Substantive Motion 1, Carter moves for judgment that Adair's involved claim 24 is unpatentable for failure to comply with the requirements of 35 U.S.C. § 135(b)(1). However, regardless of whether Adair claim 24 satisfies the written description requirement, Adair claim 24 is barred under § 135(b) and adverse judgment should be entered against Adair.

B. Adair Cannot Rely On Involved Claim 24 Itself For Written Description

Adair presented its first version of involved claim 24 in a preliminary amendment at the time of filing the '261 application (*i.e.*, November 21, 2005).[1] (MF 35-36). Thus, while Adair's involved '261 application is described as being a continuation of U.S. Patent Application No. 08/846,658 ("the '658 application"), claim 24 is not part of the disclosure of the '658 application. Thus, claim 24 itself cannot serve as a basis for satisfying the written description requirement. *Turbocare Division of Demag Delaval Turbomachinary Corporation v. General Electric Company*, 264 F.3d 1111, 1120, 60 U.S.P.Q.2d 1017, 1024 (Fed. Cir. 2001). See also 37 C.F.R.

[1] Adair thereafter amended claim 24. Adair's involved claim 24 was presented on September 9, 2009. (MF 37). A comparison of the 2005 version of claim 24 and the 2009 version of involved claim 24 is provided in Appendix 3.

§ 1.63(d)(1)(iii), which requires that "[t]he specification and drawings filed in the continuation or divisional application contain no matter that would have been new matter in the prior application."[2] Accordingly, any written description for claim 24 must be found elsewhere in the involved Adair '261 specification.

C. Adair Involved Claim 24 - Claim Construction

Adair involved claim 24 reads as follows (MF 37):

> A humanised antibody comprising a heavy chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises a non-human amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof, as numbered according to Kabat.

Given its broadest reasonable interpretation, claim 24 encompasses, *inter alia*, a framework region comprising a non-human amino acid substitution at <u>any one</u> of the human framework residues 23, 24, 49, 71, 73, and 78, and <u>any combination of these recited residues</u>. (MF 39-42). Claim 24 thus encompasses a multitude of different combinations of non-human and human amino acid residues at the recited positions, including:

- <u>non-human</u> (donor) amino acids at positions 71, 73 and 78 and <u>human</u> (acceptor) amino acids at positions 23, 24, and 49 (MF 40);
- <u>non-human</u> (donor) amino acids at positions 23 and 71 and <u>human</u> (acceptor) amino acids at positions 24, 49, 73 and 78 (MF 41); or

[2] Adair did not present a newly executed declaration at the time of filing the '261 application but, rather, relied on the inventor declaration from the parent application to purportedly satisfy the requirements of 37 C.F.R. § 1.63. (MF 34).

- non-human (donor) amino acids at position 23 and human (acceptor) amino acids at positions 24, 49, 71, 73 and 78 (MF 42).

However, as explained below, the express teachings of the involved Adair specification and positions advanced by Adair in *ex parte* prosecution clearly establish that none of the above-identified combinations (as well as many other combinations) within the scope of Adair claim 24 is described, literally or otherwise, by the involved Adair specification. Accordingly, claim 24 is unpatentable to Adair under 35 U.S.C. § 112, first paragraph, for lack of written description.

D. Adair Disclosures Relevant To Recited Residues 23, 24, 49, 71, 73, 78

1. The General Teachings Of The Adair Specification Are Ambiguous

The involved Adair specification contains two passages that, if read alone or in combination without reference to the remainder of the Adair specification, lead to a myriad of possible interpretations. First, the Abstract of Adair's specification reads, in part (MF 9):

> CDR-grafted antibody heavy and light chains comprise acceptor framework and donor antigen binding regions, the heavy chains comprising donor residues at at least one of positions (6, 23) and/or (24, 48) and/or (49, 71) and/or (73, 75) and/or (76) and/or (78) and (88) and/or (91).

Second, page 6, lines 31-37, of the Adair specification reads (MF 10):

> Accordingly, in a first aspect the invention provides a CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

While both of these passages contain the same words and residue numbers, there is considerable ambiguity as to the meaning and scope of each passage, alone and in combination.

- In instances in the Abstract where two positions are set forth in parentheses (*e.g.,* "(6, 23)," "(24, 48)," *etc.*), it is unclear whether one or both positions must be donor residues.

- It is not clear whether the passage at page 6, which does not contain parenthetical descriptions, is intended to describe a different concept than what is described in the Abstract.
- With respect to both passages, the use of the term "and/or" appears to suggest that changes at each one of the series may be required (*i.e.*, 6 + (23 and/or 24) + (48 and/or 49), *etc.*). The alternative reading of this passage would render the term "and/or" superfluous (*i.e.*, to mean any of 6, 23, 24, 49, *etc*.). This reading also ignores the plural reference to "residues" in both passages (*i.e.*, "… comprising donor residues at…").

The Examiner struggled with each of these issues when attempting to interpret the same language when it was presented as part of Adair's original U.S. claims. In particular, original claim 1 of Adair's U.S. Patent Application 07/743,329 ("the '329 application") tracked the text in the Summary of Invention section and read as follows (MF 4 and 6):

> 1. A CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

The Examiner rejected claim 1 (among others) under 35 U.S.C. § 112, second paragraph, characterizing as indefinite the recitation "at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91." (MF 4 and 22). In particular, the Examiner stated it was unclear whether the heavy chain,

a. had at least one of 6, 23, 24, 48, 49, 71, 73, 75, 76, 78, 88, or 91, or alternatively,

b. had at least one of (6) or (23 and/or 24) or (48 and/or 49) or (71 and/or 73) or (75 and/or 76 and/or 78 and 88 and/or 91), or alternatively,

c. had at least one of (6, 23) and/or (24, 48) and/or (49, 71) and/or (73, 75) and 76 and/or (78 and 88) and/or (91).

In response, Adair did not challenge the Examiner's conclusions but, rather, cancelled claim 1 and replaced it with claims reciting specific positions in the framework region that were required to be donor residues. (MF 25).

2. Additional Teachings In The Adair Specification

The broad and ambiguous passages in the Abstract and at page 6 of the Adair specification stand in sharp contrast to the way heavy chain substitutions are described in the remainder of the Adair specification.

The first clear example of Adair's description of its invention appears immediately after the ambiguous language on page 6. Specifically, at page 7, lines 1-5, Adair describes as a "preferred" embodiment a heavy chain framework region which contains donor residues at positions 23, 24, 49, 71, 73 and 78 or alternatively, at positions 23, 24 and 49. (MF 11). Adair explains at this location in the specification that substitutions at positions 71, 73, and 78 must be either all acceptor or all donor residues, while the residues at positions 23, 24, and 49 must always be donor residues. (MF 11).

At page 16, line 30 to page 19, line 9, Adair describes this "preferred protocol" for obtaining CDR-grafted antibodies. (MF 12). For heavy chains, Adair's protocol provides the following instruction: "Choose donor residues at <u>all</u> of positions 23, 24, 49, 71, 73 and 78 of the heavy chain or <u>all</u> of positions 23, 24 and 49 (71, 73 and 78 are always either <u>all donor</u> or <u>all acceptor</u>). (Emphasis added; MF 13)." Again, the Adair disclosure does not describe unrestricted combinations of acceptor/donor residues at any of the positions 23, 24, 49, 71, 73 and 78, as recited in Adair involved claim 24. Rather, Adair teaches two minimum <u>sets</u> of

residue substitutions are required. The first set must consist of donor residues at positions 23, 24 and 49. The second set concerns residues 71, 73 and 78. For this second set, two options are allowed; either 71, 73 and 78 are all from the donor antibody, or 71, 73 and 78 are all from the acceptor (*i.e.*, the human framework region).

Adair's description thus affirmatively rules out modifications to the human framework region that would involve inserting one donor residue at any of positions 23, 24, 49, 71, 73 or 78 where all other residues at these positions are acceptor (*e.g.*, human residues). It also rules out substitutions involving combinations of two, four or five donor residues at these positions. Indeed, it rules out any substitution involving less than 3 residues (*i.e.*, to insert donor residues at positions 23, 24 and 49 where all of the residues at positions 71, 73 and 78 are acceptor), and, in the alternative, permits only one other set of substitutions involving 6 residues (*i.e.*, residues 23, 24, 49 plus the scenario where 71, 73 and 78 are all donor residues).

These additional teachings make clear that Adair did not consider its invention to be any possible combination of acceptor/donor residues at positions 6, 23, 24, 48, 49, 71, 73, 75, 76, 78, 88, or 91 much less unrestricted combinations of acceptor/donor residues at positions 23, 24, 49, 71, 73 and 78, as presently recited in Adair claim 24. Thus, Adair involved claim 24 literally conflicts with Adair's written description, and, therefore, is not patentable to Adair.

E. Prosecution History Regarding Residues 23, 24, 49, 71, 73, 78

As discussed above, the Adair specification contains broad and ambiguous disclosures regarding acceptor/donor residue substitutions, followed by a more detailed description of mandatory rules regarding specific acceptor/donor residue combinations. Although Adair uses the terminology "preferred" in the context of the setting forth these rules, both the Adair specification and the subsequent prosecution history show that Adair believed following these

rules was necessary both to distinguish its invention from prior publications of other scientists and to establish support for its claims.

For example, at pages 4-6 of the specification, Adair provides a discussion of "recent" disclosures by Queen *et al.* relating to CDR-grafted antibodies and the substitution of acceptor framework residues with donor residues. (MF 7). At page 6, lines 22-28, the Adair specification states: "This has enabled us to establish a protocol for obtaining satisfactory CDR-grafted products which may be applied very widely irrespective of the level of homology between the donor immunoglobulin and acceptor framework. <u>The set of residues which we have identified as being of critical importance does not coincide with the residues identified by Queen….</u>" (Emphasis added; MF 8).

In an amendment filed on January 19, 1993, in the '329 application, Adair relied upon the recitation of donor residues at positions 23 <u>and</u> 24 as grounds to distinguish over Riechmann *et al*., which disclosed acceptor residues at positions 23 and 24. (MF 26). In the same amendment, Adair argued its claims included limitations that distinguished the claimed antibody chains over the humanized antibody disclosed in Queen *et al*. (MF 27).

In the January 1993 amendment, Adair also relied on its "protocol" to respond to enablement rejections that had been imposed on claims that recited, *inter alia,* changes to residues 71, 73 and 78 of the heavy chain. Adair stated (MF 21 and 28):

> In contrast, the teaching in the present application can be applied without any undue experimentation to any antibody. All that is required is experimentation following a protocol which is clearly set out in the description, in particular at page 16, line 30 to page 19, line 9. …
>
> There is then no need to carryout computer modeling to determine which donor residues to substitute in to the acceptor sequence. <u>The protocol in the present application provides the teaching directly</u>. It instructs the skilled person to compare the two sequences and change certain specified residues in the acceptor sequence to donor residues.

> …Thus, producing recombinant chains and testing them for affinity merely involves routine experimentation following a protocol which is clearly defined in the application. [Emphasis added].

In an amendment filed on April 7, 1993, Adair amended a claim reciting residues 71, 73 and 78, stating the following (MF 30):

> In claim 67, it has been specified that residues 71, 73 and 78 are all donor residues in order to ensure that claim 67 is novel over the anti-TAC antibody disclosed by Queen. This anti-TAC antibody has an acceptor residue at residue 73. However, as can be seen from page 7, lines 1 to 5, the Applicant considers that in general, residues 71, 73 and 78 can be either all donor or all acceptor.

In the April 1993 amendment, Adair again pointed to its protocol setting forth rules for substitutions to support its newly proposed claims. In particular, Adair explained that its rules specified only two alternatives for substitutions within the set of residues consisting of residues 23, 24, 49, 71, 73 and 78 (MF 30):

> It is stated on page 7, lines 1 to 5, that residues 71, 73 and 78 should all be either acceptor or donor. Claims 73, 80, 87, 94 and 101 cover the first alternative and claims 74, 81, 88, 95 and 102 cover the second alternative. [Emphasis added].

As Adair stated, the claims defining Adair's first alternative (*i.e.*, claims 73, 80, 87, 94 and 101) require donor residues to be inserted only at positions 23, 24 and 49, while the claims defining its second alternative require donor residues to be included at positions 23, 24, 49, 71, 73 and 78. Thus, Adair represented to the PTO that its specification defined two alternatives as to changes to the heavy chain: one involving insertion of donor residues at three locations (*i.e.*, where 71, 73 and 78 are all acceptor), and the other involving insertion of donor residues at six locations (*i.e.*, where 71, 73 and 78 are all donor). (MF 30).

It is noted that Adair's arguments often use terms such as "in general" and "can be" and "should be" when drawing substance from the rules set forth in the Adair specification. Such

attorney argument is not consistent with the express teachings of the Adair specification and is not a substitute for written description in an originally filed application.

In an amendment filed on September 9, 1993, (*i.e.*, after the rejection of its original U.S. claims), Adair cancelled its original claims in Adair's PCT benefit application/EP Patent Application No. 91901433.2 and submitted a substitute set of claims, stating (MF 5 and 31):

> 2.10. In new claim 1, it has been specified that residues 71, 73 and 78 are all donor residues in order to ensure that new claim 1 is novel over the anti-TAC antibody disclosed in PNAS-USA, 86, 10029-10033, 1989 (Queen) (cited in the International Search Report). This anti-TAC antibody has an acceptor residue at residue 73. However, as can be seen from page 7, lines 1 to 5, the Applicant considers that in general, residues 71, 73 and 78 can be either all donor or all acceptor.

In an amendment filed on February 7, 1994, Adair again pointed to its "protocol" to respond to the Examiner's repeated enablement rejections, stating (MF 32):

> It is specifically stated in the application that the present protocol represents a departure from the procedures of Reichmann [sic] and Queen, at least. Thus, the skilled person would not rely on Reichmann [sic] and Queen as teachings relevant to whether the present description is enabling.
>
> It is submitted that the skilled person would rely on the clear teaching given in the application and find that it is enabling. The specification plainly sets out what actions need to be taken. It is presumed that the Examiner agrees that the skilled person could have taken those actions. The application also sets out that, contrary to the teachings of Reichmann and Queen, the protocol is generally applicable. The application further shows that it had been successfully implemented. Thus, it is submitted that the skilled person would find that the present application is properly enabled the full extent of the claims.

Thus, throughout examination of its related '329 application, Adair consistently relied upon the "protocol" as providing the basis for distinguishing claims specifying substitutions at positions corresponding to those recited in Adair's involved claim 24 over the prior art and for supporting enablement and description of these claims. (MF 20-33).

The express teachings of the Adair specification and Adair's representations regarding the same provide compelling evidence that Adair did not consider its invention to encompass any

combination of acceptor/donor residues at positions 23, 24, 49, 71, 73 and 78, as involved Adair claim 24 specifies, and, therefore, Adair claim 24 is not patentable under 35 U.S.C. § 112, first paragraph. *Adang v. Umbeck*, 2007 U.S. App. LEXIS 25198 (Fed. Cir. 2007); *Univ. of Rochester v. G.D. Searle & Co.*, 358 F.3d 916, 927 (Fed. Cir. 2004); *PIN/NIP, Inc. v. Platte Chem. Co.*, 304 F.3d 1235, 1247-48 (Fed. Cir. 2002); *In re Curtis*, 354 F.3d 1347, 1353-54 (Fed. Cir. 2004).

F. Case Law Compels The Conclusion That Adair's Involved Claim 24 Lacks Written Description Support

A claim must be supported by an adequate written description of the invention. *Ariad Pharms., Inc. v. Eli Lilly and Co.*, 598 F.3d 1336, 94 U.S.P.Q.2d 1161 (Fed. Cir. 2010)(en banc). "To satisfy the written description requirement, a patent applicant must 'convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention. The invention is, for purposes of the 'written description' inquiry, whatever is now claimed.'" *ICU Med., Inc., v. Alaris Med. Sys., Inc*, 558 F.3d 1368 (Fed. Cir. 2009)(citing *Vas-Cath Inc. v. Mahurkar*, 935 F.2d 1555, 1563-64, 19 U.S.P.Q.2d 1111 (Fed.Cir.1991)). The description of the invention in the disclosure is what must be assessed. See *Carnegie Mellon University v. Hoffman La-Roche, Inc.*, 545 F.3d. 1115, 88 USPQ2d 1233 (Fed. Cir. 2008)("The basic function of a patent specification is to disclose an invention. It has long been the case that a patentee 'can lawfully claim only what he has invented and described, and if he claims more his patent is void.' (citing *O'Reilly v. Morse*, 56 U.S. (15 How.) 62, 121, 14 L.Ed. 601 (1853)).")

A broad claim is not patentable when the entirety of the specification clearly indicates that the invention is of a much narrower scope. *Carnegie Mellon,* 545 F.3d at 1127, citing *Gentry Gallery, Inc. v. Berkline Corp.*, 134 F.3d 1473 (Fed.Cir.1998). This is precisely the circumstance presented by Adair's involved claim. Adair's disclosure plainly envisioned a far more restricted invention that what Adair involved claim 24 now defines. First, Adair's

specification requires mandatory changes to the residues at locations 23, 24 and 49 of the human heavy chain framework region to incorporate the donor residue at each of those locations. Second, Adair's specification mandates a rule for a second set of residues at positions 71, 73 and 78, *i.e.*, these residues must all be donor or must all be acceptor. Thus, as Adair itself acknowledged during prosecution of its related '329 application, there are two alternative "minimum" sets of changes to heavy chain residues; one involving changes at three residue positions (*i.e.*, 23, 24 and 49, because residue positions 71, 73 and 78 are all to remain "acceptor" residues), and a second involving six residue positions (*i.e.*, 23, 24, and 49, plus the "donor" residues at all of positions 71, 73 and 78). Despite this description of minimum requirements, Adair's involved claim 24 allows for substitutions ranging from a single donor residue among the six recited positions, donor residues at any number of residue positions up to six, and any permutation of possible acceptor/donor substitutions at these six positions, including substitutions that violate Adair express rules regarding residues at positions 23, 24, 49, 71, 73 and 78. The stark contrast between the scope of the Adair specification and the scope of Adair's involved claim 24 compels a determination that claim 24 is not patentable under 35 U.S.C. § 112, first paragraph.

IV. CONCLUSION

Adair's involved claim 24 lacks written description and, therefore, is not patentable under 35 U.S.C. § 112, first paragraph. Judgment should be entered against Adair.

Respectfully submitted,

May 28, 2010

/Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.
Registration No. 40,491
Counsel for Party Carter

CERTIFICATE OF FILING

The undersigned certifies that a copy of the paper entitled **"CARTER SUBSTANTIVE MOTION 2"** was filed this 28th day of May, 2010, via Interference Web Portal (https://acts.uspto.gov/ifiling/), with:

The Board of Patent Appeals and Interferences
Madison Building East, 9th Floor
600 Dulany Street
Alexandria, VA 22314
Tel: 571-272-4683
Fax: 571-273-0042
E-mail: BoxInterferences@USPTO.GOV

May 28, 2010 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

CERTIFICATE OF SERVICE

The undersigned hereby certifies that a copy of the paper entitled **"CARTER SUBSTANTIVE MOTION 2"** was served this 28th day of May, 2010, via Interference Web Portal (https://acts.uspto.gov/ifiling/), on the Attorney of Record for Adair:

Doreen Yatko Trujillo, Esq.
Cozen O'Connor P.C.
1900 Market Street, 7th Floor
Philadelphia, PA 19103
Tel.: 215-665-6593
Fax: 215-701-2005
E-mail: dtrujillo@cozen.com

May 28, 2010 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

Appendix 1

EVIDENCE

I. Exhibits Cited

The following exhibits are cited in support of this motion:

Ex. 2002 U.S. Patent Application No. 11/284,261 to Adair *et al*., filed November 21, 2005.

Ex. 2003 Preliminary Amendment and Request for Interference Under 37 C.F.R. § 42.202 [sic], filed November 21, 2005, in U.S. Patent Application No. 11/284,261 to Adair *et al*.

Ex. 2004 Request for Reconsideration, filed September 9, 2009, in U.S. Patent Application No. 11/284,261 to Adair *et al*.

Ex. 2005 PCT Application No. PCT/GB90/02017 to Adair *et al*., filed December 21, 1990, published as WO 91/09967 on July 11, 1991 ("the PCT Application").

Ex. 2006 U.S. Patent Application No. 07/743,329 to Adair *et al*., filed September 17, 1991.

Ex. 2007 Response to Office Action filed January 19, 1993, in U.S. Patent Application No. 07/743,329 to Adair *et al*.

Ex. 2008 Amendment filed April 7, 1993, in U.S. Patent Application No. 07/743,329 to Adair *et al*.

Ex. 2009 Letter regarding amendments filed September 9, 1993, in European Patent Application No. 91901433.2 to Adair *et al*.

Ex. 2010 Amendment filed February 7, 1994, in U.S. Patent Application No.

07/743,329 to Adair *et al*.

Ex. 2011 Riechmann *et al.*, *Nature*, Vol. 332, pp. 323-327 (March 1988).

Ex. 2023 Queen *et al.*, *Proc. Natl. Acad. Sci. USA*, Vol. 86, pp. 10029-10033 (December 1989).

Ex. 2028 Office Action mailed September 7, 1993, in U.S. Patent Application No. 07/743,329 to Adair *et al.*

Ex. 2036 Great Britain Application No. 8928874.0 to Adair *et al*., filed December 21, 1989 ("the UK Application").

Ex. 2037 Computer generated comparison (using Workshare™ Professional 5.2 SR2 software) of the typewritten text of the UK Application to the typewritten text of the PCT Application.

Ex. 2038 Office Action mailed November 18, 1992, in U.S. Patent Application No. 07/743,329 to Adair *et al*.

Ex. 2039 Examiner Interview Summary Record dated January 27, 1993, in U.S. Patent Application No. 07/743,329 to Adair *et al*.

II. Papers Cited

The following papers are cited in support of this motion:

Paper No. 5 Adair Clean Copy of Claims filed February 16, 2010.

III. Appendices Cited

The following papers are cited in support of this motion:

Appendix 1 Evidence.

Appendix 2	Statement of Material Facts Relied Upon in Motion.
Appendix 3	Claim chart comparing Adair claim 24 presented in 2005 and Adair involved claim 24.

Appendix 2

STATEMENT OF MATERIAL FACTS RELIED UPON IN MOTION

1. On December 21, 1989, Adair filed Great Britain Application GB 8928874.0 ("the UK Application"). (Ex. 2036).

2. On December 21, 1990, Adair filed PCT Application PCT/GB90/02017 ("the PCT Application"). (Ex. 2005).

3. Exhibit 2037 is a computer generated comparison (using Workshare™ Professional 5.2 SR2 software) of the typewritten text of the UK Application to the typewritten text of the PCT Application. The last page of Exhibit 2037 contains a color-coded legend for identifying deletions, additions, and movement of text.

4. On September 17, 1991, Adair entered the U.S. national stage by filing U.S. Patent Application No. 07/743,329 ("the '329 application"). (Ex. 2006).

5. Adair's '329 application contained claims 1-23, which are identical to claims 1-23 as originally filed with Adair's PCT application. (Ex. 2005, pp. 67-70 and Ex. 2006, pp. 67-70).

6. Original claim 1 of the Adair '329 application reads as follows:

> 1. A CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91. [Ex. 2006, p. 67].

7. At pages 4-6 of the specification, Adair provides a discussion of "recent" disclosures by Queen *et al*. relating to CDR-grafted antibodies and the substitution of acceptor framework residues with donor residues. (Ex. 2002, pp. 4-6).

8. At page 6, lines 22-28, the Adair specification states:

This has enabled us to establish a protocol for obtaining satisfactory CDR-grafted products which may be applied very widely irrespective of the level of homology between the donor immunoglobulin and acceptor framework. The set of residues which we have identified as being of critical importance does not coincide with the residues identified by Queen…." [Ex. 2002, p. 6, lns. 22-28].

9. The Abstract of Adair's involved specification reads, in part, as follows:

CDR-grafted antibody heavy and light chains comprise acceptor framework and donor antigen binding regions, the heavy chains comprising donor residues at at least one of positions (6, 23) and/or (24, 48) and/or (49, 71) and/or (73, 75) and/or (76) and/or (78) and (88) and/or (91). [Ex. 2002, Abstract].

10. At page 6, lines 31-37, the Adair specification reads as follows:

Accordingly, in a first aspect the invention provides a CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91. [Ex. 2002, p. 6, lns. 31-37].

11. At page 7, lines 1-5, the Adair specification reads as follows:

In preferred embodiments, the heavy chain framework comprises donor residues at positions 23, 24, 49, 71, 73 and 78 or at positions 23, 24 and 49. The residues at positions 71, 73 and 78 of the heavy chain framework are preferably either all acceptor or all donor residues. [Ex. 2002, p. 7, lns. 1-5].

12. At page 16, line 30 to page 19, line 9, Adair describes its "preferred protocol" for obtaining CDR-grated antibodies. (Ex. 2002, p. 16, ln. 30 to p. 19, ln. 9).

13. At page 17, lines 27-30, the involved Adair specification reads as follows under a section titled "Protocol":

2. Heavy Chain
2.1 Choose donor residues at <u>all</u> of positions 23, 24, 49, 71, 73 and 78 of the heavy chain or <u>all</u> of positions 23, 24 and 49 (71, 73 and 78 are <u>always</u> either <u>all</u> donor or <u>all</u> acceptor). [Ex. 2002, p. 17, lns. 25-30; Emphasis added].

14. At page 17, lines 32-35, the involved Adair specification states:

2.2. Check that the following have the same amino acid in donor and acceptor sequences, and if not preferably choose the donor: 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107. [Ex. 2002, p. 17, lns. 32-35].

15. At pages 19-23 of its involved specification, Adair offers a "rationale" for its protocol. (Ex. 2002, pp. 19-23).

16. At page 20, line 27, the involved Adair specification states "Heavy Chain - Key residues are 23, 71 and 73." (Ex. 2002, p. 20, ln. 27).

17. At page 21, line 9, for the "packing residues near the CDRs," the involved Adair specification states "Heavy Chain - Key residues are 24, 49 and 78." (Ex. 2002, p. 21, ln. 9).

18. At page 48, lines 25-27, the involved Adair specification explains: "the presence of the 6, 23 and 24 changes are important to maintain a binding affinity similar to that of the murine antibody." (Ex. 2002, p. 48, lns. 25-27).

19. At page 52, lines 25-29, the Adair involved specification states:

> These and other results lead us to the conclusion that of the 11 mouse framework residues used in the gH341A (JA185) construct, it is important to retain mouse residues at all of positions 6, 23, 24, 48 and 49, and possibly for maximum binding affinity at 71, 73 and 78. [Ex. 2002, p. 52, lns. 25-29].

20. On November 18, 1992, the U.S. Patent and Trademark Office entered a non-final office action rejecting Adair's original claims 1-23 on various grounds. (Ex. 2038).

21. At page 5 of the November 1992 office action, the Examiner rejected claims 1-5 under 35 U.S.C. § 112, first paragraph as not being enabled. In particular, the Examiner stated that practicing the invention as claimed would require undue experimentation relative to the teachings of the Adair specification. (Ex. 2038, p. 5).

22. At page 6 of the November 1992 office action, the Examiner rejected claims 1-5 under 35 U.S.C. § 112, second paragraph, as being indefinite in their recitation of "at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91" because it was unclear whether the heavy chain,

a. had at least one of 6, 23, 24, 48, 49, 71, 73, 75, 76, 78, 88, or 91, or alternatively,

b. had at least one of (6) or (23 and/or 24) or (48 and/or 49) or (71 and/or 73) or (75 and/or 76 and/or 78 and 88 and/or 91), or alternatively,

c. had at least one of (6, 23) and/or (24, 48) and/or (49, 71) and/or (73, 75) and 76 and/or (78 and 88) and/or (91). (Ex. 2038, p. 6).

23. At pages 7-12 of the November 1992 office action, the Examiner rejected Adair's claims under 102/103 in view of Riechmann *et al*., *Nature*, Vol. 332, pp. 323-327 (March 1988) and Queen *et al*., *Proc. Natl. Acad. Sci. USA*, Vol. 86, pp. 10029-10033 (December 1989) . (Ex. 2038, pp. 7-12; Ex. 2011, and Ex. 2023).

24. On January 19, 1993, Adair responded to the November 1992 Office action. (Ex. 2007).

25. In the January 1993 amendment, Adair responded to the rejection of claims under 35 U.S.C. § 112, second paragraph, by cancelling claims 1-12. (Ex. 2007, pp. 29-32).

26. In the January 19, 1993, amendment, Adair responded to the rejection of claims under 35 U.S.C. § 102(b) in view of Riechmann *et al.* as follows:

> In Part A of this rejection, claims 1, 5, 6-8, and 12-22 were rejected as anticipated by Riechmann et al. The Examiner stated that claim 1 and claim 6 were interpreted to mean that the framework has donor residues in at least one of any of positions 6, 23, 24, 48, 49, 71, 73, 75, 76, 78, 88, or 91 in the heavy chain and (1, 3, 46, or 47) or 46, 48, 58, or 71) in the light chain, and thus, the teachings of Riechmann et al. anticipate the invention as claimed.
>
> The Examiner contends that the original claims lacked novelty over Riechmann et al. Claims 1, 5, 6-8, 12 and 22 have been cancelled without prejudice and submitted as new claims that more distinctly point out certain aspects of the present invention.

> In present claims 24 and 25, it is specified that residues 23 and 24 in the heavy chain should be donor residues. However, as can be seen from Fig. 1, panel (a) in Riechmann et al., in the recombinant antibody shown there, residues 23 and 24 are acceptor residues. [Ex. 2007, p. 32-33].

27. In the January 19, 1993, response, Adair responded to the rejection of claims under 35 U.S.C. § 102(b) in view of Queen *et al.* as follows:

> In Part B of the rejection, the Examiner rejected claims 1-6 and 12-22 as anticipated by Queen et al.
>
> Claims 1-6, 12-20 and 22 have been cancelled without prejudice and submitted as new claims that more distinctly point out certain aspects of the present invention.
>
> In present claims 24 and 25, it is specified that residues 48, 66, 67, 68, 93, 103 to 108 and 110 should all be acceptor residues. However, in Queen et al., as can be seen from Fig. 2B, in these positions Queen et al. uses donor, rather than acceptor, residues. It should again be borne in mind that Queen et al. does <u>not</u> use the Kabat numbering and it is therefore necessary to look carefully at the disclosure in Queen et al. before it is possible to come to any final conclusion. [Emphasis by Adair].
>
> In present claim 38, it is specified that residue 71 should be a donor residue. However, as can be seen from Fig. 2A of Queen et al., in that position Queen et al. uses an acceptor, rather than a donor residue.
>
> Applicants' claimed antigen-binding molecules are thus not anticipated by Queen et al. Withdrawal of this entire 35 USC § 102 (b) rejection is respectfully requested. [Ex. 2007, pp. 33-34].

28. At pages 26-28 of its January 19, 1993, response, Adair responded to the § 112, first paragraph rejection by arguing, *inter alia*, as follows:

> In contrast, the teaching in the present application can be applied without undue experimentation to any antibody. All that is required is experimentation following a protocol which is clearly set out in the description, in particular at page 16, line 30 to page 19, line 9. In order to follow this protocol, as a first step, it is necessary to determine the amino acid sequence of the donor chain. The sequence of the acceptor chain will already be known, for instance from a sequence data base.
>
> There is then no need to carry out computer modeling to determine which donor residues to substitute into the acceptor sequence. The protocol in the

> present application provides the teaching directly. It instructs the skilled person to compare the two sequences and change certain specified residues in the acceptor sequence to donor residues.
>
> Moreover, the present application provides a hierarchical structure of residues which can be considered. Thus, if changing the residues at the top of the structure does not provide adequate affinity, then a lower level of residues are considered, and so on until acceptable affinity is obtained.
>
> […]
>
> It is submitted that this identifies where the present invention makes a significant departure from the prior art. The prior art indicates that each antibody has to be treated individually. In contrast, the present invention teaches that, by following the protocol set forth in the present application, it is possible to reshape any antibody. [Ex. 2007, pp. 26-28].

29. An Examiner Interview Summary Record dated January 27, 1993, states "applicant suggests that the 'comprising' in eg clm 24 is not to be taken as 'comprising' more residues than those in clm, i.e. claimed residues are not to be considered open ended. Applicant indicated they would clarify the latter issue. Queen does not teach changing residues: 73HC; 38HC; 71 on LC # 1 on LC + #4 on LC, 36 on LC 46 on LC." (Ex. 2039, p. 4; Emphasis by Examiner).

30. On April 7, 1993, Adair made the following statements in an amendment:

> Having considered the Examiner's concerns that the language of the claims might be indefinite, because it was not clear whether the specified residues were the only or the minimum number of residues to be donor residues, the Applicants have amended the claims. In all the claims it is made clear that there is a minimum number of residues which have to be donor residues and a minimum number which have to be acceptor residues. Those residues which are not specified in the claims may be either donor or acceptor. [Ex. 2008, p. 13; Emphasis by Adair].
>
> In claim 67, it has been specified that residues 71, 73 and 78 are all donor residues in order to ensure that claim 67 is novel over the anti-TAC antibody disclosed by Queen. This anti-TAC antibody has an acceptor residue at residue 73. However, as can be seen from page 7, lines 1 to 5, the Applicant considers that in general, residues 71, 73 and 78 can be either all donor or all acceptor. [Ex. 2008, p. 14].

It is stated on page 7, lines 1 to 5, that residues 71, 73 and 78 should all be either acceptor or donor. Claims 73, 80, 87, 94 and 101 cover the first alternative and claims 74, 81, 88, 95 and 102 cover the second alternative. [Ex. 2008, p. 15].

31. On September 9, 1993, in the Adair PCT/EP Patent Application 91901433.2, Adair filed an amendment deleting original claims 1-23 and replacing them with new claims 1-20 and made the following statements:

2.10. In new claim 1, it has been specified that residues 71, 73 and 78 are all donor residues in order to ensure that new claim 1 is novel over the anti-TAC antibody disclosed in PNAS-USA, 86, 10029-10033, 1989 (Queen) (cited in the International Search Report). This anti-TAC antibody has an acceptor residue at residue 73. However, as can be seen from page 7, lines 1 to 5, the Applicant considers that in general, residues 71, 73 and 78 can be either all donor or all acceptor. [Ex. 2009, p. 3].

32. On February 7, 1994, Adair filed an amendment in the '329 application responding to the office action mailed on September 7, 1993 (Ex. 2028), wherein Adair stated:

It is specifically stated in the application that the present protocol represents a departure from the procedures of Reichmann [sic] and Queen, at least. Thus, the skilled person would not rely on Reichmann [sic] and Queen as teachings relevant to whether the present description is enabling.

It is submitted that the skilled person would rely on the clear teaching given in the application and find that it is enabling. The specification plainly sets out what actions need to be taken. It is presumed that the Examiner agrees that the skilled person could have taken those actions. The application also sets out that, contrary to the teachings of Reichmann and Queen, the protocol is generally applicable. The application further shows that it had been successfully implemented. Thus, it is submitted that the skilled person would find that the present application is properly enabled the full extent of the claims. [Ex. 2010, pp. 11-12].

33. In the February 7, 1994, amendment, Adair made the following statements:

At a very helpful interview held at the beginning of 1993, there was some discussion of the word "comprising" as used in the claims under consideration at that time. In those claims, it was only specified that certain residues should be donor residues. [Emphasis by Adair]. It was considered that it was not clear whether these were the only residues which could be donor residues. The alternative view was that these were only the minimum number of residues which must be donor but that any of the other residues could also be donor.

If the second line of interpretation were taken, the claims could be read to cover a situation in which all except one of the residues in the variable domain were donor residues. [Emphasis by Adair]. In this case, the claims could then be interpreted to cover a structure similar to a "chimeric" antibody comprising a donor variable domain and a human constant region. Such chimeric antibodies were already well known at the priority date.

It plainly is not the intention of the Applicants to claim chimeric antibodies or any similar structures. As can be seen from the description, the superhumanised antibodies of the present invention are compared to the prior art chimeric antibodies. Moreover, the present invention was intended to deal with the problem of chimeric antibodies in that chimeric antibodies were believed to be too "foreign" because of the presence of the complete donor variable domain.

For the above reasons, it is clear that the wording of the claims needed to be changed so that the Applicants' intention of excluding chimeric antibodies was made effective. The language now present in the claims puts this intention clearly into effect.

As to support for this wording, the Examiner is referred firstly to page 16, under the heading "Protocol". It can be seen from this paragraph that the first step in the process involves the choice of an appropriate acceptor chain variable domain. This acceptor domain must be of known sequence. Thus, the protocol starts with a variable domain in which all the residues are acceptor residues. In the sentence bridging pages 16 and 17, it is stated that:

> "The CDR-grafted chain is then designed starting from the basis of the acceptor sequence". [Emphasis by Adair].

On page 17, in the middle paragraph, it is stated that:

> "The positions at which donor residues are to be substituted for acceptor in the framework are then chosen as follows ...."

This again shows that, unless a residue is chosen for substitution, it will remain as in the acceptor sequence.

It must also be borne in mind that the purpose of the invention is to obviate some of the disadvantages of prior art proposals. The proposal of using chimeric antibodies had the disadvantage that they were more "foreign" than desirable. The problem of making CDR-grafted antibodies was that they generally did not provide good recovery of affinity. Thus, the aim of the present invention was to minimise as far as possible the "foreign" nature of the antibody while maximising as far as possible its affinity.

Bearing the passages referred to above and the aim of the invention in mind, it would have been abundantly clear to the skilled person reading the application that as many residues as possible should remain as acceptor residues. If this were not the case, it could hardly be said that the composite chain is based on the acceptor sequence.

The skilled person reading the application can plainly see that certain residues have been considered for changing from acceptor to donor. These are clearly set out in the description. It would be plain to the skilled person that all other residues should not be considered for changing at all. It would therefore be obvious that any residue which is not specified as being under consideration for changing must remain as in the acceptor chain.

It may be that there is no explicit statement in the description that the specified residues should remain as in the acceptor chain. However, the disclosure in a specification is not limited to the explicit disclosure but also includes that which is implicit. It is implicit, in the recitation that the chain is based on the acceptor and that only certain residues are considered for changing, that all non-specified residues must remain as acceptor residues. Subject matter which might be fairly deduced from the disclosure is not new matter. *Acme Highway Products Corp. v. D.S. Brown Co.*, 431 F.2d 1074, 1080, 167 U.S.P.Q. 129, 132-133(6th Cir. 1970), *cert denied*, 401 U.S. 956 (1971).

Another way to look at it is to consider a different way in which the claim could be drafted. It could be specified that in the composite chain, at least a certain minimum number of residues are donor residues (as in the present claims) and at most a certain maximum number of residues are donor residues. The maximum number would be derived by listing all the residues which are considered for changing. Such an amendment would have clear explicit basis in the description because all those residues are mentioned as such. However, the effect of such an amendment would be to produce claims of exactly the same scope as the present claims. It can thus be seen that the present claims do not add subject matter but are plainly properly based on the disclosure in the description.

It is therefore submitted that the claims are fully supported by the description, are commensurate in scope with the disclosure in the description, and are properly delimited over the prior art. [Ex. 2010, pp. 3-7].

34. Adair did not present a newly executed declaration at the time of filing the ‘261 application but, rather, relied on the inventor declaration from the parent application to satisfy the requirements of 37 C.F.R. § 1.63. (Ex. 2002).

35. On November 21, 2005, Adair filed its involved application, *i.e.*, U.S. Patent Application No. 11/284,261 ("the '261 Application"). (Ex. 2002).

36. On November 21, 2005, Adair presented new claim 24 as follows:

> Claim 24 (new) A humanised antibody heavy chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises an amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof, as numbered according to Kabat. [Ex. 2003, p. 3].

37. On September 9, 2009, Adair presented its involved claim 24 in the '261 application, which reads as follows:

> Claim 24 (currently amended): A humanised antibody comprising a heavy chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises a non-human amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof, as numbered according to Kabat. [Ex. 2004, p. 2; Adair Clean Copy of Claims, Paper No. 5, p. 4].

38. Appendix 3 is a claim chart comparing Adair claim 24 as originally filed in 2005 and Adair involved claim 24.

39. Adair involved claim 24 encompasses a humanized antibody wherein the heavy chain variable domain framework region has any combination of human and non-human amino acid residues at positions 23, 24, 49, 71, 73 and 78. (Adair Clean Copy of Claims, Paper No. 5, p. 4).

40. Adair involved claim 24 encompasses a humanized antibody wherein the heavy claim variable domain framework region has non-human amino acids at positions 71, 73 and 78 and human amino acids at positions 23, 24, and 49. (Adair Clean Copy of Claims, Paper No. 5, p. 4).

41. Adair involved claim 24 encompasses a humanized antibody wherein the heavy claim variable domain framework region has non-human amino acids at positions 23 and 71 and human amino acids at positions 24, 49, 73 and 78. (Adair Clean Copy of Claims, Paper No. 5, p. 4).

42. Adair involved claim 24 encompasses a humanized antibody wherein the heavy claim variable domain framework region has non-human amino acids at position 23 and human amino acids at positions 24, 49, 71, 73 and 78. (Adair Clean Copy of Claims, Paper No. 5, p. 4).

Appendix 3

CLAIM CHART COMPARING ADAIR CLAIM 24 PRESENTED IN 2005 AND ADAIR INVOLVED CLAIM 24

Adair Claim 24 Presented in 2005	Adair Involved Claim 24
A humanised antibody heavy chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises an amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof, as numbered according to Kabat.	A humanised antibody comprising a heavy chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises a non-human amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof, as numbered according to Kabat.

Mail Stop Interference
P.O. Box 1450
Alexandria Va 22313-1450
Tel: 571-272-9797
Fax: 571-273-0042

Paper 73
Filed: 16 June 2010

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

PAUL J. **CARTER** AND LEONARD G. PRESTIA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR**, DILGEET SINGH ATHWAL, and JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

Patent Interference No. 105,744
(Technology Center 1600)

ORDER –Authorizing Oppositions – 125(a)

A conference call was held on 15 June 2010 at approximately 2:00 pm.

Participating in the call were:

(1) Oliver Ashe for Carter,

(2) Doreen Trujillo for Adair, and

(3) Sally Gardner Lane, Administrative Patent Judge.

Adair oppositions

Carter has filed two motions (Paper 71 and 72). The motions address threshold issues. Adair oppositions were not previously authorized. (Paper 23 at 3). After review of the motions, the Board has determined that it is appropriate to authorize Adair oppositions to the Carter motions. As requested by Adair, a four week time period is set for the filing of the Adair oppositions.[1,2]

As discussed during the call and as agreed to by the parties, Carter will be given a small amount of time in addition to that set out in Bd. R. 155(b)(1) to make any objections to evidence relied upon in the Adair oppositions.

No Carter reply to either of the Adair oppositions is authorized at this time.

Settlement conference

Adair noted that it has neglected to initiate the settlement conference required by the Standing Order at ¶ 126. 2. Adair indicated that it is awaiting a response from its real party in interest regarding plans for a settlement conference, however it is unlikely that settlement will occur.

Order

It is

ORDERED that Adair oppositions to Carter Motions 1 and 2 shall be filed on or before **14 July 2010**;

---

[1] Due to a health issue affecting Adair lead counsel, Adair has requested, and Carter has agreed to the request for, additional time than would ordinarily be authorized.

[2] Adair indicated that it does not wish to file a responsive motion and none is authorized. Adair should contact the Board and arrange a conference call immediately if Adair determines that it wishes to seek authorization to file a responsive motion.

FURTHER ORDERED that any Carter objections to evidence relied upon in the Adair opposition under Bd. R. 155(b) (1) shall be filed on or before **28 July 2010**; and

FURTHER ORDERED that Adair shall, within a reasonable time from the date of this Order, initiate the settlement negotiations required by the Standing Order (SO at ¶ 126.2).

/Sally Gardner Lane/
Administrative Patent Judge

cc (via electronic delivery):

Attorney for Carter:

Oliver R. Ashe, Jr., Esq.
ASHE, P.C.
11440 Isaac Newton Square North
Suite 210
Reston, VA 20190

Tel: 703-467-9001
Email: oashe@ashepc.com

Jeffrey P. Kushan, Esq.
SIDLEY AUSTIN LLP
1501 K Street, NW
Washington, DC 20005

Tel: 202-736-8914
Email: jkushan@sidley.com

Attorney for Adair:

Doreen Yatko Trujillo, Esq.
Michael B. Fein, Esq
COZEN O'CONNOR P.C.
1900 Market Street
Philadelphia, PA 19103

Tel: 215-665-5593
Email: dtrujillo@cozen.com

Filed on behalf of: **Adair**
By: Doreen Yatko Trujillo
Michael B. Fein
Cozen O'Connor P.C.
1900 Market St.
Philadelphia, PA 19103
Telephone: (215) 665-5593
Facsimile: (215) 701-2005
dtrujillo@cozen.com

Paper No:_____
Date Filed: June 28, 2010

UNITED STATES PATENT AND TRADEMARK OFFICE

_____

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

_____

PAUL J. **CARTER** AND LEONARD G. PRESTA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR,** DILJEET SINGH ATHWAL,
AND JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

Patent Interference No. 105,744
(Technology Center 1600)

JOINT STATEMENT REGARDING SETTLEMENT EFFORTS

As required by ¶ 126.4 of the Standing Order ("S.O."; Paper No. 2), the parties hereby file this joint statement advising that settlement is not likely at this time. Both parties, however, are open to future discussions.

The parties believe that the conference call with Judge Lane required under S.O. ¶ 126.2 is not necessary because the parties are not engaged in settlement discussions. However, if Judge Lane would like a teleconference, counsel for Adair will arrange to schedule one.

The undersigned has been authorized to file this paper on behalf of both parties.

Respectfully submitted,

/Doreen Yatko Trujillo/
DOREEN YATKO TRUJILLO
Registration No. 35,719
Lead Counsel for Adair

Date: June 28, 2010

Cozen O'Connor P.C.
1900 Market St.
Philadelphia, PA 19103
Telephone: (215) 665-5593
Facsimile: (215) 01-2005
dtrujillo@cozen.com

Filed on behalf of: **Adair**
By: Doreen Yatko Trujillo
Michael B. Fein
Cozen O'Connor P.C.
1900 Market St.
Philadelphia, PA 19103
Telephone: (215) 665-5593
Facsimile: (215) 701-2005
dtrujillo@cozen.com

Paper No:_____
Filed: July 14, 2010

UNITED STATES PATENT AND TRADEMARK OFFICE

_____

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

_____

PAUL J. **CARTER** AND LEONARD G. PRESTA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR,** DILJEET SINGH ATHWAL,
AND JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

Patent Interference No. 105,744
(Technology Center 1600)

ADAIR OPPOSITION 1

I. Adair Statement of the Precise Relief Requested

Adair requests that Carter Substantive Motion 1 ("CSM1") be denied. Adair's involved claim 24 is not barred by 35 U.S.C. §135(b)(1).

II. Evidence

The exhibit list is attached as Appendix 1.

III. Statement of Material Facts

The Statement of Material Facts is attached as Appendix 2.

IV. Argument

Adair will ultimately show that it is the first inventor of the invention of the Count. Specifically, claim 24 of the involved Adair application has a priority date that is almost **18 months** earlier than the earliest priority date claimed by Carter (Fact 54). Even if Adair is not entitled to its earliest priority date, its next priority date is the filing date of Adair's PCT application, which is almost **six months** before the earliest priority date claimed by Carter (Fact 51).

In CSM1, Carter alleges that Adair has not complied with 35 U.S.C. § 135(b)(1) and that, thus, Adair does not have standing to pursue this interference. As Adair shows below, Adair complied with 35 U.S.C. § 135(b)(1) and Adair does have standing to pursue this interference.

A. Adair Can Rely Upon Adair's PCT Application Claims

In footnote 5, on page 3 of CSM1, Carter alleges that Adair cannot rely on its original claims in Adair's PCT application because Adair cannot rely upon a PCT application to satisfy the requirements of 35 U.S.C. § 135(b)(1) as a matter of law. Carter, however, cites no law in support of this allegation; nor is Adair aware of any such law. Indeed, the Patent Statute

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Unless otherwise indicated, the same abbreviations as used in CSM1 are used herein.

provides that a PCT application designating the United States, which Adair's PCT application does (Fact 47), has the same effect from its filing date as a national application for patent regularly filed in the United States Patent & Trademark Office **except** as otherwise provided in Section **102(e)**; Section 135(b) is not so excepted (Fact 46). 35 U.S.C. § 363, emphasis added. Regardless, as Carter admits, the '329 application is a **national phase** of Adair's PCT application (Fact 5), which makes it entitled to the filing date of Adair's PCT application as it was filed within the requisite time frame to be so entitled (Facts 1 and 5). Accordingly, Adair can rely upon its original claims in Adair's PCT application, filed December 21, 1990 (Fact 50). Thus, Adair had claims to substantially the same subject matter as the Carter '213 patent over **12 years** before the critical date (Facts 37 and 50).

B. Adair Has Complied With 135(b)(1)

Carter advances three main arguments as to why Adair does not have standing to pursue this interference. As discussed below, each of Carter's arguments fails as lacking legal and/or factual support.

1. Adair's Is Not Required To Show That Its Pre-Critical Date Claims Are Patentable

On page 1, lines 15-16, of CSM1, Carter alleges that Adair must have presented a pre-critical date claim that is patentable to Adair. Beginning on page 4, line 21, through page 9, line 14, of CSM1, Carter continues along this vein, arguing that Adair is not "statutorily entitled" to any of its original PCT/U.S. claims. Carter relies, *inter alia*, on Adair's cancellation of its original PCT/U.S. claims as evidence that the claims were not patentable. On page 9, lines 11-14, of CSM1, Carter cites four cases allegedly to support its position that pre-critical date claims must be patentable. None of the cases cited by Carter, however, holds that the pre-critical date claims must be patentable. *See Adang v. Umbeck,* 2007 U.S. App. LEXIS 25198 (Fed. Cir.

2007); *Univ. of Rochester v. G.D. Searle & Co.,* 358 F.3d 916, 927 (Fed. Cir. 2004); *PIN/NIP, Inc. v. Platte Chem. Co.,* 304 F.3d 1235, 1247-48 (Fed. Cir. 2002); *In re Curtis,* 354 F.3d 1347, 1353-54 (Fed. Cir. 2004). Indeed, as the Board has held previously, **canceled** claims can be relied upon to provoke an interference. *See Tezuka v. Wilson*, 224 USPQ 1030 (Bd. Pat. Int. 1984). Cartter's arguments requiring patentability of pre-critical date claims are unsupported.

2. Adair's Pre-Critical Date Claims Define Substantially The Same Subject Matter As The Carter '213 Patent Claims

On page 9, lines 17-21, of CSM1, Carter argues that none of Adair's pre-critical date claims can serve as a basis for compliance with § 135(b) because they do not define the same or substantially the same subject matter as an involved Carter '213 patent claim.[2] Carter asserts that each of Adair's pre-critical date claims differ in one or more material limitations relative to the Carter '213 patent claims. Carter, however, does not specify which material limitation(s) of the Carter '213 patent claims are lacking in Adair's pre-critical date claims. Rather, Carter circuitously argues that, because Adair's original PCT/U.S. claims were alleged to be indefinite, and Adair has not argued that Carter's involved claims are indefinite, Adair's original claims must differ from the Carter '213 patent claims in ways having "patentable significance (*i.e.*, in material limitations)." (CSM1, page 9, line 22, through page 10, line 2.)

Adair's response is that the burden is upon Carter, as the proponent of the motion, to identify which material limitations are lacking. Carter has not met its burden and, thus, this

[2] Carter repeatedly intimates throughout CSM1 that the claims need to be *identical* under 35 U.S.C. § 135(b). Section 135(b) does not, however, require that the claims be identical. Section 135(b) only requires that the **subject matter** of the claims be the **same or substantially the same** (Fact 45). Carter is wrong on both fronts.

motion should be dismissed outright. Nonetheless, Adair maintains that its pre-critical date claims are to substantially the same subject matter as the Carter '213 patent claims. Although Adair focused upon claims to the light chain in provoking this interference (*see* Fact 44), Adair also had heavy chain claims to substantially the same invention. Specifically, original claim 1 of Adair's PCT application recited the following:

> 1. A **CDR**-grafted **antibody heavy chain** having a **variable region domain** comprising acceptor **framework** and donor **antigen binding** regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or **24**, 48 and/or 49, 71 and/or **73**, 75 and/or **76** and/or **78** and 88 and/or 91.

(Fact 61, emphasis added.) Involved claim 66 of the Carter '213 patent recites the following:

> A humanized **antibody heavy chain variable domain** comprising non-human Complementarity Determining Region (**CDR**) amino acid residues which **bind antigen** incorporated into a human antibody **variable domain**, and further comprising a **Framework** Region (FR) amino acid substitution at a site selected from the group consisting of: **24H**, **73H, 76H, 78H**, and 93H, utilizing the numbering system set forth in Kabat.

(Fact 65, emphasis added.) Recitations which are the same in both original claim 1 of Adair's PCT application and claim 66 of the Carter '213 patent are highlighted in bold. Although original claim 1 of Adair's PCT application did not limit the donor residues to non-human as claim 66 of the Carter '213 patent does , dependent claim 16 did (Facts 44 and 62). Although original claim 1 of Adair's PCT application did not limit the framework to human, dependent claim 16 did (Facts 44 and 62). As is abundantly clear from the foregoing, original claim 16 of Adair's PCT application, as depending from claim 1, effectively contains **all** limitations of involved claim 66 of the Carter '213 patent.

Carter attempts to dismiss Adair's **original** pre-critical date claims on the basis of patentability, instead focusing upon certain of Adair's **non-original** pre-critical date claims

(CSM1, p. 10, l. 10, through p.12, l. 30). Carter then dismisses Adair's non-original pre-critical date claims by arguing that Adair's non-original pre-critical date claims recite positions that all must be donor residues, thereby distinguishing such claims from the Carter '213 patent claims. Carter never asserts, however, that Adair's non-original pre-critical date claims are patentable. Evidently, Carter holds Adair's original pre-critical date claims to a higher standard than the non-original pre-critical date claims. Regardless, as discussed above, Carter cites no support for its requirement for patentability of pre-critical date claims.

3. Materiality Of A Claim Limitation For Purposes Of Section 135(b) Is To Be Determined In View Of The Carter '213 Patent Claims

On page 13, lines 1-18, of CSM1, Carter argues that Adair's involved claim 24 is not entitled to the benefit of any pre-critical date claims because involved claim 24 lacks material limitations present in Adair's non-original pre-critical date claims. Again, Carter dismisses Adair's original pre-critical date claims as unpatentable. First, as noted above, Carter has not argued that Adair's non-original pre-critical date claims were patentable. Second, the test whether or not a limitation is material for purposes of § 135(b) is to be determined in view of the **patent** claims in interference. All material limitations of the **patent** claims must be present in, or necessarily result from, the limitations of both Adair's pre-critical date and post critical-date claims. *See In re* Berger, 279 F.3d 975, 61 USPQ2d 1523 (Fed. Cir. 2002), citing *Corbett v. Chisolm*, 568 F.2d 759, 765-766, 196 USPQ 337, 342 (CCPA 1977).

The question to be asked, thus, is did the applicant add, or remove, a limitation to its claim after the critical date that was necessary to the patentability of the claims of the **Carter '213 patent**, not did the applicant add, or remove, a limitation necessary to the patentability of **its** own claim. Adair contends that when the materiality test is properly applied, it is clear that neither the original Adair claims present in Adair's PCT application, nor claim 24 involved in the

present interference, lacks any material limitations of the Carter '213 patent claims (Facts 44 and 61-62), nor has Carter argued the same. Indeed, if the test were to be applied as Carter asserts, it is difficult to see how one could ever provoke an interference, if one has made any claim amendments during prosecution of any application in its priority chain.

Further, if the test were to be applied as Carter asserts, a motion under § 135(b) effectively becomes a motion for failure to comply with written description under § 112, first paragraph. A review of Carter's first two substantive motions in this interference bears this out - the Statements of Material Facts are nearly identical. But, the question of whether or not there is written descriptive support for **a** claimed invention (under § 112, first paragraph) is very different from the question of whether or not **two** claimed inventions are to the same or substantially the same subject matter (under § 135(b)).

V. Conclusion

Adair requests that Carter Substantive Motion 1 be denied on the merits.

Respectfully submitted,

/Doreen Yatko Trujillo/
DOREEN YATKO TRUJILLO
Registration No. 35,719
Lead Counsel for Adair

Date: July 14, 2010
Cozen O'Connor P.C.
1900 Market St.
Philadelphia, PA 19103
Telephone: (215) 665-5593
Facsimile: (215) 701-2005
dtrujillo@cozen.com

APPENDIX 1

Carter Exhibits Relied Upon:

Ex. 2001 – U.S. Patent No. 6,407,213 to Carter *et al.,* issued June 18, 2002.

Ex. 2002 – U.S. Patent Application No. 11/284,261 to Adair *et al.,* filed November 21, 2005.

Ex. 2005 – PCT Application No. PCT/GB90/02017 to Adair *et al.,* filed December 21, 1990, published as WO 91/09967 on July 11, 1991.

Ex. 2006 -- U.S. Patent Application No. 07/743,329 to Adair *et al.,* filed September 17, 1991.

Ex. 2036 -- Great Britain Application No. 8928874.0 to Adair *et al.,* filed December 21, 1989.

APPENDIX 2

ADAIR RESPONSE TO CARTER STATEMENT OF MATERIAL FACTS

1. On December 21, 1989, Adair filed Great Britain Application No. 8928874.0 ("Adair UK Application"). (Ex. 2036).

ADMITTED

2. On December 21, 1990, Adair filed PCT/GB90/02017("Adair's PCT application"), which contained claims 1-23. (Ex. 2005, pp. 67-70).

ADMITTED

3. Exhibit 2037 is a computer generated comparison (using WorkshareTM Professional 5.2 SR2 software) of the typewritten text of the UK Application to the typewritten text of the PCT Application. The last page of Exhibit 2037 contains a color-coded legend for identifying deletions, additions, and movement of text.

UNABLE TO ADMIT OR DENY

4. The PCT Application contains a section titled "Protocol" that is not contained in the UK Application. (Ex. 2005, pp. 16-19; Ex. 2036; and Ex. 2037, pp. 10-11).

DENIED

5. On September 17, 1991, Adair entered the U.S. national stage by filing U.S. Patent Application No. 07/743,329 ("the `329 application"), claiming benefit to Adair's PCT application. (Ex. 2006).

ADMITTED

6. Adair's U.S. `329 application contained claims 1-23, which are identical to claims 1-23 as originally filed with Adair's PCT application. (Ex. 2005, pp. 67-70 and Ex. 2006, pp. 67-70).

ADMITTED

7. At page 6 of its involved specification, Adair stated:

> We have further investigated the preparation of CDR-grafted humanized antibody molecules and have identified a hierarchy of positions within the framework of the variable regions (i.e., outside both the Kabat CDRs and structural loops of the variable regions) at which the amino acid identities of the residues are important for obtaining CDR-grafted products with satisfactory binding affinity. This has enabled us to establish a protocol for obtaining satisfactory CDR-grafted products which may be applied very widely irrespective of the level of homology between donor immunoglobulin and acceptor framework. The set of residues which we have identified as being of critical importance does not coincide with the residues identified by Queen et al (9). [Ex. 2002, p. 6, lns. 15-28].

ADMITTED

8. At page 6, lines 31-37, the Adair specification reads as follows:

> Accordingly, in a first aspect the invention provides a CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91. [Ex. 2002, p. 6, lns. 31-37].

ADMITTED

9. At page 7, lines 1-5, the Adair specification reads as follows:

> In preferred embodiments, the heavy chain framework comprises donor residues at positions 23, 24, 49, 71, 73 and 78 or at positions 23, 24 and 49. The residues at positions 71, 73 and 78 of the heavy chain framework are preferably either all acceptor or all donor residues. [Ex. 2002, p. 7, lns. 1-5].

ADMITTED

10. At page 17, lines 27-30, the involved Adair specification reads as follows under a section titled "Protocol":

> 2.1 Choose donor residues at all of positions 23, 24, 49, 71, 73 and 78 of the heavy chain or all of positions 23, 24 and 49 (71, 73 and 78 are always either all donor or all acceptor). [Ex. 2002, p. 17, lns. 27-30].

ADMITTED

11. On November 18, 1992, the U.S. Patent and Trademark Office ("the USPTO") entered a non-final office action rejecting claims Adair's claims 1-23 on several statutory grounds. (Ex. 2038).

UNABLE TO ADMIT OR DENY. ADAIR DOESN'T KNOW WHEN THE OFFICE ACTION WAS ENTERED.

12. On November 18, 1992, the USPTO rejected the original Adair `329 claims 1-12, 17 and 22-23 under 35 U.S.C. § 101 for lack of utility. (Ex. 2038, pp. 1-3).

DENIED

13. On November 18, 1992, the USPTO rejected the original Adair `329 claims 1-16 and 22-23 under 35 U.S.C. § 112, first paragraph, for failing to adequately teach how to make and use the claimed invention. (Ex. 2038, pp. 3-6).

DENIED

14. On November 18, 1992, the USPTO rejected the original Adair `329 claims 1-23 were rejected under 35 U.S.C. § 112, second paragraph, for failing to particularly point out and distinctly claim the subject matter which Adair regarded as its invention. (Ex. 2038, pp. 6-7).

ADMITTED

15. On November 18, 1992, the USPTO rejected the original Adair `329 claims 1, 5, 6-8, and 12-22 under 35 U.S.C. § 102(b) as being anticipated by Riechmann *et al., Nature,* Vol. 332, pp. 323-327 (March 1988). (Ex. 2038, pp. 7-9 and Ex. 2011).

ADMITTED

16. On November 18, 1992, the USPTO rejected the original Adair `329 claims 1-6 and 12-22 under 35 U.S.C. § 102(b) as being anticipated by Queen *et al., Proc. Natl. Acad. Sci. 9 USA,* Vol. 86, pp. 10029-10033 (December 1989). (Ex. 2038, pp. 9-10 and Ex. 2023).

ADMITTED

17. On November 18, 1992, the USPTO rejected the original Adair `329 claims 1-21 under 35 U.S.C. § 103 as being obvious over Riechmann *et al.* and Queen *et al.*. (Ex. 2038, pp. 10-12).

ADMITTED

18. At pages 3-6 of the November 1992 office action, the Examiner rejected claims 1-16 and 22-23 for lack of enablement under § 112, first paragraph, on the grounds that **,** *inter alia,* the specification did not support making the range of residue changes recited in the claims and that the effects of the residue changes as described in Adair's original claims could not readily be predicted. (Ex. 2038, pp. 3-6).

DENIED

19. At page 6 of the November 1992 office action, the Examiner rejected claims 1-5 under 35 U.S.C. § 112, second paragraph, as being indefinite in their recitation of "at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91" because it was unclear to the Examiner whether the heavy chain,

a. had at least one of 6, 23, 24, 48, 49, 71, 73, 75, 76, 78, 88, or 91, or alternatively,

b. had at least one of (6) or (23 and /or 24) or (48 and/or 49) or (71 and/or 73) or (75 and/or 76 and/or 78 and 88 and/or 91), or alternatively,

c. had at least one (6, 23) and/or (24, 48) and/or (49 , 71) and/or (73, 75) and 76 and/or (78 and 88) and/or (91). (Ex. 2038, p. 6).

DENIED

20. At page 9 of the November 1992 office action, the Examiner rejected Adair's claims in view of Riechmann *et al.,* noting that the Examiner interpreted the claims to mean that

the framework has donor residues at at least one of any of positions 6, 23, 24**,** 48, 49, 71, 73, 75, 76, 78, 88, or 91 in the heavy chain. (Ex. 2038, p. 9).

ADMITTED

21. In a January 19, 1993 amendment , Adair responded to the November 1992 Office action by cancelling original claims 1-20, 22 and 23, amending original claim 21, and adding new claims 24-66. (Ex. 2007, pp. 1-13).

DENIED

22. In the January 1993 amendment, Adair stated the following:

> In contrast, the teaching in the present application can be applied without any undue experimentation to any antibody. All that is required is experimentation following a protocol which is clearly set out in the description, in particular at page 16, line 30 to page 19, line 9... .
>
> There is then no need to carryout computer modeling to determine which donor residues to substitute in to the acceptor sequence. The protocol in the present application provides the teaching directly. It instructs the skilled person to compare the two sequences and change certain specified residues in the acceptor sequence to donor residues.
>
> ...Thus, producing recombinant chains and testing them for affinity merely involves routine experimentation following a protocol which is clearly defined in the application. [Ex. 2007, pp. 26-27; Emphasis added].

ADMITTED

23. In the January 1993 amendment, Adair stated the following:

> It is submitted that this identifies where the present invention makes a significant departure from the prior art. The prior art indicates that each antibody has to be treated individually. In contrast, the present invention teaches that, by following the protocol set forth in the present application, it is possible to reshape any antibody. [Ex. 2007, p. 28].

ADMITTED

24. In the January 1993 amendment, Adair responded to the rejection of claims under 35 U.S.C. § 112, second paragraph, by cancelling claims 1-12. (Ex. 2007, pp. 29-32).

DENIED

25. In the January 1993 amendment, Adair responded to the rejection of claims under 35 U.S.C. § 102(b) in view of Riechmann *et al.* as follows:

> In Part A of this rejection, claims 1, 5, 6-8, and 12-22 were rejected as anticipated by Riechmann et al. The Examiner stated that claim 1 and claim 6 were interpreted to mean that the framework has donor residues in at least one of any of positions 6, 23, 24, 48, 49, 71, 73, 75, 76, 78, 88, or 91 in the heavy chain and (1, 3, 46, or 47) or 46, 48, 58, or 71) in the light chain, and thus, the teachings of Riechmann et al. anticipate the invention as claimed.
>
> The Examiner contends that the original claims lacked novelty over Riechmann et al. Claims 1, 5, 6-8, 12 and 22 have been cancelled without prejudice and submitted as new claims that more distinctly point out certain aspects of the present invention.
>
> In present claims 24 and 25, it is specified that residues 23 and 24 in the heavy chain should be donor residues. However, as can be seen from Fig. 1, panel (a) in Riechmann et al., in the recombinant antibody shown there, residues 23 and 24 are acceptor residues. [Ex. 2007, p. 32-33].
>
> **DENIED**

26. In the January 1993 amendment, Adair responded to the rejection of claims under 35 U.S.C. § 102(b) in view of Queen *et al.* as follows:

> In Part B of the rejection, the Examiner rejected claims 1-6 and 12-22 as anticipated by Queen et al.
>
> Claims 1-6, 12-20 and 22 have been cancelled without prejudice and submitted as new claims that more distinctly point out certain aspects of the present invention.
>
> In present claims 24 and 25 , it is specified that residues 48, 66, 67, 68, 93, 103 to 108 and 110 should all be acceptor residues . However, in Queen et al., as can be seen from Fig . 2B, in these positions Queen et al. uses donor, rather than acceptor, residues . It should again be borne in mind that Queen et al. does <u>not</u> use the Kabat numbering and it is therefore necessary to look carefully at the disclosure in Queen et al. before it is possible to come to any final conclusion. [Emphasis by Adair].
>
> In present claim 38, it is specified that residue 71 should be a donor residue. However, as can be seen from Fig. 2A of Queen et al., in that position Queen et al. uses an acceptor, rather than a donor residue.

> Applicants' claimed antigen-binding molecules are thus not anticipated by Queen et al. Withdrawal of this entire 35 USC § 102 (b) rejection is respectfully requested. [Ex. 2007, pp. 33-34].

ADMITTED

27. An Examiner Interview Summary Record dated January 27, 1993, states "applicant suggests that the `comprising' in eg clm 24 is not to be taken as `comprising' more residues than those in clm, i.e. claimed residues are not to be considered open ended. Applicant indicated they would clarify the latter issue. Queen does not teach changing residues: 73HC; 38HC; 71 on LC # 1 on LC + #4 on LC, 36 on LC 46 on LC." (Ex. 2039, p. 4; Emphasis by Examiner).

ADMITTED

28. On September 9, 1993, in the Adair PCT/EP Patent Application 91901433.2, Adair filed an amendment deleting original claims 1-23 and replacing them with new claims 1-20 and made the following statements:

> 2.10. In new claim 1, it has been specified that residues 71, 73 and 78 are all donor residues in order to ensure that new claim 1 is novel over the anti-TAC antibody disclosed in PNAS-USA, 86, 10029-10033 , 1989 (Queen) (cited in the International Search Report). This anti-TAC antibody has an acceptor residue at residue 73. However, as can be seen from page 7, lines 1 to 5 , the Applicant considers that in general , residues 71, 73 and 78 can be either all donor or all acceptor. [Ex. 2009, p. 3].

ADMITTED

29. On February 7, 1994, Adair filed an amendment in the `329 application responding to an office action mailed on September 7, 1993 (Ex. 2028), wherein Adair made the following statements:

> At a very helpful interview held at the beginning of 1993, there was some discussion of the word "comprising" as used in the claims under consideration at that time. In those claims, it was only specified that certain residues should be

donor residues. [Emphasis by Adair]. It was considered that it was not clear 30 whether these were the only residues which could be donor residues. The alternative view was that these were only the minimum number of residues which must be donor but that any of the other residues could also be donor.

If the second line of interpretation were taken, the claims could be read to cover a situation in which all except one of the residues in the variable domain were donor residues. [Emphasis by Adair]. In this case, the claims could then be interpreted to cover a structure similar to a "chimeric" antibody comprising a donor variable domain and a human constant region. Such chimeric antibodies were already well known at the priority date.

It plainly is not the intention of the Applicants to claim chimeric antibodies or any similar structures. As can be seen from the description, the superhumanised antibodies of the present invention are compared to the prior art chimeric antibodies. Moreover, the present invention was intended to deal with the problem of chimeric antibodies in that chimeric antibodies were believed to be too "foreign" because of the presence of the complete donor variable domain.

For the above reasons, it is clear that the wording of the claims needed to be changed so that the Applicants' intention of excluding chimeric antibodies was made effective. The language now present in the claims puts this intention clearly into effect.

As to support for this wording, the Examiner is referred firstly to page 16, under the heading "Protocol". It can be seen from this paragraph that the first step in the process involves the choice of an appropriate acceptor chain variable domain. This acceptor domain must be of known sequence. Thus, the protocol starts with a variable domain in which all the residues are acceptor residues. In the. sentence bridging pages 16 and 17, it is stated that:

> "The CDR-grafted chain is then designed starting from the basis of the acceptor sequence". [Emphasis by Adair].

On page 17, in the middle paragraph, it is stated that:

> "The positions at which donor residues are to be substituted for acceptor in the framework are then chosen as follows ...."

This again shows that, unless a residue is chosen for substitution, it will remain as in the acceptor sequence**.**

It must also be borne in mind that the purpose of the invention is to obviate some of the disadvantages of prior art proposals. The proposal of using chimeric antibodies had the disadvantage that they were more "foreign" than desirable. The problem of making CDR-grafted antibodies was that they

generally did not provide good recovery of affinity. Thus, the aim of the present invention was to minimise as far as possible the "foreign" nature of the antibody while maximising as far as possible its affinity.

Bearing the passages referred to above and the aim of the invention in mind, it would have been abundantly clear to the skilled person reading the application that as many residues as possible should remain as acceptor residues. If this were not the case, it could hardly be said that the composite chain is based on the acceptor sequence.

The skilled person reading the application can plainly see that certain residues have been considered for changing from acceptor to donor. These are clearly set out in the description. It would be plain to the skilled person that all other residues should not be considered for changing at all. It would therefore be obvious that any residue which is not specified as being under consideration for changing must remain as in the acceptor chain.

It may be that there is no explicit statement in the description that the specified residues should remain as in the acceptor chain. However, the disclosure in a specification is not limited to the explicit disclosure but also includes that which is implicit. It is implicit, in the recitation that the chain is based on the acceptor and that only certain residues are considered for changing, that all non-specified residues must remain as acceptor residues. Subject matter which might be fairly deduced from the disclosure is not new matter. *Acme 21 Highway Products Corp. v. D.S. Brown Co.,* 431 F.2d 1074, 1080, 167 U.S.P.Q. 22 129, 132-133(6th Cir. 1970), *cert denied,* 401 U.S. 956 (1971).

Another way to look at it is to consider a different way in which the claim could be drafted. It could be specified that in the composite chain, at least a certain minimum number of residues are donor residues (as in the present claims) and at most a certain maximum number of residues are donor residues. The maximum number would be derived by listing all the residues which are considered for changing. Such an amendment would have clear explicit basis in the description because all those residues are mentioned as such. However, the effect of such an amendment would be to produce claims of exactly the same scope as the present claims. It can thus be seen that the present claims do not add subject matter but are plainly properly based on the disclosure in the description.

It is therefore submitted that the claims are fully supported by the description, are commensurate in scope with the disclosure in the description, and are properly delimited over the prior art. [Ex. 2010, pp. 3-7].

ADMITTED

30. Adair's non-original pre-critical date claims are grounded in the specific rules

governing the "hierarchy of residues" to which Adair attributed the patentability of its claims. (Ex. 2007-2010, 2012-2022, 2024-2027, 2029, 2031, and 2031-2035).

UNABLE TO ADMIT OR DENY

31. Appendix 3 is a claim chart comparing Adair claim 24 as originally filed in 2005 and Adair involved claim 24.

UNABLE TO ADMIT OR DENY

32. Appendix 4 is an accurate comparison of Adair original PCT claims 8 and 16 to Adair involved claim 24.

UNABLE TO ADMIT OR DENY

33. In an amendment filed on April 7, 1993, Adair amended a claim reciting residues 71, 73 and 78, stating the following:

> In claim 67, it has been specified that residues 71, 73 and 78 are all donor residues in order to ensure that claim 67 is novel over the anti-TAC antibody disclosed by Queen. This anti-TAC antibody has an acceptor residue at residue 73. However, as can be seen from page 7, lines 1 to 5, the Applicant considers that in general, residues 71, 73 and 78 can be either all donor or all acceptor. [Ex. 2008, p. 14].

ADMITTED

34. In the April 1993 amendment, Adair stated the following:

> It is stated on page 7, lines 1 to 5, that residues 71, 73 and 78 should all be either acceptor or donor. Claims 73, 80, 87, 94 and 101 cover the first alternative and claims 74, 81, 88, 95 and 102 cover the second alternative. [Ex. 2008, p. 15].

ADMITTED

35. In an Amendment filed on February 7, 1994, in the `329 application, Adair stated the following:

> It is specifically stated in the application that the present protocol represents a departure from the procedures of Reichmann [sic] and Queen, at least. Thus, the skilled person would not rely on Reichmann [sic] and Queen as

teachings relevant to whether the present description is enabling.

It is submitted that the skilled person would rely on the clear teaching given in the application and find that it is enabling. The specification plainly sets out what actions need to be taken. It is presumed that the Examiner agrees that the skilled person could have taken those actions. The application also sets out that, contrary to the teachings of Reichmann and Queen, the protocol is generally applicable. The application further shows that it had been successfully implemented. Thus, it is submitted that the skilled person would find that the present application is properly enabled the full extent of the claims. [Ex. 2010, pp. 11-12].

DENIED

36. Carter's involved U. S. Patent No. 6,407,213 ("the `213 patent") issued on June 18, 2002. (Ex. 2001).

ADMITTED

37. One year from the date on which the Carter `213 patent issued is June 18, 2003. (Ex. 2001).

ADMITTED

38. On November 21, 2005, Adair filed its involved application 11/284,261 ("the 261 application"). (Ex. 2002).

ADMITTED

39. On November 21, 2005, Adair presented new claim 24 as follows:

Claim 24 (new) A humanised antibody heavy chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises an amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof, as numbered according to Kabat. [Ex. 2003, p. 3].

ADMITTED

40. On September 9, 2009, Adair presented its involved claim 24 in the `261

application, which reads as follows:

> Claim 24 (currently amended): A humanised antibody comprising a heavy chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises a non-human amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof, as numbered according to Kabat. [Ex. 2004, p. 2; Adair Clean Copy of Claims, Paper No. 5, p. 4].

ADMITTED

41. None of Adair's pre-critical date claims is identical to a Carter `213 patent claim. (Ex.2001, 2005-2010, 2012-2022, 2024-2027, 2029, 2031, and 2031-2035).

UNABLE TO ADMIT OR DENY

42. None of Adair's pre-critical date claims is identical to Adair's involved claim 24. (Ex. 2005-2010, 2012-2022, 2024-2027, 2029, 2031, and 2031-2035 and Adair Clean Copy Of Claims, Paper No. 5, p. 4).

UNABLE TO ADMIT OR DENY

43. Concurrent with the filing of the `261 application, Adair filed a "Preliminary Amendment and Request for Interference under 37 CFR § 42.202 [sic]." (Ex. 2003).

ADMITTED

44. On page 4 of the 2005 amendment, Adair stated the following:

> **(b) Compliance with 35 USC § 135(b)**
> Although the present rules do not require a showing of compliance under 35 USC § 135(b), Applicants submit the following to advance the examination of the present application to allowability. [...] Claims 1-23 as filed in the PCT application are attached as Appendix A.
>
> Under 35 USC § 135(b)(1), Applicants must show that they had a claim to the same, or substantially the same, subject matter as a claim of the 213 patent within one year of the issuance of the 213 patent, or June 18, 2003. The 213 patent issued on June 18, 2002. The PCT application was filed on December 21, 1990, over 10 years earlier than the 213 patent issued. The time limit of Section 135(b)(1) has been complied with fully. See *Corbett v. Chisholm,* 196 USPQ 337

(CCPA 1977).

To meet the "same or substantially the same invention" requirement of Section 135(b)(1), Applicants must show that their claim contained all material limitations, i.e. limitations necessary to patentability, of the claim of the 213 patent alleged to be to the same, or substantially the same, invention. *Corbett v. Chisholm,* 196 USPQ 337 (C.C.P.A. 1977), *citing Wetmore v. Miller,* 477 F.2d 960,177 USPQ 699 (C.C.P.A. 19730).

As is evident from Appendix A, Applicants made a claim for the same, or substantially the same, subject matter as a claim of the 213 patent well before the issuance of the 213 patent. Claim 16 of the PCT application, as depending from claim 8, is to substantially the same subject matter as at least claim 1 of the 213 patent. For the Office's convenience, all three claims are duplicated below.

> **Claim 8 of the PCT application**: A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, 58 and 71.
>
> **Claim 16 of the PCT application**: A CDR-grafted antibody heavy or **light chain** or molecule according to any one of the preceding claims comprising human acceptor residues and non-human donor residues.
>
> **Claim 1 of the 213 patent**: A humanized antibody variable domain comprising non-human Complementarity Determining Region (CDR) amino acid residues which bind an antigen incorporated into a human antibody variable domain, and further comprising a Framework Region (FR) amino acid substitution at a site selected from the group consisting of. 4L, 38L, 43L, 44L, **58L**, 62L, 65L, 66L, 67L, 68L, 69L, 73L, 85L, 98L, 2H, 4H, 36H, 39H, 43H, 45H, 69H, 70H, 74H, and 92H, utilizing the numbering system set forth in Kabat. [Ex. 2003, pp. 4-6; Emphasis by Adair.]

ADMITTED

ADAIR STATEMENT OF ADDITIONAL MATERIAL FACTS

45. Section 135(b) of the Patent Statute does not require Adair to have a pre-critical date claim identical to a claim of the Carter '213 patent. (*See* 35 U.S.C. § 135(b) and CSM1, p. 1, lines 17-18.)

46. Section 363 of the Patent Statute gives an international application designating the United States, e.g., a Patent Cooperation Treaty ("PCT") application designating the United States, the same effect from its filing date as a national application for patent regularly filed in the United States Patent & Trademark Office except as otherwise provided in Section 102(e). (35 U.S.C. § 363.)

47. Adair's PCT application designates the United States. (Ex. 2005, first page.)

48. The application that issued as the Carter '213 patent was filed as national phase of a PCT application that was filed on June 15, 1992 ("the Carter PCT application"). (Ex. 2001, first page.)

49. The Carter PCT application was filed as a continuation-in-part of Application Serial No. 07/715,272, filed on June 14, 1991 ("the Carter '272 application"). (Ex. 2001, first page.)

50. Adair's PCT application was filed on December 21, 1990. (Fact 2.)

51. Adair's PCT application was filed almost six months before the Carter '272 application. (Facts 49 and 50.)

52. Adair's PCT application was filed almost 18 months before the Carter PCT application. (Facts 48 and 50.)

53. The Adair UK application was filed on December 21, 1989. (Fact 1.)

54. The Adair UK application was filed almost 18 months before the Carter '272 application was filed. (Facts 53 and 49.)

55. The Adair UK application was filed almost 30 months before the Carter PCT application was filed. (Facts 53 and 48.)

56. Adair's PCT application published on July 11, 1991. (Ex. 2005, first page.)

57. The Carter '272 application was filed less than one month before the Adair PCT application published. (Facts 49 and 56.)

58. On page 16, lines 23-28, the Adair specification reads as follows:

> A **preferred** protocol for obtaining CDR-grafted antibody heavy and light chains in accordance with the present invention is set out below together with the rationale by which we have derived this protocol. This protocol and rationa1e are given **without prejudice to the generality of the invention as hereinbefore described and defined**.

(Ex. 2002, p. 16, ll. 23-28, emphasis added.)

59. At page 6, lines 31-37, under the heading "Summary of the Invention," the Adair specification reads as follows:

> Accordingly, in a first aspect the invention provides a CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

(Ex. 2002, p. 6, ll. 31-37).

60. On page 17, lines 1-5, the Adair specification states that

> [i]t will be appreciated that in some cases the donor and acceptor amino acid residues may be identical at a particular position thus no change of acceptor framework residue is required.

(Ex. 2002, p. 17, ll. 1-5.)

61. Claim 1 of Adair's PCT application recites the following:

> 1. A CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of

positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

(Ex. 2005, page 67.)

62. Claim 16 of Adair's PCT application recites the following:

16. A CDR-grafted antibody heavy or light chain or molecule according to any one of the preceding claims comprising human acceptor residues and non-human donor residues.

(Ex. 2005, page 69.)

63. Claim 1 of Adair's PCT application does not require that any residues be changed to donor. (Ex. 2005, claim 1, p. 67.)

64. Original claim 1 of the '329 application does not require that any residues be changed to donor. (Ex. 2006, claim 1, p. 67.)

65. Claim 66 of the Carter '213 patent recites the following:

A humanized **antibody heavy chain variable domain** comprising non-human Complementarity Determining Region (**CDR**) amino acid residues which **bind antigen** incorporated into a human antibody **variable domain**, and further comprising a **Framework** Region (FR) amino acid substitution at a site selected from the group consisting of: **24H**, **73H, 76H, 78H**, and 93H, utilizing the numbering system set forth in Kabat.

(Carter Clean Copy of Claims, Paper No. 12, filed February 19, 2010.)

Filed on behalf of: **Adair**
By: Doreen Yatko Trujillo
Michael B. Fein
Cozen O'Connor P.C.
1900 Market St.
Philadelphia, PA 19103
Telephone: (215) 665-5593
Facsimile: (215) 701-2005
dtrujillo@cozen.com

Paper No:_____
Filed: July 14, 2010

UNITED STATES PATENT AND TRADEMARK OFFICE

_____

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

_____

PAUL J. **CARTER** AND LEONARD G. PRESTA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR,** DILJEET SINGH ATHWAL,
AND JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

Patent Interference No. 105,744
(Technology Center 1600)

ADAIR OPPOSITION 2

I. Adair Statement of the Precise Relief Requested

Adair requests that Carter Substantive Motion 2 ("CSM2") be denied. Adair's involved claim 24 is not barred under 35 U.S.C. § 112, first paragraph, for lack of written description.

II. Evidence

The exhibit list is attached as Appendix 1.

III. Statement of Material Facts

The Statement of Material Facts is attached as Appendix 2.

IV. Argument

Adair will ultimately show that it is the first inventor of the invention of the Count. Specifically, claim 24 of the '261 application has a priority date that is almost **18 months** earlier than the earliest priority date claimed by Carter (Fact 49). Even if Adair is not entitled to its earliest priority date, its next priority date is the filing date of Adair's PCT application, which is almost **six months** before the earliest priority date claimed by Carter (Fact 47).

In CSM2, Carter alleges that Adair has not complied with 35 U.S.C. § 112, first paragraph, for lack of written description and that, thus, Adair does not have standing to pursue this interference. As Adair shows below, however, Adair has complied with 35 U.S.C. § 112, first paragraph, for written description and Adair does have standing to be in this interference.

The bulk of CSM2 relies upon what is set forth as a specific protocol to support Carter's argument that there is no written descriptive support for involved claim 24 in the '261 application specification (CSM2, p. 8, l. 6, though p. 13, l. 5). Although Carter acknowledges that Adair used the terminology "preferred" in reference to the protocol, it argues that the remainder of the specification and the prosecution history show that Adair believed that

---

Unless otherwise indicated, the same abbreviations as used in CSM1 are used herein.

following the preferred protocol was necessary to distinguish its invention from the prior publications of others and to establish support for its claims (CSM2, p. 9, l. 21, through p. 10, l. 2). As discussed below, neither the remainder of the specification nor the prosecution history supports Carter's assertions.

First, the specification of the '261 application makes it clear that the invention is **not** to be limited to a preferred protocol. The specification of the '261 application contains the following text:

> A **preferred** protocol for obtaining CDR-grafted antibody heavy and light chains in accordance with the present invention is set out below together with the rationale by which we have derived this protocol. This protocol and rationa1e are given **without prejudice to the generality of the invention as hereinbefore described and defined**.

(Fact 53.) Notably, the foregoing text is not cited anywhere in CSM2. And, as admitted by Carter, on page 6, lines 31-37, under the heading "Summary of the Invention," the specification of the '261 application reads as follows:

> Accordingly, in a first aspect the invention provides a CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least **one** of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

(Fact 54, emphasis added.) The foregoing recitation clearly encompasses a framework region comprising an amino acid substitution **at any one** of residues 6, 23, 24, 48, 49, 71, 73, 75, 76, 78, 88, and 91.

As further evidence that the invention is not limited to the preferred protocol, on page 48, lines 24-27, of the '261 application, it is reported that changes at residues 6, 23, and 24, were important for maintaining a binding affinity similar to that of the murine antibody (Fact 55). Residue 49 is notably absent from the foregoing listing. On page 58, lines 1-6, of the '261

application, it is reported that the change of a **single** residue in the heavy chain, residue 73, was sufficient to generate an antibody with binding properties similar to the donor (Fact 56). Finally, the specification of the '261 application ends with the following statement:

> It will be appreciated that the foregoing examples are given by way of illustration only and are not intended to limit the scope of the claimed invention. Changes and modifications may be made to the methods described whilst still falling within the spirit and scope of the invention.

(Fact 57.) Such a recitation has been recognized by the Court of Appeals for the Federal Circuit ("Federal Circuit") to preclude limiting inventions to specific embodiments. *Pfizer Inc. v. Ranbaxy Laboratories Ltd*., 457 F3d 1284, 1290, 79 USPQ2d 1583, 1588 (Fed. Cir. 2006). Indeed, the Federal Circuit has repeatedly cautioned against unduly limiting the claims based upon embodiments in the specification. *Phillips v. AWH Corp.*, 415 F.3d 1303, 1323, 75 USPQ2d 1321, 1334 (Fed. Cir. 2005). Contrary to Carter's assertions, the "entirety" of the Adair specification did not clearly indicate the invention is of a much narrower scope.

Second, the prosecution history makes it clear that the invention is **not** to be limited to the preferred protocol. On page 11, lines 26-27, of CSM2, Carter acknowledges that Adair's arguments in the prosecution history use equivocal terms like "in general", "can be", and "should be" when drawing substance from what Carter alleges to be the "rules" set forth in the specification (CSM2, p. 11, ll. 26 -27). Carter tries to trivialize the impact of such equivocal arguments by alleging that they are not consistent with the express teachings of the specification and, thus, that they should not be substituted for written description in the originally filed application (CSM2, p. 12, ll. 1-2). As noted above, however, such arguments are consistent with the express teachings of the specification. Regardless, contrary to Carter's assertion, the foregoing clearly shows that the prosecution history contains arguments that do **not** support

Carter's position. As such, the prosecution history does not unequivocally support limiting the invention to the preferred protocol.

On page 12, ll. 27-30, of CSM2, Carter argues that Adair relied upon the preferred protocol as providing the basis for distinguishing claims specifying substitutions at positions corresponding to those recited in Adair's involved claim 24 over the prior art, i.e., Riechmann et al. (Ex. 2011) and Queen et al. (Ex. 2023). What Carter fails to appreciate, however, is that the Adair claims at the time of the rejections were not limited to making substitutions to donor, but also included the instance in which the donor and acceptor residues were the same at the recited positions, and were so interpreted by the examiner (Facts 59-64). It is clear from Carter's own arguments that the recitation of the *substitution* of a single residue in the claims would have been sufficient to overcome the cited art – e.g., residue 24 or 73 (CSM2, p. 10, ll. 11-15 and p. 4, ll. 4-10 citing Facts 26-27 and 30). If that were not the case, Carter's involved claims 66-68, 70-71, 78, and 80-82 would be invalid over the same art (Fact 65). Carter's arguments regarding such amendments are not relevant to Adair's involved claim 24, which requires a substitution at residue 23, 24, 49, 71, 73, or 78, or combinations thereof (Fact 37).

V. Conclusion

Adair requests that Carter Substantive Motion 2 be denied on the merits.

Respectfully submitted,

/Doreen Yatko Trujillo/
DOREEN YATKO TRUJILLO
Registration No. 35,719
Lead Counsel for Adair

Date: July 14, 2010
Cozen O'Connor P.C.
1900 Market St.
Philadelphia, PA 19103
Telephone: (215) 665-5593
Facsimile: (215) 701-2005
dtrujillo@cozen.com

APPENDIX 1

Carter Exhibits Relied Upon:

Ex. 2001 – U.S. Patent No. 6,407,213 to Carter *et al.,* issued June 18, 2002.

Ex. 2002 – U.S. Patent Application No. 11/284,261 to Adair *et al.,* filed November 21, 2005.

Ex. 2005 – PCT Application No. PCT/GB90/02017 to Adair *et al.,* filed December 21, 1990, published as WO 91/09967 on July 11, 1991.

Ex. 2006 -- U.S. Patent Application No. 07/743,329 to Adair *et al.,* filed September 17, 1991.

Ex. 2011 – Riechmann *et al., Nature,* Vol. 332, pp. 323-327 (March 1988).

Ex. 2023 – Queen *et al., Proc. Natl. Acad. Sci. USA,* Vol. 86, pp. 10029-10033 (December 1989).

Ex. 2036 -- Great Britain Application No. 8928874.0 to Adair *et al.,* filed December 21, 1989.

Ex. 2038 -- Office Action mailed November 18, 1992, in U.S. Patent Application No. 07/743,329 to Adair *et al.*

APPENDIX 2

ADAIR RESPONSE TO CARTER STATEMENT OF MATERIAL FACTS

1. On December 21, 1989, Adair filed Great Britain Application GB 8928874.0 ("the UK Application"). (Ex. 2036).

ADMITTED

2. On December 21, 1990, Adair filed PCT Application PCT/GB90/02017 ("the PCT Application"). (Ex. 2005).

ADMITTED

3. Exhibit 2037 is a computer generated comparison (using WorkshareTM Professional 5.2 SR2 software) of the typewritten text of the UK Application to the typewritten text of the PCT Application. The last page of Exhibit 2037 contains a color-coded legend for identifying deletions, additions, and movement of text.

UNABLE TO ADMIT OR DENY

4. On September 17, 1991, Adair entered the U.S. national stage by filing U.S. Patent Application No. 07/743,329 ("the `329 application"). (Ex. 2006).

ADMITTED

5. Adair's `329 application contained claims 1-23, which are identical to claims 1-23 as originally filed with Adair's PCT application. (Ex. 2005, pp. 67-70 and Ex. 2006, pp. 67-70).

ADMITTED

6. Original claim 1 of the Adair `329 application reads as follows:

> 1. A CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91. [Ex. 2006, p. 67].

ADMITTED

7. At pages 4-6 of the specification, Adair provides a discussion of "recent" disclosures by Queen *et al.* relating to CDR-grafted antibodies and the substitution of acceptor framework residues with donor residues. (Ex. 2002, pp. 4-6).

DENIED

8. At page 6, lines 22-28, the Adair specification states:

> This has enabled us to establish a protocol for obtaining satisfactory CDR-grafted products which may be applied very widely irrespective of the level of homology between the donor immunoglobulin and acceptor framework. The set of residues which we have identified as being of critical importance does not coincide with the residues identified by Queen...." [Ex. 2002, p. 6, lns. 22-28].

DENIED

9. The Abstract of Adair's involved specification reads, in part, as follows:

> CDR-grafted antibody heavy and light chains comprise acceptor framework and donor antigen binding regions, the heavy chains comprising donor residues at at least one of positions (6, 23) and/or (24, 48) and/or (49, 71) and/or (73, 75) and/or (76) and/or (78) and (88) and/or (91). [Ex. 2002, Abstract].

ADMITTED THAT THIS IS WHAT IS STATED IN THE SECTION DESIGNATED AS "ABSTRACT" ON BOARD ASSIGNED PAGE # 544 OF EX. 2002.

10. At page 6, lines 31-37, the Adair specification reads as follows:

> Accordingly, in a first aspect the invention provides a CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91. [Ex. 2002, p. 6, lns. 31-37].

ADMITTED

11. At page 7, lines 1-5 , the Adair specification reads as follows:

> In preferred embodiments, the heavy chain framework comprises donor residues at positions 23, 24, 49, 71, 73 and 78 or at positions 23, 24 and 49. The residues at positions 71, 73 and 78 of the heavy chain framework are preferably

either all acceptor or all donor residues. [Ex. 2002, p. 7, lns. 1-5].

ADMITTED

12. At page 16, line 30 to page 19, line 9, Adair describes its "preferred protocol" for obtaining CDR-grated antibodies. (Ex. 2002, p. 16, ln. 30 to p. 19, ln. 9).

ADMITTED

13. At page 17 , lines 27-30, the involved Adair specification reads as follows under a section titled "Protocol":

> 2. Heavy Chain
> 2.1 Choose donor residues at all of positions 23, 24, 49, 71, 73 and 78 of the heavy chain or all of positions 23, 24 and 49 (71, 73 and 78 are always either all donor or all acceptor). [Ex. 2002, p. 17, lns. 25-30; Emphasis added].

ADMITTED

14. At page 17, lines 32-35, the involved Adair specification states:

> 2.2. Check that the following have the same amino acid in donor and acceptor sequences, and if not preferably choose the donor: 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107. [Ex. 2002, p. 17, lns. 32-35].

ADMITTED

15. At pages 19-23 of its involved specification, Adair offers a "rationale" for its protocol. (Ex. 2002, pp. 19-23).

ADMITTED

16. At page 20, line 27, the involved Adair specification states "Heavy Chain - Key residues are 23, 71 and 73." (Ex. 2002, p. 20, ln. 27).

ADMITTED

17. At page 21, line 9, for the "packing residues near the CDRs," the involved Adair specification states "Heavy Chain - Key residues are 24, 49 and 78." (Ex. 2002, p. 21, ln. 9).

ADMITTED

18. At page 48, lines 25-27, the involved Adair specification explains: "the presence of the 6, 23 and 24 changes are important to maintain a binding affinity similar to that of the murine antibody ." (Ex. 2002, p. 48, lns . 25-27).

ADMITTED

19. At page 52, lines 25-29, the Adair involved specification states:

> These and other results lead us to the conclusion that of the 11 mouse framework residues used in the gH341A (JA185) construct, it is important to retain mouse residues at all of positions 6, 23, 24, 48 and 49, and possibly for maximum binding affinity at 71, 73 and 78. [Ex. 2002, p. 52, lns. 25-29].

ADMITTED

20. On November 18, 1992, the U.S. Patent and Trademark Office entered a non-final office action rejecting Adair's original claims 1-23 on various grounds. (Ex. 2038).

UNABLE TO ADMIT OR DENY. ADAIR DOES NOT KNOW WHEN THE OFFICE ACTION WAS ENTERED.

21. At page 5 of the November 1992 office action, the Examiner rejected claims 1-5 under 35 U.S.C. § 112, first paragraph as not being enabled. In particular, the Examiner stated that practicing the invention as claimed would require undue experimentation relative to the teachings of the Adair specification. (Ex. 2038, p. 5).

DENIED

22. At page 6 of the November 1992 office action, the Examiner rejected claims 1-5 under 35 U.S.C. § 112, second paragraph, as being indefinite in their recitation of "at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91" because it was unclear whether the heavy chain,

a. had at least one of 6, 23, 24, 48, 49, 71, 73, 75, 76, 78, 88, or 91, or

alternatively,

b. had at least one of (6) or (23 and/or 24) or (48 and/or 49) or (71 and/or 73)

or (75 and/or 76 and/or 78 and 88 and/or 91), or alternatively,

c. had at least one of (6, 23) and/or (24, 48) and/or (49, 71) and/or (73, 75)

and 76 and/or (78 and 88) and/or (91). (Ex. 2038, p. 6).

ADMITTED

23. At pages 7-12 of the November 1992 office action, the Examiner rejected Adair's claims under 102/103 in view of Riechmann *et al., Nature,* Vol. 332, pp. 323-327 (March 1988) and Queen *et al., Proc. Natl. Acad. Sci. USA,* Vol. 86, pp. 10029-10033 (December 1989). (Ex. 2038, pp. 7-12; Ex. 2011, and Ex. 2023).

DENIED

24. On January 19, 1993, Adair responded to the November 1992 Office action. (Ex. 2007).

ADMITTED

25. In the January 1993 amendment, Adair responded to the rejection of claims under 35 U.S.C. § 112, second paragraph, by cancelling claims 1-12. (Ex. 2007, pp. 29-32).

DENIED

26. In the January 19, 1993, amendment, Adair responded to the rejection of claims under 35 U.S.C. § 102(b) in view of Riechmann *et al.* as follows:

> In Part A of this rejection, claims 1, 5, 6-8, and 12-22 were rejected as anticipated by Riechmann et al. The Examiner stated that claim 1 and claim 6 were interpreted to mean that the framework has donor residues in at least one of any of positions 6, 23, 24, 48, 49, 71, 73, 75, 76, 78, 88, or 91 in the heavy chain and (1, 3, 46, or 47) or 46, 48, 58, or 71) in the light chain, and thus, the teachings of Riechmann et al. anticipate the invention as claimed.
>
> The Examiner contends that the original claims lacked novelty over Riechmann et al. Claims 1, 5, 6-8, 12 and 22 have been cancelled without

prejudice and submitted as new claims that more distinctly point out certain aspects of the present invention.

In present claims 24 and 25, it is specified that residues 23 and 24 in the heavy chain should be donor residues. However, as can be seen from Fig. 1, panel (a) in Riechmann et al., in the recombinant antibody shown there, residues 23 and 24 are acceptor residues. [Ex. 2007, p. 32-33].

DENIED

27. In the January 19, 1993, response, Adair responded to the rejection of claims under 35 U.S.C. § 102(b) in view of Queen *et al.* as follows:

In Part B of the rejection, the Examiner rejected claims 1-6 and 12-22 as anticipated by Queen et al.

Claims 1-6, 12-20 and 22 have been cancelled without prejudice and submitted as new claims that more distinctly point out certain aspects of the present invention.

In present claims 24 and 25, it is specified that residues 48, 66, 67, 68, 93, 103 to 108 and 110 should all be acceptor residues. However, in Queen et al., as can be seen from Fig. 213, in these positions Queen et al. uses donor, rather than acceptor, residues. It should again be borne in mind that Queen et al. does not use the Kabat numbering and it is therefore necessary to look carefully at the disclosure in Queen et al. before it is possible to come to any final conclusion. [Emphasis by Adair].

In present claim 38, it is specified that residue 71 should be a donor residue. However, as can be seen from Fig. 2A of Queen et al., in that position Queen et al. uses an acceptor, rather than a donor residue.

Applicants' claimed antigen-binding molecules are thus not anticipated by Queen et al. Withdrawal of this entire 35 USC § 102 (b) rejection is respectfully requested. [Ex. 2007, pp. 33-34].

ADMITTED

28. At pages 26-28 of its January 19, 1993 , response , Adair responded to the § 112, first paragraph rejection by arguing, *inter alia,* as follows:

In contrast, the teaching in the present application can be applied without undue experimentation to any antibody. All that is required is experimentation following a protocol which is clearly set out in the description, in particular at

page 16, line 30 to page 19, line 9. In order to follow this protocol, as a first step, it is necessary to determine the amino acid sequence of the donor chain. The sequence of the acceptor chain will already be known, for instance from a sequence data base.

There is then no need to carry out computer modeling to determine which donor residues to substitute into the acceptor sequence. The protocol in the present application provides the teaching directly. It instructs the skilled person to compare the two sequences and change certain specified residues in the acceptor sequence to donor residues.

Moreover, the present application provides a hierarchical structure of residues which can be considered. Thus, if changing the residues at the top of the structure does not provide adequate affinity, then a lower level of residues are considered, and so on until acceptable affinity is obtained.

[. . .]

It is submitted that this identifies where the present invention makes a significant departure from the prior art. The prior art indicates that each antibody has to be treated individually. In contrast, the present invention teaches that, by following the protocol set forth in the present application, it is possible to reshape any antibody. [Ex. 2007, pp. 26-28].

ADMITTED

29. An Examiner Interview Summary Record dated January 27, 1993, states "applicant suggests that the `comprising' in eg clm 24 is not to be taken as `comprising' more residues than those in c1m, i.e. claimed residues are not to be considered open ended. Applicant indicated they would clarify the latter issue. Queen does not teach changing residues: 73HC; 38HC; 71 on LC # 1 on LC + #4 on LC, 36 on LC 46 on LC." (Ex. 2039, p. 4; Emphasis by Examiner).

ADMITTED

30. On April 7, 1993, Adair made the following statements in an amendment:

Having considered the Examiner's concerns that the language of the claims might be indefinite, because it was not clear whether the specified residues were the only or the minimum number of residues to be donor residues, the Applicants have amended the claims. In all the claims it is made clear that there

is a minimum number of residues which have to be donor residues and a minimum number which have to be acceptor residues. Those residues which are not specified in the claims may be either donor or acceptor. [Ex. 2008, p. 13; Emphasis by Adair].

In claim 67, it has been specified that residues 71, 73 and 78 are all donor residues in order to ensure that claim 67 is novel over the anti-TAC antibody disclosed by Queen. This anti-TAC antibody has an acceptor residue at residue 73. However, as can be seen from page 7, lines 1 to 5, the Applicant considers that in general, residues 71, 73 and 78 can be either all donor or all acceptor. [Ex. 2008, p. 14].

It is stated on page 7, lines 1 to 5, that residues 71, 73 and 78 should all be either acceptor or donor. Claims 73, 80, 87, 94 and 101 cover the first alternative and claims 74, 81, 88, 95 and 102 cover the second alternative. [Ex. 2008, p. 15].

UNABLE TO ADMIT OR DENY

31. On September 9, 1993, in the Adair PCT/EP Patent Application 91901433.2, Adair filed an amendment deleting original claims 1-23 and replacing them with new claims 1-20 and made the following statements:

2.10. In new claim 1, it has been specified that residues 71, 73 and 78 are all donor residues in order to ensure that new claim 1 is novel over the anti-TAC antibody disclosed in PNAS-USA, 86, 10029-10033, 1989 (Queen) (cited in the International Search Report). This anti-TAC antibody has an acceptor residue at residue 73. However, as can be seen from page 7, lines 1 to 5, the Applicant considers that in general, residues 71, 73 and 78 can be either all donor or all acceptor. [Ex. 2009, p. 3].

ADMITTED

32. On February 7, 1994, Adair filed an amendment in the `329 application responding to the office action mailed on September 7, 1993 (Ex. 2028), wherein Adair stated:

It is specifically stated in the application that the present protocol represents a departure from the procedures of Reichmann [sic] and Queen, at least. Thus, the skilled person would not rely on Reichmann [sic] and Queen as teachings relevant to whether the present description is enabling.

It is submitted that the skilled person would rely on the clear teaching given in the application and find that it is enabling. The specification plainly sets out what actions need to be taken. It is presumed that the Examiner agrees that

the skilled person could have taken those actions. The application also sets out that, contrary to the teachings of Reichmann and Queen, the protocol is generally applicable. The application further shows that it had been successfully implemented. Thus, it is submitted that the skilled person would find that the present application is properly enabled the full extent of the claims. [Ex. 2010, pp. 11-12].

DENIED

33. In the February 7, 1994, amendment, Adair made the following statements:

At a very helpful interview held at the beginning of 1993, there was some discussion of the word "comprising" as used in the claims under consideration at that time. In those claims, it was only specified that certain residues should be donor residues. [Emphasis by Adair]. It was considered that it was not clear whether these were the only residues which could be donor residues. The alternative view was that these were only the minimum number of residues which must be donor but that any of the other residues could also be donor.

If the second line of interpretation were taken, the claims could be read to cover a situation in which all except one of the residues in the variable domain were donor residues. [Emphasis by Adair]. In this case, the claims could then be interpreted to cover a structure similar to a "chimeric" antibody comprising a donor variable domain and a human constant region. Such chimeric antibodies were already well known at the priority date.

It plainly is not the intention of the Applicants to claim chimeric antibodies or any similar structures. As can be seen from the description, the superhumanised antibodies of the present invention are compared to the prior art chimeric antibodies. Moreover, the present invention was intended to deal with the problem of chimeric antibodies in that chimeric antibodies were believed to be too "foreign" because of the presence of the complete donor variable domain.

For the above reasons, it is clear that the wording of the claims needed to be changed so that the Applicants' intention of excluding chimeric antibodies was made effective. The language now present in the claims puts this intention clearly into effect.

As to support for this wording, the Examiner is referred firstly to page 16, under the heading "Protocol". It can be seen from this paragraph that the first step in the process involves the choice of an appropriate acceptor chain variable domain. This acceptor domain must be of known sequence. Thus, the protocol starts with a variable domain in which all the residues are acceptor residues. In the sentence bridging pages 16 and 17, it is stated that:

"The CDR-grafted chain is then designed <u>starting from the</u>

basis of the acceptor sequence ". [Emphasis by Adair].

On page 17, in the middle paragraph, it is stated that:

"The positions at which donor residues are to be substituted for acceptor in the framework are then chosen as follows ...."

This again shows that, unless a residue is chosen for substitution, it will remain as in the acceptor sequence.

It must also be borne in mind that the purpose of the invention is to obviate some of the disadvantages of prior art proposals. The proposal of using chimeric antibodies had the disadvantage that they were more "foreign" than desirable. The problem of making CDR-grafted antibodies was that they generally did not provide good recovery of affinity. Thus, the aim of the present invention was to minimise as far as possible the "foreign" nature of the antibody while maximising as far as possible its affinity.

Bearing the passages referred to above and the aim of the invention in mind, it would have been abundantly clear to the skilled person reading the application that as many residues as possible should remain as acceptor residues. If this were not the case, it could hardly be said that the composite chain is based on the acceptor sequence.

The skilled person reading the application can plainly see that certain residues have been considered for changing from acceptor to donor. These are clearly set out in the description. It would be plain to the skilled person that all other residues should not be considered for changing at all. It would therefore be obvious that any residue which is not specified as being under consideration for changing must remain as in the acceptor chain.

It may be that there is no explicit statement in the description that the specified residues should remain as in the acceptor chain. However, the disclosure in a specification is not limited to the explicit disclosure but also includes that which is implicit. It is implicit, in the recitation that the chain is based on the acceptor and that only certain residues are considered for changing, that all non-specified residues must remain as acceptor residues. Subject matter which might be fairly deduced from the disclosure is not new matter. *Acme Highway Products Corp. v. D.S. Brown Co.,* 431 F.2d 1074, 1080, 167 U.S.P.Q. 129, 132-133(6th Cir. 1970), *cert denied,* 401 U.S. 956 (1971).

Another way to look at it.is to consider a different way in which the claim could be drafted. It could be specified that in the composite chain, at least a certain minimum number of residues are donor residues (as in the present claims) and at most a certain maximum number of residues are donor residues. The maximum number would be derived by listing all the residues which are

considered for changing. Such an amendment would have clear explicit basis in the description because all those residues are mentioned as such. However, the effect of such an amendment would be to produce claims of exactly the same scope as the present claims. It can thus be seen that the present claims do not add subject matter but are plainly properly based on the disclosure in the description.

It is therefore submitted that the claims are fully supported by the description, are commensurate in scope with the disclosure in the description, and are properly delimited over the prior art. [Ex. 2010, pp. 3-7].

ADMITTED

34. Adair did not present a newly executed declaration at the time of filing the `261 application but, rather, relied on the inventor declaration from the parent application to satisfy the requirements of 37 C.F.R. § 1.63. (Ex. 2002).

ADMITTED THAT ADAIR RELIED UPON AN INVENTOR DECLARATION FROM A PRIOR APPLICATION.

35. On November 21, 2005, Adair filed its involved application, *i.e.,* U.S. Patent Application No. 11/284,261 ("the `261 Application"). (Ex. 2002).

ADMITTED

36. On November 21, 2005, Adair presented new claim 24 as follows:

Claim 24 (new) A humanised antibody heavy chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises an amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof, as numbered according to Kabat . [Ex. 2003, p. 3].

ADMITTED

37. On September 9, 2009 , Adair presented its involved claim 24 in the `261 application, which reads as follows:

Claim 24 (currently amended): A humanised antibody comprising a heavy chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises a non-human amino acid substitution at

a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof, as numbered according to Kabat . [Ex. 2004, p. 2; Adair Clean Copy of Claims, Paper No . 5, p. 4].

ADMITTED

38. Appendix 3 is a claim chart comparing Adair claim 24 as originally filed in 2005 and Adair involved claim 24.

UNABLE TO ADMIT OR DENY

39. Adair involved claim 24 encompasses a humanized antibody wherein the heavy chain variable domain framework region has any combination of human and non-human amino acid residues at positions 23, 24, 49, 71, 73 and 78. (Adair Clean Copy of Claims, Paper No. 5, p. 4).

DENIED -- HAVING ALL HUMAN AMINO ACID RESIDUES AT THESE POSITIONS IS NOT ENCOMPASSED.

40. Adair involved claim 24 encompasses a humanized antibody wherein the heavy claim variable domain framework region has non-human amino acids at positions 71, 73 and 78 and human amino acids at positions 23, 24, and 49. (Adair Clean Copy of Claims, Paper No. 5, p. 4).

DENIED -- THERE IS NO "HEAVY CLAIM VARIABLE DOMAIN."

41. Adair involved claim 24 encompasses a humanized antibody wherein the heavy claim variable domain framework region has non-human amino acids at positions 23 and 71 and human amino acids at positions 24, 49, 73 and 78. (Adair Clean Copy of Claims, Paper No. 5, p. 4).

DENIED -- THERE IS NO "HEAVY CLAIM VARIABLE DOMAIN."

42. Adair involved claim 24 encompasses a humanized antibody wherein the heavy claim variable domain framework region has non-human amino acids at position 23 and human amino acids at positions 24, 49, 71, 73 and 78. (Adair Clean Copy of Claims, Paper No. 5, p. 4.)

DENIED -- THERE IS NO "HEAVY CLAIM VARIABLE DOMAIN."

ADAIR STATEMENT OF ADDITIONAL MATERIAL FACTS

43. The application that issued as U.S. Patent No. 6,407,213 ("the Carter '213 patent") was filed as a national phase of a PCT application filed on June 15, 1992 ("the Carter PCT application"). (Ex. 2001, first page.)

44. The Carter PCT application was filed as a continuation-in-part of Application Serial No. 07/715,272, filed on June 14, 1991 ("the Carter '272 application"). (Ex. 2001, first page.)

45. The PCT Application was filed on December 21, 1990. (Fact 2.)

46. The PCT Application was filed almost six months before the Carter '272 application. (Facts 44 and 45.)

47. The PCT Application was filed almost 18 months before the Carter PCT application. (Facts 43 and 45.)

48. The UK Application was filed on December 21, 1989. (Fact 1.)

49. The UK Application was filed almost 18 months before the Carter '272 application was filed. (Facts 44 and 48.)

50. The UK Application was filed almost 30 months before the Carter PCT application was filed. (Facts 43 and 48.)

51. The PCT Application published on July 11, 1991. (Ex. 2005, first page.)

52. The Carter '272 application was filed less than one month before the PCT Application published. (Facts 44 and 51.)

53. On page 16, lines 23-28, the involved Adair specification reads as follows:

> A **preferred** protocol for obtaining CDR-grafted antibody heavy and light chains in accordance with the present invention is set out below together with the rationale by which we have derived this protocol. This protocol and rationa1e are

given **without prejudice to the generality of the invention as hereinbefore described and defined**.

(Ex. 2002, p. 16, ll. 23-28, emphasis added.)

54. At page 6, lines 31-37, under the heading "Summary of the Invention," the involved Adair specification reads as follows:

> Accordingly, in a first aspect the invention provides a CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least **one** of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

(Ex. 2002, p. 6, ll. 31-37, emphasis added).

55. On page 48, lines 24-27, of the '261 application, it is reported that changes at residues 6, 23, and 24 were important for maintaining a binding affinity similar to that of the murine antibody. (Ex. 2002, p. 48, ll. 24-27; *see also* graft 184 in Figure 11a.)

56. On page 58, lines 1-6, of the '261 application, it is reported that the change of a **single** residue in the heavy chain, residue 73, was sufficient to generate an antibody with binding properties similar to the donor. (Ex. 2002, p. 58, ll. 31-36, emphasis added.)

57. On page 64 of the '261 application, the following is stated:

> It will be appreciated that the foregoing examples are given by way of illustration only and are not intended to limit the scope of the claimed invention. Changes and modifications may be made to the methods described whilst still falling within the spirit and scope of the invention.

(Ex. 2002, p. 64, ll. 14-18.)

58. On page 17, lines 1-5, the involved Adair specification states that

> [i]t will be appreciated that in some cases the donor and acceptor amino acid residues may be identical at a particular position thus no change of acceptor framework residue is required.

(Ex. 2002, p. 17, ll. 1-5.)

59. Claim 1 of the PCT Application does not require that any residues be changed to donor. (Ex. 2005, claim 1, p. 67.)

60. Original claim 1 of the ‘329 application does not require that any residues be changed to donor. (Ex. 2006, claim 1, p. 67.)

61. The examiner interpreted claim 1 of the ‘329 application to be anticipated by Riechmann et al. (i.e., Ex. 2011) because the examiner said the antibody of Riechmann et al. had donor residues at positions 6, 49, 76, 88, and 91 of the heavy chain. (Ex. 2038, p. 8.)

62. Riechmann et al. did not disclose changing residues 6, 49, 76, 88, and 91 of the heavy chain to donor. (Ex. 2011, p. 326, col. 1, 1st full paragraph.)

63. The examiner interpreted claim 1 of the ‘329 application to be anticipated by Queen et al. (i.e., Ex. 2023) because the examiner said the antibody of Queen et al. had donor (murine) amino acids at positions 6, 23, 24, 48, 49, 71, 73, and 78, among others. (Ex. 2038, p. 10.)

64. Queen et al. did not disclose changing residues 6, 23, 24, 39, 71, 73, or 78 of the heavy chain to donor. (Ex. 2023, e.g., Figure 2b.)

65. Claims 66-68, 70-71, 78, and 80-82 of the ‘213 patent encompass a single residue change at residues 24 or 73 of the heavy chain. (Ex. 2001, claims 66-68, 70-71, 78, and 80-82.)

Paper No. _____

Filed on behalf of: Party Carter

Filed: July 15, 2010

By: Oliver R. Ashe, Jr., Esq.
ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

Jeffrey P. Kushan, Esq.
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, DC 20005
Tel.: (202) 736-8914
Fax: (202) 736-8711
E-mail: jkushan@sidley.com

UNITED STATES PATENT AND TRADEMARK OFFICE

_____

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

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PAUL J. **CARTER** AND LEONARD G. PRESTA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR**, DILJEET SINGH ATHWAL,
AND JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

_____

Patent Interference 105,744 (SGL)
Technology Center 1600

_____

NOTICE REGARDING OBJECTIONS TO EVIDENCE
(Re: Evidence Filed With Adair Oppositions 1-2)

NOTICE REGARDING OBJECTIONS TO EVIDENCE

The Board has set an expedited schedule for consideration of Carter Motions 1 and 2. See Paper No. 23.

In the "Order - Authorizing Oppositions - 125(a)" dated June 16, 2010 (Paper No. 73), the Board extended to July 28, 2010, the time period for Carter to file any objections to evidence filed in support of Adair Oppositions 1 and 2.

Adair Oppositions 1 and 2 were filed on July 14, 2010, and were not accompanied by any new exhibits. Accordingly, Carter does not object to the admissibility of the evidence cited in support of Adair Oppositions 1 and 2.

In view of the above, Carter respectfully requests the Board to indicate whether the Board intends to authorize replies to Adair Oppositions 1 and 2, and whether the parties should plan to proceed with the filing of additional motions at Time Period 1 as presently contemplated under the scheduling order entered on April 27, 2010 (Paper No. 23).

The undersigned continues to have scheduled commitments through July 22, 2010.

Respectfully submitted,

July 15, 2010

/Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.
Registration No. 40,491
Counsel for Party Carter

ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

CERTIFICATE OF FILING

The undersigned certifies that a copy of the paper entitled **"NOTICE REGARDING OBJECTIONS TO EVIDENCE (Re: Evidence Filed With Adair Oppositions 1-2)"** was filed this 15th day of July, 2010, via Interference Web Portal (https://acts.uspto.gov/ifiling/), with:

The Board of Patent Appeals and Interferences
Madison Building East, 9th Floor
600 Dulany Street
Alexandria, VA 22314
Tel: 571-272-4683
Fax: 571-273-0042
E-mail: BoxInterferences@USPTO.GOV

July 15, 2010 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

CERTIFICATE OF SERVICE

The undersigned hereby certifies that a copy of the paper entitled **"NOTICE REGARDING OBJECTIONS TO EVIDENCE (Re: Evidence Filed With Adair Oppositions 1-2)"** was served this 15th day of July, 2010, via Interference Web Portal (https://acts.uspto.gov/ifiling/), on the Attorney of Record for Adair:

Doreen Yatko Trujillo, Esq.
Cozen O'Connor P.C.
1900 Market Street, 7th Floor
Philadelphia, PA 19103
Tel.: 215-665-6593
Fax: 215-701-2005
E-mail: dtrujillo@cozen.com

July 15, 2010 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

Paper No. _____

Filed on behalf of: Parties Carter & Adair

Filed: August 9, 2010

By: Oliver R. Ashe, Jr., Esq.
ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

Doreen Yatko Trujillo, Esq.
Michael B. Fein, Esq.
COZEN O'CONNOR P.C.
1900 Market St.
Philadelphia, PA 19103
Tel.: (215) 665-5593
Fax: (215) 701-2005
E-mail: dtrujillo@cozen.com

UNITED STATES PATENT AND TRADEMARK OFFICE

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BEFORE THE BOARD OF PATENT APPEALS
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PAUL J. **CARTER** AND LEONARD G. PRESTA
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(Application No. 11/284,261),

_____

Patent Interference 105,744 (SGL)
Technology Center 1600

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JOINT NOTICE OF EXTENSION OF TIME AND REQUEST FOR TELECONFERENCE

JOINT NOTICE OF EXTENSION OF TIME AND REQUEST FOR TELECONFERENCE

Time Period 1 is set to expire on August 9, 2010. (*See* Order - Motion Times - Bd.R. 104(c), Paper No. 23, filed April 27, 2010). In view of the particular circumstances of this interference (Interference No. 105,744) and a related Interference (Interference No. 105,762), the parties are jointly stipulating to extend Time Period 1 to **August 23, 2010**.

Specifically, in the instant interference (the '744 Interference), threshold motions and related oppositions have been filed on an expedited schedule. Time Periods 1-9 have been set for the filing of additional motions and related oppositions and replies. In a recent e-mail communication from the Board, the parties were informed that a decision on the threshold motions will be entered soon.

In the order declaring Interference No. 105,762, noting a relationship between the '744 and '762 Interferences, the Board set an accelerated schedule for the filing of motions lists and an initial teleconference with Administrative Patent Judge. In a recent e-mail communication, the Board indicated that the initial teleconference was cancelled and that an order would follow.

The decision(s) on the threshold motions may affect the substance of any additional motions to be filed in this interference. In view of the circumstances described above and the significant resources associated with the preparation and filing of additional motions, the parties have agreed to extend Time Period 1. In the event decisions on the threshold motions are not entered before **August 16, 2010**, the parties respectfully request a teleconference with the Administrative Patent Judge at her earliest convenience to discuss the status of the case and whether an additional extension of time may be necessary to allow for entry of a decision on threshold motions before Time Period 1.

The attached proposed schedule includes the times for taking action in the motions phase as set forth in the Appendix of the Order - Motion Times - Bd.R. 104(c) (Paper No. 23). The old date for Time Period 1 is crossed out and the new date is inserted by hand.

Respectfully submitted,

August 9, 2010

/Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.
Registration No. 40,491
Counsel for Party Carter
ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

/Doreen Yatko Trujillo/
Doreen Yatko Trujillo
Registration No. 35,719
Counsel for Party Adair
COZEN O'CONNOR P.C.
1900 Market St.
Philadelphia, PA 19103
Tel.: (215) 665-5593
Fax: (215) 701-2005
E-mail: dtrujillo@cozen.com

Appendix--ORDER - RULE 123(a)
(Times for substantive motions; priority deferred)

Interference 105,744

CARTER THRESHOLD MOTIONS.....**1 June 2010**

ADAIR OPPOSITION, RESPONSIVE MOTIONS.....to be set if needed

TIME PERIOD 1 (all other authorized motions).....~~**9 August 2010**~~ 23 August 2010
File motions
File (but serve one week later) priority statements

TIME PERIOD 2.....**20 September 2010**
File responsive motions (none authorized at this time)
filed in TIME PERIOD 1

TIME PERIOD 3.....**2 November 2010**
File oppositions to all motions

TIME PERIOD 4.....**14 December 2010**
File all replies

TIME PERIOD 5.....**4 January 2011**
File request for oral argument
File motions to exclude
File observations

TIME PERIOD 6.....**18 January 2011**
File oppositions to motions to exclude
File response to observations

TIME PERIOD 7.....**1 February 2011**
File replies to oppositions to motions to exclude

TIME PERIOD 8.....**8 February 2011**
File exhibits
File sets of motions
File any CD-ROMs

TIME PERIOD 9.....**to be set**
Default oral argument date (if ordered)

CERTIFICATE OF FILING

The undersigned certifies that a copy of the paper entitled **"JOINT NOTICE OF EXTENSION OF TIME AND REQUEST FOR TELECONFERENCE"** attaching revised Appendix to Paper No. 23 was filed this 9th day of August, 2010, via Interference Web Portal (https://acts.uspto.gov/ifiling/), with:

The Board of Patent Appeals and Interferences
Madison Building East, 9th Floor
600 Dulany Street
Alexandria, VA 22314
Tel: 571-272-4683
Fax: 571-273-0042
E-mail: BoxInterferences@USPTO.GOV

August 9, 2010 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

CERTIFICATE OF SERVICE

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Doreen Yatko Trujillo, Esq.
Cozen O'Connor P.C.
1900 Market Street, 7th Floor
Philadelphia, PA 19103
Tel.: 215-665-6593
Fax: 215-701-2005
E-mail: dtrujillo@cozen.com

August 9, 2010 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

Paper No. _____

Filed on behalf of: Parties Carter & Adair

Filed: August 23, 2010

By: Oliver R. Ashe, Jr., Esq.
ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

Doreen Yatko Trujillo, Esq.
Michael B. Fein, Esq.
COZEN O'CONNOR P.C.
1900 Market St.
Philadelphia, PA 19103
Tel.: (215) 665-5593
Fax: (215) 701-2005
E-mail: dtrujillo@cozen.com

UNITED STATES PATENT AND TRADEMARK OFFICE

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BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

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PAUL J. **CARTER** AND LEONARD G. PRESTA
Junior Party
(Patent 6,407,213),

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JOHN ROBERT **ADAIR**, DILJEET SINGH ATHWAL,
AND JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

_____

Patent Interference 105,744 (SGL)
Technology Center 1600

_____

SECOND JOINT NOTICE OF EXTENSION OF TIME PERIOD 1

SECOND JOINT NOTICE OF EXTENSION OF TIME PERIOD 1

Further to the Joint Notice of Extension of Time and Request for Teleconference (Paper No. 78, filed August 9, 2010), the parties have agreed to extend **Time Period 1** from August 23, 2010, to **September 6, 2010**.

The attached proposed schedule includes the times for taking action in the motions phase as set forth in the Appendix of the Order - Motion Times - Bd.R. 104(c) (Paper No. 23) and the Joint Notice of Extension of Time and Request for Teleconference (Paper No. 78). The old date for Time Period 1 is crossed out and the new date is inserted by hand.

Respectfully submitted,

August 23, 2010

/Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.
Registration No. 40,491
Counsel for Party Carter
ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

/Doreen Yatko Trujillo/
Doreen Yatko Trujillo
Registration No. 35,719
Counsel for Party Adair
COZEN O'CONNOR P.C.
1900 Market St.
Philadelphia, PA 19103
Tel.: (215) 665-5593
Fax: (215) 701-2005
E-mail: dtrujillo@cozen.com

Appendix--ORDER - RULE 123(a)
(Times for substantive motions; priority deferred)

Interference 105,744

CARTER THRESHOLD MOTIONS.....**1 June 2010**

ADAIR OPPOSITION, RESPONSIVE MOTIONS.....to be set if needed

TIME PERIOD 1 (all other authorized motions).....~~23 August 2010~~ 6 September 2010 ~~9 August 2010~~
File motions
File (but serve one week later) priority statements

TIME PERIOD 2.....**20 September 2010**
File responsive motions (none authorized at this time)
filed in TIME PERIOD 1

TIME PERIOD 3.....**2 November 2010**
File oppositions to all motions

TIME PERIOD 4.....**14 December 2010**
File all replies

TIME PERIOD 5.....**4 January 2011**
File request for oral argument
File motions to exclude
File observations

TIME PERIOD 6.....**18 January 2011**
File oppositions to motions to exclude
File response to observations

TIME PERIOD 7.....**1 February 2011**
File replies to oppositions to motions to exclude

TIME PERIOD 8.....**8 February 2011**
File exhibits
File sets of motions
File any CD-ROMs

TIME PERIOD 9.....**to be set**
Default oral argument date (if ordered)

CERTIFICATE OF FILING

The undersigned certifies that a copy of the paper entitled **"SECOND JOINT NOTICE OF EXTENSION OF TIME PERIOD 1"** attaching revised Appendix to Paper No. 23 was filed this 23rd day of August, 2010, via Interference Web Portal (https://acts.uspto.gov/ifiling/), with:

The Board of Patent Appeals and Interferences
Madison Building East, 9th Floor
600 Dulany Street
Alexandria, VA 22314
Tel: 571-272-4683
Fax: 571-273-0042
E-mail: BoxInterferences@USPTO.GOV

August 23, 2010 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

CERTIFICATE OF SERVICE

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Doreen Yatko Trujillo, Esq.
Cozen O'Connor P.C.
1900 Market Street, 7th Floor
Philadelphia, PA 19103
Tel.: 215-665-6593
Fax: 215-701-2005
E-mail: dtrujillo@cozen.com

August 23, 2010 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

Mail Stop Interference
P.O. Box 1450
Alexandria Va 22313-1450
Tel: 571-272-9797
Fax: 571-273-0042

Filed August 30, 2010

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

PAUL J. **CARTER** AND LEONARD G. PRESTA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR**, DILJEET SINGH ATHWAL, and JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261).

Patent Interference No. 105,744
(Technology Center 1600)

Before SALLY GARDNER LANE, RICHARD TORCZON, and SALLY C. MEDLEY, *Administrative Patent Judges*

LANE, *Administrative Patent Judge*

ORDER - DECISION ON MOTIONS

I. STATEMENT OF THE CASE

The interference is before a panel for consideration of non-priority motions filed by Carter. No oral argument was held.

The Interference

Parties

The Interference involves junior party Carter and senior party Adair.

Junior party Carter is involved on the basis of its patent 6,407,213 ("the Carter '213 patent"), which issued 18 June 2002, from application no. 08/146,206, filed 17 November 1993. (Paper 1 at 3.) Claims 30, 31, 60, 62, 63, 66, 67, 70, 73, and 77-81 were designated as corresponding to the Count, while claims 1-29, 32-59, 61, 64, 65 68, 69, 71, 72, 74-76, and 82 were not. (Paper 1 at 4.)

The real party-in-interest of Carter is Genentech, Inc. (Paper 10).

Senior party Adair is involved on the basis of its application 11/284,261 ("Adair '261 application"), filed 21 November 2005. (Paper 1 at 3.) Claim 24, Adair's only pending claim, was designated as corresponding to the Count. (Paper 1 at 4.)

Adair was accorded priority benefit as to the Count of 08/846,658, filed 01 May 1997; 08/303,569, filed 07 September 1994, issued as 5,859,205 on 12 January 1999; 07/743,329, filed on 17 September 1991 ("the Adair '329 application"); PCT/GB90/02017, filed 21 December 1990 ("the Adair PCT application"); and GB 8928874.0, filed 21 December 1989. (Paper 1 at 5.)

The real party-in-interest of Adair is UCB Pharma, S.A. (Paper 4.)

Subject Matter

The parties' claims are drawn to an antibody that has been "humanized," that is, it has a combination of human and non-human regions and specific amino acids. Humanization allows antibodies to be raised, in the laboratory, in non-human animals (for example, mice) against antigens of interest and then changed so that they appear to the patient's body as if they were human antibodies. Humanized antibodies are beneficial because they do not raise dangerous anti-immunoglobulin responses in human patients, as non-human antibodies can. (Carter patent col. 1, l. 52, through col. 3, l. 8.) The humanized antibody of the involved Carter and Adair claims and the Count are antibodies that have a non-human Complementarity Determining Region ("CDR"), that is the region that binds antigen, and specifically recited non-human substitutions in other regions, called the Framework Regions ("FR"), of the antibody.

II. MOTIONS

Carter filed two substantive motions, which assert "threshold" issues that end the interference if the relief requested is granted. Carter Substantive Motion 1 ("Carter Motion 1") requests that Adair claim 24 be found unpatentable under 35 U.S.C. § 135(b)(1). Carter Substantive Motion 2 ("Carter Motion 2") requests that Adair claim 24 be found unpatentable under 35 U.S.C. § 112, first paragraph, for a lack of written description in the specification. As the moving party, Carter has the burden to show that it is entitled to the relief requested in its motions. Bd. R. 208(b).

A. CARTER MOTION 1

Findings of Fact

1. The involved Carter ‘213 patent issued 18 June 2002. (Carter Ex. 2001; Carter involved ‘231 patent.)

2. The “critical date,” under 35 U.S.C. § 135(b)(1), by which Adair must have filed claims drawn to the same or substantially the same subject matter as the claims of the Carter ‘213 patent is 18 June 2003.

3. Adair filed the involved Adair ‘261 application on 21 November 2005, after the critical date. (Ex. 2002, Utility Patent Application Transmittal for Application 11/284,261.)

4. Claim 24, the only claim pending in the Adair ‘261 application was filed well after the critical date.

5. Claim 24 of the involved Adair ‘261 application recites:

> A humanised antibody comprising a heavy chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises a non-human amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof, as numbered according to Kabat.

(Paper 5.)

6. None of the claims of the Adair PCT application or the Adair ‘329 application are identical to claim 24 of the involved Adair ‘261 application. (Adair response to Carter MF 42; citing Exs. 2005-2010, 2012-2022, 2024-2027, 2029, and 2031-2035; not admitted or denied by Adair (Adair Opposition 1 at 21 (“Adair Opp. 1”)), but no claims identical to claim 24 of the involved Adair ‘261 application identified by

Adair.)

7. In its request for interference, Bd. R. 202, Adair identified claims 8 and 16 of the Adair PCT application as a basis for compliance with 35 USC §135(b).

(Ex. 2003, Adair's Preliminary Amendment and Request for Interference under 37 C.F.R. § 42.202, p. 5.)

8. Claim 8 of the Adair PCT and '261 applications recites:

> A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, 58 and 71.

(Ex. 2005, p. 68 and Ex. 2006, p. 68.)

9. Claim 16 of the Adair PCT and '329 applications recites:

> A CDR-grafted antibody heavy or light chain or molecule according to any one of the preceding claims comprising human acceptor residues and non-human donor residues.

(Ex. 2005, p. 69 and Ex. 2006, p. 69.)

10. Claim 1 of the Adair PCT and '329 applications recites:

> A CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

(Ex. 2005, p. 67 and Ex. 2006, p. 67.)

Analysis

35 U.S.C. § 135(b)(1) states that:

> [a] claim which is the same as, or for the same or substantially the same subject matter as, a claim of an issued patent may not be made in any application unless such a claim is made prior to one year from the date on which the patent was granted.

Claim 24 of Adair's involved application, which corresponds to the Count, was filed more than one year from the date on which Carter's involved patent was issued. Because of the date Adair claim 24 was filed (*see* FF 4), it is, on its face, barred under 35 USC §135(b).

The bar of 35 USC §135(b) might be avoided if Adair had filed a claim that does not differ materially from claim 24. Indeed, in its request for interference, Bd. R. 202, Adair pointed to claims 8 and 16 of its pre-critical date application to support its assertion that claim 24 is not barred under the statute. (FF 7; Ex. 2003, Adair's Preliminary Amendment and Request for Interference under 37 C.F.R. § 42.202, p. 5.)

"To establish entitlement to the earlier effective date of existing claims for purposes of the one-year bar of 35 U.S.C. § 135(b), a party must show that the later filed claim does not differ from an earlier claim in any 'material limitation,'" *In re Berger*, 279 F.3d 975, 981-82 (Fed. Cir. 2002) (quoting *Corbett v. Chisholm*, 568 F.2d 759, 765-66 (CCPA 1977)). *See also Regents of Univ. of Cal. v. Univ. of Iowa Res. Found.*, 455 F.3d 1371, 1375 (Fed. Cir. 2006) ("When a party seeks to add a new claim, or to amend an existing claim, beyond the critical date for section 135(b)(1), [the Federal Circuit] applies the material differences test discussed in opinions like *Berger* to determine if

'such a claim' is barred."). The addition of a limitation for the purpose of making a claim patentable is strong evidence that the limitation is a material one. *See Corbett*, 568 F.2d at 765 (where a party's claim lacked a method step, the court noted that the party did "not seriously contend that this [was] not a material limitation, that [was] necessary to patentability . . . ."); *see also Wetmore v. Miller*, 477 F.2d 960, 964 (CCPA 1973) ("the 'fusible' limitation of appellant's claims must be regarded as not necessary to patentability and not 'material' for present purposes [of complying with 35 U.S.C. § 135(b)]").

Carter argues that the pre-critical date claims of Adair include different material limitations than those in Adair's involved claim 24. (Carter Motion 1 at 3.)

Claim 8 of the Adair PCT application, which is identical to claim 8 of the Adair '329 application, recites:

> A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, 58 and 71.

(FF 8; Ex. 2005, p. 68; Ex. 2006, p. 68.) Claim 16 of the Adair PCT application, which is identical to claim 16 of the Adair '329 application, recites:

> A CDR-grafted antibody heavy or light chain or molecule according to anyone of the preceding claims comprising human acceptor residues and non-human donor residues.

(FF 9; Ex. 2005, p. 69; Ex. 2006, p. 69.) Thus, the claims that Adair relied upon for avoiding the 35 U.S.C. § 135(b) bar are drawn to a CDR-grafted light chain. Adair's involved claim 24, though, is drawn to a "humanized antibody comprising a heavy chain variable domain . . . ." (FF 5, Paper 5.) Involved claim 24 differs from original claims 8

and 16, by reciting a heavy chain variable domain instead of a light chain variable domain.

Adair does not dispute that claims reciting a heavy chain and claims reciting a light chain differ materially. Instead, Adair argues that Carter applied the incorrect standard for assessing whether a post-critical date claim differs materially from an earlier claim. According to Adair, the correct inquiry is whether Adair added or removed claim limitations after the critical date that were necessary to the patentability of *Carter's* claims, not Adair's own pre-critical date claims (Adair Opp. 1 at 6).

We disagree. A party seeking support from pre-critical date claims for interfering claims filed beyond the one-year bar of 35 U.S.C. § 135(b)(1) "must demonstrate that claims in [the pre-critical date] application provide pre-critical date support for the post-critical date identity between [the involved claim] and the [patentee's patent]. That demonstration necessarily entails a comparison between pre- and post-critical date claims." *Regents of Univ. of Cal.*, 455 F.3d at 1375.

Adair also argues, in response to Carter's assertion of the material differences between claims to heavy and light chains, that in addition to its claims drawn to light chains, Adair filed claims drawn to heavy chains before the critical date. Specifically, Adair cites claim 1 of its PCT application as claiming a CDR-grafted antibody heavy chain, and argues that it, together with claim 16, effectively contain all of the limitations of involved claim 66 of the Carter '213 patent. (Adair Opp. 1 at 5; *see* FF 10; Ex. 2005, p. 67; Ex. 2006, p. 67.).[1]

1 Similarly in its showing under Bd. R. 202, Adair compared its pre-critical date claims to a Carter claim but not the current Adair claim. (Ex. 2003, Adair's Preliminary Amendment and Request for

Adair has not made the correct comparison. Under the guidance provided in *Regents of University of California,* Adair's pre-critical date claims must be compared with its own current claims, not Carter's. Thus we are not persuaded by Adair's argument that it is sufficient that it had on file a claim or claims that effectively contain the limitations of an involved Carter claim.

Even when we consider claims 1 and 16 of the PCT application as they compare to Adair's current claim (and not Carter claim 66 as Adair argues), we are not convinced that Adair had a pre-critical date claim that does not differ materially from its current claim. As Carter notes, (1) claims 1 and 16 of Adair's PCT application were rejected under several statutory grounds in the Adair '329 application, including 35 U.S.C. §§ 101, 112, first and second paragraphs, 102(b), and 103(a), (*see* Ex. 2038, Office Action mailed 18 November 1992), and (2) Adair then cancelled the claims and added new ones that were eventually allowed (Ex. 2007, Amendment of 19 January 1993, p. 2). (*See* Carter Motion 1 at 5-6.)

One example of a material limitation is one that is "necessary to patentability." *See Corbett*, 568 F.2d at 765. When an applicant adds a limitation to a claim in response to a rejection and the added limitation results in allowance of the claim, the limitation is presumed to be necessary to patentability. *Cf*. *Festo Corp. v. Shoketsu Kinzoku Kogyo Kabushiki Co., Ltd.,* 535 U.S. 722, 734 (2002) (in the context of applying the doctrine of equivalents, "[a] rejection indicates that the patent examiner does not believe the original claim could be patented. While the patentee has the right to appeal, his decision to forgo an appeal and submit an amended claim is taken as a concession

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Interference under 37 C.F.R. § 42.202, p. 5.)

that the invention as patented does not reach as far as the original claim."); *see Berger*, 279 F.3d at 982 ("Inclusion of a limitation in a claim to avoid the prior art provides strong evidence of the materiality of the included limitation."). Adair does not provide any reason why the limitations that differ between involved claim 24 and original claims 1 and 16 were not necessary to the patentability of claim 24. Nor does Adair point to any other pre-critical date claim that is identical to or includes the same material limitations as its involved claim 24. (FF 6; *see* Carter MF 42, citing Exs. 2005-2010, 2013-2022, 2025-2027, 2029, and 2031-2035; not admitted or denied by Adair (Adair Opp. 1 at 21), but no claims identical to claim 24 of the involved Adair '261 application identified by Adair). We also note that as an applicant Adair could have, but did not, seek authorization to file a motion to add to its application a pre-critical date claim that interferes with the Carter claims (See Papers 23 and 73 (Orders setting times)).

Adair questions how one can provoke an interference if any claim amendments were made during prosecution under the standard stated in *Regents of University of California.* (Adair Opp. 1 at 7.) As explained in that case, "section 135(b)(1) [is] a statute of repose, placing a time limit on a patentee's exposure to an interference proceeding. *Regents Univ. of Cal.*, 455 F.3d at 1376. Despite this statute of repose, a "belated interference", i.e., based on a post-critical date claim, is appropriate in certain instances since "[t]he PTO should declare a valid interference upon receipt of a claim that satisfies section 135(b)(1), and which is otherwise patentable." (*Id.* at 1376). To insure that applicant did indeed timely present a patentable interfering claim, the post-critical date claim in interference must be materially the same as the claim that was timely presented. An applicant cannot expect to avoid the bar of §135(b) by timely

copying a claim from an issued patent when that claim is not patentable to that applicant. As the court noted, it "perceives no inequity in a construction of section 135(b)(1) that might, in some circumstances, prevent a patent applicant from relying on the filing date of a claim to which it was not statutorily entitled." (*Id.* at 1377).

We grant Carter Motion 1 and conclude that Adair involved claim 24 is barred under 35 U.S.C. § 135(b)(1).

B. CARTER MOTION 2

Carter asserts that claim 24 of Adair's involved application is unpatentable under 35 U.S.C. § 112, first paragraph, for lack of written description support.

Findings of Fact

11. Adair's specification provides a "preferred protocol" to determine which residues of a human heavy chain should be substituted for donor residues, as follows

> 2. Heavy Chain
>
> 2.1 Choose donor residues at all of positions 23, 24, 49, 71, 73 and 78 of the heavy chain or all of positions 23, 24 and 49 (71, 73 and 78 are always either all donor or all acceptor).
>
> 2.2 Check that the following have the same amino acid in donor and acceptor sequences, and if not preferably choose the donor: 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

(Ex. 2002, pp. 17-18; MF 13.)

12. Adair's specification includes the following directions regarding substituting residues of a human heavy chain for donor residues:

> "Key residues" near the surface of the heavy chain, are residues 23, 71 and 73, with residues 1, 3, and 76 reported to contribute to a lesser extent. (Ex. 2002, p. 20; MF 16.)

> "Key residues" among the "[p]acking residues" near the CDRs as 24, 49, and 78. (Ex. 2002, p. 21; MF 17.)
>
> Example 1 reports that "it is important to retain mouse residues at all of positions 6, 23, 24, 48 and 49, and possibly for maximum binding affinity at 71, 73 and 78." (Ex. 2002, p. 52; MF 19.)
>
> Example 3 reports results wherein the crystal structure of the antibody heavy chain revealed that substitution at position 73 only was found to be important for antigen binding. (Ex. 2002, pp. 57-58; MF 56.)

13. Adair's specification provides the following written description of a CDR-grafted antibody heavy chain with specified donor residues:

> Accordingly, in a first aspect the invention provides a CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

(Ex. 2002 at p. 6.)

14. Adair's specification also provides the following written description of a CDR- grafted antibody heavy chain with specified donor residues:

> In preferred embodiments, the heavy chain framework comprises donor residues at positions 23, 24, 49, 71, 73 and 78 or at positions 23, 24 and 49. The residues at positions 71, 73 and 78 of the heavy chain framework are preferably either all acceptor or all donor residues.

(Ex. 2002 at p. 7.)

15. Adair's specification states:

> A preferred protocol for obtaining CDR-grafted antibody heavy and light chains in accordance with the present invention is set out below together with the rationale by which we have derived this protocol. This protocol. and rationale are given without prejudice to the generality of the invention as hereinbefore described and defined.

(Ex. 2002, p. 16; MF 53.)

Analysis

The test for written description under 35 U.S.C. § 112, first paragraph, "is whether the disclosure of the application relied upon reasonably conveys to those skilled in the art that the inventor had possession of the claimed subject matter as of the filing date." *Ariad Pharm., Inc., v. Eli Lilly & Co.*, 598 F3d 1336, 1351 (Fed. Cir. 2010). This analysis must consider the understandings of those in the art at the time of filing, *see Bilstad v. Wakalopulos*, 386 F.3d 1116, 1125-26 (Fed. Cir. 2004), and must consider the specification as a whole, *see In re Wright,* 866 F.2d 422, 424-25 (Fed. Cir. 1989).

Claim 24 recites a humanized antibody with a heavy chain "compris[ing] a non-human amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78 and combinations thereof . . . ." (FF 5; Paper 5). As Carter asserts, the broadest reasonable interpretation of this language in claim 24 encompasses a human heavy chain with residue substitutions at any number of the six residues recited, for example at only one residue, at all six residues, or at any combination in between. (*See* Carter Motion 2 at 1 and 5-6.)

Specification

In support of its argument that Adair's specification does not provide written description support of *any* of the six residues in claim 24, Carter cites to a "preferred protocol" provided in Adair's specification. Carter asserts that this protocol limits the invention to a human heavy chain framework region with either all of residues 23, 24, and 49, or all of residues 23, 24, 29, 71, 73, and 78, but not any of the residues individually. (Carter Motion 2 at 2 and 8; FF 11; Ex. 2002, Adair Specification, pp. 17-

18.) While this portion of the Adair specification appears to exclude many of combinations of substitutions encompassed by claim 24, other portions of Adair's specification are not so limiting.

For example, elsewhere Adair's specification provides that some "key residues" for making humanized antibodies are 23, 71 and 73, while other "key residues" are 24, 49, and 78. (FF 12; Ex. 2002, pp. 20 and 21; *see* Carter Motion 2 at 3.) Carter does not point to language in this part of the specification that indicates residues 23, 24, and 49 must *all* be substituted together or that 23, 24, 49, 71, 73, and 78 must *all* be substituted together.

In addition, while Carter cites Example 1 as reporting that "it is important to retain mouse residues at all of positions 6, 23, 24, 48 and 49, and possibly for maximum binding affinity at 71, 73 and 78" (FF 12: Ex. 2002, p. 52; *see* Carter Motion 2 at 3), Example 3 reports results wherein the crystal structure of the antibody heavy chain revealed that substitution at position 73 *only* was important for antigen binding. (FF 12; Ex. 2002, pp. 57-58; *see* Adair Opposition 2 at 3-4 ("Adair Opp. 2").) Thus, not all of the examples in Adair's specification support Carter's argument of a requirement for substation of *all* residues 23, 24, and 49 or *all* of residues 23, 24, 49, 71, 73, and 78.

Carter points to the Summary of the Invention section of Adair's application, which provides that human residues of the heavy chain can be substituted for donor residues at "at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91." (Carter Motion 2 at 6; FF 13; Ex. 2002, p. 6.) According to Carter, this language does not provide written description because it is "ambiguous." (Carter Motion 2 at 6-8.) As evidence, Carter points to the rejection

under 35 U.S.C. § 112, second paragraph, of original claim 1 in the Adair '329 application, which contained this language from the Adair specification, and Adair's response canceling claim 1. (Carter Motion 2, MFs 22 and 25; Ex. 2007, p. 29-32; Ex. 2038, p. 6.)

We do not agree that the rejection under the second paragraph of § 112 necessarily shows a lack of written description support under the first paragraph of § 112. Carter's analysis lacks a consideration of the entire Adair specification and instead focuses only upon an isolated portion.

Carter points to another part of the Summary of the Invention, wherein "[i]n preferred embodiments, the heavy chain framework comprises donor residues at positions 23, 24, 49, 71, 73 and 78 or at positions 23, 24 and 49. The residues at positions 71, 73 and 78 of the heavy chain framework are preferably either all acceptor or all donor residues." (FF 14; Ex. 2002 at p. 7; *see* Carter Motion 2 at 8.) Carter characterizes this portion as providing that 71, 73, and 78 "must" be either all acceptor or all donor residues (Carter Motion 2 at 8), but the passage expressly states that positions 71, 73, and 78 are "preferably" all donor or all acceptor. Thus, this portion of Adair's specification is not as limited as Carter asserts.

It does not appear to us that, on its face, the Adair specification contains a requirement for substitution of *all* residues 23, 24, and 49 or *all* of residues 23, 24, 49, 71, 73, and 78. Carter does not direct us to the testimony or other evidence showing what the Adair specification would have conveyed to those skilled in the art at the time of filing such that we might find otherwise. "Argument of counsel cannot take the place of evidence lacking in the record." *Meitzner v. Mindick*, 549 F.2d 775, 782 (CCPA

1977).

Prosecution History

Carter also points to the prosecution of Adair's applications as evidence that claim 24 is not supported by the Adair specification. According to Carter, Adair relied on the "preferred protocol" to distinguish claims of the Adair '329 application over the prior art and to overcome rejections for lack of enablement. (Carter Motion 2 at 9-13). The rejections, amendments, and arguments relied upon by Carter were not directed to involved claim 24 and Carter does not provide a detailed analysis of the claims that were being prosecuted and their relationship to Adair's current claim 24. Thus it is difficult to understand the relevance of the rejection of these claims to involved claim 24. *See Halliburton Energy Servs., Inc. v. M-I LLC*, 514 F.3d 1244, 1250, n.2 (Fed. Cir. 2008) ("Judges are not like pigs, hunting for truffles buried in briefs." (quoting *United States v. Dunkel*, 927 F.2d 955, 956 (7th Cir. 1991)).

In addition, though Carter notes instances when Adair discussed the "preferred protocol" and other rules for determining which residues to substitute, Carter does not point to instances where Adair argues that these are the *only* disclosures in their specification. In fact, other portions of the specification indicate that this "preferred protocol" is not limiting on the invention. (*See* Adair Opp. 2 at 3-4; FFs 15 and 16; Ex. 2002, Adair Specification, pp. 16 and 64.)

Carter has not shown that Adair claim 24 lacks sufficient written description support.

III. ORDER

Upon consideration of the motions, and for the reasons given, it is

ORDERED that Carter Motion 1 for judgment that Adair claim 24 is barred under 35 U.S.C. § 135(b) is GRANTED; and

FURTHER ORDERED that Carter Motion 2 for judgment that Adair claim 24 lacks written description support is DENIED; and

FURTHER ORDERED that judgment will be entered against Adair in a separate paper.

/ss/ Sally Gardner Lane
SALLY GARDNER LANE
Administrative Patent Judge

/ss/ Richard Torczon
RICHARD TORCZON
Administrative Patent Judge

/ss/ Sally C. Medley
SALLY C. MEDLEY
Administrative Patent Judge

Counsel for Carter
Oliver R. Ashe, Jr., Esq.
Ashe, P.C.
11440 Isaac Newton Sq. North, Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

Counsel for Adair
Doreen Yatko Trujillo, Esq.
Cozen O'Connor P.C.
1900 Market St.
Philadelphia, PA 19103
Telephone: (215) 665-5593
Facsimile: (215) 701-2005
E-mail: dtrujillo@cozen.com

Mail Stop Interference
P.O. Box 1450
Alexandria, Va 22313-1450
Tel: 571-272-4683
Fax: 571-273-0042

Filed 2 September 2010

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

PAUL J. **CARTER** AND LEONARD G. PRESTIA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR**, DILGEET SINGH ATHWAL,
and JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

Patent Interference No. 105,744
(Technology Center 1600)

Before SALLY GARDNER LANE, RICHARD TORCZON, and SALLY C. MEDLEY, *Administrative Patent Judges*.

LANE, *Administrative Patent Judge*.

Judgment– Merits – Bd. R. 127

The Carter motion for judgment on the basis that the single involved Adair claim is barred under 35 U.S.C. § 135(b) was granted. (Paper 80). Because Adair no longer has an interfering claim that is not barred under 35 U.S.C. §135(b) it is appropriate to

enter judgment against Adair. *Berman v. Housey*, 291 F.3d 1345, 1351 (Fed. Cir. 2002).

It is

ORDERED that judgment on priority as to Count 1 (Paper 1 at 4), the sole count of the interference, is entered against senior party Adair;

FURTHER ORDERED that claim 24 of Adair application 11/284,261, which claim corresponds to Count 1 (Paper 1 at 4), is FINALLY REFUSED, 35 U.S.C. §135(a):

FURTHER ORDERED that if there is a settlement agreement, the parties are directed to 35 U.S.C. 135(c) and Bd. R. 205; and

FURTHER ORDERED that a copy of this judgment shall be entered into the administrative record of the Carter involved patent and application and the Adair involved application.

cc (via electronic filing):

Attorney for CARTER:

Oliver R. Ashe, Jr., Esq.
ASHE, P.C.
11440 Isaac Newton Square, North
Suite 210
Reston, VA 20190
Tel: 703-467-9001
Email: oashe@ashepc.com

Attorney for ADAIR:
Doreen Yatko Trujillo, Esq.
Michael B. Fein, Esq.
COZEN O'CONNOR P.C.
1900 Market Street
Philadelphia, PA 19103
Tel: 215-665-5593
Email: dtrujillo@cozen.com

Mail Stop Interference
P.O. Box 1450
Alexandria, VA 22313-1450
Tel: 571-272-9797
Fax: 571-273-0042

Filed September 13, 2010

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

PAUL J. **CARTER** and LEONARD G. PRESTA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR**, DILJEET SINGH ATHWAL, and JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

Patent Interference No. 105,744
(Technology Center 1600)

ORDER – Miscellaneous – 104(a)

A conference call was held on 9 September 2010 at approximately 2:00 pm. Participating in the call were:

(1) Oliver Ashe and Jeffrey Kushan for Carter,

(2) Doreen Trujillo for Adair, and

(3) Sally Gardner Lane, Administrative Patent Judge.

The purpose of the call was to discuss the filing of motions in related interference 105762. However, during the call counsel for Adair asked about the time for requesting rehearing for the Decision (Paper 80) and Judgment (Paper 81) entered 2 September 2010. As discussed during the call, because judgment was entered on the basis of the Decision, Adair has 30 days from entry of the judgment to file any request for rehearing. Bd. R. 127(d).

/Sally Gardner Lane/

Administrative Patent Judge

cc (via electronic):

Attorney for Carter:

Oliver R. Ashe, Jr., Esq.
ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
E-mail: oashe@ashepc.com

Jeffrey P. Kushan, Esq.
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, DC 20005
Tel.: (202) 736-8914
E-mail: jkushan@sidley.com

Attorney for Adair:

Doreen Yatko Trujillo
Cozen O'Connor P.C.
1900 Market St.
Philadelphia, PA 19103
Tel.: (215) 665-5593
E-mail: dtrujillo@cozen.com

Michael B. Fein
Cozen O'Connor P.C.
1900 Market St.
Philadelphia, PA 19103
Tel.: (215) 665-4622
E-mail: mfein@cozen.com

Filed on behalf of: **Adair**
By: Doreen Yatko Trujillo
Michael B. Fein
Cozen O'Connor P.C.
1900 Market St.
Philadelphia, PA 19103
Telephone: (215) 665-5593
Facsimile: (215) 701-2005
dtrujillo@cozen.com

Paper No:_____
Filed: October 1, 2010

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

PAUL J. **CARTER** AND LEONARD G. PRESTA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR,** DILJEET SINGH ATHWAL,
AND JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

Patent Interference No. 105,744
(Technology Center 1600)

ADAIR REQUEST FOR REHEARING
(regarding Carter Motion 1)

Pursuant to Bd. R. 125(c) and SO 125, Adair requests rehearing of the Board's decision regarding Carter Substantive Motion 1 ("Carter Motion 1"). In its "Order – Decision on Motions" (Paper No. 80, "the Decision"), the Board granted Carter Motion 1 requesting that Adair involved claim 24 be found unpatentable under 35 U.S.C. § 135(b)(1). Judgment was entered September 2, 2010 (Paper No. 81). This request is timely.

Adair contends, as outlined below, that the Board misapprehended and/or overlooked several factual and legal issues in the Decision. The exhibits believed to be overlooked or misapprehended by the Board are identified in Appendix 1.

On page 7, lines 9-10, the Decision states that Carter argues that the pre-critical date claims of Adair include different **material** limitations than those in Adair's involved claim 24, citing Carter Motion 1, at 3. On page 8, lines 3-4, the Decision states that Adair does not dispute that claims reciting a heavy chain and claims reciting a light chain differ **materially**. Carter, however, does not argue that claims reciting a heavy chain and claims reciting a light chain differ materially. Carter observes, rather, that heavy and light chains are different *polypeptides* and that a residue substitution in a light chain is *structurally* different from a residue substitution in a heavy chain (Carter Motion 1, p. 3, ll. 8-17). Notably, the claims of the Carter '213 patent in interference recite changes to residues in the heavy **and** the light chains (see, for example, claim 30 of Ex. 2001).

On page 9, lines 1-5, the Decision contends that Adair did not make the correct comparison under *The Regents of the Univ. of Cal. v. Univ. of Iowa Res. Found.*, 455 F.3d 1371

Unless otherwise indicated, the same abbreviations as used in the Decision are used herein.

(Fed. Cir. 2006).[2] Neither Carter nor Adair cited *Regents* in their papers (*see* Paper No. 71 -- Carter Motion 1 and Paper No. 75 -- Adair Opposition 1), nor does it appear to be applicable. In *Regents*, the **pre**-critical date claims had been copied from the patent and were canceled and replaced by another claim *after* the critical date. The Board found that the post-critical date claim contained material differences from the pre-critical date claims. The appellant did not challenge the Board's finding of material differences between the post- and pre-critical date claims. The appellant challenged, rather, whether the correct inquiry is whether the pre-critical date claims contain material differences from the post-critical date claims and not whether the pre-critical date claims are to the same invention as the patent claims. *Regents*, 455 F.3d at 1373. In the present case, the pre-critical date claims were not copied from the Carter '213 patent; the **post**-critical date claim, which eventually became Adair involved claim 24, was copied from the patent. Indeed, the court in *Regents* distinguished cases cited by the appellant in which the claims added after the critical date were copied from the patent. *Regents*, 455 F.3d at 1375.

Adair contends that the proper test to be applied to the present facts is that outlined in *In re Berger*, 279 F.3d 975 (Fed. Cir. 2002) and *Corbett v. Chisholm*, 568 F.2d 759 (CCPA 1977). In *Berger*, the issue was whether or not the **pre**-critical date claims contained all material limitations of the later claims which had been copied from the patent. The court found a limitation material because it was added by the **patentee** during prosecution to avoid the prior art.

> Because the prior art applies in like manner to the claims **as copied**, the materiality of a limitation in a claim **copied** to provoke

[2] The Decision did include parallel citations, so Adair has not.

> an interference **translates** to the copying inventor's application for purposes of assessing compliance with **35 U.S.C. § 135(b)**.

Berger, 279 F.3d at 983, emphasis added. The material differences test discussed in *Berger,* thus, focuses upon whether all material limitations of the copied **patent** claim necessarily occurred in the pre-critical date claims to determine compliance with 35 U.S.C. § 135(b).

Similarly, in *Corbett*, the court observed that

> [t]here being a material limitation of the **copied** [Chisholm patent] claim not present in Corbett's [pre-critical date] claims 24-27, they cannot be said to be directed to **substantially the same invention**.

Corbett, 568 F.2d at 766, citation omitted. Neither the Decision, nor Carter, argued that the pre-critical date claims do not contain all material limitations of the Carter '213 patent claims.

Even if *Regents* is applicable, which Adair contends it is not, it did not change the material differences test. *Regents* clearly states that the material differences test is the test discussed in opinions like *Berger*.

> When a party seeks to add a new claim, or to amend an existing claim, beyond the critical date for *section 135(b)(1)*, this court applies the material differences test discussed in opinions like *Berger* to determine if "such a claim" is barred.

Regents, 455 F.3d at 1376. As noted above, the *Berger* test compares the pre-critical date claims and the post-critical date claims, which were copied from the patent, to ensure that all material limitations of the post-critical date claims are present in the pre-critical date claims. Materiality is determined in view of the **patent** claims being copied.

The Federal Circuit does make the following statement in *Regents* in response to California's assertion that the result unfairly denied applicants access to an interference just because their **pre**-critical date claims added **to provoke the interference** lacked written descriptive support:

> … this court perceives no inequity in a construction of *section 135(b)(1)* that might, in some circumstances, prevent a patent applicant from relying on the filing date of a claim to which it was not statutorily entitled.

Regents, 455 F3d at 1376-77. This statement is cited in the Decision to support requiring that the post-critical date claim in interference be materially the same as the claim that was timely presented (the Decision, p. 10, l. 20 to p. 11, l. 4). This statement from *Regents* is not inconsistent with Adair's arguments regarding materiality, however. If, prior to the critical date, an applicant copies patent claims to provoke an interference and, after prosecution, the applicant's allowed post-critical date claims lack limitations from the pre-critical date claims that were necessary to the patentability of the **patent** claims, that applicant should not be able to rely upon the pre-critical date claims to provoke an interference with that patent. The claims are no longer to substantially the same invention as required by 35 U.S.C. § 135(b)(1).

Even if the materiality test is as asserted in the Decision, however, the Board did not properly apply the test. The Board did not determine that involved claim 24 is materially different from all pre-critical date claims. Rather, on page 9, lines 6-14, the Decision states that, even considering claims 1 and 16 of the PCT application, it is not convinced that Adair had a pre-critical date claim that does ***not*** differ materially from its involved claim, noting, *inter alia,* that the original claims were rejected under several statutory grounds and then canceled. First, canceled claims, can be relied upon for determining compliance with 35 U.S.C. § 135(b); s*ee* *Corbett*, 568 F.2d at 761 and 765 (pre-critical date claims that were canceled over 15 months after being introduced and 27 months before the patent issued were considered for compliance with 35 U.S.C. § 135(b)). Second, the Board should have to be convinced by Carter, not Adair, that **all** pre-critical date claims differ materially from involved claim 24 in order to grant Carter Motion 1, as Carter bears the burden on this motion (the Decision, p. 3, ll. 19-20). In

contravention of Bd. R. 41.121(e) and SO 121.6, Carter did not compare any pre-critical date claims other than original claims 8 and 16 to involved claim 24 (*see* Carter Motion 1, Appendix 3). Had Carter compared all pre-critical date claims to involved claim 24, the Board might not have overlooked that original pre-critical date claim 2 recites all the residues recited in involved claim 24 – i.e., residues 23, 24, 49, 71, 73, and 78, of the heavy chain (see Ex. 2005, and Appendix 2).

On page 10, lines 3-5, the Decision states that Adair does not provide any reason why the limitations that differ between involved claim 24 and original claims 1 and 16 were not necessary to the patentability of claim 24. The Decision, however, does not identify any limitations that differ between original claims 1 and 16 and involved claim 24, much less limitations that were necessary to patentability. The Decision apparently assumes that, because original claims 1 and 16 were rejected and involved claim 24 was allowed, limitations must be different and, *a priori*, must be material to patentability. And, again, the burden was upon Carter not only to show that there are differences, but also that any differences were material to patentability. Carter did not show either. Carter conclusorily stated that no pre-critical date claim was identical to or includes the same material limitations as involved claim 24 (Carter Motion 1, Fact 42). Carter dismissed the original pre-critical date claims by arguing that they were unpatentable, and that Adair could not rely upon such claims because they were unpatentable (Carter Motion 1, p. 9, ll. 8-11). Regarding the non-original pre-critical date claims, Carter simply argued that they were not supported by the specification for essentially the same reasons it asserted that involved claim 24 was not supported by the specification in Carter Motion 2 -- an argument that was clearly not accepted by the Board as it denied Carter Motion 2 – Paper No. 72 (the Decision, p. 17, ll. 5-6).

On page 10, lines 5-7, the Decision states that Adair did not point to any other pre-critical date claim that is identical to or includes the same material limitations as involved claim 24. Adair is aware of no case law requiring that the pre- and post-critical date claims be identical, nor does the Decision cite any. Further, requiring that Adair identify such a claim improperly shifts the burden on the motion to Adair, even though Carter bears the burden of proof on the motion.

On page 10, lines 5-10, the Decision states that Adair did not admit or deny Carter's Fact 42 alleging that no pre-critical date claim was identical to or includes the same material limitations as involved claim 24. In view thereof, the Decision adopts Carter Fact 42 as its own Finding of Fact 6 (the Decision, p. 4, l. 21 through p. 5, l. 1). The Decision has effectively penalized Adair for stating that it was unable to admit or deny a fact. The Standing Order, however, provides for a party to state that it is unable to admit or deny a fact, and does not state that doing so is effectively an admission of that fact. *See* SO 122.4.2.1. Regardless, as noted above, Carter did not compare any pre-critical date claims other than original claims 8 and 16 to involved claim 24 (*see* Carter Motion 1, Appendix 3), much less identify which claims were or were not identical, or which limitations were material. Under the circumstances, Adair felt the most appropriate response was to say that it was unable to admit or deny the statement, apparently to its detriment.

Finally, on page 10, lines 10-12, the Decision states that Adair could have, but did not seek, authorization to file a motion to add a pre-critical date claim that interferes with the Carter claims. As noted in *Berger*, however, the test whether or not claims interfere is not the proper test under section 135(b). *See Berger*, 279 F.3d at 982. Regardless, Adair would have to certify that it was not aware of any reason why the claim it is adding is not patentable. SO 208.5.1 and

Bd. R. § 41.208 (c). And, as the Decision noted, the original pre-critical date claims were rejected and canceled (the Decision, p. 9, ll. 6-14). Thus, it would clearly have been futile for Adair to attempt to add an original pre-critical date claim. It is not clear what the Decision concluded regarding the non-original pre-critical date claims, other than that it adopted Carter's Fact 42 as a finding of fact because Adair did not outright deny it; there was no other analysis of such claims in the Decision.

Conclusion

The Decision contains several factual and legal issues misapprehended or overlooked by the Board. Adair requests that the Decision regarding Carter Motion 1 be reconsidered and that Carter Motion 1 be denied.

Respectfully submitted,

/Doreen Yatko Trujillo/
DOREEN YATKO TRUJILLO
Registration No. 35,719
Lead Counsel for Adair

Date: October 1, 2010
Cozen O'Connor P.C.
1900 Market St.
Philadelphia, PA 19103
Telephone: (215) 665-5593
Facsimile: (215) 701-2005
dtrujillo@cozen.com

APPENDIX 1

Ex. 2001 -- U.S. Patent No. 6,407,213 to Carter ***et al.,*** issued June 18, 2002.

Ex. 2005 – PCT Application No. PCT/GB90/02017 to Adair *et al.,* filed December 21, 1990, published as WO 91/09967 on July 11, 1991.

APPENDIX 2

Claims from the PCT Application (Ex. 2005)	Adair Claim 24
1. A CDR grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91. 2. A CDR grafted heavy chain according to Claim 1 comprising donor residues at **positions 23, 24, 49, 71, 73 and 78,** or at positions 23, 24 and 49. 16. A CDR grafted antibody heavy or light chain or molecule according to anyone of the preceding claims comprising human acceptor residues and non human donor residues.	Claim 24: A humanised antibody comprising a heavy chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises a non-human amino acid substitution at **a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof,** as numbered according to Kabat.

Paper 84
Filed: 5 November 2010

Mail Stop Interference
P.O. Box 1450
Alexandria Va 22313-1450
Tel: 571-272-4683
Fax: 571-273-0042

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

PAUL J. **CARTER** AND LEONARD G. PRESTIA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR**, DILGEET SINGH ATHWAL, and JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

Patent Interference No. 105,744
(Technology Center 1600)

Before SALLY GARDNER LANE, RICHARD TORCZON, and SALLY MEDLEY, *Administrative Patent Judges*

LANE, *Administrative Patent Judge*

ORDER - DECISION ON ADIAR REQUEST FOR REHEARING

I. STATEMENT OF THE CASE

Adair filed a Request for Rehearing (Paper 83) ("Request") of our Order – Decision on Motions (Paper 80) ("Decision") granting Carter Substantive Motion 1. We considered the Request but do not modify our Decision.

II. ANALYSIS

Adair argues that we inappropriately relied on *Regents of Univ. of Cal. v. Univ. of Iowa Res. Found.,* 455 F.3d 1371 (Fed. Cir. 2006), as the standard for determining whether Adair's involved claim 24 is barred under 35 U.S.C. § 135(b)(1). (Request 2). Adair attempts to distinguish the facts of *Univ. of Cal.* from the facts of the current interference, by noting that in *Univ. of Cal*. the claim in question was copied prior to the *pre*-critical date (and then later amended), while in the current interference the claim was copied only *after* the critical date. (Request 3). According to Adair, *In re Berger*, 279 F.3d 975 (Fed. Cir. 2002), and *Corbett v. Chisholm*, 568 F.2d759 (CCPA 1977) are instructive under the current facts, instead of *Univ. of Cal.*

We disagree. *Univ. of Cal.* expressly denies that there is any difference under 35 U.S.C. § 135(b)(1) between a pre-critical date request for interference (where the copied claim would have been filed before the critical date) and a post-critical date request for interference (where the copied claim would have been filed after the critical date). *See Univ. of Cal.*, 455 F.3d at 1375 ("Section 135(b)(1) does not include any language suggesting that a pre-critical date request for interference makes any difference. Section 135(b)(1) bars any claim having a degree of identity with a claim in an issued patent unless such a claim is filed before the critical date. Thus, title 35 in this section does not demand notice of an impending interference, but instead prohibits

unsupported, post-critical date identity."); *see also id.* at 1374 ("this court does not perceive any legally significant distinctions between this case and [*Berger*]."). Thus, we did not err by relying on *Univ. of Cal.*

According to Adair, the only requirement under § 135(b)(1) is that the limitations of the copied patent claim are present in a pre-critical date claim. (Request 3-4). Both *Univ. of Cal.* and *Berger* explain that

> a copied claim may be entitled to the earlier effective date of prior claims in an application only if the copied claim does not differ from the prior claims in any material limitation. . . . The analysis focuses on the copied claim to determine whether all material limitations of the copied claim necessarily occur in the prior claims.

Berger, 279 F.3d at 982; *see also Univ. of Cal.*, 455 F.3d at 1375 (an applicant "must demonstrate that claims in [the pre-critical date] application provide pre-critical date support for the post-critical date identity between [the involved claim] and the [patentee's patent]. That demonstration necessarily entails a comparison between pre- and post-critical date claims."). We agree with Adair's statement that "the *Berger* test compares the pre-critical date claims and the post-critical date claims, which were copied from the patent, to ensure that all material limitations of the post-critical date claims are present in the pre-critical date claims" (Request 4). However, Adair has not pointed to support in *Berger* for its argument that "[m]ateriality is determined in view of the patent claims being copied" (*id.*). Even if Adair's claims do satisfy such a test for materiality, these claims must also satisfy the separate *Berger* and *University of California* requirements. *Berger* and *Univ. of Cal.* require that Adair's pre-critical date claims include all of the material limitations of its post-critical date claims to fulfill the requirement of 35 U.S.C. § 135(b)(1).

Adair also argues that we erred by not putting the burden on Carter to show that Adair's pre-critical date claims differ materially from its post-critical date claims. (Request 5-6). However, in its Motion (Paper 71), Carter showed that claim 24 (the copied claim) differs materially from those claims relied upon by Adair to meet the requirements of 35 U.S.C. § 135(b)(1), PCT claims 8 and 16 (*see* FF[1] 7, Ex. 2003, Adair's Preliminary Amendment and Request for Interference under 37 C.F.R. § 42.202, p. 5). PCT claims 8 and 16 were directed to a CDR-grafted antibody light chain, while Adair's involved claim 24 is directed to an antibody heavy chain variable domain. (*See* Decision 7-8). Carter's showing was reasonable in view of Adair's reliance on PCT claims 8 and 16. Carter met its burden for relief and shifted the burden to Adair to either show why Carter's showing was insufficient or to direct us to another pre-critical date claim that was materially the same as the copied claim.

Adair argues our Decision was incorrect in stating that a presumption of a material difference was created since Adair's involved claim 24 was added and allowed only after the pre-critical date PCT claims were rejected and cancelled (Request at 6). However, when an applicant adds a limitation to a claim in response to a rejection and the added limitation results in allowance of the claims, the limitation is presumed to be necessary to patentability. *See Corbett, 568 F.2d at 765.; Cf. Festo Corp. v. Shoketsu Kinzoku Kogyo Kabushiki Co. Ltd,* 535 U.S. 722, 734 (2002).

Adair notes, for the first time in the Request, that pre-critical date claim 2 recites all the heavy chain residues of involved claim 24. (Request 6). "Arguments not raised

[1] "FF" indicates the Findings of Fact provided in the Decision, which we incorporate into this Order.

in briefs before the Board and evidence not previously relied upon in the brief and any reply brief(s) are not permitted in the request for rehearing except [as based on recent relevant Board of Federal Circuit decisions]." 37 C.F.R. § 41.52(a)(1). Thus, we decline to consider that pre-critical date claim 2 satisfies the requirements of 35 U.S.C. § 135(b)(1). Even if we were to consider claim 2 at this point, Adair has failed to provide a sufficient comparison to show that claim 2 is materially the same as the copied claim.

In our Decision, we noted that Adair, as an applicant, could have attempted to add an original pre-critical date claim to its application if it believed that such a claim is allowable and would interfere with the Carter claims. (Decision at 10). Adair argues that "it would clearly have been futile for Adair to attempt to add an original pre-critical date claim" because "as the Decision noted, the original pre-critical date claims were rejected and canceled." (Request 8). By not arguing for the patentability of the original pre-critical date claims it relied upon for support under section 135(b)(1), Adair's position is contrary to the policy stated in *Univ. of Cal.* "prevent[ing] a patent applicant from relying on the filing date of a claim to which it is not statutorily entitled." *Univ. of Cal.*, 455 F.3d at 1377.

III. ORDER

Upon consideration of the motions, and for the reasons given, it is

ORDERED that Adair's Request that we modify our Decision is DENIED.

ss/ Sally Gardner Lane
SALLY GARDNER LANE
Administrative Patent Judge

/ss/ Richard Torczon
RICHARD TORCZON
Administrative Patent Judge

/ss/ Sally C. Medley
SALLY C. MEDLEY
Administrative Patent Judge

cc (via electronic transmission):

Counsel for Carter:

Oliver R. Ashe, Jr., Esq.
ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190

Tel: 703-467-9001
Email: oashe@ashepc.com

Jeffrey P. Kushan, Esq.
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, DC 20005

Tel: 202-736-8914
Email: jkushan@sidley.com

Counsel for Adair:

Doreen Yatko Trujillo, Esq.
Michael B. Fein, Esq.
Cozen O'Connor P.C.
1900 Market Street
Philadelphia, PA 19103

Tel: 215-665-5593
Tel: 215-665-4622
Email: dtrujillo@cozen.com
Email: mfein@cozen.com

Paper No. _____

Filed on behalf of: Party Carter

Filed: January 18, 2011

By: Oliver R. Ashe, Jr., Esq.
ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

Jeffrey P. Kushan, Esq.
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, DC 20005
Tel.: (202) 736-8914
Fax: (202) 736-8711
E-mail: jkushan@sidley.com

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

PAUL J. **CARTER** AND LEONARD G. PRESTA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR**, DILJEET SINGH ATHWAL,
AND JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

Patent Interference 105,744 (SGL)
Technology Center 1600

CARTER NOTICE OF FILING OF A NOTICE OF CROSS APPEAL
(Appeal to the Court of Appeal for the Federal Circuit)

CARTER NOTICE OF FILING OF A NOTICE OF CROSS APPEAL

Notice is hereby given to the Director of the United States Patent and Trademark Office and to the Board of Patent Appeals and Interferences that Carter filed a Notice of Cross Appeal to the Court of Appeals for the Federal Circuit relating to the Order - Decision on Motions, Paper No. 80, entered on August 30, 2010, and the Judgment, Paper No. 81, entered on September 2, 2010, in the above-captioned interference. A copy of the Notice of Cross Appeal is attached.

Respectfully submitted,

January 18, 2011

/Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.
Registration No. 40,491
Counsel for Party Carter

ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

CERTIFICATE OF FILING

The undersigned certifies that a copy of the paper entitled **"CARTER NOTICE OF FILING OF A NOTICE OF CROSS APPEAL"** was filed this 18th day of January, 2011, in the following manner:

VIA INTERFERENCE WEB PORTAL:

https://acts.uspto.gov/ifiling/
The Board of Patent Appeals and Interferences
Madison Building East, 9th Floor
600 Dulany Street
Alexandria, VA 22314
Tel: 571-272-4683
Fax: 571-273-0042
E-mail: BoxInterferences@USPTO.GOV

VIA FIRST CLASS MAIL (Postage pre-paid):

The Office of Solicitor
United States Patent and Trademark Office
P.O. Box 15667
Arlington, VA 22215

January 18, 2011 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

CERTIFICATE OF SERVICE

The undersigned hereby certifies that a copy of the paper entitled **"CARTER NOTICE OF FILING OF A NOTICE OF CROSS APPEAL"** was served this 18th day of January, 2011, via Interference Web Portal (https://acts.uspto.gov/ifiling/), on the Attorney of Record for Adair:

Doreen Yatko Trujillo, Esq.
Cozen O'Connor P.C.
1900 Market Street, 7th Floor
Philadelphia, PA 19103
Tel.: 215-665-6593
Fax: 215-701-2005
E-mail: dtrujillo@cozen.com

January 18, 2011 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

United States Court of Appeals for the Federal Circuit

JOHN ROBERT ADAIR, DILJEET SINGH ATHWAL, AND JOHN SPENCER EMTAGE,

Appellants,

v.

NOTICE OF CROSS APPEAL

PAUL J. CARTER AND LEONARD G. PRESTA,

Appellees-Cross Appellants.

PAUL J. CARTER and LEONARD G. PRESTA hereby appeal to the Court under 35 U.S.C. § 141 for review of the following Order and Judgment entered by the Board of Patent Appeals and Interferences ("the Board") in Interference No. 105,744:

- Order - Decision on Motions, Paper No. 80, entered on August 30, 2010 (to the extent the Board denied Carter Substantive Motion 2 for judgment that Adair claim 24 is unpatentable to Adair under 35 U.S.C. § 112, first paragraph, for lack of written description); and
- Judgment - Merits - Bd.R. 127, Paper No. 81, entered on September 2, 2010 (to the extent the Board did not also enter judgment against Adair claim 24 based on the relief requested in Carter Substantive Motion 2).

Copies of the Order and Judgment are enclosed.

A docketing fee in the amount of $450.00 is provided herewith.

Respectfully submitted,

January 18, 2011

Oliver R. Ashe, Jr.
ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190
Tel.: 703-467-9001
Fax: 703-467-9002

Attorney for Appellees-Cross Appellants

CERTIFICATE OF FILING

The undersigned certifies that an original and three copies of the paper entitled "**NOTICE OF CROSS APPEAL**" along with copies of the documents referred therein as being submitted and the docketing fee of $450.00 were filed this 18th day of January, 2011, by Federal Express overnight delivery service, to:

Clerk of Court
United States Court of Appeals for the Federal Circuit
717 Madison Place, N.W.
Washington, D.C. 20439

1-18-11
Date

Oliver R. Ashe, Jr.

CERTIFICATE OF SERVICE

The undersigned hereby certifies that a true and correct copy of the paper entitled "**NOTICE OF CROSS-APPEAL**" and a copy of the documents referred therein as being submitted were served this 18th day of January, 2011, by sending in the following manner:

VIA INTERFERENCE WEB PORTAL(https://acts.uspto.gov/ifiling/):

Doreen Yatko Trujillo, Esq.
Cozen O'Connor P.C.
1900 Market Street, 7th Floor
Philadelphia, PA 19103
Tel.: 215-665-5593
Fax: 215-701-2005
E-mail: dtrujillo@cozen.com

The Board of Patent Appeals and Interferences
Madison Building East, 9th Floor
600 Dulany Street
Alexandria, VA 22314
Tel.: 571-272-9797
Fax: 571-273-0042
E-mail: BoxInterfernces@USPTO.GOV

VIA FIRST CLASS MAIL (Postage pre-paid):

The Office of Solicitor
United States Patent and Trademark Office
P.O. Box 15667
Arlington, VA 22215

1-18-11
Date

Oliver R. Ashe, Jr.

Mail Stop Interference
P.O. Box 1450
Alexandria Va 22313-1450
Tel: 571-272-9797
Fax: 571-273-0042

Paper 80

Filed August 30, 2010

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

PAUL J. **CARTER** AND LEONARD G. PRESTA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR**, DILJEET SINGH ATHWAL, and JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261).

Patent Interference No. 105,744
(Technology Center 1600)

Before SALLY GARDNER LANE, RICHARD TORCZON, and SALLY C. MEDLEY,
Administrative Patent Judges

LANE, *Administrative Patent Judge*

ORDER - DECISION ON MOTIONS

I. STATEMENT OF THE CASE

The interference is before a panel for consideration of non-priority motions filed by Carter. No oral argument was held.

The Interference

Parties

The Interference involves junior party Carter and senior party Adair.

Junior party Carter is involved on the basis of its patent 6,407,213 ("the Carter '213 patent"), which issued 18 June 2002, from application no. 08/146,206, filed 17 November 1993. (Paper 1 at 3.) Claims 30, 31, 60, 62, 63, 66, 67, 70, 73, and 77-81 were designated as corresponding to the Count, while claims 1-29, 32-59, 61, 64, 65 68, 69, 71, 72, 74-76, and 82 were not. (Paper 1 at 4.)

The real party-in-interest of Carter is Genentech, Inc. (Paper 10).

Senior party Adair is involved on the basis of its application 11/284,261 ("Adair '261 application"), filed 21 November 2005. (Paper 1 at 3.) Claim 24, Adair's only pending claim, was designated as corresponding to the Count. (Paper 1 at 4.)

Adair was accorded priority benefit as to the Count of 08/846,658, filed 01 May 1997; 08/303,569, filed 07 September 1994, issued as 5,859,205 on 12 January 1999; 07/743,329, filed on 17 September 1991 ("the Adair '329 application"); PCT/GB90/02017, filed 21 December 1990 ("the Adair PCT application"); and GB 8928874.0, filed 21 December 1989. (Paper 1 at 5.)

The real party-in-interest of Adair is UCB Pharma, S.A. (Paper 4.)

Subject Matter

The parties' claims are drawn to an antibody that has been "humanized," that is, it has a combination of human and non-human regions and specific amino acids. Humanization allows antibodies to be raised, in the laboratory, in non-human animals (for example, mice) against antigens of interest and then changed so that they appear to the patient's body as if they were human antibodies. Humanized antibodies are beneficial because they do not raise dangerous anti-immunoglobulin responses in human patients, as non-human antibodies can. (Carter patent col. 1, l. 52, through col. 3, l. 8.) The humanized antibody of the involved Carter and Adair claims and the Count are antibodies that have a non-human Complementarity Determining Region ("CDR"), that is the region that binds antigen, and specifically recited non-human substitutions in other regions, called the Framework Regions ("FR"), of the antibody.

II. MOTIONS

Carter filed two substantive motions, which assert "threshold" issues that end the interference if the relief requested is granted. Carter Substantive Motion 1 ("Carter Motion 1") requests that Adair claim 24 be found unpatentable under 35 U.S.C. § 135(b)(1). Carter Substantive Motion 2 ("Carter Motion 2") requests that Adair claim 24 be found unpatentable under 35 U.S.C. § 112, first paragraph, for a lack of written description in the specification. As the moving party, Carter has the burden to show that it is entitled to the relief requested in its motions. Bd. R. 208(b).

A. CARTER MOTION 1

Findings of Fact

1. The involved Carter '213 patent issued 18 June 2002. (Carter Ex. 2001; Carter involved '231 patent.)

2. The "critical date," under 35 U.S.C. § 135(b)(1), by which Adair must have filed claims drawn to the same or substantially the same subject matter as the claims of the Carter '213 patent is 18 June 2003.

3. Adair filed the involved Adair '261 application on 21 November 2005, after the critical date. (Ex. 2002, Utility Patent Application Transmittal for Application 11/284,261.)

4. Claim 24, the only claim pending in the Adair '261 application was filed well after the critical date.

5. Claim 24 of the involved Adair '261 application recites:

> A humanised antibody comprising a heavy chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises a non-human amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof, as numbered according to Kabat.

(Paper 5.)

6. None of the claims of the Adair PCT application or the Adair '329 application are identical to claim 24 of the involved Adair '261 application. (Adair response to Carter MF 42; citing Exs. 2005-2010, 2012-2022, 2024-2027, 2029, and 2031-2035; not admitted or denied by Adair (Adair Opposition 1 at 21 ("Adair Opp. 1")), but no claims identical to claim 24 of the involved Adair '261 application identified by

Adair.)

7. In its request for interference, Bd. R. 202, Adair identified claims 8 and 16 of the Adair PCT application as a basis for compliance with 35 USC §135(b).

(Ex. 2003, Adair's Preliminary Amendment and Request for Interference under 37 C.F.R. § 42.202, p. 5.)

8. Claim 8 of the Adair PCT and '261 applications recites:

> A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, 58 and 71.

(Ex. 2005, p. 68 and Ex. 2006, p. 68.)

9. Claim 16 of the Adair PCT and '329 applications recites:

> A CDR-grafted antibody heavy or light chain or molecule according to any one of the preceding claims comprising human acceptor residues and non-human donor residues.

(Ex. 2005, p. 69 and Ex. 2006, p. 69.)

10. Claim 1 of the Adair PCT and '329 applications recites:

> A CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

(Ex. 2005, p. 67 and Ex. 2006, p. 67.)

Analysis

35 U.S.C. § 135(b)(1) states that:

> [a] claim which is the same as, or for the same or substantially the same subject matter as, a claim of an issued patent may not be made in any application unless such a claim is made prior to one year from the date on which the patent was granted.

Claim 24 of Adair's involved application, which corresponds to the Count, was filed more than one year from the date on which Carter's involved patent was issued. Because of the date Adair claim 24 was filed (*see* FF 4), it is, on its face, barred under 35 USC §135(b).

The bar of 35 USC §135(b) might be avoided if Adair had filed a claim that does not differ materially from claim 24. Indeed, in its request for interference, Bd. R. 202, Adair pointed to claims 8 and 16 of its pre-critical date application to support its assertion that claim 24 is not barred under the statute. (FF 7; Ex. 2003, Adair's Preliminary Amendment and Request for Interference under 37 C.F.R. § 42.202, p. 5.)

"To establish entitlement to the earlier effective date of existing claims for purposes of the one-year bar of 35 U.S.C. § 135(b), a party must show that the later filed claim does not differ from an earlier claim in any 'material limitation,'" *In re Berger*, 279 F.3d 975, 981-82 (Fed. Cir. 2002) (quoting *Corbett v. Chisholm*, 568 F.2d 759, 765-66 (CCPA 1977)). *See also Regents of Univ. of Cal. v. Univ. of Iowa Res. Found.*, 455 F.3d 1371, 1375 (Fed. Cir. 2006) ("When a party seeks to add a new claim, or to amend an existing claim, beyond the critical date for section 135(b)(1), [the Federal Circuit] applies the material differences test discussed in opinions like *Berger* to determine if

'such a claim' is barred."). The addition of a limitation for the purpose of making a claim patentable is strong evidence that the limitation is a material one. *See Corbett*, 568 F.2d at 765 (where a party's claim lacked a method step, the court noted that the party did "not seriously contend that this [was] not a material limitation, that [was] necessary to patentability . . . ."); *see also Wetmore v. Miller*, 477 F.2d 960, 964 (CCPA 1973) ("the 'fusible' limitation of appellant's claims must be regarded as not necessary to patentability and not 'material' for present purposes [of complying with 35 U.S.C. § 135(b)]").

Carter argues that the pre-critical date claims of Adair include different material limitations than those in Adair's involved claim 24. (Carter Motion 1 at 3.)

Claim 8 of the Adair PCT application, which is identical to claim 8 of the Adair '329 application, recites:

> A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, 58 and 71.

(FF 8; Ex. 2005, p. 68; Ex. 2006, p. 68.) Claim 16 of the Adair PCT application, which is identical to claim 16 of the Adair '329 application, recites:

> A CDR-grafted antibody heavy or light chain or molecule according to anyone of the preceding claims comprising human acceptor residues and non-human donor residues.

(FF 9; Ex. 2005, p. 69; Ex. 2006, p. 69.) Thus, the claims that Adair relied upon for avoiding the 35 U.S.C. § 135(b) bar are drawn to a CDR-grafted light chain. Adair's involved claim 24, though, is drawn to a "humanized antibody comprising a heavy chain variable domain . . . ." (FF 5, Paper 5.) Involved claim 24 differs from original claims 8

and 16, by reciting a heavy chain variable domain instead of a light chain variable domain.

Adair does not dispute that claims reciting a heavy chain and claims reciting a light chain differ materially. Instead, Adair argues that Carter applied the incorrect standard for assessing whether a post-critical date claim differs materially from an earlier claim. According to Adair, the correct inquiry is whether Adair added or removed claim limitations after the critical date that were necessary to the patentability of *Carter's* claims, not Adair's own pre-critical date claims (Adair Opp. 1 at 6).

We disagree. A party seeking support from pre-critical date claims for interfering claims filed beyond the one-year bar of 35 U.S.C. § 135(b)(1) "must demonstrate that claims in [the pre-critical date] application provide pre-critical date support for the post-critical date identity between [the involved claim] and the [patentee's patent]. That demonstration necessarily entails a comparison between pre- and post-critical date claims." *Regents of Univ. of Cal.*, 455 F.3d at 1375.

Adair also argues, in response to Carter's assertion of the material differences between claims to heavy and light chains, that in addition to its claims drawn to light chains, Adair filed claims drawn to heavy chains before the critical date. Specifically, Adair cites claim 1 of its PCT application as claiming a CDR-grafted antibody heavy chain, and argues that it, together with claim 16, effectively contain all of the limitations of involved claim 66 of the Carter '213 patent. (Adair Opp. 1 at 5; *see* FF 10; Ex. 2005, p. 67; Ex. 2006, p. 67.).[1]

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1 Similarly in its showing under Bd. R. 202, Adair compared its pre-critical date claims to a Carter claim but not the current Adair claim. (Ex. 2003, Adair's Preliminary Amendment and Request for

Adair has not made the correct comparison. Under the guidance provided in *Regents of University of California,* Adair's pre-critical date claims must be compared with its own current claims, not Carter's. Thus we are not persuaded by Adair's argument that it is sufficient that it had on file a claim or claims that effectively contain the limitations of an involved Carter claim.

Even when we consider claims 1 and 16 of the PCT application as they compare to Adair's current claim (and not Carter claim 66 as Adair argues), we are not convinced that Adair had a pre-critical date claim that does not differ materially from its current claim. As Carter notes, (1) claims 1 and 16 of Adair's PCT application were rejected under several statutory grounds in the Adair '329 application, including 35 U.S.C. §§ 101, 112, first and second paragraphs, 102(b), and 103(a), (*see* Ex. 2038, Office Action mailed 18 November 1992), and (2) Adair then cancelled the claims and added new ones that were eventually allowed (Ex. 2007, Amendment of 19 January 1993, p. 2). (*See* Carter Motion 1 at 5-6.)

One example of a material limitation is one that is "necessary to patentability." *See Corbett*, 568 F.2d at 765. When an applicant adds a limitation to a claim in response to a rejection and the added limitation results in allowance of the claim, the limitation is presumed to be necessary to patentability. *Cf. Festo Corp. v. Shoketsu Kinzoku Kogyo Kabushiki Co., Ltd.,* 535 U.S. 722, 734 (2002) (in the context of applying the doctrine of equivalents, "[a] rejection indicates that the patent examiner does not believe the original claim could be patented. While the patentee has the right to appeal, his decision to forgo an appeal and submit an amended claim is taken as a concession

---

Interference under 37 C.F.R. § 42.202, p. 5.)

that the invention as patented does not reach as far as the original claim."); *see Berger*, 279 F.3d at 982 ("Inclusion of a limitation in a claim to avoid the prior art provides strong evidence of the materiality of the included limitation."). Adair does not provide any reason why the limitations that differ between involved claim 24 and original claims 1 and 16 were not necessary to the patentability of claim 24. Nor does Adair point to any other pre-critical date claim that is identical to or includes the same material limitations as its involved claim 24. (FF 6; *see* Carter MF 42, citing Exs. 2005-2010, 2013-2022, 2025-2027, 2029, and 2031-2035; not admitted or denied by Adair (Adair Opp. 1 at 21), but no claims identical to claim 24 of the involved Adair '261 application identified by Adair). We also note that as an applicant Adair could have, but did not, seek authorization to file a motion to add to its application a pre-critical date claim that interferes with the Carter claims (See Papers 23 and 73 (Orders setting times)).

Adair questions how one can provoke an interference if any claim amendments were made during prosecution under the standard stated in *Regents of University of California.* (Adair Opp. 1 at 7.) As explained in that case, "section 135(b)(1) [is] a statute of repose, placing a time limit on a patentee's exposure to an interference proceeding. *Regents Univ. of Cal.*, 455 F.3d at 1376. Despite this statute of repose, a "belated interference", i.e., based on a post-critical date claim, is appropriate in certain instances since "[t]he PTO should declare a valid interference upon receipt of a claim that satisfies section 135(b)(1), and which is otherwise patentable." (*Id.* at 1376). To insure that applicant did indeed timely present a patentable interfering claim, the post-critical date claim in interference must be materially the same as the claim that was timely presented. An applicant cannot expect to avoid the bar of §135(b) by timely

copying a claim from an issued patent when that claim is not patentable to that applicant. As the court noted, it "perceives no inequity in a construction of section 135(b)(1) that might, in some circumstances, prevent a patent applicant from relying on the filing date of a claim to which it was not statutorily entitled." (*Id.* at 1377).

We grant Carter Motion 1 and conclude that Adair involved claim 24 is barred under 35 U.S.C. § 135(b)(1).

B. CARTER MOTION 2

Carter asserts that claim 24 of Adair's involved application is unpatentable under 35 U.S.C. § 112, first paragraph, for lack of written description support.

Findings of Fact

11. Adair's specification provides a "preferred protocol" to determine which residues of a human heavy chain should be substituted for donor residues, as follows

> 2. Heavy Chain
>
> 2.1 Choose donor residues at all of positions 23, 24, 49, 71, 73 and 78 of the heavy chain or all of positions 23, 24 and 49 (71, 73 and 78 are always either all donor or all acceptor).
>
> 2.2 Check that the following have the same amino acid in donor and acceptor sequences, and if not preferably choose the donor: 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

(Ex. 2002, pp. 17-18; MF 13.)

12. Adair's specification includes the following directions regarding substituting residues of a human heavy chain for donor residues:

> "Key residues" near the surface of the heavy chain, are residues 23, 71 and 73, with residues 1, 3, and 76 reported to contribute to a lesser extent. (Ex. 2002, p. 20; MF 16.)

"Key residues" among the "[p]acking residues" near the CDRs as 24, 49, and 78. (Ex. 2002, p. 21; MF 17.)

Example 1 reports that "it is important to retain mouse residues at all of positions 6, 23, 24, 48 and 49, and possibly for maximum binding affinity at 71, 73 and 78." (Ex. 2002, p. 52; MF 19.)

Example 3 reports results wherein the crystal structure of the antibody heavy chain revealed that substitution at position 73 only was found to be important for antigen binding. (Ex. 2002, pp. 57-58; MF 56.)

13. Adair's specification provides the following written description of a CDR-grafted antibody heavy chain with specified donor residues:

> Accordingly, in a first aspect the invention provides a CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

(Ex. 2002 at p. 6.)

14. Adair's specification also provides the following written description of a CDR- grafted antibody heavy chain with specified donor residues:

> In preferred embodiments, the heavy chain framework comprises donor residues at positions 23, 24, 49, 71, 73 and 78 or at positions 23, 24 and 49. The residues at positions 71, 73 and 78 of the heavy chain framework are preferably either all acceptor or all donor residues.

(Ex. 2002 at p. 7.)

15. Adair's specification states:

> A preferred protocol for obtaining CDR-grafted antibody heavy and light chains in accordance with the present invention is set out below together with the rationale by which we have derived this protocol. This protocol. and rationale are given without prejudice to the generality of the invention as hereinbefore described and defined.

(Ex. 2002, p. 16; MF 53.)

Analysis

The test for written description under 35 U.S.C. § 112, first paragraph, "is whether the disclosure of the application relied upon reasonably conveys to those skilled in the art that the inventor had possession of the claimed subject matter as of the filing date." *Ariad Pharm., Inc., v. Eli Lilly & Co.*, 598 F3d 1336, 1351 (Fed. Cir. 2010). This analysis must consider the understandings of those in the art at the time of filing, *see Bilstad v. Wakalopulos*, 386 F.3d 1116, 1125-26 (Fed. Cir. 2004), and must consider the specification as a whole, *see In re Wright*, 866 F.2d 422, 424-25 (Fed. Cir. 1989).

Claim 24 recites a humanized antibody with a heavy chain "compris[ing] a non-human amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78 and combinations thereof . . . ." (FF 5; Paper 5). As Carter asserts, the broadest reasonable interpretation of this language in claim 24 encompasses a human heavy chain with residue substitutions at any number of the six residues recited, for example at only one residue, at all six residues, or at any combination in between. (*See* Carter Motion 2 at 1 and 5-6.)

Specification

In support of its argument that Adair's specification does not provide written description support of *any* of the six residues in claim 24, Carter cites to a "preferred protocol" provided in Adair's specification. Carter asserts that this protocol limits the invention to a human heavy chain framework region with either all of residues 23, 24, and 49, or all of residues 23, 24, 29, 71, 73, and 78, but not any of the residues individually. (Carter Motion 2 at 2 and 8; FF 11; Ex. 2002, Adair Specification, pp. 17-

18.) While this portion of the Adair specification appears to exclude many of combinations of substitutions encompassed by claim 24, other portions of Adair's specification are not so limiting.

For example, elsewhere Adair's specification provides that some "key residues" for making humanized antibodies are 23, 71 and 73, while other "key residues" are 24, 49, and 78. (FF 12; Ex. 2002, pp. 20 and 21; *see* Carter Motion 2 at 3.) Carter does not point to language in this part of the specification that indicates residues 23, 24, and 49 must *all* be substituted together or that 23, 24, 49, 71, 73, and 78 must *all* be substituted together.

In addition, while Carter cites Example 1 as reporting that "it is important to retain mouse residues at all of positions 6, 23, 24, 48 and 49, and possibly for maximum binding affinity at 71, 73 and 78" (FF 12: Ex. 2002, p. 52; *see* Carter Motion 2 at 3), Example 3 reports results wherein the crystal structure of the antibody heavy chain revealed that substitution at position 73 *only* was important for antigen binding. (FF 12; Ex. 2002, pp. 57-58; *see* Adair Opposition 2 at 3-4 ("Adair Opp. 2").) Thus, not all of the examples in Adair's specification support Carter's argument of a requirement for substation of *all* residues 23, 24, and 49 or *all* of residues 23, 24, 49, 71, 73, and 78.

Carter points to the Summary of the Invention section of Adair's application, which provides that human residues of the heavy chain can be substituted for donor residues at "at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91." (Carter Motion 2 at 6; FF 13; Ex. 2002, p. 6.) According to Carter, this language does not provide written description because it is "ambiguous." (Carter Motion 2 at 6-8.) As evidence, Carter points to the rejection

under 35 U.S.C. § 112, second paragraph, of original claim 1 in the Adair '329 application, which contained this language from the Adair specification, and Adair's response canceling claim 1. (Carter Motion 2, MFs 22 and 25; Ex. 2007, p. 29-32; Ex. 2038, p. 6.)

We do not agree that the rejection under the second paragraph of § 112 necessarily shows a lack of written description support under the first paragraph of § 112. Carter's analysis lacks a consideration of the entire Adair specification and instead focuses only upon an isolated portion.

Carter points to another part of the Summary of the Invention, wherein "[i]n preferred embodiments, the heavy chain framework comprises donor residues at positions 23, 24, 49, 71, 73 and 78 or at positions 23, 24 and 49. The residues at positions 71, 73 and 78 of the heavy chain framework are preferably either all acceptor or all donor residues." (FF 14; Ex. 2002 at p. 7; *see* Carter Motion 2 at 8.) Carter characterizes this portion as providing that 71, 73, and 78 "must" be either all acceptor or all donor residues (Carter Motion 2 at 8), but the passage expressly states that positions 71, 73, and 78 are "preferably" all donor or all acceptor. Thus, this portion of Adair's specification is not as limited as Carter asserts.

It does not appear to us that, on its face, the Adair specification contains a requirement for substitution of *all* residues 23, 24, and 49 or *all* of residues 23, 24, 49, 71, 73, and 78. Carter does not direct us to the testimony or other evidence showing what the Adair specification would have conveyed to those skilled in the art at the time of filing such that we might find otherwise. "Argument of counsel cannot take the place of evidence lacking in the record." *Meitzner v. Mindick*, 549 F.2d 775, 782 (CCPA

1977).

Prosecution History

Carter also points to the prosecution of Adair's applications as evidence that claim 24 is not supported by the Adair specification. According to Carter, Adair relied on the "preferred protocol" to distinguish claims of the Adair '329 application over the prior art and to overcome rejections for lack of enablement. (Carter Motion 2 at 9-13). The rejections, amendments, and arguments relied upon by Carter were not directed to involved claim 24 and Carter does not provide a detailed analysis of the claims that were being prosecuted and their relationship to Adair's current claim 24. Thus it is difficult to understand the relevance of the rejection of these claims to involved claim 24. *See Halliburton Energy Servs., Inc. v. M-I LLC*, 514 F.3d 1244, 1250, n.2 (Fed. Cir. 2008) ("Judges are not like pigs, hunting for truffles buried in briefs." (quoting *United States v. Dunkel*, 927 F.2d 955, 956 (7th Cir. 1991)).

In addition, though Carter notes instances when Adair discussed the "preferred protocol" and other rules for determining which residues to substitute, Carter does not point to instances where Adair argues that these are the *only* disclosures in their specification. In fact, other portions of the specification indicate that this "preferred protocol" is not limiting on the invention. (*See* Adair Opp. 2 at 3-4; FFs 15 and 16; Ex. 2002, Adair Specification, pp. 16 and 64.)

Carter has not shown that Adair claim 24 lacks sufficient written description support.

III. ORDER

Upon consideration of the motions, and for the reasons given, it is

ORDERED that Carter Motion 1 for judgment that Adair claim 24 is barred under 35 U.S.C. § 135(b) is GRANTED; and

FURTHER ORDERED that Carter Motion 2 for judgment that Adair claim 24 lacks written description support is DENIED; and

FURTHER ORDERED that judgment will be entered against Adair in a separate paper.

/ss/ Sally Gardner Lane
SALLY GARDNER LANE
Administrative Patent Judge

/ss/ Richard Torczon
RICHARD TORCZON
Administrative Patent Judge

/ss/ Sally C. Medley
SALLY C. MEDLEY
Administrative Patent Judge

Counsel for Carter
Oliver R. Ashe, Jr., Esq.
Ashe, P.C.
11440 Isaac Newton Sq. North, Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

Counsel for Adair
Doreen Yatko Trujillo, Esq.
Cozen O'Connor P.C.
1900 Market St.
Philadelphia, PA 19103
Telephone: (215) 665-5593
Facsimile: (215) 701-2005
E-mail: dtrujillo@cozen.com

Mail Stop Interference
P.O. Box 1450
Alexandria, Va 22313-1450
Tel: 571-272-4683
Fax: 571-273-0042

Paper 81

Filed 2 September 2010

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

PAUL J. **CARTER** AND LEONARD G. PRESTIA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR**, DILGEET SINGH ATHWAL,
and JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

Patent Interference No. 105,744
(Technology Center 1600)

Before SALLY GARDNER LANE, RICHARD TORCZON, and SALLY C. MEDLEY, *Administrative Patent Judges.*

LANE, *Administrative Patent Judge.*

Judgment– Merits – Bd. R. 127

The Carter motion for judgment on the basis that the single involved Adair claim is barred under 35 U.S.C. § 135(b) was granted. (Paper 80). Because Adair no longer has an interfering claim that is not barred under 35 U.S.C. §135(b) it is appropriate to

enter judgment against Adair. *Berman v. Housey*, 291 F.3d 1345, 1351 (Fed. Cir. 2002).

It is

ORDERED that judgment on priority as to Count 1 (Paper 1 at 4), the sole count of the interference, is entered against senior party Adair;

FURTHER ORDERED that claim 24 of Adair application 11/284,261, which claim corresponds to Count 1 (Paper 1 at 4), is FINALLY REFUSED, 35 U.S.C. §135(a):

FURTHER ORDERED that if there is a settlement agreement, the parties are directed to 35 U.S.C. 135(c) and Bd. R. 205; and

FURTHER ORDERED that a copy of this judgment shall be entered into the administrative record of the Carter involved patent and application and the Adair involved application.

cc (via electronic filing):

Attorney for CARTER:

Oliver R. Ashe, Jr., Esq.
ASHE, P.C.
11440 Isaac Newton Square, North
Suite 210
Reston, VA 20190
Tel: 703-467-9001
Email: oashe@ashepc.com

Attorney for ADAIR:
Doreen Yatko Trujillo, Esq.
Michael B. Fein, Esq.
COZEN O'CONNOR P.C.
1900 Market Street
Philadelphia, PA 19103
Tel: 215-665-5593
Email: dtrujillo@cozen.com

Filed on behalf of: **Adair**
By: Doreen Yatko Trujillo
Michael B. Fein
Cozen O'Connor P.C.
1900 Market St.
Philadelphia, PA 19103
Telephone: (215) 665-5593
Facsimile: (215) 701-2005
dtrujillo@cozen.com

Paper No:_____
Filed: January 19, 2011

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

PAUL J. **CARTER** AND LEONARD G. PRESTA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR,** DILJEET SINGH ATHWAL,
AND JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

Patent Interference No. 105,744
(Technology Center 1600)

ADAIR NOTIFICATION OF NOTICE OF APPEAL

In accordance with Bd. R. 8(b) and SO ¶ 8.3, please find enclosed a copy of the Notice of Appeal to the Court of Appeals for the Federal Circuit, and accompanying papers, filed by Adair on January 4, 2011.

Respectfully submitted,

/Doreen Yatko Trujillo/
DOREEN YATKO TRUJILLO
Registration No. 35,719
Lead Counsel for Boss

Date: January 19, 2011

Cozen O'Connor P.C.
1900 Market St.
Philadelphia, PA 19103
Telephone: (215) 665-5593
Facsimile: (215) 701-2005
dtrujillo@cozen.com

Certificate of Service

This will certify that true copies of this paper and accompanying documents were served this date, January 19, 2011, via electronic mail, on the Lead Counsel for Cabilly:

Oliver R. Ashe, Jr.
ASHE, P.C.
11440 Isaac Newton Square North
Suite 210
Reston, VA 20190
Tel.: (703)467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

Date: January 19, 2011

/Doreen Yatko Trujillo/
Doreen Yatko Trujillo

UNITED STATES COURT OF APPEALS FOR THE FEDERAL CIRCUIT

JOHN ROBERT ADAIR, DILJEET SINGH ATHWAL, AND JOHN SPENCER EMTAGE Appellants vs. PAUL J. CARTER AND LEONARD G. PRESTA Appellees	**NOTICE OF APPEAL**

Appellants John Robert Adair, Diljeet Singh Athwal, and John Spencer Emtage hereby appeal to the United States Court of Appeals for the Federal Circuit from the following orders, decisions, and/or judgments rendered by the Board of Patent Appeals and Interferences in Interference No. 105,744: (i) Order -- Decision on Motions, entered August 30, 2010 (Paper No. 80); (ii) Judgment – Merits – Bd. R. 127, entered September 2, 2010 (Paper No. 81); and (iii) Order -- Decision on Adiar [sic] Request for Rehearing, entered November 5, 2010 (Paper No. 84). Copies of each are enclosed.

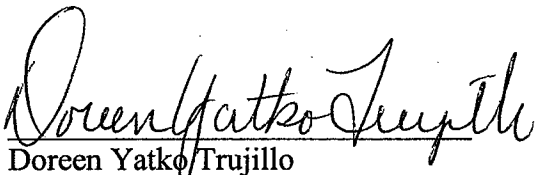
Doreen Yatko Trujillo
Registration No. 35,719
Attorney for the Appellants
Cozen O'Connor P.C.
1900 Market Street
Philadelphia, PA 19103
215-665-5593

Date: January 4, 2011

CERTIFICATE OF SERVICE

Doreen Yatko Trujillo, attorney for appellants, hereby certifies that a true and correct copy of the foregoing **Notice of Appeal, and accompanying papers,** was served this day, January 4, 2011, via Federal Express on the following:

Oliver R. Ashe, Jr.
ASHE, P.C.
1140 Isaac Newton Square North
Suite 210
Reston, VA 20190
Tel.: (703)467-9001
Fax: (703) 467-9002

BY: 
Doreen Yatko Trujillo
Registration No. 35,719

Mail Stop Interference
P.O. Box 1450
Alexandria Va 22313-1450
Tel: 571-272-9797
Fax: 571-273-0042

Filed August 30, 2010

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

PAUL J. **CARTER** AND LEONARD G. PRESTA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR**, DILJEET SINGH ATHWAL, and JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261).

Patent Interference No. 105,744
(Technology Center 1600)

Before SALLY GARDNER LANE, RICHARD TORCZON, and SALLY C. MEDLEY, *Administrative Patent Judges*

LANE, *Administrative Patent Judge*

ORDER - DECISION ON MOTIONS

I. STATEMENT OF THE CASE

The interference is before a panel for consideration of non-priority motions filed by Carter. No oral argument was held.

The Interference

Parties

The Interference involves junior party Carter and senior party Adair.

Junior party Carter is involved on the basis of its patent 6,407,213 ("the Carter '213 patent"), which issued 18 June 2002, from application no. 08/146,206, filed 17 November 1993. (Paper 1 at 3.) Claims 30, 31, 60, 62, 63, 66, 67, 70, 73, and 77-81 were designated as corresponding to the Count, while claims 1-29, 32-59, 61, 64, 65 68, 69, 71, 72, 74-76, and 82 were not. (Paper 1 at 4.)

The real party-in-interest of Carter is Genentech, Inc. (Paper 10).

Senior party Adair is involved on the basis of its application 11/284,261 ("Adair '261 application"), filed 21 November 2005. (Paper 1 at 3.) Claim 24, Adair's only pending claim, was designated as corresponding to the Count. (Paper 1 at 4.)

Adair was accorded priority benefit as to the Count of 08/846,658, filed 01 May 1997; 08/303,569, filed 07 September 1994, issued as 5,859,205 on 12 January 1999; 07/743,329, filed on 17 September 1991 ("the Adair '329 application"); PCT/GB90/02017, filed 21 December 1990 ("the Adair PCT application"); and GB 8928874.0, filed 21 December 1989. (Paper 1 at 5.)

The real party-in-interest of Adair is UCB Pharma, S.A. (Paper 4.)

Subject Matter

The parties' claims are drawn to an antibody that has been "humanized," that is, it has a combination of human and non-human regions and specific amino acids. Humanization allows antibodies to be raised, in the laboratory, in non-human animals (for example, mice) against antigens of interest and then changed so that they appear to the patient's body as if they were human antibodies. Humanized antibodies are beneficial because they do not raise dangerous anti-immunoglobulin responses in human patients, as non-human antibodies can. (Carter patent col. 1, l. 52, through col. 3, l. 8.) The humanized antibody of the involved Carter and Adair claims and the Count are antibodies that have a non-human Complementarity Determining Region ("CDR"), that is the region that binds antigen, and specifically recited non-human substitutions in other regions, called the Framework Regions ("FR"), of the antibody.

II. MOTIONS

Carter filed two substantive motions, which assert "threshold" issues that end the interference if the relief requested is granted. Carter Substantive Motion 1 ("Carter Motion 1") requests that Adair claim 24 be found unpatentable under 35 U.S.C. § 135(b)(1). Carter Substantive Motion 2 ("Carter Motion 2") requests that Adair claim 24 be found unpatentable under 35 U.S.C. § 112, first paragraph, for a lack of written description in the specification. As the moving party, Carter has the burden to show that it is entitled to the relief requested in its motions. Bd. R. 208(b).

A. CARTER MOTION 1

Findings of Fact

1. The involved Carter '213 patent issued 18 June 2002. (Carter Ex. 2001; Carter involved '231 patent.)

2. The "critical date," under 35 U.S.C. § 135(b)(1), by which Adair must have filed claims drawn to the same or substantially the same subject matter as the claims of the Carter '213 patent is 18 June 2003.

3. Adair filed the involved Adair '261 application on 21 November 2005, after the critical date. (Ex. 2002, Utility Patent Application Transmittal for Application 11/284,261.)

4. Claim 24, the only claim pending in the Adair '261 application was filed well after the critical date.

5. Claim 24 of the involved Adair '261 application recites:

> A humanised antibody comprising a heavy chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises a non-human amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof, as numbered according to Kabat.

(Paper 5.)

6. None of the claims of the Adair PCT application or the Adair '329 application are identical to claim 24 of the involved Adair '261 application. (Adair response to Carter MF 42; citing Exs. 2005-2010, 2012-2022, 2024-2027, 2029, and 2031-2035; not admitted or denied by Adair (Adair Opposition 1 at 21 ("Adair Opp. 1")), but no claims identical to claim 24 of the involved Adair '261 application identified by

Adair.)

7. In its request for interference, Bd. R. 202, Adair identified claims 8 and 16 of the Adair PCT application as a basis for compliance with 35 USC §135(b).

(Ex. 2003, Adair's Preliminary Amendment and Request for Interference under 37 C.F.R. § 42.202, p. 5.)

8. Claim 8 of the Adair PCT and '261 applications recites:

> A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, 58 and 71.

(Ex. 2005, p. 68 and Ex. 2006, p. 68.)

9. Claim 16 of the Adair PCT and '329 applications recites:

> A CDR-grafted antibody heavy or light chain or molecule according to any one of the preceding claims comprising human acceptor residues and non-human donor residues.

(Ex. 2005, p. 69 and Ex. 2006, p. 69.)

10. Claim 1 of the Adair PCT and '329 applications recites:

> A CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

(Ex. 2005, p. 67 and Ex. 2006, p. 67.)

Analysis

35 U.S.C. § 135(b)(1) states that:

> [a] claim which is the same as, or for the same or substantially the same subject matter as, a claim of an issued patent may not be made in any application unless such a claim is made prior to one year from the date on which the patent was granted.

Claim 24 of Adair's involved application, which corresponds to the Count, was filed more than one year from the date on which Carter's involved patent was issued. Because of the date Adair claim 24 was filed (*see* FF 4), it is, on its face, barred under 35 USC §135(b).

The bar of 35 USC §135(b) might be avoided if Adair had filed a claim that does not differ materially from claim 24. Indeed, in its request for interference, Bd. R. 202, Adair pointed to claims 8 and 16 of its pre-critical date application to support its assertion that claim 24 is not barred under the statute. (FF 7; Ex. 2003, Adair's Preliminary Amendment and Request for Interference under 37 C.F.R. § 42.202, p. 5.)

"To establish entitlement to the earlier effective date of existing claims for purposes of the one-year bar of 35 U.S.C. § 135(b), a party must show that the later filed claim does not differ from an earlier claim in any 'material limitation,'" *In re Berger*, 279 F.3d 975, 981-82 (Fed. Cir. 2002) (quoting *Corbett v. Chisholm*, 568 F.2d 759, 765-66 (CCPA 1977)). *See also Regents of Univ. of Cal. v. Univ. of Iowa Res. Found.*, 455 F.3d 1371, 1375 (Fed. Cir. 2006) ("When a party seeks to add a new claim, or to amend an existing claim, beyond the critical date for section 135(b)(1), [the Federal Circuit] applies the material differences test discussed in opinions like *Berger* to determine if

'such a claim' is barred."). The addition of a limitation for the purpose of making a claim patentable is strong evidence that the limitation is a material one. *See Corbett*, 568 F.2d at 765 (where a party's claim lacked a method step, the court noted that the party did "not seriously contend that this [was] not a material limitation, that [was] necessary to patentability . . . ."); *see also Wetmore v. Miller*, 477 F.2d 960, 964 (CCPA 1973) ("the 'fusible' limitation of appellant's claims must be regarded as not necessary to patentability and not 'material' for present purposes [of complying with 35 U.S.C. § 135(b)]").

Carter argues that the pre-critical date claims of Adair include different material limitations than those in Adair's involved claim 24. (Carter Motion 1 at 3.)

Claim 8 of the Adair PCT application, which is identical to claim 8 of the Adair '329 application, recites:

> A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, 58 and 71.

(FF 8; Ex. 2005, p. 68; Ex. 2006, p. 68.) Claim 16 of the Adair PCT application, which is identical to claim 16 of the Adair '329 application, recites:

> A CDR-grafted antibody heavy or light chain or molecule according to anyone of the preceding claims comprising human acceptor residues and non-human donor residues.

(FF 9; Ex. 2005, p. 69; Ex. 2006, p. 69.) Thus, the claims that Adair relied upon for avoiding the 35 U.S.C. § 135(b) bar are drawn to a CDR-grafted light chain. Adair's involved claim 24, though, is drawn to a "humanized antibody comprising a heavy chain variable domain . . . ." (FF 5, Paper 5.) Involved claim 24 differs from original claims 8

and 16, by reciting a heavy chain variable domain instead of a light chain variable domain.

Adair does not dispute that claims reciting a heavy chain and claims reciting a light chain differ materially. Instead, Adair argues that Carter applied the incorrect standard for assessing whether a post-critical date claim differs materially from an earlier claim. According to Adair, the correct inquiry is whether Adair added or removed claim limitations after the critical date that were necessary to the patentability of *Carter's* claims, not Adair's own pre-critical date claims (Adair Opp. 1 at 6).

We disagree. A party seeking support from pre-critical date claims for interfering claims filed beyond the one-year bar of 35 U.S.C. § 135(b)(1) "must demonstrate that claims in [the pre-critical date] application provide pre-critical date support for the post-critical date identity between [the involved claim] and the [patentee's patent]. That demonstration necessarily entails a comparison between pre- and post-critical date claims." *Regents of Univ. of Cal.*, 455 F.3d at 1375.

Adair also argues, in response to Carter's assertion of the material differences between claims to heavy and light chains, that in addition to its claims drawn to light chains, Adair filed claims drawn to heavy chains before the critical date. Specifically, Adair cites claim 1 of its PCT application as claiming a CDR-grafted antibody heavy chain, and argues that it, together with claim 16, effectively contain all of the limitations of involved claim 66 of the Carter '213 patent. (Adair Opp. 1 at 5; *see* FF 10; Ex. 2005, p. 67; Ex. 2006, p. 67.).[1]

---

1 Similarly in its showing under Bd. R. 202, Adair compared its pre-critical date claims to a Carter claim but not the current Adair claim. (Ex. 2003, Adair's Preliminary Amendment and Request for

Adair has not made the correct comparison. Under the guidance provided in *Regents of University of California,* Adair's pre-critical date claims must be compared with its own current claims, not Carter's. Thus we are not persuaded by Adair's argument that it is sufficient that it had on file a claim or claims that effectively contain the limitations of an involved Carter claim.

Even when we consider claims 1 and 16 of the PCT application as they compare to Adair's current claim (and not Carter claim 66 as Adair argues), we are not convinced that Adair had a pre-critical date claim that does not differ materially from its current claim. As Carter notes, (1) claims 1 and 16 of Adair's PCT application were rejected under several statutory grounds in the Adair '329 application, including 35 U.S.C. §§ 101, 112, first and second paragraphs, 102(b), and 103(a), (*see* Ex. 2038, Office Action mailed 18 November 1992), and (2) Adair then cancelled the claims and added new ones that were eventually allowed (Ex. 2007, Amendment of 19 January 1993, p. 2). (*See* Carter Motion 1 at 5-6.)

One example of a material limitation is one that is "necessary to patentability." *See Corbett*, 568 F.2d at 765. When an applicant adds a limitation to a claim in response to a rejection and the added limitation results in allowance of the claim, the limitation is presumed to be necessary to patentability. *Cf. Festo Corp. v. Shoketsu Kinzoku Kogyo Kabushiki Co., Ltd.,* 535 U.S. 722, 734 (2002) (in the context of applying the doctrine of equivalents, "[a] rejection indicates that the patent examiner does not believe the original claim could be patented. While the patentee has the right to appeal, his decision to forgo an appeal and submit an amended claim is taken as a concession

---

Interference under 37 C.F.R. § 42.202, p. 5.)

that the invention as patented does not reach as far as the original claim."); *see Berger*, 279 F.3d at 982 ("Inclusion of a limitation in a claim to avoid the prior art provides strong evidence of the materiality of the included limitation."). Adair does not provide any reason why the limitations that differ between involved claim 24 and original claims 1 and 16 were not necessary to the patentability of claim 24. Nor does Adair point to any other pre-critical date claim that is identical to or includes the same material limitations as its involved claim 24. (FF 6; *see* Carter MF 42, citing Exs. 2005-2010, 2013-2022, 2025-2027, 2029, and 2031-2035; not admitted or denied by Adair (Adair Opp. 1 at 21), but no claims identical to claim 24 of the involved Adair '261 application identified by Adair). We also note that as an applicant Adair could have, but did not, seek authorization to file a motion to add to its application a pre-critical date claim that interferes with the Carter claims (See Papers 23 and 73 (Orders setting times)).

Adair questions how one can provoke an interference if any claim amendments were made during prosecution under the standard stated in *Regents of University of California.* (Adair Opp. 1 at 7.) As explained in that case, "section 135(b)(1) [is] a statute of repose, placing a time limit on a patentee's exposure to an interference proceeding. *Regents Univ. of Cal.*, 455 F.3d at 1376. Despite this statute of repose, a "belated interference", i.e., based on a post-critical date claim, is appropriate in certain instances since "[t]he PTO should declare a valid interference upon receipt of a claim that satisfies section 135(b)(1), and which is otherwise patentable." (*Id.* at 1376). To insure that applicant did indeed timely present a patentable interfering claim, the post-critical date claim in interference must be materially the same as the claim that was timely presented. An applicant cannot expect to avoid the bar of §135(b) by timely

copying a claim from an issued patent when that claim is not patentable to that applicant. As the court noted, it "perceives no inequity in a construction of section 135(b)(1) that might, in some circumstances, prevent a patent applicant from relying on the filing date of a claim to which it was not statutorily entitled." (*Id.* at 1377).

We grant Carter Motion 1 and conclude that Adair involved claim 24 is barred under 35 U.S.C. § 135(b)(1).

B. CARTER MOTION 2

Carter asserts that claim 24 of Adair's involved application is unpatentable under 35 U.S.C. § 112, first paragraph, for lack of written description support.

Findings of Fact

11. Adair's specification provides a "preferred protocol" to determine which residues of a human heavy chain should be substituted for donor residues, as follows

> 2. Heavy Chain
>
> 2.1 Choose donor residues at all of positions 23, 24, 49, 71, 73 and 78 of the heavy chain or all of positions 23, 24 and 49 (71, 73 and 78 are always either all donor or all acceptor).
>
> 2.2 Check that the following have the same amino acid in donor and acceptor sequences, and if not preferably choose the donor: 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

(Ex. 2002, pp. 17-18; MF 13.)

12. Adair's specification includes the following directions regarding substituting residues of a human heavy chain for donor residues:

> "Key residues" near the surface of the heavy chain, are residues 23, 71 and 73, with residues 1, 3, and 76 reported to contribute to a lesser extent. (Ex. 2002, p. 20; MF 16.)

"Key residues" among the "[p]acking residues" near the CDRs as 24, 49, and 78. (Ex. 2002, p. 21; MF 17.)

Example 1 reports that "it is important to retain mouse residues at all of positions 6, 23, 24, 48 and 49, and possibly for maximum binding affinity at 71, 73 and 78." (Ex. 2002, p. 52; MF 19.)

Example 3 reports results wherein the crystal structure of the antibody heavy chain revealed that substitution at position 73 only was found to be important for antigen binding. (Ex. 2002, pp. 57-58; MF 56.)

13. Adair's specification provides the following written description of a CDR-grafted antibody heavy chain with specified donor residues:

> Accordingly, in a first aspect the invention provides a CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

(Ex. 2002 at p. 6.)

14. Adair's specification also provides the following written description of a CDR- grafted antibody heavy chain with specified donor residues:

> In preferred embodiments, the heavy chain framework comprises donor residues at positions 23, 24, 49, 71, 73 and 78 or at positions 23, 24 and 49. The residues at positions 71, 73 and 78 of the heavy chain framework are preferably either all acceptor or all donor residues.

(Ex. 2002 at p. 7.)

15. Adair's specification states:

> A preferred protocol for obtaining CDR-grafted antibody heavy and light chains in accordance with the present invention is set out below together with the rationale by which we have derived this protocol. This protocol. and rationale are given without prejudice to the generality of the invention as hereinbefore described and defined.

(Ex. 2002, p. 16; MF 53.)

Analysis

The test for written description under 35 U.S.C. § 112, first paragraph, "is whether the disclosure of the application relied upon reasonably conveys to those skilled in the art that the inventor had possession of the claimed subject matter as of the filing date." *Ariad Pharm., Inc., v. Eli Lilly & Co.*, 598 F3d 1336, 1351 (Fed. Cir. 2010). This analysis must consider the understandings of those in the art at the time of filing, *see Bilstad v. Wakalopulos*, 386 F.3d 1116, 1125-26 (Fed. Cir. 2004), and must consider the specification as a whole, *see In re Wright,* 866 F.2d 422, 424-25 (Fed. Cir. 1989).

Claim 24 recites a humanized antibody with a heavy chain "compris[ing] a non-human amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78 and combinations thereof . . . ." (FF 5; Paper 5). As Carter asserts, the broadest reasonable interpretation of this language in claim 24 encompasses a human heavy chain with residue substitutions at any number of the six residues recited, for example at only one residue, at all six residues, or at any combination in between. (*See* Carter Motion 2 at 1 and 5-6.)

Specification

In support of its argument that Adair's specification does not provide written description support of *any* of the six residues in claim 24, Carter cites to a "preferred protocol" provided in Adair's specification. Carter asserts that this protocol limits the invention to a human heavy chain framework region with either all of residues 23, 24, and 49, or all of residues 23, 24, 29, 71, 73, and 78, but not any of the residues individually. (Carter Motion 2 at 2 and 8; FF 11; Ex. 2002, Adair Specification, pp. 17-

18.) While this portion of the Adair specification appears to exclude many of combinations of substitutions encompassed by claim 24, other portions of Adair's specification are not so limiting.

For example, elsewhere Adair's specification provides that some "key residues" for making humanized antibodies are 23, 71 and 73, while other "key residues" are 24, 49, and 78. (FF 12; Ex. 2002, pp. 20 and 21; *see* Carter Motion 2 at 3.) Carter does not point to language in this part of the specification that indicates residues 23, 24, and 49 must *all* be substituted together or that 23, 24, 49, 71, 73, and 78 must *all* be substituted together.

In addition, while Carter cites Example 1 as reporting that "it is important to retain mouse residues at all of positions 6, 23, 24, 48 and 49, and possibly for maximum binding affinity at 71, 73 and 78" (FF 12: Ex. 2002, p. 52; *see* Carter Motion 2 at 3), Example 3 reports results wherein the crystal structure of the antibody heavy chain revealed that substitution at position 73 *only* was important for antigen binding. (FF 12; Ex. 2002, pp. 57-58; *see* Adair Opposition 2 at 3-4 ("Adair Opp. 2").) Thus, not all of the examples in Adair's specification support Carter's argument of a requirement for substation of *all* residues 23, 24, and 49 or *all* of residues 23, 24, 49, 71, 73, and 78.

Carter points to the Summary of the Invention section of Adair's application, which provides that human residues of the heavy chain can be substituted for donor residues at "at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91." (Carter Motion 2 at 6; FF 13; Ex. 2002, p. 6.) According to Carter, this language does not provide written description because it is "ambiguous." (Carter Motion 2 at 6-8.) As evidence, Carter points to the rejection

under 35 U.S.C. § 112, second paragraph, of original claim 1 in the Adair '329 application, which contained this language from the Adair specification, and Adair's response canceling claim 1. (Carter Motion 2, MFs 22 and 25; Ex. 2007, p. 29-32; Ex. 2038, p. 6.)

We do not agree that the rejection under the second paragraph of § 112 necessarily shows a lack of written description support under the first paragraph of § 112. Carter's analysis lacks a consideration of the entire Adair specification and instead focuses only upon an isolated portion.

Carter points to another part of the Summary of the Invention, wherein "[i]n preferred embodiments, the heavy chain framework comprises donor residues at positions 23, 24, 49, 71, 73 and 78 or at positions 23, 24 and 49. The residues at positions 71, 73 and 78 of the heavy chain framework are preferably either all acceptor or all donor residues." (FF 14; Ex. 2002 at p. 7; *see* Carter Motion 2 at 8.) Carter characterizes this portion as providing that 71, 73, and 78 "must" be either all acceptor or all donor residues (Carter Motion 2 at 8), but the passage expressly states that positions 71, 73, and 78 are "preferably" all donor or all acceptor. Thus, this portion of Adair's specification is not as limited as Carter asserts.

It does not appear to us that, on its face, the Adair specification contains a requirement for substitution of *all* residues 23, 24, and 49 or *all* of residues 23, 24, 49, 71, 73, and 78. Carter does not direct us to the testimony or other evidence showing what the Adair specification would have conveyed to those skilled in the art at the time of filing such that we might find otherwise. "Argument of counsel cannot take the place of evidence lacking in the record." *Meitzner v. Mindick*, 549 F.2d 775, 782 (CCPA

1977).

Prosecution History

Carter also points to the prosecution of Adair's applications as evidence that claim 24 is not supported by the Adair specification. According to Carter, Adair relied on the "preferred protocol" to distinguish claims of the Adair '329 application over the prior art and to overcome rejections for lack of enablement. (Carter Motion 2 at 9-13). The rejections, amendments, and arguments relied upon by Carter were not directed to involved claim 24 and Carter does not provide a detailed analysis of the claims that were being prosecuted and their relationship to Adair's current claim 24. Thus it is difficult to understand the relevance of the rejection of these claims to involved claim 24. *See Halliburton Energy Servs., Inc. v. M-I LLC*, 514 F.3d 1244, 1250, n.2 (Fed. Cir. 2008) ("Judges are not like pigs, hunting for truffles buried in briefs." (quoting *United States v. Dunkel*, 927 F.2d 955, 956 (7th Cir. 1991)).

In addition, though Carter notes instances when Adair discussed the "preferred protocol" and other rules for determining which residues to substitute, Carter does not point to instances where Adair argues that these are the *only* disclosures in their specification. In fact, other portions of the specification indicate that this "preferred protocol" is not limiting on the invention. (*See* Adair Opp. 2 at 3-4; FFs 15 and 16; Ex. 2002, Adair Specification, pp. 16 and 64.)

Carter has not shown that Adair claim 24 lacks sufficient written description support.

III. ORDER

Upon consideration of the motions, and for the reasons given, it is

ORDERED that Carter Motion 1 for judgment that Adair claim 24 is barred under 35 U.S.C. § 135(b) is GRANTED; and

FURTHER ORDERED that Carter Motion 2 for judgment that Adair claim 24 lacks written description support is DENIED; and

FURTHER ORDERED that judgment will be entered against Adair in a separate paper.

/ss/ Sally Gardner Lane
SALLY GARDNER LANE
Administrative Patent Judge

/ss/ Richard Torczon
RICHARD TORCZON
Administrative Patent Judge

/ss/ Sally C. Medley
SALLY C. MEDLEY
Administrative Patent Judge

Counsel for Carter
Oliver R. Ashe, Jr., Esq.
Ashe, P.C.
11440 Isaac Newton Sq. North, Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

Counsel for Adair
Doreen Yatko Trujillo, Esq.
Cozen O'Connor P.C.
1900 Market St.
Philadelphia, PA 19103
Telephone: (215) 665-5593
Facsimile: (215) 701-2005
E-mail: dtrujillo@cozen.com

Mail Stop Interference
P.O. Box 1450
Alexandria, Va 22313-1450
Tel: 571-272-4683
Fax: 571-273-0042

Paper 81

Filed 2 September 2010

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

PAUL J. **CARTER** AND LEONARD G. PRESTIA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR**, DILGEET SINGH ATHWAL,
and JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

Patent Interference No. 105,744
(Technology Center 1600)

Before SALLY GARDNER LANE, RICHARD TORCZON, and SALLY C. MEDLEY, *Administrative Patent Judges.*

LANE, *Administrative Patent Judge.*

Judgment– Merits – Bd. R. 127

The Carter motion for judgment on the basis that the single involved Adair claim is barred under 35 U.S.C. § 135(b) was granted. (Paper 80). Because Adair no longer has an interfering claim that is not barred under 35 U.S.C. §135(b) it is appropriate to

enter judgment against Adair. *Berman v. Housey*, 291 F.3d 1345, 1351 (Fed. Cir. 2002).

It is

ORDERED that judgment on priority as to Count 1 (Paper 1 at 4), the sole count of the interference, is entered against senior party Adair;

FURTHER ORDERED that claim 24 of Adair application 11/284,261, which claim corresponds to Count 1 (Paper 1 at 4), is FINALLY REFUSED, 35 U.S.C. §135(a):

FURTHER ORDERED that if there is a settlement agreement, the parties are directed to 35 U.S.C. 135(c) and Bd. R. 205; and

FURTHER ORDERED that a copy of this judgment shall be entered into the administrative record of the Carter involved patent and application and the Adair involved application.

cc (via electronic filing):

Attorney for CARTER:

Oliver R. Ashe, Jr., Esq.
ASHE, P.C.
11440 Isaac Newton Square, North
Suite 210
Reston, VA 20190
Tel: 703-467-9001
Email: oashe@ashepc.com

Attorney for ADAIR:
Doreen Yatko Trujillo, Esq.
Michael B. Fein, Esq.
COZEN O'CONNOR P.C.
1900 Market Street
Philadelphia, PA 19103
Tel: 215-665-5593
Email: dtrujillo@cozen.com

Mail Stop Interference
P.O. Box 1450
Alexandria Va 22313-1450
Tel: 571-272-4683
Fax: 571-273-0042

Paper 84
Filed: 5 November 2010

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

PAUL J. **CARTER** AND LEONARD G. PRESTIA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR**, DILGEET SINGH ATHWAL, and JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

Patent Interference No. 105,744
(Technology Center 1600)

Before SALLY GARDNER LANE, RICHARD TORCZON, and SALLY MEDLEY,
Administrative Patent Judges

LANE, *Administrative Patent Judge*

ORDER - DECISION ON ADIAR REQUEST FOR REHEARING

I. STATEMENT OF THE CASE

Adair filed a Request for Rehearing (Paper 83) ("Request") of our Order – Decision on Motions (Paper 80) ("Decision") granting Carter Substantive Motion 1. We considered the Request but do not modify our Decision.

II. ANALYSIS

Adair argues that we inappropriately relied on *Regents of Univ. of Cal. v. Univ. of Iowa Res. Found.*, 455 F.3d 1371 (Fed. Cir. 2006), as the standard for determining whether Adair's involved claim 24 is barred under 35 U.S.C. § 135(b)(1). (Request 2). Adair attempts to distinguish the facts of *Univ. of Cal.* from the facts of the current interference, by noting that in *Univ. of Cal.* the claim in question was copied prior to the *pre*-critical date (and then later amended), while in the current interference the claim was copied only *after* the critical date. (Request 3). According to Adair, *In re Berger*, 279 F.3d 975 (Fed. Cir. 2002), and *Corbett v. Chisholm*, 568 F.2d759 (CCPA 1977) are instructive under the current facts, instead of *Univ. of Cal.*

We disagree. *Univ. of Cal.* expressly denies that there is any difference under 35 U.S.C. § 135(b)(1) between a pre-critical date request for interference (where the copied claim would have been filed before the critical date) and a post-critical date request for interference (where the copied claim would have been filed after the critical date). *See Univ. of Cal.*, 455 F.3d at 1375 ("Section 135(b)(1) does not include any language suggesting that a pre-critical date request for interference makes any difference. Section 135(b)(1) bars any claim having a degree of identity with a claim in an issued patent unless such a claim is filed before the critical date. Thus, title 35 in this section does not demand notice of an impending interference, but instead prohibits

unsupported, post-critical date identity."); *see also id.* at 1374 ("this court does not perceive any legally significant distinctions between this case and [*Berger*]."). Thus, we did not err by relying on *Univ. of Cal.*

According to Adair, the only requirement under § 135(b)(1) is that the limitations of the copied patent claim are present in a pre-critical date claim. (Request 3-4). Both *Univ. of Cal.* and *Berger* explain that

> a copied claim may be entitled to the earlier effective date of prior claims in an application only if the copied claim does not differ from the prior claims in any material limitation. . . . The analysis focuses on the copied claim to determine whether all material limitations of the copied claim necessarily occur in the prior claims.

Berger, 279 F.3d at 982; *see also Univ. of Cal.*, 455 F.3d at 1375 (an applicant "must demonstrate that claims in [the pre-critical date] application provide pre-critical date support for the post-critical date identity between [the involved claim] and the [patentee's patent]. That demonstration necessarily entails a comparison between pre- and post-critical date claims."). We agree with Adair's statement that "the *Berger* test compares the pre-critical date claims and the post-critical date claims, which were copied from the patent, to ensure that all material limitations of the post-critical date claims are present in the pre-critical date claims" (Request 4). However, Adair has not pointed to support in *Berger* for its argument that "[m]ateriality is determined in view of the patent claims being copied" (*id.*). Even if Adair's claims do satisfy such a test for materiality, these claims must also satisfy the separate *Berger* and *University of California* requirements. *Berger* and *Univ. of Cal.* require that Adair's pre-critical date claims include all of the material limitations of its post-critical date claims to fulfill the requirement of 35 U.S.C. § 135(b)(1).

Adair also argues that we erred by not putting the burden on Carter to show that Adair's pre-critical date claims differ materially from its post-critical date claims. (Request 5-6). However, in its Motion (Paper 71), Carter showed that claim 24 (the copied claim) differs materially from those claims relied upon by Adair to meet the requirements of 35 U.S.C. § 135(b)(1), PCT claims 8 and 16 (*see* FF[1] 7, Ex. 2003, Adair's Preliminary Amendment and Request for Interference under 37 C.F.R. § 42.202, p. 5). PCT claims 8 and 16 were directed to a CDR-grafted antibody light chain, while Adair's involved claim 24 is directed to an antibody heavy chain variable domain. (*See* Decision 7-8). Carter's showing was reasonable in view of Adair's reliance on PCT claims 8 and 16. Carter met its burden for relief and shifted the burden to Adair to either show why Carter's showing was insufficient or to direct us to another pre-critical date claim that was materially the same as the copied claim.

Adair argues our Decision was incorrect in stating that a presumption of a material difference was created since Adair's involved claim 24 was added and allowed only after the pre-critical date PCT claims were rejected and cancelled (Request at 6). However, when an applicant adds a limitation to a claim in response to a rejection and the added limitation results in allowance of the claims, the limitation is presumed to be necessary to patentability. *See Corbett, 568 F.2d at 765.; Cf. Festo Corp. v. Shoketsu Kinzoku Kogyo Kabushiki Co. Ltd,* 535 U.S. 722, 734 (2002).

Adair notes, for the first time in the Request, that pre-critical date claim 2 recites all the heavy chain residues of involved claim 24. (Request 6). "Arguments not raised

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[1] "FF" indicates the Findings of Fact provided in the Decision, which we incorporate into this Order.

in briefs before the Board and evidence not previously relied upon in the brief and any reply brief(s) are not permitted in the request for rehearing except [as based on recent relevant Board of Federal Circuit decisions]." 37 C.F.R. § 41.52(a)(1). Thus, we decline to consider that pre-critical date claim 2 satisfies the requirements of 35 U.S.C. § 135(b)(1). Even if we were to consider claim 2 at this point, Adair has failed to provide a sufficient comparison to show that claim 2 is materially the same as the copied claim.

In our Decision, we noted that Adair, as an applicant, could have attempted to add an original pre-critical date claim to its application if it believed that such a claim is allowable and would interfere with the Carter claims. (Decision at 10). Adair argues that "it would clearly have been futile for Adair to attempt to add an original pre-critical date claim" because "as the Decision noted, the original pre-critical date claims were rejected and canceled." (Request 8). By not arguing for the patentability of the original pre-critical date claims it relied upon for support under section 135(b)(1), Adair's position is contrary to the policy stated in *Univ. of Cal.* "prevent[ing] a patent applicant from relying on the filing date of a claim to which it is not statutorily entitled." *Univ. of Cal.*, 455 F.3d at 1377.

III. ORDER

Upon consideration of the motions, and for the reasons given, it is

ORDERED that Adair's Request that we modify our Decision is DENIED.

ss/ Sally Gardner Lane
SALLY GARDNER LANE
Administrative Patent Judge

/ss/ Richard Torczon
RICHARD TORCZON
Administrative Patent Judge

/ss/ Sally C. Medley
SALLY C. MEDLEY
Administrative Patent Judge

cc (via electronic transmission):

Counsel for Carter:

Oliver R. Ashe, Jr., Esq.
ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190

Tel: 703-467-9001
Email: oashe@ashepc.com

Jeffrey P. Kushan, Esq.
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, DC 20005

Tel: 202-736-8914
Email: jkushan@sidley.com

Counsel for Adair:

Doreen Yatko Trujillo, Esq.
Michael B. Fein, Esq.
Cozen O'Connor P.C.
1900 Market Street
Philadelphia, PA 19103

Tel: 215-665-5593
Tel: 215-665-4622
Email: dtrujillo@cozen.com
Email: mfein@cozen.com

Appeal Nos. 2011-1212, -1213
(Interference No. 105,744)

United States Court of Appeals
for the
Federal Circuit

JOHN ROBERT ADAIR, DILJEET SINGH ATHWAL
and JOHN SPENCER EMTAGE,

Appellants,

– v. –

PAUL J. CARTER and LEONARD G. PRESTA,

Cross Appellants.

APPEAL FROM THE UNITED STATES PATENT AND TRADEMARK OFFICE,
BOARD OF PATENT APPEALS AND INTERFERENCES

BRIEF OF THE APPELLANTS JOHN ROBERT ADAIR, DILJEET SINGH ATHWAL AND JOHN SPENCER EMTAGE

DOREEN YATKO TRUJILLO
Counsel of Record
KYLE VOS STRACHE
COZEN O'CONNOR, P.C.
1900 Market Street
Philadelphia, Pennsylvania 19103
(215) 665-2000

Attorneys for Appellants John Robert Adair, Diljeet Singh Athwal and John Spencer Emtage

May 13, 2011

CERTIFICATE OF INTEREST

Counsel for the (petitioner) (appellant) (respondent) (appellee) (amicus) (name of party) APPELLANT ADAIR certifies the following (use "None" if applicable; use extra sheets if necessary):

1. The full name of every party or amicus represented by me is:

John Robert Adair, Diljeet Singh Athwal, and John Spencer Emtage

2. The name of the real party in interest (if the party named in the caption is not the real party in interest) represented by me is:

UCB Pharma S.A.

3. All parent corporations and any publicly held companies that own 10 percent or more of the stock of the party or amicus curiae represented by me are:

UCB Pharma S.A. is wholly-owned by UCB S.A.
Financiere de Tubize S.A. is a publicly owned company that owns more than 10% of the stock of UCB S.A.

4. The names of all law firms and the partners or associates that appeared for the party or amicus now represented by me in the trial court or agency or are expected to appear in this court are:

Cozen O'Connor P.C. – Doreen Yatko Trujillo, Michael B. Fein, Kyle Vos Strache

May 13, 2011
Date: May 13, 2011

Doreen Yatko Trujillo
Signature of counsel

Doreen Yatko Trujillo
Printed name of counsel

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STATEMENT OF RELATED CASES

No other appeal from the same interference was previously before this or any other appellate court. Another appeal of a final judgment of the Board of Patent Appeals and Interferences ("Board") in Interference 105,762 is before this Court. The Notice of Appeal was filed April 1, 2011. No other case is known to counsel to be pending in this or any other court that will directly affect or be directly affected by this Court's decision in the pending appeal.

STATEMENT OF JURISDICTION

1. The statutory basis for jurisdiction of the Board for application to patent interferences is 35 U.S.C. § 135(a).

2. The statutory bases for jurisdiction of this Court to hear the appeal of a decision of the Board in an interference are 28 U.S.C. § 1295(a)(4)(A) and 35 U.S.C. § 141.

3. This appeal is from a final judgment of the Board dated September 2, 2010 (A19-21), which was affirmed in the "Order -- Decision on Adiar [sic] Request for Rehearing," dated November 5, 2010 (A22-28).

4. The appeal is timely, as the Notice of Appeal was filed by Express Mail on January 4, 2011 with the United States Patent & Trademark Office ("USPTO"). The USPTO confirmed timely filing with the submission of the Certified Index on February 14, 2011, and the case was docketed at this Court on February 15, 2011. *See,* 35 U.S.C. § 142.

STATEMENT OF THE ISSUE

Whether the Board erred as a matter of law in finding that Adair's single claim involved in Interference 105,744 was barred under 35 U.S.C. § 135(b)(1). In reaching this finding, the Board:

a) required that claims filed before the critical date ("pre-critical date claims") that are relied upon to support claims filed after the critical date ("post-critical date claims") for purposes of s*ection 135(b)(1)* be shown to be patentable;

b) created a material differences test between pre- and post-critical date claims without any reference to the patent claims being copied;

c) created a presumption of material differences when pre-critical date claims have been amended or canceled; and

d) improperly shifted the burden of production to Adair.

STATEMENT OF THE CASE

This is an appeal of a final judgment of the Board in an interference between Adair and Carter awarding judgment on priority of Count 1 ("Count"), the sole count in the interference, to Carter (A19-21). Carter is in the interference based upon U.S. Patent No. 6,407,213, filed November 17, 1993 and issued June 18, 2002 ("the Carter patent") (A97). Adair is in the interference based upon U.S. Application Serial No. 11/284,261, filed November 21, 2005 ("the Adair

application") (A97). The Board decided that Adair's only claim in interference was barred under 35 U.S.C. § 135(b)(1) (A11) and entered judgment against Adair on September 2, 2010 (A19-21). Adair requested rehearing of the Board's decision on October 1, 2010 (A426-35). The Board denied Adair's request on November 5, 2010 (A22-27).

STATEMENT OF THE FACTS

Count 1, the sole count of the interference, is reproduced below:

> A humanized antibody heavy chain variable domain comprising non-human Complementarity Determining Region (CDR) amino acid residues which bind antigen incorporated into a human antibody variable domain, and further comprising a Framework Region (FR) amino acid substitution at a site selected from the group consisting of: 24H, 71H, 73H, and 78H, utilizing the numbering system set forth in Kabat.

(A98). As the Board observed, the invention of the Count is drawn to humanized antibodies, that is, antibodies that are a combination of human and non-human regions (A3). More specifically, the invention of the Count is drawn to the variable domain of the heavy chain of humanized antibodies. Naturally occurring antibodies comprise two heavy chains and two light chains, each of which has a variable domain that is involved in binding the antibody to antigen (A49). Antibodies of non-human origin are naturally antigenic in humans when used in therapy and can give rise to an undesirable anti-antibody response (A561). Humanization techniques, typically involving the use of recombinant DNA

technology, were developed to make non-human antibodies less antigenic (A561-62). The humanized antibodies of the Carter claims, the Adair claim, and the Count have non-human Complementarity Determining Regions (CDR) and human Framework Regions (FR), with a specifically recited non-human substitution in the FR, i.e., at one of residues 24, 71, 73, or 78 in the amino acid sequence using the numbering system according to Kabat (A3; A98). Such antibodies are also known as CDR-grafted antibodies (A562-65).

A. Factual Background

The Patent Statute requires that claims that are to substantially the same invention as claims in an issued patent be made prior to one year from the date on which the patent was granted.

> A claim which is the same as, or for the same or substantially the same subject matter as, a claim of an issued patent may not be made in any application unless such a claim is made prior to one year from the date on which the patent was granted.

35 U.S.C. § 135(b)(1). The "critical date" for purposes of determining compliance with 35 U.S.C. § 135(b)(1) is, thus, June 18, 2003 (A4).

Adair requested this interference in a preliminary amendment filed concurrently with the filing of the Adair application on November 21, 2005 ("Preliminary Amendment"), which was after the critical date (A653-73). Although the rules do not require Adair to do so (*see* 37 C.F.R. § 41.202(a)), Adair

showed compliance with 35 U.S.C. § 135(b)(1) in the Preliminary Amendment (A656-58). Adair contended that claim 16 as depending from claim 8 of PCT/GB90/02017, filed December 21, 1990 ("the PCT application") was to substantially the same subject matter as claim 1 of the Carter patent (A656-58). The PCT application was filed almost 12 years before the Carter patent issued and well prior to one year from the date on which the Carter patent issued. Claims 8 and 16 of the PCT application are duplicated below:

> 8. A CDR-grafted antibody **light** chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, **58**, and 71.
>
> 16. A CDR-grafted antibody heavy or light chain or molecule according to any one of the preceding claims comprising human acceptor residues and non-human donor residues.

(A748-49, emphasis added.) Claim 1 of the Carter patent is duplicated below:

> 1. A humanized antibody variable domain comprising non-human Complementarity Determining Region (CDR) amino acid residues which bind an antigen incorporated into a human antibody variable domain, and further comprising a Framework Region (FR) amino acid substitution at a site selected from the group consisting of: 4L, 38L, 43L, 44L, **58L**, 62L, 65L, 66L, 67L, 68L 69L, 73L, 85L, 98L, 2H, 4H, 36H, 39H, 43H, 45H, 69H, 70H, 74H, and 92H, utilizing the numbering system set forth in Kabat.

(A91, emphasis added.) (The "L" or "H" after a number in claim 1 of the Carter patent refers to the light chain or heavy chain, respectively (A1374).) Both claim 8 of the PCT application and claim 1 of the Carter patent cover a CDR-grafted light chain variable region in which a single residue in the light chain, i.e., residue 58, is substituted.

In the Preliminary Amendment, Adair proposed that the count of the interference be claim 24 as submitted, or claim 30 or claim 80 of the Carter patent (A669-70). Claim 24 as submitted is duplicated below:

> 24. A humanised antibody **heavy** chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises an amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof, as numbered according to Kabat.

(A655, emphasis added.) As claim 1 of the Carter patent, claims 30 and 80 recite amino acid substitutions at residues in the framework of the heavy and light chains (A92-93).

Instead of adopting Adair's proposed count, the Board devised its own count, set forth above. Claims 30, 31, 60, 62, 63, 66, 67, 70, 73, and 77-81 of the Carter patent were designated as corresponding to the Count (A98). Claim 24 of the Adair application ("Adair claim 24") was designated as corresponding to the Count (A98). Adair claim 24 is duplicated below:

> A humanised antibody **comprising a** heavy chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises **a non-human** amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof, as numbered according to Kabat.

(A199, emphasis added.) Adair claim 24 differs from claim 24 submitted in the Preliminary Amendment by the language highlighted in bold above.

Over four years after Adair first attempted to provoke an interference, the present interference was declared (A95). In the declaration of the interference, Adair was accorded priority benefit, ultimately, of GB 8928874.0, filed on December 21, 1989 ("the Adair GB") (A99). Adair was also accorded priority benefit of, *inter alia*, a Patent Cooperation Treaty ("PCT") application, PCT/GB90/02017, filed December 21, 1990 ("the PCT application") (A99). Carter was accorded priority benefit of, ultimately, U.S. Application Serial No. 07/715,272, filed June 14, 1991 ("the Carter '272 application") (A99). Carter was, thus, designated the Junior Party in the interference (A96).

As the Junior Party in the interference, the burden would have been upon Carter to prove priority of invention by a preponderance of the evidence. 37 C.F.R. § 41.207(a)(2). Notably, Carter's earliest priority date, i.e., June 14, 1991, is almost six months after the PCT application filing date, i.e., December 21, 1990, and almost 18 months after the Adair GB filing date, i.e., December 21, 1989.

Therefore, it seems unlikely that Carter could establish a conception date earlier than December 21, 1989, much less show reasonable diligence from just before December 21, 1989 to June 14, 1991. Carter did not have to do so.

In its list of proposed motions, Carter proposed filing a motion that Adair claim 24 is barred under 35 U.S.C. § 135(b)(1) ("135(b) motion") and requested that the motion be treated as a threshold issue (A266). The Standing Order in place for this interference provides that preliminary motions may be decided prior to motions for priority (see A175-76). The rules of practice for interferences also provide that certain threshold issues may be decided before others. 37 C.F.R. § 41.201. One such threshold issue is repose under 35 U.S.C. § 135(b), for claims first made after issuance of the movant's patent. 37 C.F.R. § 41.201.

The Board authorized Carter to file its 135(b) motion prior to the other authorized motions (A272). Carter filed its 135(b) motion (Carter Substantive Motion 1) on May 28, 2010 (A294). The Board authorized Adair to file an opposition to the 135(b) motion, which it did on July 14, 2010 (A367). No reply by Carter was authorized.

B. Summary Of Carter's 135(b) Motion

Carter alleged that Adair must satisfy at least three conditions to comply with 35 U.S.C. § 135(b)(1): 1) Adair must have presented a pre-critical date claim that is patentable to Adair; 2) Adair must have presented a pre-critical date claim

that defines the same or substantially the same subject matter as a claim of the Carter patent; and 3) Adair claim 24 cannot differ in any material limitation from Adair's pre-critical date claims. Carter cited four cases allegedly supporting condition one above -- *Adang v. Umbeck,* 2007 U.S. App. LEXIS 25198 (Fed. Cir. 2007); *Univ. of Rochester v. G.D. Searle & Co.,* 358 F.3d 916, 927 (Fed. Cir. 2004); *PIN/NIP, Inc. v. Platte Chem. Co.,* 304 F.3d 1235, 1247-48 (Fed. Cir. 2002); and *In re Curtis,* 354 F.3d 1347, 1353-54 (Fed. Cir. 2004) (A304). Regarding condition two, Carter argued that Adair's original pre-critical date claims submitted in the PCT application were rejected as indefinite and that the Carter patent claims are not indefinite, so the two sets of claims *must* differ in ways having patentable significance (A304-05). Carter argued that many of the non-original pre-critical date claims were determined to be not patentable, without citing any support therefor or identifying which claims (A305). Carter also argued that such claims differ from the Carter patent claims in material limitations, asserting that Adair's non-original pre-critical date claims recite positions that all must be donor, whereas the Carter patent claims do not require that each recited position be donor (A307). Finally, regarding condition 3, Carter reiterated the arguments for conditions one and two, and also argued that Adair claim 24 regards the heavy chain, whereas claims 16 and 8 of the PCT application regarded the light

chain and that Adair claim 24 and **all** of Adair's pre-critical date claims were, thus, materially different from each other (A298).

C. Summary Of Adair's Opposition

Regarding Carter's condition one, Adair argued that none of the cases Carter cited to support its assertion that the pre-critical date claims must be patentable supported the assertion and that, as the Board has held previously, canceled claims can be relied upon to provoke an interfere (A369-70). Adair cited *Tezuka v. Wilson*, 224 USPQ 1030 (Bd. Pat. Int. 1984) in support (A370). Regarding condition two, Adair argued that Carter did not specify which material limitations were lacking and, therefore, failed to meet its burden on the issue (A370-71). Adair also argued that claim 16, as depending from claim 1 of the PCT application, effectively contains all limitations of claim 66 of the Carter patent (A371). Regarding condition three, Adair argued that Carter was misapplying the materiality test (A372-73). Adair argued that the test whether or not a limitation is material for purposes of § 135(b) is to be determined in view of the **patent** claims in interference and that all material limitations of the **patent** claims must be present in, or necessarily result from, the limitations of both Adair's pre-critical date and post critical-date claims (A372). *In re Berger*, 279 F.3d 975, 61 USPQ2d 1523 (Fed. Cir. 2002) and *Corbett v. Chisolm*, 568 F.2d 759, 765-766, 196 USPQ 337, 342 (CCPA 1977) were cited in support (A372).

D. Summary Of The Board's Decision

The Board asserted that Adair did not dispute that claims reciting a heavy chain and claims reciting a light chain differ materially (A8). The Board disagreed with Adair's argument that Carter was misapplying the materiality test, but then quoted a statement from *Regents of the Univ. of Cal. v. Univ. of Iowa Res. Found.*, 455 F.3d 1371 (Fed. Cir. 2006), *reh'g en banc denied*, 2006 U.S. Appl. Lexis 27583 (Fed. Cir., Oct. 16, 2006) that seems to support Adair's interpretation instead of the Board's – i.e., that pre-critical date claims must provide support for post-critical date **identity** between the **involved claim** and the patentee's **patent** (A8, emphasis added). The Board argued that Adair's pre-critical date claims must be compared with its own claims for identity, not Carter's (A9).

The Board then considered original pre-critical date claims 1 and 16 of the PCT application as compared to Adair claim 24, without any reference to claim 66 of the Carter patent, and found that because claims 1 and 16 were rejected and ultimately canceled, they are materially different from Adair claim 24 (A9-10). The Board reached this conclusion by combining two distinct areas of case law – interference and doctrine of equivalents – to create a new presumption. The Board cited *Corbett,* 568 F.2d at 765, to show that one example of a material limitation is one that is necessary to patentability (A9). The Board relied upon *Festo Corp. v. Shoketsu Kinzoku Kogyo Kabushiki Co., Ltd.*, 535 U.S. 722 (2002) for creating a

presumption that, when an applicant adds a limitation to a claim in response to a rejection which results in allowance of the claim, that limitation was necessary to patentability, i.e., material (A9). Although the Board had just created this presumption in its decision, it faulted Adair for not showing why the limitations that differ between Adair claim 24 and original claims 1 and 16 were **not** necessary to the patentability of Adair claim 24 and stated that Adair did not point to any other pre-critical date claim that is identical to or includes the same material limitations as Adair claim 24 (A10). The Board stated that Adair could have sought authorization to file a motion to add a pre-critical date claim that interferes with the Carter claims but did not (A10).

The Board cited *Regents*, 455 F.3d at 1376, for the proposition that the USPTO should declare an interference upon receipt of a claim that satisfies 35 U.S.C. § 135(b) and is otherwise patentable (A10). Although seemingly recognizing that the two issues are separate, the Board then alleged that patentability of pre-critical date claims is required to satisfy 35 U.S.C. § 135(b), based upon the following statement in *Regents*, at 1377, – "this court perceives no inequity in a construction of *section 135(b)(1)* that might, in some circumstances, prevent a patent applicant from relying on the filing date of a claim to which it was not statutorily entitled" (A10-11).

E. Summary Of Adair's Request For Rehearing

Adair challenged the applicability of *Regents* to the present facts because, in *Regents*, the pre-critical date claims were copied from the patent whereas Adair's post-critical date claims were copied from the patent (A428). As Adair also argued, *Regents* distinguished cases in which the post-critical date claims were copied (A428). Adair asserted that the proper test is that set forth in *Berger,* 279 F.3d 975 and *Corbett*, 568 F.2d 759, and is whether or not all material limitations of the copied patent claim are present in the pre-critical date claim (A428-29). Specifically, Adair cited the following passage from *Berger:*

> Because the prior art applies in like manner to the **claims as copied**, the materiality of a limitation in a **claim copied** to provoke an interference **translates** to the copying inventor's application for purposes of assessing compliance with **35 U.S.C. § 135(b)**.

Berger, 279 F.3d at 983 (emphasis added) (A428-29). Adair also cited the following passage from *Corbett*:

> [t]here being a material limitation of the **copied** [Chisholm patent] claim not present in Corbett's [pre-critical date] claims 24-27, they cannot be said to be directed to **substantially the same invention.**

Corbett, 568 F.2d at 766 (citation omitted) (emphasis added) (A429). Adair pointed out that neither the Board, nor Carter, had argued that Adair's pre-critical date claims do not contain all material limitations of the Carter patent claims (A429).

Adair further argued that the passage from *Regents* quoted by the Board is not inconsistent with Adair's interpretation regarding the materiality test (A429-30). Adair contended that, if, after prosecution, the applicant's allowed post-critical date claims lack limitations from the pre-critical date claims that were necessary to the patentability of the **patent** claims, that applicant should not be able to rely upon the pre-critical date claims to provoke an interference with that patent (A430). Under such circumstances, the allowable post-critical date claims are no longer to substantially the same invention as the patent claims as required by 35 U.S.C. § 135(b)(1) (A430).

Adair also argued that, even if the materiality test were to be applied as the Board asserted, i.e., without reference to the patent claims being copied, the Board made several errors. First, canceled claims can be relied upon for determining compliance with 35 U.S.C. § 135(b)(1) (A430). Second, the burden should have been placed on Carter, as the movant, to show that **all** of the pre-critical date claims differed materially from Adair claim 24, not on Adair to show that none of the pre-critical date claims differed materially from Adair claim 24 (A430-31). Third, an original pre-critical date claim, claim 2 of the PCT application, recites all the residues recited in Adair claim 24, as Adair showed in an attached chart (A431, A435). Finally, Adair observed that it would have been futile to attempt to add an original pre-critical date claim because Adair would have to certify that it was not

aware of any reason the claim it was adding is not patentable considering that the original pre-critical date claims had been rejected (A432-33).

F. Summary Of The Board's Decision On Rehearing

The Board repeated its quote from *Regents* that Adair contends actually supports Adair's interpretation of the material differences test (A24). The Board then argued that Adair did not point to support in *Berger* for its argument that "[m]ateriality is determined in view of the patent claims being copied" (A24), even though Adair had provided a quote and page citation from *Berger* as noted above. The Board said that it was reasonable for Carter to rely upon only those claims that Adair had relied upon in its Preliminary Amendment and that, by doing so, Carter met its burden for relief and shifted the burden to Adair to show why Carter's showing was insufficient or to direct the Board to another pre-critical date claim that was materially the same as the copied claim (A25). Notably, the Board did not argue that claim 2 of the PCT application differs materially from Adair claim 24 but, rather, declined to consider claim 2 as being submitted too late and said that, even if it did consider claim 2, Adair failed to provide a sufficient comparison to show that claim 2 is materially the same as the copied claim (A25-26), despite the fact that Adair had provided a chart comparing the two claims. Finally, the Board argued that Adair's failure to argue the patentability of the original pre-critical date claims is contrary to what it refers to as the "policy" stated in *Regents*, i.e.,

"prevent[ing] a patent applicant from relying on the filing date of a claim to which it is not statutorily entitled" (A26).

SUMMARY OF THE ARGUMENT

The policy of *section 135(b)* is to place a time limit on a patentee's exposure to interferences. *Regents*, 455 F.3d at 1376. Where an interference is merely belated, i.e., should have been declared earlier by the USPTO, the interference should not be barred by *section 135(b)(1)*. *Id.*, at 1376. As is clear from the foregoing facts, Adair was claiming substantially the same subject matter as Carter well before the Carter patent issued. The present interference should have been declared earlier.[1] Adair, thus, should not be barred under *section 135(b)(1)*. The Board, however, seems to have a different view.

The Board has interpreted *Regents* in a manner which Adair contends is inconsistent with the case to bar Adair under *section 135(b)(1)*. First, the Board has interpreted *Regents* to require that applicants relying upon pre-critical date claims show that those pre-critical date claims are patentable (A26). But such an interpretation is not only inconsistent with *Regents*, but it is also inconsistent with precedent that is binding on this Court. Second, the Board has interpreted *Regents*

[1] Per the Manual of Patent Examination and Procedure ("MPEP"), examiners are required to perform an interference search of the comprehensive inventive features of the broadest claim prior to issuance. MPEP, § 1302.08. Notably, at the time the Carter patent issued, the Assistant Examiner on the Carter patent was examining an application to which the Adair application claims priority (*see* A34 and A1235).

as requiring a new material differences test between an applicant's pre- and post-critical date claims without any reference to the patent claims being copied (A24). Adair contends that *Regents* did not create such a test.

The Board's incorrect interpretation of *Regents* enabled it, effectively, to shift the burden of persuasion to Adair regarding Carter's 135(b) motion in contravention of the rules and Standing Order. The Board created a presumption that pre-critical date claims that are amended for any reason are materially different from post-critical date claims in its decision and then faulted Adair for not acting in a manner consistent with the presumption in its papers, which were filed before the presumption was created (A9-10). Adair would have to be prescient to have done so.

Finally, the Board inappropriately shifted the burden of production to Adair in contravention of the rules and Standing Order. The Board found that Carter met its burden of going forward by only specifically addressing two of Adair's numerous pre-critical date claims because that is all Adair addressed in its paper attempting to provoke the interference (A25). But, Adair did not have to address any claims in its paper attempting to provoke the interference. 37 C.F.R. § 41.202(a). Rather, Carter, as the movant, was required to show that none of Adair's pre-critical date claims satisfied the requirements of *section 135(b)(1)*. 37 C.F.R. § 41.208(b). Because the Board inappropriately shifted the burden of

production to Adair, it refused to consider an original pre-critical date claim that Adair argued met the Board's new materiality test, since the argument was submitted in Adair's request for rehearing (A26).

ARGUMENT

I. Standard of Review

The Board's legal conclusions are reviewed without deference; the Board's factual findings are reviewed for substantial evidence. *Hitzeman v. Rutter*, 243 F.3d 1345, 1353-54 (Fed. Cir. 2001).

II. Analysis

The Board's construction of 35 U.S.C. § 135(b)(1) is a question of law. *Regents*, 455 F.3d at 1373. For the reasons set forth below, Adair contends that the Board erred as a matter of law in its construction of *section 135(b)(1)*. The Board imposed additional requirements for compliance with *section 135(b)(1)* not supported by the statute or the case law, created a presumption that did not exist prior to its decision in this interference, and improperly shifted the burden of going forward to Adair. It is only by doing so that the Board was able to find that Adair did not comply with *section 135(b)(1)*.

A. The Board Erred By Requiring That Pre-Critical Date Claims Be Patentable

The Board asserted that this Court stated a policy in *Regents* under *section 135(b)(1)* of "prevent[ing] a patent applicant from relying on the filing date of a claim to which it is not statutorily entitled" (A26). In view of this "policy," the Board imposed a requirement upon Adair to argue the patentability of original pre-critical date claims being relied upon for support under *section 135(b)(1)* (A10-11, A26). Indeed, the Board criticized Adair for not seeking authorization to file a motion to add a pre-critical date claim that interferes with the Carter claims to the interference (A26). In such a motion, Adair would have to argue the patentability of any claim it was trying to add to the interference. 37 C.F.R. § 41.208 (c)(1).

Contrary to what the Board asserted, this Court did not state that there is a policy requiring a showing of patentability of pre-critical date claims in *Regents*. Rather, this Court stated the following:

> To the contrary, this court perceives no inequity in a construction of *section 135(b)(1)* that *might, in some circumstances*, prevent a patent applicant from relying on the filing date of a claim to which it was not statutorily entitled.

Regents, 455 F.3d at 1377 (emphasis added). The Board cropped the foregoing quote in half and then characterized it as setting forth a policy, something this Court did not do.

Regardless, the statement does not say that the Court perceives no inequity in a construction that *would, in all circumstances*, prevent an applicant from relying on the filing date of a claim to which it was not statutorily entitled, as the Board intimates. As Adair argued, an equally appropriate interpretation of this statement is that if, after prosecution, the applicant's allowed post-critical date claims lack material limitations from the pre-critical date claims, i.e., limitations that were necessary to the patentability of the **patent** claims being copied, that applicant should not be able to rely upon the pre-critical date claims to provoke an interference with that patent (A430). Under such circumstances, the allowable post-critical date claims are no longer to substantially the same subject matter as the patent claims, as is required by 35 U.S.C. § 135(b)(1) (A430). Adair's interpretation is more consistent with the policy which was stated in *Regents* – i.e., to place a time limit on a patentee's exposure to an interference proceeding. *Regents*, 455 F.3d at 1376. Such is not the present case. No one has argued that allowable Adair claim 24 is not to substantially the same invention as a claim of the Carter patent.

Further, a requirement that the pre-critical date claims be patentable is contrary to legal precedent. This Court's predecessor court considered pre-critical date claims that had been canceled over 15 months after being introduced, and 27 months before the patent issued, for compliance with *section 135(b)(1)*. *Corbett*, 568 F.2d at 761, 765. The court in *Corbett* did not comment on the patentability of

the canceled claims, nor require that they not have been rejected. Further, *Corbett* specifically approved of combining pre-critical date claims to find support for all material limitations of the patented claims for compliance with *section 135(b)*, as long as the claims being combined were to the same invention. *Id.*, 568 F.2d at 766. If one can combine claims, then patentability of individual claims is surely not relevant.

This Court is bound by precedent of the Court of Customs and Patent Appeals. *South Corp. v. United States*, 690 F.2d 1368, 1370 (Fed. Cir. 1982). Such precedent cannot be overruled by a panel of this Court. *Mothers Restaurant, Inc. v. Mama's Pizza, Inc.*, 723 F. 2d 1566, 1573 (Fed. Cir. 1983). *Regents* was a panel decision, and rehearing en banc was denied. *Regents,* 2006 U.S. Appl. Lexis 27583 (Fed. Cir., Oct. 16, 2006). Thus, even if the language in *Regents* relied upon by the Board could be interpreted to impose a requirement for patentability of pre-critical date claims, such a requirement would be inappropriate as contrary to binding precedent.

B. The Board Erred By Creating A New Material Differences Test

Section 135(b) requires that the claims being made to provoke an interference be to substantially the same subject matter as a claim of an issued patent. 35 U.S.C. § 135(b)(1). When the patent claims are copied post-critical date, as in the present case, the case law has allowed applicants trying to provoke an

interference to rely upon pre-critical date claims to show compliance with *section 135(b)(1)* as long as the pre-critical date claims contain all material limitations of the copied post-critical date claim. Materiality is to be determined in view of the patent claim being copied, as Adair has repeatedly argued (A372; A428-29). *See Berger*, 279 F.3d at 983; *Corbett*, 568 F.2d at 766.

Allegedly based upon *Berger* and *Regents*, the Board imposed a requirement that Adair's pre-critical date claims include all material limitations of the post-critical date claims, regardless of whether those limitations were material limitations of the patented claim (A24). To the extent *Regents* is found to have created such a requirement, Adair contends that *Regents* is not applicable to the present facts (A428). As noted above, Adair's **post**-critical date claims were copied from the patent. In *Regents*, the **pre**-critical date claims were copied from the patent. *Regents*, 455 F.3d at 1373. As Adair argued, *Regents* distinguished cases in which the post-critical date claims were copied from the patent (A428). *Id.*, at 1375 (distinguishing *In re Frey*, 182 F.2d 184 (CCPA 1950) and *Thompson v. Hamilton*, 152 F.2d 994 (CCPA 1946)).

Adair maintains, however, that *Regents* did not create a new test regarding materiality.[2] First, materiality was not at issue in *Regents* -- the appellant in

[2] In its initial decision on motions, the Board asserted that the new materiality test is the proper test to be applied (A9). In its decision on Adair's request for

Regents did not contest the Board's finding of material differences between the pre- and post-critical date claims, just whether or not the presence of material differences mattered. *Regents*, 455 F.3d at 1373. Second, as is clear from this Court's repeated reference to *section 135(b)(1)* throughout the opinion in *Regents,* and its distinguishing of cases in which the post-critical date claims were the ones that were copied, the reason the pre- and post-critical date claims are to be compared with one another is to ensure that the post-critical date claims are still to substantially the same subject matter as the **patent** claims. Finally, this Court said that the material differences test discussed in opinions like *Berger* is to be applied. *Id.*, at 1376. As noted above, the material differences test set forth in *Berger* is whether or not all material limitations of the patent claim are present. In *Berger*, a limitation in the copied claim that had been added by the patentee to avoid prior art was found to be material. *Berger*, 279 F.3d at 982.

> Because the prior art applies in like manner to the claims **as copied**, the materiality of a limitation in a claim **copied** to provoke an interference **translates** to the copying inventor's application for purposes of assessing compliance with 35 U.S.C. § 135(b).

Id., at 983 (emphasis added).

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rehearing, the Board asserted that the new materiality test is an additional requirement (A24). Adair contends that both assertions are wrong.

C. The Board Erred By Creating A Presumption That Any Differences Between Adair's Pre- and Post-critical Date Claims Are Material

Adair maintains that it does not need to show that its pre-critical date claims have all material limitations of its post-critical date claim without reference to the Carter patent claims. Nonetheless, in response to Carter's implication that claims to heavy chain (e.g., Adair claim 24) are different from claims to light chain (e.g., original claim 8 of the PCT application)(A298), Adair pointed out that original pre-critical date claim 1 of the PCT application recited heavy chain (A371). Claim 16 as depending upon claim 1 of the PCT application, thus, is to substantially the same invention as claim 66 of the Carter patent (A371). The Board did not challenge Adair's argument that original pre-critical date claim 16 as depending from claim 1 of the PCT application was to the same patentable subject matter as the Carter patent claims (A9). Rather, the Board said that it was not convinced that Adair had a pre-critical date claim that does not differ materially from Adair claim 24, noting that claims 1 and 16 of the PCT application had been rejected during prosecution and were canceled (A9).

Compounding the other two errors discussed above – i.e., requiring that the pre-critical date claims be patentable, and that there be no material differences between the pre- and post-critical date claims without reference to the patent claims being copied -- the Board created a presumption that a limitation added in response to a rejection that results in allowance is necessary to patentability and,

thus, material (A9-10). The Board created this presumption for the first time in its decision, and did so by combining two very divergent cases -- the *Corbett* and *Festo* cases discussed above (A9). As the Board acknowledged, however, *Festo* addresses infringement, i.e., the doctrine of equivalents, not interferences (A9). Adair contends that the combination of the two cases is, thus, inappropriate. Regardless, even in the context of the doctrine of equivalents, *Festo* does not create a presumption that a limitation was necessary to patentability. *Festo*, 535 U.S. at 734.

The patent rules provide that the burden of proof on a motion is on the movant. 37 C.F.R. § 41.208(b). The burden of proof for the 135(b) motion, thus, lay with Carter, not Adair. In view of their newly created presumption, the Board faulted Adair for not providing any reason why the limitations that differ between original pre-critical date claims 1 and 16 and Adair claim 24 were **not** material, for not pointing to another pre-critical date claim that is identical to or includes the **same** material limitations as Adair claim 24, and for not seeking authorization to file a motion to add a pre-critical date claim that interferes with the Carter claims (A10). The effect of the Board's fabricated presumption, thus, was to shift the burden of persuasion to Adair, particularly the requirement to move to resubmit a pre-critical date claim. As noted above, Adair would have to argue the patentability of such a claim. 37 C.F.R. § 41.208(c)(1).

D. The Board Erred By Shifting The Burden Of Production To Adair

Even assuming that the materiality test is as propounded by the Board, the burden was upon Carter to show that **all** of Adair's pre-critical date claims, i.e., those pursued during the more than 12-year period from December 21, 1990 through June 12, 2003, differed materially from Adair claim 24. 37 C.F.R. § 41.208(b) ("To be sufficient, a motion must provide a showing, supported with appropriate evidence, such that, if unrebutted, it would justify the relief sought. The burden of proof is on the movant."). Carter did not do so. Instead, Carter only specifically addressed the two claims Adair raised in its Preliminary Amendment to provoke the interference, and made sweeping conclusory statements regarding all others (A298; A308; A324). Carter had not specifically compared any other pre-critical date claims to Adair claim 24, in contravention of both the rules and the Standing Order (A430-31).

In its request for rehearing, Adair argued that Carter had not met its burden on the 135(b) motion and that, because of Carter's failure to meet its burden, the Board overlooked that claim 2 of the PCT application recites all residues recited in Adair claim 24 (A430-31). The Board responded that Carter's showing was reasonable in view of Adair's reliance on the two claims in its Preliminary Amendment (A25). Further, the Board said that the showing was sufficient to shift the burden to Adair to either show why Carter's showing was insufficient or to

direct the Board to another pre-critical date claim that was materially the same as the copied claim (A25). Consequently, the Board treated Adair's arguments regarding claim 2 of the PCT application as an untimely submission under 37 C.F.R. § 41.52(a)(1) and declined to consider whether the claim satisfied the requirements of *section 135(b)(1)* (A25-26). Thirty-seven C.F.R. § 41.52(a)(1), however, applies to *ex parte* appeals, not interferences (copy attached in Addendum).

Regardless, the Board erred in finding that Carter's showing was sufficient to shift the burden of production to Adair. Contrary to what the Board alleges, Carter's showing was not reasonable. The rules do not require that applicants wishing to provoke an interference show compliance with 35 U.S.C. § 135(b)(1). *See* 37 C.F.R. § 41.202(a). Adair, thus, did not have to argue that **any** pre-critical date claims were not materially different from Adair claim 24 to provoke the interference. In an abundance of caution, however, Adair argued that at least one of its pre-critical date claims -- claim 16 as depending from claim 8 of the PCT application -- was to substantially the subject matter as the Carter patent claims (A656-58). Adair evidently did so to its detriment. The Board should have denied Carter's motion outright. Instead, it shifted the burden of production to Adair.

Seemingly recognizing that its burden shifting was inappropriate, the Board alleged that, even if it were to consider claim 2 of the PCT application at this point,

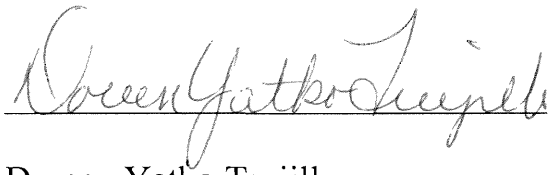
Adair had failed to provide a sufficient comparison to show that it is materially the same as the copied claim (A26). Adair is not sure what more it could have done. Adair argued that claim 2 of the PCT application recited all the residues recited in Adair claim 24, and included a chart in the appendix to its request for rehearing showing the same in bolded text (A431; A435). The chart included claims 1 and 16 of the PCT application, thereby showing that **all** limitations of Adair claim 24 were found in the pre-critical date claims (A435). Had the Board considered claim 2 of the PCT application, Adair would have prevailed even under the Board's erroneous analysis.

CONCLUSION AND STATEMENT OF RELIEF SOUGHT

Adair contends that the Board erred as a matter of law in finding that Adair did not comply with 35 U.S.C. § 135(b)(1). Adair respectfully requests that this Court reverse the Board's decision and deny Carter Substantive Motion 1.

Respectfully Submitted,

Dated: May 13, 2011



Doreen Yatko Trujillo
Kyle Vos Strache
Cozen O'Connor, P.C.
1900 Market St.
Philadelphia, PA 19103
215-665-2000

Attorneys for Appellants
John Robert Adair, Diljeet Singh Athwal,
And John Spencer Emtage

Mail Stop Interference
P.O. Box 1450
Alexandria Va 22313-1450
Tel: 571-272-9797
Fax: 571-273-0042

Filed August 30, 2010

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

PAUL J. **CARTER** AND LEONARD G. PRESTA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR**, DILJEET SINGH ATHWAL, and JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261).

Patent Interference No. 105,744
(Technology Center 1600)

Before SALLY GARDNER LANE, RICHARD TORCZON, and SALLY C. MEDLEY, *Administrative Patent Judges*

LANE, *Administrative Patent Judge*

ORDER - DECISION ON MOTIONS

I. STATEMENT OF THE CASE

The interference is before a panel for consideration of non-priority motions filed by Carter. No oral argument was held.

The Interference

Parties

The Interference involves junior party Carter and senior party Adair.

Junior party Carter is involved on the basis of its patent 6,407,213 ("the Carter '213 patent"), which issued 18 June 2002, from application no. 08/146,206, filed 17 November 1993. (Paper 1 at 3.) Claims 30, 31, 60, 62, 63, 66, 67, 70, 73, and 77-81 were designated as corresponding to the Count, while claims 1-29, 32-59, 61, 64, 65 68, 69, 71, 72, 74-76, and 82 were not. (Paper 1 at 4.)

The real party-in-interest of Carter is Genentech, Inc. (Paper 10).

Senior party Adair is involved on the basis of its application 11/284,261 ("Adair '261 application"), filed 21 November 2005. (Paper 1 at 3.) Claim 24, Adair's only pending claim, was designated as corresponding to the Count. (Paper 1 at 4.)

Adair was accorded priority benefit as to the Count of 08/846,658, filed 01 May 1997; 08/303,569, filed 07 September 1994, issued as 5,859,205 on 12 January 1999; 07/743,329, filed on 17 September 1991 ("the Adair '329 application"); PCT/GB90/02017, filed 21 December 1990 ("the Adair PCT application"); and GB 8928874.0, filed 21 December 1989. (Paper 1 at 5.)

The real party-in-interest of Adair is UCB Pharma, S.A. (Paper 4.)

Subject Matter

The parties' claims are drawn to an antibody that has been "humanized," that is, it has a combination of human and non-human regions and specific amino acids. Humanization allows antibodies to be raised, in the laboratory, in non-human animals (for example, mice) against antigens of interest and then changed so that they appear to the patient's body as if they were human antibodies. Humanized antibodies are beneficial because they do not raise dangerous anti-immunoglobulin responses in human patients, as non-human antibodies can. (Carter patent col. 1, l. 52, through col. 3, l. 8.) The humanized antibody of the involved Carter and Adair claims and the Count are antibodies that have a non-human Complementarity Determining Region ("CDR"), that is the region that binds antigen, and specifically recited non-human substitutions in other regions, called the Framework Regions ("FR"), of the antibody.

II. MOTIONS

Carter filed two substantive motions, which assert "threshold" issues that end the interference if the relief requested is granted. Carter Substantive Motion 1 ("Carter Motion 1") requests that Adair claim 24 be found unpatentable under 35 U.S.C. § 135(b)(1). Carter Substantive Motion 2 ("Carter Motion 2") requests that Adair claim 24 be found unpatentable under 35 U.S.C. § 112, first paragraph, for a lack of written description in the specification. As the moving party, Carter has the burden to show that it is entitled to the relief requested in its motions. Bd. R. 208(b).

A. CARTER MOTION 1

Findings of Fact

1. The involved Carter '213 patent issued 18 June 2002. (Carter Ex. 2001; Carter involved '231 patent.)

2. The "critical date," under 35 U.S.C. § 135(b)(1), by which Adair must have filed claims drawn to the same or substantially the same subject matter as the claims of the Carter '213 patent is 18 June 2003.

3. Adair filed the involved Adair '261 application on 21 November 2005, after the critical date. (Ex. 2002, Utility Patent Application Transmittal for Application 11/284,261.)

4. Claim 24, the only claim pending in the Adair '261 application was filed well after the critical date.

5. Claim 24 of the involved Adair '261 application recites:

> A humanised antibody comprising a heavy chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises a non-human amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof, as numbered according to Kabat.

(Paper 5.)

6. None of the claims of the Adair PCT application or the Adair '329 application are identical to claim 24 of the involved Adair '261 application. (Adair response to Carter MF 42; citing Exs. 2005-2010, 2012-2022, 2024-2027, 2029, and 2031-2035; not admitted or denied by Adair (Adair Opposition 1 at 21 ("Adair Opp. 1")), but no claims identical to claim 24 of the involved Adair '261 application identified by

Adair.)

7. In its request for interference, Bd. R. 202, Adair identified claims 8 and 16 of the Adair PCT application as a basis for compliance with 35 USC §135(b).

(Ex. 2003, Adair's Preliminary Amendment and Request for Interference under 37 C.F.R. § 42.202, p. 5.)

8. Claim 8 of the Adair PCT and '261 applications recites:

> A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, 58 and 71.

(Ex. 2005, p. 68 and Ex. 2006, p. 68.)

9. Claim 16 of the Adair PCT and '329 applications recites:

> A CDR-grafted antibody heavy or light chain or molecule according to any one of the preceding claims comprising human acceptor residues and non-human donor residues.

(Ex. 2005, p. 69 and Ex. 2006, p. 69.)

10. Claim 1 of the Adair PCT and '329 applications recites:

> A CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

(Ex. 2005, p. 67 and Ex. 2006, p. 67.)

Analysis

35 U.S.C. § 135(b)(1) states that:

> [a] claim which is the same as, or for the same or substantially the same subject matter as, a claim of an issued patent may not be made in any application unless such a claim is made prior to one year from the date on which the patent was granted.

Claim 24 of Adair's involved application, which corresponds to the Count, was filed more than one year from the date on which Carter's involved patent was issued. Because of the date Adair claim 24 was filed (*see* FF 4), it is, on its face, barred under 35 USC §135(b).

The bar of 35 USC §135(b) might be avoided if Adair had filed a claim that does not differ materially from claim 24. Indeed, in its request for interference, Bd. R. 202, Adair pointed to claims 8 and 16 of its pre-critical date application to support its assertion that claim 24 is not barred under the statute. (FF 7; Ex. 2003, Adair's Preliminary Amendment and Request for Interference under 37 C.F.R. § 42.202, p. 5.)

"To establish entitlement to the earlier effective date of existing claims for purposes of the one-year bar of 35 U.S.C. § 135(b), a party must show that the later filed claim does not differ from an earlier claim in any 'material limitation,'" *In re Berger*, 279 F.3d 975, 981-82 (Fed. Cir. 2002) (quoting *Corbett v. Chisholm*, 568 F.2d 759, 765-66 (CCPA 1977)). *See also Regents of Univ. of Cal. v. Univ. of Iowa Res. Found.*, 455 F.3d 1371, 1375 (Fed. Cir. 2006) ("When a party seeks to add a new claim, or to amend an existing claim, beyond the critical date for section 135(b)(1), [the Federal Circuit] applies the material differences test discussed in opinions like *Berger* to determine if

'such a claim' is barred."). The addition of a limitation for the purpose of making a claim patentable is strong evidence that the limitation is a material one. *See Corbett*, 568 F.2d at 765 (where a party's claim lacked a method step, the court noted that the party did "not seriously contend that this [was] not a material limitation, that [was] necessary to patentability . . . ."); *see also Wetmore v. Miller*, 477 F.2d 960, 964 (CCPA 1973) ("the 'fusible' limitation of appellant's claims must be regarded as not necessary to patentability and not 'material' for present purposes [of complying with 35 U.S.C. § 135(b)]").

Carter argues that the pre-critical date claims of Adair include different material limitations than those in Adair's involved claim 24. (Carter Motion 1 at 3.)

Claim 8 of the Adair PCT application, which is identical to claim 8 of the Adair '329 application, recites:

> A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, 58 and 71.

(FF 8; Ex. 2005, p. 68; Ex. 2006, p. 68.) Claim 16 of the Adair PCT application, which is identical to claim 16 of the Adair '329 application, recites:

> A CDR-grafted antibody heavy or light chain or molecule according to anyone of the preceding claims comprising human acceptor residues and non-human donor residues.

(FF 9; Ex. 2005, p. 69; Ex. 2006, p. 69.) Thus, the claims that Adair relied upon for avoiding the 35 U.S.C. § 135(b) bar are drawn to a CDR-grafted light chain. Adair's involved claim 24, though, is drawn to a "humanized antibody comprising a heavy chain variable domain . . . ." (FF 5, Paper 5.) Involved claim 24 differs from original claims 8

and 16, by reciting a heavy chain variable domain instead of a light chain variable domain.

Adair does not dispute that claims reciting a heavy chain and claims reciting a light chain differ materially. Instead, Adair argues that Carter applied the incorrect standard for assessing whether a post-critical date claim differs materially from an earlier claim. According to Adair, the correct inquiry is whether Adair added or removed claim limitations after the critical date that were necessary to the patentability of *Carter's* claims, not Adair's own pre-critical date claims (Adair Opp. 1 at 6).

We disagree. A party seeking support from pre-critical date claims for interfering claims filed beyond the one-year bar of 35 U.S.C. § 135(b)(1) "must demonstrate that claims in [the pre-critical date] application provide pre-critical date support for the post-critical date identity between [the involved claim] and the [patentee's patent]. That demonstration necessarily entails a comparison between pre- and post-critical date claims." *Regents of Univ. of Cal.*, 455 F.3d at 1375.

Adair also argues, in response to Carter's assertion of the material differences between claims to heavy and light chains, that in addition to its claims drawn to light chains, Adair filed claims drawn to heavy chains before the critical date. Specifically, Adair cites claim 1 of its PCT application as claiming a CDR-grafted antibody heavy chain, and argues that it, together with claim 16, effectively contain all of the limitations of involved claim 66 of the Carter '213 patent. (Adair Opp. 1 at 5; *see* FF 10; Ex. 2005, p. 67; Ex. 2006, p. 67.).[1]

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1 Similarly in its showing under Bd. R. 202, Adair compared its pre-critical date claims to a Carter claim but not the current Adair claim. (Ex. 2003, Adair's Preliminary Amendment and Request for

Adair has not made the correct comparison. Under the guidance provided in *Regents of University of California,* Adair's pre-critical date claims must be compared with its own current claims, not Carter's. Thus we are not persuaded by Adair's argument that it is sufficient that it had on file a claim or claims that effectively contain the limitations of an involved Carter claim.

Even when we consider claims 1 and 16 of the PCT application as they compare to Adair's current claim (and not Carter claim 66 as Adair argues), we are not convinced that Adair had a pre-critical date claim that does not differ materially from its current claim. As Carter notes, (1) claims 1 and 16 of Adair's PCT application were rejected under several statutory grounds in the Adair '329 application, including 35 U.S.C. §§ 101, 112, first and second paragraphs, 102(b), and 103(a), (*see* Ex. 2038, Office Action mailed 18 November 1992), and (2) Adair then cancelled the claims and added new ones that were eventually allowed (Ex. 2007, Amendment of 19 January 1993, p. 2). (*See* Carter Motion 1 at 5-6.)

One example of a material limitation is one that is "necessary to patentability." *See Corbett*, 568 F.2d at 765. When an applicant adds a limitation to a claim in response to a rejection and the added limitation results in allowance of the claim, the limitation is presumed to be necessary to patentability. *Cf. Festo Corp. v. Shoketsu Kinzoku Kogyo Kabushiki Co., Ltd.*, 535 U.S. 722, 734 (2002) (in the context of applying the doctrine of equivalents, "[a] rejection indicates that the patent examiner does not believe the original claim could be patented. While the patentee has the right to appeal, his decision to forgo an appeal and submit an amended claim is taken as a concession

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Interference under 37 C.F.R. § 42.202, p. 5.)

that the invention as patented does not reach as far as the original claim."); *see Berger*, 279 F.3d at 982 ("Inclusion of a limitation in a claim to avoid the prior art provides strong evidence of the materiality of the included limitation."). Adair does not provide any reason why the limitations that differ between involved claim 24 and original claims 1 and 16 were not necessary to the patentability of claim 24. Nor does Adair point to any other pre-critical date claim that is identical to or includes the same material limitations as its involved claim 24. (FF 6; *see* Carter MF 42, citing Exs. 2005-2010, 2013-2022, 2025-2027, 2029, and 2031-2035; not admitted or denied by Adair (Adair Opp. 1 at 21), but no claims identical to claim 24 of the involved Adair '261 application identified by Adair). We also note that as an applicant Adair could have, but did not, seek authorization to file a motion to add to its application a pre-critical date claim that interferes with the Carter claims (See Papers 23 and 73 (Orders setting times)).

Adair questions how one can provoke an interference if any claim amendments were made during prosecution under the standard stated in *Regents of University of California.* (Adair Opp. 1 at 7.) As explained in that case, "section 135(b)(1) [is] a statute of repose, placing a time limit on a patentee's exposure to an interference proceeding. *Regents Univ. of Cal.*, 455 F.3d at 1376. Despite this statute of repose, a "belated interference", i.e., based on a post-critical date claim, is appropriate in certain instances since "[t]he PTO should declare a valid interference upon receipt of a claim that satisfies section 135(b)(1), and which is otherwise patentable." (*Id.* at 1376). To insure that applicant did indeed timely present a patentable interfering claim, the post-critical date claim in interference must be materially the same as the claim that was timely presented. An applicant cannot expect to avoid the bar of §135(b) by timely

copying a claim from an issued patent when that claim is not patentable to that applicant. As the court noted, it "perceives no inequity in a construction of section 135(b)(1) that might, in some circumstances, prevent a patent applicant from relying on the filing date of a claim to which it was not statutorily entitled." (*Id.* at 1377).

We grant Carter Motion 1 and conclude that Adair involved claim 24 is barred under 35 U.S.C. § 135(b)(1).

B. CARTER MOTION 2

Carter asserts that claim 24 of Adair's involved application is unpatentable under 35 U.S.C. § 112, first paragraph, for lack of written description support.

Findings of Fact

11. Adair's specification provides a "preferred protocol" to determine which residues of a human heavy chain should be substituted for donor residues, as follows

> 2. Heavy Chain
>
> 2.1 Choose donor residues at all of positions 23, 24, 49, 71, 73 and 78 of the heavy chain or all of positions 23, 24 and 49 (71, 73 and 78 are always either all donor or all acceptor).
>
> 2.2 Check that the following have the same amino acid in donor and acceptor sequences, and if not preferably choose the donor: 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

(Ex. 2002, pp. 17-18; MF 13.)

12. Adair's specification includes the following directions regarding substituting residues of a human heavy chain for donor residues:

> "Key residues" near the surface of the heavy chain, are residues 23, 71 and 73, with residues 1, 3, and 76 reported to contribute to a lesser extent. (Ex. 2002, p. 20; MF 16.)

"Key residues" among the "[p]acking residues" near the CDRs as 24, 49, and 78. (Ex. 2002, p. 21; MF 17.)

Example 1 reports that "it is important to retain mouse residues at all of positions 6, 23, 24, 48 and 49, and possibly for maximum binding affinity at 71, 73 and 78." (Ex. 2002, p. 52; MF 19.)

Example 3 reports results wherein the crystal structure of the antibody heavy chain revealed that substitution at position 73 only was found to be important for antigen binding. (Ex. 2002, pp. 57-58; MF 56.)

13. Adair's specification provides the following written description of a CDR-grafted antibody heavy chain with specified donor residues:

> Accordingly, in a first aspect the invention provides a CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

(Ex. 2002 at p. 6.)

14. Adair's specification also provides the following written description of a CDR- grafted antibody heavy chain with specified donor residues:

> In preferred embodiments, the heavy chain framework comprises donor residues at positions 23, 24, 49, 71, 73 and 78 or at positions 23, 24 and 49. The residues at positions 71, 73 and 78 of the heavy chain framework are preferably either all acceptor or all donor residues.

(Ex. 2002 at p. 7.)

15. Adair's specification states:

> A preferred protocol for obtaining CDR-grafted antibody heavy and light chains in accordance with the present invention is set out below together with the rationale by which we have derived this protocol. This protocol. and rationale are given without prejudice to the generality of the invention as hereinbefore described and defined.

(Ex. 2002, p. 16; MF 53.)

Analysis

The test for written description under 35 U.S.C. § 112, first paragraph, "is whether the disclosure of the application relied upon reasonably conveys to those skilled in the art that the inventor had possession of the claimed subject matter as of the filing date." *Ariad Pharm., Inc., v. Eli Lilly & Co.*, 598 F3d 1336, 1351 (Fed. Cir. 2010). This analysis must consider the understandings of those in the art at the time of filing, *see Bilstad v. Wakalopulos*, 386 F.3d 1116, 1125-26 (Fed. Cir. 2004), and must consider the specification as a whole, *see In re Wright,* 866 F.2d 422, 424-25 (Fed. Cir. 1989).

Claim 24 recites a humanized antibody with a heavy chain "compris[ing] a non-human amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78 and combinations thereof . . . ." (FF 5; Paper 5). As Carter asserts, the broadest reasonable interpretation of this language in claim 24 encompasses a human heavy chain with residue substitutions at any number of the six residues recited, for example at only one residue, at all six residues, or at any combination in between. (*See* Carter Motion 2 at 1 and 5-6.)

Specification

In support of its argument that Adair's specification does not provide written description support of *any* of the six residues in claim 24, Carter cites to a "preferred protocol" provided in Adair's specification. Carter asserts that this protocol limits the invention to a human heavy chain framework region with either all of residues 23, 24, and 49, or all of residues 23, 24, 29, 71, 73, and 78, but not any of the residues individually. (Carter Motion 2 at 2 and 8; FF 11; Ex. 2002, Adair Specification, pp. 17-

18.) While this portion of the Adair specification appears to exclude many of combinations of substitutions encompassed by claim 24, other portions of Adair's specification are not so limiting.

For example, elsewhere Adair's specification provides that some "key residues" for making humanized antibodies are 23, 71 and 73, while other "key residues" are 24, 49, and 78. (FF 12; Ex. 2002, pp. 20 and 21; *see* Carter Motion 2 at 3.) Carter does not point to language in this part of the specification that indicates residues 23, 24, and 49 must *all* be substituted together or that 23, 24, 49, 71, 73, and 78 must *all* be substituted together.

In addition, while Carter cites Example 1 as reporting that "it is important to retain mouse residues at all of positions 6, 23, 24, 48 and 49, and possibly for maximum binding affinity at 71, 73 and 78" (FF 12: Ex. 2002, p. 52; *see* Carter Motion 2 at 3), Example 3 reports results wherein the crystal structure of the antibody heavy chain revealed that substitution at position 73 *only* was important for antigen binding. (FF 12; Ex. 2002, pp. 57-58; *see* Adair Opposition 2 at 3-4 ("Adair Opp. 2").) Thus, not all of the examples in Adair's specification support Carter's argument of a requirement for substation of *all* residues 23, 24, and 49 or *all* of residues 23, 24, 49, 71, 73, and 78.

Carter points to the Summary of the Invention section of Adair's application, which provides that human residues of the heavy chain can be substituted for donor residues at "at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91." (Carter Motion 2 at 6; FF 13; Ex. 2002, p. 6.) According to Carter, this language does not provide written description because it is "ambiguous." (Carter Motion 2 at 6-8.) As evidence, Carter points to the rejection

under 35 U.S.C. § 112, second paragraph, of original claim 1 in the Adair '329 application, which contained this language from the Adair specification, and Adair's response canceling claim 1. (Carter Motion 2, MFs 22 and 25; Ex. 2007, p. 29-32; Ex. 2038, p. 6.)

We do not agree that the rejection under the second paragraph of § 112 necessarily shows a lack of written description support under the first paragraph of § 112. Carter's analysis lacks a consideration of the entire Adair specification and instead focuses only upon an isolated portion.

Carter points to another part of the Summary of the Invention, wherein "[i]n preferred embodiments, the heavy chain framework comprises donor residues at positions 23, 24, 49, 71, 73 and 78 or at positions 23, 24 and 49. The residues at positions 71, 73 and 78 of the heavy chain framework are preferably either all acceptor or all donor residues." (FF 14; Ex. 2002 at p. 7; *see* Carter Motion 2 at 8.) Carter characterizes this portion as providing that 71, 73, and 78 "must" be either all acceptor or all donor residues (Carter Motion 2 at 8), but the passage expressly states that positions 71, 73, and 78 are "preferably" all donor or all acceptor. Thus, this portion of Adair's specification is not as limited as Carter asserts.

It does not appear to us that, on its face, the Adair specification contains a requirement for substitution of *all* residues 23, 24, and 49 or *all* of residues 23, 24, 49, 71, 73, and 78. Carter does not direct us to the testimony or other evidence showing what the Adair specification would have conveyed to those skilled in the art at the time of filing such that we might find otherwise. "Argument of counsel cannot take the place of evidence lacking in the record." *Meitzner v. Mindick*, 549 F.2d 775, 782 (CCPA

1977).

Prosecution History

Carter also points to the prosecution of Adair's applications as evidence that claim 24 is not supported by the Adair specification. According to Carter, Adair relied on the "preferred protocol" to distinguish claims of the Adair '329 application over the prior art and to overcome rejections for lack of enablement. (Carter Motion 2 at 9-13). The rejections, amendments, and arguments relied upon by Carter were not directed to involved claim 24 and Carter does not provide a detailed analysis of the claims that were being prosecuted and their relationship to Adair's current claim 24. Thus it is difficult to understand the relevance of the rejection of these claims to involved claim 24. *See Halliburton Energy Servs., Inc. v. M-I LLC*, 514 F.3d 1244, 1250, n.2 (Fed. Cir. 2008) ("Judges are not like pigs, hunting for truffles buried in briefs." (quoting *United States v. Dunkel*, 927 F.2d 955, 956 (7th Cir. 1991)).

In addition, though Carter notes instances when Adair discussed the "preferred protocol" and other rules for determining which residues to substitute, Carter does not point to instances where Adair argues that these are the *only* disclosures in their specification. In fact, other portions of the specification indicate that this "preferred protocol" is not limiting on the invention. (*See* Adair Opp. 2 at 3-4; FFs 15 and 16; Ex. 2002, Adair Specification, pp. 16 and 64.)

Carter has not shown that Adair claim 24 lacks sufficient written description support.

III. ORDER

Upon consideration of the motions, and for the reasons given, it is

ORDERED that Carter Motion 1 for judgment that Adair claim 24 is barred under 35 U.S.C. § 135(b) is GRANTED; and

FURTHER ORDERED that Carter Motion 2 for judgment that Adair claim 24 lacks written description support is DENIED; and

FURTHER ORDERED that judgment will be entered against Adair in a separate paper.

/ss/ Sally Gardner Lane
SALLY GARDNER LANE
Administrative Patent Judge

/ss/ Richard Torczon
RICHARD TORCZON
Administrative Patent Judge

/ss/ Sally C. Medley
SALLY C. MEDLEY
Administrative Patent Judge

Counsel for Carter
Oliver R. Ashe, Jr., Esq.
Ashe, P.C.
11440 Isaac Newton Sq. North, Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

Counsel for Adair
Doreen Yatko Trujillo, Esq.
Cozen O'Connor P.C.
1900 Market St.
Philadelphia, PA 19103
Telephone: (215) 665-5593
Facsimile: (215) 701-2005
E-mail: dtrujillo@cozen.com

Mail Stop Interference
P.O. Box 1450
Alexandria, Va 22313-1450
Tel: 571-272-4683
Fax: 571-273-0042

Paper 81

Filed 2 September 2010

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

PAUL J. **CARTER** AND LEONARD G. PRESTIA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR**, DILGEET SINGH ATHWAL,.
and JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

Patent Interference No. 105,744
(Technology Center 1600)

Before SALLY GARDNER LANE, RICHARD TORCZON, and SALLY C. MEDLEY, *Administrative Patent Judges.*

LANE, *Administrative Patent Judge.*

Judgment– Merits – Bd. R. 127

The Carter motion for judgment on the basis that the single involved Adair claim is barred under 35 U.S.C. § 135(b) was granted. (Paper 80). Because Adair no longer has an interfering claim that is not barred under 35 U.S.C. §135(b) it is appropriate to

enter judgment against Adair. *Berman v. Housey*, 291 F.3d 1345, 1351 (Fed. Cir. 2002).

It is

ORDERED that judgment on priority as to Count 1 (Paper 1 at 4), the sole count of the interference, is entered against senior party Adair;

FURTHER ORDERED that claim 24 of Adair application 11/284,261, which claim corresponds to Count 1 (Paper 1 at 4), is FINALLY REFUSED, 35 U.S.C. §135(a):

FURTHER ORDERED that if there is a settlement agreement, the parties are directed to 35 U.S.C. 135(c) and Bd. R. 205; and

FURTHER ORDERED that a copy of this judgment shall be entered into the administrative record of the Carter involved patent and application and the Adair involved application.

cc (via electronic filing):

Attorney for CARTER:

Oliver R. Ashe, Jr., Esq.
ASHE, P.C.
11440 Isaac Newton Square, North
Suite 210
Reston, VA 20190
Tel: 703-467-9001
Email: oashe@ashepc.com

Attorney for ADAIR:
Doreen Yatko Trujillo, Esq.
Michael B. Fein, Esq.
COZEN O'CONNOR P.C.
1900 Market Street
Philadelphia, PA 19103
Tel: 215-665-5593
Email: dtrujillo@cozen.com

Mail Stop Interference
P.O. Box 1450
Alexandria Va 22313-1450
Tel: 571-272-4683
Fax: 571-273-0042

Paper 84
Filed: 5 November 2010

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

PAUL J. **CARTER** AND LEONARD G. PRESTIA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR**, DILGEET SINGH ATHWAL, and JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

Patent Interference No. 105,744
(Technology Center 1600)

Before SALLY GARDNER LANE, RICHARD TORCZON, and SALLY MEDLEY,
Administrative Patent Judges

LANE, *Administrative Patent Judge*

ORDER - DECISION ON ADIAR REQUEST FOR REHEARING

I. STATEMENT OF THE CASE

Adair filed a Request for Rehearing (Paper 83) ("Request") of our Order – Decision on Motions (Paper 80) ("Decision") granting Carter Substantive Motion 1. We considered the Request but do not modify our Decision.

II. ANALYSIS

Adair argues that we inappropriately relied on *Regents of Univ. of Cal. v. Univ. of Iowa Res. Found.*, 455 F.3d 1371 (Fed. Cir. 2006), as the standard for determining whether Adair's involved claim 24 is barred under 35 U.S.C. § 135(b)(1). (Request 2). Adair attempts to distinguish the facts of *Univ. of Cal.* from the facts of the current interference, by noting that in *Univ. of Cal.* the claim in question was copied prior to the *pre*-critical date (and then later amended), while in the current interference the claim was copied only *after* the critical date. (Request 3). According to Adair, *In re Berger*, 279 F.3d 975 (Fed. Cir. 2002), and *Corbett v. Chisholm*, 568 F.2d759 (CCPA 1977) are instructive under the current facts, instead of *Univ. of Cal.*

We disagree. *Univ. of Cal.* expressly denies that there is any difference under 35 U.S.C. § 135(b)(1) between a pre-critical date request for interference (where the copied claim would have been filed before the critical date) and a post-critical date request for interference (where the copied claim would have been filed after the critical date). *See Univ. of Cal.*, 455 F.3d at 1375 ("Section 135(b)(1) does not include any language suggesting that a pre-critical date request for interference makes any difference. Section 135(b)(1) bars any claim having a degree of identity with a claim in an issued patent unless such a claim is filed before the critical date. Thus, title 35 in this section does not demand notice of an impending interference, but instead prohibits

unsupported, post-critical date identity."); *see also id.* at 1374 ("this court does not perceive any legally significant distinctions between this case and [*Berger*]."). Thus, we did not err by relying on *Univ. of Cal.*

According to Adair, the only requirement under § 135(b)(1) is that the limitations of the copied patent claim are present in a pre-critical date claim. (Request 3-4). Both *Univ. of Cal.* and *Berger* explain that

> a copied claim may be entitled to the earlier effective date of prior claims in an application only if the copied claim does not differ from the prior claims in any material limitation. . . . The analysis focuses on the copied claim to determine whether all material limitations of the copied claim necessarily occur in the prior claims.

Berger, 279 F.3d at 982; *see also Univ. of Cal.*, 455 F.3d at 1375 (an applicant "must demonstrate that claims in [the pre-critical date] application provide pre-critical date support for the post-critical date identity between [the involved claim] and the [patentee's patent]. That demonstration necessarily entails a comparison between pre- and post-critical date claims."). We agree with Adair's statement that "the *Berger* test compares the pre-critical date claims and the post-critical date claims, which were copied from the patent, to ensure that all material limitations of the post-critical date claims are present in the pre-critical date claims" (Request 4). However, Adair has not pointed to support in *Berger* for its argument that "[m]ateriality is determined in view of the patent claims being copied" (*id.*). Even if Adair's claims do satisfy such a test for materiality, these claims must also satisfy the separate *Berger* and *University of California* requirements. *Berger* and *Univ. of Cal.* require that Adair's pre-critical date claims include all of the material limitations of its post-critical date claims to fulfill the requirement of 35 U.S.C. § 135(b)(1).

Adair also argues that we erred by not putting the burden on Carter to show that Adair's pre-critical date claims differ materially from its post-critical date claims. (Request 5-6). However, in its Motion (Paper 71), Carter showed that claim 24 (the copied claim) differs materially from those claims relied upon by Adair to meet the requirements of 35 U.S.C. § 135(b)(1), PCT claims 8 and 16 (*see* FF[1] 7, Ex. 2003, Adair's Preliminary Amendment and Request for Interference under 37 C.F.R. § 42.202, p. 5). PCT claims 8 and 16 were directed to a CDR-grafted antibody light chain, while Adair's involved claim 24 is directed to an antibody heavy chain variable domain. (*See* Decision 7-8). Carter's showing was reasonable in view of Adair's reliance on PCT claims 8 and 16. Carter met its burden for relief and shifted the burden to Adair to either show why Carter's showing was insufficient or to direct us to another pre-critical date claim that was materially the same as the copied claim.

Adair argues our Decision was incorrect in stating that a presumption of a material difference was created since Adair's involved claim 24 was added and allowed only after the pre-critical date PCT claims were rejected and cancelled (Request at 6). However, when an applicant adds a limitation to a claim in response to a rejection and the added limitation results in allowance of the claims, the limitation is presumed to be necessary to patentability. *See Corbett, 568 F.2d at 765.; Cf. Festo Corp. v. Shoketsu Kinzoku Kogyo Kabushiki Co. Ltd,* 535 U.S. 722, 734 (2002).

Adair notes, for the first time in the Request, that pre-critical date claim 2 recites all the heavy chain residues of involved claim 24. (Request 6). "Arguments not raised

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[1] "FF" indicates the Findings of Fact provided in the Decision, which we incorporate into this Order.

in briefs before the Board and evidence not previously relied upon in the brief and any reply brief(s) are not permitted in the request for rehearing except [as based on recent relevant Board of Federal Circuit decisions]." 37 C.F.R. § 41.52(a)(1). Thus, we decline to consider that pre-critical date claim 2 satisfies the requirements of 35 U.S.C. § 135(b)(1). Even if we were to consider claim 2 at this point, Adair has failed to provide a sufficient comparison to show that claim 2 is materially the same as the copied claim.

In our Decision, we noted that Adair, as an applicant, could have attempted to add an original pre-critical date claim to its application if it believed that such a claim is allowable and would interfere with the Carter claims. (Decision at 10). Adair argues that "it would clearly have been futile for Adair to attempt to add an original pre-critical date claim" because "as the Decision noted, the original pre-critical date claims were rejected and canceled." (Request 8). By not arguing for the patentability of the original pre-critical date claims it relied upon for support under section 135(b)(1), Adair's position is contrary to the policy stated in *Univ. of Cal.* "prevent[ing] a patent applicant from relying on the filing date of a claim to which it is not statutorily entitled." *Univ. of Cal.*, 455 F.3d at 1377.

III. ORDER

Upon consideration of the motions, and for the reasons given, it is

ORDERED that Adair's Request that we modify our Decision is DENIED.

ss/ Sally Gardner Lane
SALLY GARDNER LANE
Administrative Patent Judge

/ss/ Richard Torczon
RICHARD TORCZON
Administrative Patent Judge

/ss/ Sally C. Medley
SALLY C. MEDLEY
Administrative Patent Judge

cc (via electronic transmission):

Counsel for Carter:

Oliver R. Ashe, Jr., Esq.
ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190

Tel: 703-467-9001
Email: oashe@ashepc.com

Jeffrey P. Kushan, Esq.
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, DC 20005

Tel: 202-736-8914
Email: jkushan@sidley.com

Counsel for Adair:

Doreen Yatko Trujillo, Esq.
Michael B. Fein, Esq.
Cozen O'Connor P.C.
1900 Market Street
Philadelphia, PA 19103

Tel: 215-665-5593
Tel: 215-665-4622
Email: dtrujillo@cozen.com
Email: mfein@cozen.com

Code of Federal Regulations

37

Revised as of July 1, 2010

Patents, Trademarks, and Copyrights

Containing a codification of documents of general applicability and future effect

As of July 1, 2010

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Office of the Federal Register
National Archives and Records
Administration

A Special Edition of the Federal Register

whole or in part. Affirmance of a rejection of a claim constitutes a general affirmance of the decision of the examiner on that claim, except as to any rejection specifically reversed.

(b) *Remand.* The Board may remand an application to the examiner. If in response to a remand for further consideration of a rejection, the examiner enters an examiner's answer, within two months the appellant shall exercise one of the following two options to avoid abandonment of the application or termination of a reexamination proceeding:

(1) *Request to reopen prosecution.* Request that prosecution be reopened before the examiner by filing a reply under §1.111 of this title with or without amendment or submission of evidence. Any amendment or evidence must be responsive to the remand or issues discussed in the examiner's answer. A request that complies with this paragraph will be entered and the application or patent under reexamination will be reconsidered by the examiner under the provisions of §1.112 of this title. A request under this paragraph will be treated as a request to dismiss the appeal.

(2) *Request to re-docket the appeal.* The appellant may request that the Board re-docket the appeal (*see* §41.35(a) of this subpart) and file a reply brief as set forth in §41.41 of this subpart. A reply brief may not be accompanied by any amendment or evidence. A reply brief which is accompanied by an amendment or evidence will be treated as a request to reopen prosecution pursuant to paragraph (b)(1) of this section.

(c) *Remand not final action.* Whenever a decision of the Board includes a remand, the decision shall not be considered a final decision of the Board. When appropriate, upon conclusion of proceedings on remand before the examiner, the Board may enter an order making its decision final.

(d) *New ground of rejection.* Should the Board have a basis not involved in the appeal for rejecting any pending claim, it may enter a new ground of rejection. A new ground of rejection shall be considered an interlocutory order and shall not be considered a final decision. If the Board enters a new ground of rejection, within two months appellant must exercise one of the following two options with respect to the new ground of rejection to avoid dismissal of the appeal as to any claim subject to the new ground of rejection:

(1) *Reopen prosecution.* Submit an amendment of the claims subject to a new ground of rejection or new evidence relating to the new ground of rejection or both, and request that the matter be reconsidered by the examiner. The application or reexamination proceeding on appeal will be remanded to the examiner. A new ground of rejection by the Board is binding on the examiner unless, in the opinion of the examiner, the amendment or new evidence overcomes the new ground of rejection. In the event the examiner maintains the new ground of rejection, appellant may again appeal to the Board.

(2) *Request for rehearing.* Submit a request for rehearing pursuant to §41.52 of this subpart relying on the Record.

(e) *Recommendation.* In its opinion in support of its decision, the Board may include a recommendation, explicitly designated as such, of how a claim on appeal may be amended to overcome a specific rejection. When the Board makes a recommendation, appellant may file an amendment or take other action consistent with the recommendation. An amendment or other action, otherwise complying with statutory patentability requirements, will overcome the specific rejection. An examiner, however, upon return of the application or reexamination proceeding to the jurisdiction of the examiner, may enter a new ground of rejection of a claim amended in conformity with a recommendation, when appropriate.

(f) *Request for briefing and information.* The Board may enter an order requiring appellant to brief matters or supply information or both that the Board believes would assist in deciding the appeal. Appellant will be given a non-extendable time period within which to respond to the order. Failure of appellant to timely respond to the order may result in dismissal of the appeal in whole or in part.

(g) *Extension of time to take action.* A request for an extension of time to respond to a request for briefing and information under paragraph (f) of this section is not authorized. A request for an extension of time to respond to Board action under paragraphs (b) and (d) of this section shall be presented as a petition under §41.3 of this part.

§ 41.52 Rehearing.

(a)(1) Appellant may file a single request for rehearing within two months of the date of the original decision of the Board. No request for rehearing from a decision on rehearing will be permitted, unless the rehearing decision so modified the original decision as to become, in effect, a new decision, and the Board states that a second request for rehearing would be permitted. The request for rehearing must state with particularity the points believed to have been misapprehended or overlooked by the Board. Arguments not raised in the briefs before the Board and evidence not previously relied upon in the brief and any reply brief(s) are not permitted in the request for rehearing except as permitted by paragraphs (a)(2) and (a)(3) of this section. When a request for rehearing is made,

the Board shall render a decision on the request for rehearing. The decision on the request for rehearing is deemed to incorporate the earlier opinion reflecting its decision for appeal, except for those portions specifically withdrawn on rehearing, and is final for the purpose of judicial review, except when noted otherwise in the decision on rehearing.

(2) Upon a showing of good cause, appellant may present a new argument based upon a recent relevant decision of either the Board or a Federal Court.

(3) New arguments responding to a new ground of rejection made pursuant to § 41.50(b) are permitted.

(b) Extensions of time under § 1.136(a) of this title for patent applications are not applicable to the time period set forth in this section. See § 1.136(b) of this title for extensions of time to reply for patent applications and § 1.550(c) of this title for extensions of time to reply for *ex parte* reexamination proceedings.

EFFECTIVE DATE NOTE: At 73 FR 32977, June 10, 2008, § 41.52 was revised, effective December 10, 2008. Per a subsequent final rule published at 73 FR 74972, Dec. 10, 2008, the effective date of this rule was delayed indefinitely.

For the convenience of the user, the revised text is set forth as follows:

§ 41.52 Rehearing.

(a) *Request for rehearing authorized.* An appellant may file a single request for rehearing.

(b) *Time for filing request for rehearing.* Any request for rehearing must be filed within two months from the date of the decision mailed by the Board.

(c) *Extension of time to file request for rehearing.* A request for an extension of time shall be presented as a petition under § 41.3 of this part.

(d) *Content of request for rehearing.* The form of a request for rehearing is governed by the requirements of § 41.37(v) of this subpart, except that a request for rehearing may not exceed 10 pages, excluding any table of contents, table of authorities, and signature block. A request to exceed the page limit shall be made by petition under § 41.3 at least ten calendar days before the request for rehearing is due. A request for rehearing must contain, under appropriate headings and in the order indicated, the following items:

(1) Table of contents—*see* § 41.37(i) of this subpart.

(2) Table of authorities—*see* § 41.37(j) of this subpart.

(3) [Reserved]

(4) Argument—*see* paragraph (f) of this section.

(e) [Reserved]

(f) *Argument.* A request for rehearing shall state with particularity the points believed to have been misapprehended or overlooked by the Board. In filing a request for rehearing, the argument shall adhere to the following format: "On page x, lines y-z of the Board's opinion, the Board states that (set out what was stated). The point misapprehended or overlooked was made to the Board in (identify paper, page and line where argument was made to the Board) or the point was first made in the opinion of the Board. The response is (state response)." As part of each response, appellant shall refer to the page number and line or drawing number of a document in the Record. A general restatement of the case will not be considered an argument that the Board has misapprehended or overlooked a point. A new argument cannot be made in a request for rehearing, except:

(1) *New ground of rejection.* Appellant may respond to a new ground of rejection entered pursuant to § 41.50(d)(2) of this subpart.

(2) *Recent legal development.* Appellant may rely on and call the Board's attention to a recent court or Board opinion which is relevant to an issue decided in the appeal.

(g) *No amendment or new evidence.* No amendment or new evidence may accompany a request for rehearing.

(h) *Decision on rehearing.* A decision will be rendered on a request for rehearing. The decision on rehearing is deemed to incorporate the underlying decision sought to be reheard except for those portions of the underlying decision specifically modified on rehearing. A decision on rehearing is final for purposes of judicial review, except when otherwise noted in the decision on rehearing.

§ 41.54 Action following decision.

After decision by the Board, the proceeding will be returned to the examiner, subject to appellant's right of appeal or other review, for such further action by appellant or by the examiner, as the condition of the proceeding may require, to carry into effect the decision.

EFFECTIVE DATE NOTE: At 73 FR 32977, June 10, 2008, § 41.54 was revised, effective December 10, 2008. Per a subsequent final rule published at 73 FR 74972, Dec. 10, 2008, the effective date of this action was delayed indefinitely.

For the convenience of the user, the revised text is set forth as follows:

CERTIFICATE OF SERVICE

United States Court of Appeals
for the Federal Circuit
No. 2011-1212,-1213

----------)
John Robert Adair, Appellants,

v.

Paul J. Carter, Cross Appellants.
----------)

I, Elissa Matias, being duly sworn according to law and being over the age of 18, upon my oath depose and say that:

Counsel Press was retained by COZEN O'CONNOR, Attorneys for Appellants to print this document. I am an employee of Counsel Press.

On the **13th of May 2011**, I served 2 copies of the **Brief of the Appellants John Robert Adair, Diljeet Singh Athwal and John Spencer Emtage** upon :

Oliver R. Ashe, Jr.
ASHE, P.C.
11440 Isaac Newton Square North
Suite 210
Reston, VA 20190
Tel: 703-467-9001
Fax: 703-467-9002

via Federal Express,

Unless otherwise noted, 12 copies have been delivered to the Court on the same date via Federal Express.

Elissa Matias

May 13, 2011

UNITED STATES COURT OF APPEALS FOR THE FEDERAL CIRCUIT

2011-1212, -1213
(Interference No. 105,744)

JOHN ROBERT ADAIR, DILJEET SINGH ATHWAL,
and JOHN SPENCER EMTAGE,
Appellants,

v.

PAUL J. CARTER and LEONARD G. PRESTA,
Cross Appellants.

Appeals from the United States Patent and Trademark Office, Board of Patent Appeals and Interferences

---

UNOPPOSED MOTION TO DISMISS CARTER'S CROSS-APPEAL

Oliver R. Ashe, Jr.
ASHE, P.C.
11440 Isaac Newton Square North, # 210
Reston, VA 20190
(703) 467-9001

Jeffrey P. Kushan
Rachel H. Townsend
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, DC 20005
(202) 736-8000

Attorneys for Cross-Appellants, Carter *et al.*

June 9, 2011

CERTIFICATE OF INTEREST

Counsel for the Cross-Appellants certifies the following:

1. The full name of every party or amicus represented by me is:

PAUL J. CARTER and LEONARD G. PRESTA

2. The name of the real party in interest represented by me is:

GENENTECH, INC.

3. All parent corporations and any publicly held companies that own 10 percent or more of the stock of the party or amicus curiae represented by me are:

ROCHE HOLDINGS, INC.

4. The names of all law firms and the partners or associates that appeared for the party or amicus now represented by me in the trial court or agency or are expected to appear in this court are:

Oliver R. Ashe, Jr. of ASHE, P.C.
Jeffrey P. Kushan and Rachel H. Townsend of SIDLEY AUSTIN, LLP

Dated: June 9, 2011

Oliver R. Ashe, Jr.

In accordance with Rule 42(b), Cross-Appellants PAUL J. CARTER and LEONARD G. PRESTA ("Carter") move the Court to dismiss Carter's cross-appeal filed on January 18, 2011, and assigned Appeal No. 2011-1213.

In support of this motion, Carter states as follows:

1. Carter filed two substantive motions with the Board of Patent Appeals and Interferences, which asserted "threshold" issues that if decided in Carter's favor would end the interference. The first motion requested that Adair claim 24 be found to be barred under 35 U.S.C. § 135(b)(1). The second motion requested that Adair claim 24 be found unpatentable under 35 U.S.C. § 112, first paragraph, for lack of written description. On August 30, 2010, the Board granted Carter's first motion concluding that Adair's involved claim 24 is barred under 35 U.S.C. § 135(b)(1). The Board denied Carter's second motion. The Board entered judgment against Adair on September 2, 2010, "[b]ecause Adair no longer has an interfering claim that is not barred under 35 U.S.C. § 135(b)." Adair's request for rehearing, filed October 1, 2010, was denied by the Board on November 5, 2010.

2. On January 4, 2011, Appellants JOHN ROBERT ADAIR, DILJEET SINGH ATHWAL, and JOHN SPENCER EMTAGE ("Adair") filed its notice of appeal of the Board's adverse decision that its claim 24 was barred under 35 U.S.C. § 135(b).

3. On January 18, 2011, Carter filed a notice of cross-appeal of the Board's adverse decision denying Carter's motion that Adair's claim 24 was unpatentable under 35 U.S.C. § 112, first paragraph, for lack of written description.

4. Adair requested and was granted a twenty-five day extension of time extending the time to file its principal brief from April 18, 2011, to May 13, 2011. Adair filed its principal brief on May 13, 2011.

5. On March 24, 2011, this Court issued a precedential order in *Aventis Pharma S.A. v. Hospira,* Nos. 2011-1018, -1047 (Fed. Cir. March 24, 2011). In that order, the Court held that Apotex's additional claims for invalidity and claims of non-infringement to the same claims did not expand the scope of the judgment in Apotex's favor and thus were improper grounds for cross-appeal. Slip op. at 4-5. The Court did go on to note, however, that Apotex could "consistent with our practice and precedent, raise these arguments in its appellees' brief if it so chooses." *Id.* at 5.

6. The *Aventis* order does not address the specific circumstance wherein a claim has been held to be barred under 35 U.S.C. § 135(b) but a motion asserting the unpatentability of the same claim under 35 U.S.C. § 112, first paragraph, has been denied. Nevertheless, in view of the Court's concern expressed in *Aventis* as to whether an issue on cross-appeal would expand the scope of the judgment, Carter withdraws its cross-appeal relating to the issue of whether Adair's claim 24

is unpatentable under 35 U.S.C. § 112, first paragraph, for lack of written description. The parties agree that this motion does not preclude Carter from raising the issue of the unpatentability of Adair claim 24 under 35 U.S.C. § 112, first paragraph, in its responsive brief in Appeal No. 2011-1212 as an alternative ground for affirmance of the Board's entry of judgment against Adair.

7. Adair consents to the withdrawal of the cross-appeal and each party has agreed to bear its own costs on the cross-appeal.

For the foregoing reasons, Carter's cross-appeal should be dismissed. A proposed order with service list is attached.

Respectfully submitted,

June 9, 2011

Oliver R. Ashe, Jr.
ASHE, P.C.
11440 Isaac Newton Square North, # 210
Reston, VA 20190
(703) 467-9001

Jeffrey P. Kushan
Rachel H. Townsend
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, DC 20005
(202) 736-8000
Attorneys for Cross-Appellants, Carter *et al.*

CERTIFICATE OF FILING

The undersigned certifies that an original and three copies of the paper entitled "**UNOPPOSED MOTION TO DISMISS CARTER"S CROSS-APPEAL**" was filed this 9th day of June, 2011, by Federal Express overnight delivery service, to:

Clerk of Court
United States Court of Appeals for the Federal Circuit
717 Madison Place, N.W.
Washington, D.C. 20439

6-9-11
Date

Oliver R. Ashe, Jr.

CERTIFICATE OF SERVICE

The undersigned hereby certifies that a true and correct copy of the paper entitled "**UNOPPOSED MOTION TO DISMISS CARTER"S CROSS-APPEAL**" was served this 9th day of June, 2011, by sending in the following manner:

VIA INTERFERENCE WEB PORTAL(https://acts.uspto.gov/ifiling/):

Doreen Yatko Trujillo, Esq.
Cozen O'Connor P.C.
1900 Market Street, 7th Floor
Philadelphia, PA 19103
Tel.: 215-665-5593
Fax: 215-701-2005
E-mail: dtrujillo@cozen.com

The Board of Patent Appeals and Interferences
Madison Building East, 9th Floor
600 Dulany Street
Alexandria, VA 22314
Tel.: 571-272-9797
Fax: 571-273-0042
E-mail: BoxInterfernces@USPTO.GOV

VIA FIRST CLASS MAIL (Postage pre-paid):

The Office of Solicitor
United States Patent and Trademark Office
P.O. Box 15667
Arlington, VA 22215

6-9-11
Date

Oliver R. Ashe, Jr.

UNITED STATES COURT OF APPEALS FOR THE FEDERAL CIRCUIT

2011-1212, -1213
(Interference No. 105,744)

JOHN ROBERT ADAIR, DILJEET SINGH ATHWAL,
and JOHN SPENCER EMTAGE,
Appellants,

v.

PAUL J. CARTER and LEONARD G. PRESTA,
Cross Appellants.

Appeals from the United States Patent and Trademark Office, Board of Patent Appeals and Interferences

ORDER

Upon consideration of the Unopposed Motion to Dismiss Carter's Cross-Appeal filed by Cross-Appellants PAUL J. CARTER and LEONARD G. PRESTA,

IT IS ORDERED THAT:

1) The unopposed motion be GRANTED, and the Clerk of the Court dismiss the cross-appeal assigned Appeal No. 2011-1213.

2) Each side shall bear its own costs.

FOR THE COURT:

Date: _____ _____

Copies to:

Oliver R. Ashe, Jr.
ASHE, P.C.
11440 Isaac Newton Square North, # 210
Reston, VA 20190
Tel.: 703-467-9001
Counsel for Cross-Appellants PAUL J. CARTER and LEONARD G. PRESTA

Doreen Yatko Trujillo, Esq.
Cozen O'Connor P.C.
1900 Market Street, 7th Floor
Philadelphia, PA 19103
Tel.: 215-665-5593
Counsel for Appellants JOHN ROBERT ADAIR, DILJEET SINGH ATHWAL, and JOHN SPENCER EMTAGE

The Office of Solicitor
United States Patent and Trademark Office
P.O. Box 15667
Arlington, VA 22215

The Board of Patent Appeals and Interferences
Madison Building East, 9th Floor
600 Dulany Street
Alexandria, VA 22314
Tel.: 571-272-9797

UNITED STATES COURT OF APPEALS FOR THE FEDERAL CIRCUIT

2011-1212, -1213
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JOHN ROBERT ADAIR, DILJEET SINGH ATHWAL,
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Cross Appellants.

Appeals from the United States Patent and Trademark Office, Board of Patent Appeals and Interferences

---

UNOPPOSED MOTION OF CROSS APPELLANTS, PAUL J. CARTER AND LEONARD G. PRESTA, FOR A THIRTY-DAY EXTENSION OF TIME TO FILE ITS OPENING BRIEF

Oliver R. Ashe, Jr.
ASHE, P.C.
11440 Isaac Newton Square North, # 210
Reston, VA 20190
(703) 467-9001

Jeffrey P. Kushan
Rachel H. Townsend
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, DC 20005
(202) 736-8000

Attorneys for Cross-Appellants, Carter *et al.*

June 15, 2011

CERTIFICATE OF INTEREST

Counsel for the Cross-Appellants certifies the following:

1. The full name of every party or amicus represented by me is:

PAUL J. CARTER and LEONARD G. PRESTA

2. The name of the real party in interest represented by me is:

GENENTECH, INC.

3. All parent corporations and any publicly held companies that own 10 percent or more of the stock of the party or amicus curiae represented by me are:

ROCHE HOLDINGS, INC.

4. The names of all law firms and the partners or associates that appeared for the party or amicus now represented by me in the trial court or agency or are expected to appear in this court are:

Oliver R. Ashe, Jr. of ASHE, P.C.
Jeffrey P. Kushan and Rachel H. Townsend of SIDLEY AUSTIN, LLP

Dated: June 15, 2011

Oliver R. Ashe, Jr.

UNOPPOSED MOTION OF CROSS APPELLANTS, PAUL J. CARTER AND LEONARD G. PRESTA, FOR A THIRTY-DAY EXTENSION OF TIME TO FILE ITS OPENING BRIEF

Pursuant to Fed. R. App. P. 26(b), Cross-Appellants PAUL J. CARTER AND LEONARD G. PRESTA ("Carter") respectfully requests that this Court grant a thirty (30) day extension of time to and including July 27, 2011, within which to file its opening brief in the above-identified case.

The date that Carter's opening brief is currently due is June 27, 2011. Carter has not previously sought any extension of time in this appeal and is filing this motion at least seven days before the brief due date. Counsel for JOHN ROBERT ADAIR, DILJEET SINGH ATHWAL, and JOHN SPENCER EMTAGE has represented that it does not oppose this motion.

There is good cause for this motion as explained below. Carter's lead attorney on this appeal, Oliver R. Ashe, Jr., is lead and backup lead counsel on five interference proceedings presently before the Board of Patent Appeals and Interferences ("the Board"). Two of these interference proceedings are in fully active motions phases and the schedules are not amenable to significant alterations. Mr. Ashe is responsible for preparing a number of motions to be filed at the Board, including motions due on Wednesday, June 15, 2011, in Interference No. 105,792, motions due on June 24, 2011, in Interference No. 105,771, and responsive motions due on July 15, 2011, in Interference No. 105,771. In addition, due to

longstanding plans for a family vacation, Mr. Ashe will be away from the office from June 25, 2011, through July 10, 2011.

Additionally, one of Carter's other appellate counsel, Jeffery P. Kushan, has a variety of professional commitments that has limited and will continue to limit the time that he is able to devote to the assistance of the preparation and review of Carter's brief. Mr. Kushan is one of the attorneys responsible for preparing and filing expert reports on July 1, 2011 in a case docketed in the District of Delaware. In addition, Mr. Kushan has a longstanding speaking engagement on June 21. And due to a longstanding professional commitment and planned vacation, Mr. Kushan will be away from the office from June 22 through June 28.

Accordingly, Carter needs additional time to prepare its brief. For the purposes of Fed. Cir. R. 27(a)(8), it is not believed that any of the above facts are subject to dispute. However, for the purposes of Fed. Cir. R. 26(b)(5), Carter hereby submits declarations of counsel showing good cause for the extension.

For the reasons set forth herein, it is respectfully requested that Carter's unopposed motion to extend the due date for its brief in the above appeal by thirty (30) days to and including July 27, 2011, be granted.

A proposed order granting the relief requested in this motion with service list is attached.

Respectfully submitted,

June 15, 2011

Oliver R. Ashe, Jr.
ASHE, P.C.
11440 Isaac Newton Square North, # 210
Reston, VA 20190
(703) 467-9001

Jeffrey P. Kushan
Rachel H. Townsend
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, DC 20005
(202) 736-8000
Attorneys for Cross-Appellants, Carter *et al.*

CERTIFICATE OF FILING

The undersigned certifies that an original and three copies of the paper entitled "**UNOPPOSED MOTION FOR CROSS APPELLANTS, PAUL J. CARTER AND LEONARD G. PRESTA, FOR A THIRTY-DAY EXTENSION OF TIME TO FILE ITS OPENING BRIEF** " was filed this 15th day of June, 2011, by Hand-Delivery, to:

Clerk of Court
United States Court of Appeals for the Federal Circuit
717 Madison Place, N.W.
Washington, D.C. 20439

6-15-11
Date

Oliver R. Ashe, Jr.

CERTIFICATE OF SERVICE

The undersigned hereby certifies that a true and correct copy of the paper entitled "**UNOPPOSED MOTION FOR CROSS APPELLANTS, PAUL J. CARTER AND LEONARD G. PRESTA, FOR A THIRTY-DAY EXTENSION OF TIME TO FILE ITS OPENING BRIEF**" was served this 15th day of June, 2011, by sending in the following manner:

VIA INTERFERENCE WEB PORTAL(https://acts.uspto.gov/ifiling/):

Doreen Yatko Trujillo, Esq.
Cozen O'Connor P.C.
1900 Market Street, 7th Floor
Philadelphia, PA 19103
Tel.: 215-665-5593
Fax: 215-701-2005
E-mail: dtrujillo@cozen.com

The Board of Patent Appeals and Interferences
Madison Building East, 9th Floor
600 Dulany Street
Alexandria, VA 22314
Tel.: 571-272-9797
Fax: 571-273-0042
E-mail: BoxInterfernces@USPTO.GOV

6-15-11
Date

Oliver R. Ashe, Jr.

UNITED STATES COURT OF APPEALS FOR THE FEDERAL CIRCUIT

2011-1212, -1213
(Interference No. 105,744)

JOHN ROBERT ADAIR, DILJEET SINGH ATHWAL,
and JOHN SPENCER EMTAGE,
Appellants,

v.

PAUL J. CARTER and LEONARD G. PRESTA,
Cross Appellants.

Appeals from the United States Patent and Trademark Office, Board of Patent Appeals and Interferences

ORDER

Upon consideration of the Unopposed Motion for Extension of Time filed by Cross-Appellants PAUL J. CARTER and LEONARD G. PRESTA,

IT IS ORDERED THAT:

1) The unopposed motion be GRANTED, and the Clerk of the Court note this extension on the docket.

2) The principal brief of Cross-Appellants PAUL J. CARTER and LEONARD G. PRESTA shall be due on July 27, 2011.

FOR THE COURT:

Date: _____ _____

Service List:

Oliver R. Ashe, Jr.
ASHE, P.C.
11440 Isaac Newton Square North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Counsel for Cross Appellants, Carter *et al.*

Doreen Yatko Trujillo, Esq.
Kyle Vos Strache, Esq.
Cozen O'Connor P.C.
1900 Market Street, 7th Floor
Philadelphia, PA 19103
Tel.: 215-665-5593
Counsel for Appellees, Adair *et al.*

The Board of Patent Appeals and Interferences
Madison Building East, 9th Floor
600 Dulany Street
Alexandria, VA 22314
Tel.: 571-272-9797

UNITED STATES COURT OF APPEALS FOR THE FEDERAL CIRCUIT

2011-1212, -1213
(Interference No. 105,744)

JOHN ROBERT ADAIR, DILJEET SINGH ATHWAL,
and JOHN SPENCER EMTAGE,
Appellants,

v.

PAUL J. CARTER and LEONARD G. PRESTA,
Cross-Appellants.

Appeals from the United States Patent and Trademark Office, Board of Patent Appeals and Interferences

Declaration of Oliver R. Ashe, Jr.

1. I am lead counsel for Cross-Appellants PAUL J. CARTER and LEONARD G. PRESTA ("Carter").

2. This appeal was docketed in this Court on February 15, 2011, which made Appellants JOHN ROBERT ADAIR, DILJEET SINGH ATHWAL, and JOHN SPENCER EMTAGE ("Adair")'s opening brief due on April 18, 2011. Adair requested and was granted an extension of time in which to file its opening brief. Adair filed that brief on May 13, 2011. Based on the May 13 filing of Adair's brief, Carter's brief is due on June 27, 2011.

3. I am lead and backup lead counsel on five interference proceedings presently before the Board of Patent Appeals and Interferences ("the Board"). Two of these interference proceedings are in fully active motions phases and the schedules are not amenable to significant alterations. I am responsible for preparing a number of motions to be filed at the Board, including motions due on Wednesday, June 15, 2011, in Interference No. 105,792, and motions due on June 24, 2011, in Interference No. 105,771, and responsive motions due on July 15, 2011, in Interference No. 105,771. In addition, due to longstanding plans for a family vacation, I will be away from the office from June 25, 2011, through July 10, 2011.

4. While significant efforts have been made to avoid having to seek an extension in this case, it has now been determined that an extension of time of thirty (30) days to and including July 27, 2011, would allow adequate time for me to coordinate the drafting, reviewing and filing of Carter's brief.

Pursuant to 29 U.S.C. §1746, I declare under penalty of perjury that the foregoing is true and correct.

June 15, 2011

Oliver R. Ashe, Jr.

UNITED STATES COURT OF APPEALS FOR THE FEDERAL CIRCUIT

2011-1212, -1213
(Interference No. 105,744)

JOHN ROBERT ADAIR, DILJEET SINGH ATHWAL,
and JOHN SPENCER EMTAGE,
Appellants,

v.

PAUL J. CARTER and LEONARD G. PRESTA,
Cross-Appellants.

Declaration of Jeffrey P. Kushan

1. I am co-counsel for Cross-Appellants PAUL J. CARTER AND LEONARD G. PRESTA ("Carter").

2. This appeal was docketed in this Court on February 15, 2011, which made Appellants JOHN ROBERT ADAIR, DILJEET SINGH ATHWAL, and JOHN SPENCER EMTAGE ("Adair") opening brief due on April 18, 2011. Adair requested and was granted an extension of time in which to file its opening brief. Adair filed that brief on May 13, 2011. Based on the May 13 filing of Adair's brief, Carter's brief is due on June 27, 2011.

3. Various professional commitments have limited and will continue to limit the time that I am able to devote to the assistance of the preparation and review of

Carter's opening brief. Among other matters, I am counsel for Alza Corporation and Ortho-McNeil-Janssen Pharmaceuticals, Inc. in *Alza Corp. v. Kremers Urban, LLC.*, CA No. 10-23-LPS (D. Del.) and am one of the attorneys responsible for preparing and filing expert reports in that case on July 1, 2011. I also have a speaking engagement on June 21 for which I will be out of the office. In addition, due to a longstanding professional commitment and family vacation, I will be away from the office from June 22 through June 28.

4. As a result of these and other commitments, and despite diligent efforts, it will not be possible for me to assist in the preparation and filing of Carter's opening brief in this matter by June 27, 2011. An extension of time of thirty (30) days to and including July 27, 2011, would allow adequate time for counsel to coordinate the drafting, reviewing and filing of Carter's brief.

Pursuant to 29 U.S.C. §1746, I declare under penalty of perjury that the foregoing is true and correct

June 15, 2011

Jeffrey P. Kushan

NOTE: This order is nonprecedential.

United States Court of Appeals for the Federal Circuit

JOHN ROBERT ADAIR, DILJEET SINGH ATHWAL, AND JOHN SPENCER EMTAGE,
Appellants,

v.

PAUL J. CARTER AND LEONARD G. PRESTA,
Appellees.

2011-1212
(Interference No. 105,744)

Appeal from the United States Patent & Trademark Office, Board of Patent Appeals and Interferences.

JOHN ROBERT ADAIR, DILJEET SINGH ATHWAL, AND JOHN SPENCER EMTAGE,
Appellees,

v.

PAUL J. CARTER AND LEONARD G. PRESTA,
Appellants.

2011-1213
(Interference No. 105,744)

Appeal from the United States Patent & Trademark Office, Board of Patent Appeals and Interferences.

ORDER

Upon consideration of the cross-appellants' unopposed motion for voluntary dismissal of cross-appeal, 2011-1213, pursuant to Fed. R. App. P. 42(b),

IT IS ORDERED THAT:

The motion is granted. The revised caption in 2011-1212 is reflected above.

(2) Each side shall bear its own costs in 2011-1213.

FOR THE COURT

JUL 6 2011
Date

/s/ Jan Horbaly
Jan Horbaly
Clerk

cc: Doreen Yatko Trujillo, Esq.
Oliver R. Ashe, Jr., Esq.

s24

ISSUED AS A MANDATE (as to 2011-1213 only): JUL 6 2011

2011-1212
(Interference No. 105,744)

UNITED STATES COURT OF APPEALS FOR THE FEDERAL CIRCUIT

JOHN ROBERT ADAIR, DILJEET SINGH ATHWAL,
and JOHN SPENCER EMTAGE,

Appellants,

v.

PAUL J. CARTER and LEONARD G. PRESTA,

Cross Appellants.

Appeals from the United States Patent and Trademark Office, Board of Patent Appeals and Interferences.

REPLY BRIEF OF THE APPELLANTS JOHN ROBERT ADAIR, DILJEET SINGH ATHWAL, and JOHN SPENCER EMTAGE

Doreen Yatko Trujillo
(Counsel of Record)
Kyle Vos Strache
Cozen O'Connor, P.C.
1900 Market St.
Philadelphia, PA 19103
215-665-2000

Attorneys for Appellants
John Robert Adair, Diljeet Singh Athwal,
And John Spencer Emtage

Dated: August 15, 2011

CERTIFICATE OF INTEREST

Counsel for the (petitioner) (appellant) (respondent) (appellee) (amicus) (name of party) APPELLANT ADAIR certifies the following (use "None" if applicable; use extra sheets if necessary):

1. The full name of every party or amicus represented by me is:

John Robert Adair, Diljeet Singh Athwal, and John Spencer Emtage

2. The name of the real party in interest (if the party named in the caption is not the real party in interest) represented by me is:

UCB Pharma S.A.

3. All parent corporations and any publicly held companies that own 10 percent or more of the stock of the party or amicus curiae represented by me are:

UCB Pharma S.A. is wholly-owned by UCB S.A.
Financiere de Tubize S.A. is a publicly owned company that owns more than 10% of the stock of UCB S.A.

4. The names of all law firms and the partners or associates that appeared for the party or amicus now represented by me in the trial court or agency or are expected to appear in this court are:

Cozen O'Connor P.C. – Doreen Yatko Trujillo, Michael B. Fein, Kyle Vos Strache

August 15, 2011
Date: August 15, 2011

Doreen Yatko Trujillo
Signature of counsel

Doreen Yatko Trujillo
Printed name of counsel

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INTRODUCTION

Carter dedicated over seven pages of its 57-page brief (twice as long as Adair's principal brief) arguing, essentially, that Adair's claims should be limited by its specification, and that the specification requires multiple framework residues to be changed to donor, i.e., to be non-human (Red Br. 11-18). Adair is unsure why Carter dedicated such a major portion of its brief to an argument not relevant to the issues on appeal. Nonetheless, Carter is misrepresenting Adair's specification. Adair's specification is not as limiting as Carter alleges – the specification does not require multiple framework residues to be changed (A565). Carter is relying upon what is clearly delineated as a "preferred" protocol in arguing that the specification is so limited (Red Br. 12; A576). Further, *In re* Berger, 279 F.3d 975 (Fed. Cir. 2002) disapproves of focusing upon the specification for satisfying *§ 135(b)*. *Id.*, at 983.

Citing an irrelevant patent issued to Adair, Carter also advances the disingenuous argument that changing multiple residues to donor was necessary for Adair to overcome the prior art (Red Br. 18). But Carter's claims do not recite changing multiple residues (A91-3). If the recitation of multiple residues was not necessary for Carter's claims to overcome the prior art, then it is difficult to see how it would be necessary for Adair claim 24.

Neither of the foregoing arguments is relevant to the basis for this appeal.

I. The Basis For This Appeal

The basis for this appeal is the correct interpretation of 35 U.S.C. § 135(b). Specifically, does *§ 135(b)* require applicants to show, in an interference based upon a claim that was submitted post-critical date, not only that the claim has pre-critical date support for its post-critical date identity with a claim of the patent, but also an additional requirement, as Carter and the Board allege, that the claim does not differ from the pre-critical date claim in virtually any respect? By presuming that any change to pre-critical date claims is material and suggesting (repeatedly) that Adair could have moved to add claims **identical** to pre-critical date claims in the involved application, the Board is essentially requiring a showing that the post-critical date claim does not differ from the pre-critical date claim in any respect, thereby setting forth a standard that is not only inconsistent with legal precedent, but is also impossible for applicants to meet.

As anyone who has prosecuted an application before the USPTO[1] knows, particularly in the field of biotechnology, originally-filed claims are rarely, if ever, allowed. Indeed, if they are, applicants are concerned that they did not claim broadly enough. Thus, the fact that an applicant chooses to amend the claims upon rejection, or even cancel them in favor of different claims, is not a concession of unpatentability *per se*, particularly for applications filed after June 7, 1995, but

[1] Unless otherwise indicated, the same abbreviations as were used in the principal brief are used here.

more a reflection of a desire to get allowable claims in a reasonable time frame. An appeal of a rejection can take years to be resolved, particularly if the appeal has to be taken to this Court.

II. 35 U.S.C. § 135(b) Does Not Require An Additional Comparison Between Pre- And Post-Critical Date Claims Without Reference To The Patent Claims Being Copied For Interference

Adair contends that this Court did not impose an additional requirement in *Regents of the Univ. of Cal. v. Univ. of Iowa Res. Found.,* 455 F.3d 1371 (Fed. Cir. 2006), *reh'g en banc denied*, 2006 U.S. Appl. Lexis 27583 (Fed. Cir., Oct. 16, 2006) that pre- and post-critical date claims do not differ from each other in any respect, irrespective of whether or not both contain all material limitations of the patent claim. In *Regents*, this Court stated that *§ 135(b)* prohibits unsupported post-critical date **identity** with a **patent** claim, that one must show pre-critical date support for the post-critical date **identity** between the post-critical date claim in interference and a **patent** claim, and that this demonstration entails a comparison between the pre- and post-critical date claims. *Id.*, at 1375, emphasis added. Accordingly, the pre-critical date claim must have all material limitations of the post-critical date claim, with materiality being assessed in view of the patent claim. This analysis is all that the precedent cited throughout *Regents*, i.e., *Berger*, 279 F.3d 975 and *Corbett v. Chisholm*, 568 F.2d 759 (CCPA 1977), required. In *Berger*, a limitation added by the patentee was considered material.

> The Board found the "circumferential groove" limitation to be material because it was added by Muller [the patentee] during prosecution to avoid prior art. We agree with the Board's determination of materiality.

Berger, 279 F.3d at 982. Similarly, in Chisholm, materiality was assessed in view of the patent claim.

> Turning to a comparison of Chisholm patent claim 1 and claims 24-27, we agree with the conclusion of the board that these claims, even considered as a group, do not recite Chisholm's claimed squeezing step (b). Corbett does not seriously contend that this is not a material limitation, that is, necessary to patentability. . . There being a material limitation of the copied claim not present in Corbett's claims 24-27, they cannot be said to be directed to substantially the same invention.

Corbett, 568 F.2d at 765-66. The pre-critical date claim does not need to have all limitations of the post-critical date claim, then, just those limitations that were material to the **patented** claim.

Indeed, every express limitation is not material under *§ 135(b)*. *Stalego v. Heymes*, 263 F.2d 334, 339 (CCPA 1959). A review of *Berger* reveals that the "circumferential groove" was not the only difference between the post-critical date claim that was copied from the Muller patent and the pre-critical date claim. The pre-critical date claim also did not contain a recitation of a pull tab. *Berger*, 279 F.3d 977-78.

When the post-critical date claim contains all material limitations of the patented claim the comparison becomes, in essence, a comparison between the pre-

critical date claims and the patent claims. *See Berger*, 279 F.3d at 982-83 and *Corbett*, 568 F.2d at 763,765-66. The Court in *Regents* also stated, however, that there is a distinction between comparing pre- and post-critical date claims with one another and comparing pre-critical date claims with the patented claims. *Regents*, 455 F.3d at 1375. The Board, and Carter, has interpreted this statement in *Regents* to mean that there is an additional requirement that the pre-critical date claims contain, essentially, all the limitations of the post-critical date claims, and *vice versa*, irrespective of whether both contain all material limitations of the patent claims. Adair, however, cannot reconcile this interpretation with the purpose of *§ 135(b)*, nor the Court's statements in *Regents* regarding the purpose of *§ 135(b)*, i.e., prohibiting unsupported post-critical date identity with the patent claim, nor the Court's repeated references to *Berger* and *Corbett*. *Regents*, 455 F.3d 1374-75.

An alternative interpretation proffered by Adair in this interference is that if, after prosecution, the applicant's allowed post-critical date claims lack material limitations from the pre-critical date claims, i.e., limitations that were necessary to the patentability of the patent claims, the applicant should not be allowed in the interference, as the claims are no longer to substantially the same invention (Br. 14). Under such circumstances, it would not be sufficient to compare the pre-critical date claims to the patent claims alone. Consistent with this view, the Court distinguished cases in which the post-critical date claims were the ones copied

from the patent. *Regents*, 455 F.3d at 1375.

This interpretation seems to be the most consistent with the whole of the Court's decision but, unfortunately, it is not consistent with the underlying facts as Adair interprets them. A review of the underlying decision of the Board in *Regents* suggests that the post-critical date claim had all the material limitations of the patented claim. *Univ. of Iowa Res. Found. v. Regents of the Univ. of Cal.,* Interf. No. 105,171, slip op. at 3 and 6 (B.P.A.I. March 10, 2005) (Board Decision) Perhaps, however, the Court took the appellant in *Regents* at its word that there were material differences between the post- and pre-critical date claims, and assumed that the post-critical date claims were no longer to the same invention as the patent claims.

Assuming Adair's alternative interpretation is correct, *Regents* is not applicable to the current facts. Adair first requested this interference post-critical date. Even if applicable, Adair maintains that *Regents* did not create an additional test for materiality completely divorced from the patent claims for purposes of compliance with *§ 135(b)*.

Carter argues, incredulously, that this Court found the limitations of the patent claims to be irrelevant in *Regents* because "the relevant question for the issue of repose is whether the later claim is entitled to the effective date of the earlier claim . . . which is essential to establishing that the *same* interference could

have been declared earlier" (Red Br. 43, emphasis in original). Adair questions how the limitations of the patent claims can ever be irrelevant under a statute that requires that a claim that is to substantially the same subject matter as a claim of an issued **patent** be submitted within a specified time frame. 35 U.S.C. § 135(b). Further, *§ 135(b)* does not require that the **same** interference could have been declared earlier, just that **an** interference could have been declared earlier.

Adair contends that both Carter and the Board are confounding the analysis for determining effective filing date for purposes of 35 U.S.C. § 120 with the analysis for determining effective filing date for purposes of 35 U.S.C. § 135(b). But these sections of the Patent Statute serve distinct purposes and have very different requirements. This distinction was recognized in *Berger* which refers to "the earlier effective filing date of those prior claims **for purposes of satisfying 35 U.S.C. § 135(b)**." *Berger*, 279 F.3d at 982 (emphasis added). For example, *§ 120* allows an application for patent to rely upon the filing date of an earlier filed application if the invention is disclosed in the earlier application in the manner provided by the first paragraph of 35 U.S.C. § 112. 35 U.S.C. § 120. Accordingly, under *§ 120*, one must show, *inter alia*, written descriptive support for the recitations in the claims in earlier applications, and one can look to the specification for such support. Contrastingly, *§135(b)* makes no reference to the first paragraph of 35 U.S.C. § 112, nor to the benefit of a filing date, and focuses

upon the claims alone. All that *§ 135(b)* requires is that a claim that is to substantially the same subject matter, not exactly the same subject matter, as a claim of the patent be made prior to one year from the date the patent was granted. 35 U.S.C. § 135(b). The Board and Carter, however, are requiring applicants to show, allegedly under *§ 135(b)*, written descriptive support for all recitations in the post-critical date claims in the pre-critical date claims themselves.

One source of the confusion may be the apparent discrepancy in the various reported versions of a statement in *Berger*, 279 F.3d at 982. The Lexis® and Westlaw® electronic databases report the statement as the following:

> This is a distinctly different question from whether claims made for purposes of interference by different parties are directed to **interfering** subject matter.

Other electronic databases, as well as the book version of the reporter, report the statement as the following:

> This is a distinctly different question from whether claims made for purposes of interference by different parties are directed **to the same or substantially the same** subject matter.

The differences between the two are highlighted in bold. Notably, the immediately preceding sentence in *Berger* sets forth what must be shown under *§ 135(b)*. *Id.*, 279 F.3d at 981-2. As discussed above, *§ 135(b)* recites the language "the same or substantially the same subject matter." 35 U.S.C. § 135(b). Adair contends that the correct version is the first one because interfering subject matter under (prior)

37 C.F.R. § 1.601 was being distinguished from the requirements under *§ 135(b)* in *Berger*. *Id.* Under the latter version, showing that claims are to the same or substantially the same subject matter is being distinguished from showing that claims are to the same or substantially the same subject matter.

III. Even If An Additional Comparison Under *§135(b)* Is Required, Adair Claim 24 Satisfies It

As the Board, and Carter, repeatedly asserted, a limitation that is necessary to patentability is material (see, for example, Red Br. 36). Adair claim 24, having been indicated as allowable, is presumptively patentable (Red Br. 26). If Adair claim 24 is lacking limitations from the earlier claims, then, those limitations could not have been material. Regardless, as Adair argued in its request for rehearing, claim 2 of the PCT application recites all the residues recited as alternatives in Adair claim 24 (Br. 14; A431, A435). As shown in the appendix to Adair's request for rehearing, claim 16 of the PCT application, as depending from claim 2 of the PCT application, thus, contains all material limitations of Adair claim 24, and *vice versa*.[2] The Board declined to consider claim 2 of the PCT application,

---

[2] Carter also argues that the Board noted that Adair did not make a sufficient comparison to show that claim 2 is materially the same as the copied claim, evidently in reference to claim 66 of the Carter patent (Red Br. 24). In its initial decision, however, the Board argued that Adair was not to compare its pre-critical date claims to the patent claims under *Regents* (A9). Regardless, if Adair claim 24 contains all material limitations of claim 66 of the Carter patent, which neither the Board nor Carter has argued to the contrary, and claim 16 of the PCT application, as depending from claim 2, contains all material limitations of Adair claim 24,

however, citing a rule related to *ex parte* appeals, not interferences (A26).

In defense of the Board's declination, Carter asserts that Adair has been prosecuting this portfolio for over 14 years and had ample opportunity to explain why claim 2 provided the requisite pre critical date support under *§ 135(b)* (Red Br. 51). Carter's assertion is flawed. Adair had only been trying to provoke an interference with the Carter patent since November 21, 2005 (Br. 2-3). Adair would have had no reason to raise the issue before then. Further, as Adair has repeatedly pointed out, the rules do not require Adair to show compliance with *§ 135(b)* to provoke an interference (Br. 4-5). Carter keeps faulting Adair for not raising an issue that Adair was not required to raise. Notably, nothing prevented the USPTO from raising *§135(b)* as a basis for rejection during that five-year period. *See Berger*, 279 F.3d at 981.

Carter also asserts the Adair's submission of arguments regarding claim 2 of the PCT application were belated (Red Br. 52-3). Carter notes that the Board's Standing Order explains that the Board will not consider evidence presented belatedly in a reply (Red Br. 52). Carter is completely disregarding that the burden was on Carter, as the movant, to make out a *prima facie* case, not on Adair. All Adair needed to do in its opposition was address the arguments raised by Carter. Carter did not cite *Regents* in its motion to support its arguments that Adair claim

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claim 16 of the PCT application, as depending from claim 2, must contain all material limitations of claim 66 of the Carter patent.

24 differed materially from Adair's non-original, pre-critical date claims (A308). In its opposition, then, Adair focused upon arguing that Carter was applying the incorrect materiality test (372). *Regents* was first raised by the Board in its decision (A6). Adair's first chance to address the Board's interpretation of *Regents*, thus, was in its request for rehearing.

Carter also argues that 37 C.F.R. § 41.125(c)(3) prevents a party from raising a matter on rehearing that was not previously addressed by requiring a party to show all matters believed to have been overlooked and to show where the matter was previously addressed in the motion, opposition, or reply (Red Br. 52). First, the Board did not raise this section of the regulations in its decision. Second, Adair complied with 37 C.F.R. § 41.125(c)(3). Adair pointed out that the Board had overlooked claim 2 of the PCT application because Carter failed to meet its burden of addressing each pre-critical date claim in its motion, which Adair had argued in its opposition (A430-1; A370-1).

Regardless, the Board did not raise the Standing Order or 37 C.F.R. § 41.125(c)(3) when it declined to consider claim 2 of the PCT application. Rather, the Board cited a rule relating to *ex parte* appeals (A26). Adair pointed out this further legal error in its brief (Br. 27). Carter faults Adair for not arguing that the Board's declination was arbitrary or unreasonable or otherwise an abuse of discretion (Red Br. 51). Adair did not make such arguments because Adair

contends it was legal error for the Board to apply the wrong regulation. Nonetheless, an abuse of discretion can be established if the exercise of discretion is based upon **an error of law**. *Novo Nordisk of North America, Inc. v. Genentech, Inc.*, 77 F.3d 1364, 1367 (Fed. Cir. 1996) (emphasis added).

IV. No Precedent Requires Patentability of Pre-critical Date Claims Under *§ 135(b)*

Carter maintains that pre-critical date claims must be patentable (Red Br. 37). Notably, Carter could not point to any legal precedent supporting its position that *§ 135(b)* requires pre-critical date claims to be patentable. Instead, Carter could only argue that the absence of observations in *Corbett* regarding the requirement of patentability of pre-critical date claims cannot be used as precedent that patentability of a pre-critical date claim is **not** a factor in a *§ 135(b)* determination (Red Br. 40-1, emphasis added). But Carter is wrong regarding the absence of observations in *Corbett* regarding the requirement of patentability of pre-critical date claims. In *Corbett*, four sets of pre-critical date claims (or 12 claims) were being analyzed to determine support for the post-critical date claim copied from the patent in interference. *Corbett*, 568 F.2d at 759-63. The court indicated that one claim (which made up one of the sets) was allowed. *Id.*, at 763. The court, thus, did make observations about the patentability of the pre-critical date claims, but clearly did not consider it a factor in its *§ 135(b)* analysis.

Carter's error regarding *Corbett* appears to be based upon a misreading of

the facts. Carter asserts that patentability was not an issue addressed by the court in *Corbett* because the pre-critical date claims and post-critical date claims were identical (Red Br. 40). Carter is wrong. One set of pre-critical date claims (four claims) was cancelled even before the involved patent had issued, so there clearly could not be any post-critical date claims identical to those claims. *Corbett* at 761.

Regardless, the fact that Carter could not point to any precedent in **support** of a requirement of showing patentability supports Adair's contention that such a requirement by the Board is legal error.

V. The Board Cannot Create Substantive Law

Adair maintains that the burden was upon Carter, as the movant, to show that **no** Adair pre-critical date claim supports the identity between the patent claim and the post-critical date claim. If the application claims priority to several applications and spans over 12 years of prosecution, as in the present case, the burden on the patentee can be quite onerous. No matter how onerous the patentee's burden may be, however, the Board does not get to shift the burden of persuasion to Adair through its creation of a presumption, particularly one as far-reaching as the one created here – i.e., that a cancelled pre-critical date claim is, *a priori*, materially different from the post-critical date claim. In support of the presumption created by the Board, Carter argues that courts routinely draw from related legal doctrines to support their decisions. The Board, however, is not a

court of law, and does not get to create substantive law. *See Merck & Co., Inc. v. Kessle*r, 80 F.3d 1543, 1549–50 (Fed. Cir. 1996) ("[T]he broadest of the PTO's rulemaking powers—35 U.S.C. §6(a)—authorizes the Commissioner to promulgate regulations directed only to 'the conduct of proceedings in the [PTO]'; it does not grant the Commissioner the authority to issue substantive rules.")

VI. The Board Improperly Shifted The Burden Of Production To Adair

In addition to creating the presumption, the Board inappropriately shifted the burden of production to Adair. Citing 37 C.F.R. § 41.208(b), Carter contends that the USPTO's regulations only require a "**demonstration** that if unrebutted would justify the relief sought" by the movant to make out a *prima facie* showing (Red Br. 47, emphasis added). The cited rule does not require a mere demonstration, however, but rather a "showing." 37 C.F.R. § 41.208(b). As Adair stated previously, the burden was upon Carter to **show** that none of Adair's pre-critical date claims could be relied upon under *§ 135(b)* (Br. 17), not to **demonstrate** that some of Adair's pre-critical date claims could not be relied upon. Under Carter's analysis, demonstrating that **two** patent claims out of many are invalid would be sufficient to shift the burden of production to the patentee to show that **all** of its claims are valid. It is doubtful that Carter would argue that the burden should be shifted under such circumstances.

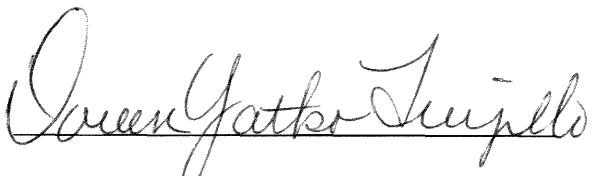
Carter further contends that the showing by Carter was completely reasonable in view of the page limitations for briefs in an interference. Carter alleges that it would have been impossible for Carter to separately address each of Adair's pre-critical date claims in the 25-page limit (Red Br. 48). Of course, Carter could have asked for a waiver of the page limit. Regardless, Adair is aware of no precedent excusing a party from meeting their burden because of a page limit.

CONCLUSION AND STATEMENT OF RELIEF SOUGHT

Adair contends that the Board erred as a matter of law in finding that Adair claim 24 does not comply with 35 U.S.C. § 135(b)(1). Adair respectfully requests that this Court reverse the Board's decision and deny Carter Substantive Motion 1.

Respectfully Submitted,

Dated: August 15, 2011



Doreen Yatko Trujillo
Kyle Vos Strache
Cozen O'Connor, P.C.
1900 Market St.
Philadelphia, PA 19103
215-665-2000

Attorneys for Appellants
John Robert Adair, Diljeet Singh Athwal,
And John Spencer Emtage

CERTIFICATE OF SERVICE

United States Court of Appeals
for the Federal Circuit
No. **2011-1212 (Interference No. 105,744)**
----------)
John Robert Adair, Diljeet Singh Athwal, and
John Spencer Emtage

Appellants,

v.

Paul J. Carter and Leonard G. Presta,

Cross Appellants.
----------)

I, Elissa Matias being duly sworn according to law and being over the age of 18, upon my oath depose and say that:

Counsel Press was retained by COZEN O'CONNOR, Attorneys for Appellants to print this document. I am an employee of Counsel Press.

On the **15th Day of August, 2011**, I served the within **Reply Brief of Appellants John Robert Adair, Diljeet Singh Athwal, and John Spencer Emtage** upon:

Oliver R. Ashe, Jr.
ASHE, P.C.
11440 Isaac Newton Square North
Suite 210
Reston, VA 20190
Tel: 703-467-9001
Fax: 703-467-9002

via Federal Express, overnight delivery by causing 2 true copies of each, enclosed in a properly addressed wrapper, to be deposited in an official depository of FedEx.

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August 15, 2011

United States Court of Appeals for the Federal Circuit

(Interference No. 105,744)

JOHN ROBERT ADAIR, DILJEET SINGH ATHWAL, AND JOHN SPENCER EMTAGE,
Appellants,

v.

PAUL J. CARTER AND LEONARD G. PRESTA,
Appellees.

2011-1212

Appeal from the United States Patent and Trademark Office, Board of Patent Appeals and Interferences.

Decided: February 7, 2012

DOREEN YATKO TRUJILLO, Cozen O'Connor, P.C., of Philadelphia, Pennsylvania, argued for appellants. With her on the brief was KYLE VOS STRACHE.

OLIVER R. ASHE, JR., Ashe, P.C., of Reston, Virginia, argued for appellees. Of counsel on the brief were JEFFREY P. KUSHAN and RACHEL H. TOWNSEND, Sidley Austin, LLP, of Washington, DC.

Before RADER, *Chief Judge*, LINN and MOORE, *Circuit Judges*.

LINN, *Circuit Judge*.

Appellants John Robert Adair, Diljeet Singh Athwal, and John Spencer Emtage (collectively, "Adair") appeal a decision of the Board of Patent Appeals and Interferences ("Board") holding that Adair's single claim involved in Interference 105,744 with junior party Paul J. Carter and Leonard G. Presta (collectively, "Carter") was barred under 35 U.S.C. § 135(b)(1). Because the Board properly determined that Adair's claim was barred under § 135(b)(1), this court affirms.

I. BACKGROUND

On November 21, 2005, Adair filed U.S. Application Serial No. 11/284,261 ("'261 Application") with the United States Patent and Trademark Office ("PTO"). In a preliminary amendment filed concurrently with this application, Adair requested an interference based on Carter's U.S. Patent No. 6,407,213 ("'213 Patent"). The only count of the interference is drawn to humanized antibodies. More specifically, the count involves non-human amino acid substitutions on specific residues of the heavy chain variable domain (an antibody comprises two light chains and two heavy chains, each with a "constant" and "variable" domain). On February 2, 2010, the Board declared the interference, identifying the claims in the count to be claims 30, 31, 60, 62, 63, 66, 67, 70, 73, 77-81 of the '213 Patent and claim 24 of the '261 Application. *Carter v. Adair*, Interference No. 105,744, Declaration of Interference at 4 (Feb. 2, 2010). The Board awarded Adair priority benefit to PCT/GB90/02017 ("PCT Application"), filed December 21, 1990, which claims priority to a British application filed by Adair on December 21, 1989.

Claim 66 of Carter's '213 Patent, representative of the claims in the count and the basis for an interference-in-fact, recites:

> **66.** A humanized antibody heavy chain variable domain comprising non-human Complementarity Determining Region (CDR) amino acid residues which bind antigen incorporated into a human antibody variable domain, and further comprising a Framework Region (FR) amino acid substitution at a site selected from the group consisting of: 24H [H=heavy], 73H, 76H, 78H, and 93H, utilizing the numbering system set forth in Kabat.

'213 Patent col.88 l.66-col.89 l.6.

Corresponding claim 24 in Adair's '261 Application recites:

> 24. A humanised antibody *comprising a* heavy chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises a *non-human* amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof, as numbered according to Kabat.

'261 Application, Preliminary Amendment and Request for Interference dated Nov. 21, 2005 at 3, *as amended by* Amendment of Sept. 9, 2009 at 4 (added language emphasized).

Because Adair's claim 24 was not presented to the PTO prior to June 18, 2003, one year from issuance of the Carter '213 Patent (the "critical date") as required by 35 U.S.C. § 135(b)(1), Adair relied on pre-critical date claims

1 and 16 of the PCT Application and corresponding U.S. national stage Application No. 07/743,329 ("'329 Application") to avoid the bar of § 135(b)(1). Claims 1 and 16 recite:

> 1. A CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.
>
> 16. A CDR-grafted antibody heavy or light chain or molecule according to any one of the preceding claims comprising human acceptor residues and non-human donor residues.

PCT Application at 67-69. Adair originally relied on claim 8 of the PCT Application, but because that claim related to light chains, Adair later abandoned that argument. In its request for rehearing before the Board, Adair argued for the first time that claim 2 of the PCT Application also provided pre-critical date support for claim 24, but the Board declined to consider this argument for the first time on rehearing. *Carter v. Adair*, Interference No. 105,774, Decision on Request for Rehearing at 4-5 (Nov. 5, 2010) ("*Rehearing*").

At the national stage, the examiner originally rejected each of Adair's PCT claims under one or more of the following sections: 101, 102(b), 103, and 112 first and second paragraphs. '329 Application, Office Action of November 18, 1992. Adair cancelled the PCT claims and added claims 23-66, later cancelled by an amendment adding claims 67-119 requiring multiple amino acid substitutions at specific locations in the heavy chain. '329

Application, Amendments of January 19, 1993 and April 16, 1993.

The Board rejected Adair's argument that claims 1 and 16 in the PCT Application provide pre-critical date support for claim 24 in the '261 Application because: (1) the PCT claims were not patentable to Adair; (2) Adair added limitations to overcome the examiner's rejection; and accordingly, (3) material differences presumptively existed between the post- and pre-critical date claims that Adair failed to rebut. *Carter v. Adair*, Interference No. 105,774, Decision on Motions at 9-10 (Aug. 30, 2010) ("*Decision*"). Citing *Regents of the University of California v. University of Iowa Research Foundation*, 455 F.3d 1371, 1377 (Fed. Cir. 2006), the Board stated that "[a]n applicant cannot expect to avoid the bar of § 135(b) by timely copying a claim from an issued patent when that claim is not patentable to that applicant." *Decision* at 10-11. On rehearing, the Board rejected Adair's assertion that materiality must be "determined in view of the patent claims being copied" and declined to compare Adair's post- or pre-critical date claims with copied claim 66 from Carter's '213 Patent. *Rehearing* at 3. Adair appeals, and this court has jurisdiction pursuant to 28 U.S.C. § 1295(a)(4)(A).

II. DISCUSSION

A. Standard of Review

"We review the Board's construction of 35 U.S.C. § 135(b)(1) de novo, as statutory interpretation is a question of law." *In re Berger*, 279 F.3d 975, 980 (Fed. Cir. 2002).

B. Analysis

Adair argues that the Board erred by failing to assess material differences "in view of the patent claim being copied [claim 66 from Carter's '213 Patent]." Appellant Br. 22. According to Adair, this court's precedent does not endorse a test that allows the Board to completely ignore copied claim 66 from Carter's '213 Patent when assessing the material differences between the post- and pre-critical date claims. Adair argues that the materiality test from *Berger* and *Regents* requires an assessment of material limitations based on the "identity" between the post-critical date claim and copied claim 66 from Carter's '213 Patent—in other words, in view of the "count"—and not based on the post-critical date claim standing alone. *See Regents*, 455 F.3d at 1375 ("[A]s this court's precedent explains, California must demonstrate that claims in the '191 application provide pre-critical date support for the *post-critical date identity* between claim 205 [the post-critical date claim] and the '646 patent [the issued patent]." (emphasis added)); *Berger*, 279 F.3d at 983.

Carter counters that the question of "[w]hether there is a sufficient degree of identity between pre- and post-critical date claims for compliance with § 135(b) is an inquiry that is distinct and independent" from any comparison with the patent claims copied. Appellee Br. 33. According to Carter, the Board correctly interpreted § 135(b)(1) in holding that "establishing support for post-critical date claims does not entail looking at material limitations of the patented claims." *Id.* 42.

This court agrees with Carter. Section 135(b)(1) states:

> A claim which is the same as, or for the same or substantially the same subject matter as, a claim of an issued patent may not be made in any appli-

> cation unless such a claim is made prior to one year from the date on which the patent was granted.

35 U.S.C. § 135(b)(1). Notwithstanding the seemingly strict language of the statute, a limited exception to this one year bar exists "where the copier had already been claiming substantially the same invention as the patentee" during the critical time period. *Corbett v. Chisholm*, 568 F.2d 759, 765 (CCPA 1977).

i.

In *Corbett*, the post-critical date claims "correspond[ed] exactly" with issued "Chisholm patent" claim 1. 568 F.2d at 759. The Board rejected Corbett's post-critical date claims under § 135(b)(1). *Id.* Corbett relied upon several groups of pre-critical date claims from the application and a predecessor application in an attempt to avoid the § 135(b) bar. *Id.* at 761-63. On appeal, this court compared the "copied claim" with the pre-critical date claims and affirmed the Board's finding that material differences precluded Corbett from relying on any of the pre-critical date claims to overcome the § 135(b) bar. *Id.* at 765-66. In identifying certain limitations of Chisholm patent claim 1 as "material," the court was simply noting the material differences that existed between that claim as copied by Corbett after the critical date and those pre-critical date claims Corbett was relying on to overcome the § 135(b) bar. The court did not establish any rule requiring some sort of threshold assessment of which limitations of the copied patent claim are material before determining whether material differences exist between post- and pre-critical date claims. In making this comparison, the court referenced Chisholm patent claim 1 only because that was the post-critical date claim.

Similarly, in *Berger*, the post-critical date claim was copied directly from and identical to issued "Muller patent" claim 1. 279 F.3d at 978. The examiner rejected Berger's pre-critical date claims 1-6 for indefiniteness and other grounds, and rejected post-critical date claim 7 under § 135(b)(1). *Id.* at 979. The Board rejected Berger's argument that claims 1-6 provided pre-critical date support for claim 7 because it found material differences between the "copied claim" and the pre-critical date claims, and this court affirmed. *Id.* at 982 ("The Board found the 'circumferential groove' limitation to be material because it was added by Muller during prosecution to avoid prior art. We agree with the Board's determination of materiality."). Again, the court in *Berger* referenced the issued Muller patent claim 1 only because the post-critical date claim, claim 7, was a direct copy of the patent claim. *Id.* at 981-83. This court affirmed the Board's analysis based only on the material differences between the *post- and pre-critical date claims*. *Id.* at 983 ("Because Berger's *original claims 1-6* [the pre-critical date claims] *do not include a material limitation of Berger claim 7* [the post-critical date claim], copied claim 7 is not entitled to the earlier effective date of those original claims for purposes of satisfying § 135(b)." (emphasis added)).

In *Regents*, this court expressly approved an analysis of material differences based solely on a comparison of the post- and pre-critical date claims in order to obtain the benefit of the earlier filing date:

> The Board compared claim 205 [the post-critical date claim] with claims 202-203 . . . and then with claim 204 [collectively, the pre-critical date claims]. The Board found that California's claim 205 contained material differences from claims 202-204. Therefore, claim 205 could not benefit from the earlier filing date of those claims. . . . On

> appeal, California does not contest the Board's finding of material differences between claim 205 and claims 202-204. Instead, California challenges the Board's conclusion that the correct inquiry under § 135(b)(1) asks whether claims 202-204 contain material differences from claim 205 and not whether claims 202-204 are to the same invention as claims in the '646 patent.

455 F.3d at 1373. The court in *Regents* rejected California's argument, explaining that "the relationship between the post- and pre-critical date claims . . . is not only relevant, but dispositive of the section 135(b)(1) question." *Id.* at 1374. Adair's arguments in this case are similar to California's arguments in *Regents*, where the court held that there is no requirement that the Board reference the issued patent claim(s) in the count to assess the material differences between the post- and pre-critical date claims. *Id.* at 1374-76.

The statement in *Regents* that the applicant's earlier filed claims must "provide pre-critical date support for the *post-critical date identity* between [the post-critical date claim] and the [issued patent]" to avoid the § 135(b)(1) bar, 455 F.3d at 1375 (emphasis added), does not require the Board to assess material differences in view of the issued patent claim(s) in the count. *See Berger*, 279 F.3d at 982. The question of material differences between post- and pre-critical date claims for purposes of overcoming a § 135(b) bar "is a distinctly different question from whether claims . . . are directed to the same or substantially the same subject matter" for purposes of provoking an interference. *Id.* As explained in *Regents*, § 135(b) is a statute of repose, intended to "limit[] the patentee's vulnerability to a declaration of an interference" by "limit[ing] the window of time in which the cause of the interference can occur." 455 F.3d at 1376. When a material difference exists between the post- and pre-critical

date claims, a belated interference is improper because it would be a "*different interference*[]" than that which "should have been earlier declared by the PTO." *Id.* (emphasis added).

For these reasons, this court holds that to overcome a § 135(b) bar for a post-critical date claim, an applicant must show that such claim is not materially different from a pre-critical date claim present in the application or any predecessor thereto in order to obtain the benefit of the earlier filing date. Any claims filed within the critical period, whether or not later cancelled, may provide pre-critical date support for the later filed patent claim(s), so long as the pre-critical date claims are not materially different from the later filed claim(s). *Corbett*, 568 F.2d at 765-66; *see also Regents*, 455 F.3d at 1373; *Berger*, 279 F.3d at 981-82.

Here, the Board found material differences between post-critical date claim 24 of the '261 Application and pre-critical date claims 1 and 16 of the PCT Application based on the prosecution history of the '261 Application. During prosecution, Adair added several limitations to claim 24—limitations not present in claims 1 and 16 of the PCT Application—to avoid examiner rejections during prosecution. *Decision* at 9. Adair failed to rebut the Board's finding with any evidence that the differences between claim 24 and claims 1 and 16 of the PCT Application were immaterial. *Id.* at 10. Adair criticizes the Board for failing to consider claim 66 from Carter's '213 Patent in assessing material differences. But, for the reasons explained above, an assessment of claim 66 was not necessary. What was required in determining whether the § 135(b) bar might be overcome was an assessment of the material differences between the post- and pre-critical date claims, which is precisely what the Board did.

ii.

Adair also contends that the Board erred in applying *Festo Corp. v. Shoketsu Kinzoku Kogyo Kabushiki Co.*, 535 U.S. 722, 734 (2002) in the context of an interference to conclude that a limitation added to a claim in response to a rejection that results in allowance is presumed to be necessary to patentability and therefore "material." Adair asserts that the burden of proof for the § 135(b) motion lay with Carter, and thus Adair cannot be faulted "for not providing any reason why the limitations that differ . . . were not material." Appellant Br. 25. Carter counters that "the Board's presumption of material differences is firmly grounded in the law." Appellee Br. 44. *See Parks v. Fine*, 773 F.2d 1577, 1579 (Fed. Cir. 1985); *Corbett*, 568 F.2d at 765.

Carter is correct. When an applicant adds limitations in response to an examiner's rejection, and those limitations result in allowance, there exists a well established presumption that those limitations are necessary to patentability and thus material. *See Festo*, 535 U.S. at 734; *Corbett*, 568 F.2d at 765. This presumption applies with equal force in the interference context. *Parks*, 773 F.2d at 1579 (holding in an interference case that "[t]he insertion of [a] limitation to overcome the examiner's rejection is *strong, if not conclusive, evidence of materiality*" (emphasis added)). Here, because Adair cancelled claims 1 and 16 of the PCT Application in response to the examiner's rejections, and added limitations into what eventually became claim 24 of the '261 Application to secure allowance, the Board properly presumed material differences between Adair's post- and pre-critical date claims. Adair failed to rebut this presumption.

iii.

Adair argues that the Board erred by establishing an absolute requirement that the pre-critical date claims be patentable to the applicant for the applicant to rely on those claims to avoid the § 135(b) bar. Carter counters that the Board did not articulate such a requirement, but even if it did, the requirement is appropriate. The Board quoted language from *Regents*, where this court stated that it "perceives no inequity in a construction of section 135(b)(1) that might, in some circumstances, prevent a patent applicant from relying on the filing date of a claim to which it was not statutorily entitled." *Regents*, 455 F.3d at 1377.

The court in *Regents* did not articulate a per se patentability requirement for an applicant to rely on pre-critical date claims, but rather observed that where material limitations are added to overcome an examiner's rejection after the critical date, there is "no inequity" in finding the later added claims barred under § 135(b)(1). Adair is correct that cancelled claims may be relied upon to avoid the § 135(b) bar. *See Corbett*, 568 F.2d at 765 ("The words 'prior to' in the present code clearly point to a 'critical date' prior to which . . . the copier had to be claiming the invention, whether or not the claims were subsequently cancelled."). Adair is incorrect, however, in contending that the Board established any absolute requirement that the pre-critical date claims must have been patentable to Adair. Even if it did, the error would have been harmless because the Board found material differences between the post- and pre-critical date claims, which Adair failed to rebut.

iv.

Finally, Adair argues that the Board abused its discretion in failing to consider claim 2 of the PCT Application as pre-critical date support for claim 24. The Board did not abuse its discretion in declining to consider claim 2 of the PCT Application for the first time on rehearing. 37 C.F.R. § 41.125(c), governing rehearing before the Board, provides that "[t]he burden of showing a decision should be modified lies with the party attacking the decision [and t]he request must specifically identify . . . (ii) The place *where the matter was previously addressed* in a motion, opposition, or reply." 37 C.F.R. § 41.125(c)(3) (emphasis added). Because Adair failed to previously address claim 2 prior to its petition for rehearing, the Board properly refused to consider it on rehearing.

III. CONCLUSION

For the foregoing reasons, this court affirms the decision of the Board.

AFFIRMED

2011-1212
(Interference No. 105,744)

UNITED STATES COURT OF APPEALS FOR THE FEDERAL CIRCUIT

JOHN ROBERT ADAIR, DILJEET SINGH ATHWAL,
and JOHN SPENCER EMTAGE,

Appellants,

v.

PAUL J. CARTER and LEONARD G. PRESTA,

Appellees.

Appeals from the United States Patent and Trademark Office, Board of Patent Appeals and Interferences.

PETITION FOR PANEL REHEARING of APPELLANTS JOHN ROBERT ADAIR, DILJEET SINGH ATHWAL, and JOHN SPENCER EMTAGE

Doreen Yatko Trujillo
(*Counsel of Record)*
Kyle Vos Strache
Cozen O'Connor, P.C.
1900 Market St.
Philadelphia, PA 19103
215-665-2000

Attorneys for Appellants John Robert Adair,
Diljeet Singh Athwal,
and John Spencer Emtage

Dated: March 7, 2012

CERTIFICATE OF INTEREST

Counsel for the (petitioner) (appellant) (respondent) (appellee) (amicus) (name of party) APPELLANT ADAIR certifies the following (use "None" if applicable; use extra sheets if necessary):

1. The full name of every party or amicus represented by me is:

John Robert Adair, Diljeet Singh Athwal, and John Spencer Emtage

2. The name of the real party in interest (if the party named in the caption is not the real party in interest) represented by me is:

UCB Pharma S.A.

3. All parent corporations and any publicly held companies that own 10 percent or more of the stock of the party or amicus curiae represented by me are:

UCB Pharma S.A. is wholly-owned by UCB S.A.
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4. The names of all law firms and the partners or associates that appeared for the party or amicus now represented by me in the trial court or agency or are expected to appear in this court are:

Cozen O'Connor P.C. – Doreen Yatko Trujillo, Michael B. Fein, Kyle Vos Strache

March 7, 2012
Date

Doreen Yatko Trujillo
Signature of counsel

Doreen Yatko Trujillo
Printed name of counsel

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POINTS OF LAW OR FACT OVERLOOKED OR MISAPPREHENDED BY THE PANEL

1. The panel's adoption of Carter's position that "establishing support for post-critical date claims does not entail looking at material limitations of the patented claims" misapprehends, and appears to directly conflict with, 35 U.S.C. § 135(b) and binding precedent of this Court.
2. The panel's assertion that the Board found that Adair added limitations to its post-critical date claim not present in its pre-critical date claims to avoid examiner rejections during prosecution is factually incorrect and not supported by the record.
3. The panel's requirement that Adair rebut a factual finding and a presumption before either was levied against Adair and, in the case of the presumption, before it was even created, is factually and legally impossible.
4. The panel's failure to address the differences between the two reported versions of *In re Berger* overlooks the fact that the two versions yield different results and, therefore, leaves a conflict unresolved.

ARGUMENT

In a precedential opinion, the panel affirmed the Board of Patent Appeals and Interferences ("Board") finding that Adair's claim involved in the interference, claim 24, was barred under 35 U.S.C. § 135(b). Slip Op. at 13. As indicated

above, and discussed in more detail below, the panel misapprehended or overlooked several points of law and fact in its opinion.

1. The panel's adoption of Carter's position that "establishing support for post-critical date claims does not entail looking at material limitations of the patented claims" misapprehends, and appears to directly conflict with, 35 U.S.C. § 135(b) and binding precedent of this Court.

Section 135(b) requires that a claim to, at least, substantially the same subject matter as **a claim of an issued patent** be made prior to one year from the date on which the patent was granted. 35 U.S.C. § 135(b) (emphasis added). As the panel noted "a limited exception to this one year bar statute exists 'where the copier had already been claiming substantially the same invention as the **patentee**' during the critical time period." Slip Op. at 7 (citing *Corbett v. Chisholm*, 568 F.2d 759, 765 (CCPA 1977)) (emphasis added). Section 135(b) does not require that the claim be identical to a claim of an issued patent, or that it be to the same subject matter as a claim of an issued patent, just that it be to substantially the same subject matter as a claim of an issued patent. A claim is to substantially the same subject matter as a claim of an issued patent if it has all material limitations of the patent claim, i.e., all limitations necessary to patentability of the patent claim. *Corbett* at 765-66.

Notwithstanding the foregoing, the panel adopted Carter's position that, to establish pre-critical date support for post-critical date claims, one does not need to consider the material limitations of the patented claims at all. Under the panel's

analysis, one only looks at the pre- and post-critical date claims of the provocateur of the interference. Under such an analysis, the pre-critical date claim could be lacking a material limitation of the patent claim, yet the interference could still proceed. Alternatively, as in the present case, the pre-critical date claims could contain all material limitations of the patent claim, which is all that § 135(b) requires, yet the interference will not proceed. The panel's adoption of the position that one does not need to consider the material limitations of the patent claims at all not only misapprehends § 135(b) and binding precedent of this Court, but it also appears to be in direct conflict with both.

In support of its position, the panel stated that the court in *Corbett* did not establish any rule requiring a threshold assessment of which limitations of the copied patent claim are material and "referenced Chisholm patent claim 1 only because that was the post-critical date claim." Slip. Op at 7. The panel's statement is not consistent with *Corbett*. The court in *Corbett* not only referred to the patented claim, but it also referred to Figures 1 and 4 of the patent to support its conclusion that the **patentee** contemplated sufficiently severe reduction and expansion steps. *Corbett* at 760. Both steps were considered to be material by the court in its assessment of compliance with § 135(b). *Id.*, at 765-6. Thus, the court in *Corbett* clearly made a threshold assessment of materiality based upon the patent claim. Notably, the court in *Corbett* did not argue that the limitations were

material simply because they were added by **Corbett**, the provocateur of the interference, to its own pre-critical date claims.

The panel made a similar assertion regarding *In re Berger*, 279 F.3d 975 (Fed. Cir. 2002). The panel stated that the Court in *Berger* "referenced the issued Muller patent claim 1 only because the post-critical date claim, claim 7, was a direct copy of the patent claim." Slip Op. at 8 (citing *Berger* at 981-83). Again, the panel's statement is not consistent with *Berger*. The Court in *Berger* did not merely reference the patent claim; it referenced the prosecution history of the patent claim. The Court in *Berger* found that the limitation "circumferential groove" in the copied claim, i.e., the post-critical date claim, was material "because it was added by Muller [the patentee] during prosecution to avoid prior art." *Berger* at 982. The Court in *Berger* did not argue that the "circumferential groove" limitation was material because it was added by **Berger**, the provocateur of the interference, during prosecution of its own claims but, rather, because the limitation was added by **Muller**, the patentee, during prosecution of the patent claims.

To the extent the panel may take the position that the situation is different when the post-critical date claim is not identical to the patent claim, Adair directs the panel to *Parks v. Fine*, 773 F.2d 1577 (Fed. Cir. 1985). In *Parks*, the post-critical date claim was **not** identical to the patented claim. *Id.* at 1578. Once

again, however, the Court assessed materiality of a limitation based upon the patented claim. "The record establishes that the 'absence of a catalyst' limitation in the **Parks patent** claims and the contested counts is material. **Parks** inserted this limitation in his claims in response to, and to avoid, a rejection by the examiner." *Id.* at 1579 (emphasis added). The Court did not find that the limitation was material simply because it was added to the pre-critical date claims of **Fine**, the provocateur of the interference, but rather because it was added by **Parks**, the patentee, during prosecution of the patent claims.[1]

The only precedent arguably consistent with the panel's position is *Regents of the Univ. of Cal. v. Univ. of Iowa Res. Found.*, 455 F.3d 1371 (Fed. Cir. 2006) *reh'g en banc denied*, 2006 U.S. Appl. Lexis 27583 (Fed. Cir. Oct. 16, 2006).[2] *Regents* is cited as approving an analysis of material differences based **solely** upon a comparison of post- and pre-critical date claims. Slip Op. at 8 (emphasis added). To the extent *Regents* approved such an analysis, however, it is not consistent with the prior binding precedent of this Court as discussed above, i.e., *Corbett*, *Berger*,

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[1] The panel relied upon *Parks* to support the levying of a presumption regarding materiality based upon Adair's prosecution, but seems to have overlooked the fact that the passage it relied upon was referring to what occurred during prosecution of the patent claim. Slip Op. at 11.

[2] *Regents* cites the correct standard for assessing compliance with § 135(b) -- "[A]s this court's **precedent** explains, California must demonstrate that claims in the '191 application provide pre-critical date support for the *post-critical date **identity*** between [the post-critical date claim] and the [issued patent]" -- but apparently did not apply it. *Id.* at 1375 (emphasis in bold added; emphasis in italics in Slip Op. at 9).

and *Parks*. Binding precedent cannot be overruled by a panel decision; binding precedent can only be overruled *en banc*. *Mothers Restaurant, Inc. v. Mama's Pizza, Inc.*, 723 F.2d 1566, 1573 (Fed. Cir. 1983).

2. The panel's assertion that the Board found that Adair added limitations to its post-critical date claim not present in its pre-critical date claims to avoid examiner rejections during prosecution is factually incorrect and not supported by the record.

Citing the Board's decision, the panel stated that one of the reasons the Board rejected Adair's arguments that claims 1 and 16 of the PCT Application provide pre-critical date support for claim 24 was because Adair added limitations to overcome the examiner's rejection. Slip. Op. at 5, 10. The Board, however, never stated that Adair had **added limitations** to claims 1 and 16, just that there were limitations that differed between involved claim 24 and claims 1 and 16:

> Adair does not provide any reason why the limitations that differ between involved claim 24 and original claims 1 and 16 were not necessary to the patentability of claim 24.

A10. Nor did the Board state what limitations allegedly differed between the two sets of claims; the Board simply levied a presumption of materiality based upon the cancellation of claims 1 and 16 after rejection. A9-10. Regardless, such a finding is not supported by the record. A comparison between claim 24 and claims 1 and 16 of the PCT Application reveals that **all** limitations of claim 24 are recited in

claims 1 and 16, including the two words emphasized by the panel. *See* Slip Op. at 3-4.

Further, the presumption of materiality levied by the Board, and approved by the panel, is based upon a fiction that the amendments to claim 24 on September 9, 2009 were in response to rejections levied almost 16 years earlier against different claims. A9-10; Slip Op. at 3, 11. The rejections being relied upon were levied November 18, 1992 against, among others, claims 1 and 16 of the PCT Application. A9. Claims 1 and 16 of the PCT Application were cancelled shortly thereafter, i.e., on January 19, 1993. A9. Claim 24 was added on November 21, 2005 to provoke the interference. Blue Br. at 4, 6. Claim 24 was clearly not amended on September 9, 2009 in response to a rejection levied almost 16 years earlier. By relying upon the presumption, the panel is disregarding the facts in favor of a fiction.

Indeed, it is difficult to understand how a presumption could ever be levied when, as a panel of this Court recently confirmed, multiple pre-critical date claims can be relied upon to show support for the post-critical date claim. *See Pioneer v. Monsanto,* No. 2011-1285, 2012 WL 612800 (Fed. Cir. February 28, 2012). If multiple claims can be relied upon to show support for post-critical date claims, then what happens to an individual claim, i.e., whether it was rejected or not, cannot be relevant. Consistent with this, *Pioneer* contained no analysis of what

happened to the pre-critical date claims during prosecution to arrive at the post-critical date claim, even though a review of the underlying facts reveals that the provocateur had admitted that at least one recitation in the post-critical date claim was added to overcome a rejection over the prior art. *Id.*[3]

3. The panel's requirement that Adair rebut a factual finding and a presumption before either was levied against Adair and, in the case of the presumption, before it was even created, is factually and legally impossible.

The panel criticized Adair for not rebutting the finding regarding pre-critical date claims 1 and 16 of the PCT Application with any evidence that the differences were immaterial. Slip Op. at 10. The panel made the same assertion regarding the presumption. Slip Op. at 11. First, the Board never identified which differences were material; instead the Board levied a presumption of materiality based on alleged differences. Second, both the finding and presumption were levied for the first time in the Board's decision. Indeed, the presumption was **created** for the

[3] Several differences between the post-critical date claim and the pre-critical date claims are evident in *Pioneer*, even when the pre-critical date claims are combined. In particular, the recitation "transformed cell" is completely absent from the pre-critical date claims. See *Pioneer*; Slip Op. at 7. Indeed, Monsanto, the provocateur of the interference, admitted that the "transformed cell" recitation was added to overcome an obviousness rejection. *Monsanto v. Pioneer,* Interference No. 105,728, Monsanto Opposition 1, Appendix 2 (March 25, 2010) (Material Facts 24 and 26-28 admitted by Monsanto); *Monsanto v. Pioneer,* Interference No. 105,728, Pioneer Motion 1, Appendix 2 (February 24, 2010). Yet, the Board did not even discuss materiality in its decision. *Monsanto v. Pioneer*, Interference 105,728, Decision, Bd. R. 125 (April 22, 2010). (All of the foregoing papers from Interference No. 105,728 are available on the United States Patent & Trademark Office's website, in the Interference Portal.)

first time in the Board's decision. Adair could not have rebutted either one before it was levied, which means that Adair could not have rebutted either one before it filed its request for rehearing.

The panel also asserted, however, that the Board did not abuse its discretion in refusing to consider a rebuttal argument Adair made in it its request for rehearing, i.e., that claim 2 of the PCT Application contained all material limitations of claim 24. Slip Op. at 13. The panel cited the rule governing rehearings before the Board that requires the requestor to show where it previously addressed a matter in a motion, opposition, or reply. *Id.* At the time Adair filed its opposition, however, no finding or presumption existed. Adair could not have addressed a finding or a presumption before it was levied. The panel has imposed a standard which is impossible for Adair to meet and has left Adair without any legal recourse. At a minimum, the panel should have considered claim 2 of the PCT Application, or remanded the matter to the Board to do so.

4. The panel's failure to address the differences between the two reported versions of *In re Berger* overlooks the fact that the two versions yield different results and, therefore, leaves a conflict unresolved.

As Adair pointed out in its reply brief, there is a discrepancy in the various reported versions of a statement in *Berger*. The Lexis® and Westlaw® electronic databases report the statement as follows:

> This is a distinctly different question from whether claims made for purposes of interference by different parties are directed to **interfering subject matter**.

Other electronic databases, as well as the book version of the Federal Reporter, report the statement as follows:

> This is a distinctly different question from whether claims made for purposes of interference by different parties are directed **to the same or substantially the same subject matter**.

Berger at 982. The differences between the two are highlighted in bold. *See* Gray Br. at 8. Without addressing the discrepancy, the panel relies upon the latter version to support its contention that material differences between post- and pre-critical date claims for purposes of overcoming a § 135(b) is a distinctly different question from whether the claims are directed to substantially the same subject matter. Slip Op. at 9. The Court should grant rehearing not only to clarify this conflict in the reported versions of *Berger*, but also because the outcome of the present appeal is clearly affected by which version is being relied upon -- the first version does not support the panel's contention.

Adair maintains that the correct version is the first one. As Adair argued previously, the sentence immediately preceding the statement in question sets forth what must be shown under § 135(b). Gray Br. at 8-9 and *Berger* at 981-82. As discussed above, § 135(b) recites the language "the same or substantially the same subject matter." 35 U.S.C. § 135(b). The sentence immediately following the

passage states that the "comparison standard of 37 C.F.R. § 1.601(n) was formulated not to determine the effective date of a claim in one party's application for compliance with § 135(b), but instead to define the extent of **interfering subject matter** as between applications of potentially conflicting parties." *Id.* at 982 (emphasis added). In the statement in question, then, interfering subject matter under (prior) 37 C.F.R. § 1.601(n) was being distinguished from the requirements under § 135(b), which is consistent with the first reported version. *Id* at 981-82. Further, under the second reported version, showing that claims are to the *same or substantially the same subject matter* is being distinguished from showing that claims are to the *same or substantially the same subject matter,* which is a distinction without a difference.

CONCLUSION

The petition for panel rehearing should be granted.

Respectfully Submitted,

Dated: March 7, 2012

Doreen Yatko Trujillo
Kyle Vos Strache
Cozen O'Connor, P.C.
1900 Market St.
Philadelphia, PA 19103
215-665-2000

Attorneys for Appellants
John Robert Adair, Diljeet Singh Athwal,
And John Spencer Emtage

ADDENDUM

United States Court of Appeals for the Federal Circuit

(Interference No. 105,744)

JOHN ROBERT ADAIR, DILJEET SINGH ATHWAL, AND JOHN SPENCER EMTAGE,
Appellants,

v.

PAUL J. CARTER AND LEONARD G. PRESTA,
Appellees.

2011-1212

Appeal from the United States Patent and Trademark Office, Board of Patent Appeals and Interferences.

Decided: February 7, 2012

DOREEN YATKO TRUJILLO, Cozen O'Connor, P.C., of Philadelphia, Pennsylvania, argued for appellants. With her on the brief was KYLE VOS STRACHE.

OLIVER R. ASHE, JR., Ashe, P.C., of Reston, Virginia, argued for appellees. Of counsel on the brief were JEFFREY P. KUSHAN and RACHEL H. TOWNSEND, Sidley Austin, LLP, of Washington, DC.

Before RADER, *Chief Judge*, LINN and MOORE, *Circuit Judges*.

LINN, *Circuit Judge*.

Appellants John Robert Adair, Diljeet Singh Athwal, and John Spencer Emtage (collectively, "Adair") appeal a decision of the Board of Patent Appeals and Interferences ("Board") holding that Adair's single claim involved in Interference 105,744 with junior party Paul J. Carter and Leonard G. Presta (collectively, "Carter") was barred under 35 U.S.C. § 135(b)(1). Because the Board properly determined that Adair's claim was barred under § 135(b)(1), this court affirms.

I. BACKGROUND

On November 21, 2005, Adair filed U.S. Application Serial No. 11/284,261 ("'261 Application") with the United States Patent and Trademark Office ("PTO"). In a preliminary amendment filed concurrently with this application, Adair requested an interference based on Carter's U.S. Patent No. 6,407,213 ("'213 Patent"). The only count of the interference is drawn to humanized antibodies. More specifically, the count involves non-human amino acid substitutions on specific residues of the heavy chain variable domain (an antibody comprises two light chains and two heavy chains, each with a "constant" and "variable" domain). On February 2, 2010, the Board declared the interference, identifying the claims in the count to be claims 30, 31, 60, 62, 63, 66, 67, 70, 73, 77-81 of the '213 Patent and claim 24 of the '261 Application. *Carter v. Adair*, Interference No. 105,744, Declaration of Interference at 4 (Feb. 2, 2010). The Board awarded Adair priority benefit to PCT/GB90/02017 ("PCT Application"), filed December 21, 1990, which claims priority to a British application filed by Adair on December 21, 1989.

Claim 66 of Carter's '213 Patent, representative of the claims in the count and the basis for an interference-in-fact, recites:

> **66.** A humanized antibody heavy chain variable domain comprising non-human Complementarity Determining Region (CDR) amino acid residues which bind antigen incorporated into a human antibody variable domain, and further comprising a Framework Region (FR) amino acid substitution at a site selected from the group consisting of: 24H [H=heavy], 73H, 76H, 78H, and 93H, utilizing the numbering system set forth in Kabat.

'213 Patent col.88 l.66-col.89 l.6.

Corresponding claim 24 in Adair's '261 Application recites:

> **24.** A humanised antibody *comprising a* heavy chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises a *non-human* amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof, as numbered according to Kabat.

'261 Application, Preliminary Amendment and Request for Interference dated Nov. 21, 2005 at 3, *as amended by* Amendment of Sept. 9, 2009 at 4 (added language emphasized).

Because Adair's claim 24 was not presented to the PTO prior to June 18, 2003, one year from issuance of the Carter '213 Patent (the "critical date") as required by 35 U.S.C. § 135(b)(1), Adair relied on pre-critical date claims

1 and 16 of the PCT Application and corresponding U.S. national stage Application No. 07/743,329 ("'329 Application") to avoid the bar of § 135(b)(1). Claims 1 and 16 recite:

> 1. A CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.
>
> 16. A CDR-grafted antibody heavy or light chain or molecule according to any one of the preceding claims comprising human acceptor residues and non-human donor residues.

PCT Application at 67-69. Adair originally relied on claim 8 of the PCT Application, but because that claim related to light chains, Adair later abandoned that argument. In its request for rehearing before the Board, Adair argued for the first time that claim 2 of the PCT Application also provided pre-critical date support for claim 24, but the Board declined to consider this argument for the first time on rehearing. *Carter v. Adair*, Interference No. 105,774, Decision on Request for Rehearing at 4-5 (Nov. 5, 2010) ("*Rehearing*").

At the national stage, the examiner originally rejected each of Adair's PCT claims under one or more of the following sections: 101, 102(b), 103, and 112 first and second paragraphs. '329 Application, Office Action of November 18, 1992. Adair cancelled the PCT claims and added claims 23-66, later cancelled by an amendment adding claims 67-119 requiring multiple amino acid substitutions at specific locations in the heavy chain. '329

Application, Amendments of January 19, 1993 and April 16, 1993.

The Board rejected Adair's argument that claims 1 and 16 in the PCT Application provide pre-critical date support for claim 24 in the '261 Application because: (1) the PCT claims were not patentable to Adair; (2) Adair added limitations to overcome the examiner's rejection; and accordingly, (3) material differences presumptively existed between the post- and pre-critical date claims that Adair failed to rebut. *Carter v. Adair*, Interference No. 105,774, Decision on Motions at 9-10 (Aug. 30, 2010) ("*Decision*"). Citing *Regents of the University of California v. University of Iowa Research Foundation*, 455 F.3d 1371, 1377 (Fed. Cir. 2006), the Board stated that "[a]n applicant cannot expect to avoid the bar of § 135(b) by timely copying a claim from an issued patent when that claim is not patentable to that applicant." *Decision* at 10-11. On rehearing, the Board rejected Adair's assertion that materiality must be "determined in view of the patent claims being copied" and declined to compare Adair's post- or pre-critical date claims with copied claim 66 from Carter's '213 Patent. *Rehearing* at 3. Adair appeals, and this court has jurisdiction pursuant to 28 U.S.C. § 1295(a)(4)(A).

II. DISCUSSION

A. Standard of Review

"We review the Board's construction of 35 U.S.C. § 135(b)(1) de novo, as statutory interpretation is a question of law." *In re Berger*, 279 F.3d 975, 980 (Fed. Cir. 2002).

B. Analysis

Adair argues that the Board erred by failing to assess material differences "in view of the patent claim being copied [claim 66 from Carter's '213 Patent]." Appellant Br. 22. According to Adair, this court's precedent does not endorse a test that allows the Board to completely ignore copied claim 66 from Carter's '213 Patent when assessing the material differences between the post- and pre-critical date claims. Adair argues that the materiality test from *Berger* and *Regents* requires an assessment of material limitations based on the "identity" between the post-critical date claim and copied claim 66 from Carter's '213 Patent—in other words, in view of the "count"—and not based on the post-critical date claim standing alone. *See Regents*, 455 F.3d at 1375 ("[A]s this court's precedent explains, California must demonstrate that claims in the '191 application provide pre-critical date support for the *post-critical date identity* between claim 205 [the post-critical date claim] and the '646 patent [the issued patent]." (emphasis added)); *Berger*, 279 F.3d at 983.

Carter counters that the question of "[w]hether there is a sufficient degree of identity between pre- and post-critical date claims for compliance with § 135(b) is an inquiry that is distinct and independent" from any comparison with the patent claims copied. Appellee Br. 33. According to Carter, the Board correctly interpreted § 135(b)(1) in holding that "establishing support for post-critical date claims does not entail looking at material limitations of the patented claims." *Id.* 42.

This court agrees with Carter. Section 135(b)(1) states:

> A claim which is the same as, or for the same or substantially the same subject matter as, a claim of an issued patent may not be made in any appli-

> cation unless such a claim is made prior to one year from the date on which the patent was granted.

35 U.S.C. § 135(b)(1). Notwithstanding the seemingly strict language of the statute, a limited exception to this one year bar exists "where the copier had already been claiming substantially the same invention as the patentee" during the critical time period. *Corbett v. Chisholm*, 568 F.2d 759, 765 (CCPA 1977).

i.

In *Corbett*, the post-critical date claims "correspond[ed] exactly" with issued "Chisholm patent" claim 1. 568 F.2d at 759. The Board rejected Corbett's post-critical date claims under § 135(b)(1). *Id.* Corbett relied upon several groups of pre-critical date claims from the application and a predecessor application in an attempt to avoid the § 135(b) bar. *Id.* at 761-63. On appeal, this court compared the "copied claim" with the pre-critical date claims and affirmed the Board's finding that material differences precluded Corbett from relying on any of the pre-critical date claims to overcome the § 135(b) bar. *Id.* at 765-66. In identifying certain limitations of Chisholm patent claim 1 as "material," the court was simply noting the material differences that existed between that claim as copied by Corbett after the critical date and those pre-critical date claims Corbett was relying on to overcome the § 135(b) bar. The court did not establish any rule requiring some sort of threshold assessment of which limitations of the copied patent claim are material before determining whether material differences exist between post- and pre-critical date claims. In making this comparison, the court referenced Chisholm patent claim 1 only because that was the post-critical date claim.

Similarly, in *Berger*, the post-critical date claim was copied directly from and identical to issued "Muller patent" claim 1. 279 F.3d at 978. The examiner rejected Berger's pre-critical date claims 1-6 for indefiniteness and other grounds, and rejected post-critical date claim 7 under § 135(b)(1). *Id.* at 979. The Board rejected Berger's argument that claims 1-6 provided pre-critical date support for claim 7 because it found material differences between the "copied claim" and the pre-critical date claims, and this court affirmed. *Id.* at 982 ("The Board found the 'circumferential groove' limitation to be material because it was added by Muller during prosecution to avoid prior art. We agree with the Board's determination of materiality."). Again, the court in *Berger* referenced the issued Muller patent claim 1 only because the post-critical date claim, claim 7, was a direct copy of the patent claim. *Id.* at 981-83. This court affirmed the Board's analysis based only on the material differences between the *post- and pre-critical date claims. Id.* at 983 ("Because Berger's *original claims 1-6* [the pre-critical date claims] *do not include a material limitation of Berger claim 7* [the post-critical date claim], copied claim 7 is not entitled to the earlier effective date of those original claims for purposes of satisfying § 135(b)." (emphasis added)).

In *Regents*, this court expressly approved an analysis of material differences based solely on a comparison of the post- and pre-critical date claims in order to obtain the benefit of the earlier filing date:

> The Board compared claim 205 [the post-critical date claim] with claims 202-203 . . . and then with claim 204 [collectively, the pre-critical date claims]. The Board found that California's claim 205 contained material differences from claims 202-204. Therefore, claim 205 could not benefit from the earlier filing date of those claims. . . . On

> appeal, California does not contest the Board's finding of material differences between claim 205 and claims 202-204. Instead, California challenges the Board's conclusion that the correct inquiry under § 135(b)(1) asks whether claims 202-204 contain material differences from claim 205 and not whether claims 202-204 are to the same invention as claims in the '646 patent.

455 F.3d at 1373. The court in *Regents* rejected California's argument, explaining that "the relationship between the post- and pre-critical date claims . . . is not only relevant, but dispositive of the section 135(b)(1) question." *Id.* at 1374. Adair's arguments in this case are similar to California's arguments in *Regents*, where the court held that there is no requirement that the Board reference the issued patent claim(s) in the count to assess the material differences between the post- and pre-critical date claims. *Id.* at 1374-76.

The statement in *Regents* that the applicant's earlier filed claims must "provide pre-critical date support for the *post-critical date identity* between [the post-critical date claim] and the [issued patent]" to avoid the § 135(b)(1) bar, 455 F.3d at 1375 (emphasis added), does not require the Board to assess material differences in view of the issued patent claim(s) in the count. *See Berger*, 279 F.3d at 982. The question of material differences between post- and pre-critical date claims for purposes of overcoming a § 135(b) bar "is a distinctly different question from whether claims . . . are directed to the same or substantially the same subject matter" for purposes of provoking an interference. *Id.* As explained in *Regents*, § 135(b) is a statute of repose, intended to "limit[] the patentee's vulnerability to a declaration of an interference" by "limit[ing] the window of time in which the cause of the interference can occur." 455 F.3d at 1376. When a material difference exists between the post- and pre-critical

date claims, a belated interference is improper because it would be a "*different interference*[]" than that which "should have been earlier declared by the PTO." *Id.* (emphasis added).

For these reasons, this court holds that to overcome a § 135(b) bar for a post-critical date claim, an applicant must show that such claim is not materially different from a pre-critical date claim present in the application or any predecessor thereto in order to obtain the benefit of the earlier filing date. Any claims filed within the critical period, whether or not later cancelled, may provide pre-critical date support for the later filed patent claim(s), so long as the pre-critical date claims are not materially different from the later filed claim(s). *Corbett*, 568 F.2d at 765-66; *see also Regents*, 455 F.3d at 1373; *Berger*, 279 F.3d at 981-82.

Here, the Board found material differences between post-critical date claim 24 of the '261 Application and pre-critical date claims 1 and 16 of the PCT Application based on the prosecution history of the '261 Application. During prosecution, Adair added several limitations to claim 24—limitations not present in claims 1 and 16 of the PCT Application—to avoid examiner rejections during prosecution. *Decision* at 9. Adair failed to rebut the Board's finding with any evidence that the differences between claim 24 and claims 1 and 16 of the PCT Application were immaterial. *Id.* at 10. Adair criticizes the Board for failing to consider claim 66 from Carter's '213 Patent in assessing material differences. But, for the reasons explained above, an assessment of claim 66 was not necessary. What was required in determining whether the § 135(b) bar might be overcome was an assessment of the material differences between the post- and pre-critical date claims, which is precisely what the Board did.

ii.

Adair also contends that the Board erred in applying *Festo Corp. v. Shoketsu Kinzoku Kogyo Kabushiki Co.*, 535 U.S. 722, 734 (2002) in the context of an interference to conclude that a limitation added to a claim in response to a rejection that results in allowance is presumed to be necessary to patentability and therefore "material." Adair asserts that the burden of proof for the § 135(b) motion lay with Carter, and thus Adair cannot be faulted "for not providing any reason why the limitations that differ . . . were not material." Appellant Br. 25. Carter counters that "the Board's presumption of material differences is firmly grounded in the law." Appellee Br. 44. *See Parks v. Fine*, 773 F.2d 1577, 1579 (Fed. Cir. 1985); *Corbett*, 568 F.2d at 765.

Carter is correct. When an applicant adds limitations in response to an examiner's rejection, and those limitations result in allowance, there exists a well established presumption that those limitations are necessary to patentability and thus material. *See Festo*, 535 U.S. at 734; *Corbett*, 568 F.2d at 765. This presumption applies with equal force in the interference context. *Parks*, 773 F.2d at 1579 (holding in an interference case that "[t]he insertion of [a] limitation to overcome the examiner's rejection is *strong, if not conclusive, evidence of materiality*" (emphasis added)). Here, because Adair cancelled claims 1 and 16 of the PCT Application in response to the examiner's rejections, and added limitations into what eventually became claim 24 of the '261 Application to secure allowance, the Board properly presumed material differences between Adair's post- and pre-critical date claims. Adair failed to rebut this presumption.

iii.

Adair argues that the Board erred by establishing an absolute requirement that the pre-critical date claims be patentable to the applicant for the applicant to rely on those claims to avoid the § 135(b) bar. Carter counters that the Board did not articulate such a requirement, but even if it did, the requirement is appropriate. The Board quoted language from *Regents*, where this court stated that it "perceives no inequity in a construction of section 135(b)(1) that might, in some circumstances, prevent a patent applicant from relying on the filing date of a claim to which it was not statutorily entitled." *Regents*, 455 F.3d at 1377.

The court in *Regents* did not articulate a per se patentability requirement for an applicant to rely on pre-critical date claims, but rather observed that where material limitations are added to overcome an examiner's rejection after the critical date, there is "no inequity" in finding the later added claims barred under § 135(b)(1). Adair is correct that cancelled claims may be relied upon to avoid the § 135(b) bar. *See Corbett*, 568 F.2d at 765 ("The words 'prior to' in the present code clearly point to a 'critical date' prior to which . . . the copier had to be claiming the invention, whether or not the claims were subsequently cancelled."). Adair is incorrect, however, in contending that the Board established any absolute requirement that the pre-critical date claims must have been patentable to Adair. Even if it did, the error would have been harmless because the Board found material differences between the post- and pre-critical date claims, which Adair failed to rebut.

iv.

Finally, Adair argues that the Board abused its discretion in failing to consider claim 2 of the PCT Application as pre-critical date support for claim 24. The Board did not abuse its discretion in declining to consider claim 2 of the PCT Application for the first time on rehearing. 37 C.F.R. § 41.125(c), governing rehearing before the Board, provides that "[t]he burden of showing a decision should be modified lies with the party attacking the decision [and t]he request must specifically identify . . . (ii) The place *where the matter was previously addressed* in a motion, opposition, or reply." 37 C.F.R. § 41.125(c)(3) (emphasis added). Because Adair failed to previously address claim 2 prior to its petition for rehearing, the Board properly refused to consider it on rehearing.

III. CONCLUSION

For the foregoing reasons, this court affirms the decision of the Board.

AFFIRMED

CERTIFICATE OF SERVICE

United States Court of Appeals
for the Federal Circuit
No. **2011-1212 (Interference No. 105,744)**
----------)
John Robert Adair, Diljeet Singh Athwal, and
John Spencer Emtage

Appellants,

v.

Paul J. Carter and Leonard G. Presta,

Appellees.
----------)

I, Robyn Cocho, being duly sworn according to law and being over the age of 18, upon my oath depose and say that:

Counsel Press was retained by COZEN O'CONNOR, Attorneys for Appellants to print this document. I am an employee of Counsel Press.

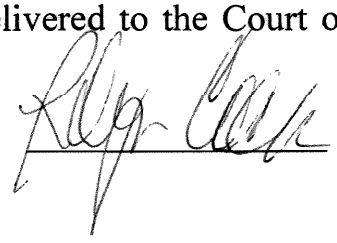
On the **7th Day of March, 2012**, I served the within **Petition for Panel Rehearing of Appellants John Robert Adair, Diljeet Singh Athwal, and John Spencer Emtage** upon:

Oliver R. Ashe, Jr.
ASHE, P.C.
11440 Isaac Newton Square North
Suite 210
Reston, VA 20190
Tel: 703-467-9001
Fax: 703-467-9002

via Federal Express, overnight delivery by causing 2 true copies of each, enclosed in a properly addressed wrapper, to be deposited in an official depository of FedEx.

Unless otherwise noted, 19 copies have been delivered to the Court on the same date via Federal Express

March 7, 2012



11/284,261

United States Court of Appeals for the Federal Circuit

2011-1212
(Interference No. 105,744)

JOHN ROBERT ADAIR, DILJEET SINGH ATHWAL,
and JOHN SPENCER EMTAGE,

Appellants,

v.

PAUL J. CARTER and LEONARD G. PRESTA,

Appellees.

Judgment

ON APPEAL from the United States Patent and Trademark Office, Board of Patent Appeals and Interferences

in CASE NO(S). Interference No. 105,744

This CAUSE having been heard and considered, it is

ORDERED and ADJUDGED:

AFFIRMED

ENTERED BY ORDER OF THE COURT

DATED FEB - 7 2012

Jan Horbaly, Clerk

CERTIFIED COPY
I HEREBY CERTIFY THIS DOCUMENT
IS A TRUE AND CORRECT COPY
OF THE ORIGINAL ON FILE.
UNITED STATES COURT OF APPEALS
FOR THE FEDERAL CIRCUIT
Date: 4/18/12

ISSUED AS A MANDATE: APR - 2 2012

PETITIONER'S EXHIBITS

Exhibit 1095 Page 1836 of 1849

United States Court of Appeals for the Federal Circuit

(Interference No. 105,744)

JOHN ROBERT ADAIR, DILJEET SINGH ATHWAL, AND JOHN SPENCER EMTAGE,
Appellants,

v.

PAUL J. CARTER AND LEONARD G. PRESTA,
Appellees.

2011-1212

Appeal from the United States Patent and Trademark Office, Board of Patent Appeals and Interferences.

Decided: February 7, 2012

DOREEN YATKO TRUJILLO, Cozen O'Connor, P.C., of Philadelphia, Pennsylvania, argued for appellants. With her on the brief was KYLE VOS STRACHE.

OLIVER R. ASHE, JR., Ashe, P.C., of Reston, Virginia, argued for appellees. Of counsel on the brief were JEFFREY P. KUSHAN and RACHEL H. TOWNSEND, Sidley Austin, LLP, of Washington, DC.

Before RADER, *Chief Judge*, LINN and MOORE, *Circuit Judges*.

LINN, *Circuit Judge*.

Appellants John Robert Adair, Diljeet Singh Athwal, and John Spencer Emtage (collectively, "Adair") appeal a decision of the Board of Patent Appeals and Interferences ("Board") holding that Adair's single claim involved in Interference 105,744 with junior party Paul J. Carter and Leonard G. Presta (collectively, "Carter") was barred under 35 U.S.C. § 135(b)(1). Because the Board properly determined that Adair's claim was barred under § 135(b)(1), this court affirms.

I. BACKGROUND

On November 21, 2005, Adair filed U.S. Application Serial No. 11/284,261 ("'261 Application") with the United States Patent and Trademark Office ("PTO"). In a preliminary amendment filed concurrently with this application, Adair requested an interference based on Carter's U.S. Patent No. 6,407,213 ("'213 Patent"). The only count of the interference is drawn to humanized antibodies. More specifically, the count involves non-human amino acid substitutions on specific residues of the heavy chain variable domain (an antibody comprises two light chains and two heavy chains, each with a "constant" and "variable" domain). On February 2, 2010, the Board declared the interference, identifying the claims in the count to be claims 30, 31, 60, 62, 63, 66, 67, 70, 73, 77-81 of the '213 Patent and claim 24 of the '261 Application. *Carter v. Adair*, Interference No. 105,744, Declaration of Interference at 4 (Feb. 2, 2010). The Board awarded Adair priority benefit to PCT/GB90/02017 ("PCT Application"), filed December 21, 1990, which claims priority to a British application filed by Adair on December 21, 1989.

Claim 66 of Carter's '213 Patent, representative of the claims in the count and the basis for an interference-in-fact, recites:

> **66.** A humanized antibody heavy chain variable domain comprising non-human Complementarity Determining Region (CDR) amino acid residues which bind antigen incorporated into a human antibody variable domain, and further comprising a Framework Region (FR) amino acid substitution at a site selected from the group consisting of: 24H [H=heavy], 73H, 76H, 78H, and 93H, utilizing the numbering system set forth in Kabat.

'213 Patent col.88 l.66-col.89 l.6.

Corresponding claim 24 in Adair's '261 Application recites:

> **24.** A humanised antibody *comprising a* heavy chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises a *non-human* amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof, as numbered according to Kabat.

'261 Application, Preliminary Amendment and Request for Interference dated Nov. 21, 2005 at 3, *as amended by* Amendment of Sept. 9, 2009 at 4 (added language emphasized).

Because Adair's claim 24 was not presented to the PTO prior to June 18, 2003, one year from issuance of the Carter '213 Patent (the "critical date") as required by 35 U.S.C. § 135(b)(1), Adair relied on pre-critical date claims

1 and 16 of the PCT Application and corresponding U.S. national stage Application No. 07/743,329 ("'329 Application") to avoid the bar of § 135(b)(1). Claims 1 and 16 recite:

> 1. A CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.
>
> 16. A CDR-grafted antibody heavy or light chain or molecule according to any one of the preceding claims comprising human acceptor residues and non-human donor residues.

PCT Application at 67-69. Adair originally relied on claim 8 of the PCT Application, but because that claim related to light chains, Adair later abandoned that argument. In its request for rehearing before the Board, Adair argued for the first time that claim 2 of the PCT Application also provided pre-critical date support for claim 24, but the Board declined to consider this argument for the first time on rehearing. *Carter v. Adair*, Interference No. 105,774, Decision on Request for Rehearing at 4-5 (Nov. 5, 2010) ("*Rehearing*").

At the national stage, the examiner originally rejected each of Adair's PCT claims under one or more of the following sections: 101, 102(b), 103, and 112 first and second paragraphs. '329 Application, Office Action of November 18, 1992. Adair cancelled the PCT claims and added claims 23-66, later cancelled by an amendment adding claims 67-119 requiring multiple amino acid substitutions at specific locations in the heavy chain. '329

Application, Amendments of January 19, 1993 and April 16, 1993.

The Board rejected Adair's argument that claims 1 and 16 in the PCT Application provide pre-critical date support for claim 24 in the '261 Application because: (1) the PCT claims were not patentable to Adair; (2) Adair added limitations to overcome the examiner's rejection; and accordingly, (3) material differences presumptively existed between the post- and pre-critical date claims that Adair failed to rebut. *Carter v. Adair*, Interference No. 105,774, Decision on Motions at 9-10 (Aug. 30, 2010) ("*Decision*"). Citing *Regents of the University of California v. University of Iowa Research Foundation*, 455 F.3d 1371, 1377 (Fed. Cir. 2006), the Board stated that "[a]n applicant cannot expect to avoid the bar of § 135(b) by timely copying a claim from an issued patent when that claim is not patentable to that applicant." *Decision* at 10-11. On rehearing, the Board rejected Adair's assertion that materiality must be "determined in view of the patent claims being copied" and declined to compare Adair's post- or pre-critical date claims with copied claim 66 from Carter's '213 Patent. *Rehearing* at 3. Adair appeals, and this court has jurisdiction pursuant to 28 U.S.C. § 1295(a)(4)(A).

II. DISCUSSION

A. Standard of Review

"We review the Board's construction of 35 U.S.C. § 135(b)(1) de novo, as statutory interpretation is a question of law." *In re Berger*, 279 F.3d 975, 980 (Fed. Cir. 2002).

B. Analysis

Adair argues that the Board erred by failing to assess material differences "in view of the patent claim being copied [claim 66 from Carter's '213 Patent]." Appellant Br. 22. According to Adair, this court's precedent does not endorse a test that allows the Board to completely ignore copied claim 66 from Carter's '213 Patent when assessing the material differences between the post- and pre-critical date claims. Adair argues that the materiality test from *Berger* and *Regents* requires an assessment of material limitations based on the "identity" between the post-critical date claim and copied claim 66 from Carter's '213 Patent—in other words, in view of the "count"—and not based on the post-critical date claim standing alone. *See Regents*, 455 F.3d at 1375 ("[A]s this court's precedent explains, California must demonstrate that claims in the '191 application provide pre-critical date support for the *post-critical date identity* between claim 205 [the post-critical date claim] and the '646 patent [the issued patent]." (emphasis added)); *Berger*, 279 F.3d at 983.

Carter counters that the question of "[w]hether there is a sufficient degree of identity between pre- and post-critical date claims for compliance with § 135(b) is an inquiry that is distinct and independent" from any comparison with the patent claims copied. Appellee Br. 33. According to Carter, the Board correctly interpreted § 135(b)(1) in holding that "establishing support for post-critical date claims does not entail looking at material limitations of the patented claims." *Id.* 42.

This court agrees with Carter. Section 135(b)(1) states:

> A claim which is the same as, or for the same or substantially the same subject matter as, a claim of an issued patent may not be made in any appli-

> cation unless such a claim is made prior to one year from the date on which the patent was granted.

35 U.S.C. § 135(b)(1). Notwithstanding the seemingly strict language of the statute, a limited exception to this one year bar exists "where the copier had already been claiming substantially the same invention as the patentee" during the critical time period. *Corbett v. Chisholm*, 568 F.2d 759, 765 (CCPA 1977).

i.

In *Corbett*, the post-critical date claims "correspond[ed] exactly" with issued "Chisholm patent" claim 1. 568 F.2d at 759. The Board rejected Corbett's post-critical date claims under § 135(b)(1). *Id.* Corbett relied upon several groups of pre-critical date claims from the application and a predecessor application in an attempt to avoid the § 135(b) bar. *Id.* at 761-63. On appeal, this court compared the "copied claim" with the pre-critical date claims and affirmed the Board's finding that material differences precluded Corbett from relying on any of the pre-critical date claims to overcome the § 135(b) bar. *Id.* at 765-66. In identifying certain limitations of Chisholm patent claim 1 as "material," the court was simply noting the material differences that existed between that claim as copied by Corbett after the critical date and those pre-critical date claims Corbett was relying on to overcome the § 135(b) bar. The court did not establish any rule requiring some sort of threshold assessment of which limitations of the copied patent claim are material before determining whether material differences exist between post- and pre-critical date claims. In making this comparison, the court referenced Chisholm patent claim 1 only because that was the post-critical date claim.

Similarly, in *Berger*, the post-critical date claim was copied directly from and identical to issued "Muller patent" claim 1. 279 F.3d at 978. The examiner rejected Berger's pre-critical date claims 1-6 for indefiniteness and other grounds, and rejected post-critical date claim 7 under § 135(b)(1). *Id.* at 979. The Board rejected Berger's argument that claims 1-6 provided pre-critical date support for claim 7 because it found material differences between the "copied claim" and the pre-critical date claims, and this court affirmed. *Id.* at 982 ("The Board found the 'circumferential groove' limitation to be material because it was added by Muller during prosecution to avoid prior art. We agree with the Board's determination of materiality."). Again, the court in *Berger* referenced the issued Muller patent claim 1 only because the post-critical date claim, claim 7, was a direct copy of the patent claim. *Id.* at 981-83. This court affirmed the Board's analysis based only on the material differences between the *post- and pre-critical date claims*. *Id.* at 983 ("Because Berger's *original claims 1-6* [the pre-critical date claims] *do not include a material limitation of Berger claim 7* [the post-critical date claim], copied claim 7 is not entitled to the earlier effective date of those original claims for purposes of satisfying § 135(b)." (emphasis added)).

In *Regents*, this court expressly approved an analysis of material differences based solely on a comparison of the post- and pre-critical date claims in order to obtain the benefit of the earlier filing date:

> The Board compared claim 205 [the post-critical date claim] with claims 202-203 . . . and then with claim 204 [collectively, the pre-critical date claims]. The Board found that California's claim 205 contained material differences from claims 202-204. Therefore, claim 205 could not benefit from the earlier filing date of those claims. . . . On

> appeal, California does not contest the Board's finding of material differences between claim 205 and claims 202-204. Instead, California challenges the Board's conclusion that the correct inquiry under § 135(b)(1) asks whether claims 202-204 contain material differences from claim 205 and not whether claims 202-204 are to the same invention as claims in the '646 patent.

455 F.3d at 1373. The court in *Regents* rejected California's argument, explaining that "the relationship between the post- and pre-critical date claims . . . is not only relevant, but dispositive of the section 135(b)(1) question." *Id.* at 1374. Adair's arguments in this case are similar to California's arguments in *Regents*, where the court held that there is no requirement that the Board reference the issued patent claim(s) in the count to assess the material differences between the post- and pre-critical date claims. *Id.* at 1374-76.

The statement in *Regents* that the applicant's earlier filed claims must "provide pre-critical date support for the *post-critical date identity* between [the post-critical date claim] and the [issued patent]" to avoid the § 135(b)(1) bar, 455 F.3d at 1375 (emphasis added), does not require the Board to assess material differences in view of the issued patent claim(s) in the count. *See Berger*, 279 F.3d at 982. The question of material differences between post- and pre-critical date claims for purposes of overcoming a § 135(b) bar "is a distinctly different question from whether claims . . . are directed to the same or substantially the same subject matter" for purposes of provoking an interference. *Id.* As explained in *Regents*, § 135(b) is a statute of repose, intended to "limit[] the patentee's vulnerability to a declaration of an interference" by "limit[ing] the window of time in which the cause of the interference can occur." 455 F.3d at 1376. When a material difference exists between the post- and pre-critical

date claims, a belated interference is improper because it would be a "*different interference*[]" than that which "should have been earlier declared by the PTO." *Id.* (emphasis added).

For these reasons, this court holds that to overcome a § 135(b) bar for a post-critical date claim, an applicant must show that such claim is not materially different from a pre-critical date claim present in the application or any predecessor thereto in order to obtain the benefit of the earlier filing date. Any claims filed within the critical period, whether or not later cancelled, may provide pre-critical date support for the later filed patent claim(s), so long as the pre-critical date claims are not materially different from the later filed claim(s). *Corbett*, 568 F.2d at 765-66; *see also Regents*, 455 F.3d at 1373; *Berger*, 279 F.3d at 981-82.

Here, the Board found material differences between post-critical date claim 24 of the '261 Application and pre-critical date claims 1 and 16 of the PCT Application based on the prosecution history of the '261 Application. During prosecution, Adair added several limitations to claim 24—limitations not present in claims 1 and 16 of the PCT Application—to avoid examiner rejections during prosecution. *Decision* at 9. Adair failed to rebut the Board's finding with any evidence that the differences between claim 24 and claims 1 and 16 of the PCT Application were immaterial. *Id.* at 10. Adair criticizes the Board for failing to consider claim 66 from Carter's '213 Patent in assessing material differences. But, for the reasons explained above, an assessment of claim 66 was not necessary. What was required in determining whether the § 135(b) bar might be overcome was an assessment of the material differences between the post- and pre-critical date claims, which is precisely what the Board did.

ii.

Adair also contends that the Board erred in applying *Festo Corp. v. Shoketsu Kinzoku Kogyo Kabushiki Co.*, 535 U.S. 722, 734 (2002) in the context of an interference to conclude that a limitation added to a claim in response to a rejection that results in allowance is presumed to be necessary to patentability and therefore "material." Adair asserts that the burden of proof for the § 135(b) motion lay with Carter, and thus Adair cannot be faulted "for not providing any reason why the limitations that differ . . . were not material." Appellant Br. 25. Carter counters that "the Board's presumption of material differences is firmly grounded in the law." Appellee Br. 44. *See Parks v. Fine*, 773 F.2d 1577, 1579 (Fed. Cir. 1985); *Corbett*, 568 F.2d at 765.

Carter is correct. When an applicant adds limitations in response to an examiner's rejection, and those limitations result in allowance, there exists a well established presumption that those limitations are necessary to patentability and thus material. *See Festo*, 535 U.S. at 734; *Corbett*, 568 F.2d at 765. This presumption applies with equal force in the interference context. *Parks*, 773 F.2d at 1579 (holding in an interference case that "[t]he insertion of [a] limitation to overcome the examiner's rejection is *strong, if not conclusive, evidence of materiality*" (emphasis added)). Here, because Adair cancelled claims 1 and 16 of the PCT Application in response to the examiner's rejections, and added limitations into what eventually became claim 24 of the '261 Application to secure allowance, the Board properly presumed material differences between Adair's post- and pre-critical date claims. Adair failed to rebut this presumption.

iii.

Adair argues that the Board erred by establishing an absolute requirement that the pre-critical date claims be patentable to the applicant for the applicant to rely on those claims to avoid the § 135(b) bar. Carter counters that the Board did not articulate such a requirement, but even if it did, the requirement is appropriate. The Board quoted language from *Regents*, where this court stated that it "perceives no inequity in a construction of section 135(b)(1) that might, in some circumstances, prevent a patent applicant from relying on the filing date of a claim to which it was not statutorily entitled." *Regents*, 455 F.3d at 1377.

The court in *Regents* did not articulate a per se patentability requirement for an applicant to rely on pre-critical date claims, but rather observed that where material limitations are added to overcome an examiner's rejection after the critical date, there is "no inequity" in finding the later added claims barred under § 135(b)(1). Adair is correct that cancelled claims may be relied upon to avoid the § 135(b) bar. *See Corbett*, 568 F.2d at 765 ("The words 'prior to' in the present code clearly point to a 'critical date' prior to which . . . the copier had to be claiming the invention, whether or not the claims were subsequently cancelled."). Adair is incorrect, however, in contending that the Board established any absolute requirement that the pre-critical date claims must have been patentable to Adair. Even if it did, the error would have been harmless because the Board found material differences between the post- and pre-critical date claims, which Adair failed to rebut.

iv.

Finally, Adair argues that the Board abused its discretion in failing to consider claim 2 of the PCT Application as pre-critical date support for claim 24. The Board did not abuse its discretion in declining to consider claim 2 of the PCT Application for the first time on rehearing. 37 C.F.R. § 41.125(c), governing rehearing before the Board, provides that "[t]he burden of showing a decision should be modified lies with the party attacking the decision [and t]he request must specifically identify . . . (ii) The place *where the matter was previously addressed* in a motion, opposition, or reply." 37 C.F.R. § 41.125(c)(3) (emphasis added). Because Adair failed to previously address claim 2 prior to its petition for rehearing, the Board properly refused to consider it on rehearing.

III. CONCLUSION

For the foregoing reasons, this court affirms the decision of the Board.

AFFIRMED