GP 1816

#23 4/24/96

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of **Carter et al.** Serial No.: 08/146,206 Filed: November 17, 1993 For: **METHOD FOR MAKING HUMANIZED ANTIBODIES**	Group Art Unit: 1816 Examiner: D. Adams CERTIFICATE OF MAILING I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on March 27, 1996 Susan Tafoya

NOTICE OF APPEAL

Box AF
Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Applicant hereby appeals to the Board of Appeals and Interferences from the decision dated October 27, 1995, of the Primary Examiner finally rejecting claims 1-12, 15 and 19-25.

The Commissioner is hereby authorized to charge Deposit Account No. 07-0630 in the amount of $290 to cover the fees for this appeal and to charge the deposit account for any further fees in regard to this patent application. A duplicate copy of this Notice is enclosed for this purpose.

A copy of a document pursuant to 37 C.F.R. § 10.9(b) is attached as proof of the authorization of the undersigned to prosecute the above-mentioned application. The original of this document is on file in the Office of Enrollment and Discipline.

Respectfully submitted,
GENENTECH, INC.

Date: March 27, 1996

By: _____
Wendy M. Lee

460 Pt. San Bruno Blvd.
So. San Francisco, CA 94080-4990
Phone: (415) 225-1994
Fax: (415) 952-9881

Corres. and Mail
BOX AF

#22

Patent Docket P0709P1 4/24/96

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of **Carter et al.** Serial No.: 08/146,206 Filed: November 17, 1993 For: **METHOD FOR MAKING HUMANIZED ANTIBODIES**	Group Art Unit: 1816 Examiner: D. Adams CERTIFICATE OF MAILING I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on March 27, 1996 Susan Tafoya

PETITION AND FEE FOR TWO MONTH EXTENSION OF TIME
(37 CFR 1.136(a))

Box AF
Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Applicant petitions the Commissioner of Patents and Trademarks to extend the time for response to the Final Office Action dated October 27, 1995 for two (2) months, from January 27, 1996 to March 27, 1996. The extended time for response does not exceed the statutory period.

Please charge Deposit Account No. 07-0630 in the amount of $380.00 to cover the cost of the extension. Any deficiency or overpayment should be charged or credited to this deposit account. A duplicate of this sheet is enclosed.

A copy of a document pursuant to 37 C.F.R. § 10.9(b) is attached as proof of the authorization of the undersigned to prosecute the above-mentioned application. The original of this document is on file in the Office of Enrollment and Discipline.

Respectfully submitted,
GENENTECH, INC.

Date: March 27, 1996

By: _____
Wendy M. Lee

460 Pt. San Bruno Blvd.
So. San Francisco, CA 94080-4990
Phone: (415) 225-1994
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UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

LIMITED RECOGNITION UNDER 37 CFR § 10.9(b)

Wendy M. Lee is hereby given limited recognition under 37 CFR § 10.9(b) as an employee of Genentech, Inc., to prepare and prosecute patent applications and to represent patent applicants wherein Genentech, Inc., is the assignee of record of the entire interest. This limited recognition shall expire on the date appearing below, or when whichever of the following events first occurs prior to July 15, 1996: (i) Wendy M. Lee ceases to lawfully reside in the United States, (ii) Wendy M. Lee's employment with Genentech, Inc., ceases or is terminated, or (iii) Wendy M. Lee ceases to remain or reside in the United States on a H-1B visa.

This document constitutes proof of such recognition. The original of this document is on file in the Office of Enrollment and Discipline of the U.S. Patent and Trademark Office.

Expires: July 15, 1996

Karen L. Bovard, Director
Office of Enrollment and Discipline

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 DOCKETT NO.

EXAMINER	
ART UNIT	PAPER NUMBER
	21

DATE MAILED:

EXAMINER INTERVIEW SUMMARY RECORD

All participants (applicant, applicant's representative, PTO personnel):

(1) Ms Wendy M. Lee (3) _____

(2) Donald E Adams (4) _____

Date of Interview 4/8/96

Type: ☐ Telephonic ☒ Personal (copy is given to ☐ applicant ☒ applicant's representative).

Exhibit shown or demonstration conducted: ☐ Yes ☐ No. If yes, brief description: N/A

Agreement ☐ was reached with respect to some or all of the claims in question. ☒ was not reached.

Claims discussed: All generally

Identification of prior art discussed: All cited Art.

Description of the general nature of what was agreed to if an agreement was reached, or any other comments: discussed problems w/spec-identified a difference in format between PTO & Generated copies. Discussed Kabat Consensus Sequence & How The method taught by prior art refs teach away from using A Consensus Sequence. Suggested adding claim 19 originally not entered as a new claim #26 & Correcting all previous dependency on claim 19 To claim 26

(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.)

☐ 1. It is not necessary for applicant to provide a separate record of the substance of the interview.

Unless the paragraph below has 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.

☐ 2. 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. Applicant is not relieved from providing a separate record of the substance of the interview unless box 1 above is also checked.

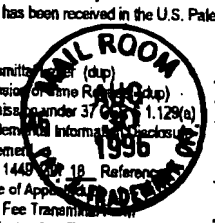
Donald E Adams
Examiner's Signature

PTOL-413 (REV. 2 -93)

In re Application of: Paul J. Carter et al.
Serial No.: 08/146,206
Filed On: 17 November 1993
Mailed On: 27 August 1996

Docket No.: P0709P1
By: Wendy M. Lee
Reg. No.: P-40,378

The following has been received in the U.S. Patent Office on the date stamped:



X	Transmittal Letter (dup)	___	Notice To File Missing Parts (dup)
X	Extension of Time Request (dup)	___	Declaration/Power of Atty
X	Submission under 37 CFR 1.129(a)	___	Assignment
X	Supplemental Information Disclosure Statement	___	Recordation Form
X	Form 1449/PTO 1__ References	___	Drawings: __ Sheets ___ Informal ___ Formal
___	Notice of Appeal	___	Certificate re: Sequence Listing
___	Issue Fee Transmittal	___	Sequence Listing & Diskette
X	Certificate of Mailing	___	Fees: $__
___	Certificate of Express Mailing Express Mail Label No.:	___	Other:

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1816 Examiner: D. Adams CERTIFICATE OF MAILING I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on August 27, 1996 Duane Alexander Vick Duane Alexander Vick

SUBMISSION UNDER 37 CFR §1.129(a)

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

The accompanying papers are being filed in response to the Office Action mailed October 27, 1995 issuing a final rejection of the claims pending in the application. On March 27, 1996, Applicants filed a Notice of Appeal. Submitted herewith is a three month extension of time for making this submission.

The present submission, in the form of a Supplemental Information Disclosure Statement, is being submitted under Section 1.129(a) along with the fee set forth in Section 1.17(r).

Respectfully submitted,
GENENTECH, INC.

Date: August 27, 1996

By: _____
Wendy M. Lee
Reg. No. P-40,378

460 Pt. San Bruno Blvd.
So. San Francisco, CA 94080 4990
Phone: (415) 225-1994
Fax: (415) 952-9881

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1816 Examiner: D. Adams CERTIFICATE OF MAILING I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on August 27, 1996 Duane Alexander Vick Duane Alexander Vick

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Applicants submit herewith patents, publications or other information (attached hereto and listed on the attached Form PTO-1449) of which they are aware, which they believe may be material to the examination of this application and in respect of which there may be a duty to disclose in accordance with 37 CFR §1.56.

This Information Disclosure Statement:

(a) [] accompanies the new patent application submitted herewith. 37 CFR §1.97(a).

(b) [] is filed within three months after the filing date of the application or within three months after the date of entry of the national stage of a PCT application as set forth in 37 CFR§1.491.

(c) [] as far as is known to the undersigned, is filed before the mailing date of a first Office action on the merits.

(d) [] is filed after the first Office Action and more than three months after the application's filing date or PCT national stage date of entry filing but, as far as is known to the undersigned, prior to the mailing date of either a final rejection or a notice of allowance, whichever occurs first, and is accompanied by either the fee ($220) set forth in 37 CFR §1.17(p) or a certification as specified in 37 CFR §1.97(e), as checked below. Should any fee be due, the U.S. Patent and Trademark Office is hereby authorized to charge Deposit Account No. 07-0630 in the amount of $220.00 to cover the cost of this Information Disclosure Statement. Any deficiency or overpayment should be charged or credited to this deposit account. **A duplicate of this sheet is enclosed.**

Revised (10/20/95)

(c) [ ] is filed after the mailing date of either a final rejection or a notice of allowance, whichever occurred first, and is accompanied by the fee ($130) set forth in 37 CFR §1.17(i)(1) and a certification as specified in 37 CFR §1.97(e), as checked below. **This document is to be considered as a petition requesting consideration of the information disclosure statement.** The U.S. Patent and Trademark Office is hereby authorized to charge Deposit Account No. 07-0630 in the amount of $130.00 to cover the cost of this Information Disclosure Statement. Any deficiency or overpayment should be charged or credited to this deposit account. **A duplicate of this sheet is enclosed.**

(d) [X] is filed after the mailing date of a final rejection, but a request to withdraw the finality thereof under 37 CFR § 1.129(a) is submitted herewith. The U.S. Patent and Trademark Office is hereby authorized to charge Deposit Account No. 07-0630 to cover the cost of this Information Disclosure Statement in the event that any fees are due. **A duplicate of this sheet is enclosed.**

[If either of boxes (d) or (e) is checked above, the following "certification" under 37 CFR §1.97(e) may need to be completed.] The undersigned certifies that:

[ ] Each item of information contained in the information disclosure statement was cited in a communication mailed from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of this information disclosure statement.

[ ] No item of information contained in this information disclosure statement was cited in a communication mailed from a foreign patent office in a counterpart foreign application or, to the knowledge of the undersigned after making reasonable inquiry, was known to any individual designated in 37 CFR §1.56(c) more than three months prior to the filing of this information disclosure statement.

A list of the patent(s) or publication(s) is set forth on the attached Form PTO-1449 (Modified).

A copy of the items on PTO-1449 is supplied herewith:

[X] each [ ] none [ ] only those listed below:

Those patent(s) or publication(s) which are marked with an asterisk (*) in the attached PTO-1449 form are not supplied because they were previously cited by or submitted to the Office in a prior application Serial No. 07/715,272, filed 14 June 1991 and relied upon in this application for an earlier filing date under 35 USC §120.

A concise explanation of relevance of the items listed on PTO-1449 is:

[X] not given

[ ] given for each listed item

[ ] given for only non-English language listed item(s) [Required]

[ ] in the form of an English language copy of a Search Report from a foreign patent office, issued in a counterpart application, which refers to the relevant portions of the references.

The Examiner is reminded that a "concise explanation of the relevance" of the submitted prior art "may be nothing more than identification of the particular figure or paragraph of the patent or publication which has some relation to the claimed invention," MPEP §609.

While the information and references disclosed in this Information Disclosure Statement may be "material" pursuant to 37 CFR §1.56, it is not intended to constitute an admission that any patent, publication or other information referred to therein is "prior art" for this invention unless specifically designated as such.

In accordance with 37 CFR §1.97(g), the filing of this Information Disclosure Statement shall not be construed to mean that a search has been made or that no other material information as defined in 37 CFR §1.56(a) exists. It is submitted that the Information Disclosure Statement is in compliance with 37 CFR §1.98 and MPEP §609 and the Examiner is respectfully requested to consider the listed references.

Respectfully submitted,
GENENTECH, INC.

Date: August 27, 1996

By: _____
Wendy M. Lee
Reg. No. P-40,378

460 Pt. San Bruno Blvd.
So. San Francisco, CA 94080-4990
Phone: (415) 225-1994
Fax: (415) 952-9881

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1816 Examiner: D. Adams CERTIFICATE OF MAILING I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on August 27, 1996 Duane Alexander Vick

PETITION AND FEE FOR THREE MONTH EXTENSION OF TIME
(37 CFR 1.136(a))

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Applicant petitions the Commissioner of Patents and Trademarks to extend the time for response to the Notice of Appeal dated 3/27/96 for 3 month(s) from 5/27/96 to 8/27/96. The extended time for response does not exceed the statutory period.

Please charge Deposit Account No. 07-0630 in the amount of $ 900.00 to cover the cost of the extension. Any deficiency or overpayment should be charged or credited to this deposit account. A duplicate of this sheet is enclosed.

Respectfully submitted,
GENENTECH, INC.

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By: _____
Wendy M. Lee
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Fax: (415) 952-9881

Revised (10/17/96)

GP 1816
#29
Dr. Plunkett
9/14/96

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1816 Examiner: D. Adams SEP 10 1996 GROUP 1800 CERTIFICATE OF MAILING I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on August 27, 1996 Duane Alexander Vick Duane Alexander Vick

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

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Revised (10/20/95)

(e) [] is filed after the mailing date of either a final rejection or a notice of allowance, whichever occurred first, and is accompanied by the fee ($130) set forth in 37 CFR §1.17(i)(1) **and** a certification as specified in 37 CFR §1.97(e), as checked below. **This document is to be considered as a petition requesting consideration of the information disclosure statement.** The U.S. Patent and Trademark Office is hereby authorized to charge Deposit Account No. 07-0630 in the amount of $130.00 to cover the cost of this Information Disclosure Statement. Any deficiency or overpayment should be charged or credited to this deposit account. **A duplicate of this sheet is enclosed.**

(f) [X] is filed after the mailing date of a final rejection, but a request to withdraw the finality thereof under 37 CFR § 1.129(a) is submitted herewith. The U.S. Patent and Trademark Office is hereby authorized to charge Deposit Account No. 07-0630 to cover the cost of this Information Disclosure Statement in the event that any fees are due. **A duplicate of this sheet is enclosed.**

[If either of boxes (d) or (e) is checked above, the following "certification" under 37 CFR §1.97(e) may need to be completed.] The undersigned certifies that:

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[] given for each listed item

[] given for only non-English language listed item(s) [Required]

[] in the form of an English language copy of a Search Report from a foreign patent office, issued in a counterpart application, which refers to the relevant portions of the references.

Revised (10/20/95)

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While the information and references disclosed in this Information Disclosure Statement may be "material" pursuant to 37 CFR §1.56, it is not intended to constitute an admission that any patent, publication or other information referred to therein is "prior art" for this invention unless specifically designated as such.

In accordance with 37 CFR §1.97(g), the filing of this Information Disclosure Statement shall not be construed to mean that a search has been made or that no other material information as defined in 37 CFR §1.56(a) exists. It is submitted that the Information Disclosure Statement is in compliance with 37 CFR §1.98 and MPEP §609 and the Examiner is respectfully requested to consider the listed references.

Respectfully submitted,
GENENTECH, INC.

Date: August 27, 1996 By: _____
Wendy M. Lee
Reg. No. P-40,378

460 Pt. San Bruno Blvd.
So. San Francisco, CA 94080-4990
Phone: (415) 225-1994
Fax: (415) 952-9881

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1816 Examiner: D. Adams **CERTIFICATE OF MAILING** I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on August 27, 1996 Duane Alexander Vick Duane Alexander Vick

PETITION AND FEE FOR THREE MONTH EXTENSION OF TIME
(37 CFR 1.136(a))

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

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AG70175 09712/96 08146206 07-0630 070 117 900.00CH

#26

FAX TRANSMISSION

GENENTECH, INC.
460 PT. SAN BRUNO BLVD.
SOUTH SAN FRANCISCO, CA 94080-4990
415-225-1994
FAX: 415-952-9881

OFFICIAL

26

To:	**Examiner Chris Eisenschenk** **Group 1816** Tel: (703) 308-0452 U.S. Patent and Trademark Office Washington, D.C. 20231	**Date:**	December 3, 1996
Fax #:	703-308-4242	**Pages:**	9 , including this cover sheet.
From:	Wendy M. Lee		
Subject:	U.S. Serial No. 08/146,206 Our Docket No. P0709P1		

COMMENTS:

PLEASE DELIVER THESE DOCUMENTS DIRECTLY TO EXAMINER EISENSCHENK.

DEC 03 1996

12/03/1996

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, DC 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
08/146,206	11/1/93	CARTER P	709P1

18M1/1223

JANET E. HASAK
GENENTECH, INC.
460 POINT SAN BRUNO BOULEVARD
SOUTH SAN FRANCISCO CA 94080-4990

EXAMINER	
NOLAN, P	
ART UNIT	**PAPER NUMBER**
1816	#27

DATE MAILED: 12/23/96

6407213

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

PTO-90C (REV. 2/95) 1-File Copy

☆U.S.GOVERNMENT PRINTING OFFICE 1995-319-826

Office Action Summary	Application No. 08/146,206	Applicant(s) Carter et al.
	Examiner Patrick Nolan	Group Art Unit 1816

☒ Responsive to communication(s) filed on *Dec 3, 1996* .

☐ This action is **FINAL.**

☐ Since this application is 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.

A shortened statutory period for response to this action is set to expire *3* month(s), ~~or thirty days, whichever is longer~~, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

☒ Claim(s) *1-12, 15, and 19-25* is/are pending in the application.

Of the above, claim(s) _____ is/are withdrawn from consideration.

☐ Claim(s) _____ is/are allowed.

☒ Claim(s) *1-12, 15, and 19-25* is/are rejected.

☐ Claim(s) _____ is/are objected to.

☐ Claims _____ are subject to restriction or election requirement.

Application Papers

☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on _____ is ☐ approved ☐ disapproved.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been

☐ received.

☐ received in Application No. (Series Code/Serial Number) _____ .

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____ .

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☐ Notice of References Cited, PTO-892

☒ Information Disclosure Statement(s), PTO-1449, Paper No(s). *19, 24,26*

☐ Interview Summary, PTO-413

☐ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

--- SEE OFFICE ACTION ON THE FOLLOWING PAGES ---

1. Claims 1-12, 15 and 19-25 are pending.

2. Claims 19-21 stand 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.

Claims 19-21 are substantial duplicates of claim 1. There appears to be no difference in scope between these claims, see MPEP 706.03(k).

3. 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) or (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.

4. 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 C.F.R. § 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.

5. Claims 1, 2, 4-12, 15, and renumbered claims 19-22 and 24-25 stand rejected under 35 U.S.C. § 103 as being unpatentable over Winter [EP 0239400], Riechmann et al. [Nature 332:323-327 (1988)]

and Queen et al. [Proc. Natl. Acad. Sci. 86:10029-10033 (1989)], all of record for the same reasons set forth in paper No. 18.

Briefly the claims are drawn to a method for producing humanized antibodies and humanized antibodies. Winter, teaches the production of altered, chimeric, antibodies by replacing the complementarily determining regions (CDRs), see abstract. Winter, teaches the requirements for CDR fusions, see page 6 to page 8, line 29. Particularly, page 8, lines 11-18, where Winter, teaches that "merely by replacing one or more CDRs with complementary CDRs may not always result in a functional altered antibody..... it will be well within the competence of the man skilled in the art, either by carrying out routine experimentation or by trail and error testing to obtain a functional altered antibody. Note at page 8, last full paragraph that Winter states that framework region replacement and sequence changing may be necessary to obtain a functional humanized antibody. On page 9, lines 13-16, Winter suggests that the antibodies would be of importance for use in human therapy. Winter, teaches a method of producing the antibody, see page 10, paragraph 3 to page 15, paragraph 2. Consistent with Winter, Riechmann et al. teach a method of reshaping human antibodies for therapy by CDR grafting, see whole document and Queen et al. teach the humanization of antibodies by CDR grafting, see entire document. Riechmann et al. teach altering the sequence of the antibody to restore packing or to increase binding affinity, see page 326, first column, first full paragraph. Queen et al. teach the use of computer modeling to assist in the production of humanized antibodies, specifically to predict which amino acids to change thereby effecting molecular interactions, note that of the amino acids predicted to change include those identified by applicant in claims 7 and 10. A person of ordinary skill in the art would have realized that dependent upon the framework region selected and the sequence of the CDR regions amino acid changes would need to be made and they would depend upon the precise amino acid interactions of the polypeptide. The combination of Winter, Riechmann et al. and Queen et al. teach a comprehensive method for producing humanized antibodies which include the steps outlined in applicant's claims. Therefore, it would have been prima facia obvious to a person of ordinary skill in the art at the time the invention was made to take the combined teachings of Winter, Riechmann et al. and Queen et al. to produce a method of making a humanized antibody and to have a humanized antibody for either diagnostic or therapeutic use.

Applicant's arguments filed 6/12/95 have been fully considered but they are not persuasive. Applicant argues that the claimed invention is distinct from that taught by the above combination of

references because a consensus sequence is used and further modifications are not necessary. Applicant further argues that the combination of references do not teach a humanized antibody with reduced immunogenicity.

Regarding the consensus sequence, the combination of references teach the human framework regions having a significantly high degree of sequence homology (conservative regions). Queen et al. in particular point to Kabat as demonstrating that this was known in the art well in advance of applicant's filing date, see reference 38, cited by Queen et al. In essence there is no functional/structural distinction from what applicant has claimed and that taught by the combination of references. Ex parte C, 27 U.S.P.Q.2d 1492 (BPAI 1993). Applicants recitation of Co et al. is unclear, it was not used in the prior art rejection. Applicant then points to several other references concluding that the techniques of the prior art and the technique of the instant application are "certainly different". However, the minor differences between the prior art and the claimed invention are obvious differences. Modifications in the framework regions which affect the proximity or orientation of the V_L-V_H interface regions is the same as substituting that FR residue from the import regions that is involved in the effects set forth in paragraph (f) of claim 1. The combination of references clearly teach reduced immunogenicity associated with the humanized antibody. See e.g. Riechmann et al. page 323, column 2, lines 5-8. Applicant's comments have been fully considered and were as a whole not found persuasive.

6. Claims 1, 2, 4-12 and 15, and renumbered claims 19-22 and 24-25 stand rejected under 35 U.S.C. § 103 as being unpatentable over Winter [EP 0239400], Riechmann et al. [Nature 332:323-327 (1988)] and Queen et al. [Proc. Natl. Acad. Sci. 86:10029-10033 (1989)] in view of In re Durden 226 U.S.P.Q. 359 (Fed. Cir. 1985), all of record, for the same same reasons set forth in paper No. 18.

Briefly the claims are drawn to a method for producing humanized antibodies and humanized antibodies. As discussed above the combination of Winter, Riechmann et al. and Queen et al. teach humanized antibodies and methods for their production. Applicant's claimed invention does not appear to differ from what has previously known in the art.

Applicant cites the above comments in their response to this rejection.

Applicant's comments were fully considered as described above and

were not found persuasive, to the extent that they apply to this rejection.

7. Claim 3 and renumbered claim 23 stand rejected under 35 U.S.C. § 103 as being unpatentable over Winter [EP 0239400], Riechmann et al. [Nature 332:323-327 (1988)] and Queen et al. [Proc. Natl. Acad. Sci. 86:10029-10033 (1989)] as applied to claims 1, 2, 4-12 and 15 and further in view of Roitt [Immunology, published 1985, by Gower Medical Publishing Ltd. (London, England) page 5.5], all of record for the same reasons set forth in paper No. 18.

Briefly the claim is drawn to a method for producing humanized antibodies having the additional steps of searching the import variable domain sequence for glycosylation sites, determining if any such glycosylation site is reasonable expected to affect the antigen binding or affinity of the antibody and if so substituting the glycosylation site into the consensus sequence. As discussed above the combination of Winter, Riechmann et al. and Queen et al. teach humanized antibodies and methods of producing humanized antibodies. The combination of Winter, Riechmann et al. and Queen et al. do not teach the importance of carbohydrate residues. However, Roitt teaches that antibodies contain carbohydrate residues in the variable region. A person of ordinary skill in the art would realize that carbohydrate residues can produce steric modifications in the folding characteristics of polypeptides. Therefore it would have been prima facia obvious to a person of ordinary skill in the art at the time the invention was made to include a step in the method taught by the combination of Winter, Riechmann et al. and Queen et al. which determines if the presence of carbohydrate residues occur in the variable region that can affect antigen binding and then include in the antibody sequence the appropriate glycosylation signal, by adding the appropriate consensus sequence. A person of ordinary skill in the art would have been motivated to add the additional step of identifying glycosylation that may affect antigen binding to ensure that the antibody produced will have the appropriate binding affinity. A person of ordinary skill in the art would have been motivated to produce such an method to produce antibodies having diagnostic or therapeutic utility.

> *The bulk of applicant's argument is that the references relied on in the above rejection do not render the invention obvious and Roitt adds nothing to these references to overcome the deficiency.*

From the above discussion, the references used render the claimed invention obvious. Roitt fulfills the deficiency of the references

discussed above to the extent that Roitt teaches antibodies contain carbohydrate residues in the variable region. A person of ordinary skill in the art would realize that carbohydrate residues can produce steric modifications in the folding characteristics of polypeptides.

THE FOLLOWING REJECTIONS ARE NEW GROUNDS OF REJECTIONS

Double Patenting

The non-statutory double patenting rejection, whether of the obviousness-type or non-obviousness-type, is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent. *In re Thorington,* 418 F.2d 528, 163 USPQ 644 (CCPA 1969); *In re Vogel,* 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Van Ornum,* 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Longi,* 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); and *In re Goodman,* 29 USPQ2d 2010 (Fed. Cir. 1993).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(b) and (c) may be used to overcome an actual or provisional rejection based on a non-statutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.78(d). Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

8. Claims 1-12, 15 and 19-25 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-12, 15 and 19 of copending application Serial No. 08/439,004. Although the conflicting claims are not identical, they are not patentably distinct from each other because the invention claimed in claims 1-12, 15 and 19 of copending application Serial No. 08/439,004 encompasses the invention claimed in claims 1-12, 15 and 19, of the instant application.

This is a *provisional* obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Claim Rejections - 35 USC § 102

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 --
> e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent.

9. Claims 1-12, 15 and 19-25 are rejected under 35 U.S.C. § 102(e) as being anticipated by U.S. Patent 5,530,101 (82).

Claims 1-2 and 19-25:

The '101 patent teaches methods for the production of humanized antibodies wherein the CDR amino acid sequences from the import (i.e. donor) are exchanged for the human (i.e. acceptor) CDR amino acid sequences (abstract, in particular). The '101 patent teaches alignment of import and human framework regions and selection of substituted human framework antibody residues based on the following effects; the import framework residue non-covalently binds antigen directly (i.e. Category three, column 14, in particular), interacts with a CDR (i.e. Category three or four, column 14-15, in particular), or participates in the V_L-V_H interface (i.e. Category 3,4 or 5, column 14-15, in particular).

The '101 patent teaches that if a residues is exposed on the surface of the domain (i.e. interacts with CDR) and doesn't have one of the effects of step f in claim 1, then to leave the human residue intact (column 13-14, in particular). The term "consensus" has been interpreted to include the aligning of murine import framework residues to human acceptor framework residues, in addition to the aligning of all human framework resiudues and compiling a single "consensus" human framework to be used as a template in every humanized antibody. Since "consensus" has limitless interpretations as vaguely defined in the specification, the prior art reads on the claimed invention.

Claims 3 and 4:

The additional step of determining whether or not a substituted residue is glycosylated is determined by the residue makeup of the import peptide, a fact well known in the art prior to

the invention and therefore lends no patentable import to the invention.

Claim 5:

The '101 patent teaches retaining those residues that are highly conserved (i.e. not rare) in the human framework region (Category 2 and 5, Column 14-16, in particular).

Claims 6-8:

The '101 patent teaches which human and import residues are likely to be selected for substitution. In addition the '101 patent teaches corresponding import for human substitution at specific sites (Column 15, in particular).

Claim 9:

The '101 patent teaches a method employing a consensus human variable domain based on human variable domains and additionally variable domains from species other than human (Column 13, in particular).

Claims 10-12:

The '101 patent teaches a humanized antibody variable domain having a non-human CDR incorporated into a human antibody variable domain, wherein the improvement comprises the substitution of only specific corresponding human and import amino acid residues (column 15, in particular).

Claim 15:

The '101 patent teaches a method for engineering a humanized antibody comprising introducing residues from an import antibody variable domain into an amino acid sequence representing a consensus of mammalian antibody variable domain sequences (column 12-13, in particular).

The prior art teachings anticipate the claimed invention.

10. The references crossed out in the form PTO-1449 filed on 12/3/96 are the duplicates of the references stated in the formn PTO-1449 filed 8/30/96.

11. The lengthy specification has not been checked to the extent necessary to determine the presence of all possible minor errors. Applicants cooperation is requested in correcting any errors of which applicant may become aware of in the specification.

12. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Patrick

Nolan whose telephone number is (703) 305-1987. The examiner can normally be reached on Monday through Friday from 8:30 am to 4:30 pm.

13. If attempts to reach the examiner are unsuccessful, the examiner's supervisor, Christina Chan, can be reached at (703) 305-3973. The FAX number for our group, 1816, is (703) 305-7939. Any inquiry of a general nature relating to the status of this application or proceeding should be directed to the Group receptionist, whose telephone number is (703) 308-0196.

Patrick J. Nolan, Ph.D.
December 19, 1996

Christina Chan

CHRISTINA Y. CHAN
SUPERVISORY PATENT EXAMINER
GROUP 1800

[redacted] Duplicate of [redacted] Received 12/3/96

DDS received on 08/30/96

Sheet 1 of 1

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

LIST OF DISCLOSURES CITED BY APPLICANT

(Use several sheets if necessary)

Atty Docket No.	Serial No.
P07090T	08/146,206
Applicant: Carter et al.	
Filing Date: 17 Nov 1993	Group: ~~1806~~ 1816

U.S. PATENT DOCUMENTS

Examiner Initials		Document Number	Date	Name	Class	Subclass	Filing Date
PN	81	5,225,539 7-6-93	06.07.93	Winter, G.	C07K	15/28	25.10.91 10-25-91
PN	82	5,530,101 6-25-96	25.06.96	Queen et al.	A61K	39/395	19.12.90 12-19-90

FOREIGN PATENT DOCUMENTS

Examiner Initials		Document Number	Date	Country	Class	Subclass	Translation Yes	Translation No
PN	83	85058/91 2-20	92 30.03.92	AUSTRALIA	C07K	15/13		
	84	328,404 A1 8-16	89 16.08.89	EPO	A61K	39/395		
	85	451,216 B1 4-24	96 24.01.96	EPO	C12P	21/08		
	86	WO 91/09966 7-11-	91 11.07.91	PCT	C12P	21/08		
	87	WO 91/09968 7-11-	91 11.07.91	PCT	C12P	21/08		
PN	88	WO 92/11018 9-7-	92 09.09.92	PCT	A61K	35/14		

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

PN	89	Carter et al., "High level escherichia coli expression and production of a bivalent humanized antibody fragment." Bio/Technology 10:163-167 (1992)
	90	Foote et al., "Antibody Framework Residues Affecting the Conformation of the Hypervariable Loops" J. Mol. Biol. 224:487-499 (1992)
	91	Foote, J., "Humanized Antibodies" Nova acta Leopoldina 61(269):101-110 (1989)
	92	Kabat et al., "Sequences of Proteins of Immunological Interest", Bethesda, MD:National Institute of Health pps. 14-32 (1983)
	93	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)
	94	Maeda et al., "Construction of Reshaped Human Antibodies with HIV-neutralizing Activity" Hum. Antibod. Hybridomas 2:124-134 (July 1991)
	95	Riechmann et al, "Expression of an Antibody Fv Fragment in Myeloma Cells" J. Mol. Biol. 203:825-828 (1988)
	96	Routledge et al., "A Humanized Monovalent CD3 Antibody which Can Activate Homologous Complement" European Journal of Immunology 21:2717-2725 (1991)
	97	Rheinman et al., "Construction, Expression and Characterization of Humanized Antibodies Directed Against the Human α/β T Cell Receptor" J. Immunol. 147(12):4366-4373 (December 15, 1991)
PN	98	Tempest et al., "Reshaping a Human Monoclonal Antibody to Inhibit Human Respiratory Syncytial Virus Infection In Vivo" Bio/Technology 9:266-271 (March 1991)

Examiner: Patrick J. Nolan Date Considered: 12/16/96

*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.

USCOMM-DC 80-398

Patent Docket P0709P1

#29

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1816 Examiner: P. Nolan

CERTIFICATE OF MAILING
I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on

June 23, 1997

Sandra K. T. Sullivan

AMENDMENT TRANSMITTAL

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Transmitted herewith is an amendment in the above-identified application.

The fee has been calculated as shown below.

	Claims Remaining After Amendment		Highest No. Previously Paid For	Present Extra	Rate	Additional Fees
Total	31	-	24	7	x 22 =	$154.00
Independent	7	-	10	0	x 80 =	$0.00
__ First Presentation of Multiple Dependent Claims					+ 260 =	
					Total Fee Calculation	$154.00

_____ No additional fee is required.

X The Commissioner is hereby authorized to charge Deposit Account No. 07-0630 in the amount of $154.00. **A duplicate copy of this transmittal is enclosed.**

X Petition for Extension of Time is enclosed.

The Commissioner is hereby authorized to charge any additional fees required under 37 CFR 1.16 and 1.17, or credit overpayment to Deposit Account No. 07-0630. **A duplicate copy of this sheet is enclosed.**

Respectfully submitted,
GENENTECH, INC.

Date: June 23, 1997

By: Janet E. Hasak
Janet Hasak
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1816

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1816 Examiner: P. Nolan CERTIFICATE OF MAILING I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on June 23, 1997 Sandra K. T. Sullivan

PETITION AND FEE FOR THREE MONTH EXTENSION OF TIME
(37 CFR 1.136(a))

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Applicant petitions the Commissioner of Patents and Trademarks to extend the time for response to the OFFICIAL ACTION dated 23 December 1996 for three month(s) from 23 March 1997 to 23 June 1997. The extended time for response does not exceed the statutory period.

Please charge Deposit Account No. 07-0630 in the amount of $930.00 to cover the cost of the extension. Any deficiency or overpayment should be charged or credited to this deposit account. A duplicate of this sheet is enclosed.

Respectfully submitted,
GENENTECH, INC.

Date: June 23, 1997

By: Janet E. Hasak
Janet Hasak
Reg. No. 28,616
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1816

#29/E

C Griffin

8/1/97

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: 17 November 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1816 Examiner: P. Nolan RECEIVED JUL 2 1997 GROUP 1800 CERTIFICATE OF MAILING I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on June 23, 1997 Sandra K. T. Sullivan

TP 7/31/97

AMENDMENT UNDER 37 C.F.R. §1.111

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

In response to the Office Action dated December 23, 1996, the period for response having been extended as a result of the enclosed Petition for a three-month Extension of Time and requisite fee, Applicants respectfully request reconsideration of the above-identified application in view of the following amendments and remarks.

IN THE CLAIMS:

1. (Twice Amended) A method for making a humanized antibody comprising amino acid sequences of a non-human, import antibody and a human antibody, comprising the steps of:

(a) obtaining the amino acid sequences of at least a portion of an import heavy chain variable domain and of a consensus human variable domain of a human heavy chain immunoglobulin subgroup;

F 1

(b) identifying Complementarity Determining Region (CDR) amino acid sequences in the import variable domain and the consensus human variable domain;

(c) substituting an import CDR amino acid sequence for the corresponding consensus human CDR amino acid sequence;

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(d) aligning the amino acid sequences of a Framework Region (FR) of the import variable domain and a corresponding FR of the consensus human variable domain;

(e) identifying import FR residues in the aligned FR sequences that are non-homologous to the corresponding consensus FR residues;

(f) determining if the non-homologous import FR residue is 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 by affecting the proximity or orientation of the V_L and V_H regions with respect to one another; and

(g) for any non-homologous import FR residue which is expected to have at least one of these effects, substituting that residue for the corresponding amino acid residue in the consensus FR .

6. (Twice Amended) The method of claim 1, wherein the corresponding consensus FR residues substituted in step (g) are selected from the group consisting of 4L, [35L,] 36L, 38L, 43L, 44L, 46L, 58L, 62L, [64L,] 65L, 66L, 67L, 68L, 69L, 70L, [71L,] 73L, 85L, 87L, 98L, 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, [and] 78H and 92H.

7. (Twice Amended) A method comprising providing at least a portion of an import, non-human heavy chain variable domain amino acid sequence having a Complementarity Determining Region (CDR) and a Framework Region (FR), obtaining the amino acid sequence of at least a portion of a consensus human variable domain of a human heavy chain immunoglobulin subgroup having a CDR and a FR, substituting the non-human CDR for the human CDR in the consensus human variable domain, and substituting a non-human amino acid residue for the consensus amino acid residue at at least one of the following sites:
4L, [35L,] 36L, 38L, 43L, 44L, 46L, 58L, 62L, [64L,] 65L, 66L, 67L, 68L, 69L, 70L, [71L,] 73L, 85L, 87L, 98L, 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 69H, 68H, 70H, 73H, 74H, 75H, 76H, [and] 78H or 92H.

Please cancel claim 9, without prejudice.

10. (Twice Amended) A humanized antibody variable domain having a non-human Complementarity Determining Region (CDR) incorporated into a [consensus] human antibody variable domain, wherein an [human] amino acid residue has been substituted [by a non-] for the human amino acid residue at a site selected from the group consisting of: 4L, [35L,] 36L, 38L, 43L, 44L, 46L, 58L, 62L, [64L,] 65L, 66L, 67L, 68L, 69L, 70L, [71L,] 73L, 85L, 87L, 98L, 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, [and] 78H and 92H.

15. (Twice Amended) A method for engineering a humanized antibody comprising introducing amino acid residues from a non-human, import heavy chain variable domain into a consensus human variable domain of a human heavy chain immunoglobulin subgroup.

Please cancel claims 19-21, without prejudice.

22. (Amended) A humanized antibody comprising a consensus human variable domain of a human heavy chain immunoglobulin subgroup wherein the amino acid residues forming the Complementarity Determining Regions (CDRs) thereof comprise non-human import antibody amino acid residues.

In claim 25, line 1, please replace "about 7" with --about 5--.

Please add the following claims:

--26. The humanized antibody of claim 22 wherein the human heavy chain immunoglobulin subgroup is V_H subgroup III.

27. The humanized antibody of claim 26 wherein the consensus human variable domain comprises the amino acid sequence of SEQ ID NO:4.

28. The humanized antibody of claim 22 further comprising a consensus human light chain variable domain comprising the amino acid sequence of SEQ ID NO:3 wherein the amino acid residues forming the CDRs of the light chain variable domain comprise non-human import antibody amino acid residues.

29. The humanized antibody of claim 23 wherein the FR residue noncovalently binds antigen directly.

30. The humanized antibody of claim 23 wherein the FR residue interacts with a CDR.

31. The humanized antibody of claim 23 wherein the FR residue comprises a glycosylation site which affects the antigen binding or affinity of the antibody.

32. The humanized antibody of claim 23 wherein the FR residue 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.

33. The humanized antibody of claim 22 which comprises one or more CDR residues from the consensus human variable domain.

34. The humanized antibody of claim 22 which binds antigen more tightly than the non-human antibody.

35. The humanized antibody of claim 22 which mediates antigen dependent cellular cytotoxicity (ADCC) to a greater extent than the non-human antibody.

36. The humanized antibody of claim 35 which is an IgG.

37. The humanized antibody of claim 36 which has an IgGγ1 constant region, wherein residue 359 of the constant region is D and residue 361 of the constant region is L.

38. A method for making a humanized antibody comprising amino acid sequences of a non-human antibody and of a human antibody, comprising the steps of aligning the amino acid sequence of a Framework Region (FR) of the non-human antibody and the corresponding amino acid sequence of a FR of the human antibody, identifying non-human antibody residue(s) in the aligned FR sequences that are non-homologous to the corresponding human antibody residue(s); and if any such non-homologous residue(s) is/are exposed on the surface of the variable domain, providing the corresponding human antibody residue(s) in the humanized antibody.--

REMARKS

Amendments

Claims 1, 7, 15 and **22** have been revised herein to refer to a consensus human variable domain of a "human heavy chain immunoglobulin subgroup," as supported, for example, on page 15, lines 18-25 and page 64, line 33 through to page 65, line 2 of the specification. Basis for heavy chain variable domain in **claims 1, 7** and **15** is found on at least page 11, line 9 of the specification. **Claims 6, 7** and **10** have been amended to include FR substitutions as in the claims as originally filed. **Claim 10** has been amended to have wording as in the claim as originally filed, and basis for the revision to **claim 25** is found, for example, in Table 3 in Example 1.

Claims 26-38 have been added herein and find basis at least as follows: claims **26 and 27** (page 15, lines 18-25 and page 64, line 33 through to page 65, line 2); **claim 28** (page 15, lines 18-21); **claims 29-32** (part f of claim 1 and originally filed, now canceled claim 3); **claim 33** (page 27, lines 1-8; page 27 lines 8-9 and page 65, lines 5-9); **claim 34** (page 68, lines 25-27 and Table 3 on page 65 with respect to Kd values for the murine antibody and two humanized variants huMAb4D5-6 and huMAb4D5-8); **claim 35** (page 69, lines 32-34 and Table 4 on page 74); **claim 36** (page 11, lines 11-14); **claim 37** (page 65, line 29 through to page 66, line 1); and **claim 38** (claims 1 and 10, and originally filed, now canceled claim 2).

In that the amendments do not introduce new matter, their entry is respectfully requested.

Section 112, second paragraph

Claims 19-21 are rejected under 35 USC §112, second paragraph, as substantial duplicates of claim 1. In the interest of expediting examination, and without acquiescing in the rejection, claims 19-21 have been canceled, thus rendering this rejection moot.

§103

Claims 1, 2, 4-12, 15 and renumbered claims 19-22 and 24-25 stand rejected under 35 USC §103 as unpatentable over EP239,400A2 (Winter patent application); Riechmann *et al. Nature* **332**:323-327 (1988); and Queen *et al. PNAS, USA* **86**:10029-10033 (1989). The Examiner states that Applicants' arguments filed 6/12/95 are not considered to be persuasive. Concerning the consensus sequence, the Examiner alleges that "the combination of references teach [the] human framework regions having a significantly high degree of sequence homology (conservative regions)" and states that Queen *et al.* point to Kabat as demonstrating that this was known in the art. The Examiner urges that "In essence there is no functional/structural distinction from what applicant has claimed and that taught by the combination of references." The Examiner contends that modifications in the framework regions which affect the proximity or orientation of the V_L-V_H interface regions are the same as substituting that FR residue from the import regions that is involved in the effects set forth in paragraph (f) of claim 1. According to the Examiner, the references, *e.g.,* Riechmann *et al.*, teach reduced immunogenicity associated with the humanized antibody.

Applicants respectfully traverse this rejection as it may apply to the claims as amended herein.

With respect to the cited references, Applicants point out that the Winter patent application fails to disclose or suggest the use of a consensus human variable domain in antibody humanization. On the contrary, the heavy chain framework region of the humanized B1-8 antibody of Example 1 and of the humanized anti-lysozyme antibody D1.3 of Example 2 was derived from the human myeloma heavy chain NEWM (see page 17, lines 1-2 and lines 9-10 on page 26), which was chosen because the crystallographic structure thereof was known (see page 17, lines 2-3). The light chains of the B1-8 and D1.3 antibodies were never humanized in EP 239,400 A2.

Furthermore, only the CDRs were transferred in the Examples of this patent application; none of the non-human FR residues were incorporated into the engineered molecule.

Using the same strategy as disclosed in the Winter patent application, Riechmann and his colleagues made a humanized heavy chain variable domain which had the framework regions of human NEWM alternating with the CDRs of the rat CAMPATH-1 antibody. Thus, the same heavy chain framework region as disclosed in the Winter patent application was used once again, in view of the availability of a crystallographic structure for it (see page 325, second to last paragraph of Riechmann *et al.*). In this respect, Riechmann *et al.* fails to disclose or suggest the use of a "consensus human variable domain of a human heavy chain immunoglobulin subgroup" (*e.g.,* human heavy chain immunoglobulin V_H subgroup III (claim 26) having the amino acid sequence of SEQ ID NO:4 (claim 27), for example) for providing the framework region of the heavy chain variable domain of the humanized antibody. For humanization of the light chain of the rat CAMPATH-1 antibody, Riechmann *et al.* states that a framework sequence based on the human REI light chain variable domain (for which a crystallographic structure was available) was used (see, Figure 1 legend and page 325, second column). Applicants have now learnt that the humanized light chain gene of the CAMPATH-1 antibody in Riechmann *et al.* was converted from an anti-lysozyme construct (see page 108 of Foote, J., *Nova acta Leopoldina* NF **61**(**269**):103-110 (1989), of record). Foote's anti-lysozyme construct was prepared by combining CDR sequences from the kappa light chain of the anti-lysozyme antibody with consensus human kappa frameworks (see page 106, third paragraph of Foote, *supra*).

Queen *et al.* teaches that human framework regions used in humanization must be chosen to maximize homology with the murine antibody in order to avoid introducing "distortions into the CDRs" (see page 10031, column 2, paragraph 2). Using their "best-fit" approach, Queen *et al.* used the heavy and light chain variable regions of the human Eu antibody to form the framework of their humanized anti-Tac antibody. There is no mention of a consensus human variable domain for providing the framework region of the humanized antibody. In fact, Queen *et al.* taught away from the instantly claimed invention, in that they proposed that the framework region sequence of the humanized antibody be derived from a single human antibody amino acid sequence which was as homologous as possible to the non-human sequence to be humanized.

Therefore, according to the teachings of Queen *et al.,* human framework region sequences needed to be tailored to each non-human antibody to be humanized. Furthermore, this reference taught that the heavy chain and light chain used for humanization should be derived from the same human antibody.

Applicants submit that the invention recited in independent **claims 1, 7, 15 and 22** herein differs from the teachings of each of the cited references in that it provides humanized antibodies wherein the heavy chain framework region of the humanized antibody is provided by a consensus human variable domain of a human heavy chain immunoglobulin subgroup, such as the V_H subgroup III consensus human variable domain, *e.g.*, of SEQ ID NO:4. The references cited by the Office fail to disclose or suggest the use of such a heavy chain consensus human variable domain.

First, Applicants will comment on the statement by the Examiner that "there is no functional/structural distinction from what applicant has claimed and that taught by the combination of references." As noted above, independent claims 1, 7, 15 and 22 herein recite a "consensus human variable domain of a human heavy chain immunoglobulin subgroup." As noted on page 15, lines 15-25 of the application, consensus sequences (*i.e.,* most commonly occurring residue or pair of residues) of human heavy chain immunoglobulin subgroups are compiled in Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, Fourth Edition, U.S. Dept. of Health & Human Services, pubs., (1987). Kabat *et al.* grouped various heavy and light chain variable domains according to their amino acid sequence identity to form several human immunoglobulin *subgroups, i.e.*, human kappa light chains subgroups I to IV, human lambda light chains subgroups I to VI and human heavy chains subgroups I to III (see pages 41-76 and 160-175 of Kabat *et al.*, copies attached). The "occurrences of most common amino acid" (*i.e.,* "consensus human variable domain" of the instant claims) at each position of the variable domain are provided in the second to last column for each immunoglobulin subgroup in Kabat *et al.* The cited references fail to disclose or suggest the use of a consensus human variable domain of a human heavy chain immunoglobulin subgroup having such an amino acid sequence in antibody humanization. Thus, Applicants submit that the heavy chain framework region of the claims herein, in fact, is structurally distinct from the framework regions of the cited references.

Second, with respect to the Examiner's comment that a modification in the framework regions which affects the proximity or orientation of the V_L-V_H interface regions is the same as substituting that FR residue from the import regions that is involved in the effects set forth in paragraph (f) of claim 1, Applicants respectfully invite the Office to point out where exactly the references teach the invention set forth in part (f)(3) of claim 1.

Finally, concerning the allegation that Riechmann *et al.* teaches reduced immunogenicity associated with the humanized antibody, Applicants enclose a copy of Isaacs *et al. The Lancet* **340**:748-752 (1992). Isaacs *et al.* demonstrate that three out of four patients treated with Riechmann's humanized CAMPATH-1H antibody developed antiglobulins that were able to inhibit the binding of CAMPATH-1H to its antigen (see first paragraph of the discussion on page 751 of this reference). On the contrary, repeated administration (*i.e.*, loading dose and 10 weekly doses) of the humanized anti-HER2 antibody (huMAb4D5-8) of Example 1 of the instant application has not lead to an immunogenic response in patients treated therewith (*i.e.* no antibodies against rhuMAb HER2 were detected in any patients). See abstract of Baselga *et al.*, *J. Clin. Oncol.* **14**(**3**):737-744 (1996), copy attached. Likewise, multidose administrations of an anti-IgE antibody humanized according to the teachings of the instant application and having a consensus human variable domain as claimed herein, did not induce a human antihuman antibody response in any of the patients treated therewith (see column 1, last paragraph on page 311 of Shields *et al.*, *Int. Arch. Allergy Immunol.* **107**:308-312 (1995), copy attached). These data point to the functional distinctness of the claimed consensus human variable domain.

In addition to the desirable lack of immunogenicity of the claimed humanized antibodies, as is apparent from the examples, the binding affinity of an antibody humanized using the claimed method is essentially retained and in some instances is *improved* in the humanized antibody compared to the non-human antibody from which it was derived. As shown, for example, in Table 3 of Example 1, anti-HER2 humanized variants huMAb4D5-6 and huMAb4D5-8 had binding affinities which were superior to the murine antibody from which they were derived. This could not have been predicted from the prior art, especially from Queen *et al.*, which advocated

the best-fit method (see above) and incorporated many (*i.e.*, 15; see Figure 2) murine residues back into the humanized sequence to generate a "high affinity" humanized antibody. The above-mentioned anti-HER2 variants, on the other hand, had only five FR substitutions and were not generated using the "best-fit" method said to be essential by Queen *et al.*

The instantly claimed invention has other novel and non-obvious features. For example, claim 2 involves retaining the human residue, where the corresponding non-homologous import residue is exposed on the surface of the domain. The cited references fail to describe anywhere such a step. Claim 3 is independently patentable, as will be elaborated below. Claim 4 involves replacing consensus glycosylation sites which are not present in the import sequence with the corresponding import residue. The references are silent as to such a step. Similarly, the references fail to describe the additional step of claim 5 of the instant application. Also, the FR residues which can be substituted as now listed in claims 6, 7 and 10 are not disclosed in the cited references. Thus, Applicants submit that the invention recited in the claims of the instant application is clearly non obvious over the cited references.

Accordingly, Applicants request that the above section 103 rejection be withdrawn.

§103 - *In re Durden*

Claims 1, 2, 4-12 and 15 and renumbered claims 19-22 and 24-25 stand rejected under 35 USC §103 as being unpatentable over the Winter patent application, Riechmann *et al.* and Queen *et al.* in view of *In re Durden* 226 USPQ 359 (Fed. Cir. 1985).

The Examiner states that the claimed methods for producing humanized antibodies and for humanization do not appear to differ from what was disclosed in the references. For the reasons given in the previous section, Applicants submit that the instantly claimed methods for humanization and the humanized antibodies are clearly different from what was disclosed in the cited references, especially with respect to the consensus human variable domain forming the FR of the humanized antibody.

Further, the Examiner is respectfully referred to the recent CAFC decisions of In re Brouwer, 37 USPQ2d 1663 (Fed. Cir. 1996) and In re Ochiai, 37 USPQ2d 1127 (Fed. Cir. 1995). These cases stand for the proposition that a *prima facie* case of obviousness cannot be based on Durden, but rather needs to rest on particularized findings. It was held in Brouwer that there are no Durden obviousness rejections *per se*, only sec. 103 obviousness rejections. In the case of the instant claims, where the particular end product is unobvious, these cases hold that the method of making them is also unobvious. In this regard, the Examiner is referred to the Official Gazette notice of 3/26/96, copy enclosed, which establishes guidelines for PTO personnel and the public on the proper consideration of method claims in light of these cases. In this Notice, it is stated that:

> [I]nterpreting a claimed invention as a whole requires consideration of all claim limitations. Thus, language in a process claim which recites making or using a nonobvious product must be treated as a material limitation, and a motivation to make or use the nonobvious product must be present in the prior art for a § 103 rejection to be sustained.
>
> In light of Ochiai and Brouwer, Office personnel will consider all claim limitations when analyzing process claims which make or use nonobvious products under § 103. Office personnel will focus on treating claims as a whole and follow the analysis set forth in Graham v. John Deere, 383 U.S. 1, 148 USPQ 459 (1966). (emphasis in original)

Therefore, since there is no motivation in the cited art, as a whole, to make or use the nonobvious product, the claimed methods herein are non-obvious, and Applicants respectfully request that this rejection be reconsidered and withdrawn.

§103 - Claims 3 and 23

Claim 3 and renumbered claim 23 stand rejected under 35 USC §103 as being unpatentable over the Winter patent application, Riechmann *et al.* and Queen *et al.* as applied to claims 1, 2, 4-12, and 15 and further in view of Roitt *et al., Immunology* Gower Medical Publishing Ltd., London, England, pg. 5.5 (1985) for the same reasons set forth in Paper #18.

Applicants submit that claim 3 and FR substitution (c) of claim 23 clearly would not have been obvious in light of the cited references. The three primary references have been discussed

above. Roitt *et al.* merely shows that IgA1 immunoglobulins may possibly have carbohydrate units in their variable domains. No such carbohydrate or oligosaccharide units are depicted in the diagrams of IgD and IgE variable domains in this reference. This reference is not concerned with antibody humanization, much less how to deal with glycosylation sites in humanization. In fact, the 4D5 antibody referred to in Example 1 is fairly unusual in that it has a glycosylation site in its variable region (*i.e.*, residue number 65 of the light chain). As far as Applicants are aware, the instant application teaches, for the first time, how to deal with glycosylation sites in antibody humanization.

Accordingly, Applicants submit that claim 3 and FR substitution (c) of claim 23 are clearly not obvious in light of the references cited and therefore respectfully request that the §103 rejection be withdrawn.

Provisional double patenting rejection

Claims 1-12, 15 and 19-25 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-12, 15 and 19 of copending application Serial No. 08/439,004. Given the provisional nature of this rejection, Applicants respectfully request that it be held in abeyance pending resolution as to allowable subject matter in this application or in the application on which this provisional rejection is based.

§102

Claims 1-12, 15 and 19-25 are rejected under 35 USC §102(e) as being anticipated by US Patent 5,530,101 (the "101 patent"). With respect to claims 1-2 and 19-25, the Examiner is of the view that the 101 patent teaches methods for the production of humanized antibodies wherein the CDR amino acid sequences from the import/donor are exchanged for the human/acceptor CDR amino acid sequences, as well as the alignment of import and human framework regions and selection of substituted human framework antibody residues based on the following effects; the import framework residue noncovalently binds antigen directly, interacts with a CDR, or participates in the V_L-V_H interface. The Examiner asserts that the 101 patent teaches that, if a residue is exposed on the surface of the domain and does not have one of the effects of step (f) of claim 1, one should leave the human residue intact. The Examiner states

that the term "consensus" has been interpreted to include the aligning of murine import framework residues to human acceptor framework residues, in addition to the aligning of all human framework residues and compiling a single "consensus" human framework. The Examiner comments separately on claims 3 and 4, 5, 6-8, 9, 10-12 and 15 and contends that these claims are also anticipated by the 101 patent.

Applicants submit that the instantly claimed invention is not anticipated by the 101 patent for the reasons that follow.

The 101 patent fails to teach the use, in antibody humanization, of a consensus human variable domain, such as that of a human heavy chain immunoglobulin subgroup, as set forth in independent **claims 1, 7, 15 and 22** herein. As to claim 1 (and FR substitution (d) of claim 23), the 101 patent further fails to teach the step of identifying and altering FR residues that participate in the interface between the light chain variable domain and the heavy chain variable domain of an antibody (*i.e.*, the "V_L-V_H interface"). The Examiner takes the view that categories 3, 4 and 5 in columns 14 and 15 of the 101 patent teach selection and substitution of such FR residues, but Applicants respectfully disagree. The FR residues to be identified in categories 3, 4 and 5 of the 101 patent are those which "interact with amino acids in the CDR's", "interact directly with the antigen" or are "rare" for human sequences. There is no explicit teaching in the 101 patent as to category (f)(3) of claim 1 or FR substitution (d) of claim 23 herein.

Hence, Applicants submit that independent claims 1, 7, 15 and 22 as well as FR substitution (d) of claim 23 are clearly novel over the 101 patent.

As to the other rejected claims, Applicants submit that they are further novel over the 101 patent for the reasons which follow.

Claim 2 is concerned with determining whether non-homologous residues are exposed on the surface of the domain or buried within it. Where the non-homologous residue is exposed, the human residue is retained. Applicants submit that determining whether a residue is exposed on the surface of a domain or buried within it as recited in claim 2 is not the same as determining

whether a residue "interacts with a CDR". Applicants contend that the 101 patent in columns 13-14 does not teach the additional step of claim 2 of the instant application.

With respect to **claims 3 and 4** (as well as FR substitution (c) of claim 23), Applicants submit that since the Examiner has failed to show where the 101 patent mentions glycosylation, let alone the invention recited in claims 3 and 4 and part (c) of claim 23, these claims must be novel over the 101 patent. If this rejection is to be maintained, Applicants request that the Examiner point out specifically where the 101 patent teaches the method steps of claims 3 and 4 and part (c) of claim 23 herein.

As to **claim 5**, this refers to a step wherein non-homologous residues are identified and the human residue is used, where it represents a residue which is highly conserved across all species at that site. Category 2 in column 14 of the 101 patent refers, on the other hand, to using the "donor amino acid rather than the acceptor". Category 5 in the paragraph bridging columns 15-16 of the 101 patent suggests that neither the donor nor the acceptor residue be used where the donor and acceptor residues are "rare". Clearly, the 101 patent fails to anticipate the method of claim 5 herein.

Turning now to **claims 6-8**, the residues specifically mentioned as candidates for substitution in column 15 of the 101 patent (to which the Examiner refers) have been removed from claim 6 and claim 7 (on which claim 8 depends).

Concerning **claim 9**, Applicants submit that the 101 patent fails to enable the consensus human variable domain of this claim, but nevertheless the rejection is moot, due to the cancellation of claim 9.

With respect to **claims 10-12**, the residue positions mentioned in column 15 of the 101 patent have been removed from claim 10 (on which claims 11 and 12 depend).

As to **claims 19-21**, Applicants submit that these claims are novel over the 101 patent, but they were canceled, and thus the §102 rejection is moot insofar as it applies to these claims.

Finally, with respect to **claims 24-25**, Applicants submit that the Examples of the 101 patent require many more FR substitutions than "about 1 to about 5" as recited in these claims.

Applicants submit that, for the reasons given above, **claims 1-12, 15** and **19-25** are clearly novel over the 101 patent, and therefore respectfully request that this rejection be reconsidered and withdrawn.

Applicants believe that the amendments and comments here put this case in condition for allowance. Nevertheless, should the Examiner have any further comments or questions, he is invited to call Wendy Lee at (415) 225-1994 concerning these.

Respectfully submitted,
GENENTECH, INC.

Date: June 23, 1997

By: Janet E. Hasak
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Enclosures:
Isaacs *et al.*
Baselga *et al.*
Shields *et al.*
Kabat *et al.*
OG Notice of 3/26/96

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

08/146,206

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKETT NO.

EXAMINER	
ART UNIT	PAPER NUMBER

DATE MAILED:

EXAMINER INTERVIEW SUMMARY RECORD

All participants (applicant, applicant's representative, PTO personnel):

(1) PATRICIC NOLAN (3) CHRIS EISENSCHENK

(2) WENDY LEE (4)

Date of Interview 7/23/97

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: All

Identification of prior art discussed:

Description of the general nature of what was agreed to if an agreement was reached, or any other comments: It was disussed that Applicant define "consensus" by Framework region residues.

(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.)

☑ 1. It is not necessary for applicant to provide a separate record of the substance of the interview.

Unless the paragraph below has 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.

☐ 2. 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. Applicant is not relieved from providing a separate record of the substance of the interview unless box 1 above is also checked.

Patrick J. Nolan
Examiner's Signature

#30
10/7/97

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: 17 November 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1816 Examiner: P. Nolan **CERTIFICATE OF HAND DELIVERY** I hereby certify that this correspondence is being delivered to Receptionist, Group 1800 of the United States Patent and Trademark Office, Washington, D.C. 20231 on September 2, 1997 Martin P. Hoffman Printed Name: MARTIN P. HOFFMAN

SUPPLEMENTAL AMENDMENT

Assistant Commissioner of Patents
Washington, D.C. 20231
Sir:

Please amend the application in the following respects:

IN THE SPECIFICATION:

On page 9, line 1, please replace "muMAb4d5" with --muMAb4D5--.

On page 9, lines 24, 29, 30 and 31, please replace "huxCD3v9" with --huxCD3v1--.

On page 9, line 30, please replace "20" with --26--.

On page 9, line 33, please replace "(○)" with --(●)--.

On page 84, line 29, please replace "(Fig. 5)" with --(SEQ ID NO:20)--.

On page 90, please substitute the "SEQUENCE LISTING" with the enclosed paper copy of the "SEQUENCE LISTING".

REMARKS

This amendment is prepared for the purposes of introducing a substitute sequence listing into the application. Applicants have found that SEQ ID NO:20 from the previously submitted sequence listing corresponds to the heavy chain variable domain sequence of huxCD3v9 (see page 84, line 29), whereas Figure 5 shows the sequence of huxCD3v1. The description of Figure 5 on page 9 has been corrected in this respect and the sequence of huxCD3v1 in Figure 5 is included in the substitute sequence listing as SEQ ID NO:26. Further typographical errors in lines 1 and 33 on page 9 are corrected herein. Furthermore, page 84, line 29 now refers to SEQ ID NO:20, the huxCD3v9 heavy chain variable domain sequence. In accordance with 37 C.F.R. §§1.821(f) and (g), the undersigned hereby states that the content of the paper and the computer readable sequence listings is the same. I further state that this submission includes no new matter.

Respectfully submitted,

GENENTECH, INC.

By: Wendy M. Lee

Wendy M. Lee
Reg. No. 40,378

Date: August 29, 1997

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South San Francisco, CA 94080-4990
Phone: (415) 225-1994

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Carter, Paul J.
Presta, Leonard G.

(ii) TITLE OF INVENTION: Method for Making Humanized Antibodies

(iii) NUMBER OF SEQUENCES: 26

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Genentech, Inc.
(B) STREET: 1 DNA Way
(C) CITY: South San Francisco
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 94080

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 3.5 inch, 1.44 Mb floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: WinPatin (Genentech)

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: 08/146206
(B) FILING DATE: 17-Nov-1993
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 07/715272
(B) FILING DATE: 14-JUN-1991

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Lee, Wendy M.
(B) REGISTRATION NUMBER: 40,378
(C) REFERENCE/DOCKET NUMBER: P0709P1

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 650/225-1994
(B) TELEFAX: 650/952-9881

(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
95 100 105

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

F1 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

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
95 100 105

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
95 100 105

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

F1

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 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

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
95 100 105

Ala Met Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
110 115 120

(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                 100                 105

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

f1

(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                 100                 105

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

(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

F1

(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

(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                 100                 105

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
            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                 100                 105
```

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
95 100 105

F1

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

Ser Ala Val Tyr Tyr Cys Ala Arg Ser Gly Tyr Tyr Gly Asp Ser
95 100 105

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

F1 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
95 100 105

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

#33

Patent Docket P0709P1

10/7/97

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1816 Examiner: P. Nolan CERTIFICATE OF HAND DELIVERY I hereby certify that this correspondence is being delivered to Receptionist, Group 1800 of the United States Patent and Trademark Office, Washington, D.C. 20231 on Sept. 2 ~~August~~ ___, 1997 MARTIN P. HOFFMAN Printed Name Martin P. Hoffman

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

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Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Applicants submit herewith patents, publications or other information (attached hereto and listed on the attached Form PTO-1449) of which they are aware, which they believe may be material to the examination of this application and in respect of which there may be a duty to disclose in accordance with 37 CFR §1.56.

This Information Disclosure Statement:

(a) [ ] accompanies the new patent application submitted herewith. 37 CFR §1.97(a).

(b) [ ] is filed within three months after the filing date of the application or within three months after the date of entry of the national stage of a PCT application as set forth in 37 CFR§1.491.

(c) [ ] as far as is known to the undersigned, is filed before the mailing date of a first Office action on the merits.

(d) [X] is filed after the first Office Action and more than three months after the application's filing date or PCT national stage date of entry filing but, as far as is known to the undersigned, prior to the mailing date of either a final rejection or a notice of allowance, whichever occurs first, and is accompanied by either the fee ($230) set forth in 37 CFR §1.17(p) **or** a certification as specified in 37 CFR §1.97(e), as checked below. Should any fee be due, the U.S. Patent and Trademark Office is hereby authorized to charge Deposit Account No. 07-0630 in the amount of $220.00 to cover the cost of this Information Disclosure Statement. Any deficiency or overpayment should be charged or credited to this deposit account. **A duplicate of this sheet is enclosed.**

(e) [ ] is filed after the mailing date of either a final rejection or a notice of allowance, whichever occurred first, and is accompanied by the fee ($130) set forth in 37 CFR §1.17(i)(1) **and** a certification as specified in 37 CFR §1.97(e), as checked below. **This document is to be considered as a petition requesting consideration of the information disclosure statement**. The U.S. Patent and Trademark Office is hereby authorized to charge Deposit Account No. 07-0630 in the amount of $130.00 to cover the cost of this Information Disclosure Statement. Any deficiency or overpayment should be charged or credited to this deposit account. **A duplicate of this sheet is enclosed.**

[If either of boxes (d) or (e) is checked above, the following "certification" under 37 CFR §1.97(e) may need to be completed.] The undersigned certifies that:

[ ] Each item of information contained in the information disclosure statement was cited in a communication mailed from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of this information disclosure statement.

[ ] No item of information contained in this information disclosure statement was cited in a communication mailed from a foreign patent office in a counterpart foreign application or, to the knowledge of the undersigned after making reasonable inquiry, was known to any individual designated in 37 CFR §1.56(c) more than three months prior to the filing of this information disclosure statement.

A list of the patent(s) or publication(s) is set forth on the attached Form PTO-1449 (Modified).

A copy of the items on PTO-1449 is supplied herewith:

[x] each [ ] none [ ] only those listed below:

Those patent(s) or publication(s) which are marked with an asterisk (*) in the attached PTO-1449 form are not supplied because they were previously cited by or submitted to the Office in a prior application Serial No. ___ ___, filed _____and relied upon in this application for an earlier filing date under 35 USC §120.

A concise explanation of relevance of the items listed on PTO-1449 is:

[x] not given

[ ] given for each listed item

[ ] given for only non-English language listed item(s) [Required]

[ ] in the form of an English language copy of a Search Report from a foreign patent office, issued in a counterpart application, which refers to the relevant portions of the references.

The Examiner is reminded that a "concise explanation of the relevance" of the submitted prior art "may be nothing more than identification of the particular figure or paragraph of the patent or publication which has some relation to the claimed invention," MPEP §609.

While the information and references disclosed in this Information Disclosure Statement may be "material" pursuant to 37 CFR §1.56, it is not intended to constitute an admission that any patent, publication or other information referred to therein is "prior art" for this invention unless specifically designated as such.

In accordance with 37 CFR §1.97(g), the filing of this Information Disclosure Statement shall not be construed to mean that a search has been made or that no other material information as defined in 37 CFR §1.56(a) exists. It is submitted that the Information Disclosure Statement is in compliance with 37 CFR §1.98 and MPEP §609 and the Examiner is respectfully requested to consider the listed references.

Respectfully submitted,

GENENTECH, INC.

Date: August 29, 1997

By: _____

Wendy M. Lee
Reg. No. 40,378

460 Pt. San Bruno Blvd.
So. San Francisco, CA 94080-4990
Phone: (415) 225-1994
Fax: (415) 952-9881

#33 Attach

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

LIST OF DISCLOSURES CITED BY APPLICANT

(Use several sheets if necessary)

Atty Docket No.	Serial No.
P0709P1	08/146,206
Applicant	
Carter et al.	
Filing Date	**Group**
17 Nov 1993	1806

U.S. PATENT DOCUMENTS

Examiner Initials		Document Number	Date	Name	Class	Subclass	Filing Date
TDS	100	4,845,198	04.07.89	Urdal et al.	530	388.22	
	101	5,132,405	21.07.92	Huston et al.	530	387.3	
	102	5,558,864	24.09.96	Bendig et al.	424	133.1	
	103	5,585,089	17.12.96	Queen et al.	424	133.1	

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	106	365,209 A2	25.04.90	EPO				
	107	365,997 A2	02.05.90	EPO				
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	110	WO 87/02671	07.05.87	PCT				
	111	WO 88/09344	01.12.88	PCT				
	112	WO 91/07500	30.05.91	PCT				
	113	WO 92/01047	23.01.92	PCT				
	114	WO 92/04380	19.03.92	PCT				

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Examiner M. T. DAVIS Date Considered 12/05/01

*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.

USCOMM-DC 80-398.

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

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P0709P1	08/146,206
Applicant	
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Filing Date	**Group**
17 Nov 1993	1806

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Examiner	Date Considered
M. T. DAVIS	12/05/01

*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.

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Carter et al.	
Filing Date	**Group**
17 Nov 1993	1806

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Examiner	Date Considered
M. T. DAVIS	12/01/01

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P0709P1	08/146,206
Applicant	
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Filing Date	**Group**
17 Nov 1993	1806

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USCOMM-DC 80-398.

#3 (Supp) 10/7/97

Patent Docket P0709P1

OFFICIAL PLEASE ENTER P.N. 10-7-97

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1816 Examiner: P. Nolan CERTIFICATE OF HAND DELIVERY I hereby certify that this correspondence is being delivered to Receptionist, Group 1800 of the United States Patent and Trademark Office, Washington, D.C. 20231 on October 7, 1997 R. H. Mitchell Printed Name R. H. Mitchell

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Applicants submit herewith patents, publications or other information (attached hereto and listed on the attached Form PTO-1449) of which they are aware, which they believe may be material to the examination of this application and in respect of which there may be a duty to disclose in accordance with 37 CFR §1.56.

This Information Disclosure Statement:

(a) [ ] accompanies the new patent application submitted herewith. 37 CFR §1.97(a).

(b) [ ] is filed within three months after the filing date of the application or within three months after the date of entry of the national stage of a PCT application as set forth in 37 CFR§1.491.

(c) [ ] as far as is known to the undersigned, is filed before the mailing date of a first Office action on the merits.

(d) [X] is filed after the first Office Action and more than three months after the application's filing date or PCT national stage date of entry filing but, as far as is known to the undersigned, prior to the mailing date of either a final rejection or a notice of allowance, whichever occurs first, and is accompanied by either the fee ($230) set forth in 37 CFR §1.17(p) **or** a certification as specified in 37 CFR §1.97(e), as checked below. Should any fee be due, the U.S. Patent and Trademark Office is hereby authorized to charge Deposit Account No. 07-0630 in the amount of $220.00 to cover the cost of this Information Disclosure Statement. Any deficiency or overpayment should be charged or credited to this deposit account. **A duplicate of this sheet is enclosed.**

(e) [ ] is filed after the mailing date of either a final rejection or a notice of allowance, whichever occurred first, and is accompanied by the fee ($130) set forth in 37 CFR §1.17(i)(1) **and** a certification as specified in 37 CFR §1.97(e), as checked below. **This document is to be considered as a petition requesting consideration of the information disclosure statement**. The U.S. Patent and Trademark Office is hereby authorized to charge Deposit Account No. 07-0630 in the amount of $130.00 to cover the cost of this Information Disclosure Statement. Any deficiency or overpayment should be charged or credited to this deposit account. **A duplicate of this sheet is enclosed.**

[If either of boxes (d) or (e) is checked above, the following "certification" under 37 CFR §1.97(e) may need to be completed.] The undersigned certifies that:

[ ] Each item of information contained in the information disclosure statement was cited in a communication mailed from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of this information disclosure statement.

[ ] No item of information contained in this information disclosure statement was cited in a communication mailed from a foreign patent office in a counterpart foreign application or, to the knowledge of the undersigned after making reasonable inquiry, was known to any individual designated in 37 CFR §1.56(c) more than three months prior to the filing of this information disclosure statement.

A list of the patent(s) or publication(s) is set forth on the attached Form PTO-1449 (Modified).

A copy of the items on PTO-1449 is supplied herewith:

[x] each [ ] none [ ] only those listed below:

Those patent(s) or publication(s) which are marked with an asterisk (*) in the attached PTO-1449 form are not supplied because they were previously cited by or submitted to the Office in a prior application Serial No. ___, filed _____and relied upon in this application for an earlier filing date under 35 USC §120.

A concise explanation of relevance of the items listed on PTO-1449 is:

[x] not given

[ ] given for each listed item

[ ] given for only non-English language listed item(s) [Required]

[ ] in the form of an English language copy of a Search Report from a foreign patent office, issued in a counterpart application, which refers to the relevant portions of the references.

The Examiner is reminded that a "concise explanation of the relevance" of the submitted prior art "may be nothing more than identification of the particular figure or paragraph of the patent or publication which has some relation to the claimed invention," MPEP §609.

While the information and references disclosed in this Information Disclosure Statement may be "material" pursuant to 37 CFR §1.56, it is not intended to constitute an admission that any patent, publication or other information referred to therein is "prior art" for this invention unless specifically designated as such.

In accordance with 37 CFR §1.97(g), the filing of this Information Disclosure Statement shall not be construed to mean that a search has been made or that no other material information as defined in 37 CFR §1.56(a) exists. It is submitted that the Information Disclosure Statement is in compliance with 37 CFR §1.98 and MPEP §609 and the Examiner is respectfully requested to consider the listed references.

Respectfully submitted,
GENENTECH, INC.

Date: October 6, 1997 By: _____
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FORM PTO-1449 U.S. Dept. of Commerce Patent and Trademark Office

Atty Docket No.	Serial No.
P0709P1	08/146,206
Applicant: Carter and Presta	
Filing Date: 17 Nov 1993	Group: ~~1806~~ 1816

LIST OF DISCLOSURES CITED BY APPLICANT

(Use several sheets if necessary)

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PN	1	4,816,567	~~28.03.89~~ 3-28-89	Cabilly et al.			

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	5	WO 89/06692	~~7-25-89~~ ~~27.07.89~~	PCT				
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M. T. DAVIS 12/05-/01

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Adams	10/25/95

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M.T. DAUS 12/05/01

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Examiner: Adams

Date Considered: 10/25/95

*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.

M. T. DAVIS 12/05/01

USCOMM-DC 80-398.

Patent Docket P0709P1 #32

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1816 Examiner: P. Nolan **CERTIFICATE OF HAND DELIVERY** I hereby certify that this correspondence is being delivered to Receptionist, Group 1800 of the United States Patent and Trademark Office, Washington, D.C. 20231 on October 7, 1997 R. H. Mitchell Printed Name R. H. Mitchell

AMENDMENT TRANSMITTAL

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Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Transmitted herewith is an amendment in the above-identified application.

The fee has been calculated as shown below.

	Claims Remaining After Amendment		Highest No. Previously Paid For	Present Extra	Rate	Additional Fees
Total	35	-	31	4	x 88 =	$88.00
Independent	8	-	10	0	x 80 =	$0.00
__ First Presentation of Multiple Dependent Claims					+ 260 =	
					Total Fee Calculation	$88.00

_____ No additional fee is required.
__X__ The Commissioner is hereby authorized to charge Deposit Account No. 07-0630 in the amount of $88.00. **A duplicate copy of this transmittal is enclosed.**
_____ Petition for Extension of Time is enclosed.

The Commissioner is hereby authorized to charge any additional fees required under 37 CFR 1.16 and 1.17, or credit overpayment to Deposit Account No. 07-0630. **A duplicate copy of this sheet is enclosed.**

Respectfully submitted,
GENENTECH, INC.

Date: October 6, 1997

By: W M Lee
Wendy M. Lee
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#32/8

Patent Docket P0709P1

10/7/97

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: 17 November 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1816 Examiner: P. Nolan **CERTIFICATE OF HAND DELIVERY** I hereby certify that this correspondence is being delivered to Receptionist, Group 1800 of the United States Patent and Trademark Office, Washington, D.C. 20231 on October ___, 1997 Printed Name:

SUPPLEMENTAL AMENDMENT UNDER 37 C.F.R. §1.111

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Applicants respectfully request reconsideration of the above-identified application in view of the following amendments and remarks.

IN THE SPECIFICATION:

On page 8, lines 25-27 and page 15, lines 23-24, please replace the sequence in its entirety with the following sequence --

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYAMSWVRQAPGKGLEWVAVISENGSDTYYADS
VKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCARDRGGAVSYFDVWGQGTLVTVSS--

On page 9, line 30, please replace "huκI" with --hulll--.

IN THE CLAIMS:

10. (Three times amended) A humanized antibody variable domain having a non-human Complementarity Determining Region (CDR) incorporated into a human antibody variable domain, wherein an amino acid residue has been substituted for the human amino acid residue at a site selected from the group consisting of:

4L, [36L], 38L, 43L, 44L, 46L, 58L, 62L, 65L, 66L, 67L, 68L, 69L, [70L,] 73L, 85L, [87L,] 98L, 2H,

4H, [24H,] 36H, [37H,] 39H, 43H, 45H, [49H, 68H,] 69H, 70H, [73H,] 74H, 75H, 76H, 78H and 92H.

Please add the following claims:

--39. A humanized heavy chain variable domain comprising FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, wherein FR1-4 comprise the four framework regions of a consensus human variable domain of a human heavy chain immunoglobulin subgroup and CDR1-3 comprise the three complementarity determining regions (CDRs) of a nonhuman import antibody, and further wherein consensus human framework region (FR) residues have been replaced by nonhuman import residues where the FR residue (a) noncovalently binds antigen directly; (b) interacts with a CDR; (c) comprises a glycosylation site which affects the antigen binding or affinity of the antibody; or (d) participates in the V_L - V_H interface.

40. The humanized heavy chain variable domain of claim 39 wherein the human heavy chain immunoglobulin subgroup is V_H subgroup III.

41. The humanized heavy chain variable domain of claim 40 wherein:
FR1 of the consensus human variable domain comprises the amino acid sequence:
EVQLVESGGGLVQPGGSLRLSCAAS (SEQ ID NO:27);
FR2 of the consensus human variable domain comprises the amino acid sequence:
WVRQAPGKGLEWVA (SEQ ID NO:28);
FR3 of the consensus human variable domain comprises the amino acid sequence:
RFTISRDDSKNTLYLQMNSLRAEDTAVYYCAR (SEQ ID NO:29); and
FR4 of the consensus human variable domain comprises the amino acid sequence:
WGQGTLVTVSS (SEQ ID NO:30).

42. The humanized antibody of claim 22 which lacks immunogenicity upon repeated administration to a human patient in order to treat a chronic disease in that patient.--

REMARKS

A. Amendments

The undersigned confirms having met with Examiners Nolan and Eisenschenk in the interview 7/23/97 and takes this opportunity to thank the Examiners for the courtesies extended in the interview. Claims 39-41 have been added herein which use language as proposed by Examiner Nolan in the interview. Independent claim 39 is similar to a combination of presently pending claims 22 and 23. Basis for the language "FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, wherein FR1-4 comprise the four framework regions of a consensus human variable domain of a human heavy chain immunoglobulin subgroup and CDR1-3 comprise the three complementarity determining regions (CDRs) of a nonhuman import antibody" in claim 39 is found on page 1, lines 28-30 and page 25, lines 28-29, for example. Claim 40 finds specification basis on at least page 15, line 18. Claim 41 finds specification support in Figure 1B with respect to the framework regions of the HUV_HIII consensus sequence therein. Claim 42 has also been added and finds specification basis on at least page 60, lines 25-32 and page 70, lines 6-8. With respect to the amendments to the specification, the sequence on pages 8 and 15 has been corrected (see Section B of this amendment) and the typographical error with respect to the Fig. 5 sequence has been corrected herein. In that the amendments do not introduce new matter, their entry is respectfully requested.

B. Substitute Sequence Listing

A further substitute sequence listing is submitted herewith. Applicants have found that SEQ ID NO:4 in the previous sequence listings did not correspond to the HUV_HIII consensus sequence of Fig. 1B (see page 9, lines 1-2) and hence SEQ ID NO:4 in the attached substitute sequence listing has been corrected accordingly. Furthermore, SEQ ID NO:4 is hereby corrected on pages 8 and 15 of the application. In addition, separate sequence identifiers (SEQ ID NO's 27-30) have been given to the FR1-4 sequences in claim 41 added herein. In accordance with 37 C.F.R. §§1.821(f) and (g), the undersigned hereby states that the content of the paper and the computer readable sequence listings is the same. I further state that this submission includes no new matter.

C. Antibodies humanized according to the teachings of the instant application

As discussed in the interview, the consensus human variable domain of the instant claims has been used to humanize a number of antibodies, including:

1. *Anti-p185^{HER2} antibodies*. See Example 1 of the application, including Table 3 on page 72 (which describes humanized variants huMAb4D5-1-8) and page 65, lines 1-4 (concerning the use of a consensus human variable domain as recited in the claims herein). huMAb4D5-6 and huMAb4D5-8 had binding affinities which were suprisingly *superior* to that of the nonhuman antibody (muMAb4D5); see second to last column of Table 3. Repeated administration of the humanized anti-p185^{HER2} antibody huMAb4D5-8 has not lead to an immunogenic response in cancer patients treated therewith. See abstract of Baselga *et al.*, *J. Clin. Oncol.* 14(3):737-744 (1996), of record.

2. *Anti-CD3 antibodies*. See Example 3 on pages 79-88 of the application; and Fig. 5 as well as page 9, lines 25-31 concerning the use of a consensus human variable domain as claimed herein. [Note: In the Fig. 5 V_H consensus sequence (hulll), the last residue of FR2 is S, *i.e.* A→S, and eighth residue of FR3 is N, *i.e.* D→N, because of changes in 1987 to 1991 consensus sequence of Kabat *et al.*; such an equivalent consensus sequence and other changes in consensus sequences that result from the addition of further human antibody sequences to subsequent antibody compilations by Kabat *et al.* are clearly encompassed by the claims herein]. Humanized anti-CD3 variant (v1) was found to enhance the cytotoxic effects of activated human cytotoxic T lymphocytes (CTL) 4-fold against SK-BR-3 tumor cells overexpressing p185^{HER2} (page 81, lines 1-4). Variants of the humanized v1 antibody were made (v6 to v12; see page 82, line 22 and page 84, line 17 through to page 85, line 2 and page 86, lines 17-31), including the most potent variant, v9, which bound Jurkat cells almost as efficiently as the chimeric BsF(ab')$_2$ (page 86, lines 20-22).

3. *Anti-CD18 antibody*. See Example 4 on page 89 of the application and Figs. 6A and 6B with respect to a consensus human variable domain as claimed in the instant application. The binding affinity of the humanized anti-CD18 antibody (pH52-8.0/pH52-9.0; see Figs. 6A and 6B of

the application) was similar to the nonhuman H52 antibody; *i.e.* the humanized antibody has an affinity of 3.9 ± 0.9nM and murine H52 antibody has an affinity of 1.5 ± 0.3nM.

4. *Anti-IgE antibodies*. See Presta *et al. J. Immunol.* 151(5)2623-2632 (1993), of record. Use of a consensus human variable domain of the claims of the instant application is disclosed on page 2624 (column 1, first and third full paragraphs) and in Fig. 1. A number of humanized variants were made (see full paragraph 2 in column 1 on page 2624), including F(ab)-12 with only five framework region substitutions which exhibited binding comparable to the murine antibody (paragraph 2 on page 2631). Multidose administrations of full length anti-IgE variant 12 did not induce a human antihuman antibody response in allergic patients treated therewith (see column 1, last paragraph on page 311 of Shields *et al.*, *Int. Arch. Allergy Immunol.* 107:308-312 (1995), of record).

5. *Anti-CD11a antibodies*. See Werther *et al. J. Immnol.* 157:4986-4995 (1996), of record. Use of a consensus human variable domain as taught and claimed in the instant application is discussed in the first sentence of the Results section on page 4988 and in Fig. 1 (see note in paragraph 2 above, with respect to changes in 1987 to 1991 consensus sequences. Eight humanized variants were made (see Table 1 on page 4989), including HuIgG1 which had an apparent Kd similar to the parent murine antibody and comparable activity to the murine antibody in the cell adhesion and mixed leukocyte reaction (MLR) assays (see paragraph briging columns 1-2 on page 4993).

6. *Anti-VEGF antibodies*. See Presta *et al.* "Humanization of an anti-VEGF monoclonal antibody for the therapy of solid tumors and other disorders" *Cancer Research*, in press, pps. 1-32 of the manuscript, of record. The first paragraph on page 12 refers to the use of a consensus human variable domain as in the claims of this application. With respect to the consensus sequence in the figure on page 32 of the manuscript, see note in paragraph 2 above concerning change in 1987 to 1991 consensus sequences. As shown in Table 1 on page 29, twelve humanized anti-VEGF antibodies were made. The humanized antibody 12-IgG1 acquired the binding properties and biological activities of a high-affinity murine anti-VEGF MAb (see page 16,

last paragraph of this reference).

D. FR substitutions by Queen *et al.*

With respect to pending claim 10 herein reciting substitutions at specified sites in the V_H and V_L framework regions, as discussed at the interview, Queen *et al. PNAS, USA* 86:10029-10033 (1989) and US Patent 5,530,101 (the "101 patent") (cited by the office in the previous office action) use sequential numbering for the variable domain residues of the antibodies described in these references, whereas the claims of the instant application use Kabat numbering for the framework region residues (see page 14, lines 6-22 of the instant application). As requested by the Examiner in the interview, alignments of heavy chain variable domain (Exhibit A) and light chain variable domain (Exhibit B) sequences of the 101 patent (including the sequences for the murine and humanized anti-Tac antibody of Queen *et al.*) with sequential and Kabat residue numbering are attached. "murx" refers to the murine antibody sequence; "hzx" refers to the humanized antibody sequence; "H" is used for heavy chain variable domain sequences and "L" for light chain variable domain sequences. The sites at which the 101 patent refers to FR substitutions are:

Anti-Tac antibody (Figs. 1A and 1B of 101 patent)			
V_H FR substitions		V_L FR substitutions	
Sequential numbering	**Kabat numbering**	**Sequential numbering**	**Kabat numbering**
27H	27H	48L	48L
30H	30H	60L	60L
48H	48H	63L	63L
67H	66H		
68H	67H		
93H	89H		
95H	91H		
98H	94H		

107H	103H		
108H	104H		
109H	105H		
111H	107H		
Fd79 antibody (Figs. 2A and 2B of 101 patent)			
V_H FR substitions		**V_L FR substitutions**	
Sequential numbering	**Kabat numbering**	**Sequential numbering**	**Kabat numbering**
82H	81H	9L	9L
97H	93H	45L	41L
112H	103H	46L	42L
		53L	49L
		81L	77L
		83L	79L
Fd138-80 antibody (Figs. 3A and 3B of 101 patent)			
V_H FR substitions		**V_L FR substitutions**	
Sequential numbering	**Kabat numbering**	**Sequential numbering**	**Kabat numbering**
27H	27H	36L	36L
30H	30H	48L	48L
37H	37H	63L	63L
48H	48H	87L	87L
67H	66H		
68H	67H		
93H	89H		
98H	94H		

111H	103H		
112H	104H		
113H	105H		
115H	107H		
M195 antibody (Figs. 4A and 4B of the 101 patent)			
V_H FR substitions		**V_L FR substitutions**	
Sequential numbering	**Kabat numbering**	**Sequential numbering**	**Kabat numbering**
27H	27H	10L	10L
30H	30H	40L	36L
48H	48H	52L	48L
67H	66H	67L	63L
68H	67H	74L	70L
93H	89H	110L	106L
95H	91H		
98H	94H		
106H	103H		
107H	104H		
108H	105H		
110H	107H		
mik-β1 antibody (Figs. 5A and 5B of the 101 patent)			
V_H FR substitions		**V_L FR substitutions**	
Sequential numbering	**Kabat numbering**	**Sequential numbering**	**Kabat numbering**
1H	1H	13L	13L
29H	29H	41L	42L

30H	30H	70L	71L
49H	49H		
72H	72H		
73H	73H		
84H	82bH		
89H	86H		
90H	87H		
CMV5 antibody (Figs. 6A and 6B of the 101 patent)			
V_H FR substitions		**V_L FR substitutions**	
Sequential numbering	**Kabat numbering**	**Sequential numbering**	**Kabat numbering**
5H	5H	49L	49L
24H	24H		
27H	27H		
28H	28H		
30H	30H		
69H	68H		
80H	79H		
97H	93H		
AF2 antibody (Figs. 44A and 44B of the 101 patent)			
V_H FR substitions		**V_L FR substitutions**	
Sequential numbering	**Kabat numbering**	**Sequential numbering**	**Kabat numbering**
27H	27H	48L	48L
28H	28H	63L	63L
30H	30H	70L	70L

93H	89H		
95H	91H		
98H	94H		
107H	103H		
108H	104H		
109H	105H		
111H	107H		

Should the Examiner have any comments or questions concerning this amendment, he is invited to call Wendy Lee at (650) 225-1994 concerning these.

Respectfully submitted,
GENENTECH, INC.

Date: October 6, 1997

By: _____
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EXHIBIT A

Alignment of heavy chains from '101 patent

```
sequential 1        10        20        30        40        50
Kabat      1        10        20        30        40        50
           .        .         .         .         .         .
murxTacH   QVQLQQSGAELAKPGASVKWSCKASGYTFTSYRMHWVKQRPGQGLEWIGY
hzxTacH    QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSYTMHWVRQAPGQGLEWIGY
EuH        QVQLVQSGAEVKKPGSSVKVSCKASGGTFSRSAIIWVRQAPGQGLEWMGG
murxMikH   QVQLKQSGPGLVQPSQSLSITCTVSGFSVTSYGVHWIRQSPGKGLEWLGV
hzxMikH    EVQLLESGGGLVQPGQSLRLSCAASGFTVTSYGVHWVRQAPGKGLEWVGV
LayH       AVQLLESGGGLVQPGGSLRLSCAASGFTFSASAMSWVRQAPGKGLEWVAW
murxAF2H   QVQLQQPGADLVMPGAPVKLSCLASGYIFTSSWINWVKQRPGRGLEWIGR
hzxAF2H    QVQLVQSGAEVKKPGSSVKVSCKASGYIFTSSWINWVRQAPGQGLEWMGR
murxCMV5H  EVQLQQSGPELVKPGASMKISCKASVYSFTGYTMNWVKQSHGQNLEWIGL
hzxCMV5H   QVQLVQSGAEVKKPGSSVRVSCKASGYSFTGYTMNWVRQAPGKGLEWVGL
murxFd138H QVQLQQSDAELVKPGASVKISCKVSGYTFTDHTIHWMKQRPEQGLEWFGY
hzxFd138H  QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHTIHWMRQAPGQGLEWFGY
murxFd79H  EMILVESGGGLVKPGASLKLSCAASGFTFSNYGLSWVRQTSDRRLEWVAS
hzxFd79H   EVQLLESGGGLVQPGGSLRLSCAASGFTFSNYGLSWVRQAPGKGLEWVAS
murxM195H  EVQLQQSGPELVKPGASVKISCKASGYTFTDYNMHWVKQSHGKSLEWIGY
hzxM195H   QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDYNMHWVRQAPGQGLEWIGY

sequential          60        70        80        90
Kabat         a     60        70        80  abc   90
                    .         .         .         .
murxTacH   INPSTGYTEYNQKFKDKATLTADKSSSTAYMQLSSLTFEDSAVYYCARG
hzxTacH    INPSTGYTEYNQKFKDKATITADESTNTAYMELSSLRSEDTAVYYCARG
EuH        IVPMFGPPNYAQKFQGRVTITADESTNTAYMELSSLRSEDTAFYFCAGG
murxMikH   IW-SGGSTDYNAAFISRLTISKDNSKSQVFFKVNSLQPADTAIYYCARA
hzxMikH    IW-SGGSTDYNAAFISRFTISRDNSKNTLYLQMNSLQAEDTAIYYCARA
LayH       KYENGNDKHYADSVNGRFTISRNDSKNTLYLQMNGLQAEVSAIYYCARD
murxAF2H   IDPSDGEVHYNQDFKDKATLTVDKSSSTAYIQLNSLTSEDSAVYYCARG
hzxAF2H    IDPSDGEVHYNQDFKDRVTITADESTNTAYMELSSLRSEDTAVYYCARG
murxCMV5H  INPYNGGTSYNQKFKGKATLYVDKSSNTAYMELLSLTSADSAVYYCTRR
hzxCMV5H   INPYNGGTSYNQKFKGRVTVSLKPSFNQAYMELSSLFSEDTAVYYCTRR
murxFd138H IYPRDGHTRYSEKFKGKATLTADKSASTAYMHLNSLTSEDSAVYFCARG
hzxFd138H  IYPRDGHTRYAEKFKGKATITADESTNTAYMELSSLRSEDTAVYFCARG
murxFd79H  ISRGGGRIYSPDNLKGRFTISRDDAKNTLYLQMSSLKSEDTALYYCLRE
hzxFd79H   ISRGGGRIYSPDNLKGRFTISRNDSKNTLYLQMNSLQAEDTALYYCLRE
murxM195H  IYPYNGGTGYNQKFKSKATLTVDNSSSTAYMDVRSLTSEDSAVYYCARG
hzxM195H   IYPYNGGTGYNQKFKSKATITADESTNTAYMELSSLRSEDTAVYYCARG
```

EXHIBIT A
(cont.)

```
sequential                  110
Kabat              103      110
                    •        •
murxTacH    GGV------FDYWGQGTTLTVSS
hzxTacH     GGV------FDYWGQGTLVTVSS
EuH         YGIYS----PEEYNGGLVTVSS
murxMikH    GDYNYDG--FAYWGQGTLVTVSA
hzxMikH     GDYNYDG--FAYWGQGTLVTVSS
LayH        AGPYVSPTFFAHWGQGTLVTVSS
murxAF2H    FLPW-----FADWGQGTLVTVSA
hzxAF2H     FLPW-----FADWGQGTLVTVSS
murxCMV5H   GFRDYS---MDYWGQGTSVTVSS
hzxCMV5H    GFRDYS---MDYWGQGTSVTVSS
murxFd138H  RDSRERNG-FAYWGQGTLVTVS-
hzxFd138H   RDSRERNG-FAYWGQGTLVTVSS
murxFd79H   GIYYADYGFFDVWGTGTTVIVSS
hzxFd79H    GIYYADYGFFDVWGQGTLVTVSS
murxM195H   RPA------MDYWGQGTSVTVSS
hzxM195H    RPA------MDYWGQGTLVTVSS
```

EXHIBIT B

Alignment of light chains from '101 patent

```
sequential  1        10        20        30              40
Kabat       1        10        20        30             40
            •        •         •         •              •
murxTacL    QIVLTQSPAIMSASPGEKVTITCSASSSIS-----YMHWFQQKPGTSPKL
hzxTacL     DIQMTQSPSTLSASVGDRVTITCSASSSIS-----YMHWYQQKPGKAPKL
EuL         DIQMTQSPSTLSASVGDRVTITCRASQSINT----WLAWYQQKPGKAPKL
murxMikL    QIVLTQSPAIMSASPGEKVTMTCSGSSSVS-----FMYWYQQRPGSSPRL
hzxMikL     DIQMTQSPSSLSASVGDRVTITCSGSSSVS-----FMYWYQQKPGKAPKL
LayL        DIQMTQSPSSLSVSVGDRVTITCQASQNVNA----YLNWYQQKPGLAPKL
murxAF2L    NIVMTQSPKSMYVSIGERVTLSCKASENVDT----YVSWYQQKPEQSPKL
hzxAF2L     DIQMTQSPSTLSASVGDRVTITCKASENVDT----YVSWYQQKPGKAPKL
murxCMV5L   DIVLTQSPATLSVTPGDSVSLSCRASQSISN----NLHWYQQKSHESPRL
hzxCMV5L    EIVLTQSPGTLSLSPGERATLSCRASQSISN----NLHWYQQKPGQAPRL
murxFd138L  DIVMTQSHKFMSTSVGDRVSITCKASQDVGS----AVVWHQQKSGQSPKL
hzxFd138L   DIQMTQSPSTLSASVGDRVTITCKASQDVGS----AVVWHQQKPGKAPKL
murxFd79L   DIVLTQSPASLAVSLGQRATISCRASQSVSTSTYNYMHWYQQKPGQPPKL
hzxFd79L    EIVMTQSPATLSVSPGERATLSCRASQSVSTSTYNYMHWYQQKPGQSPRL
murxM195L   DIVLTQSPASLAVSLGQRATISCRASESVDNYGISFMNWFQQKPGQPPKL
hzxM195L    DIQMTQSPSSLSASVGDRVTITCRASESVDNYGISFMNWFQQKPGKAPKL

sequential    50        60        70        80        90
Kabat         50        60        70        80        90
              •         •         •         •         •
murxTacL    WIYTTSNLASGVPARFSGSGSGTSYSLTISRMEAEDAATYYCHQRSTYPL
hzxTacL     LIYTTSNLASGVPARFSGSGSGTEFTLTISSLQPDDFATYYCHQRSTYPL
EuL         LMYKASSLESGVPSRFIGSGSGTEFTLTISSLQPDDFATYYCQQYNSDSK
murxMikL    LIYDTSNLASGVPVRFSGSGSGTSYSLTISRMEAEDAATYYCQQWSTYPL
hzxMikL     LIYDTSNLASGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQQWSTYPL
LayL        LIYGASTREAGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQYNNWPP
murxAF2L    LIYGASNRYTGVHDRFTGSGSATDFTLTISSVQAEDLADYHCGQSYNYPF
hzxAF2L     LIYGASNRYTGVPSRFSGSGSGTDFTLTISSLQPDDFATYYCGQSYNYPF
murxCMV5L   LIKYASQSISGIPSRFSGSGSGTDFTLSVNGVETEDFGMYFCQQSNSWPH
hzxCMV5L    LIKYASQSISGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQSNSWPH
murxFd138L  LIYWASTRHTGVPDRFTGSGSGTDFTLTITNVQSEDLADYFCQQYSIFPL
hzxFd138L   LIYWASTRHTGVPSRFTGSGSGTEFTLTISSLQPDDFATYFCQQYSIFPL
murxFd79L   LIKYASNLESGVPARFSGSGFGTDFTLNIHPVEEEDTVTYYCQHSWEIPY
hzxFd79L    LIKYASNLESGIPARFSGSGSGTEFTLTISRLESEDFAVYYCQHSWEIPY
murxM195L   LIYAASNQGSGVPARFSGSGSGTDFSLNIHPMEEDDTAMYFCQQSKEVPW
hzxM195L    LIYAASNQGSGVPSRFSGSGSGTDFTLNISSLQPDDFATYYCQQSKEVPW
```

EXHIBIT B
(cont.)

```
sequential     100
Kabat          100
                •
murxTacL    TFGSGTKLELK
hzxTacL     TFGQGTKVEVK
EuL         MFGQGTKVEVK
murxMikL    TFGAGTKLELK
hzxMikL     TFGQSTKVEVK
LayL        TFGQGTKVEVK
murxAF2L    TFGSGTKLEIK
hzxAF2L     TFGQGTKVEVK
murxCMV5L   TFGGGTKLEIK
hzxCMV5L    TFGQGTKVEIK
murxFd138L  TFGAGTRLELK
hzxFd138L   TFGQGTKVEVK
murxFd79L   TFGGGTKLEIK
hzxFd79L    TFGQGTRVEIK
murxM195L   TFGGGTKLEIK
hzxM195L    TFGQGTKVEIK
```

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Carter, Paul J.
Presta, Leonard G.

(ii) TITLE OF INVENTION: Method for Making Humanized Antibodies

(iii) NUMBER OF SEQUENCES: 30

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Genentech, Inc.
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(E) COUNTRY: USA
(F) ZIP: 94080

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 3.5 inch, 1.44 Mb floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: WinPatin (Genentech)

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: 08/146206
(B) FILING DATE: 17-Nov-1993
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 07/715272
(B) FILING DATE: 14-JUN-1991

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Lee, Wendy M.
(B) REGISTRATION NUMBER: 40,378
(C) REFERENCE/DOCKET NUMBER: P0709P1

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 650/225-1994
(B) TELEFAX: 650/952-9881

(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
95 100 105

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

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
95 100 105

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

2

```
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                 100                 105

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                 100                 105

Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
                 110                 115                 120
```

(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                  100                 105

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                  100                 105

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

(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:

(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                  100                 105

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
        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                  100                 105
```

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

(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:

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
95 100 105

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

Ser Ala Val Tyr Tyr Cys Ala Arg Ser Gly Tyr Tyr Gly Asp Ser
95 100 105

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
95 100 105

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
95 100 105

Ser Gly Leu Tyr Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val
110 115 120

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
95 100 105

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

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                 100                 105

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

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

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                  100                 105

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                 100                 105

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

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                 100                 105

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:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```
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
                 20                  25
```

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

```
Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala
  1               5                  10              14
```

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

```
Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Leu Tyr Leu
  1               5                  10                  15

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                 20                  25                  30
```

Ala Arg
32

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
1 5 10 11

This Raw Listing contains the General Information Section and up to the first 5 pages.

ENTERED

SEQUENCE LISTING

(1) General Information:

(i) APPLICANT: Carter, Paul J.
Presta, Leonard G.

(ii) TITLE OF INVENTION: Method for Making Humanized Antibodies

(iii) NUMBER OF SEQUENCES: 26

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Genentech, Inc.
(B) STREET: 1 DNA Way
(C) CITY: South San Francisco
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 94080

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 3.5 inch, 1.44 Mb floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: WinPatin (Genentech)

(vi) CURRENT APPLICATION DATA:
--> **(A) APPLICATION NUMBER: 08/146206**
(B) FILING DATE: 17-Nov-1993
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 07/715272
(B) FILING DATE: 14-JUN-1991

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Lee, Wendy M.
(B) REGISTRATION NUMBER: 40,378
(C) REFERENCE/DOCKET NUMBER: P0709P1

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 650/225-1994
(B) TELEFAX: 650/952-9881

(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                 100                 105

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
```

```
100   Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
101                    80                  85                  90
102
103   Thr Ala Val Tyr Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr
104                    95                 100                 105
105
106   Ala Met Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
107                   110                 115                 120
108
109  (2) INFORMATION FOR SEQ ID NO:3:
110
111     (i) SEQUENCE CHARACTERISTICS:
112         (A) LENGTH: 109 amino acids
113         (B) TYPE: Amino Acid
114         (D) TOPOLOGY: Linear
115
116    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:
117
118   Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
119     1               5                  10                  15
120
121   Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser
122                    20                  25                  30
123
124   Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
125                    35                  40                  45
126
127   Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser
128                    50                  55                  60
129
130   Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
131                    65                  70                  75
132
133   Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
134                    80                  85                  90
135
136   Tyr Asn Ser Leu Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu
137                    95                 100                 105
138
139   Ile Lys Arg Thr
140                109
141
142  (2) INFORMATION FOR SEQ ID NO:4:
143
144     (i) SEQUENCE CHARACTERISTICS:
145         (A) LENGTH: 120 amino acids
146         (B) TYPE: Amino Acid
147         (D) TOPOLOGY: Linear
148
149    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
150
151   Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
152     1               5                  10                  15
```

```
153
154   Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
155                    20                  25                  30
156
157   Asp Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
158                    35                  40                  45
159
160   Glu Trp Val Ala Val Ile Ser Glu Asn Gly Gly Tyr Thr Arg Tyr
161                    50                  55                  60
162
163   Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser
164                    65                  70                  75
165
166   Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
167                    80                  85                  90
168
169   Thr Ala Val Tyr Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr
170                    95                 100                 105
171
172   Ala Met Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
173                   110                 115                 120
174
175  (2) INFORMATION FOR SEQ ID NO:5:
176
177     (i) SEQUENCE CHARACTERISTICS:
178         (A) LENGTH: 109 amino acids
179         (B) TYPE: Amino Acid
180         (D) TOPOLOGY: Linear
181
182    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
183
184   Asp Ile Val Met Thr Gln Ser His Lys Phe Met Ser Thr Ser Val
185     1               5                  10                  15
186
187   Gly Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asp Val Asn
188                    20                  25                  30
189
190   Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly His Ser Pro Lys
191                    35                  40                  45
192
193   Leu Leu Ile Tyr Ser Ala Ser Phe Arg Tyr Thr Gly Val Pro Asp
194                    50                  55                  60
195
196   Arg Phe Thr Gly Asn Arg Ser Gly Thr Asp Phe Thr Phe Thr Ile
197                    65                  70                  75
198
199   Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln
200                    80                  85                  90
201
202   His Tyr Thr Thr Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu
203                    95                 100                 105
204
205   Ile Lys Arg Ala
```

206 109

207

208 (2) INFORMATION FOR SEQ ID NO:6:

209

210 (i) SEQUENCE CHARACTERISTICS:
211 (A) LENGTH: 120 amino acids
212 (B) TYPE: Amino Acid
213 (D) TOPOLOGY: Linear

214

215 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

216

217 Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly
218 1 5 10 15

219

220 Ala Ser Leu Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys
221 20 25 30

222

223 Asp Thr Tyr Ile His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu
224 35 40 45

225

226 Glu Trp Ile Gly Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr
227 50 55 60

228

229 Asp Pro Lys Phe Gln Asp Lys Ala Thr Ile Thr Ala Asp Thr Ser
230 65 70 75

231

232 Ser Asn Thr Ala Tyr Leu Gln Val Ser Arg Leu Thr Ser Glu Asp
233 80 85 90

234

235 Thr Ala Val Tyr Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr
236 95 100 105

237

238 Ala Met Asp Tyr Trp Gly Gln Gly Ala Ser Val Thr Val Ser Ser
239 110 115 120

240

241 (2) INFORMATION FOR SEQ ID NO:7:

242

243 (i) SEQUENCE CHARACTERISTICS:
244 (A) LENGTH: 27 base pairs
245 (B) TYPE: Nucleic Acid
246 (C) STRANDEDNESS: Single
247 (D) TOPOLOGY: Linear

248

249 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

250

251

252 TCCGATATCC AGCTGACCCA GTCTCCA 27

253

254 (2) INFORMATION FOR SEQ ID NO:8:

255

256 (i) SEQUENCE CHARACTERISTICS:
257 (A) LENGTH: 31 base pairs
258 (B) TYPE: Nucleic Acid

SEQUENCE VERIFICATION REPORT
PATENT APPLICATION *US/08/146,206B*

DATE: 10/08/97
TIME: 13:19:59

INPUT SET: S20851.raw

Line	Error	Original Text
27	Wrong application Serial Number	(A) APPLICATION NUMBER: 08/146206

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
08/146,206	11/17/93	CARTER P	709P1

18M1/1223

JANET E. HASAK
GENENTECH, INC.
460 POINT SAN BRUNO BOULEVARD
SOUTH SAN FRANCISCO CA 94080-4990

EXAMINER	
NOLAN, P	35
ART UNIT	**PAPER NUMBER**
1816	

DATE MAILED: 12/23/97

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary	Application No. 08/146,206	Applicant(s) Carter et al.
	Examiner Patrick J. Nolan	Group Art Unit 1816

☒ Responsive to communication(s) filed on *6-27-97, 9-1-97 and 10-7-97*.

☒ This action is **FINAL.**

☐ Since this application is 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.

A shortened statutory period for response to this action is set to expire *3* month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

☒ Claim(s) *1-8, 10-12, 15, and 22-42* is/are pending in the application.

Of the above, claim(s) _____ is/are withdrawn from consideration.

☐ Claim(s) _____ is/are allowed.

☒ Claim(s) *1-8, 10-12, 15, and 22-41* is/are rejected.

☒ Claim(s) *42* is/are objected to.

☐ Claims _____ are subject to restriction or election requirement.

Application Papers

☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on _____ is ☐approved ☐disapproved.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been

☐ received.

☐ received in Application No. (Series Code/Serial Number) _____.

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____.

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☒ Notice of References Cited, PTO-892

☐ Information Disclosure Statement(s), PTO-1449, Paper No(s). _____

☐ Interview Summary, PTO-413

☐ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

--- SEE OFFICE ACTION ON THE FOLLOWING PAGES ---

Serial No. 08/146,206

Art Unit 1816

1. Claims 1-8, 10-12, 15 and 22-42 are pending.

Double Patenting

2. The non-statutory double patenting rejection, whether of the obviousness-type or non-obviousness-type, is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent. *In re Thorington,* 418 F.2d 528, 163 USPQ 644 (CCPA 1969); *In re Vogel,* 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Van Ornum,* 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Longi,* 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); and *In re Goodman,* 29 USPQ2d 2010 (Fed. Cir. 1993).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(b) and (c) may be used to overcome an actual or provisional rejection based on a non-statutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.78(d). Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

3. Claims 1-12, 15 stand 19-25 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-12, 15 and 19 of copending application Serial No. 08/439,004.

Applicant's request these rejection be held in abeyance until the prosecution of the two pending cases are completed.

Claim Rejections - 35 USC § 102

4. 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 --
>
> e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section

371(c) of this title before the invention thereof by the applicant for patent.

5. Claims 1-8, 10-12, 15 and 22-24 stand rejected under 35 U.S.C. § 102(e) as being anticipated by U.S. Patent 5,530,101 (82).

Applicant's arguments filed 6-23-97 have been fully considered but are not found persuasive.

6. Applicant argues that the '101 patent does not teach the determination of residues which will disrupt the V_L-V_H interface as part of their method to make a humanized antibody.

However, Applicant's claims are drawn to using one of the following effects recited in claim 1 and 23, part (f), not all three.

7. Applicant argues that the determination of residues being exposed to the CDR region is not the same as the '101 teaching of whether the residue "interacts with a CDR".

Protein chemistry dictates that for an amino acid residue to interact with another amino acid residues it needs to be exposed to it.

8. Applicant argues that since the '101 patent does not specifically teach glycosylation of the residue being a factor for selection it cannot be used as a prior art reference.

The teaching of glycosylation effects on amino acid residues, is of record, as taught by Roitt et al., submitted in the last office action. Roitt is an educational textbook demonstrating concepts well known to those in the art.

9. Applicant argues that claims drawn to specific residue changes have been amended to distinguish the claims from the '101 patent. Applicant has also demonstrated the numbering difference between the '101 patent and the current application.

If applicant wishes to distinguish over the prior art, they may do so by claiming the actual numbering system used in the actual claim.

The following new grounds of rejections are necessitated by the amendments filed 6-27-97, 9-1-97 and 10-7-97.

10. Claims 22-25, 38, and 39 are rejected under 35 U.S.C. § 102(e) as being anticipated by U.S. Patent 5,693,762 (A).

The '762 patent teaches the aligning of heavy chain immunoglobulin regions for the creation of a consensus sequence to be used in making a humanized antibody (column 13, lines 4-26 and claims 7-9 and 20, in particular). The '762 patent also teaches that in selecting which consensus framework sequence to be used, the acceptor immunoglobulin most likely should be as homologous to the donor sequence as possible (i.e. same isotype) (column 13).

The prior art teachings anticipate the claimed invention.

Claim Rejections - 35 USC § 103

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) or (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(a), 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 35 U.S.C. 103(c) and potential 35 U.S.C. 102(f) or (g) prior art under 35 U.S.C. 103(a).

11. Claims 26-36 and 40-41 are rejected under 35 U.S.C. § 103 as being unpatentable over U.S. Patent 5,693,762 (A), in view of Kabat et al.

The `762 patent has been discussed supra. The claimed

invention differs from the prior art teachings only by the recitation the Ig gamma isotype sequences used to make a consensus heavy chain framework region.

However, Kabat et al., teach the sequences of all known Ig gamma subtypes.

Therefore it would have been prima facie obvious to one of skill in the art at the time the invention was made to use the teachings of the '762 patent and align all of the known Ig gamma heavy chains for the creation of a consensus sequence with the expectation that said consensus sequence immunoglobulin would have a smaller chance of changing the an amino acid near the CDR's that distorts their conformation, as taught by the '762 patent (column 13).

12. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL.** See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

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.

13. If attempts to reach the examiner are unsuccessful, the examiner's supervisor, Christina Chan, can be reached at (703) 305-3973. The FAX number for our group, 1816, is (703) 305-7939. Any inquiry of a general nature relating to the status of this application or proceeding should be directed to the Group receptionist, whose telephone number is (703) 308-0196.

Patrick J. Nolan, Ph.D.
December 19, 1997

F.C. Eisenschenk
Primary Examiner
December 19, 1997

Notice of References Cited

Application No.	Applicant(s)		
08/146,206	Carter et al.		
Examiner	Group Art Unit	Page 1 of 1	
Patrick J. Nolan	1816		

U.S. PATENT DOCUMENTS

		DOCUMENT NO.	DATE	NAME	CLASS	SUBCLASS
	A	5,693,762	12-2-97	Queen et al.	530	387.2
	B					
	C					
	D					
	E					
	F					
	G					
	H					
	I					
	J					
	K					
	L					
	M					

FOREIGN PATENT DOCUMENTS

		DOCUMENT NO.	DATE	COUNTRY	NAME	CLASS	SUBCLASS
	N						
	O						
	P						
	Q						
	R						
	S						
	T						

NON-PATENT DOCUMENTS

		DOCUMENT (Including Author, Title, Source, and Pertinent Pages)	DATE
	U		
	V		
	W		
	X		

TDais 12/05/01

Gp 1644

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1644 Examiner: P. Nolan RECEIVED APR 16 1998 CERTIFICATE OF MAILING I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on April 8, 1998 Nicole Kehoe

NOTICE OF CHANGE OF ADDRESS AND AREA CODE

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Please direct all future communications in connection with the above referenced patent application to:

Genentech, Inc.
1 DNA Way
South San Francisco, CA 94080-4990

Please also note the change in area code from 415 to 650 (see below).

Respectfully submitted,
GENENTECH, INC.

Date: April 7, 1998

By: _____
Wendy M. Lee
Reg. No. 40,378

1 DNA Way
So. San Francisco, CA 94080-4990
Phone: (650) 225-1994
Fax: (650) 952-9881

GP1644

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1644 Examiner: P. Nolan CERTIFICATE OF MAILING I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on June 23, 1998 Yvonne E. Carter

NOTICE OF APPEAL

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Applicant hereby appeals to the Board of Appeals and Interferences from the decision dated 23 December 1997, of the Primary Examiner finally rejecting claims 1-8, 10-12, 15, and 22-41 and objecting to claim 42.

The Commissioner is hereby authorized to charge Deposit Account No. 07-0630 in the amount of $310 to cover the fees for this appeal and to charge the deposit account for any further fees in regard to this patent application. **A duplicate copy of this Notice is enclosed for this purpose.**

07/01/1998 SSANDARA 00000105 070630 08146206
01 FC:119 310.00 CH

Respectfully submitted,
GENENTECH, INC.

Date: June 23, 1998

By: _____
Richard B. Love
Reg. No. 34,659

RECEIVED
JUL 6 1998
GROUP 1600

1 DNA Way
So. San Francisco, CA 94080-4990
Phone: (650) 225-1994
Fax: (650) 952-9881

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1644 Examiner: P. Nolan CERTIFICATE OF MAILING I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on June 23, 1998 Yvonne E. Carter

PETITION AND FEE FOR THREE MONTH EXTENSION OF TIME
(37 CFR 1.136(a))

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Applicant petitions the Commissioner of Patents and Trademarks to extend the time for response to the FINAL OFFICE ACTION dated 23 December 1997 for three month(s) from 23 March 1998 to 23 June 1998. The extended time for response does not exceed the statutory period.

Please charge Deposit Account No. 07-0630 in the amount of $950.00 to cover the cost of the extension. Any deficiency or overpayment should be charged or credited to this deposit account. A duplicate of this sheet is enclosed.

07/01/1998 SSANDARA 00000105 070630 08146206
02 FC:117 950.00 CH

Respectfully submitted,
GENENTECH, INC.

Date: June 23, 1998

By: _____
Richard B. Love
Reg. No. 34,659

1 DNA Way
So. San Francisco, CA 94080-4990
Phone: (650) 225-1994
Fax: (650) 952-9881

#33

In re Application of Paul J. Carter et al.
Serial No.: 08/146,206
Filed On: November 17, 1993
Mailed On: 23 June 1998

Docket No.: P0709P1
By: Richard B. Love
Reg. No.: 34,659

LS

The following has been received in the U.S. Patent Office on the date stamped:

- X Petition to Extent Time for Three Months
- X Notice of Appeal Transmittal
- X Fees $ 1,260.00
- X Postcard

UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE OFFICE OF ENROLLMENT AND DISCIPLINE

LIMITED RECOGNITION UNDER 37 CFR § 10.9(b)

Wendy M. Lee is hereby given limited recognition under 37 CFR § 10.9(b) as an employee of Genentech, Inc. to prepare and prosecute patent applications and to represent patent applicants wherein Genentech, Inc. is the assigneed of record of the entire interest. This limited recognition shall expire on the date appearing below, or when whichever of the following events first occurs prior to the date appearing below: (i) Wendy M. Lee ceases to lawfully reside in the United States, (ii) Wendy M. Lee's employment with Genentech, Inc. ceases or is terminated, or (iii) if Wendy M. Lee ceases to remain or reside in the United States on a H-1 visa.

This document constitutes proof of such recognition. The original of this document is on file in the Office of Enrollment and Discipline of the U.S. Patent and Trademark Office.

EXPIRES: DECEMBER 9, 1995

Cameron Weiffenbach, Director
Office of Enrollment and Discipline

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1644 Examiner: P. Nolan CERTIFICATE OF MAILING I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on June 23, 1998 Yvonne E. Carter

#38 Supp 8-17-98 FORMAL PLEASE ENTER P.N. 8-13-98

PETITION AND FEE FOR THREE MONTH EXTENSION OF TIME
(37 CFR 1.136(a))

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Applicant petitions the Commissioner of Patents and Trademarks to extend the time for response to the FINAL OFFICE ACTION dated 23 December 1997 for three month(s) from 23 March 1998 to 23 June 1998. The extended time for response does not exceed the statutory period.

Please charge Deposit Account No. 07-0630 in the amount of $950.00 to cover the cost of the extension. Any deficiency or overpayment should be charged or credited to this deposit account. A duplicate of this sheet is enclosed.

08/19/1998 DLYONS 00000007 070630 08146206

01 FC:117 950.00 CH
02 FC:119 310.00 CH

Respectfully submitted,

GENENTECH, INC.

Date: June 23, 1998

By: _____
Richard B. Love
Reg. No. 34,659

1 DNA Way
So. San Francisco, CA 94080-4990
Phone: (650) 225-1994
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Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1644 Examiner: P. Nolan CERTIFICATE OF MAILING I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on June 23, 1998 Yvonne E. Carter

NOTICE OF APPEAL

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Applicant hereby appeals to the Board of Appeals and Interferences from the decision dated 23 December 1997, of the Primary Examiner finally rejecting claims 1-8, 10-12, 15, and 22-41 and objecting to claim 42.

The Commissioner is hereby authorized to charge Deposit Account No. 07-0630 in the amount of $310 to cover the fees for this appeal and to charge the deposit account for any further fees in regard to this patent application. A duplicate copy of this Notice is enclosed for this purpose.

Respectfully submitted,
GENENTECH, INC.

Date: June 23, 1998

By: _____
Richard B. Love
Reg. No. 34,659

1 DNA Way
So. San Francisco, CA 94080-4990
Phone: (650) 225-1994
Fax: (650) 952-9881

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

08/146,206

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKETT NO.

EXAMINER

ART UNIT	PAPER NUMBER
	37

DATE MAILED:

EXAMINER INTERVIEW SUMMARY RECORD

All participants (applicant, applicant's representative, PTO personnel):

(1) Wendy Lee (3) _____

(2) Patrick Nolan (4) _____

Date of interview 8-13-98

Type: ☒ Telephonic ☒ Personal (copy is given to ☐ applicant ☒ applicant's representative).

Exhibit shown or demonstration conducted: ☒ Yes ☐ No. If yes, brief description: Wall street Journal article

Agreement ☐ was reached with respect to some or all of the claims in question. ☒ was not reached.

Claims discussed: Newly Proposed Claims Faxed 8-10-98

Identification of prior art discussed: Green Patent 5,693,762

Description of the general nature of what was agreed to if an agreement was reached, or any other comments: Discussed unexpected results to overcome 103 rejections

(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.)

☒ 1. It is not necessary for applicant to provide a separate record of the substance of the interview.

Unless the paragraph below has 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.

☐ 2. 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. Applicant is not relieved from providing a separate record of the substance of the interview unless box 1 above is also checked.

Patrick J. Nolan
Examiner's Signature

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AF / Gpu 1644

Patent Docket P0709P10 $

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1644 Examiner: P. Nolan RECEIVED SEP 0 1 1998 GROUP 1800

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on

August 24, 1996

Wendy M. Lee

AMENDMENT TRANSMITTAL

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Transmitted herewith is an Amendment under 37 C.F.R. §1.129(a) in the above-identified application.

The fee has been calculated as shown below.

	Claims Remaining After Amendment		Highest No. Previously Paid For	Present Extra	Rate	Additional Fees
Total	72	-	35	37	x 22 =	$814.00
Independent	7	-	10	0	x 78 =	$0.00
__ First Presentation of Multiple Dependent Claims					+ 250 =	
					Total Fee Calculation	$814.00

__X__ Amendment under 37 C.F.R. §1.129(a) submitted with fee of $750.00 pursuant to 37 C.F.R. §1.17(r)

__X__ The Commissioner is hereby authorized to charge Deposit Account No. 07-0630 in the amount of $1,564.00 pursuant to 37 C.F.R. §1.17(r). **A duplicate copy of this transmittal is enclosed.**

__X__ A Declaration of Steven Shak with Exhibits A-F is enclosed.

__X__ A Supplemental Information Disclosure Statement, PTO-1449 Form, and copies of Refs. 218-224 are enclosed.

The Commissioner is hereby authorized to charge any additional fees required under 37 CFR 1.16 and 1.17, or credit overpayment to Deposit Account No. 07-0630. **A duplicate copy of this sheet is enclosed.**

Respectfully submitted,
GENENTECH, INC.

Date: August 24, 1998

By: _____
Wendy M. Lee
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#39 Amdt H (Rule 1.116) Grahm 08/03/98

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1644 Examiner: P. Nolan CERTIFICATE OF MAILING I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on August 24, 1998 Wendy M. Lee

AMENDMENT UNDER 37 C.F.R. §1.129(a)

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

This paper is being filed in response to the Office Action mailed December 23, 1997. In the Office Action, the Examiner issued a final rejection of claims 1-8, 10-12, 15 and 22-41 and objected to claim 42. Applicants filed a Notice of Appeal on June 23, 1998. Applicants have not yet filed an Appeal Brief. Accordingly, the present response is being submitted under Section 1.129(a) along with the fee set forth in Section 1.17(r). In that August 23, 1998 fell on a Sunday, this amendment is timely filed.

Entry of the following amendment is respectfully requested:

IN THE CLAIMS:

Please cancel claims 1-8, 10-12, 15 and 22-42 without prejudice or disclaimer of the subject matter claimed therein.

Please add the following claims:

--43. (New) A humanized antibody variable domain comprising a non-human Complementarity Determining Region (CDR) incorporated into a human antibody variable domain, and further comprising an 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.

44. (New) The humanized variable domain of claim 43 wherein the substituted residue is the residue found at the corresponding location of the non-human antibody from which the non-human CDR was obtained.

45. (New) The humanized variable domain of claim 43 wherein no human Framework Region (FR) residue other than those set forth in the group has been substituted.

46. (New) The humanized variable domain of claim 43 wherein the human antibody variable domain is a consensus human variable domain.

47. (New) The humanized variable domain of claim 43 wherein the residue at site 4L has been substituted.

48. (New) The humanized variable domain of claim 43 wherein the residue at site 38L has been substituted.

49. (New) The humanized variable domain of claim 43 wherein the residue at site 43L has been substituted.

50. (New) The humanized variable domain of claim 43 wherein the

residue at site 44L has been substituted.

51. (New) The humanized variable domain of claim 43 wherein the residue at site 58L has been substituted.

52. (New) The humanized variable domain of claim 43 wherein the residue at site 62L has been substituted.

53. (New) The humanized variable domain of claim 43 wherein the residue at site 65L has been substituted.

54. (New) The humanized variable domain of claim 43 wherein the residue at site 66L has been substituted.

55. (New) The humanized variable domain of claim 43 wherein the residue at site 67L has been substituted.

56. (New) The humanized variable domain of claim 43 wherein the residue at site 68L has been substituted.

57. (New) The humanized variable domain of claim 43 wherein the residue at site 69L has been substituted.

58. (New) The humanized variable domain of claim 43 wherein the residue at site 73L has been substituted.

59. (New) The humanized variable domain of claim 43 wherein the residue at site 85L has been substituted.

60. (New) The humanized variable domain of claim 43 wherein the residue at site 98L has been substituted.

61. (New) The humanized variable domain of claim 43 wherein the

residue at site 2H has been substituted.

62. (New) The humanized variable domain of claim 43 wherein the residue at site 4H has been substituted.

63. (New) The humanized variable domain of claim 43 wherein the residue at site 36H has been substituted.

64. (New) The humanized variable domain of claim 43 wherein the residue at site 39H has been substituted.

65. (New) The humanized variable domain of claim 43 wherein the residue at site 43H has been substituted.

66. (New) The humanized variable domain of claim 43 wherein the residue at site 45H has been substituted.

67. (New) The humanized variable domain of claim 43 wherein the residue at site 69H has been substituted.

68. (New) The humanized variable domain of claim 43 wherein the residue at site 70H has been substituted.

69. (New) The humanized variable domain of claim 43 wherein the residue at site 74H has been substituted.

70. (New) The humanized variable domain of claim 43 wherein the residue at site 92H has been substituted.

71. (New) An antibody comprising the humanized variable domain of claim 43.

72. (New) An antibody which binds $p185^{HER2}$ and comprises a

humanized antibody variable domain comprising a non-human Complementarity Determining Region (CDR) 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.

73. (New) The antibody of claim 72 wherein the substituted residue is the residue found at the corresponding location of the non-human antibody from which the non-human CDR was obtained.

74. (New) The antibody of claim 72 wherein no human Framework Region (FR) residue other than those set forth in the group has been substituted.

75. (New) The antibody of claim 72 wherein the human antibody variable domain is a consensus human variable domain.

76. (New) The antibody of claim 72 wherein the residue at site 4L has been substituted.

77. (New) The antibody of claim 72 wherein the residue at site 38L has been substituted.

78. (New) The antibody of claim 72 wherein the residue at site 43L has been substituted.

79. (New) The antibody of claim 72 wherein the residue at site 44L has been substituted.

80. (New) The antibody of claim 72 wherein the residue at site 46L has been substituted.

39 30
81. (New) The antibody of claim 72 wherein the residue at site 58L has been substituted.

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82. (New) The antibody of claim 72 wherein the residue at site 62L has been substituted.

41 30
83. (New) The antibody of claim 72 wherein the residue at site 65L has been substituted.

42 30
84. (New) The antibody of claim 72 wherein the residue at site 66L has been substituted.

43 30
85. (New) The antibody of claim 72 wherein the residue at site 67L has been substituted.

44 30
86. (New) The antibody of claim 72 wherein the residue at site 68L has been substituted.

45 30
87. (New) The antibody of claim 72 wherein the residue at site 69L has been substituted.

H 1 contd

46 30
88. (New) The antibody of claim 72 wherein the residue at site 73L has been substituted.

47 30
89. (New) The antibody of claim 72 wherein the residue at site 85L has been substituted.

48 30
90. (New) The antibody of claim 72 wherein the residue at site 98L has been substituted.

49 30
91. (New) The antibody of claim 72 wherein the residue at site 2H has been substituted.

92. (New) The antibody of claim 72 wherein the residue at site 4H has been substituted.

93. (New) The antibody of claim 72 wherein the residue at site 36H has been substituted.

94. (New) The antibody of claim 72 wherein the residue at site 39H has been substituted.

95. (New) The antibody of claim 72 wherein the residue at site 43H has been substituted.

96. (New) The antibody of claim 72 wherein the residue at site 45H has been substituted.

97. (New) The antibody of claim 72 wherein the residue at site 69H has been substituted.

98. (New) The antibody of claim 72 wherein the residue at site 70H has been substituted.

H1 cont'd

99. (New) The antibody of claim 72 wherein the residue at site 74H has been substituted.

100. (New) The antibody of claim 72 wherein the residue at site 75H has been substituted.

101. (New) The antibody of claim 72 wherein the residue at site 76H has been substituted.

102. (New) The antibody of claim 72 wherein the residue at site 78H has been substituted.

61 30

103. (New) The antibody of claim 72 wherein the residue at site 92H has been substituted.

104. (New) A humanized antibody variable domain comprising a non-human Complementarity Determining Region (CDR) 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.

SUB I3

105. (New) An antibody which lacks significant immunogenicity upon repeated administration to a human patient in order to treat a chronic disease in that patient and comprises (a) non-human Complementarity Determining Region (CDR) 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.

SUB I4

H1 cont'd

106. (New) An antibody which lacks significant immunogenicity upon repeated administration to a human patient in order to treat a chronic disease in that patient and comprises a consensus human variable domain 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 comprising a Framework Region (FR) substitution where the substituted FR residue: (a) noncovalently binds antigen directly; (b) interacts with a CDR; (c) comprises a glycosylation site which affects the antigen binding or affinity of the antibody; or (d) participates in the V_L-V_H interface by

SUB I5

H

affecting the proximity or orientation of the V_L and V_H regions with respect to one another.

107. (New) The antibody of claim 106 comprising a non-human FR residue which noncovalently binds antigen directly.

108. (New) The antibody of claim 106 comprising a non-human FR residue which interacts with a CDR.

109. (New) The antibody of claim 106 comprising a non-human FR residue which comprises a glycosylation site which affects the antigen binding or affinity of the antibody.

110. (New) The antibody of claim 106 comprising a non-human FR residue which 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.

111. (New) A humanized antibody comprising a consensus human variable domain of human V_H subgroup III, wherein amino acid residues forming Complementarity Determining Regions (CDRs) thereof comprise non-human antibody amino acid residues, and further comprising a Framework Region (FR) substitution where the substituted FR residue: (a) noncovalently binds antigen directly; (b) interacts with a CDR; (c) comprises 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.

112. (New) The humanized antibody of claim 111 which lacks significant immunogenicity upon repeated administration to a human patient in order to treat a chronic disease in that patient.

113. (New) A humanized variant of a non-human parent antibody which binds an antigen with better affinity than the parent antibody and comprises a consensus human variable domain 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 comprising a Framework Region (FR) substitution where the substituted FR residue: (a) noncovalently binds antigen directly; (b) interacts with a CDR; (c) comprises 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.

114. (New) The humanized variant of claim 113 which binds the antigen at least about 3-fold more tightly than the parent antibody.--

REMARKS

The undersigned confirms having met with Examiner Nolan in the personal interview on August 13, 1998 and thanks the Examiner for the courtesies extended in the interview. In the interview, the undersigned pointed out that claim 42 was not rejected, but was objected-to in the above-noted final Office Action. However, the basis for the objection was not elaborated in the body of the Office Action. The Examiner indicated that claim 42 was objected to for depending on a rejected claim (*i.e.* claim 22). Other issues discussed in the interview will be mentioned herein-below where appropriate.

Amendments

The previously pending claims are cancelled herein without prejudice and without disclaimer of the subject matter claimed

therein and without acquiescing in any rejection or objection raised by the Office. Applicants reserve the right to pursue continuing application(s) directed to cancelled claims. The claims herein correspond to those discussed in the interview and are believed to be allowable.

Former claim/specification basis for each of the claims added herein can be found at least as follows:

Claims 43 and 47-70 - claim 10 as amended 10-7-97; and page 6, lines 21-22 for "utilizing the numbering system set forth in Kabat"
Claim 44 - original claim 11
Claim 45 - original claim 12
Claim 46 - language from claim 1
Claim 71 - page 11, lines 3-4
Claims 72 and 76-103 - claim 10 as amended 10-7-97; page 63, line 21 for "antibody which binds $p185^{HER2}$"; and page 6, lines 21-22 for "utilizing the numbering system set forth in Kabat"
Claim 73 - original claim 11
Claim 74 - original claim 12
Claim 75 - language from claim 1
Claim 104 - claim 10 as amended 10-7-97; claim 1 for "consensus human variable domain"; and page 6, lines 21-22 for "utilizing the numbering system set forth in Kabat"
Claim 105 - claims 10 and 42 from the amendment 10-7-97; and page 6, lines 21-22 for "utilizing the numbering system set forth in Kabat"
Claim 106 - combination of claims 22, 23 and 42
Claims 107-110 - claim 23
Claim 111 - combination of claims 22, 23 and 26
Claim 112 - claim 42
Claim 113 - claims 22 and 23; page 71, lines 1-2 and Table 3 on

page 72 showing humanized variants with improved binding affinity compared to the murine parent antibody.
Claim 114 - page 71, lines 1-2

In that the claims do not introduce new matter, their entry is respectfully requested.

Information Disclosure

1. In the above-mentioned interview, the undersigned inquired as to the status of the IDS carried to the PTO September 1997 citing references 100-207. The Examiner indicated he had this IDS and the references and would consider them with respect to the above application. Applicants await receipt of a copy of the initialed PTO-1449 form indicating consideration of the cited art.

2. A further supplemental IDS is submitted herewith. Applicants respectfully request consideration of the art cited in this supplemental IDS with respect to the instant application.

Provisional Double Patenting Rejection

Claims 1-12, 15 and 19-25 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-12, 15 and 19 of copending application Serial No. 08/439,004. This rejection is moot as USSN 08/439,004 is now abandoned.

Section 102(e) - US Patent 5,530,101

Claims 1-8, 10-12, 15 and 22-24 are rejected under 35 USC §102(e) as being anticipated by US Patent 5,530,101 ("the '101 patent")

With respect to claim 10, the Examiner states in item 9 of the Office Action that the claim may be distinguished over the prior art by claiming the actual numbering system used in the actual

claim. In order to expedite prosecution, Applicants have followed the Examiner's suggestion and recite the numbering system of Kabat in independent claims 43, 72, 104 and 105 herein for claim precision.

Further patentable features in these claims and the claims which depend thereon include, without limitation: the target antigen $p185^{HER2}$ in claim 72 (which is not taught in the '101 patent); a consensus human variable domain which, as will be explained below, is not taught or enabled by the '101 patent; and the antibody which lacks significant immunogenicity upon repeated administration to a human patient in order to treat a chronic disease in that patient (see comments below).

Applicants submit that independent claims 43, 72, 104 and 105 herein as well as the claims which depend thereon are patentable over the cited art.

Reconsideration and withdrawal of the Section 102 rejection is respectfully requested.

Section 102(e) - US Patent 5,693,762

Claims 22-25, 38 and 39 are rejected under 35 USC §102(e) as being anticipated by US Patent 5,693,762 ("the '762 patent").

The Examiner asserts that the '762 patent taught the aligning of heavy chain immunoglobulin regions for the creation of a consensus sequence to be used in making a humanized antibody and that the acceptor immunoglobulin most likely should be as homologous to the donor sequence as possible (*i.e.* same isotype).

Applicants submit that the '762 patent does not anticipate the instant invention.

Importantly, the '762 patent did not in fact teach a consensus human variable domain as the term is used in the present application.

Applicants contend that the phrase "consensus framework from many human antibodies" in line 7 of column 13 in the '762 patent which is cited by the Office, was not intended to refer to a "consensus human variable domain" as in the present application (*i.e.* a sequence representing the most frequently occurring amino acid residues at each location in all immunoglobulins of any particular subclass; see page 14, lines 29-31 of the instant application). Applicants submit that the '762 patent was using the phrase "consensus framework from many human antibodies" synonymously with a framework "from a particular human immunoglobulin that is unusually homologous to the donor immunoglobulin to be humanized". If one reads lines 4-26 in column 13 of the '762 patent and, indeed, the entire patent, it becomes clear that the method for humanizing advocated therein involved selecting an immunoglobulin framework sequence from a single human immunoglobulin which was unusually homologous to the donor immunoglobulin to be humanized and this is what was actually done in the working examples. It is apparent then that the phrase "consensus framework from many human antibodies" was used in the '762 patent as another way of saying "a framework from a particular human immunoglobulin that is unusually homologous to the donor immunoglobulin to be humanized", *i.e.*, a framework from a particular human immunoglobulin which "agrees" with the donor immunoglobulin when the sequences are aligned.

Thus, Applicants submit that the '762 patent did not teach or enable a consensus human variable domain as described in the present application, much less a "consensus human variable domain of a human heavy chain immunoglobulin subgroup." Accordingly,

reconsideration and withdrawal of the rejection is respectfully requested.

As to rejected claim 38, this relates to the method of "veneering" or "resurfacing" an antibody. As discussed in the above-mentioned interview, this approach was not taught in the '762 patent.

Applicants respectfully request reconsideration and withdrawal of the Section 102(e) rejection in view of the above.

Section 103

Claims 26-36 and 40-41 are rejected under 35 USC §103 as being unpatentable over the '762 patent in view of Kabat *et al*.

The Examiner asserts that the claimed invention differs from the prior art teachings only by recitation of Ig gamma isotype sequences used to make a consensus heavy chain framework region. The Examiner cites Kabat as teaching the sequences of all known Ig gamma subtypes and contends that it would have been *prima facie* obvious at the time the invention was made to use the teachings of the '762 patent and align all of the known Ig gamma heavy chains for the creation of a consensus sequence with the expectation that such consensus sequence immunoglobulin would have a smaller chance of changing an amino acid near the CDRs that distorts their conformation as allegedly taught in column 13 of the '762 patent.

Applicants submit that the instant invention is patentable over the cited art.

With respect to the Examiner's combining of the '762 patent and Kabat, Applicants submit that the rejection is made impermissibly using hindsight reconstruction of the present invention. "One cannot use hindsight reconstruction to pick and choose among

isolated disclosures in the prior art to depreciate the claimed invention." *In re Fine* 837 F2d 1071, 1075 (Fed. Cir. 1988).

In particular, as noted above, the term "consensus framework from many human antibodies" in the '762 patent was not intended to refer to a sequence representing the most frequently occurring amino acid residues at each location in all immunoglobulins of any particular subclass as in the present application. Thus, Applicants submit that the '762 patent would not have provided any motivation to make a consensus human variable domain as in the present application.

With respect to the Examiner's assertion that "the claimed invention differs from the prior art teachings only by recitation of Ig gamma isotype sequences used to make a consensus heavy chain framework region", Applicants believe that the Examiner has misunderstood the selection invention involving a "V_H subgroup III" consensus sequence. As opposed to a collection of antibodies with the same "isotype" due to the amino acid sequence of their heavy chain constant region (page 11 of the application), V_H subgroup III represents a subclass of antibodies grouped together because of their heavy chain variable domain sequences. For this reason alone, Applicants submit that the Examiner has failed to establish a *prima facie* case of obviousness.

Moreover, Applicants submit that there was nothing in the cited art to suggest combining Kabat with the '762 patent. In particular, the term "consensus" is not used in Kabat. Kabat refers to "occurrences of most common amino acid" for various heavy or light chain immunoglobulin subgroups. Without knowing about the invention of the present application, Applicants contend that those skilled in the art would not have been motivated to combine the mention of "consensus framework from many human antibodies" in the '762 patent with Kabat's disclosure of "occurrences of most common

amino acid", especially since, as elaborated above, the '762 patent did not intend the term "consensus framework" to refer to "occurrences of most common amino acid".

This further illustrates that the Examiner is using impermissible hindsight to combine the references.

Moreover, Applicants are able to show that the '762 patent would have taught away from the instantly claimed invention. In particular, the '762 patent states that one must select a framework from a particular human immunoglobulin that is unusually homologous to the donor immunoglobulin in order to reduce the chance of distorting the conformation of the CDR's (see column 13 of the '762 patent). This has been termed the "best-fit" method of humanization.

On the contrary, the instant invention does not rely on selection of an unusually homologous framework from a single human antibody; a consensus human variable domain comprising the most frequently occurring amino acid residues at each location in human immunoglobulins is used as the framework region.

Whereas the '762 patent requires at least 65% homology between the human "acceptor" framework region (FR) sequence and murine "donor" FR sequence (see column 13, lines 33-36) to avoid distorting the conformation of the CDRs, Applicants have generated humanized antibodies using the V_H subgroup III consensus sequence having low FR homology to murine donor antibody FR sequences.

For example, in contrast to the teachings of the '762 patent, Applicants have shown that FR homologies as low as 53% for an anti-CD18 antibody (Example 4 on page 89 of the present application); 57% for an anti-IgE antibody [Presta *et al. J. Immunol.*

151(5):2623-2632 (1993) (of record)]; 57% for an anti-CD11a antibody [Werther *et al. J. Immunol.* 157:4986-4995 (1996) (of record)]; 61% for an anti-VEGF antibody [Presta *et al. Cancer Research* 57(20):4593-4599(1997)(copy attached)] and 63% for an anti-HER2 antibody1 (Example 1 herein) have resulted in humanized antibodies with strong binding affinities.

Applicants submit that the '762 patent would have lead those skilled in the art away from the instantly claimed invention because they would have feared that this would result in "distortions in the CDR's" of the humanized antibody so produced.

In further support of the patentability of the instant claims, Applicants will now show that the claimed invention can produce humanized antibodies with at least three unexpected and useful properties. Unexpected results provide objective evidence of non-obviousness. *Specialty Composites v. Cabot Corp.*, 845 F. 2d 981, 6 USPQ 2d 1601 (Fed. Cir. 1988).

The unexpected properties to be demonstrated include: lack of significant immunogenicity of the claimed humanized antibodies upon repeated administration to a human patient, *e.g.*, to treat a chronic disease in the patient; binding affinities superior to those of the non-human parent antibody; and the ability to use the same consensus human variable domain to make many strong affinity antibodies, thus avoiding tailoring each human FR to each non-human antibody to be humanized.

In order to demonstrate that lack of significant immunogenicity upon repeated administration of the humanized antibody to a human

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1 In the case of the anti-HER2 antibody, surprisingly, the humanized antibody had improved binding affinity relative to the murine parent antibody. This unexpected result will be discussed in more detail below.

patient could not have been predicted for the instantly claimed humanized antibodies, Applicants refer to Isaacs *et al. The Lancet* 340:748-752 (1992) (of record). Isaacs *et al.* demonstrate that three out of four patients treated with humanized CAMPATH-1H antibody (*i.e.* the antibody humanized in Riechmann) developed antiglobulins that were able to inhibit the binding of CAMPATH-1H to its antigen (see first paragraph of the discussion on page 751 of this reference).

On the contrary, the instant application describes humanized antibodies which lack significant immunogenicity upon repeated administration to a human patient in order to treat a chronic disease in that patient. Therefore, the instantly claimed antibodies are useful, among other things, for treating chronic disorders such as cancer.

As suggested by the Examiner in the interview, Applicants attach a Declaration under 37 CFR §1.132 by Dr. Steven Shak. In his declaration, Dr. Shak discusses human clinical data which demonstrates the lack of significant immunogenicity of humanized antibodies of the present application. Dr. Shak is a very experienced clinician with over 20 years experience as is evident from his curriculum vitae attached as Exhibit A to his declaration.

Dr. Shak explains in paragraph 2 of his declaration that the instant application describes humanized antibodies which were anticipated to lack significant immunogenicity upon repeated administration to a human patient in order to treat a chronic disease in that patient.

Dr. Shak further states that the humanized anti-HER2 antibody, huMAb4D5-8 (HERCEPTIN®), disclosed in Example 1 of the above-

identified patent application has been repeatedly administered to patients in breast cancer clinical trials (paragraph 3 of the declaration). Using an ELISA to detect antibodies to HERCEPTIN® antibody in the serum of treated patients, Dr. Shak reports in paragraph 4 that only one patient out of the 885 patients evaluated as of December 31, 1997 had detectable human antihuman antibodies (HAHA).

Dr. Shak further reviews in paragraphs 5-7 of his declaration human clinical data relating to a humanized variant of a murine anti-IgE antibody which was humanized according to the teachings of the present application. Dr. Shak explains that human patients suffering from allergic rhinitis and asthma (both chronic diseases) have received repeated administrations of the humanized anti-IgE antibody (rhuMAb-E25), but no patients were found to have HAHA to rhuMAb-E25. This is particularly impressive given that the patients who were treated with rhuMAb-E25 were hyper-reactive to foreign antigens.

Dr. Shak states in the final two paragraphs of his declaration that no significant immunogenic response has been observed in patients treated with two further antibodies which were humanized according to the teachings of the present application; *i.e.*, anti-VEGF and anti-CD11a (paragraphs 8 and 9 of the declaration). The patients received multiple doses of these two antibodies.

Accordingly, Applicants submit that it is apparent that the instant specification describes humanized antibodies which lack significant immunogenicity upon repeated administration to a human patient in order to treat a chronic disease in that patient.

In accordance with a recommendation of the Examiner in the interview, for clarity reasons, independent claim 106 herein

includes functional language concerning the unexpected lack of significant immunogenicity of the antibody claimed therein.

In addition to the above-discussed unexpected result pertaining to lack of immunogenicity of the humanized antibodies of the present invention, binding affinity is essentially retained and in some instances is surprisingly improved in the humanized antibody compared to the non-human parent antibody. As shown, for example, in the second to last column of Table 3 on page 72, anti-HER2 humanized variants huMAb4D5-6 and huMAb4D5-8 had binding affinities which were superior to the non-human parent antibody. This could not have been predicted from the prior art, especially from the '762 patent, which advocated the best-fit method (see above) to generate a "high affinity" humanized antibody. The above-mentioned anti-HER2 variants on the other hand were not generated using the "best-fit" method said to be essential in the '762 patent.

As suggested by the Examiner in the interview, claim 113 herein refers to this unexpected property of the humanized variant in that claim (*i.e.* a variant which binds an antigen with better affinity than the non-human parent antibody).

The '762 patent fails to teach humanized antibodies which bind antigen with better affinity than the parent antibody. The reported affinity comparisons in the '762 patent are summarized here for the Examiner's convenience:

- The humanized anti-Tac antibody in Example 1 of the '762 patent allegedly had "approximately the same" binding affinity as the murine parent anti-Tac antibody (lines 25-31 in column 41). The corresponding scientific publication, Queen *et al. PNAS (USA)* 86:10029-10033 (1989) (of record) states that the humanized

anti-Tac antibody actually had an affinity about 1/3 that of murine anti-Tac (see the abstract).

- The humanized mik-β1 humanized antibody of Example 5 had a binding affinity 2-fold worse than the mouse mik-β1 antibody (lines 50-52 in column 52 and Figure 28).

- The humanized Fd79 antibody of the '762 patent apparently displayed a 2-fold decrease in affinity and the affinity of the humanized Fd138-80 antibody was apparently "comparable" to that of the murine antibody (lines 42-46 in column 56).

- The humanized M195 antibody is stated to have an "affinity the same as the mouse M195 antibody to within experimental error" (lines 31-32 in column 60).

- In the line bridging columns 63-64, the humanized CMV5 antibody is stated to have "approximately the same binding affinity as mouse CV5".

- Finally, lines 9-11 in column 67 state that "Mouse AF2 and humanized AF2 will compete similarly, showing that their binding affinities for γ-IFN are approximately the same".

Hence, the '762 patent, in addition to its deficiencies with respect to the use of a consensus human variable domain as in the present application, fails to report any humanized antibody with better binding affinity than the non-human parent antibody.

With respect to another unexpected feature of the present invention, Applicants have shown that a consensus human variable domain of a human heavy chain immunoglobulin subgroup can be used to generate many different strong affinity humanized antibodies, including the following:

(a) anti-HER2 (4D5) [see Example 1 of the application];
(b) anti-CD3 [see Example 3 of the application];
(c) anti-CD18 [see Example 4 of the application];
(d) anti-IgE [see Presta *et al. J. Immunol.* 151(5):2623-2632 (1993) (of record)];
(e) anti-CD11a [see Werther *et al. J. Immunol.* 157:4986-4995 (1996) (of record)]; and
(f) anti-VEGF [see Presta *et al. Cancer Research* 57(20): 4593-4599 (1997) (copy attached]

This could not have been predicted based on the teachings of the '762 patent, since this reference taught that an individual human framework region needed to be tailored to each non-human antibody to be humanized (see comments above).

In summary then, Applicants submit that the cited art is deficient in teaching the instantly claimed humanized antibodies and the unexpected results of the present invention.

Turning now to claim 111 herein, this claim recites the selection invention concerning a "V_H subgroup III" consensus sequence. Applicants submit that this claim is independently patentable.

In particular, there is no suggestion in the cited art to use the particular V_H subgroup III consensus sequence.

In fact, the '762 patent taught away from this consensus sequence by advocating the "best-fit" method of humanization using the most homologous human framework for humanization. As noted above, the V_H subgroup III consensus sequence lacks significant homology to the various non-human antibodies humanized according to the teachings of the present invention. Even if (which is strongly

denied), the '762 patent had intended the phrase "consensus framework from many human antibodies" in column 13 thereof to mean a consensus human variable domain as contemplated in the present application, there is nothing in the '762 patent to indicate that a useful consensus sequence is that of a human heavy chain immunoglobulin subgroup in Kabat, let alone V_H subgroup III. For example, even though the V_H subgroup I FR in Kabat was more homologous (67% homology) to the murine anti-HER2 antibody 4D5 in Example 1 than the V_H subgroup III FR (63% homology), the inventors did not use the more homologous consensus sequence. Notwithstanding this, humanized anti-HER2 antibodies produced using this low homology human FR bound target antigen with <u>better</u> affinity than the non-human parent antibody (see comments above).

Moreover, Applicants have subsequently found that V_H subgroup III consensus sequence surprisingly has the same amino acid sequence as the human germline sequence YAC-5 in Fig. 2 of Cook *et al.*, *Nature Genetics* 7:162-168 (1994) (of record). This subsequent finding supports Applicants' observations that antibodies humanized using this FR sequence are non-immunogenic in humans.

In summation then, Applicants submit that there is nothing in the cited references to teach selection of a V_H subgroup III consensus sequence as in claim 111 for forming the V_H FR template of the humanized antibody, much less the advantages associated with such a consensus sequence. Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

Applicants believe that this case is now in condition for allowance and look forward to receiving early notification of same. If there are outstanding issues however, Applicants invite the Examiner to call the undersigned at the number noted below.

Respectfully submitted,

GENENTECH, INC.

Date: August 24, 1998 By: _____

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#40 Sodium 09/03/98

PATENT
Docket P709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

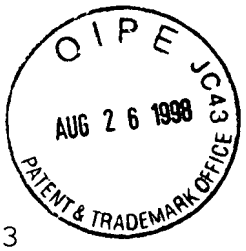
In re Application of

Carter et al.

Serial. No. 08/146,206

Filed: 17 November 1993

For: Method for Making Humanized Antibodies



Group Art Unit: 1644

Examiner: P. Nolan

DECLARATION UNDER 37 CFR §1.132

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

I, STEVEN SHAK, do hereby declare and say as follows:

1. I obtained my M.D. degree in 1977 from New York University (NYU) School of Medicine. Following this, I was a Teaching Assistant and then an Assistant Professor of Medicine and Pharmacology at NYU School of Medicine. Since 1986, I have been employed as a Scientist at Genentech, Inc. Presently, I am the Clinical Team Leader for the therapeutic antibody, anti-HER2. A complete listing of my professional experience, project management experience, education, postdoctoral training, certification and licensure, honors and awards, and publications is found in my curriculum vitae attached as Exhibit A.

2. In my capacity as anti-HER2 Clinical Team Leader, I am familiar with human clinical data relating to the humanized anti-HER2 antibody, huMAb4D5-8 (HERCEPTIN®), disclosed in Example 1 of the above-identified patent application. As explained on page 70,

lines 7-9 of the above application, a humanized variant of the murine anti-HER2 antibody was made which was intended to lack significant immunogenicity upon repeated administration to a human patient in order to treat a chronic disease in that patient.

3. The HERCEPTIN® antibody has been administered to patients in breast cancer clinical trials using a dosing protocol which involves an initial loading dose of 4mg/kg of intravenous (IV) HERCEPTIN® antibody then weekly doses of 2mg/kg (IV) each. Patients have been treated with HERCEPTIN® antibody as a single agent or HERCEPTIN® antibody concomitantly with either (a) cyclophosphamide and doxorubicin or epirubicin (AC) or (b) paclitaxel (TAXOL®).

4. The presence of antibodies to HERCEPTIN® antibody in the serum of treated patients has been determined by enzyme-linked immunosorbent assay (ELISA). As of December 31, 1997, there is only one case of human antihuman antibodies (HAHA) in 885 patients evaluated. This one patient received nine weekly infusions of HERCEPTIN® antibody and discontinued the study on day 65 due to disease progression. At the termination evaluation, antibody measurements were suggestive of antibody formation against the $F(ab')_2$ portion of the HERCEPTIN® antibody. Antibody formation in this one case was not associated with severe allergic symptoms.

5. I have also reviewed human clinical data in relation to a humanized variant of the murine antibody MaE11 which binds IgE. MaE11 was humanized using a consensus human variable domain of a human heavy chain immunoglobulin subgroup [see Figure 1 of Presta *et al. J. Immunol.* 151(5):2623-2632 (1993), Exhibit B attached].

6. Recombinant humanized MaE11 (rhuMAb-E25) has been administered intravenously (IV) or subcutaneously (SQ) to human

patients suffering from allergic rhinitis and asthma. One hundred eighty one subjects with a documented history of seasonal allergic rhinitis or rhinoconjunctivitis received an initial IV loading dose followed by SQ or IV administrations of rhuMAb-E25 on days 7, 14, 28, 42, 56, 70 and 84 [Abstract of Casale *et al. J. Allergy Clin. Immunol.* 100(1):110-121 (1997); Exhibit C attached]. Nineteen allergic asthmatic subjects received rhuMAB-E25 IV the day after the baseline airway allergen challenge and at weekly intervals for eight weeks [Abstract and Figure 1 of Fahy *et al. Am J. Respir. Crit. Care Med.* 155:1828-1834 (1997); Exhibit D]. Potential HAHA in the serum of treated patients were assayed as described in Casale *et al.* and Fahy *et al.*

7. As reported on page 116 of Casale *et al.* and page 1830 of Fahy *et al.*, no patients were found to have HAHA to rhuMAb-E25.

8. I am also aware that we have not observed a significant immunogenic response in patients receiving multiple doses of a humanized anti-VEGF antibody for inhibiting VEGF-induced angiogenesis. The humanized antibody is question is a variant of murine anti-VEGF antibody A.4.6.1, and was humanized using a consensus human variable domain of a human heavy chain immunoglobulin subgroup [Figure 1 on page 4596 of Presta *et al. Cancer Research* 57(20):4593-4599 (1997); Exhibit E attached].

9. Finally, I have been told that no significant immunogenicity has been associated with repeated administration of a humanized anti-CD11a antibody to psoriasis patients. The humanized anti-CD11a antibody with which the psoriasis patients have been treated was prepared from the murine MHM24 antibody using a consensus human variable domain of a human heavy chain immunoglobulin subgroup [Figure 1 of Werther *et al. J. Immunol.* 157(11):4986-4995(1996), Exhibit F attached].

I declare further 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 issuing thereon.

Dated: 8/24/98

STEVEN SHAK

CURRICULUM VITAE

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Professional Experience:

1998-	Staff Clinical Scientist, Genentech, Inc.
1996-98	Senior Clinical Scientist, Genentech, Inc.
1989-96	Director, Departments of Immunobiology, Pulmonary Research, and Pathology, Genentech, Inc.
1986-89	Scientist, Genentech, Inc.
1984-86	Assistant Professor of Medicine and Pharmacology New York University School of Medicine
1978-80	Teaching Assistant, Department of Medicine New York University School of Medicine

Project Management:

1996-	Anti-HER2 Clinical Team Leader
1996-97	Anti-VEGF Clinical Team Leader
1996-	Chair, Clinical Assessment Committee
1993-96	Chair, Genentech-GenVec Research Committee
1993-	Board of Directors, Genentech Endowment for Cystic Fibrosis
1991-96	Research Representative on Clinical Research Advisory Committee
1995-96	DNase SLE Biology Team Leader
1992-94	DNase Pulmozyme Chronic Bronchitis Team Leader

1988-91	DNase Pulmozyme Project Team Leader

Education:

1973-77	M.D., New York University School of Medicine
1969-73	B.A., Amherst College

Postdoctoral Training:

Research:	
1981-84	University of California, San Francisco Cardiovascular Research Institute Rosalyn Russell Arthritis Research Laboratory Chief: Ira M. Goldstein, M.D.
Fellowship:	
1980-84	University of California, San Francisco Cardiovascular Research Institute Subspeciality: Pulmonary Medicine Chairmen: John F. Murray, M.D. and Jay A. Nadel, M.D.
Residency:	
1977-80	Bellevue Hospital Specialty: Internal Medicine Chairman: Saul J. Farber, M.D.

Certification and Licensure:

1982	Diplomate, Pulmonary Disease
1980	Diplomate, American Board of Internal Medicine
1980	Licensed, California (current)
1978	Licensed, New York State

Honors and Awards:

1995	Prix Gallien, Portugal for "Pulmozyme Discovery and Development"
1995	"Parenting Achievement Award," Parenting Magazine
1993	Distinguished Corporate Scientist Award, Cystic Fibrosis

	Foundation
1992	CF Achievement Award, Cystic Fibrosis Research, Inc.
1985	J. Burns Amberson Award, NY Lung Association
1980	Medical School Pulmonary Faculty Training Award National Institutes of Health
1977	Alpha Omega Alpha
1974	Valentine Mott Award in Anatomy and Cell Biology
1973	Summa Cum Laude
1973	Phi Beta Kappa
1973	Sigma Xi
1973	Howard Waters Doughty Prize in Chemistry

Personal:

Born: July 21, 1950, Elizabeth, NJ
Married, two children
Social Security No.: 145-42-8006

Publications:

I. Book Chapters.

1. SHAK S, Goldstein IM: The major pathway for leukotriene B_4 catabolism in human polymorphonuclear leukocytes involves ω-oxidation by a cytochrome P-450 enzyme. In PROSTAGLANDINS, LEUKOTRIENES, AND LIPOXINS. (JM Bailey, ed.) Plenum Publishing Corporation, New York, 1985.

2. SHAK S: Leukotriene B_4 catabolism: Quantitation of leukotriene B_4 and its ω-oxidation prducts by reversed phase high-performance liquid chromatography. METHODS IN ENZYMOLOGY. Vol. 141. Cellular Regulators (AR Means and PM Conn, eds.) Academic Press, Florida, pp. 355-371, 1987.

3. SHAK S: Molecular mechanisms for the catabolism of leukotriene B_4. In ADVANCES IN INFLAMMATION RESEARCH. Vol. 12. (A Lewis, ed.) Raven Press, Ltd., New York, pp. 111-124, 1988.

4. Goldstein IM, SHAK S: Humoral and cellular mediators of host defenses. In TEXTBOOK OF RESPIRATORY MEDICINE. (JF Murray and JA Nadel, eds.) W.B. Saunders Company, Philadelphia, pp. 358-373, 1988.

5. Goldstein IM, SHAK S: Host defenses in the lung: Neutrophils, complement, and other humoral mediators. In TEXTBOOK OF RESPIRATORY MEDICINE. (JF Murray and JA Nadel, eds.) W.B. Saunders Company, Philadelphia, pp. 402-418, 1994.

6. S SHAK: Mucins and lung secretions. In THE LUNG--SCIENTIFIC FOUNDATIONS. (RG Crystal, JB West, ER Weibel, and PJ Barnes, eds.) Lippincott-Raven Publishers, Philadelphia, pp. 479-486.

II. Articles

1. SHAK, S, Perez HD, Goldstein IM: A novel dioxygenation product of arachidonic acid posseses potent chemotactic activity for human polymorphonuclear leukocytes. THE JOURNAL OF BIOLOGICAL CHEMISTRY, 258:14948-14953, 1983.

2. Perez HD, Bissell DM, Roll FJ, SHAK S, Goldstein IM: A possible explanation for leukocytic infiltration of the liver in acute alcoholic hepatitis: Ethanol-induced generation by hepatocytes of a lipid chemotactic factor. TRANSACTIONS OF THE ASSOCIATION OF AMERICAN PHYSICIANS. 96:56-64, 1983.

3. Charo, IF, SHAK S, Darasek MA, Davison PM, Goldstein IM: Prostaglandin I_2 is not a major metabolite of arachidonic acid in cultured endothelial cells from human foreskin microvessels. THE JOURNAL OF CLINICAL INVESTIGATION. 74:914-919, 1984.

4. Perez HD, Roll JF, Bissell DM, SHAK S, Goldstein IM: Ethanol induces isolated rat hepatocytes to generate chemotactic activity for polymorphonuclear leukocytes. THE JOURNAL OF CLINICAL INVESTIGATION. 74:1350-1357, 1984.

5. SHAK S, Goldstein IM: ω-Oxidation is the major pathway for the catabolism of leukotriene B_4 in human polymorphonuclear leukocytes. THE JOURNAL OF BIOLOGICAL CHEMISTRY. 259:10181-10187, 1984.

6. SHAK S, Goldstein IM: Carbon monoxide inhibits ω-oxidation of leukotriene B_4 by human polymorphonuclear leukocytes: Evidence that catabolism of leukotriene B_4 is mediated by a cytochorme P-450 enzyme. BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS. 123:475-481, 1984.

7. SHAK S, Reich N, Goldstein IM, Ortiz de Montellano PM: Leukotriene B_4 ω-hydroxylase in human polymorphonuclear leukocytes: Suicidal inactivation by acetylenic fatty acids. THE JOURNAL OF BIOLOGICAL CHEMISTRY. 260:13023-13028, 1985.

8. SHAK S, Goldstein IM: Leukotriene B_4 ω-hydroxylase in human polymorphonuclear leukocytes: Partial purification and identification as a cytochrome P-450. THE JOURNAL OF CLINICAL INVESTIGATION. 76:1218-1228, 1985.

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10. Kruskal BA, SHAK S, Maxfield FR: Spreading of human neutrophils is immediately preceded by a large increase in cytoplasmic free calcium concentration. PROCEEDINGS OF THE NATIONAL ACADEMY OF THE SCIENCES USA. 83:2919-2923, 1986.

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13. SHAK S, Capon DJ, Hellmiss R, Marsters SA, Baker CL: Recombinant human DNase I reduces the viscosity of cystic fibrosis sputum. PROCEEDINGS OF THE NATIONAL ACADAMY OF SCIENCES, USA. 87:9188-9192, 1990.

14. Aitken ML, Burke W, McDonald G, SHAK S, Montgomery AB, Smith A: Recombinant human DNase inhalation in normal and patients with cystic fibrosis: A phase I study. THE JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION. 267:1947-1951, 1992.

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19. Sinicropi D, Baker DL, Prince WS, Shiffer K, SHAK S: Colorimetric determination of DNase I activity with a DNA-methyl green substrate. ANALYTICAL BIOCHEMISTRY. 222:351-358, 1994.

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21. Zahm JM, Girod de Bentzmann S, Deneuville E, Perrot-Minnot C, Dabadie A, Pennaforte F, Roussey M, SHAK S, Puchelle E: Dose-dependent in vitro effect of recombinant human DNase on rheological and transport properties of cystic fibrosis respiratory mucus. EUROPEAN RESPIRATORY JOURNAL. 8:381-6, 1995.

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#40 09/02/98

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1644 Examiner: P. Nolan **CERTIFICATE OF MAILING** I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on August 24, 1998 Wendy M. Lee

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Applicants submit herewith patents, publications or other information (attached hereto and listed on the attached Form PTO-1449) of which they are aware, which they believe may be material to the examination of this application and in respect of which there may be a duty to disclose in accordance with 37 CFR §1.56.

This Information Disclosure Statement:

(a) [] accompanies the new patent application submitted herewith. 37 CFR §1.97(a).

(b) [] is filed within three months after the filing date of the application or within three months after the date of entry of the national stage of a PCT application as set forth in 37 CFR§1.491.

(c) [] as far as is known to the undersigned, is filed before the mailing date of a first Office action on the merits.

(d) [] is filed after the first Office Action and more than three months after the application's filing date or PCT national stage date of entry filing but, as far as is known to the undersigned, prior to the mailing date of either a final rejection or a notice of allowance, whichever occurs first, and is accompanied by either the fee ($240) set forth in 37 CFR §1.17(p) **or** a statement as specified in 37 CFR §1.97(e), as checked below. Should any fee be due, the U.S. Patent and Trademark Office is hereby authorized to charge Deposit Account No. 07-0630 in the amount of $240.00 to cover

the cost of this Information Disclosure Statement. Any deficiency or overpayment should be charged or credited to this deposit account. **A duplicate of this sheet is enclosed.**

(e) [] is filed after the mailing date of either a final rejection or a notice of allowance, whichever occurred first, and is accompanied by the fee ($130) set forth in 37 CFR §1.17(i) **and** a statement as specified in 37 CFR §1.97(e), as checked below. **This document is to be considered as a petition requesting consideration of the information disclosure statement.** The U.S. Patent and Trademark Office is hereby authorized to charge Deposit Account No. 07-0630 in the amount of $130.00 to cover the cost of this Information Disclosure Statement. Any deficiency or overpayment should be charged or credited to this deposit account. **A duplicate of this sheet is enclosed.**

(f) [x] is filed after the mailing date of a final rejection, but a request to withdraw the finality thereof under 37 CFR § 1.129(a) is submitted herewith. The U.S. Patent and Trademark Office is hereby authorized to charge Deposit Account No. 07-0630 to cover the cost of this Information Disclosure Statement in the event that any fees are due. **A duplicate of this sheet is enclosed.**

[If either of boxes (d) or (e) is checked above, the following statement under 37 CFR §1.97(e) may need to be completed.] The undersigned states that:

[] Each item of information contained in the information disclosure statement was cited in a communication mailed from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of this information disclosure statement.

[] No item of information contained in this information disclosure statement was cited in a communication mailed from a foreign patent office in a counterpart foreign application and, to the knowledge of the undersigned after making reasonable inquiry, was known to any individual designated in 37 CFR §1.56(c) more than three months prior to the filing of this information disclosure statement.

A list of the patent(s) or publication(s) is set forth on the attached Form PTO-1449 (Modified).

A copy of the items on PTO-1449 is supplied herewith:

[] each [] none [x] only those listed below:

5,677,171

5,772,997

Brown, Jr. et al.

Mathieson et al.

Presta et al.

Casale et al.

Fahy et al.

A concise explanation of relevance of the items listed on PTO-1449 is:

[x] not given

[] given for each listed item

[] given for only non-English language listed item(s) [Required]

[] in the form of an English language copy of a Search Report from a foreign patent office, issued in a counterpart application, which refers to the relevant portions of the references.

The Examiner is reminded that a "concise explanation of the relevance" of the submitted prior art "may be nothing more than identification of the particular figure or paragraph of the patent or publication which has some relation to the claimed invention," MPEP §609.

While the information and references disclosed in this Information Disclosure Statement may be "material" pursuant to 37 CFR §1.56, it is not intended to constitute an admission that any patent, publication or other information referred to therein is "prior art" for this invention unless specifically designated as such.

In accordance with 37 CFR §1.97(g), the filing of this Information Disclosure Statement shall not be construed to mean that a search has been made or that no other material information as defined in 37 CFR §1.56(a) exists. It is submitted that the Information Disclosure Statement is in compliance with 37 CFR §1.98 and MPEP §609 and the Examiner is respectfully requested to consider the listed references.

Respectfully submitted,
GENENTECH, INC.

Date: August 24, 1998

By: _____
Wendy M. Lee
Reg. No. 40,378

1 DNA Way
So. San Francisco, CA 94080-4990
Phone: (650) 225-1994
Fax: (650) 952-9881

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, DC 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.

EXAMINER	
ART UNIT	PAPER NUMBER

DATE MAILED: #41

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.

EXAMINER	
ART UNIT	PAPER NUMBER

DATE MAILED:

INTERVIEW SUMMARY

All participants (applicant, applicant's representative, PTO personnel):

(1) Minh-Tam Davis (3) Wendy Lee

(2) Lisa Teisse (4)

Date of Interview 10/16/01

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. ☐ was not reached.

Claim(s) discussed: all pending claims

Identification of prior art discussed:

Description of the general nature of what was agreed to if an agreement was reached, or any other comments: 1/112, 2nd, 1st para issue of cls 43-105 with respect to binding of cDR, 2/112, 2nd para issue of cls 101-112 with respect to "significant" immunogenicity

(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.)

1. ☐ It is not necessary for applicant to provide a separate record of the substance of the interview.

Unless the paragraph above has 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. (See MPEP Section 713.04). If a response to the last Office action has are ready been filed, APPLICANT IS GIVEN ONE MONTH FROM THIS INTERVIEW DATE TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW.

2. ☐ 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. Applicant is not relieved from providing a separate record of the interview unless box 1 above is also checked.

Examiner Note: You must sign this form unless it is an attachment to another form.

FORM **PTOL-413** (REV.1-96)

Minh Tam Davis

Official Document

#42

GENENTECH, INC.

1 DNA Way, South San Francisco, CA 94080-4990 Tel: 650-225-1994 Fax: 650-952-9881

FAX TRANSMISSION COVER SHEET

Date: November 6, 1998

To: Lila Feisee ~~Examiner M.T. Davis~~ Group Art Unit: 1642 of US PTO

Fax: (703) 308-~~4426~~ 0294

Re: U.S. Ser. No 08/146,206 filed November 17, 1993 (Attorney Docket No.: P0709P1)

Sender: Wendy M. Lee

CERTIFICATION OF FACSIMILE TRANSMISSION
I hereby certify that this paper is being facsimile transmitted to the Patent and Trademark Office on the date shown below.
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Signature Date

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Comments:

42/I

11/6/98

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1644 Examiner: Tam Davis

SUPPLEMENTAL AMENDMENT

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Further to the amendment dated August 24, 1998, Applicants request that the above-identified application be amended as follows:

IN THE CLAIMS:

Please amend claims 43, 72, 104-106 and 112 as follows:

43. (Amended) A humanized antibody variable domain comprising a non-human Complementarity Determining Region (CDR) which binds an antigen incorporated into a human antibody variable domain, and further comprising an 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.

I,1 and J1

72. (Amended) An antibody which binds $p185^{HER2}$ and comprises a humanized antibody variable domain comprising a non-human Complementarity Determining Region (CDR) which binds $p185^{HER2}$ incorporated into a human antibody variable domain, and further

I2 and J3

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.

104. (Amended) A humanized antibody variable domain comprising a non-human Complementarity Determining Region (CDR) which binds 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.

105. (Amended) An antibody which lacks [significant] 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 and comprises a non-human Complementarity Determining Region (CDR) which binds 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.

106. (Amended) An antibody which lacks [significant] 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 and comprises a consensus human

variable domain 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 comprising a Framework Region (FR) substitution where the substituted FR residue: (a) noncovalently binds antigen directly; (b) interacts with a CDR; (c) comprises 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.

112. (Amended) The humanized antibody of claim 111 which lacks [significant] 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.

REMARKS

The undersigned confirms having met with Examiners Davis and Feisee in the interview October 16, 1998. In that interview, the Examiners suggested that independent claims 43, 72, 104 and 105 be amended for claim precision to refer to a CDR which binds an antigen. Without acquiescing in any objection or rejection and purely to facilitate allowance, claims 43, 104 and 105 have been revised herein as recommended by the Office to refer to a CDR "which binds an antigen" and claim 72 refers to a CDR "which binds $p185^{HER2}$".

Moreover, the Examiners proposed in the interview that, for clarity reasons, claims 105, 106 and 112 (refering to antibodies with diminished immunogenicity) be revised to refer to an antibody which "lacks immunogenicity compared to a non-human

parent antibody". Without acquiescing in any objection or rejection and purely to facilitate allowance, Applicants have adopted the language proposed by the Office. Hence, the instantly claimed antibodies display significantly reduced immunogenicity upon repeated administration to a human patient in order to treat a chronic disease in that patient (see page 70, lines 6-8 of the instant application), as opposed to the immunogenicity observed with the prior art humanized antibody in Isaacs *et al.*, *The Lancet* 340:748-752 (1992) (see first paragraph on page 19 of the amendment dated August 24, 1998).

Applicants look forward to early receipt of a notice of allowance in the above application.

Respectfully submitted,
GENENTECH, INC.

Date: November 6, 1998

By: _____
Wendy M. Lee
Reg. No. 40,378

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#44

Official Document - GENENTECH, INC.

1 DNA Way, South San Francisco, CA 94080-4990 Tel: 650-225-7039 Fax: 650-952-9881

FAX TRANSMISSION COVER SHEET

Date: January 15, 1999

To: Examiner Julie Reeves Group Art Unit: 1642 of US PTO

Fax: (703) 308-4426

Re: U.S. Ser. No 08/146,206 filed November 17, 1993 (Attorney Docket No.: P0709P1)

Sender: Wendy M. Lee

CERTIFICATION OF FACSIMILE TRANSMISSION
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Ann Savelli 1/15/99
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Comments:

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1642 Examiner: J. Reeves

AMENDMENT TRANSMITTAL

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Transmitted herewith is an amendment in the above-identified application.

The fee has been calculated as shown below

	Claims Remaining After Amendment		Highest No. Previously Paid For	Present Extra	Rate	Additional Fees
Total	86	-	72	14	$18	$252.00
Independent	9	-	7	2	$78	$156.00
__ Multiple dependent claim(s), if any					$260	$0.00
					Total Fee Calculation	$408.00

_____ No additional fee is required.
_____ x The Commissioner is hereby authorized to charge Deposit Account No. 07-0630 in the amount of $408.00. A duplicate copy of this transmittal is enclosed.
_____ Petition for Extension of Time is enclosed.

The Commissioner is hereby authorized to charge any additional fees required under 37 CFR 1.16 and 1.17, or credit overpayment to Deposit Account No. 07-0630. A duplicate copy of this sheet is enclosed.

Respectfully submitted,
GENENTECH, INC.

Date: January 15, 1999

By. _____
Wendy M. Lee
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1 DNA Way
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1/15/99

paper #44 Amdt J

Patent Docket P0709P1

1/15/99 JK

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1642 Examiner: Julie Reeves

SUPPLEMENTAL AMENDMENT

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Please amend the claims as indicated below. Pending claims which are not amended herein are marked "(Reiterated)" for the Examiner's convenience.

1 ~~43~~. (TWICE AMENDED) A humanized antibody variable domain comprising [a] non-human Complementarity Determining Region (CDR) amino acid residues which bind[s] an antigen incorporated into a human antibody variable domain, and further comprising ~~an~~ 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.

K J1

2 ~~44~~. (AMENDED) The humanized variable domain of claim ~~43~~ 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 [was] obtained.

J2

45. (Reiterated) The humanized variable domain of claim 43 wherein no human Framework Region (FR) residue other than those set forth in the group has been substituted.

Duplicate

/cc

J

46. (Reiterated) The humanized variable domain of claim 43 wherein the human antibody variable domain is a consensus human variable domain.

47. (Reiterated) The humanized variable domain of claim 43 wherein the residue at site 4L has been substituted.

48. (Reiterated) The humanized variable domain of claim 43 wherein the residue at site 38L has been substituted.

49. (Reiterated) The humanized variable domain of claim 43 wherein the residue at site 43L has been substituted.

50. (Reiterated) The humanized variable domain of claim 43 wherein the residue at site 44L has been substituted.

51. (Reiterated) The humanized variable domain of claim 43 wherein the residue at site 58L has been substituted.

52. (Reiterated) The humanized variable domain of claim 43 wherein the residue at site 62L has been substituted.

53. (Reiterated) The humanized variable domain of claim 43 wherein the residue at site 65L has been substituted.

54. (Reiterated) The humanized variable domain of claim 43 wherein the residue at site 66L has been substituted.

55. (Reiterated) The humanized variable domain of claim 43 wherein the residue at site 67L has been substituted.

56. (Reiterated) The humanized variable domain of claim 43 wherein the residue at site 68L has been substituted.

57. (Reiterated) The humanized variable domain of claim 43 wherein the residue at site 69L has been substituted.

58. (Reiterated) The humanized variable domain of claim 43 wherein the residue at site 73L has been substituted.

59. (Reiterated) The humanized variable domain of claim 43 wherein the residue at site 85L has been substituted.

60. (Reiterated) The humanized variable domain of claim 43 wherein the residue at site 98L has been substituted.

61. (Reiterated) The humanized variable domain of claim 43 wherein the residue at site 2H has been substituted.

62. (Reiterated) The humanized variable domain of claim 43 wherein the residue at site 4H has been substituted.

63. (Reiterated) The humanized variable domain of claim 43 wherein the residue at site 36H has been substituted.

64. (Reiterated) The humanized variable domain of claim 43 wherein the residue at site 39H has been substituted.

65. (Reiterated) The humanized variable domain of claim 43 wherein the residue at site 43H has been substituted.

66. (Reiterated) The humanized variable domain of claim 43 wherein the residue at site 45H has been substituted.

67. (Reiterated) The humanized variable domain of claim 43 wherein the residue at site 69H has been substituted.

68. (Reiterated) The humanized variable domain of claim 43 wherein the residue at site 70H has been substituted.

69. (Reiterated) The humanized variable domain of claim 43 wherein the residue at site 74H has been substituted.

70. (Reiterated) The humanized variable domain of claim 43 wherein the residue at site 92H has been substituted.

71. (Reiterated) An antibody comprising the humanized variable domain of claim 43.

J3

72. (TWICE AMENDED) An antibody which binds $p185^{HER2}$ and comprises a humanized antibody variable domain, wherein the humanized antibody variable domain comprises [comprising a] non-human Complementarity Determining Region (CDR) amino acid residues which bind[s] $p185^{HER2}$ 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.

J4

73. (AMENDED) The antibody of claim 72 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 [was] obtained.

74. (Reiterated) The antibody of claim 72 wherein no human Framework Region (FR) residue other than those set forth in the group has been substituted.

75. (Reiterated) The antibody of claim 72 wherein the human antibody variable domain is a consensus human variable domain.

76. (Reiterated) The antibody of claim 72 wherein the residue at site 4L has been substituted.

77. (Reiterated) The antibody of claim 72 wherein the residue at site 38L has been substituted.

78. (Reiterated) The antibody of claim 72 wherein the residue at site 43L has been substituted.

79. (Reiterated) The antibody of claim 72 wherein the residue at site 44L has been substituted.

80. (Reiterated) The antibody of claim 72 wherein the residue at site 46L has been substituted.

81. (Reiterated) The antibody of claim 72 wherein the residue at site 58L has been substituted.

82. (Reiterated) The antibody of claim 72 wherein the residue at site 62L has been substituted.

83. (Reiterated) The antibody of claim 72 wherein the residue at site 65L has been substituted.

84. (Reiterated) The antibody of claim 72 wherein the residue at site 66L has been substituted.

85. (Reiterated) The antibody of claim 72 wherein the residue at site 67L has been substituted.

86. (Reiterated) The antibody of claim 72 wherein the residue at site 68L has been substituted.

87. (Reiterated) The antibody of claim 72 wherein the residue at site 69L has been substituted.

88. (Reiterated) The antibody of claim 72 wherein the residue at site 73L has been substituted.

89. (Reiterated) The antibody of claim 72 wherein the residue at site 85L has been substituted.

90. (Reiterated) The antibody of claim 72 wherein the residue at site 98L has been substituted.

91. (Reiterated) The antibody of claim 72 wherein the residue at site 2H has been substituted.

92. (Reiterated) The antibody of claim 72 wherein the residue at site 4H has been substituted.

93. (Reiterated) The antibody of claim 72 wherein the residue at site 36H has been substituted.

94. (Reiterated) The antibody of claim 72 wherein the residue at site 39H has been substituted.

95. (Reiterated) The antibody of claim 72 wherein the residue at site 43H has been substituted.

96. (Reiterated) The antibody of claim 72 wherein the residue at site 45H has been substituted.

97. (Reiterated) The antibody of claim 72 wherein the residue at site 69H has been substituted.

98. (Reiterated) The antibody of claim 72 wherein the residue at site 70H has been substituted.

99. (Reiterated) The antibody of claim 72 wherein the residue at site 74H has been substituted.

100. (Reiterated) The antibody of claim 72 wherein the residue at site 75H has been substituted.

101. (Reiterated) The antibody of claim 72 wherein the residue at site 76H has been substituted.

102. (Reiterated) The antibody of claim 72 wherein the residue at site 78H has been substituted.

103. (Reiterated) The antibody of claim 72 wherein the residue at site 92H has been substituted.

J5

104. (TWICE AMENDED) A humanized antibody variable domain comprising [a] non-human Complementarity Determining Region (CDR) amino acid residues which bind[s] 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.

J6

105. (TWICE AMENDED) [An] 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 [and], wherein the humanized antibody comprises [a] non-human Complementarity Determining Region (CDR) amino acid residues which bind[s] 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.

106. (TWICE AMENDED) [An] 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[and], wherein the humanized antibody comprises a consensus human variable domain 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 [comprising] comprises a Framework Region (FR) substitution where the substituted FR residue: (a) noncovalently binds antigen directly; (b) interacts with a CDR; (c) [comprises] 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.

107. (AMENDED) The humanized antibody of claim 106 comprising a non-human FR residue which noncovalently binds antigen directly.

108. (AMENDED) The humanized antibody of claim 106 comprising a non-human FR residue which interacts with a CDR.

109. (AMENDED) The humanized antibody of claim 106 comprising a non-human FR residue which [comprises] introduces a glycosylation site which affects the antigen binding or affinity of the antibody.

110. (AMENDED) The humanized antibody of claim 106 comprising a non-human FR residue which participates in the V_L-V_H interface by affecting the proximity or orientation of the V_L-V_H regions with respect to one another.

111. (AMENDED) A humanized antibody comprising a consensus human variable domain of human V_H subgroup III, wherein amino acid residues forming Complementarity Determining Regions (CDRs) thereof comprise non-human antibody amino acid residues, and further comprising a Framework Region (FR) substitution where the substituted FR residue: (a) noncovalently binds antigen directly; (b) interacts with a CDR; (c) [comprises] 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.

112. (Reiterated) The humanized antibody of claim 111 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.

113. (AMENDED) A humanized variant of a non-human parent antibody which binds an antigen with better affinity than the parent antibody and comprises a consensus human variable domain 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 [comprising] comprises a Framework Region (FR) substitution where the substituted FR residue: (a) noncovalently binds antigen directly; (b) interacts with a CDR; (c) [comprises] 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.

J9

114. (AMENDED) The humanized variant of claim 113 which binds the antigen at least about 3-fold more tightly than the parent antibody binds antigen.

Please add the following claims to the above-identified application:

--115. (NEW) 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 an 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.

J10

116. (NEW) The humanized variable domain of claim 115 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.

117. (NEW) The humanized variable domain of claim 115 wherein no human Framework Region (FR) residue other than those set forth in the group has been substituted.

118. (NEW) The humanized variable domain of claim 115 wherein the human antibody variable domain is a consensus human variable domain.

119. (NEW) The humanized variable domain of claim 115 wherein the residue at site 24H has been substituted.

120. (NEW) The humanized variable domain of claim 115 wherein the residue at site 73H has been substituted.

121. (NEW) The humanized variable domain of claim 115 wherein the residue at site 76H has been substituted.

122. (NEW) The humanized variable domain of claim 115 wherein the residue at site 78H has been substituted.

123. (NEW) The humanized variable domain of claim 115 wherein the residue at site 93H has been substituted.

J10

124. (NEW) The humanized variable domain of claim 115 which further comprises an amino acid substitution at site 71H.

125. (NEW) The humanized variable domain of claim 115 which further comprises amino acid substitutions at sites 71H and 73H.

126. (NEW) The humanized variable domain of claim 115 which further comprises amino acid substitutions at sites 71H, 73H and 78H.

127. (NEW) An antibody comprising the humanized variable domain of claim 115.

128. (NEW) 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 a Framework Region (FR) 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 humanized variant binds the antigen more tightly than the parent antibody

binds ag.

#45/AL
12/11/98

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1642 Examiner: J. Reeves

CERTIFICATE OF HAND DELIVERY
I hereby certify that this correspondence is being hand delivered in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on
February 1, 1999
R. H. Mitchell

COMMUNICATION

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Further to the Supplemental Amendment fax-filed on January 15, 1999, please find enclosed priority documents USSN 07/290,975 and USSN 07/310,252 for the "PDL Patents" as promised on page 11 of that amendment.

Applicants further submit herewith a Supplemental Information Disclosure Statement. In this respect, Applicants bring to the Examiner's attention a Celltech press release entitled: "Celltech Antibody Technology Platform Further Strengthened Through New Patents in US and Europe." (Exhibit A attached) This press release refers to an allowed US "Adair" patent application. Applicants believe this US Adair patent application corresponds to WO91/09967 (of record) and EP 460,167 B1 (copy attached).

Should the Examiner have questions concerning this communication, she is invited to call the undersigned.

Respectfully submitted,
GENENTECH, INC.

Date: January 29, 1999

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Celltech Antibody Technology Platform Further Strengthened Through New Patents in US and Europe

SLOUGH, U.K., Sept. 26 /PRNewswire/ -- Celltech announced today that the U.S. Patent Office has allowed one of its key patent applications covering engineered human antibodies. The grant of this "Adair" patent will occur in early 1997 and will expire in 2014. This broad product patent covers a key approach to the construction of new human antibodies which is essential in order to achieve full therapeutic activity. It covers all antibodies which have been constructed using this approach. A corresponding patent has already been granted in Europe, although it is anticipated that the financial benefit to Celltech from the U.S. patent will be more significant in the near term because of the numbers of antibodies in late-stage development in the U.S.

The "Adair" patent is an important new element in Celltech's technology platform, and complements previous Celltech patents in the field of antibody engineering. It covers all of Celltech's own antibodies currently in clinical development, thus substantially extending their period of patent protection. In addition the patent covers a range of antibodies under development by other companies. This would result in royalty revenues should these products reach the market.

There are already a number of process patents covering the manufacture of engineered antibodies including those granted to Celltech, Genentech, the Medical Research Council and Protein Design Laboratories. Celltech has agreements in place with Genentech and the Medical Research Council relating to the commercial exploitation of some of these patents. Celltech pursues the strategy of licensing its existing antibody patents to any interested party for products which are not directly competitive with Celltech's own products. This policy will be pursued with the new "Adair" patent and all licensees who have directly licensed pre-existing patents from Celltech (in particular the "Boss" antibody engineering patents) will be offered favorable terms for the "Adair" patent.

Commenting on the news today, Dr. Peter Fellner, CEO, said, "Celltech has built a very valuable platform technology in the field of antibody engineering and the grant of this patent will further strengthen our position. We expect a continued growth in royalty revenues from our licensed patents which will make a significant contribution to the profitability of the company. The potential in this area can be seen from the growing success of ReoPro(TM) (Lilly/Centocor). Royalties on the sales of this product are paid to both Genentech and Celltech."
SOURCE Celltech Therapeutics Ltd.

/NOTE TO EDITORS:

1. The Adair product patent covers any antibody in which the antigen binding regions from a donor antibody have been transferred to the framework of a human antibody, and specifies certain requirements in specific amino acid residues within the product which are necessary to recover full antigen binding activity of the newly created antibody.

2. Antibodies are natural proteins which bind tightly and specifically to antigens. This binding property is particularly important in providing a defense mechanism against infectious organisms such as bacteria and viruses. For some time, scientists have been able to produce antibodies in the

laboratory and their availability has had a profound impact on diagnostic medicine. In contrast, they have had little impact on therapeutic medicine. The reason for this is that the first antibodies were derived from animal sources. When these animal antibodies were injected into humans they induced a significant immune response which led to either adverse reactions or a rapid loss of therapeutic efficacy. More recently techniques have been developed to produce engineered human antibodies which are virtually identical to natural human antibodies. The main advantage of these antibodies is that they do not cause a significant immune response in man and they are very well tolerated. Because of their good tolerance, their binding properties are being used in a wide variety of therapeutic applications in areas such as blockade of receptor functions in heart disease, neutralization of cytokine in rheumatoid arthritis and killing of cancer cells./

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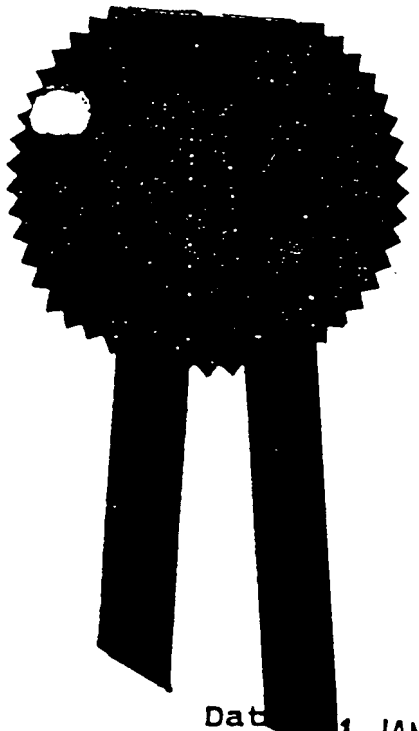
TITLE

NOVEL IL-2 RECEPTOR-SPECIFIC HUMAN IMMUNOGLOBULINS



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By authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

C. A. Lane

Certifying Officer

Date 31 JAN 1990

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NOVEL IL-2 RECEPTOR-SPECIFIC HUMAN IMMUNOGLOBULINS

Field of the Invention

The present invention relates generally to the combination of recombinant DNA and monoclonal antibody technologies for developing novel therapeutic agents and, more particularly, to the production of non-immunogenic antibodies specific for the human interleukin-2 receptor and their uses.

Background of the Invention

In mammals, the immune response is mediated by two types of cells that interact specifically with foreign material, i.e., antigens. One of these cell types, B-cells, are responsible for the production of antibodies. The second cell class, T-cells, include a wide variety of cellular subsets controlling the in vivo function of both B-cells and a wide variety of other hematopoietic cells, including T-cells.

One way in which T-cells exert this control is through the production of a lymphokine known as interleukin-2 (IL-2), originally named T-cell growth factor. IL-2's prime function appears to be the stimulation and maintenance of T-cells. Indeed, some immunologists believe that IL-2 may be at the center of the entire immune response (see, Farrar, J., et al., Immunol. Rev. 63:129-166 (1982), which is incorporated herein by reference).

To exert its biological effects, IL-2 interacts with a speciric high-affinity membrane receptor (Greene, W., et al., Progress in Hematology XIV, E. Brown, Ed., Grune and Statton, New York (1986), at pgs. 283 ff). The human IL-2 receptor is a complex multichain glycoprotein, with one chain, known as the Tac peptide, being about 55kD in size (see, Leonard, W., et al., J. Biol. Chem. 260:1872 (1985), which is incorporated herein by reference). A gene encoding this protein has been isolated, and predicts a 272 amino acid peptide, including a 21 amino acid signal peptide (see,

Leonard, W., et al., Nature 311: 626 (1984)). The 219 NH_2-terminal amino acids of the p55 Tac protein apparently comprise an extracellular domain (see, Leonard, W., et al., Science, 230:633-639 (1985), which is incorporated herein by reference).

Much of the elucidation of the human IL-2 receptor's structure and function is due to the development of specifically reactive monoclonal antibodies. In particular, one mouse monoclonal antibody, known as anti-Tac (Uchiyama, et al., J. Immunol. 126:1393 (1981)) has shown that IL-2 receptors can be detected on T-cells, but also on cells of the monocyte-macrophage family, Kupffer cells of the liver, Langerhans' cells of the skin and, of course, activated T-cells. Importantly, resting T-cells, B-cells or circulating machrophages typically do not display the IL-2 receptor (Herrmann, et al., J. Exp. Med. 162:1111 (1985)).

The anti-Tac monoclonal antibody has also been used to define lymphocyte functions that require IL-2 interaction, and has been shown to inhibit various T-cell functions, including the generation of cytotoxic and suppressor T lymphocytes in cell culture. Also, based on studies with anti-Tac and other antibodies, a variety of disorders are now associated with improper IL-2 receptor expression by T-cells, in particular adult T-cell leukemia.

More recently, the IL-2 receptor has been shown to be an ideal target for novel therapeutic approaches to T-cell mediated diseases. It has been proposed that IL-2 receptor specific antibodies, such as the anti-Tac monoclonal antibody, can be used either alone or as an immunoconjugate (e.g., with Ricin A, isotopes and the like) to effectively remove cells bearing the IL-2 receptor. These agents can, for example, theoretically eliminate IL-2 receptor-expressing leukemic cells, certain B-cells, or activated T-cells involved in a disease state, yet allow the retention of mature normal T-cells and their precursors to ensure the capability of mounting a normal T-cell immune response as needed. In general, most other T-cell specific agents can destroy essentially all peripheral T-cells, which limits the

agents' therapeutic efficacy. Overall, the use of appropriate monoclonal antibodies specific for the IL-2 receptor may have therapeutic utility in autoimmune diseases, organ transplantation and any unwanted response by activated T-cells. Indeed, clinical trials have been initiated using, e.g., anti-Tac antibodies (see, generally, Waldman, T., et al., Cancer Res. 45:625 (1985) and Waldman, T., Science 232:727-732 (1986), both of which are incorporated herein by reference).

Unfortunately, the use of the anti-Tac and other non-human monoclonal antibodies have certain drawbacks, particularly in repeated therapeutic regimens as explained below. Mouse monoclonal antibodies, for example, do not fix human complement well, and lack other important immunoglobulin functional characteristics when used in humans.

Perhaps more importantly, anti-Tac and other non-human monoclonal antibodies contain substantial stretches of amino acid sequences that will be immunogenic when injected into a human patient. Numerous studies have shown that, after injection of a foreign antibody, the immune response elicited by a patient against an antibody can be quite strong, essentially eliminating the antibody's therapeutic utility after an initial treatment. Moreover, as increasing numbers of different mouse or other antigenic (to humans) monoclonal antibodies can be expected to be developed to treat various diseases, after the first and second treatments with any different non-human antibodies, subsequent treatments even for unrelated therapies can be ineffective or even dangerous in themselves.

While the production of so-called "chimeric antibodies" (e.g., mouse variable regions joined to human constant regions) has proven somewhat successful, a significant immunogenicity problem remains. In general, the production of human immunoglobulins reactive with the human IL-2 receptor, as with many human antigens, has been extremely difficult using typical human monoclonal antibody production techniques. Similarly, utilizing recombinant DNA

technology to produce so-called "humanized" antibodies (see, e.g., EPO Publication No. 0239400), provides uncertain results, in part due to unpredictable binding affinities.

Thus, there is a need for improved forms of human-like immunoglobulins specific for the human IL-2 receptor that are substantially non-immunogenic in humans, yet easily and economically produced in a manner suitable for therapeutic formulation and other uses. The present invention fulfills these and other needs.

Summary of the Invention

The present invention provides novel compositions useful, for example, in the treatment of T-cell mediated human disorders, the compositions containing human-like immunoglobulins specifically capable of blocking the binding of human IL-2 to its receptor and/or capable of binding to the p55 Tac protein on human IL-2 receptors. The immunoglobulins can have two pairs of light chain/heavy chain complexes, typically at least one pair having chains comprising mouse complementarity determining regions functionally joined to human framework region segments. For example, mouse complementarity determining regions, with or without additional naturally-associated mouse amino acid residues, can be used to produce human-like antibodies capable of binding to the human IL-2 receptor at affinity levels stronger than about 10^8 M^{-1}.

The immunoglobulins, including binding fragments and other derivatives thereof, of the present invention may be produced readily by a variety of recombinant DNA techniques, with ultimate expression in transfected cells, preferably immortalized eukaryotic cells, such as myeloma or hybridoma cells. Polynucleotides comprising a first sequence coding for human-like immunoglobulin framework regions and a second sequence set coding for the desired immunoglobulin complementarity determining regions can be produced synthetically or by combining appropriate cDNA and genomic DNA segments.

The human-like immunoglobulins may be utilized alone in substantially pure form, or complexed with a cytotoxic agent, such as a radionuclide, a ribosomal inhibiting protein or a cytotoxic agent active at cell surfaces. All of these compounds will be particularly useful in treating T-cell mediated disorders. The human-like immunoglobulins or their complexes can be prepared in a pharmaceutically accepted dosage form, which will vary depending on the mode of administration.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Comparison of sequences of anti-Tac heavy chain (upper lines) and Eu heavy chain (lower lines). The 1-letter code for amino acids is used. The first amino acid on each line is numbered at the left. Identical amino acids in the two sequences are connected by lines. The 3 CDRs are underlined. Other amino acid positions for which the anti-Tac amino acid rather than the Eu amino acid was used in the humanized anti-Tac heavy chain are denoted by an *.

Figure 2. Comparison of sequences of anti-Tac light chain (upper lines) and Eu light chain (lower lines). The single-letter code for amino acids is used. The first amino acid on each line is numbered at the left. Identical amino acids in the two sequences are connected by lines. The 3 CDRs are underlined. Other amino acid positions for which the anti-Tac amino acid rather than the Eu amino acid was used in the humanized anti-Tac heavy chain are denoted by an *.

Figure 3. Nucleotide sequence of the gene for the humanized anti-Tac heavy chain variable region gene. The translated amino acid sequence for the part of the gene encoding protein is shown underneath the nucleotide sequence. The nucleotides TCTAGA at the beginning and end of the gene are Xba I sites. The mature heavy chain sequence begins with amino acid #20 Q.

Figure 4. Nucleotide sequence of the gene for the humanized anti-Tac light chain variable region gene. The translated amino acid sequence for the part of the gene encoding protein is shown underneath the nucleotide sequence. The nucleotides TCTAGA at the beginning and end of the gene are Xba I sites. The mature light chain sequence begins with amino acid #21 D.

Figure 5. A. Sequences of the four oligonucleotides used to synthesize the humanized anti-Tac heavy chain gene, printed 5' to 3'. B. Relative positions of the oligonucleotides. The arrows point in the 3' direction for each oligonucleotide.

Figure 6. (A) Sequences of the four oligonucleotides used to synchesize the humanized anti-Tac light chain gene, printed 5' to 3'. (B) Relative positions of the oligonucleotides. The arrows point in the 3' direction for each oligonucleotide. The position of a Hind III site in the overlap of JFD2 and JFD3 is shown.

Figure 7. Schematic diagram of the plasmid pHuGTAC1 used to express the humanized anti-Tac heavy chain. Relevant restriction sites are shown, and coding regions of the heavy chain are displayed as boxes. The direction of transcription from the immunoglobulin (Ig) promoter is shown by an arrow. E_H = heavy chain enhancer, Hyg = hygromycin resistance gene.

Figure 8. Schematic diagram of the plasmid pHuLTAC used to express the humanized anti-Tac light chain. Relevant restriction sites are shown, and coding regions of the light chain are displayed as boxes. The direction of transcription from the Ig promoter is shown by an arrow.

Figure 9. Fluorocytometry of HUT-102 and Jurkat cells stained with anti-Tac antibody or humanized anti-Tac antibody followed respectively by fluorescein-conjugated goat anti-mouse Ig antibody or goat anti-human Ig antibody, as labeled. In each panel, the dotted curve shows the results when the first antibody was omitted, and the solid curve the results when first and second (conjugated) antibodies were included as described.

Figure 10. (A) Fluorocytometry of HUT-102 cells stained with 0-40 ng of anti-Tac as indicated, then with biotinylated anti-Tac, and then with phycoerythrin-conjugated avidin. (B) Fluorocytometry of HUT-102 cells stained with the indicated antibody, then with biotinylated anti-Tac, and then with phycoerythrin-conjugated avidin.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, human-like immunoglobulins specifically reactive with the IL-2 receptor on human T-cells are provided. These immunoglobulins, which have binding affinities of at least about 10^8 M^{-1}, and preferably 10^9 M^{-1} to 10^{10} M^{-1} or stronger, are capable of, e.g., blocking the binding of IL-2 to human IL-2 receptors. The human-like immunoglobulins will have a human-like framework and can have complementarity determining regions (CDR's) from an immunoglobulin, typically a mouse immunoglobulin, specifically reactive with an epitope on p55 Tac protein. The immunoglobulins of the present invention, which can be produced economically in large quantities, find use, for example, in the treatment of T-cell mediated disorders in human patients by a variety of techniques.

The basic antibody structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25kD) and one "heavy" chain (about 50-70kD). The NH_2-terminus of each chain begins a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The COOH terminus of each chain defines a constant region primarily responsible for effector function.

Light chains are classified as either kappa or lambda. Heavy chains are classified (and subclassified) as gamma, mu, alpha, delta, or epsilon, and define the antibody's isotype as IgG, IgM, IgA, IgD and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 12 more amino acids. (See, generally, Fundamental Immunology, Paul, W., Ed., Chapter 7, pgs. 131-166, Raven Press, N.Y. (1984), which is incorporated herein by reference.)

The variable regions of each light/heavy chain pair form the antibody binding site. The chains all exhibit the same general structure of relatively conserved framework

regions joined by three hypervariable regions, also called CDR's (see, "Sequences of Proteins of Immunological Interest," Kabat, E., et al., U.S. Department of Health and Human Services, (1983); and Cholthia and Lesk, J. Mol. Biol., 196:901-917 (1987), which are incorporated herein by reference). The CDR's from the two chains of each pair are aligned by the framework regions, enabling binding to a specific epitope.

As used herein, the term "immunoglobulin" refers to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes. The immunoglobulins may exist in a variety of forms besides antibodies; including, for example, Fv, Fab, and $F(ab)_2$, as well as in single chains (e.g., Huston, et al., Proc. Nat. Acad. Sci. U.S.A., 85:5879-5883 (1988) and Bird, et al., Science, 242:423-426 (1988), which are incorporated herein by reference). (See, generally, Hood, et al., "Immunology", Benjamin, N.Y., 2nd ed. (1984), and Hunkapiller and Hood, Nature, 323:15-16 (1986), which are incorporated herein by reference).

Chimeric antibodies are antibodies whose light and heavy chain genes have been constructed, typically by genetic engineering, from immunoglobulin gene segments belonging to different species. For example, the variable (V) segments of the genes from a mouse monoclonal antibody may be joined to human constant (C) segments, such as γ_1 and γ_3. A typical therapeutic chimeric antibody is thus a hybrid protein consisting of the V or antigen-binding domain from a mouse antibody and the C or effector domain from a human antibody (e.g., A.T.C.C. Accession No. CRL 9688 secretes an anti-Tac chimeric antibody), although other mammalian species may be used.

As used herein, the term "framework region" refers to those portions of immunoglobulin light and heavy chain variable regions that are relatively conserved (i.e., other

than the CDR's) among different immunoglobulins in a single species, as defined by Kabat, et al., op. cit. As used herein, a "human-like framework region" is a framework region that in each existing chain comprises at least about 70 or more amino acid residues, typically 75 to 85 or more residues, identical to those in human immunoglobulins.

As used herein, the term "human-like immunoglobulin" refers to an immunoglobulin comprising a human-like framework and in which any constant region present is substantially homologous to a human immunoglobulin constant region, i.e., at least about 85-90%, preferably about 95% identical. Hence, all parts of a human-like immunoglobulin, except possibly the CDR's, are substantially homologous to corresponding parts of one or more native human immunoglobulin sequences. For example, a human-like immunoglobulin would not encompass a chimeric mouse variable region/human constant region antibody.

Human-like antibodies have at least three potential advantages over mouse or and in some cases chimeric antibodies for use in human therapy:

1) because the effector portion is human, it may interact better with the other parts of the human immune system (e.g., destroy the target cells more efficiently by complement-dependent cytotoxicity (CDC) or antibody-dependent cellular cytotoxicity (ADCC)).

2) The human immune system should not recognize the framework or C region of the human-like antibody as foreign, and therefore the antibody response against such an injected antibody should be less than against a totally foreign mouse antibody or a partially foreign chimeric antibody.

3) Injected mouse antibodies have been reported to have a half-life in the human circulation much shorter than the half-life of normal antibodies (Shaw, D., et al., J. Immunol. 138:4534-4538 (1987)). Injected human-like

antibodies will presumably have a half-life essentially identical to naturally occurring human antibodies, allowing smaller and less frequent doses to be given.

In one aspect, the present invention is directed to recombinant DNA segments encoding the heavy and/or light chain CDR's from an immunoglobulin capable of binding to a desired epitope on the human IL-2 receptor, such as the anti-Tac monoclonal antibody. The DNA segments encoding these regions will typically be joined to DNA segments encoding appropriate human-like framework regions. The preferred DNA sequences, which on expression code for the polypeptide chains comprising the anti-Tac heavy and light chain hypervariable regions (with human-like framework regions), are shown in Figures 1 and 2, respectively. Due to codon degeneracy and non-critical amino-acid substitutions, other DNA sequences can be readily substituted for those sequences, as detailed below.

The DNA segments will typically further include an expression control DNA sequence operably linked to the human-like antibody coding sequences, including naturally-associated or heterologous promoter regions. Preferably, the expression control sequences will be eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells, but control sequences for prokaryotic hosts may also be used. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences, and, as desired, the collection and purification of the light chains, heavy chains, light/heavy chain dimers or intact antibodies, binding fragments or other immunoglobulin forms may follow.

Human constant region DNA sequences can be isolated in accordance with well known procedures from a variety of human cells, but preferably immortalized B-cells (see, Kabat op. cit. and WP87/02671). The CDR's for producing the immunoglobulins of the present invention will be similarly derived from monoclonal antibodies capable of binding to the

human IL-2 receptor and produced in any convenient mammalian source, including, mice, rats, rabbits, or other veterbrate capable of producing antibodies by well known methods. Suitable source cells for the DNA sequences and host cells for immunoglobulin expression and secretion can be obtained from a number of sources, such as the American Type Culture Collection ("Catalogue of Cell Lines and Hybridomas," Fifth edition (1985) Rockville, Maryland, U.S.A., which is incorporated herein by reference).

In addition to the human-like immunoglobulins specifically described herein, other "substantially homologous" modified immunoglobulins can be readily designed and manufactured utilizing various recombinant DNA techniques well known to those skilled in the art. For example, the framework regions can vary from the sequences in Figures 3 and 4 at the primary structure level by several amino acid substitutions, terminal and intermediate additions and deletions, and the like. Moreover, a variety of different human framework regions may be used singly or in combination as a basis for the human-like immunoglobulins of the present invention. In general, modifications of the genes may be readily accomplished by a variety of well-known techniques, such as site-directed mutagenesis (see, Gillman and Smith, Gene 8:81-97 (1979) and Roberts, S. et al, Nature 328:731-734 (1987), both of which are incorporated herein by reference).

Alternatively, polypeptide fragments comprising only a portion of the primary antibody structure may be produced, which fragments possess one or more immunoglobulin activities (e.g., complement fixation activity). Also because like many genes, the immunoglobulin-related genes contain separate functional regions, each having one or more distinct biological activities, the genes may be fused to functional regions from other genes (e.g., enzymes, see, commonly assigned U.S.S.N. 132,387, filed Dec. 15, 1987, which is incorporated herein by reference) to produce fusion proteins (e.g., immunotoxins) having novel properties.

The nucleic acid sequences of the present invention capable of ultimately expressing the desired human-like antibodies can be formed from a variety of different polynucleotides (genomic or cDNA, RNA, synthetic oligonucleotides, etc.) and components (e.g., V, J, D, and C regions), as well as by a variety of different techniques. Joining appropriate genomic sequences is presently the most common method of production, but cDNA sequences may also be utilized (see, European Patent Publication No. 0239400 and Reichmann, L., et al., Nature 332:323-327 (1988), both of which are incorporated herein by reference).

As stated previously, the DNA sequences will be expressed in hosts after the sequences have been operably linked to (i.e., positioned to ensure the functioning of) an expression control sequence. These expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression vectors will contain selection markers, e.g., tetracycline or neomycin, to permit detection of those cells transformed with the desired DNA sequences (see, e.g., U.S. Patent 4,704,362, which is incorporated herein by reference).

E. coli is one prokaryotic host useful particularly for cloning the DNA sequences of the present invention. Other microbial hosts suitable for use include bacilli, such as Bacillus subtilus, and other enterobacteriaceae, such as Salmonella, Serratia, and various Pseudomonas species. In these prokaryotic hosts, one can also make expression vectors, which will typically contain expression control sequences compatible with the host cell (e.g., an origin of replication). In addition, any number of a variety of well-known promoters will be present, such as the lactose promoter system, a tryptophan (trp) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. The promoters will typically control expression, optionally with an operator sequence, and have ribosome binding site sequences and the like, for initiating and completing transcription and translation.

Other microbes, such as yeast, may also be used for expression. Saccharomyces is a preferred host, with suitable vectors having expression control sequences, such as promoters, including 3-phosphoglycerate kinase or other glycolytic enzymes, and an origin of replication, termination sequences and the like as desired.

In addition to microorganisms, mammalian tissue cell culture may also be used to express and produce the polypeptides of the present invention (see, Winnacker, "From Genes to Clones," VCH Publishers, N.Y., N.Y. (1987), which is incorporated herein by reference). Eukaryotic cells are actually preferred, because a number of suitable host cell lines capable of secreting intact immunoglobulins have been developed in the art, and include the CHO cell lines, various COS cell lines, HeLa cells, myeloma cell lines, etc, but preferably transformed B-cells or hybridomas. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter, an enhancer (Queen, C., et al., Immunol. Rev. 89:49-68 (1986), which is incorporated herein by reference), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Preferred expression control sequences are promoters derived from immunoglobulin genes, SV40, Adenovirus, Bovine Papilloma Virus, and the like.

The vectors containing the DNA segments of interest (e.g., the heavy and light chain encoding sequences and expression control sequences) can be transferred into the host cell by well-known methods, which vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment or electroporation may be used for other cellular hosts. (See, generally, Maniatis, et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, (1982), which is incorporated herein by reference.)

Once expressed, the whole antibodies, their dimers, individual light and heavy chains, or other immunoglobulin

forms of the present invention can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like (see, generally, Scopes, R., Protein Purification, Springer-Verlag, N.Y. (1982)). Substantially pure immunoglobulins of at least about 90 to 95% homogeneity are preferred, and 98 to 99% or more homogeneity most preferred, for pharmaceutical uses. Once purified, partially or to homogeneity as desired, the polypeptides may then be used therapeutically (including extracorporeally) or in developing and performing assay procedures, immunofluorescent stainings, and the like. (See, generally, Immunological Methods, Vols. I and II, Lefkovits and Pernis, eds., Academic Press, New York, N.Y. (1979 and 1981)).

The antibodies of the present invention will typically find use individually in treating a T-cell mediated disease state. Generally, where the cell linked to a disease has been identified as IL-2 receptor bearing, then the human-like antibodies capable of blocking the binding of IL-2 to the human IL-2 receptor are suitable (see, U.S.S.N. 085,707, entitled "Treating Human Malignancies and Disorders," which is incorporated herein by reference).

For example, typical disease states suitable for treatment include graft versus host disease and transplant rejection in patients undergoing an organ transplant, such as heart, lungs, kidneys, liver, etc. Other diseases include autoimmune diseases, such as Type I diabetes, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, and myasthenia gravis.

The human-like antibodies of the present invention may also be used in combination with other antibodies, particularly human monoclonal antibodies reactive with other markers on cells responsible for the disease. For example, suitable T-cell markers can include those grouped into the so-called "Clusters of Differentiation," as named by the First International Leukocyte Differentiation Workshop,

Leukocyte Typing, Bernard, et al., Eds., Springer-Verlag, N.Y. (1984), which is incorporated herein by reference.

The antibodies can also be used as separately administered compositions given in conjunction with chemotherapeutic or immunosuppressive agents. Typically, the agents will include cyclosporin A or a purine analog (e.g., methotrexate, 6-mercaptopurine, or the like), but numerous additional agents (e.g., cyclophosphamide, prednisone, etc.) well-known to those skilled in the art may also be utilized.

A preferred pharmaceutical composition of the present invention comprises the use of the subject antibodies in immunotoxins. Immunotoxins are characterized by two components and are particularly useful for killing selected cells in vitro or in vivo. One component is a cytotoxic agent which is usually fatal to a cell when attached or absorbed. The second component, known as the "delivery vehicle," provides a means for delivering the toxic agent to a particular cell type, such as cells comprising a carcinoma. The two components are commonly chemically bonded together by any of a variety of well-known chemical procedures. For example, when the cytotoxic agent is a protein and the second component is an intact immunoglobulin, the linkage may be by way of heterobifunctional cross-linkers, e.g., SPDP, carbodiimide, glutaraldehyde, or the like. Production of various immunotoxins is well-known with the art, and can be found, for example in "Monoclonal Antibody-Toxin Conjugates: Aiming the Magic Bullet," Thorpe et al, Monoclonal Antibodies in Clinical Medicine, Academic Press, pp. 168-190 (1982), which is incorporated herein by reference.

A variety of cytotoxic agents are suitable for use in immunotoxins. Cytotoxic agents can include radionuclides, such as Iodine-131, Yttrium-90, Rhenium-188, and Bismuth-212; a number of chemotherapeutic drugs, such as vindesine, methotrexate, adriamycin, and cisplatinm; and cytotoxic proteins such as ribosomal inhibiting proteins like pokeweed antiviral protein, Pseudomonas exotoxin A, ricin, diphtheria toxin, ricin A chain, etc., or an agent active at the cell surface, such as the phospholipase enzymes (e.g.,

phospholipase C). (See, generally, commonly assigned U.S.S.N. _____ (Townsend and Townsend Docket No. 11823-7-2) filed concurrently herewith, "Chimeric Toxins," Olsnes and Phil, Pharmac. Ther., 25:355-381 (1982), and "Monoclonal Antibodies for Cancer Detection and Therapy," eds. Baldwin and Byers, pp. 159-179, 224-266, Academic Press (1985), all of which are incorporated herein by reference.)

The delivery component of the immunotoxin will include the human-like immunoglobulins of the present invention. Intact immunoglobulins or their binding fragments, such as Fab, are preferably used. Typically, the antibodies in the immunotoxins will be of the human IgM or IgG isotype, but other mammalian constant regions may be utilized as desired.

The human-like antibodies and pharmaceutical compositions thereof of this invention are particularly useful for parenteral administration, i.e., subcutaneously, intramuscularly or intravenously. The compositions for parenteral administration will commonly comprise a solution of the antibody or a cocktail thereof dissolved in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used, e.g., water, buffered water, 0.4% saline, 0.3% glycine and the like. These solutions are sterile and generally free of particulate matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, etc. The concentration of antibody in these formulations can vary widely, i.e., from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

Thus, a typical pharmaceutical composition for intramuscular injection could be made up to contain 1 ml sterile buffered water, and 50 mg of antibody. A typical composition for intravenous infusion could be made up to contain 250 ml of sterile Ringer's solution, and 150 mg of antibody. Actual methods for preparing parenterally administrable compositions will be known or apparent to those skilled in the art and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pennsylvania (1980), which is incorporated herein by reference.

The antibodies of this invention can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional immune globulins and art-known lyophilization and reconstitution techniques can be employed. It will be appreciated by those skilled in the art that lyophilization and reconstitution can lead to varying degrees of antibody activity loss (e.g., with conventional immune globulins, IgM antibodies tend to have greater activity loss than IgG antibodies) and that use levels may have to be adjusted to compensate.

The compositions containing the present human-like antibodies or a cocktail thereof can be administered for prophylactic and/or therapeutic treatments. In therapeutic application, compositions are administered to a patient already suffering from a disease, in an amount sufficient to cure or at least partially arrest the disease and its complications. An amount adequate to accomplish this is defined as a "therapeutically effective dose." Amounts effective for this use will depend upon the severity of the infection and the general state of the patient's own immune system, but generally range from about 1 to about 200 mg of antibody per dose, with dosages of from 5 to 25 mg per patient being more commonly used. It must be kept in mind that the materials of this invention may generally be employed in serious disease states, that is life-threatening or potentially life-threatening situations. In such cases,

in view of the minimization of extraneous substances and the lower probability of "foreign substance" rejections which are achieved by the present human-like antibodies of this invention, it is possible and may be felt desirable by the treating physician to administer substantial excesses of these antibodies.

In prophylactic applications, compositions containing the present antibodies or a cocktail thereof are administered to a patient not already in a disease state to enhance the patient's resistance. Such an amount is defined to be a "prophylactically effective dose." In this use, the precise amounts again depend upon the patient's state of health and general level of immunity, but generally range from 0.1 to 25 mg per dose, especially 0.5 to 2.5 mg per patient. A preferred prophylactic use is for the prevention of kidney transplant rejection.

Single or multiple administrations of the compositions can be carried out with dose levels and pattern being selected by the treating physician. In any event, the pharmaceutical formulations should provide a quantity of the antibody(ies) of this invention sufficient to effectively treat the patient.

Human-like antibodies of the present invention can further find a wide variety of utilities <u>in vitro</u>. By way of example, the antibodies can be utilized for T-cell typing, for isolating specific IL-2 receptor bearing cells or fragments of the receptor, for vaccine preparation, or the like.

For diagnostic purposes, the antibodies may either be labeled or unlabeled. Unlabeled antibodies can be used in combination with other labeled antibodies (second antibodies) that are reactive with the human-like antibody, such as antibodies specific for human immunoglobulin constant regions. Alternatively, the antibodies can be directly labeled. A wide variety of labels may be employed, such as radionuclides, fluors, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, ligands (particularly haptens),

etc. Numerous types of immunoassays are available and are well known to those skilled in the art.

Kits can also be supplied for use with the subject antibodies in the protection against or detection of a cellular activity or for the presence of a selected antigen. Thus, the subject antibody composition of the present invention may be provided, usually in a lyophilized form in a container, either alone or in conjunction with additional antibodies specific for the desired cell type. The antibodies, which may be conjugated to a label or toxin, or unconjugated, are included in the kits with buffers, such as Tris, phosphate, carbonate, etc., stabilizers, biocides, inert proteins, e.g., serum albumin, or the like, and a set of instructions for use. Generally, these materials will be present in less than about 5% wt. based on the amount of active antibody, and usually present in total amount of at least about 0.001% wt. based again on the antibody concentration. Frequently, it will be desirable to include an inert extender or excipient to dilute the active ingredients, where the excipient may be present in from about 1 to 99% wt. of the total composition. Where a second antibody capable of binding to the chimeric antibody is employed in an assay, this will usually be present in a separate vial. The second antibody is typically conjugated to a label and formulated in an analogous manner with the antibody formulations described above.

The following examples are offered by way of illustration, not by limitation.

EXPERIMENTAL

Design of genes for human-like light and heavy chains

The sequence of the human antibody Eu (Sequences of Proteins of Immunological Interest, Kabat, E., et al., U.S. Dept. of Health and Human Services, 1983) was used to provide the framework of the humanized antibody, because the amino acid sequence of the heavy chain of anti-Tac is more homologous to the heavy chain of this antibody than to any other heavy chain sequence in the National Biomedical Foundation Protein Identification Resource.

To select the sequence of the humanized heavy chain, the anti-Tac heavy chain sequence (see, commonly assigned U.S.S.N.'s 186,862 and 223,037, which are incorporated herein by reference) was aligned with the sequence of the Eu heavy chain (Figure 1). At each position, the Eu amino acid was selected for the humanized sequence, unless that position fell in any one of the following categories, in which case the anti-Tac amino acid was selected.

(1) The position fell within a complementarity determining region (CDR), as defined by Kabat, et al., op. cit. (amino acids 31-35, 50-66, 99-106);

(2) The Eu amino acid was unusual for human heavy chains at that position, whereas the anti-Tac amino acid was typical for human heavy chains at that position (amino acids 27, 93, 95, 98, 107-109, 111);

(3) The position was immediately adjacent to a CDR in the amino acid sequence of the anti-Tac heavy chain (amino acids 30 and 67).

(4) 3-dimensional modeling of the anti-Tac antibody suggested that the amino acid was physically close to the antigen binding region (amino acids 48 and 68).

Some amino acids fell in more than one of these categories but are only listed in one.

To select the sequence of the humanized light chain, the anti-Tac light chain sequence was aligned with the sequence of the Eu light chain (Figure 2). The Eu amino acid was selected at each position, unless the position again fell into one of the categories (1) - (4), (with light chain replacing heavy chain in the category definitions):

(1) CDRs (amino acids 24-34, 50-56, 89-97).

(2) Anti-Tac amino acid more typical than Eu (amino acids 48 and 63).

(3) Adjacent to CDRs (no amino acids; Eu and anti-Tac were already the same at all these positions).

(4) Possible 3-dimensional proximity to binding region (amino acid 60).

The actual nucleotide sequence of the heavy (Figure 3) and light chain (Figure 4) genes were selected as follows:

(1) the nucleotide sequences code for the amino acid sequences chosen as described above.

(2) 5' of these coding sequences, the nucleotide sequences code for a leader (signal) sequence, namely the leader of the light chain of the antibody MOPC 63 and the leader of the heavy chain of the antibody PCH 108A (Kabat et al., op. cit.). These leader sequences were chosen as typical of antibodies.

(3) 3' of the coding sequences, the nucleotide sequences are the sequences that follow the mouse light chain J5 segment and the mouse heavy chain J2 segment, which are part of the anti-Tac sequences. These sequences are included because they contain splice donor signals.

(4) At each end of the sequence is an Xba I site to allow cutting at the Xba I sites and cloning into the Xba I site of a vector.

Construction of humanized light and heavy chain genes

To synthesize the heavy chain, four oligonucleotides HES12, HES13, HES14, HES15 (Figure 5A) were synthesized using an Applied Biosystems 380B DNA synthesizer. Two of the oligonucleotides are part of each strand of the heavy chain, and each oligonucleotide overlaps the next one by about 20 nucleotides to allow annealing (Figure 5B). Together, the oligonucleotides cover the entire humanized heavy chain (Figure 3) with a few extra nucleotides at each end to allow cutting at the Xba I sites. The oligonucleotides were purified from polyacrylamide gels.

Each oligonucleotide was phosphorylated using ATP and T4 polynucleotide kinase by standard procedures (see, Maniatis, op. cit.). To anneal the phosphorylated oligonucleotides, they were suspended together in 40 ul of TA (33 mM Tris acetate, pH 7.9, 66 mM potassium acetate, 10 mM magnesium acetate) at a concentration of about 3.75 uM each, heated to 95 deg for 4 min. and cooled slowly to 4 deg. To synthesize the complete gene from the oligonucleotides by synthesizing the opposite strand of each oligonucleotide (Figure 5B), the following components were added in a final volume of 100ul:

10 ul	annealed oligonucleotides
0.16 mM each	deoxyribonucleotide
0.5 mM	ATP
0.5 mM	DTT
100 ug/ml	BSA
3.5 ug/ml	T4 g43 protein (DNA polymerase)
25 ug/ml	T4 g44/62 protein (polymerase accessory protein)
25 ug/ml	45 protein (polymerase accessory protein)

The mixture was incubated at 37 deg for 30 min. Then 10 u of T4 DNA ligase was added and incubation at 37 deg resumed for 30 min. The polymerase and ligase were inactivated by incubation of the reaction at 70 deg for

15 min. To digest the gene with Xba I, to the reaction was added 50 ul of 2x TA containing BSA at 200 ug/ml and DTT at 1 mM, 43 ul of water, and 50 u of Xba I in 5 ul. The reaction was incubated for 3 hr at 37 deg, and run on a gel. The 431 bp Xba I fragment was purified from a gel and cloned into the Xba I site of the plasmid pUC19 by standard methods. Four plasmid isolates were purified and sequenced using the dideoxy method. One of these had the correct sequence (Figure 3).

To synthesize the light chain, four oligonucleotides JFD1, JFD2, JFD3, JFD4 (Figure 6A) were synthesized. Two of the oligonucleotides are part of each strand of the light chain, and each oligonucleotide overlaps the next one by about 20 nucleotides to allow annealing (Figure 6B). Together, the oligonucleotides cover the entire humanized light chain (Figure 4) with a few extra nucleotides at each end to allow cutting at the Xba I sites. The oligonucleotides were purified from polyacrylamide gels.

The light chain gene was synthesized from these olignucleotides in two parts. 0.5 ug each of JFD1 and JFD2 were combined in 20 ul sequenase buffer (40 mM Tris-HCl, pH 7.5, 20 mM magnesium chloride, 50 mM sodium chloride), heated at 70 deg for 3 min and allowed to cool slowly to 23 deg in order for the oligonucleotides to anneal. JFD3 and JFD4 were treated in the same way. Each reaction was made 10 mM in DTT and 0.5 mM in each deoxyribonucleotide and 6.5 u of sequenase (US Biochemicals) was added, in a final volume of 24 ul, and incubated for 1 hr at 37 deg to synthesize the opposite strands of the oligonucleotides. Xba I and Hind III were added to each reaction to digest the DNA (there is a Hind III site in the region where JFD2 and JFD3 overlap and therefore in each of the synthesized DNAs; Figure 6B). The reactions were run on polyacrylamide gels, and the Xba I - Hind III fragments were purified and cloned into pUC18 by standard methods. Several plasmid isolates for each fragment were sequenced by the dideoxy method, and correct ones chosen.

Construction of plasmids to express humanized light and heavy chains

The heavy chain Xba I fragment was isolated from the pUC19 plasmid in which it had been inserted and then inserted into the Xba I site of the vector pVγ1 (see, commonly assigned U.S.S.N. 223,037) in the correct orientation by standard methods, to produce the plasmid pHuGTAC1 (Figure 7). This plasmid will express high levels of a complete heavy chain when transfected into an appropriate host cell.

The two light chain Xba I - Hind III fragments were isolated from the pUC18 plasmids in which they had been inserted. The vector plasmid pVκ1 (see, commonly assigned U.S.S.N. 223,037) was cut with Xba I, dephosphorylated and ligated with the two fragments by standard methods. The desired reaction product has the circular form: vector - Xba I - fragment 1 - Hind III - fragment 2 - Xba I - vector. Several plasmid isolates were analyzed by restriction mapping and sequencing, and one with this form chosen. This plasmid, pHuLTAC (Figure 8), therefore contains the complete humanized light chain (Figure 4) and will express high levels of the light chain when transfected into an appropriate host cell.

Synthesis and affinity of humanized antibody

The plasmids pHuGTAC1 and pHuLTAC were transfected into mouse Sp2/0 cells, and cells that integrated the plasmids were selected on the basis of resistance to mycophenolic acid and/or hygromycin B conferred by the gpt and hyg genes on the plasmids (Figures 7,8) by standard methods. To verify that these cells secreted antibody that binds to the IL-2 receptor, supernatant from the cells was incubated with HUT-102 cells that are known to express the IL-2 receptor. After washing, the cells were incubated with fluorescein-conjugated goat anti-human antibody, washed, and analyzed for fluorescence on a FACSCAN cytofluorometer. The results (Figure 9A), clearly show that the humanized antibody binds to these cells, but not to Jurkat T-cells that do not express the IL-2 receptor (Figure 9D). As controls, the

original mouse anti-Tac antibody was also used to stain these cells (Figure 9B,C), giving similar results.

For further experiments, cells producing the humanized antibody were injected into mice, and the resultant ascites collected. Humanized antibody was purified to substantial homogeneity from the ascites by passage through an affinity column of goat anti-human immunoglobulin antibody, prepared on an Affigel-10 support (Bio-Rad Laboratories, Inc., Richmond, CA) according to standard techniques. To determine the affinity of the humanized antibody relative to the original anti-Tac antibody, a competitive binding experiment was performed. About 5×10^5 HUT-102 cells were incubated with known quantities (10 - 40 ng) of the anti-Tac antibody and the humanized anti-Tac antibody for 10 min at 4 deg. Then 100 ng of biotinylated anti-Tac was added to the cells and incubated for 30 min at 4 deg. This quantity of anti-Tac had previously been determined to be sufficient to saturate the binding sites on the cells, but not to be in large excess. Then the cells were washed twice with 2 ml of phosphate buffered saline (PBS) containing 0.1% sodium azide. The cells were then incubated for 30 min at 4 deg with 250 ng of phycoerythrin-conjugated avidin, which bound to the biotinylated anti-Tac already bound to the cells. The cells were washed again as above, fixed in PBS containing 1% paraformaldehyde, and analyzed for fluorescence on a FACSCAN cytofluorometer.

Use of increasing amounts (10 - 40 ng) of the anti-Tac antibody as competitor in the first step decreased the amount of biotinylated anti-Tac that could bind to the cells in the second step, and therefore the amount of phycoerythrin-conjugated avidin that bound in the last step, thus decreasing fluorescence (Figure 10A). Equivalent amounts (20 ng) of anti-Tac, and humanized anti-Tac used as competitor decreased the fluorescence to approximately the same degree (Figure 10B). This shows that these antibodies have approximately the same affinity, because if one had greater affinity, it would have more effectively competed

with the biotinylated anti-Tac, thus decreasing fluorescence more.

Biological properties of the humanized antibody

For optimal use in treatment of human disease, the humanized antibody should be able to destroy T-cells in the body that express the IL-2 receptor. One mechanism by which antibodies may destroy target cells is antibody-dependent cell-mediated cytotoxicity, abbreviated ADCC (Fundamental Immunology, Paul, W., Ed., Raven Press, New York (1984), at pg. 681), in which the antibody forms a bridge between the target cell and an effector cell such as a macrophage that can lyse the target. To determine whether the humanized antibody and the original mouse anti-Tac antibody can mediate ADCC, a chromium release assay was performed by standard methods. Specifically, human leukemia HUT-102 cells, which express the IL-2 receptor, were incubated with ^{51}Cr to allow them to absorb this radionuclide. The HUT-102 cells were then incubated with an excess of either anti-Tac or humanized anti-Tac antibody. The HUT-102 cells were next incubated for 4 hrs with either a 30:1 or 100:1 ratio of effector cells, which were normal purified human peripheral blood mononuclear cells that had been activated by incubation for about 20 hrs with human recombinant IL-2. Release of ^{51}Cr, which indicated lysis of the target HUT-102 cells, was measured and the background subtracted (Table 1). The results show that at either ratio of effector cells, anti-Tac did not lyse a significant number of the target cells (less than 5%), while the humanized antibody did (more than 20%). Hence, the humanized antibody is likely to be more efficacious than the original mouse antibody in treating T-cell leukemia or other T-cell mediated diseases.

TABLE 1

Percent ^{51}Cr release after ADCC

	Effector: Target ratio	
	30:1	100:1
Antibody		
Anti-Tac	4%	< 1%
Humanized anti-Tac	24%	23%

From the foregoing, it will be appreciated that the human-like immunoglobulins of the present invention offer numerous advantages of other human IL-2 receptor-specific antibodies. In comparison to anti-Tac mouse monoclonal antibodies, the present human-like immunoglobulin can be more economically produced and contain substantially less foreign amino acid sequences. This reduced likelihood of antigenicity after injection into a human patient represents a significant therapeutic improvement.

Although the present invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the appended claims.

WE CLAIM:

1. A composition comprising a substantially pure human-like immunoglobulin specifically reactive with p55 Tac protein.

2. A composition according to Claim 1, wherein the immunoglobulin comprises two pairs of light/heavy chain dimers, wherein each chain comprises a variable region and a constant region.

3. A composition according to Claim 2, wherein a variable region of at least one chain comprises at least about 75 amino acids from a human immunoglobulin variable region framework.

4. A composition comprising a substantially pure human-like immunoglobulin capable of inhibiting binding of human interleukin-2 (IL-2) to a human IL-2 receptor.

5. A composition according to Claims 1 or 4, wherein the immunoglobulin exhibits a binding affinity to a human IL-2 receptor of about 10^8 M^{-1} or stronger.

6. A composition according to Claims 1 or 4, wherein the immunoglobulin comprises complementarity determining regions from one immunoglobulin and framework regions from at least one different immunoglobulin.

7. A recombinant immunoglobulin composition comprising a human-like framework and one or more foreign complementarity determining regions not naturally associated with the framework, wherein said immunoglobulin is capable of binding to a human interleukin-2 receptor.

8. A composition according to Claim 7, wherein one or more of the foreign CDR's are substantially homologous to a CDR from an immunoglobulin reactive with human p55 Tac protein.

9. A composition according to Claim 7, wherein all of the foreign CDR's are located on heavy chains of the immunoglobulin.

10. A composition according to Claim 7, wherein the immunoglobulin is an IgG_1 immunoglobulin isotype.

11. A composition according to Claim 7, wherein the mature light and heavy variable region protein sequences are substantially homologous to the sequences in Figures 3 and 4.

12. A human-like immunoglobulin having two pairs of light chain/heavy chain dimers and capable of specifically reacting with an epitope on a human interleukin-2 receptor with an affinity of at least about 10^8 M^{-1}, said light and heavy chains comprising complementarity determining regions (CDR's) and human-like framework regions, wherein the CDR's are from different immunoglobulin molecules than the framework regions.

13. An immunoglobulin according to Claim 12, which binds to an epitope located on a p55 Tac protein.

14. An immunoglobulin according to Claim 12, which is capable of blocking the binding of interleukin-2 (IL-2) to human IL-2 receptors.

15. An immunoglobulin according to Claim 12, wherein the human-like framework regions comprise amino acids sequences from at least two human immunoglobulins.

16. An immunoglobulin according to Claim 12, wherein the CDR's are from a mouse immunoglobulin.

17. A humanized immunoglobulin capable of binding to human interleukin-2 receptors, said immunoglobulin comprising one or more complementarity determining regions (CDR's) from anti-Tac antibody in a human-like framework.

18. A humanized immunoglobulin according to Claim 17, wherein the human framework is substantially homologous to an Eu immunoglobulin framework.

19. A humanized immunoglobulin according to Claim 17, having a mature heavy chain variable sequence as shown in Figure 3, and a mature light chain sequence as shown in Figure 4.

20. A humanized immunoglobulin according to Claim 17 which is capable of blocking the binding of IL-2 to interleukin-2 receptors on human T-cells.

21. A method of treating T-cell mediated disorders in a human patient, said method comprising administering to said patient a therapeutically effective dose of an immunoglobulin according to Claims 1, 5, 12, or 17.

22. An immunoglobulin according to Claims 1, 5, 12, or 17 which was produced in a myeloma or hybridoma cell.

23. A human-like immunoglobulin heavy chain comprising a human-like heavy chain framework region and a hypervariable region which is substantially identical to a monoclonal antibody heavy chain hypervariable region secreted by the cell line designated A.T.C.C. Accession No. CRL 9688.

24. A human-like immunoglobulin light chain comprising a human light chain framework region and a hypervariable region which is substantially identical to a monoclonal antibody light chain hypervariable region secreted by the cell line designated A.T.C.C. Accession No. CRL 9688.

25. A polynucleotide molecule comprising a first sequence coding for human-like immunoglobulin framework regions and a second sequence coding for one or more mouse immunoglobulin complementarity determining regions, wherein upon expression said polynucleotide encodes an immunoglobulin specifically reactive with p55 Tac protein and capable of blocking the binding of interleukin-2 (IL-2) to the IL-2 receptor on human T-cells.

26. A cell line transfected with a polynucleotide of Claim 25.

NOVEL IL-2 RECEPTOR-SPECIFIC HUMAN IMMUNOGLOBULINS

ABSTRACT OF THE DISCLOSURE

Human-like immunoglobulins specifically reactive with human IL-2 receptors are prepared employing recombinant DNA technology for use in, e.g., treatment of T-cell mediated disorders.

ATTORNEY DOCKET NO.

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; I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

NOVEL IL-2 RECEPTOR-SPECIFIC HUMAN IMMUNOGLOBULINS

the specification of which ☒ is attached hereto or ☐ was filed on _____ as Application Serial No. _____ and was amended on _____ (if applicable).

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 Title 37, Code of Federal Regulations, §1.56(a). I claim foreign priority benefits under Title 35, United States Code, §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 the application on which priority is claimed:

Prior Foreign Application(s)

COUNTRY	APPLICATION NUMBER	DATE OF FILING	PRIORITY CLAIMED UNDER 35 U.S.C. 119
			Yes____ No____
			Yes____ No____

I claim the benefit under Title 35, United States Code, §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 Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §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.	DATE OF FILING	STATUS
		☐ Patented ☐ Pending ☐ Abandoned
		☐ Patented ☐ Pending ☐ Abandoned

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) who are partners and associates in the firm of Townsend and Townsend to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. William M. Smith, Reg. No. 30,223
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I further 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 issuing thereon.

Signature of Inventor 201	Signature of Inventor 202	Signature of Inventor 203
	Harold E. Selick	
Date: 12/28/88	Date: 12/28/88	Date:

T&T 14A (12 87)

Atty. Docket [illegible]

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS
(37 CFR 1.9(f) and 1.27(c)) — SMALL BUSINESS CONCERN

Applicant or Patentee: Cary L. Queen and Harold Edwin Selick
Serial No.: _____ Filing Date: December 28, 1988
Patent No.: _____ Issued: _____
For: NOVEL IL-2 RECEPTOR-SPECIFIC HUMAN IMMUNOGLOBULINS

I hereby declare that I am

[ ] the owner of the small business concern identified below:
[X] an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN Protein Design Labs, Inc., a Delaware Corporation
ADDRESS OF CONCERN 3181 Porter Drive
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I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled NOVEL IL-2 RECEPTOR-SPECIFIC HUMAN IMMUNOGLOBULINS by inventor(s) Cary L. Queen and Harold Edwin Selick described in

[X] the application filed herewith
[ ] application serial no. _____, filed _____
[ ] patent no. _____, issued _____

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I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

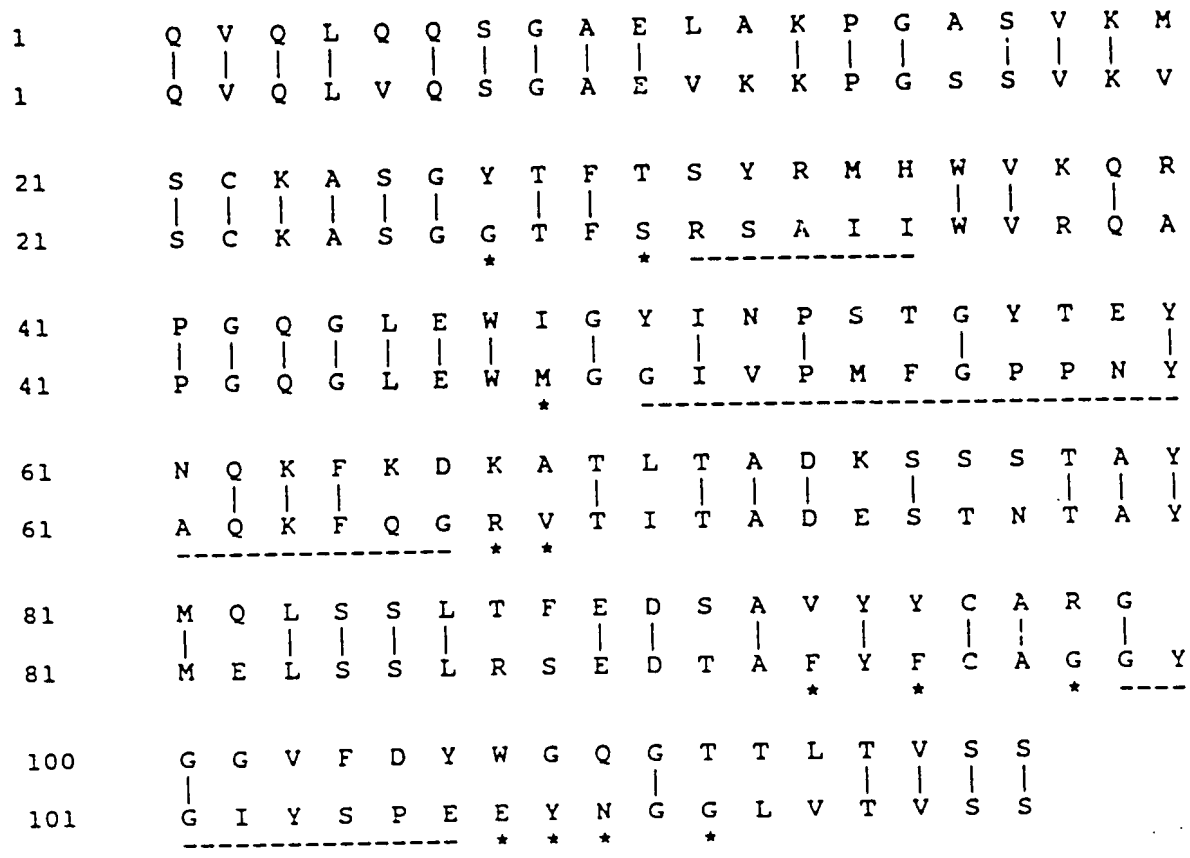
I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, 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, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Laurence Jay Korn
TITLE OF PERSON OTHER THAN OWNER President
ADDRESS OF PERSON SIGNING 3181 Porter Drive, Palo Alto, California 94304

SIGNATURE Laurence Jay Korn DATE 28/12/88

PAT 39

FIGURE 1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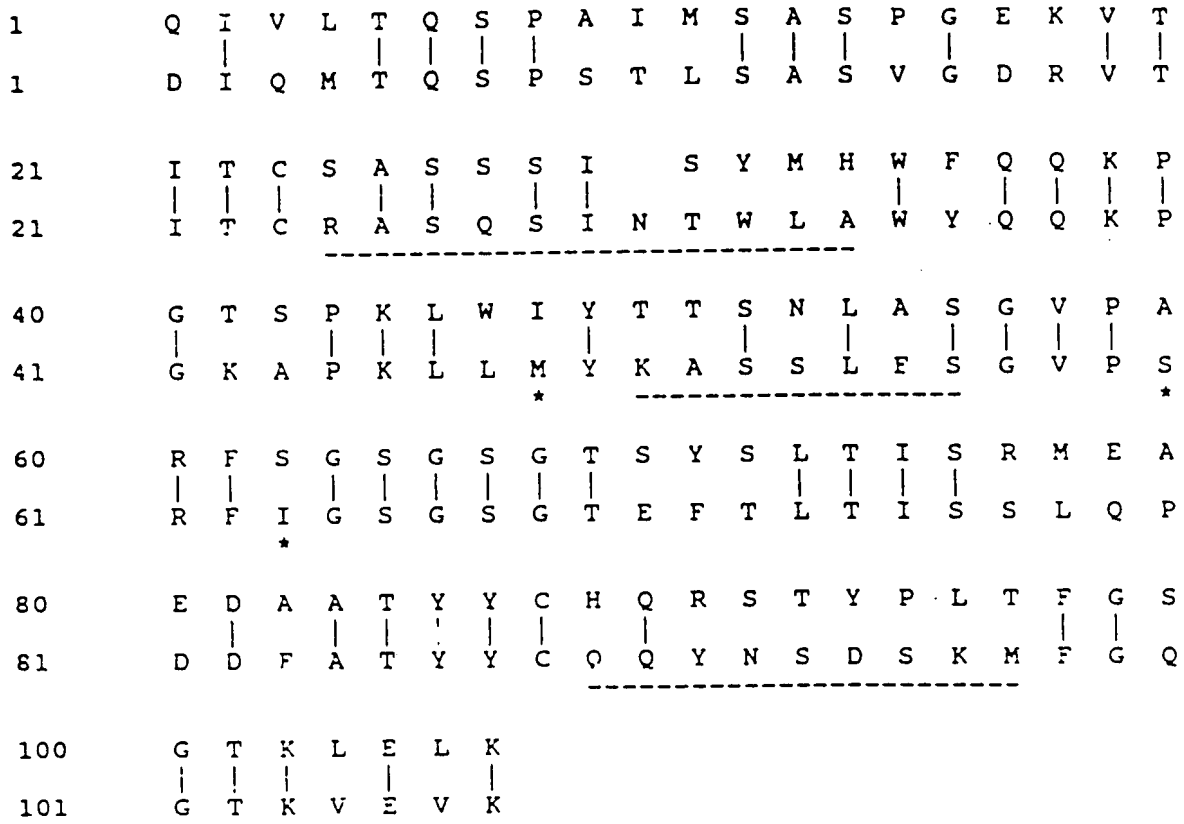
21 S C K A S G G T F S R S A I I W V R Q A
41 P G Q G L E W I G Y I N P S T G Y T E Y
41 P G Q G L E W M G G I V P M F G P P N Y
61 N Q K F K D K A T L T A D K S S S T A Y
61 A Q K F Q G R V T I T A D E S T N T A Y
81 M Q L S S L T F E D S A V Y Y C A R G
81 M E L S S L R S E D T A F Y F C A G G Y
100 G G V F D Y W G Q G T T L T V S S
101 G I Y S P E E Y N G G L V T V S S

FIGURE 2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1 D I Q M T Q S P S T L S A S V G D R V T
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21 I T C R A S Q S I N T W L A W Y Q Q K P
40 G T S P K L W I Y T T S N L A S G V P A
41 G K A P K L L M Y K A S S L E S G V P S
60 R F S G S G S G T S Y S L T I S R M E A
61 R F I G S G S G T E F T L T I S S L Q P
80 E D A A T Y Y C H Q R S T Y P L T F G S
81 D D F A T Y Y C Q Q Y N S D S K M F G Q
100 G T K L E L K
101 G T K V E V K

FIGURE 3

```
        10         20         30         40         50         60
TCTAGATGGGATGGAGCTGGATCTTTCTCTTCCTCCTGTCAGGTACCGCGGGCGTGCACT
      M  G  W  S  W  I  F  L  F  L  L  S  G  T  A  G  V  H

        70         80         90        100        110        120
CTCAGGTCCAGCTTGTCCAGTCTGGGGCTGAACTCAAGAAACCTGGCTCGAGCGTGAAGG
S  Q  V  Q  L  V  Q  S  G  A  E  V  K  K  P  G  S  S  V  K

       130        140        150        160        170        180
TCTCCTGCAAGGCTTCTGGCTACACCTTTACTAGCTACAGGATGCACTGGGTAAGGCAGG
V  S  C  K  A  S  G  Y  T  F  T  S  Y  R  M  H  W  V  R  Q

       190        200        210        220        230        240
CCCCTGGACAGGGTCTGGAATGGATTGGATATATTAATCCGTCGACTGGGTATACTGAAT
A  P  G  Q  G  L  E  W  I  G  Y  I  N  P  S  T  G  Y  T  E

       250        260        270        280        290        300
ACAATCAGAAGTTCAAGGACAAGGCAACAATTACTGCAGACGAATCCACCAATACAGCCT
Y  N  Q  K  F  K  D  K  A  T  I  T  A  D  E  S  T  N  T  A

       310        320        330        340        350        360
ACATGGAACTGAGCAGCCTGAGATCTGAGGACACCGCAGTCTATTACTGTGCAAGAGGGG
Y  M  E  L  S  S  L  R  S  E  D  T  A  V  Y  Y  C  A  R  G

       370        380        390        400        410        420
GGGGGGTCTTTGACTACTGGGGCCAAGGAACCCTGGTCACAGTCTCCTCAGGTGAGTCCT
G  G  V  F  D  Y  W  G  Q  G  T  L  V  T  V  S  S

       430
TAAAACCTCTAGA
```

FIGURE 4

```
        10         20         30         40         50         60
TCTAGATGGAGACCGATACCCTCCTGCTATGGGTCCTCCTGCTATGGGTCCCAGGATCAA
      M  E  T  D  T  L  L  L  W  V  L  L  L  W  V  P  G  S

        70         80         90        100        110        120
CCGGAGATATTCAGATGACCCAGTCTCCATCTACCCTCTCTGCTAGCGTCGGGGATAGGG
T  G  D  I  Q  M  T  Q  S  P  S  T  L  S  A  S  V  G  D  R

       130        140        150        160        170        180
TCACCATAACCTGCTCTGCCAGCTCAAGTATAAGTTACATGCACTGGTACCAGCAGAAGC
V  T  I  T  C  S  A  S  S  S  I  S  Y  M  H  W  Y  Q  Q  K

       190        200        210        220        230        240
CAGGCAAAGCTCCCAAGCTTCTAATTTATACCACATCCAACCTGGCTTCTGGAGTCCCTG
P  G  K  A  P  K  L  L  I  Y  T  T  S  N  L  A  S  G  V  P

       250        260        270        280        290        300
CTCGCTTCAGTGGCAGTGGATCTGGGACCGAGTTCACCCTCACAATCAGCTCTCTGCAGC
A  R  F  S  G  S  G  S  G  T  E  F  T  L  T  I  S  S  L  Q

       310        320        330        340        350        360
CAGATGATTTCGCCACTTATTACTGCCATCAAAGGAGTACTTACCCACTCACGTTCGGTC
P  D  D  F  A  T  Y  Y  C  H  Q  R  S  T  Y  P  L  T  F  G

       370        380        390        400
AGGGGACCAAGGTGGAGGTCAAACGTAAGTACACTTTTCTAGA
Q  G  T  K  V  E  V  K
```

FIGURE 5

A

HES12 AGCTTCTAGATGGGATGGAGCTGGATCTTTCTCTTCCTCCTGTCAGGTACCGCGGGCGTG
CACTCTCAGGTCCAGCTTGTCCAGTCTGGGGCTGAAGTCAAGAAACCTGGCTCGAGCGTG
AAGGTC

HES13 CCCAGTCGACGGATTAATATATCCAATCCATTCCAGACCCTGTCCAGGGGCCTGCCTTAC
CCAGTGCATCCTGTAGCTAGTAAAGGTGTAGCCAGAAGCCTTGCAGGAGACCTTCACGCT
CGAGCCAGG

HES14 TATATTAATCCGTCGACTGGGTATACTGAATACAATCAGAAGTTCAAGGACAAGGCAACA
ATTACTGCAGACGAATCCACCAATACAGCCTACATGGAACTGAGCAGCCTGAGATCTGAG
GACA

HES15 ATATCGTCTAGAGGTTTTAAGGACTCACCTGAGGAGACTGTGACCAGGGTTCCTTGGCCC
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AGGCTGCT

B

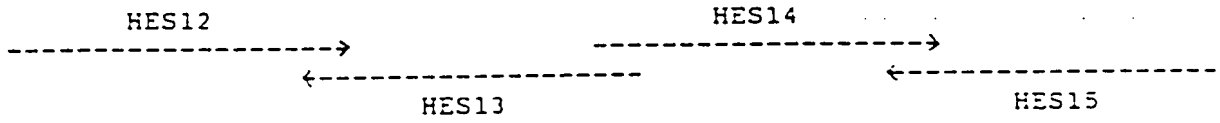


FIGURE 6

A

JFD1 CAAATCTAGATGGAGACCGATACCCTCCTGCTATGGGTCCTCCTGCTATGGGTCCCAGGA
TCAACCGGAGATATTCAGATGACCCAGTCTCCATCTACCCTCTCTGCTAGCGTCGGGGAT

JFD2 ATAAATTAGAAGCTTGGGAGCTTTGCCTGGCTTCTGCTGGTACCAGTGCATGTAACTTAT
ACTTGAGCTGGCAGAGCAGGTTATGGTGACCCTATCCCCGACGCTAGCAGAGAG

JFD3 GCTCCCAAGCTTCTAATTTATACCACATCCAACCTGGCTTCTGGAGTCCCTGCTCGCTTC
AGTGGCAGTGGATCTGGGACCGAGTTCACCCTCACAATCAGCTCTCTGCAGCCAGATGAT
TTC

JFD4 TATATCTAGAAAAGTGTACTTACGTTTGACCTCCACCTTGGTCCCCTGACCGAACGTGAG
TGGGTAAGTACTCCTTTGATGGCAGTAATAAGTGGCGAAATCATCTGGCTGCAGAGAGCT
GA

B

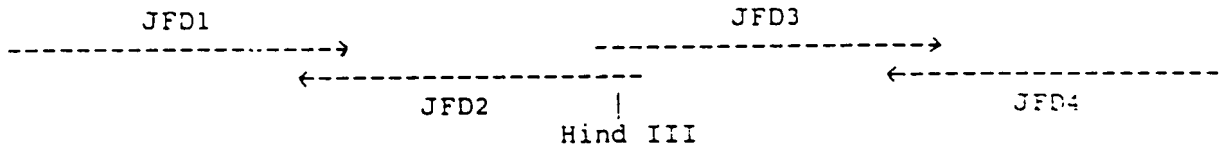


FIGURE 7

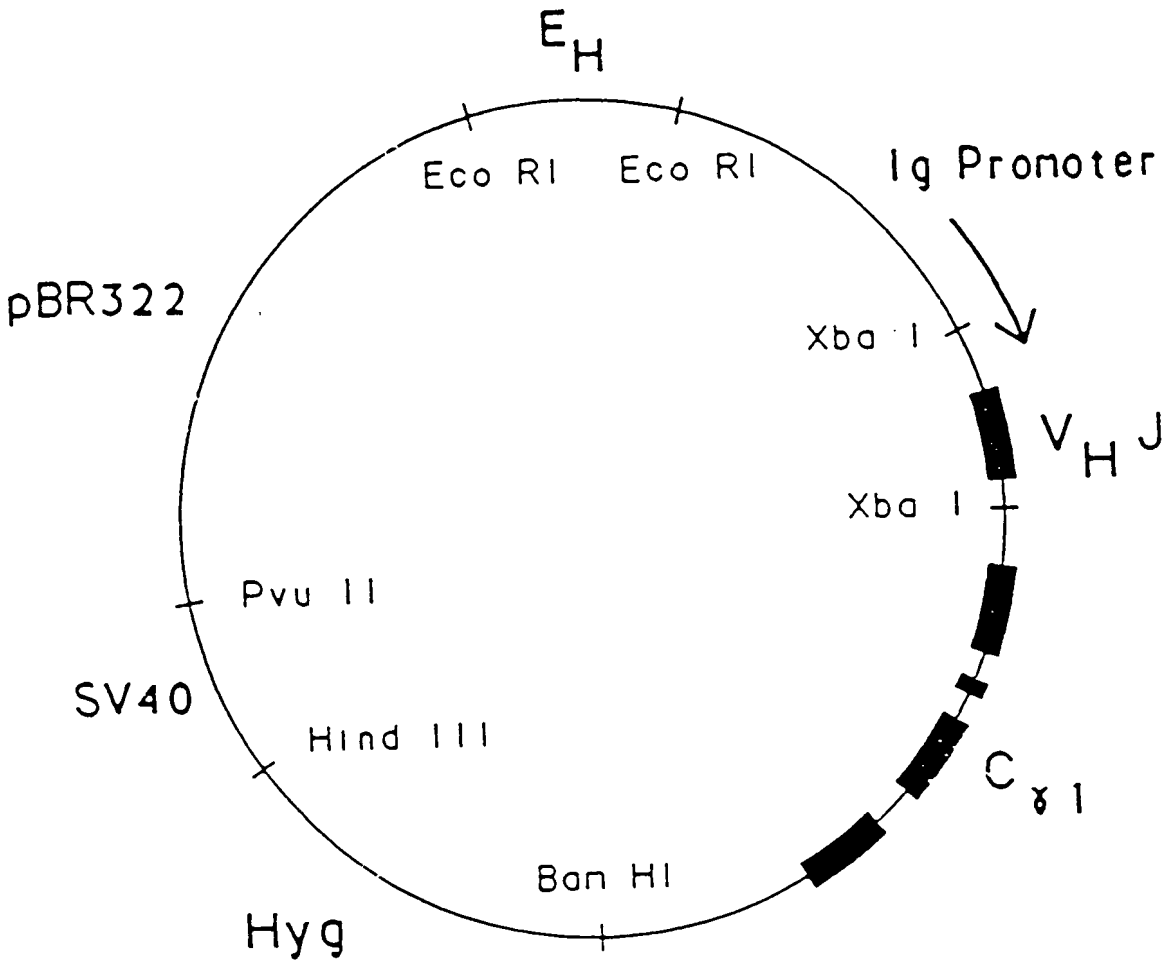

E H
Eco RI
Eco RI
Ig Promoter
pBR322
Xba I
V H J
Xba I
Pvu II
SV40
Hind III
C γ 1
Bam HI
Hyg

FIGURE 8

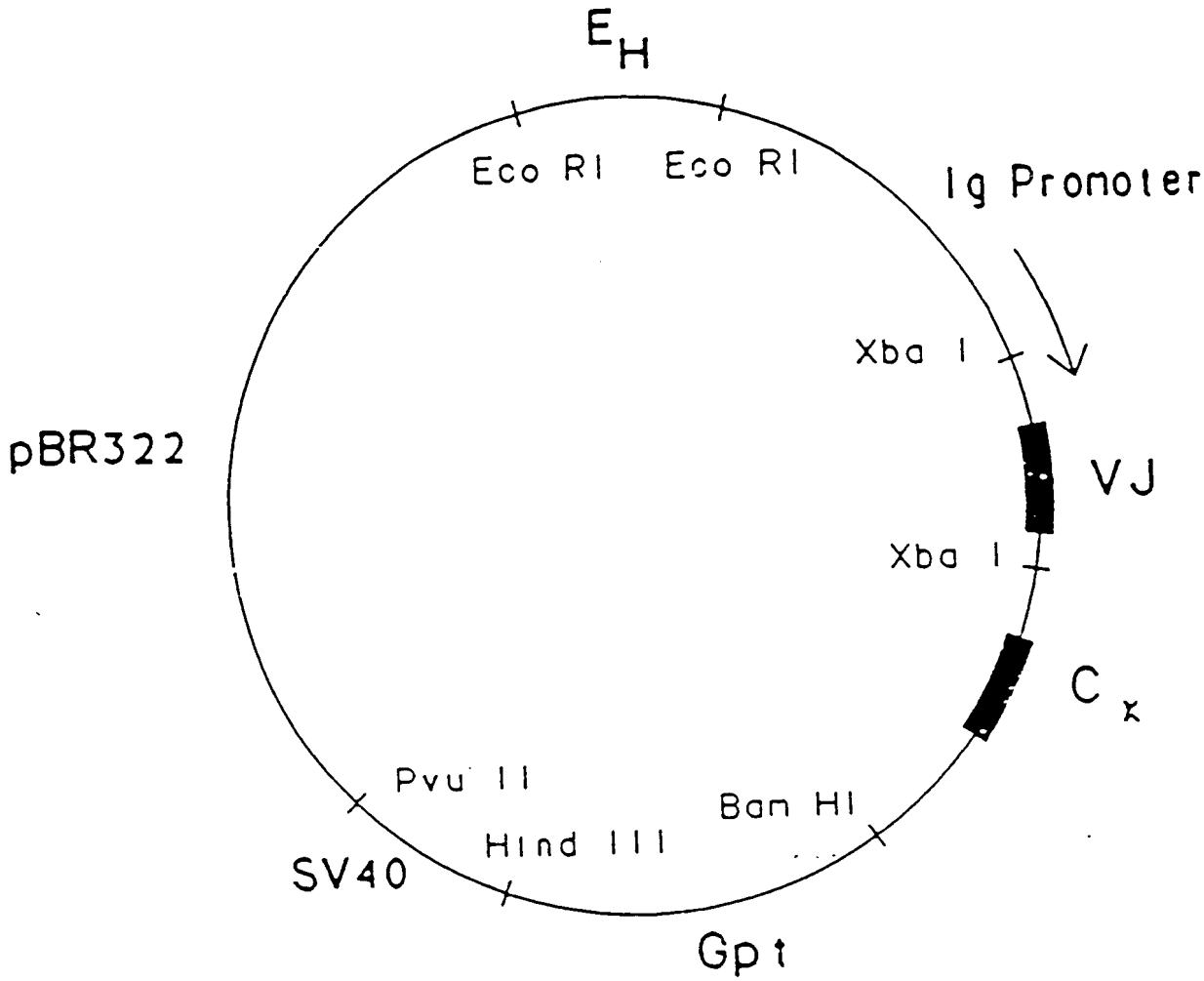
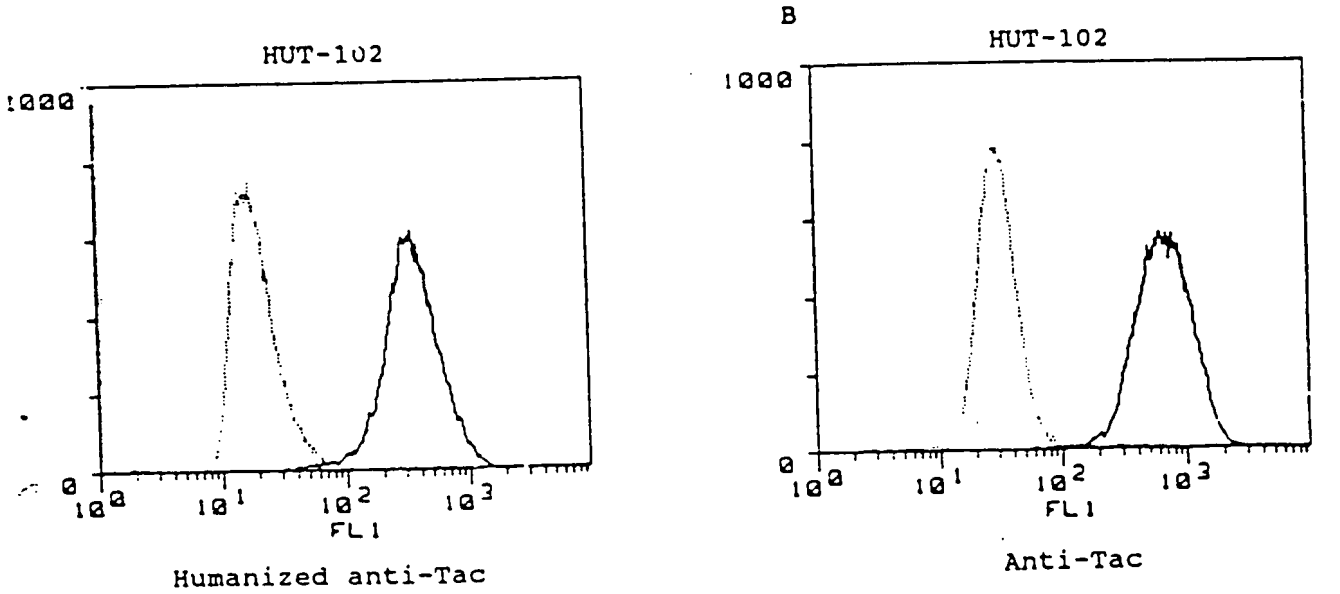

E H
Eco RI
Eco RI
Ig Promoter
Xba I
pBR322
VJ
Xba I
C x
Pvu II
Ban HI
Hind III
SV40
Gpt

FIGURE 9


B
HUT-102
1000
0
10^0
10^1
10^2
10^3
FL1
Humanized anti-Tac
HUT-102
1000
0
10^0
10^1
10^2
10^3
FL1
Anti-Tac
Jurkat
1000
0
10^0
10^1
10^2
10^3
FL1
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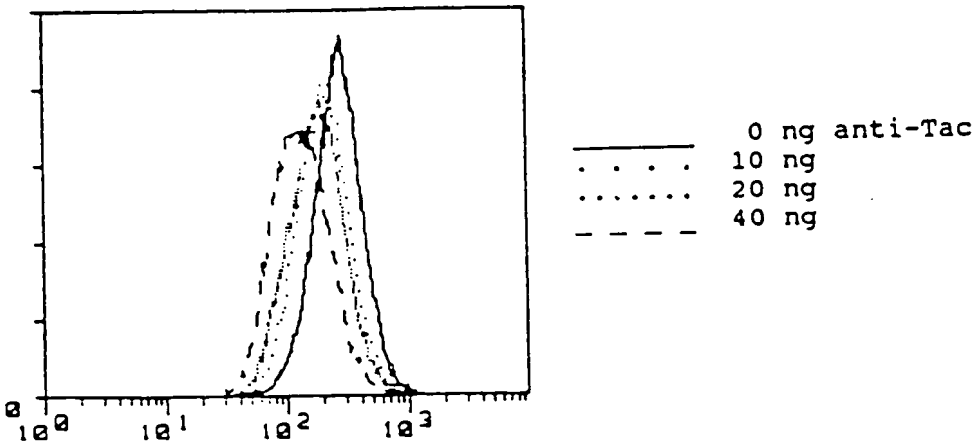
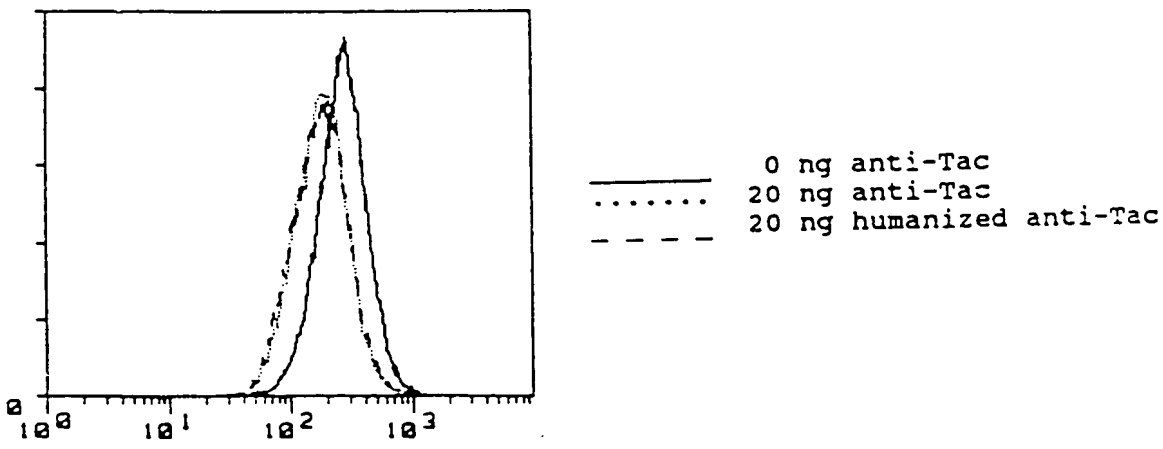
10^2
10^3
FL1
Humanized anti-Tac

FIGURE 10

A


0
10^0
10^1
10^2
10^3
0 ng anti-Tac
10 ng
20 ng
40 ng

B


0
10^0
10^1
10^2
10^3
0 ng anti-Tac
20 ng anti-Tac
20 ng humanized anti-Tac

SERIAL NUMBER (Series of 1987)	PATENT DATE	PATENT NUMBER
07/310252		

SERIAL NUMBER	FILING DATE	CLASS	SUBCLASS	GROUP ART UNIT	EXAMINER
07/310,252	02/13/89	435		135	

APPLICANTS

CARY L. QUEEN, PALO ALTO, CA; HAROLD E. SELICK, BELMONT, CA.

CONTINUING DATA********
VERIFIED THIS APPLN IS A CIP OF 07/290,975 12/28/88

REC'D 28 DEC 1989
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FOREIGN/PCT APPLICATIONS********
VERIFIED

PRIORITY DOCUMENT

FOREIGN FILING LICENSE GRANTED 03/03/89 ***** SMALL ENTITY *****

Foreign priority claimed / 35 USC 119 conditions met / Verified and Acknowledged (Examiner's Initials)	AS FILED	STATE OR COUNTRY	SHEETS DRWGS.	TOTAL CLAIMS	INDEP. CLAIMS	FILING FEE RECEIVED	ATTORNEY'S DOCKET NO.
☐yes ☐no / ☐yes ☐no	→	CA	10	28	3	$ 279.00	113239

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TITLE
DESIGNING IMPROVED HUMANIZED IMMUNOGLOBULINS

...COMM. Pat. & TM Office — PTO-436L (rev. 10-78)

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By authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

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...ITION ...S EXH...TS
...Date ... FEB 1990

PATENT APPLICATION SERIAL NO. 07/310252

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

S 00016 02/17 09 310252 20 1430 [illegible] 291 [illegible]

DESIGNING IMPROVED HUMANIZED IMMUNOGLOBULINS

CROSS-REFERENCE TO RELATED APPLICATION

This is a continuation-in-part application of commonly assigned patent application U.S.S.N. 290,975, filed December 28, 1988, which is incorporated herein by reference.

Field of the Invention

The present invention relates generally to the combination of recombinant DNA and monoclonal antibody technologies for developing novel therapeutic agents and, more particularly, to the production of non-immunogenic antibodies having strong affinity for a predetermined antigen.

Background of the Invention

The advent of monoclonal antibody technology in the mid 1970's heralded a new age of medicine. For the first time, researchers and clinicians had access to essentially unlimited quantities of uniform antibodies capable of binding to a predetermined antigenic site and having various immunological effector functions. These proteins, known as "monoclonal antibodies" were thought to hold great promise in, e.g., the removal of harmful cells in vivo. Indeed, the clinical value of monoclonal antibodies seemed limitless for this use alone.

Unfortunately, the development of appropriate therapeutic products based on these proteins has been severely hampered by a number of drawbacks inherent in monoclonal antibody production. For example, most monoclonal antibodies are mouse derived, and thus do not fix human complement well. They also lack other important immunoglobulin functional characteristics when used in humans.

Perhaps most importantly, non-human monoclonal antibodies contain substantial stretches of amino acid sequences that will be immunogenic when injected into a human

patient. Numerous studies have shown that after injection of a foreign antibody, the immune response mounted by a patient can be quite strong, essentially eliminating the antibody's therapeutic utility after an initial treatment. Moreover, as increasing numbers of different mouse or other antigenic (to humans) monoclonal antibodies can be expected to developed to treat various diseases, after the first or second treatments with any non-human antibodies, subsequent treatments, even for unrelated therapies, can be ineffective or even dangerous in themselves.

While the production of so called "chimeric antibodies" (e.g., mouse variable regions joined to human constant regions) has proven somewhat successful, a significant immunogenicity problem remains. Moreover, efforts to immortalize human B-cells or generate human hybridomas capable of producing human immunoglobulins against a desired antigen have been generally unsuccessful, particularly with many important human antigens. Most recently, recombinant DNA technology has been utilized to produce immunoglobulins which have human framework regions combined with complementarity determining regions (CDR's) from a donor mouse or rat immunoglobulin (see, e.g., EPO Publication No. 0239400, which is incorporated herein by reference). These new proteins are called "humanized immunoglobulins" and the process by which the donor immunoglobulin is converted into a human-like immunoglobulin by combining its CDR's with a human framework is called "humanization". Humanized antibodies are important because they bind to the same antigen as the original antibodies, but are less immunogenic when injected into humans.

However, a major problem with present humanization procedures has been a loss of affinity for the antigen, usually by at least 2 to 3-fold (Jones et al., Nature, 321:522-525 (1986)) and in some instances as much as 10-fold or more, especially when the antigen is a protein (Verhoeyen et al., Science, 239:1534-1536 (1988)). Loss of any affinity is, of course, highly undesirable. At the least, it means that more of the humanized antibody will have to be injected

into the patient, at higher cost and greater risk of adverse effects. Even more critically, an antibody with reduced affinity may have poorer biological functions, such as complement lysis, antibody-dependent cellular cytotoxicity, or virus neutralization. For example, the loss of affinity in the partially humanized antibody HuVHCAMP may have caused it to lose all ability to mediate complement lysis (see, Riechmann et al., Nature, 332:323-327 (1988); Table 1).

Thus, there is a need for improved means for producing humanized antibodies specifically reactive with strong affinity to a predetermined antigen. These humanized immunoglobulins should remain substantially non-immunogenic in humans, yet be easily and economically produced in a manner suitable for therapeutic formulation and other uses. The present invention fulfills these and other needs.

Summary of the Invention

The present invention provides novel methods for designing humanized immunoglobulin chains having one or more complementarity determining regions (CDR's) from a donor immunoglobulin and a framework region from a human immunoglobulin, the preferred methods comprising first comparing the framework or variable region amino acid sequence of the donor immunoglobulin to corresponding sequences in a collection of human immunoglobulin chains, and selecting as the human immunoglobulin one of the more homologous sequences from the collection. The human immunoglobulin, or acceptor immunoglobulin, sequence is typically selected from a collection of at least 10 to 20 immunoglobulin chain sequences, and usually will have the highest homology to the donor immunoglobulin sequence of any sequence in the collection. The human immunoglobulin framework sequence will typically have about 65 to 70% homology or more to the donor immunoglobulin framework sequences. The donor immunoglobulin may be either a heavy chain or light chain (or both), and the human collection will contain the same kind of chain. A humanized light and heavy chain can be used to form a complete humanized immunoglobulin

or antibody, having two light/heavy chain pairs, with or without partial or full-length human constant regions and other proteins.

In another embodiment of the present invention, either in conjunction with the above comparison step or separately, additional amino acids in an acceptor immunoglobulin chain may be replaced with amino acids form the CDR-donor immunoglobulin chain. More specifically, further optional substitutions of a human framework amino acid of the acceptor immunoglobulin with a corresponding amino acid from a donor immunoglobulin will be made at positions in the immunoglobulins where:

(a) the amino acid in the human framework region of an acceptor immunoglobulin is rare for that position and the corresponding amino acid in the donor immunoglobulin is common for that position in human immunoglobulin sequences; or

(b) the amino acid is immediately adjacent to one of the CDR's; or

(c) the amino acid is predicted to be within about 3Å of the CDR's in a three-dimensional immunoglobulin model and capable of interacting with the antigen or with the CDR's of the humanized immunoglobulin.

The humanized immunoglobulin chain will typically comprise at least about 3 amino acids from the donor immunoglobulin in addition to the CDR's, usually at least one of which is immediately adjacent to a CDR in the donor immunoglobulin. The heavy and light chains may each be designed by using any one or all three of the position criteria.

When combined into an intact antibody, the humanized light and heavy chains of the present invention will be substantially non-immunogenic in humans and retain substantially the same affinity as the donor immunoglobulin to the antigen (such as a protein or other compound containing an epitope). These affinity levels can vary from about 10^8 M^{-1} or higher, and may be within about 4 fold of the donor immunoglobulin's original affinity to the antigen.

Once designed, the immunoglobulins, including binding fragments and other immunoglobulin forms, of the present invention may be produced readily by a variety of recombinant DNA or other techniques. Preferably, polynucleotides encoding the desired amino acid sequences are produced synthetically or by joining appropriate nucleic acid sequences for expression in a suitable host (e.g., cell culture). The humanized immunoglobulins will be particularly useful in treating human disorders susceptible to monoclonal antibody therapy, but find a variety of other uses as well.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Comparison of sequences of anti-Tac heavy chain (upper lines) and Eu heavy chain (lower lines). The 1-letter code for amino acids is used. The first amino acid on each line is numbered at the left. Identical amino acids in the two sequences are connected by lines. The 3 CDR's are underlined. Other amino acid positions for which the anti-Tac amino acid rather than the Eu amino acid was used in the humanized anti-Tac heavy chain are denoted by an *.

Figure 2. Comparison of sequences of anti-Tac light chain (upper lines) and Eu light chain (lower lines). The single-letter code for amino acids is used. The first amino acid on each line is numbered at the left. Identical amino acids in the two sequences are connected by lines. The 3 CDR's are underlined. Other amino acid positions for which the anti-Tac amino acid rather than the Eu amino acid was used in the humanized anti-Tac heavy chain are denoted by an *.

Figure 3. Nucleotide sequence of the gene for the humanized anti-Tac heavy chain variable region gene. The translated amino acid sequence for the part of the gene encoding protein is shown underneath the nucleotide sequence. The nucleotides TCTAGA at the beginning and end of the gene are Xba I sites. The mature heavy chain sequence begins with amino acid #20 Q.

Figure 4. Nucleotide sequence of the gene for the humanized anti-Tac light chain variable region gene. The translated amino acid sequence for the part of the gene encoding protein is shown underneath the nucleotide sequence. The nucleotides TCTAGA at the beginning and end of the gene are Xba I sites. The mature light chain sequence begins with amino acid #21 D.

Figure 5. A. Sequences of the four oligonucleotides used to synthesize the humanized anti-Tac heavy chain gene, printed 5' to 3'. B. Relative positions of the oligonucleotides. The arrows point in the 3' direction for each oligonucleotide.

Figure 6. (A) Sequences of the four oligonucleotides used to synthesize the humanized anti-Tac light chain gene, printed 5' to 3'. (B) Relative positions of the oligonucleotides. The arrows point in the 3' direction for each oligonucleotide. The position of a Hind III site in the overlap of JFD2 and JFD3 is shown.

Figure 7. Schematic diagram of the plasmid pHuGTAC1 used to express the humanized anti-Tac heavy chain. Relevant restriction sites are shown, and coding regions of the heavy chain are displayed as boxes. The direction of transcription from the immunoglobulin (Ig) promoter is shown by an arrow. E_H = heavy chain enhancer, Hyg = hygromycin resistance gene.

Figure 8. Schematic diagram of the plasmid pHuLTAC used to express the humanized anti-Tac light chain. Relevant restriction sites are shown, and coding regions of the light chain are displayed as boxes. The direction of transcription from the Ig promoter is shown by an arrow.

Figure 9. Fluorocytometry of HUT-102 and Jurkat cells stained with anti-Tac antibody or humanized anti-Tac antibody followed respectively by fluorescein-conjugated goat anti-mouse Ig antibody or goat anti-human Ig antibody, as labeled. In each panel, the dotted curve shows the results when the first antibody was omitted, and the solid curve the results when first and second (conjugated) antibodies were included as described.

Figure 10. (A) Fluorocytometry of HUT-102 cells stained with 0-40 ng of anti-Tac as indicated, then with biotinylated anti-Tac, and then with phycoerythrin-conjugated avidin. (B) Fluorocytometry of HUT-102 cells stained with the indicated antibody, then with biotinylated anti-Tac, and then with phycoerythrin-conjugated avidin.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, novel means of designing humanized immunoglobulins capable of specifically binding to a predetermined antigen with strong affinity are provided. These improved methods produce immunoglobulins that are substantially non-immunogenic in humans but have binding affinities of at least about 10^8 M^{-1}, preferably 10^9 M^{-1} to 10^{10} M^{-1}, or stronger. The humanized immunoglobulins will have a human framework and have one or more complementary determining regions (CDR's), plus a limited number of other amino acids, from a donor immunoglobulin specifically reactive with an antigen. The immunoglobulins can be produced economically in large quantities and find use, for example, in the treatment of various human disorders by a variety of techniques.

In order that the invention may be more completely understood, several definitions are set forth. As used herein, the term "immunoglobulin" refers to a protein having one or more polypeptides substantially encoded by immunoglobulin genes. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Full-length immunoglobulin "light chains" (about 25 Kd, about 214 amino acids) are encoded by a variable region gene at the NH2-terminus (about 110 amino acids) and a kappa or lambda constant region gene at the COOH - terminus. Full-length immunoglobulin "heavy chains" (about 50 Kd, about 446 amino acids), are similarly encoded by a variable region gene (encoding about 116 amino acids) and one of the other

aforementioned constant region genes, e.g., gamma (encoding about 330 amino acids).

One form of immunoglobulin constitutes the basic structural unit of an antibody. This form is a tetramer and consists of two identical pairs of immunoglobulin chains, each pair having one light and one heavy chain. In each pair, the light and heavy chain variable regions are together responsible for binding to an antigen, and the constant regions are responsible for the antibody effector functions. In addition to antibodies, immunoglobulins may exist in a variety of other forms (including less than full-length that retain the desired activities), including, for example, Fv, Fab, and F(ab')2, as well as single chain antibodies (e.g., Huston et al., Proc. Nat. Acad. Sci. U.S.A., 85:5879-5883 (1988) and Bird et al., Science, 242:423-426 (1988), which are incorporated herein by reference). (See, generally, Hood et al., "Immunology", Benjamin, N.Y., 2nd ed. (1984), and Hunkapiller and Hood, Nature, 323:15-16 (1986), which are incorporated herein by reference).

An immunoglobulin light or heavy chain variable region consists of a "framework" region interrupted by three hypervariable regions, also called CDR's. The extent of the framework region and CDR's have been precisely defined (see, "Sequences of Proteins of Immunological Interest," E. Kabat et al., U.S. Department of Health and Human Services, (1983); which is incorporated herein by reference). The sequences of the framework regions of different light or heavy chains are relatively conserved within a species. The framework region of an antibody, that is the combined framework regions of the constituent light and heavy chains, serves to position and align the CDR's. The CDR's are primarily responsible for binding to an epitope of an antigen.

Chimeric antibodies are antibodies whose light and heavy chain genes have been constructed, typically by genetic engineering, from immunoglobulin variable and constant region genes belonging to different species. For example, the variable segments of the genes from a mouse monoclonal antibody may be joined to human constant segments, such as

gamma 1 and gamma 3. A typical therapeutic chimeric antibody is thus a hybrid protein composed of the variable or antigen-binding domain from a mouse antibody and the constant or effector domain from a human antibody (e.g., A.T.C.C. Accession No. CRL 9688 secretes an anti-Tac chimeric antibody), although other mammalian species may be used.

As used herein, the term "humanized" immunoglobulin refers to an immunoglobulin comprising a substantially human framework region and one or more CDR's from a non-human (usually a mouse or rat) immunoglobulin. The non-human immunoglobulin providing the CDR's is called the "donor" and a human immunoglobulin providing the framework is called the "acceptor". Constant regions need not be present, but if they are, they must be substantially homologous to human immunoglobulin constant regions, i.e., at least about 85-90%, preferably about 95% or more identical. Hence, all parts of a humanized immunoglobulin, except possibly the CDR's, are substantially homologous to corresponding parts of natural human immunoglobulin sequences. A "humanized antibody" is an antibody comprising a humanized light chain and a humanized heavy chain immunoglobulin. For example, a humanized antibody would not encompass a typical chimeric antibody as defined above, e.g., because the entire variable region of a chimeric antibody is non-human. One says that the donor antibody has been "humanized", by the process of "humanization", because the resultant humanized antibody is expected to bind to the same antigen as the donor antibody that provides the CDR's.

Humanized immunoglobulins, including humanized antibodies, have been constructed by means of genetic engineering. Most humanized immunoglobulins that have been previously described (Jones et al., op. cit.; Verhoeyen et al., op. cit.; Riechmann et al., op. cit.) have comprised a framework that is identical to the framework of a particular human immunoglobulin chain, the acceptor, and three CDR's from a non-human donor immunoglobulin chain. In one case (Riechmann et al., op. cit.), two additional amino acids in the framework were changed to be the same as amino acids in

other human framework regions. The present invention includes criteria by which a limited number of amino acids in the framework of a humanized immunoglobulin chain are chosen to be the same as the amino acids at those positions in the donor rather than in the acceptor, in order to increase the affinity of an antibody comprising the humanized immunoglobulin chain.

The present invention is based in part on the model that two contributing causes of the loss of affinity in prior means of producing humanized antibodies (using as examples mouse antibodies as the source of CDR's) are:

(1) When the mouse CDR's are combined with the human framework, the amino acids in the framework close to the CDR's become human instead of mouse. Without intending to be bound by theory, we believe that these changed amino acids may slightly distort the CDR's, because they create different electrostatic or hydrophobic forces than in the donor mouse antibody, and the distorted CDR's may not make as effective contacts with the antigen as the CDR's did in the donor antibody;

(2) Also, amino acids in the original mouse antibody that are close to, but not part of, the CDR's (i.e., still part of the framework), may make contacts with the antigen that contribute to affinity. These amino acids are lost when the antibody is humanized, because all framework amino acids are made human.

To avoid these problems, and to produce humanized antibodies that have a very strong affinity for a desired antigen, the present invention uses the following four criteria for designing humanized immunoglobulins. These criteria may be used singly, or when necessary in combination, to achieve the desired affinity or other characteristics.

Criterion I: As acceptor, use a framework from a particular human immunoglobulin that is unusually homologous to the donor immunoglobulin to be humanized, or use a consensus framework from many human antibodies. For example,

comparison of the sequence of a mouse heavy (or light) chain variable region against human heavy (or light) variable regions in a data bank (for example, the National Biomedical Research Foundation Protein Identification Resource) shows that the extent of homology to different human regions varies greatly, typically from about 40% to about 60-70%. By choosing as the acceptor immunoglobulin one of the human heavy (respectively light) chain variable regions that is most homologous to the heavy (respectively light) chain variable region of the donor immunoglobulin, fewer amino acids will be changed in going from the donor immunoglobulin to the humanized immunoglobulin. Hence, and again without intending to be bound by theory, it is believed that there is a smaller chance of changing an amino acid near the CDR's that distorts their conformation. Moreover, the precise overall shape of a humanized antibody comprising the humanized immunoglobulin chain may more closely resemble the shape of the donor antibody, also reducing the chance of distorting the CDR's.

Typically, one of the 3-5 most homologous heavy chain variable region sequences in a representative collection of at least about 10 to 20 distinct human heavy chains will be chosen as acceptor to provide the heavy chain framework, and similarly for the light chain. Preferably, one of the 1-3 most homologous variable regions will be used. The selected acceptor immunoglobulin chain will most preferably have at least about 65% homology in the framework region to the donor immunoglobulin.

Regardless of how the acceptor immunoglobulin is chosen, higher affinity may be achieved by selecting a small number of amino acids in the framework of the humanized immunoglobulin chain to be the same as the amino acids at those positions in the donor rather than in the acceptor. The following criteria define what amino acids may be so selected. Preferably, at most or all amino acid positions satisfying one of these criteria, the donor amino acid will in fact be selected.

Criterion II: If an amino acid in the framework of the human acceptor immunoglobulin is unusual (i.e., "rare", which as used herein indicates an amino acid occurring at that position in no more than about 10% of human heavy (respectively light) chain V region sequences in a representative data bank), and if the donor amino acid at that position is typical for human sequences (i.e., "common", which as used herein indicates an amino acid occurring in at least about 25% of sequences in a representative data bank), then the donor amino acid rather than the acceptor may be selected. This criterion helps ensure that an atypical amino acid in the human framework does not disrupt the antibody structure. Moreover, by replacing an unusual amino acid with an amino acid from the donor antibody that happens to be typical for human antibodies, the humanized antibody may be made less immunogenic.

Criterion III: In the positions immediately adjacent to the 3 CDR's in the humanized immunoglobulin chain, the donor amino acid rather than acceptor amino acid may be selected. These amino acids are particularly likely to interact with the amino acids in the CDR's and, if chosen from the acceptor, distort the donor CDR's and reduce affinity. Moreover, the adjacent amino acids may interact directly with the antigen (Amit et al., Science, 233, 747-753 (1986), which is incorporated herein by reference) and selecting these amino acids from the donor may be desirable to keep all the antigen contacts that provide affinity in the original antibody.

Criterion IV: A 3-dimensional model, typically of the original donor antibody, shows that certain amino acids outside of the CDR's are close to the CDR's and have a good probability of interacting with amino acids in the CDR's by hydrogen bonding, Van der Waals forces, hydrophobic interactions, etc. At those amino acid positions, the donor amino acid rather than the acceptor immunoglobulin amino acid may be selected. Amino acids according to this criterion will

generally have a side chain atom within about 3 angstrom units of some site in the CDR's and must contain atoms that could interact with the CDR atoms according to established chemical forces, such as those listed above. Computer programs to create models of proteins such as antibodies are generally available and well known to those skilled in the art (see, Loew et al., Int. J. Quant. Chem., Quant. Biol. Symp., 15:55-66 (1988); Bruccoleri et al., Nature, 335, 564-568 (1988); Chothia et al., Science, 233:755-758 (1986), all of which are incorporated herein by reference). These do not form part of the invention. Indeed, because all antibodies have similar structures, the known antibody structures, which are available from the Brookhaven Protein Data Bank, can be used if necessary as rough models of other antibodies. Commercially available computer programs can be used to display these models on a computer monitor, to calculate the distance between atoms, and to estimate the likelihood of different amino acids interacting (see, Ferrin et al., J. Mol. Graphics, 6:13-27 (1988)).

Humanized antibodies generally have at least three potential advantages over mouse or in some cases chimeric antibodies for use in human therapy:

1) Because the effector portion is human, it may interact better with the other parts of the human immune system (e.g., destroy the target cells more efficiently by complement- dependent cytotoxicity (CDC) or antibody-dependent cellular cytotoxicity (ADCC)).

2) The human immune system should not recognize the framework or constant region of the humanized antibody as foreign, and therefore the antibody response against such an injected antibody should be less than against a totally foreign mouse antibody or a partially foreign chimeric antibody.

3) Injected mouse antibodies have been reported to have a half-life in the human circulation much shorter than the half-life of normal antibodies (D. Shaw et al., J. Immunol., 138:4534-4538 (1987)). Injected humanized antibodies will presumably have a half-life more similar to

naturally occurring human antibodies, allowing smaller and less frequent doses to be given.

In one aspect, the present invention is directed to designing humanized immunoglobulins that are produced by expressing recombinant DNA segments encoding the heavy and light chain CDR's from a donor immunoglobulin capable of binding to a desired antigen, such as the human IL-2 receptor, to DNA segments encoding acceptor human framework regions. Exemplary DNA sequences designed in accordance with the present invention and, which on expression code for the polypeptide chains comprising heavy and light chain CDR's with substantially human framework regions, are shown in Figures 3 and 4, respectively. Due to codon degeneracy and non-critical amino acid substitutions, other DNA sequences can be readily substituted for those sequences, as detailed below. In general, the criteria of the present invention find applicability to designing substantially any humanized immunoglobulin.

The DNA segments will typically further include an expression control DNA sequence operably linked to the humanized immunoglobulin coding sequences, including naturally-associated or heterologous promoter regions. Preferably, the expression control sequences will be eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells, but control sequences for prokaryotic hosts may also be used. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences, and, as desired, the collection and purification of the humanized light chains, heavy chains, light/heavy chain dimers or intact antibodies, binding fragments or other immunoglobulin forms may follow (see, S. Beychok, Cells of Immunoglobulin Synthesis, Academic Press, N.Y., (1979), which is incorporated herein by reference.

Human constant region DNA sequences can be isolated in accordance with well known procedures from a variety of human cells, but preferably immortalized B-cells (see, Kabat

op. cit. and WP87/02671). The CDR's for producing the immunoglobulins of the present invention will be similarly derived from monoclonal antibodies capable of binding to the predetermined antigen, such as the human IL-2 receptor, and produced by well known methods in any convenient mammalian source including, mice, rats, rabbits, or other vertebrate capable of producing antibodies. Suitable source cells for the constant region and framework DNA sequences, and host cells for immunoglobulin expression and secretion, can be obtained from a number of sources, such as the American Type Culture Collection ("Catalogue of Cell Lines and Hybridomas," Fifth edition (1985) Rockville, Maryland, U.S.A., which is incorporated herein by reference).

In addition to the humanized immunoglobulins specifically described herein, other "substantially homologous" modified immunoglobulins to the native sequences can be readily designed and manufactured utilizing various recombinant DNA techniques well known to those skilled in the art. For example, the framework regions can vary specifically from the sequences in Figures 3 and 4 at the primary structure level by several amino acid substitutions, terminal and intermediate additions and deletions, and the like. Moreover, a variety of different human framework regions may be used singly or in combination as a basis for the humanized immunoglobulins of the present invention. In general, modifications of the genes may be readily accomplished by a variety of well-known techniques, such as site-directed mutagenesis (see, Gillman and Smith, Gene, 8:81-97 (1979) and S. Roberts et al., Nature, 328:731-734 (1987), both of which are incorporated herein by reference).

Alternatively, polypeptide fragments comprising only a portion of the primary antibody structure may be produced, which fragments possess one or more immunoglobulin activities (e.g., complement fixation activity). Also because like many genes, the immunoglobulin-related genes contain separate functional regions, each having one or more distinct biological activities, the genes may be fused to functional regions from other genes (e.g., enzymes, see,

commonly assigned U.S.S.N. 132,387, filed Dec. 15, 1987, which is incorporated herein by reference) to produce fusion proteins (e.g., immunotoxins) having novel properties. The nucleic acid sequences of the present invention capable of ultimately expressing the desired humanized antibodies can be formed from a variety of different polynucleotides (genomic or cDNA, RNA, synthetic oligonucleotides, etc.) and components (e.g., V, J, D, and C regions), as well as by a variety of different techniques. Joining appropriate genomic sequences is presently the most common method of production, but cDNA sequences may also be utilized (see, European Patent Publication No. 0239400 and L. Reichmann et al., Nature, 332:323-327 (1988), both of which are incorporated herein by reference).

As stated previously, the DNA sequences will be expressed in hosts after the sequences have been operably linked to (i.e., positioned to ensure the functioning of) an expression control sequence. These expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression vectors will contain selection markers, e.g., tetracycline or neomycin, to permit detection of those cells transformed with the desired DNA sequences (see, e.g., U.S. Patent 4,704,362, which is incorporated herein by reference).

E. coli is one prokaryotic host useful particularly for cloning the DNA sequences of the present invention. Other microbial hosts suitable for use include bacilli, such as Bacillus subtilus, and other enterobacteriaceae, such as Salmonella, Serratia, and various Pseudomonas species. In these prokaryotic hosts, one can also make expression vectors, which will typically contain expression control sequences compatible with the host cell (e.g., an origin of replication). In addition, any number of a variety of well-known promoters will be present, such as the lactose promoter system, a tryptophan (trp) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. The promoters will typically control expression, optionally with an operator sequence, and have ribosome binding site

sequences and the like, for initiating and completing transcription and translation.

Other microbes, such as yeast, may also be used for expression. Saccharomyces is a preferred host, with suitable vectors having expression control sequences, such as promoters, including 3-phosphoglycerate kinase or other glycolytic enzymes, and an origin of replication, termination sequences and the like as desired.

In addition to microorganisms, mammalian tissue cell culture may also be used to express and produce the polypeptides of the present invention (see, Winnacker, "From Genes to Clones," VCH Publishers, N.Y., N.Y. (1987), which is incorporated herein by reference). Eukaryotic cells are actually preferred, because a number of suitable host cell lines capable of secreting intact immunoglobulins have been developed in the art, and include the CHO cell lines, various COS cell lines, HeLa cells, myeloma cell lines, etc, but preferably transformed B-cells or hybridomas. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter, an enhancer (Queen et al., Immunol. Rev., 89:49-68 (1986), which is incorporated herein by reference), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Preferred expression control sequences are promoters derived from immunoglobulin genes, SV40, Adenovirus, Bovine Papilloma Virus, and the like.

The vectors containing the DNA segments of interest (e.g., the heavy and light chain encoding sequences and expression control sequences) can be transferred into the host cell by well-known methods, which vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment or electroporation may be used for other cellular hosts. (See, generally, Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, (1982), which is incorporated herein by reference.)

Once expressed, the whole antibodies, their dimers, individual light and heavy chains, or other immunoglobulin forms of the present invention, can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like (see, generally, R. Scopes, "Protein Purification", Springer-Verlag, N.Y. (1982)). Substantially pure immunoglobulins of at least about 90 to 95% homogeneity are preferred, and 98 to 99% or more homogeneity most preferred, for pharmaceutical uses. Once purified, partially or to homogeneity as desired, the polypeptides may then be used therapeutically (including extracorporeally) or in developing and performing assay procedures, immunofluorescent stainings, and the like. (See, generally, Immunological Methods, Vols. I and II, Lefkovits and Pernis, eds., Academic Press, New York, N.Y. (1979 and 1981)).

The antibodies of the present invention will typically find use individually in treating substantially any disease susceptible to monoclonal antibody-based therapy. In particular, the immunoglobulins can be used for passive immunization or the removal of unwanted cells or antigens, such as by complement mediated lysis, all without substantial immune reactions (e.g., anaphylactic shock) associated with many prior antibodies. For example, where the cell linked to a disease has been identified as IL-2 receptor bearing, then humanized antibodies that bind to the human IL-2 receptor are suitable (see, U.S.S.N. 085,707, entitled "Treating Human Malignancies and Disorders," which is incorporated herein by reference). For such a humanized immunoglobulin, typical disease states suitable for treatment include graft versus host disease and transplant rejection in patients undergoing an organ transplant, such as heart, lungs, kidneys, liver, etc. Other diseases include autoimmune diseases, such as Type I diabetes, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, and myasthenia gravis.

The method of producing humanized antibodies of the present invention can be used to humanize a variety of donor

antibodies, especially monoclonal antibodies reactive with markers on cells responsible for a disease. For example, suitable antibodies bind to antigens on T-cells, such as those grouped into the so-called "Clusters of Differentiation," as named by the First International Leukocyte Differentiation Workshop, Leukocyte Typing, Bernard et al., Eds., Springer- Verlag, N.Y. (1984), which is incorporated herein by reference.

The antibodies of the present invention can also be used as separately administered compositions given in conjunction with chemotherapeutic or immunosuppressive agents. Typically, the agents will include cyclosporin A or a purine analog (e.g., methotrexate, 6-mercaptopurine, or the like), but numerous additional agents (e.g., cyclophosphamide, prednisone, etc.) well-known to those skilled in the art may also be utilized.

A preferred pharmaceutical composition of the present invention comprises the use of the subject antibodies in immunotoxins. Immunotoxins are characterized by two components and are particularly useful for killing selected cells in vitro or in vivo. One component is a cytotoxic agent which is usually fatal to a cell when attached or absorbed. The second component, known as the "delivery vehicle," provides a means for delivering the toxic agent to a particular cell type, such as cells comprising a carcinoma. The two components are commonly chemically bonded together by any of a variety of well-known chemical procedures. For example, when the cytotoxic agent is a protein and the second component is an intact immunoglobulin, the linkage may be by way of heterobifunctional cross-linkers, e.g., SPDP, carbodiimide, glutaraldehyde, or the like. Production of various immunotoxins is well-known with the art, and can be found, for example in "Monoclonal Antibody-Toxin Conjugates: Aiming the Magic Bullet," Thorpe et al., Monoclonal Antibodies in Clinical Medicine, Academic Press, pp. 168-190 (1982), which is incorporated herein by reference.

A variety of cytotoxic agents are suitable for use in immunotoxins. Cytotoxic agents can include radionuclides,

such as Iodine-131, Yttrium-90, Rhenium-188, and Bismuth-212; a number of chemotherapeutic drugs, such as vindesine, methotrexate, adriamycin, and cisplatinm; and cytotoxic proteins such as ribosomal inhibiting proteins like pokeweed antiviral protein, Pseudomonas exotoxin A, ricin, diphtheria toxin, ricin A chain, etc., or an agent active at the cell surface, such as the phospholipase enzymes (e.g., phospholipase C). (See, generally, commonly assigned U.S.S.N. 290,968 (Townsend and Townsend Docket No. 11823-7-2) filed in U.S.P.T.O. on December 28, 1988, "Chimeric Toxins," Olsnes and Phil, Pharmac. Ther., 25:355-381 (1982), and "Monoclonal Antibodies for Cancer Detection and Therapy," eds. Baldwin and Byers, pp. 159-179, 224-266, Academic Press (1985), all of which are incorporated herein by reference.)

The delivery component of the immunotoxin will include the humanized immunoglobulins of the present invention. Intact immunoglobulins or their binding fragments, such as Fab, are preferably used. Typically, the antibodies in the immunotoxins will be of the human IgM or IgG isotype, but other mammalian constant regions may be utilized as desired.

For diagnostic purposes, the antibodies may either be labeled or unlabeled. Unlabeled antibodies can be used in combination with other labeled antibodies (second antibodies) that are reactive with the humanized antibody, such as antibodies specific for human immunoglobulin constant regions. Alternatively, the antibodies can be directly labeled. A wide variety of labels may be employed, such as radionuclides, fluors, enzymes, enzyme substrates, enzyme co-factors, enzyme inhibitors, ligands (particularly haptens), etc. Numerous types of immunoassays are available and are well known to those skilled in the art.

The following examples are offered by way of illustration, not by limitation.

EXPERIMENTAL

Design of genes for humanized light and heavy chains

The sequence of the human antibody Eu (Sequences of Proteins of Immunological Interest, E. Kabat et al., U.S. Dept. of Health and Human Services, 1983) was used to provide the framework of the humanized antibody, because the amino acid sequence of the heavy chain variable region of anti-Tac is more homologous to the heavy chain of this antibody than to any other complete heavy chain variable region sequence in the National Biomedical Foundation Protein Identification Resource.

To select the sequence of the humanized heavy chain, the anti-Tac heavy chain sequence (see, commonly assigned U.S.S.N.'s 186,862 and 223,037, which are incorporated herein by reference) was aligned with the sequence of the Eu heavy chain (Figure 1). At each position, the Eu amino acid was selected for the humanized sequence, unless that position fell in any one of the following categories, in which case the anti-Tac amino acid was selected:

(1) The position fell within a complementarity determining region (CDR), as defined by Kabat, et al., op. cit. (amino acids 31-35, 50-66, 99-106);

(2) The Eu amino acid was rare for human heavy chains at that position, whereas the anti-Tac amino acid was common for human heavy chains at that position (amino acids 27, 93, 95, 98, 107-109, 111);

(3) The position was immediately adjacent to a CDR in the amino acid sequence of the anti-Tac heavy chain (amino acids 30 and 67); or

(4) 3-dimensional modeling of the anti-Tac antibody suggested that the amino acid was physically close to the antigen binding region (amino acids 48 and 68).

Amino acid #27 is listed in category (4) because the acceptor Eu amino acid Gly is rare, and the donor anti-Tac amino acid Tyr is chemically similar to the amino acid Phe, which is common, but the substitution was actually made because #27

also fell in category (4). Although some amino acids fell in more than one of these categories, they are only listed in one. Categories (2) - (4) correspond to criteria (2) - (4) described above.

To select the sequence of the humanized light chain, the anti-Tac light chain sequence was aligned with the sequence of the Eu light chain (Figure 2). The Eu amino acid was selected at each position, unless the position again fell into one of the categories (1) - (4) (with light chain replacing heavy chain in the category definitions):

(1) CDR's (amino acids 24-34, 50-56, 89-97);

(2) Anti-Tac amino acid more typical than Eu (amino acids 48 and 63);

(3) Adjacent to CDR's (no amino acids; Eu and anti-Tac were already the same at all these positions); or

(4) Possible 3-dimensional proximity to binding region (amino acid 60).

The actual nucleotide sequence of the heavy (Figure 3) and light chain (Figure 4) genes were selected as follows:

(1) The nucleotide sequences code for the amino acid sequences chosen as described above;

(2) 5' of these coding sequences, the nucleotide sequences code for a leader (signal) sequence, namely the leader of the light chain of the antibody MOPC 63 and the leader of the heavy chain of the antibody PCH 108A (Kabat et al., op. cit.). These leader sequences were chosen as typical of antibodies;

(3) 3' of the coding sequences, the nucleotide sequences are the sequences that follow the mouse light chain J5 segment and the mouse heavy chain J2 segment, which are part of the anti-Tac sequences. These sequences are included because they contain splice donor signals; and

(4) At each end of the sequence is an Xba I site to allow cutting at the Xba I sites and cloning into the Xba I site of a vector.

Construction of humanized light and heavy chain genes

To synthesize the heavy chain, four oligonucleotides HES12, HES13, HES14, HES15 (Figure 5A) were synthesized using an Applied Biosystems 380B DNA synthesizer. Two of the oligonucleotides are part of each strand of the heavy chain, and each oligonucleotide overlaps the next one by about 20 nucleotides to allow annealing (Figure 5B). Together, the oligonucleotides cover the entire humanized heavy chain variable region (Figure 3) with a few extra nucleotides at each end to allow cutting at the Xba I sites. The oligonucleotides were purified from polyacrylamide gels.

Each oligonucleotide was phosphorylated using ATP and T4 polynucleotide kinase by standard procedures (see, Maniatis, op. cit.). To anneal the phosphorylated oligonucleotides, they were suspended together in 40 ul of TA (33 mM Tris acetate, pH 7.9, 66 mM potassium acetate, 10 mM magnesium acetate) at a concentration of about 3.75 uM each, heated to 95 deg for 4 min. and cooled slowly to 4 deg. To synthesize the complete gene from the oligonucleotides by synthesizing the opposite strand of each oligonucleotide (Figure 5B), the following components were added in a final volume of 100ul:

10 ul	annealed oligonucleotides
0.16 mM each	deoxyribonucleotide
0.5 mM	ATP
0.5 mM	DTT
100 ug/ml	BSA
3.5 ug/ml	T4 g43 protein (DNA polymerase)
25 ug/ml	T4 g44/62 protein (polymerase accessory protein)
25 ug/ml	45 protein (polymerase accessory protein)

The mixture was incubated at 37 deg for 30 min. Then 10 u of T4 DNA ligase was added and incubation at 37 deg resumed for 30 min. The polymerase and ligase were inactivated by incubation of the reaction at 70 deg for

15 min. To digest the gene with Xba I, to the reaction was added 50 ul of 2x TA containing BSA at 200 ug/ml and DTT at 1 mM, 43 ul of water, and 50 u of Xba I in 5 ul. The reaction was incubated for 3 hr at 37 deg, and run on a gel. The 431 bp Xba I fragment was purified from a gel and cloned into the Xba I site of the plasmid pUC19 by standard methods. Four plasmid isolates were purified and sequenced using the dideoxy method. One of these had the correct sequence (Figure 3).

To synthesize the light chain, four oligonucleotides JFD1, JFD2, JFD3, JFD4 (Figure 6A) were synthesized. Two of the oligonucleotides are part of each strand of the light chain, and each oligonucleotide overlaps the next one by about 20 nucleotides to allow annealing (Figure 6B). Together, the oligonucleotides cover the entire humanized light chain variable region (Figure 4) with a few extra nuclectides at each end to allow cutting at the Xba I sites. The oligonucleotides were purified from polyacrylamide gels.

The light chain gene was synthesized from these oligonucleotides in two parts. 0.5 ug each of JFD1 and JFD2 were combined in 20 ul sequenase buffer (40 mM Tris-HCl, pH 7.5, 20 mM magnesium chloride, 50 mM sodium chloride), heated at 70 deg for 3 min and allowed to cool slowly tc 23 deg in order for the oligonucleotides to anneal. JFD3 and JFD4 were treated in the same way. Each reaction was made 10 mM in DTT and 0.5 mM in each deoxyribonucleotide and 6.5 u of sequenase (US Biochemicals) was added, in a final volume of 24 ul, and incubated for 1 hr at 37 deg to synthesize the opposite strands of the oligonucleotides. Xba I and Hind III were added to each reaction to digest the DNA (there is a Hind III site in the region where JFD2 and JFD3 overlap and therefore in each of the synthesized DNAs; Figure 6B). The reactions were run on polyacrylamide gels, and the Xba I - Hind III fragments were purified and cloned into pUC18 by standard methods. Several plasmid isolates for each fragment were sequenced by the dideoxy method, and correct ones chosen.

Construction of plasmids to express humanized light and heavy chains

The heavy chain Xba I fragment was isolated from the pUC19 plasmid in which it had been inserted and then inserted into the Xba I site of the vector pVγ1 (see, commonly assigned U.S.S.N. 223,037) in the correct orientation by standard methods, to produce the plasmid pHuGTAC1 (Figure 7). This plasmid will express high levels of a complete heavy chain when transfected into an appropriate host cell.

The two light chain Xba I - Hind III fragments were isolated from the pUC18 plasmids in which they had been inserted. The vector plasmid pVκ1 (see, commonly assigned U.S.S.N. 223,037) was cut with Xba I, dephosphorylated and ligated with the two fragments by standard methods. The desired reaction product has the circular form: vector - Xba I - fragment 1 - Hind III - fragment 2 - Xba I - vector. Several plasmid isolates were analyzed by restriction mapping and sequencing, and one with this form chosen. This plasmid, pHuLTAC (Figure 8), therefore contains the complete humanized light chain (Figure 4) and will express high levels of the light chain when transfected into an appropriate host cell.

Synthesis and affinity of humanized antibody

The plasmids pHuGTAC1 and pHuLTAC were transfected into mouse Sp2/0 cells, and cells that integrated the plasmids were selected on the basis of resistance to mycophenolic acid and/or hygromycin B conferred by the gpt and hyg genes on the plasmids (Figures 7,8) by standard methods. To verify that these cells secreted antibody that binds to the IL-2 receptor, supernatant from the cells was incubated with HUT-102 cells that are known to express the IL-2 receptor. After washing, the cells were incubated with fluorescein-conjugated goat anti-human antibody, washed, and analyzed for fluorescence on a FACSCAN cytofluorometer. The results (Figure 9A), clearly show that the humanized antibody binds to these cells, but not to Jurkat T-cells that do not express the IL-2 receptor (Figure 9D). As controls, the

original mouse anti-Tac antibody was also used to stain these cells (Figure 9B,C), giving similar results.

For further experiments, cells producing the humanized antibody were injected into mice, and the resultant ascites collected. Humanized antibody was purified to substantial homogeneity from the ascites by passage through an affinity column of goat anti-human immunoglobulin antibody, prepared on an Affigel-10 support (Bio-Rad Laboratories, Inc., Richmond, CA) according to standard techniques. To determine the affinity of the humanized antibody relative to the original anti-Tac antibody, a competitive binding experiment was performed. About 5×10^5 HUT-102 cells were incubated with known quantities (10 - 40 ng) of the anti-Tac antibody and the humanized anti-Tac antibody for 10 min at 4 deg. Then 100 ng of biotinylated anti-Tac was added to the cells and incubated for 30 min at 4 deg. This quantity of anti-Tac had previously been determined to be sufficient to saturate the binding sites on the cells, but not to be in large excess. Then the cells were washed twice with 2 ml of phosphate buffered saline (PBS) containing 0.1% sodium azide. The cells were then incubated for 30 min at 4 deg with 250 ng of phycoerythrin-conjugated avidin, which bound to the biotinylated anti-Tac already bound to the cells. The cells were washed again as above, fixed in PBS containing 1% paraformaldehyde, and analyzed for fluorescence on a FACSCAN cytofluorometer.

Use of increasing amounts (10 - 40 ng) of the anti-Tac antibody as competitor in the first step decreased the amount of biotinylated anti-Tac that could bind to the cells in the second step, and therefore the amount of phycoerythrin-conjugated avidin that bound in the last step, thus decreasing fluorescence (Figure 10A). Equivalent amounts (20 ng) of anti-Tac, and humanized anti-Tac used as competitor decreased the fluorescence to approximately the same degree (Figure 10B). This shows that these antibodies have approximately the same affinity, because if one had greater affinity, it would have more effectively competed

with the bictinylated anti-Tac, thus decreasing fluorescence more.

Biological properties of the humanized antibody

For optimal use in treatment of human disease, the humanized antibody should be able to destroy T-cells in the body that express the IL-2 receptor. One mechanism by which antibodies may destroy target cells is antibody-dependent cell-mediated cytotoxicity, abbreviated ADCC (Fundamental Immunology, Paul, W., Ed., Raven Press, New York (1984), at pg. 681), in which the antibody forms a bridge between the target cell and an effector cell such as a macrophage that can lyse the target. To determine whether the humanized antibody and the original mouse anti-Tac antibody can mediate ADCC, a chromium release assay was performed by standard methods. Specifically, human leukemia HUT-102 cells, which express the IL-2 receptor, were incubated with ^{51}Cr to allow them to absorb this radionuclide. The HUT-102 cells were then incubated with an excess of either anti-Tac or humanized anti-Tac antibody. The HUT-102 cells were next incubated for 4 hrs with either a 30:1 or 100:1 ratio of effector cells, which were normal purified human peripheral blood mononuclear cells that had been activated by incubation for about 20 hrs with human recombinant IL-2. Release of ^{51}Cr, which indicated lysis of the target HUT-102 cells, was measured and the background subtracted (Table 1). The results show that at either ratio of effector cells, anti-Tac did not lyse a significant number of the target cells (less than 5%), while the humanized antibody did (more than 20%). Hence, the humanized antibody is likely to be more efficacious than the original mouse antibody in treating T-cell leukemia or other T-cell mediated diseases.

TABLE 1

Percent ^{51}Cr release after ADCC

Antibody	Effector: Target ratio 30:1	100:1
Anti-Tac	4%	< 1%
Humanized anti-Tac	24%	23%

Although the present invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the appended claims.

WE CLAIM:

1. A method of designing a humanized immunoglobulin (Ig) chain having one or more complementarity determining regions (CDR's) from a donor Ig and a framework region from a human Ig, said method comprising: comparing the framework or variable region amino acid sequence of the donor Ig with corresponding sequences in a collection of human Ig chains; and selecting to provide the human Ig framework one of the about three most homologous sequences from the collection.

2. A method according to Claim 1, wherein the human Ig sequence is selected from a collection of at least about ten to twenty Ig chain sequences.

3. A method according to Claim 1, wherein the human Ig chain sequence selected has the highest homology in the collection to the donor Ig sequence.

4. A method according to Claim 1, wherein the human Ig framework sequence selected is at least about 65% homologous to the donor Ig framework sequence.

5. A method according to Claim 1, wherein the immunoglobulin chain is a heavy chain.

6. A method according to Claim 1, wherein the humanized Ig chain comprises a human constant region.

7. An immunoglobulin comprising two light/heavy chain pairs, wherein at least one chain is designed in accordance with Claim 1.

8. A method of designing a humanized immunoglobulin chain having a framework region from a human acceptor immunoglobulin and complementarity determining regions (CDR's) from a donor immunoglobulin capable of binding to an antigen, said method comprising the steps of substituting at least one human framework amino acid of the acceptor immunoglobulin with a corresponding amino acid from the donor immunoglobulin at a position in the immunoglobulins where:

(a) the amino acid in the human framework region of the acceptor immunoglobulin is rare for said position and the corresponding amino acid in the donor immunoglobulin is common for said position in human immunoglobulin sequences; or

(b) the amino acid is immediately adjacent to one of the CDR's; or

(c) the amino acid is predicted to have a side chain atom within about 3Å of the CDR's in a three-dimensional immunoglobulin model and to be capable of interacting with the antigen or with the CDR's of the humanized immunoglobulin.

9. A method according to Claim 8, wherein the humanized immunoglobulin chain comprises in addition to the CDR's at least three amino acids from the donor immunoglobulin chosen by criteria (a), (b) or (c).

10. A method according to Claim 9, wherein at least one of the amino acids substituted from the donor is immediately adjacent a CDR.

11. A method according to Claim 9, wherein said humanized immunoglobulin chain is a heavy chain.

12. An immunoglobulin comprising two light/heavy chain pairs, wherein at least one chain is designed in accordance with Claim 8.

13. An immunoglobulin according to Claim 12, which is specifically reactive with an antigen at an affinity of at least about 10^8 M^{-1} or stronger.

14. An immunoglobulin according to Claim 12, wherein the designed chain is a light chain comprising about 214 amino acids.

15. An immunoglobulin according to Claim 12, wherein the designed chain is a heavy chain comprising about 446 amino acids.

16. A DNA sequence which upon expression encodes a humanized immunoglobulin chain according to Claim 1 or Claim 8.

17. A method for improving the affinity of a humanized immunoglobulin (Ig) to an antigen, by replacing amino acids of the human Ig framework with amino acids from the donor Ig framework at positions where:

(a) the amino acid in the human framework region of the first immunoglobulin is rare for said position and the corresponding amino acid in the donor immunoglobulin is common for said position in human immunoglobulin sequences; or

(b) the amino acid is immediately adjacent to one of the CDR's; or

(c) the amino acid is predicted to have a side chain atom within about 3Å of the CDR's in a three-dimensional immunoglobulin model and to be capable of interacting with the antigen or the CDR's of the humanized immunoglobulin.

18. A method according to Claim 17, wherein the additional amino acids comprise up to three amino acids, each of which is immediately adjacent to one of the CDR's in the second Ig.

19. A method according to Claim 17, wherein the additional amino acids comprise one amino acid immediately adjacent to a CDR.

20. A method according to Claim 17, wherein the additional amino acids comprise at least two amino acids from the donor Ig which are predicted by modelling to be capable of interacting with the antigen or the CDR's.

21. A method according to Claim 20, wherein said two or more amino acids are predicted to be within about 3Å of the donor Ig CDR's.

22. A method according to Claim 17, wherein the humanized Ig has an affinity to the antigen within about 2 to 3 fold of the donor Ig.

23. A method according to Claim 17, wherein the antigen is a protein.

24. A method of producing a humanized immunoglobulin containing a heavy chain and a light chain designed in accordance with Claim 17, said method comprising:

culturing a host capable of expressing said heavy chain, said light chain, or both, under conditions suitable for production of said chains; and

recovering from the culture said humanized immunoglobulin.

25. A polynucleotide composition comprising a DNA sequence coding for a humanized immunoglobulin designed in accordance with Claim 17.

26. A method of producing an improved humanized immunoglobulin comprising expressing the polynucleotide composition of Claim 25.

27. A cell transformed with a polynucleotide composition according to Claim 25.

28. A composition comprising a humanized immunoglobulin secreted by a cell line according to Claim 24.

DESIGNING IMPROVED HUMANIZED IMMUNOGLOBULINS

ABSTRACT OF THE DISCLOSURE

Novel methods for designing humanized immunoglobulins having one or more complementarity determining regions (CDR's) from a donor immunoglobulin and a framework region from a human immunoglobulin comprising first comparing the framework or variable region amino acid sequence of the donor immunoglobulin to corresponding sequences in a collection of human immunoglobulin chains, and selecting as the human immunoglobulin one of the more homologous sequences from the collection. Each humanized immunoglobulin chain may comprise about 3 or more amino acids from the donor immunoglobulin in addition to the CDR's, usually at least one of which is immediately adjacent to a CDR in the donor immunoglobulin. The heavy and light chains may each be designed by using any one or all three additional position criteria. When combined into an intact antibody, the humanized immunoglobulins of the present invention will be substantially non-immunogenic in humans and retain substantially the same affinity as the donor immunoglobulin to the antigen, such as a protein or other compound containing an epitope.

11923-9
ATTORNEY DOCKET NO

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. I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

DESIGNING IMPROVED HUMANIZED IMMUNOGLOBULINS

the specification of which ☒ is attached hereto or ☐ was filed on _____ as Application Serial No. _____ and was amended on _____ (if applicable).

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 Title 37, Code of Federal Regulations, §1.56(a). I claim foreign priority benefits under Title 35, United States Code, §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 the application on which priority is claimed.

Prior Foreign Application(s)

COUNTRY	APPLICATION NUMBER	DATE OF FILING	PRIORITY CLAIMED UNDER 35 U.S.C. 119
			Yes____ No____
			Yes____ No____

I claim the benefit under Title 35, United States Code, §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 Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §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.	DATE OF FILING	STATUS
290,975	December 28, 1988	☐ Patented ☒ Pending ☐ Abandoned
		☐ Patented ☐ Pending ☐ Abandoned

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) who are partners and associates in the firm of Townsend and Townsend to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

William M. Smith, Reg. No. 30,223
James M. Heslin, Reg. No. 29,541
Steve W. Parmelee, Reg. No. 31,990

SEND CORRESPONDENCE TO:	DIRECT TELEPHONE CALLS TO (name, registration number, and telephone number)
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202	FULL NAME OF INVENTOR	Last Name: Selick	First Name: Harold		Middle Name or Initial: Edwin
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203	FULL NAME OF INVENTOR	Last Name:	First Name:		Middle Name or Initial:
	RESIDENCE & CITIZENSHIP	City:	State or Foreign Country:		Country of Citizenship:
	POST OFFICE ADDRESS	Post Office Address:	City:	State or Country:	Zip Code:

I further 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 issuing thereon.

Signature of Inventor 201	Signature of Inventor 202	Signature of Inventor 203
Cary Queen	Harold P. Selick	
Date: 2/13/89	Date: 2/10/89	Date:

BT (AA) 12-87

TOWNSEND AND TOWNSEND

Atty. Docket No. [illegible]

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS
(37 CFR 1.9(f) and 1.27(c)) – SMALL BUSINESS CONCERN

Applicant or Patentee: Cary L. Queen and Harold Edwin Selick
Serial No.: Not yet assigned Filing Date: February 13, 1989
Patent No.: Issued:
For: DESIGNING IMPROVED HUMANIZED IMMUNOGLOBULINS

I hereby declare that I am

[ ] the owner of the small business concern identified below:
[XX] an official of the small business concern empowered to act on behalf of the concern identified below

NAME OF CONCERN: PROTEIN DESIGN LABS, INC.
ADDRESS OF CONCERN: 3181 Porter Drive
Palo Alto, California 94304

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled DESIGNING IMPROVED HUMANIZED IMMUNOGLOBULINS by (inventor(s)) Cary L. Queen and Harold Edwin Selick described in

[XX] the application filed herewith
[ ] application serial no. , filed
[ ] patent no. , issued

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9(d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

NAME
ADDRESS
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

NAME
ADDRESS
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

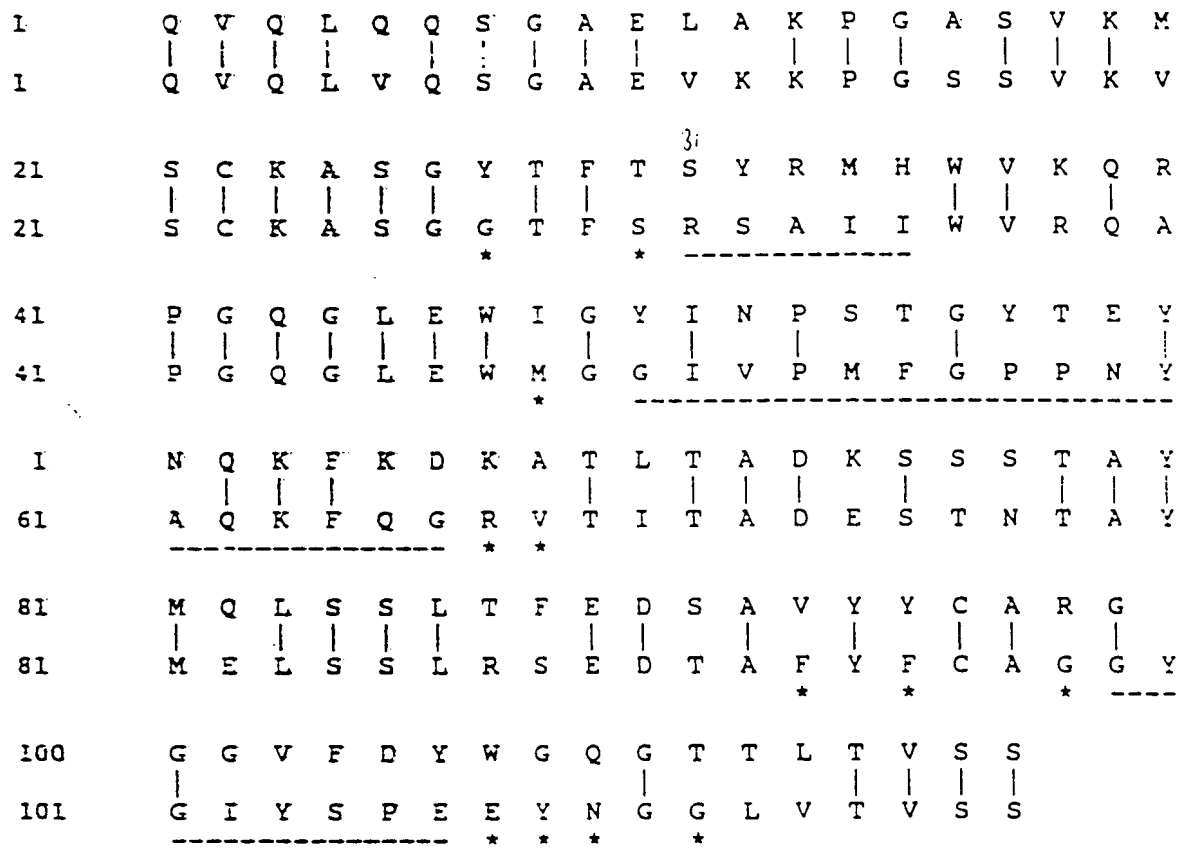
I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

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, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Shirley L. Clayton
TITLE OF PERSON OTHER THAN OWNER Chief Financial Officer
ADDRESS OF PERSON SIGNING Protein Design Labs, Inc., 3181 Porter Drive, Palo Alto, CA 94304

SIGNATURE Shirley L. Clayton DATE 2/13/89

FIGURE 1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81 M Q L S S L T F E D S A V Y Y C A R G
81 M E L S S L R S E D T A F Y F C A G G Y
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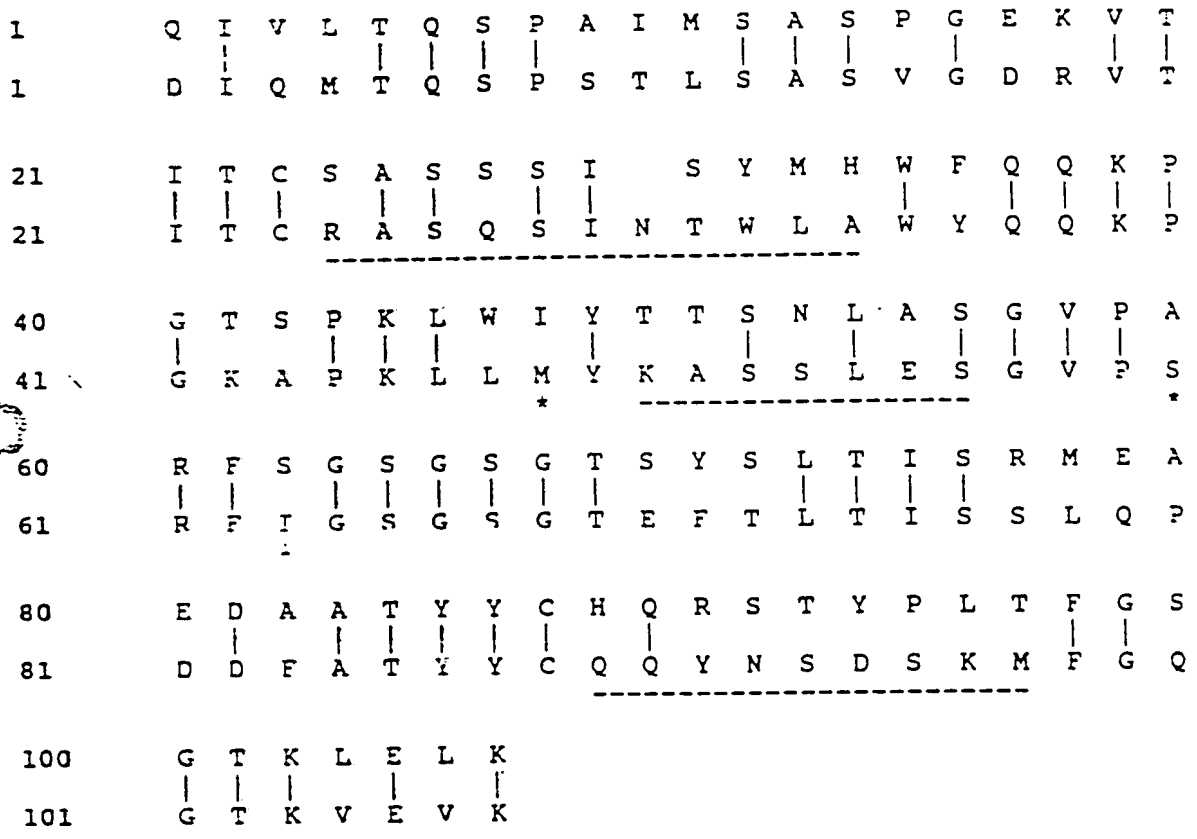
FIGURE 2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21 I T C R A S Q S I N T W L A W Y Q Q K P
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41 G K A P K L L M Y K A S S L E S G V P S
60 R F S G S G S G T S Y S L T I S R M E A
61 R F I G S G S G T E F T L T I S S L Q P
80 E D A A T Y Y C H Q R S T Y P L T F G S
81 D D F A T Y Y C Q Q Y N S D S K M F G Q
100 G T K L E L K
101 G T K V E V K

FIGURE 3

```
         10         20         30         40         50         60
TCTAGATGGGATGGAGCTGGATCTTTCTCTTCCTCCTGTCAGGTACCGCGGGCGTGCACT
      M  G  W  S  W  I  F  L  F  L  L  S  G  T  A  G  V  H

         70         80         90        100        110        120
CTCAGGTCCAGCTTGTCCAGTCTGGGGCTGAAGTCAAGAAACCTGGCTCGAGCGTGAAGG
S  Q  V  Q  L  V  Q  S  G  A  E  V  K  K  P  G  S  S  V  K

        130        140        150        160        170        180
TCTCCTGCAAGGCTTCTGGCTACACCTTTACTAGCTACAGGATGCACTGGGTAAGGCAGG
V  S  C  K  A  S  G  Y  T  F  T  S  Y  R  M  H  W  V  R  Q

        190        200        210        220        230        240
CCCCTGGACAGGGTCTGGAATGGATTGGATATATT. ATCCGTCGACTGGGTATACTGAAT
A  P  G  Q  G  L  E  W  I  G  Y  I  N  P  S  T  G  Y  T  E

        250        260        270        280        290        300
ACAATCACAAGTTCAAGGACAAGGCAACAATTACTGCAGACGAATCCACCAATACAGCCT
Y  N  Q  K  F  K  D  K  A  T  I  T  A  D  E  S  T  N  T  A

        310        320        330        340        350        360
ACATGGAACTGAGCAGCCTGAGATCTGAGGACACCGCAGTCTATTACTGTGCAAGAGGGG
Y  M  E  L  S  S  L  R  S  E  D  T  A  V  Y  Y  C  A  R  G

        370        380        390        400        410        420
GGGGGGTCTTTGACTACTGGGGCCAAGGAACCCTGGTCACAGTCTCCTCAGGTGAGTCCT
G  G  V  F  D  Y  W  G  Q  G  T  L  V  T  V  S  S

        430
TAAAACCTCTAGA
```

FIGURE 4

```
        10         20         30         40         50         60
TCTAGATGGAGACCGATACCCTCCTGCTATGGGTCCTCCTGCTATGGGTCCCAGGATCAA
      M  E  T  D  T  L  L  L  W  V  L  L  L  W  V  P  G  S

        70         80         90        100        110        120
CCGGACATATTCAGATGACCCAGTCTCCATCTACCCTCTCTGCTAGCGTCGGGGATAGGG
T  G  D  I  Q  M  T  Q  S  P  S  T  L  S  A  S  V  G  D  R

       130        140        150        160        170        180
TCACCATAACCTGCTCTGCCAGCTCAAGTATAAGTTACATGCACTGGTACCAGCAGAAGC
V  T  I  T  C  S  A  S  S  S  I  S  Y  M  H  W  Y  Q  Q  K

       190        200        210        220        230        240
CAGGCAAAGCTCCCAAGCTTCTAATTTATACCACATCCAACCTGGCTTCTGGAGTCCCTG
P  G  K  A  P  K  L  L  I  Y  T  T  S  N  L  A  S  G  V  P

       250        260        270        280        290        300
CTCGCTTCAGTGGCAGTGGATCTGGGACCGAGTTCACCCTCACAATCAGCTCTCTGCAGC
A  R  F  S  G  S  G  S  G  T  E  F  T  L  T  I  S  S  L  Q

       310        320        330        340        350        360
CAGATGATTTCGCCACTTATTACTGCCATCAAAGGAGTACTTACCCACTCACGTTCGGTC
P  D  D  F  A  T  Y  Y  C  H  Q  R  S  T  Y  P  L  T  F  G

       370        380        390        400
AGGGGACCAAGGTGGAGGTCAAACGTAAGTACACTTTTCTAGA
Q  G  T  K  V  E  V  K
```

FIGURE 5

A

HES12 AGCTTCTAGATGGGATGGAGCTGGATCTTTCTCTTCCTCCTGTCAGGTACCGCGGGCGTG
CACTCTCAGGTCCAGCTTGTCCAGTCTGGGGCTGAAGTCAAGAAACCTGGCTCGAGCGTG
AAGGTC

HES13 CCCAGTCGACGGATTAATATATCCAATCCATTCCAGACCCTGTCCAGGGGCCTGCCTTAC
CCAGTGCATCCTGTAGCTAGTAAAGGTGTAGCCAGAAGCCTTGCAGGAGACCTTCACGCT
CGAGCCAGG

HES14 TATATTAATCCGTCGACTGGGTATACTGAATACAATCAGAAGTTCAAGGACAAGGCAACA
ATTACTGCAGACGAATCCACCAATACAGCCTACATGGAACTGAGCAGCCTGAGATCTGAG
GACA

HES15 ATATCGTCTAGAGGTTTTAAGGACTCACCTGAGGAGACTGTGACCAGGGTTCCTTGGCCC
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AGGCTGCT

B

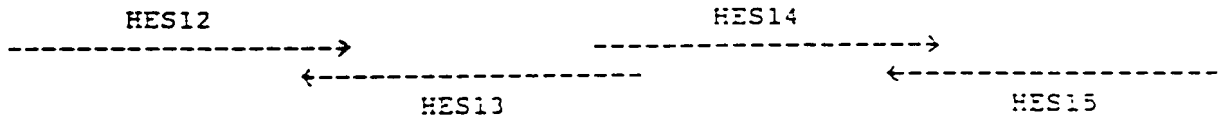

FIGURE 6

A

JFD1 CAAATCTAGATGGAGACCGATACCCTCCTGCTATGGGTCCTCCTGCTATGGGTCCCAGGA
TCAACCGGAGATATTCAGATGACCCAGTCTCCATCTACCCTCTCTGCTAGCGTCGGGGAT

JFD2 ATAAATTAGAAGCTTGGGAGCTTTGCCTGGCTTCTGCTGGTACCAGTGCATGTAACTTAT
ACTTGAGCTGGCAGAGCAGGTTATGGTGACCCTATCCCCGACGCTAGCAGAGAG

JFD3 GCTCCCAAGCTTCTAATTTATACCACATCCAACCTGGCTTCTGGAGTCCCTGCTCGCTTC
AGTGGCAGTGGATCTGGGACCGAGTTCACCCTCACAATCAGCTCTCTGCAGCCAGATGAT
TTC

JFD4 TATATCTAGAAAAGTGTACTTACGTTTGACCTCCACCTTGGTCCCCTGACCGAACGTGAG
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GA

B

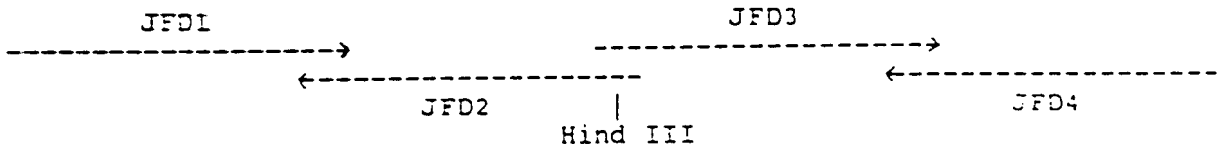


FIGURE 7

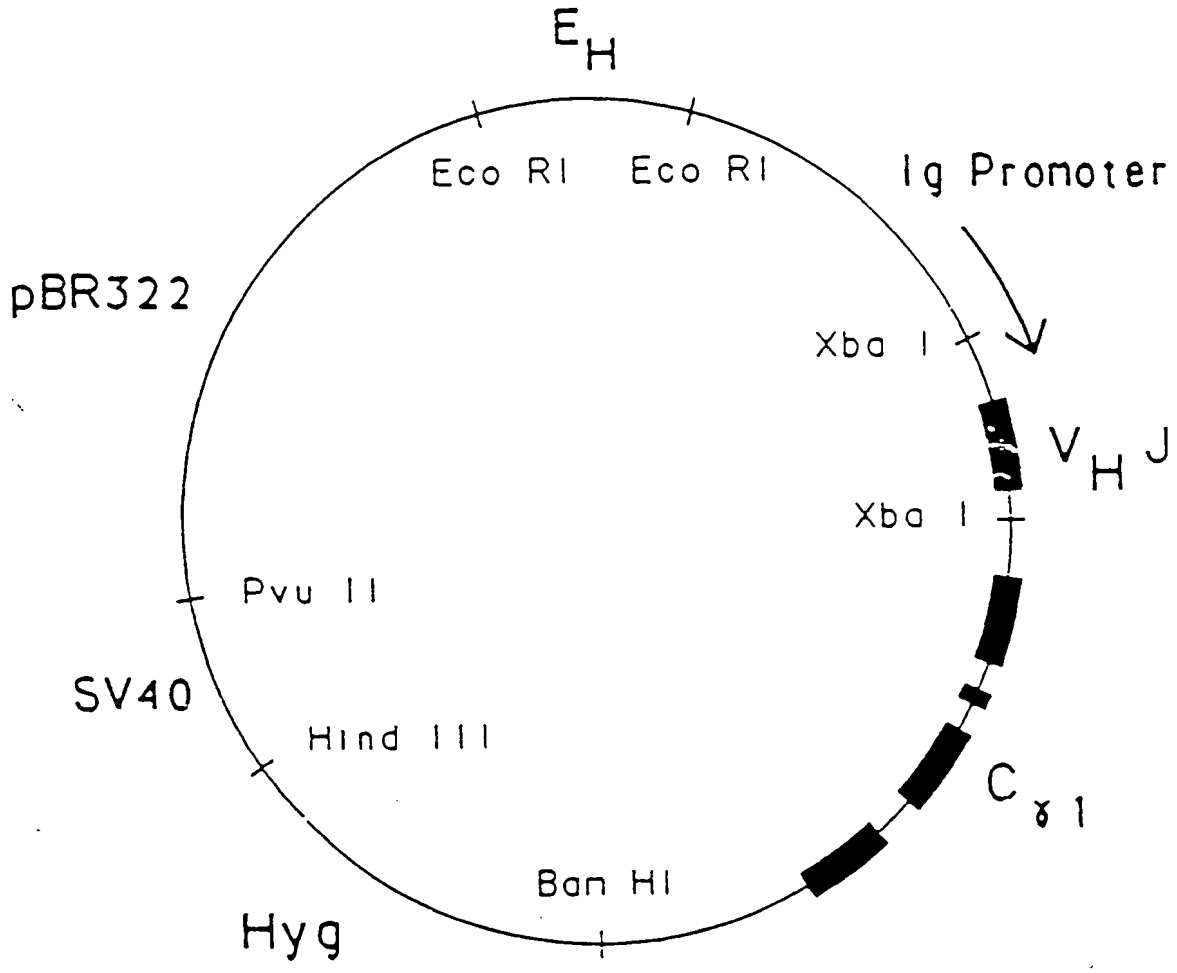
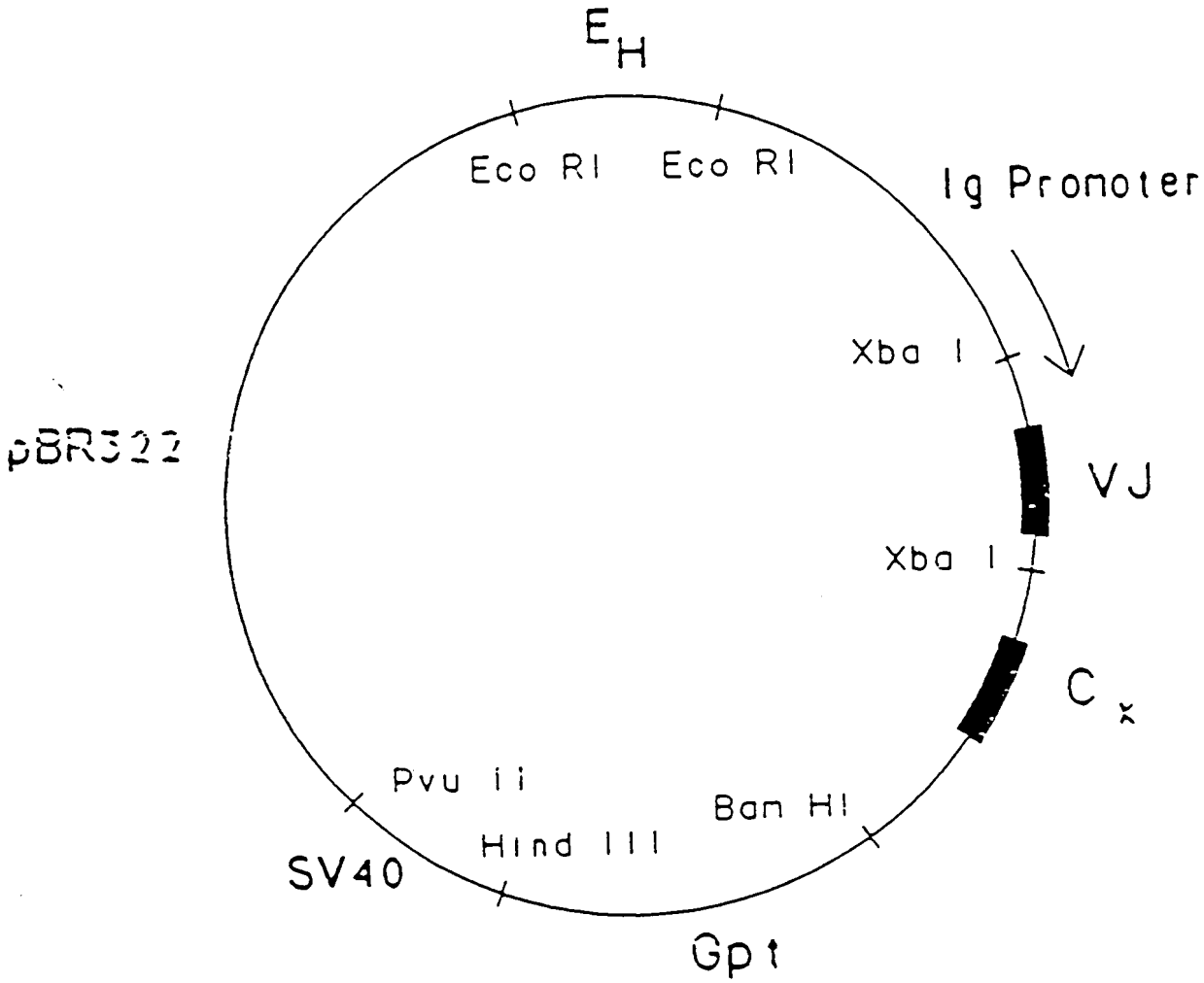

E_H
Eco RI
Eco RI
Ig Promoter
pBR322
Xba I
V_H J
Xba I
Pvu II
SV40
Hind III
$C_{\gamma 1}$
Ban HI
Hyg

FIGURE 8


E H
Eco RI
Eco RI
Ig Promoter
Xba I
VJ
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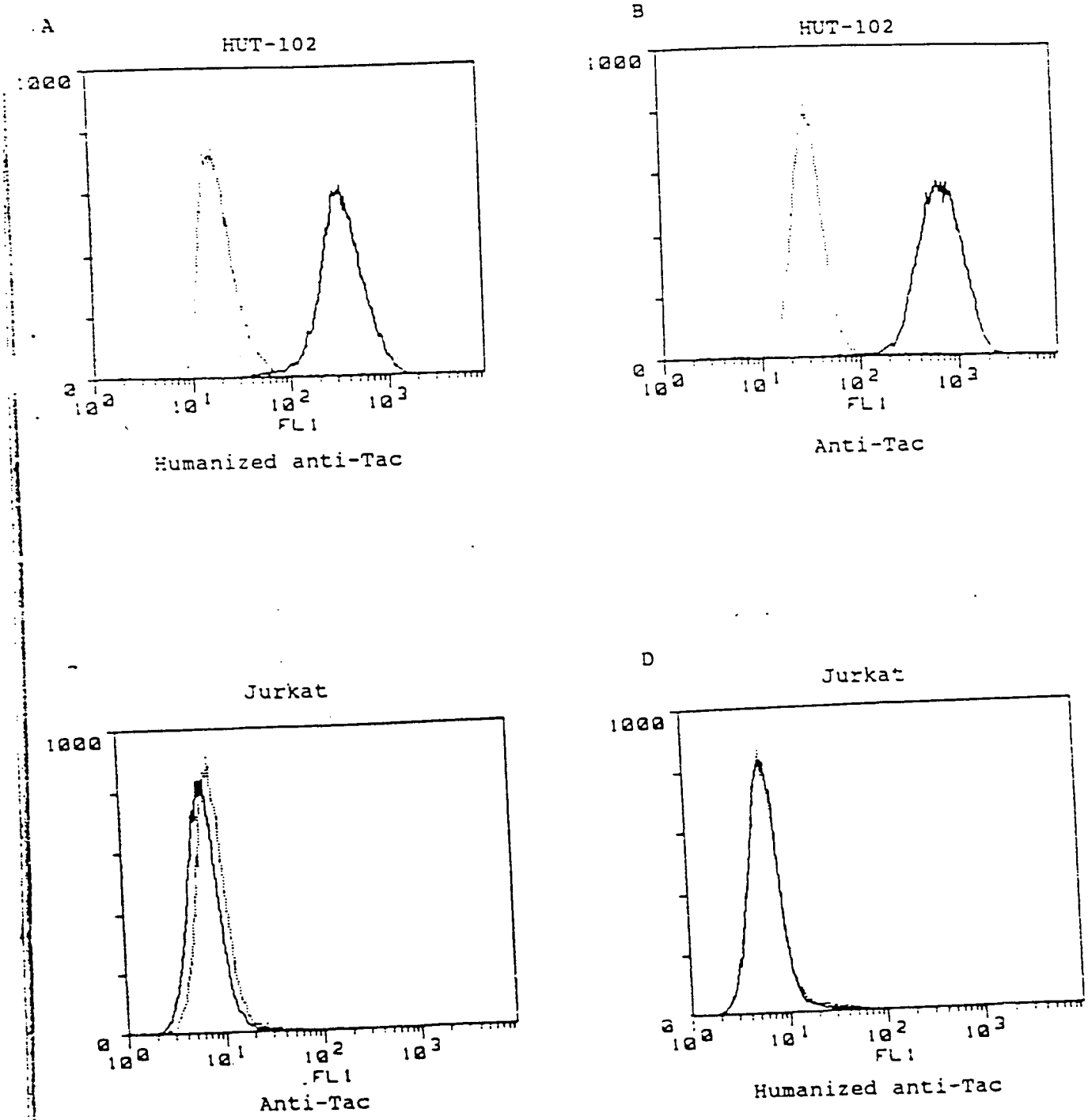
pBR322
Pvu II
Ban HI
Hind III
SV40
Gpt

FIGURE 9


A
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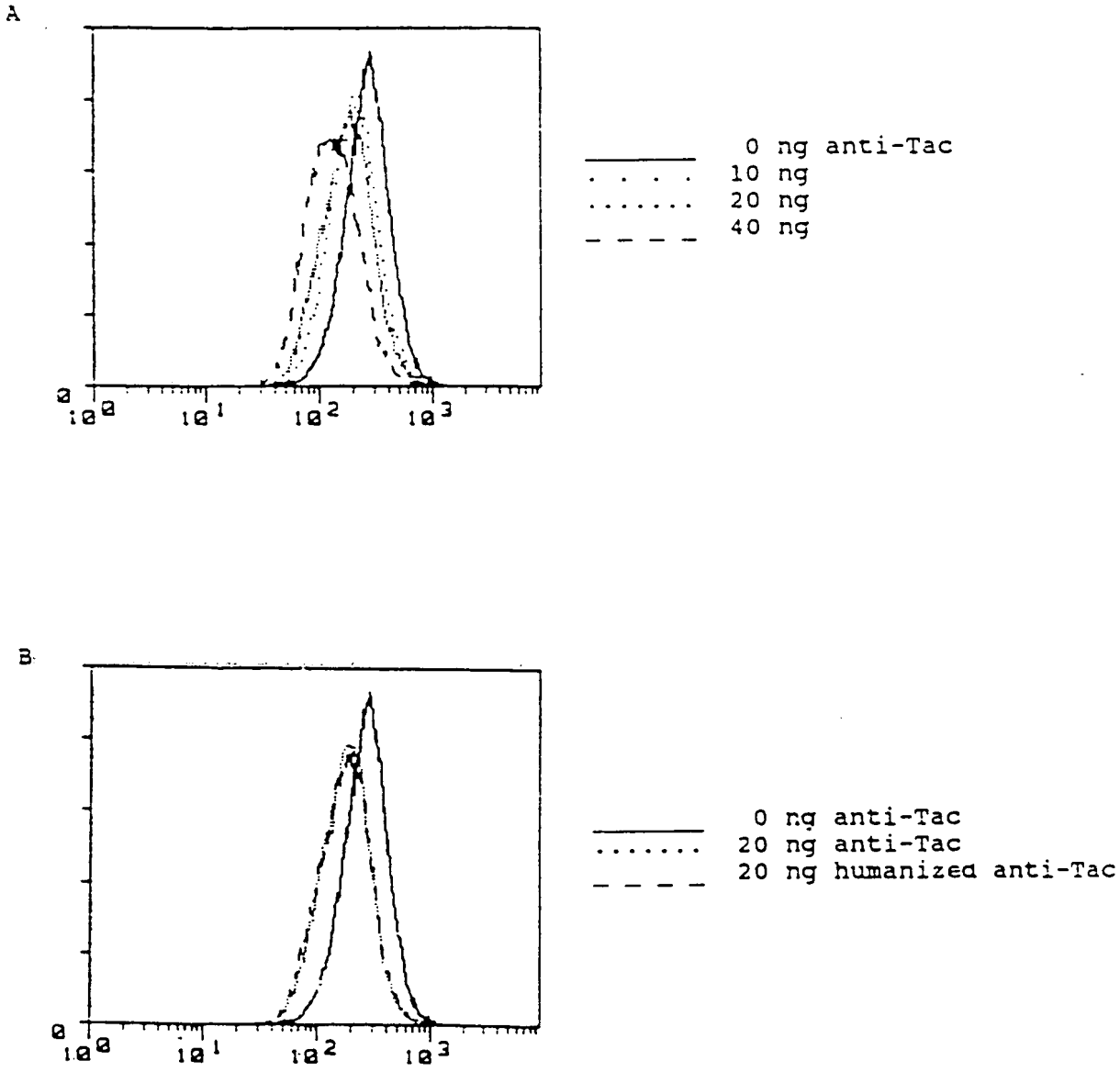
10^0
10^1
10^2
10^3
FL1
Humanized anti-Tac
B
HUT-102
1000
0
10^0
10^1
10^2
10^3
FL1
Anti-Tac
C
Jurkat
1000
0
10^0
10^1
10^2
10^3
FL1
Anti-Tac
D
Jurkat
1000
0
10^0
10^1
10^2
10^3
FL1
Humanized anti-Tac

FIGURE 10


A
0
10^0
10^1
10^2
10^3
0 ng anti-Tac
10 ng
20 ng
40 ng
B
0
10^0
10^1
10^2
10^3
0 ng anti-Tac
20 ng anti-Tac
20 ng humanized anti-Tac

#46/2R
02/11/99

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1642 Examiner: J. Reeves CERTIFICATE OF HAND DELIVERY I hereby certify that this correspondence is beinghand delivered in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on February 1, 1999 R. H. Mitchell

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

03/26/1999 TGRAY1 00000002 070630 08146206
01 FC:126 240.00 CH

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Applicants submit herewith patents, publications or other information (attached hereto and listed on the attached Form PTO-1449) of which they are aware, which they believe may be material to the examination of this application and in respect of which there may be a duty to disclose in accordance with 37 CFR §1.56.

This Information Disclosure Statement:

(a) ( ) accompanies the new patent application submitted herewith. 37 CFR §1.97(a).

(b) ( ) is filed within three months after the filing date of the application or within three months after the date of entry of the national stage of a PCT application as set forth in 37 CFR§1.491.

(c) ( ) as far as is known to the undersigned, is filed before the mailing date of a first Office action on the merits.

(d) ( ) is filed after the first Office Action and more than three months after the application's filing date or PCT national stage date of entry filing but, as far as is known to the undersigned, prior to the mailing date of either a final rejection or a notice of allowance, whichever occurs first, and is accompanied by either the fee ($240) set forth in 37 CFR §1.17(p) or a statement as specified in 37 CFR §1.97(e), as checked below. Should any fee be due, the U.S. Patent and Trademark Office is hereby authorized to charge Deposit Account No. 07-0630 in the amount of $240.00 to cover the cost of this Information Disclosure Statement. Any deficiency or overpayment

should be charged or credited to this deposit account. A duplicate of this sheet is enclosed.

(e) 0 is filed after the mailing date of either a final rejection or a notice of allowance, whichever occurred first, and is accompanied by the fee ($130) set forth in 37 CFR §1.17(i) and a statement as specified in 37 CFR §1.97(e), as checked below. This document is to be considered as a petition requesting consideration of the information disclosure statement. The U.S. Patent and Trademark Office is hereby authorized to charge Deposit Account No. 07-0630 in the amount of $130.00 to cover the cost of this Information Disclosure Statement. Any deficiency or overpayment should be charged or credited to this deposit account. A duplicate of this sheet is enclosed.

(f) (x) is filed after the mailing date of a final rejection, but a request to withdraw the finality thereof under 37 CFR § 1.129(a) was submitted on August 24, 1998. The U.S. Patent and Trademark Office is hereby authorized to charge Deposit Account No. 07-0630 to cover the cost of this Information Disclosure Statement in the event that any fees are due. A duplicate of this sheet is enclosed.

(If either of boxes (d) or (e) is checked above, the following statement under 37 CFR §1.97(e) may need to be completed.) The undersigned states that:

0 Each item of information contained in the information disclosure statement was cited in a communication mailed from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of this information disclosure statement.

0 No item of information contained in this information disclosure statement was cited in a communication mailed from a foreign patent office in a counterpart foreign application and, to the knowledge of the undersigned after making reasonable inquiry, was known to any individual designated in 37 CFR §1.56(c) more than three months prior to the filing of this information disclosure statement.

A list of the patent(s) or publication(s) is set forth on the attached Form PTO-1449 (Modified).

A copy of the items on PTO-1449 is supplied herewith:

(x) each 0 none 0 only those listed below:

A concise explanation of relevance of the items listed on PTO-1449 is:

(x) not given

0 given for each listed item

0 given for only non-English language listed item(s) (Required)

0 in the form of an English language copy of a Search Report from a foreign patent

office, issued in a counterpart application, which refers to the relevant portions of the references.

The Examiner is reminded that a "concise explanation of the relevance" of the submitted prior art "may be nothing more than identification of the particular figure or paragraph of the patent or publication which has some relation to the claimed invention," MPEP §609.

While the information and references disclosed in this Information Disclosure Statement may be "material" pursuant to 37 CFR §1.56, it is not intended to constitute an admission that any patent, publication or other information referred to therein is "prior art" for this invention unless specifically designated as such.

In accordance with 37 CFR §1.97(g), the filing of this Information Disclosure Statement shall not be construed to mean that a search has been made or that no other material information as defined in 37 CFR §1.56(a) exists. It is submitted that the Information Disclosure Statement is in compliance with 37 CFR §1.98 and MPEP §609 and the Examiner is respectfully requested to consider the listed references.

Respectfully submitted,
GENENTECH, INC.

Date: January 29, 1999 By: _____
Wendy M. Lee
Reg. No. 40,378

1 DNA Way
So. San Francisco, CA 94080-4990
Phone: (650) 225-1994
Fax: (650) 952-9881

Paper # 47
1642

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1642 Examiner: J. Reeves **CERTIFICATE OF MAILING** I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail with sufficient postage in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on March 9, 1999 Ann Savelli

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

Assistant Commissioner of Patents
Washington, D.C. 20231

Applicants submit herewith patents, publications or other information (attached hereto and listed on the attached Form PTO-1449) of which they are aware, which they believe may be material to the examination of this application and in respect of which there may be a duty to disclose in accordance with 37 CFR §1.56.

This Information Disclosure Statement:

(a) ( ) accompanies the new patent application submitted herewith. 37 CFR §1.97(a).

(b) ( ) is filed within three months after the filing date of the application or within three months after the date of entry of the national stage of a PCT application as set forth in 37 CFR§1.491.

(c) ( ) as far as is known to the undersigned, is filed before the mailing date of a first Office action on the merits.

(d) ( ) is filed after the first Office Action and more than three months after the application's filing date or PCT national stage date of entry filing but, as far as is known to the undersigned, prior to the mailing date of either a final rejection or a notice of allowance, whichever occurs first, and is accompanied by either the fee ($240) set forth in 37 CFR §1.17(p) or a statement as specified in 37 CFR §1.97(e), as checked below. Should any fee be due, the U.S. Patent and Trademark Office is hereby authorized to charge Deposit Account No. 07-0630 in the amount of $240.00 to cover

the cost of this Information Disclosure Statement. Any deficiency or overpayment should be charged or credited to this deposit account. A duplicate of this sheet is enclosed.

(e) 0 is filed after the mailing date of either a final rejection or a notice of allowance, whichever occurred first, and is accompanied by the fee ($130) set forth in 37 CFR §1.17(i) and a statement as specified in 37 CFR §1.97(e), as checked below. This document is to be considered as a petition requesting consideration of the information disclosure statement. The U.S. Patent and Trademark Office is hereby authorized to charge Deposit Account No. 07-0630 in the amount of $130.00 to cover the cost of this Information Disclosure Statement. Any deficiency or overpayment should be charged or credited to this deposit account. A duplicate of this sheet is enclosed.

(f) (x) is filed after the mailing date of a final rejection, but a request to withdraw the finality thereof under 37 CFR § 1.129(a) was submitted on August 24, 1998. The U.S. Patent and Trademark Office is hereby authorized to charge Deposit Account No. 07-0630 to cover the cost of this Information Disclosure Statement in the event that any fees are due. A duplicate of this sheet is enclosed.

(If either of boxes (d) or (e) is checked above, the following statement under 37 CFR §1.97(e) may need to be completed.) The undersigned states that:

0 Each item of information contained in the information disclosure statement was cited in a communication mailed from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of this information disclosure statement.

(x) No item of information contained in this information disclosure statement was cited in a communication mailed from a foreign patent office in a counterpart foreign application and, to the knowledge of the undersigned after making reasonable inquiry, was known to any individual designated in 37 CFR §1.56(c) more than three months prior to the filing of this information disclosure statement.

A list of the patent(s) or publication(s) is set forth on the attached Form PTO-1449 (Modified).

A copy of the items on PTO-1449 is supplied herewith:

(x) each 0 none 0 only those listed below:

A concise explanation of relevance of the items listed on PTO-1449 is:

(x) not given

0 given for each listed item

0 given for only non-English language listed item(s) (Required)

0 in the form of an English language copy of a Search Report from a foreign patent office, issued in a counterpart application, which refers to the relevant portions of the references.

The Examiner is reminded that a "concise explanation of the relevance" of the submitted prior art "may be nothing more than identification of the particular figure or paragraph of the patent or publication which has some relation to the claimed invention," MPEP §609.

While the information and references disclosed in this Information Disclosure Statement may be "material" pursuant to 37 CFR §1.56, it is not intended to constitute an admission that any patent, publication or other information referred to therein is "prior art" for this invention unless specifically designated as such.

In accordance with 37 CFR §1.97(g), the filing of this Information Disclosure Statement shall not be construed to mean that a search has been made or that no other material information as defined in 37 CFR §1.56(a) exists. It is submitted that the Information Disclosure Statement is in compliance with 37 CFR §1.98 and MPEP §609 and the Examiner is respectfully requested to consider the listed references.

Respectfully submitted,

GENENTECH, INC.

Date: March 9, 1999

By: _____

Wendy M. Lee
Reg. No. 40,378

1 DNA Way
So. San Francisco, CA 94080-4990
Phone: (650) 225-1994
Fax: (650) 952-9881

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
08/146,206	11/17/93	CARTER P	709P1

HM22/0329

GENENTECH, INC.
1 DNA WAY
SOUTH SAN FRANCISCO CA 94080-4990

EXAMINER	
REEVES, J	

ART UNIT	PAPER NUMBER
1642	48

DATE MAILED: 03/29/99

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary	Application No. 08/146,206	Applicant(s) Carter et al
	Examiner Julie E. Reeves, Ph.D.	Group Art Unit 1642

☒ Responsive to communication(s) filed on *Aug 26, 1998*.

☐ This action is **FINAL**.

☐ Since this application is 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.

A shortened statutory period for response to this action is set to expire *zero* month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

☒ Claim(s) *43-128* is/are pending in the application.

Of the above, claim(s) _____ is/are withdrawn from consideration.

☐ Claim(s) _____ is/are allowed.

☐ Claim(s) _____ is/are rejected.

☐ Claim(s) _____ is/are objected to.

☒ Claims *43-128* are subject to restriction or election requirement.

Application Papers

☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on _____ is ☐approved ☐disapproved.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been

☐ received.

☐ received in Application No. (Series Code/Serial Number) _____.

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____.

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☐ Notice of References Cited, PTO-892

☐ Information Disclosure Statement(s), PTO-1449, Paper No(s). _____

☒ Interview Summary, PTO-413 Paper # 43

☐ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

--- SEE OFFICE ACTION ON THE FOLLOWING PAGES ---

1. Restriction is required under 35 U.S.C. 121 and 372.

2. This application contains claims directed to more than one species of the generic invention. These species are deemed to lack unity of invention because they are not so linked as to form a single general inventive concept under PCT Rule 13.1.

The species are as follows:

Species A: 4L

Species B: 38L

Species C: 43L

Species D: 44L

Species E: 46L

Species F: 58L

Species G: 62L

Species H: 65L

Species I: 66L

Species J: 67L

Species K: 68L

Species L: 69L

Species M: 73L

Species N 85L

Species O: 98L

Species P: 2H

Species Q: 4H

Species R: 36H

Species S: 39H

Species T: 43H

Species U: 45H

Species V: 69H

Species W: 70H

Species X 74H

Species Y 75H

Species Z: 76H

Species AA: 78H

Species BB: 92H

Species CC: noncovalently binds antigen directly

Species DD: interacts with a CDR

Species EE: comprises a glycosylation site which affects the antigen binding or affinity of the antibody

Species FF: participates in the VL-VH interface by affecting the proximity or orientation of the VL and VH regions with respect to one another.

Species GG 24H

Species HH 73H

Species II 76H

Species JJ 78H

Species KK 93H

Applicant is required, in reply to this action, to elect a single species to which the claims shall be restricted if no generic claim is finally held to be allowable. The reply must also identify the claims readable on the elected species, including any claims subsequently added. An argument that a claim is allowable or that all claims are generic is considered non-responsive unless accompanied by an election.

Upon the allowance of a generic claim, applicant will be entitled to consideration of claims to additional species which are written in dependent form or otherwise include all the limitations of an allowed generic claim as provided by 37 CFR 1.141. If claims are added after the election, applicant must indicate which are readable upon the elected species. MPEP § 809.02(a).

3. The claims are deemed to correspond to the species A-BB listed above in the following manner: Claims 47-70 and claims 76-103 are limited to one of Species A-BB, respectively. Claims 107-110 are limited to one of the species CC-FF, respectively.

The following claim(s) are generic:

Claims 43-46, 71-75, 104-105 are generic for Species A-BB.

Claims 106, 111-114, 128 are generic for Species CC-FF.

Claims 115-118, 124-126 are generic for Species GG-KK

4. The species listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: each of the particular amino acid substitution positions recited in Species A-BB or GG-KK or each of the functional definitions of amino acid substitution changes recited in Species CC-FF result in different primary amino acid structure which would result in different secondary, tertiary, and quaternary structure yielding a protein with different biological, physiological and immunological properties, including different immunogenicity and antigen binding functions. Further, species EE, for example, recites the addition of a glycosylation site, which would involve the presence of a carbohydrate moiety and its affect on amino acid structure. The examination of all species would require the consideration of different patentability issues.

Applicant is advised that the reply to this requirement to be complete must include an election of the invention to be examined even though the requirement be traversed (37 CFR 1.143).

5. Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a petition under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

6. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Julie E. Reeves, Ph.D. whose telephone number is (703) 308-7553.

Julie E. Reeves, Ph.D.

JULIE REEVES
PATENT EXAMINER

Interview Summary

Application No.	Applicant(s)
08/146,206	Carter et al
Examiner	**Group Art Unit**
Julie E. Reeves, Ph.D.	1642

All participants (applicant, applicant's representative, PTO personnel):

(1) *Julie E. Reeves, Ph.D.* (3) _____

(2) *Wendy Lee* (4) _____

Date of Interview *Jan 7, 1999*

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. ☒ was not reached.

Claim(s) discussed: *all pending*

Identification of prior art discussed:
none

Description of the general nature of what was agreed to if an agreement was reached, or any other comments:
Applicant indicated that they intend to file a supplemental amdt.

(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 amendents which would render the claims allowable is available, a summary thereof must be attached.)

1. ☐ It is not necessary for applicant to provide a separate record of the substance of the interview.

Unless the paragraph above has 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. (See MPEP Section 713.04). If a response to the last Office action has already been filed, APPLICANT IS GIVEN ONE MONTH FROM THIS INTERVIEW DATE TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW.

2. ☐ 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. Applicant is not relieved from providing a separate record of the interview unless box 1 above is also checked.

J. Reeves
JULIE REEVES
PATENT EXAMINER

Examiner Note: You must sign and stamp this form unless it is an attachment to a signed Office action.

#49

Official Document - GENENTECH, INC.

1 DNA Way, South San Francisco, CA 94080-4990 Tel: 650-225-7039 Fax: 650-952-9881

FAX TRANSMISSION COVER SHEET

Date: April 9, 1999

To: Examiner J. Reeves — Group Art Unit: 1642 of US PTO

Fax: (703)308-4426

Re: U.S. Ser. No 08/146,206 filed November 17, 1993 (Attorney Docket No.: P0709P1)

Sender: Wendy M. Lee

CERTIFICATION OF FACSIMILE TRANSMISSION
I hereby certify that this paper is being facsimile transmitted to the Patent and Trademark Office on the date shown below.
Ann Savelli
Type or print name of person signing certification
Ann Savelli 4/9/99
Signature Date

YOU SHOULD RECEIVE 2 PAGES, INCLUDING THIS COVER SHEET. IF YOU DO NOT RECEIVE ALL THE PAGES, PLEASE CALL 650-225-7039

Comments:

Patent Docket P0709P1

#49

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1642 Examiner: J. Reeves

Response to Restriction Requirement

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Responsive to the Office Action dated March 29, 1999 and pursuant to the telephonic conversation between the undersigned and Examiner Reeves of today's date, Applicants hereby elect the species 78H ("Species AA" and "Species JJ"), with traverse. Claims readable on the elected species include claims 72-75, 102, 104, 105, 115-118, 122 and 124-127. Applicants traverse the restriction requirement to the extent that 37 CFR 1.129(b)(1) states that in applications such as the present application (which had been pending for at least three years as of June 8, 1995 taking into account reference made in the application under 35 USC 120 to USSN 07/715,272 filed June 14, 1991), "no requirement for restriction or for the filing of divisional applications shall be made or maintained in the application after June 8, 1995".

Respectfully submitted,
GENENTECH, INC.

Date: April 9, 1999

By: _____
Wendy M. Lee
Reg. No. 40,378

1 DNA Way
So. San Francisco, CA 94080-4990
Phone: (650) 225-1994
Fax: (650) 952-9881

Paper #30
6/11/99

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1642 Examiner: Julie Burke

COMMUNICATION

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

As requested by Examiner Julie Burke enclosed is the specification for USSN 07/715,272 (now abandoned) which is the priority document for the above-identified patent application.

Respectfully submitted,
GENENTECH, INC.

By: _____
Wendy M. Lee
Reg. No. 40,378

Date: June 9, 1999

1 DNA Way
So. San Francisco, CA 94080-4990
Phone: (650) 225-1994
Fax: (650) 952-9881

Interview Summary

Application No.	Applicant(s)
08/146,206	Carter et al
Examiner	**Group Art Unit**
Julie E. Burke, (Reeves), Ph.D.	1642

All participants (applicant, applicant's representative, PTO personnel):

(1) *Julie E. Burke, (Reeves), Ph.D.* (3) _____

(2) *Wendy Lee* (4) _____

Date of Interview *16 Jul 1999*

Type: [X] Telephonic [ ] Personal (copy is given to [ ] applicant [ ] applicant's representative).

Exhibit shown or demonstration conducted: [ ] Yes [X] No. If yes, brief description:

Agreement [ ] was reached. [X] was not reached.

Claim(s) discussed: *all pending*

Identification of prior art discussed:
none in detail

Description of the general nature of what was agreed to if an agreement was reached, or any other comments:
Examiner phoned to say the claims 43-44, 46-73, 75-105, 115-127 are in condition for allowance; claims 45, 74, 117 are objected to for not further limiting the independent claims; claims 111-112 are double patenting with claims reciting the VH subgroup III heavy chain consensus region, as allowed in 08/437,642, accordingly a terminal disclaimer is necessary for the allowance of claims 111-112; claims 106-110, 113-114 and 128 need further prosecution. Applicant elected to not procede with the allowance at this time. A supplemental amdt will be filed today and an interview has been scheduled 23rd August.

(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 amendents which would render the claims allowable is available, a summary thereof must be attached.)

1. [ ] It is not necessary for applicant to provide a separate record of the substance of the interview.

Unless the paragraph above has 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. (See MPEP Section 713.04). If a response to the last Office action has already been filed, APPLICANT IS GIVEN ONE MONTH FROM THIS INTERVIEW DATE TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW.

2. [ ] 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. Applicant is not relieved from providing a separate record of the interview unless box 1 above is also checked.

JBurke

JULIE BURKE
PRIMARY EXAMINER

Examiner Note: You must sign and stamp this form unless it is an attachment to a signed Office action.

7/16/99 *Official Document* - GENENTECH, INC. #51

1 DNA Way, South San Francisco, CA 94080-4990 Tel: 650-225-7039 Fax: 650-952-9881

FAX TRANSMISSION COVER SHEET

Date: July 16, 1999

To: Examiner Julie Burke — Group Art Unit: 1642 of US PTO

Fax: (703) 308-4426

Re: U.S. Ser. No 08/146,206 filed November 17, 1993 (Attorney Docket No.: P0709P1)

Sender: Wendy M. Lee

CERTIFICATION OF FACSIMILE TRANSMISSION
I hereby certify that this paper is being facsimile transmitted to the Patent and Trademark Office on the date shown below.

Wendy Lee
Type or print name of person signing certification

Signature

7/16/99
Date

YOU SHOULD RECEIVE 2 PAGE(S), INCLUDING THIS COVER SHEET. IF YOU DO NOT RECEIVE ALL THE PAGES, PLEASE CALL 650-225-7039

Comments:

Paper #51 Amd + K

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE 7/16/99 9L

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1642 Examiner: J. Burke

SUPPLEMENTAL AMENDMENT

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Further to the Supplemental Amendment dated January 15, 1999, please amend the present application as follows:

IN THE CLAIMS:

In line 3 of claims 43 and 115, please replace "further comprising an" with --further comprising a Framework Region (FR)--.

In line 4 of claim 72 please replace "further comprises an" with --further comprises a Framework Region (FR)--.

REMARKS

For claim precision, claims 43, 72 and 115 now refer to a Framework Region (FR) substitution, which provides anticedence for Framework Region (FR) in the claims which depend thereon.

Respectfully submitted,
GENENTECH, INC.

Date: July 16, 1999

By: _____
Wendy M. Lee
Reg. No. 40,378

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Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1644 Examiner: Julie Burke

CERTIFICATE OF FACSIMILE TRANSMISSION

Aug. 30, 1999 : Date of Transmission

I hereby certify that this correspondence, consisting of a Supplemental Amendment, is being facsimile transmitted to the Assistant Commissioner of Patents, Washington, D.C. 20231.

Ann Savelli

Ann Savelli

SUPPLEMENTAL AMENDMENT

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Further to the Supplemental Amendment dated July 16, 1999, please amend the present application as follows:

IN THE CLAIMS:

Please cancel claims 106-112, without prejudice.

In claim 113, line 9, after "one another", please insert --, wherein the humanized variant binds antigen up to about 3-fold more tightly than the parent antibody binds antigen--.

In claim 114, line 1, please delete "at least".

In claim 128, line 7, please insert --up to about 3-fold-- before "more tightly".

REMARKS

The undersigned confirms having met with Examiners Burke and Feisee in the interview August 23, 1999, and takes this opportunity to thank them for the courtesies extended in that interview.

As requested by Examiner Burke in the above interview, claims 113 and 128 have been revised, for claim precision, to refer to the humanized variant which binds antigen up to about 3-fold better than the parent antibody. Claims 113-114 and 128 have been revised herein in order to facilitate allowance of the present application and without acquiescing in any rejection. Basis for the revisions of these claims is found on at least page 70, lines 31-32 and in Table 3 on page 72. Aside from humanized anti-HER2 variants huMAb4D5-6 and huMAb4D5-8 in the present application, it is noted that humanized M195 has an affinity which is about 3-fold better than the parent antibody as recited in claim 128 (see first line on page 1153 of Co *et al. J. Immunol.* 148:1149-1154 (1992) (of record); and Caron *et al. Cancer Research* 52:6761-6767 (1992) (of record)).

To avoid the obviousness-type double patenting rejection of claim 111 over claim 47 of co-pending application USSN 08/437,642, Applicants have cancelled claims 111-112 herein, without prejudice to filing a continuing application directed thereto. In addition, in order to simplify prosecution, and without acquiescing in any objection or rejection, claims 106-110 have been cancelled. Applicants reserve the right to

file a continuing application directed to claims 106-110.

Examiner Burke suggested that claims 45, 74 and 117 be cancelled as not further limiting the independent claims on which they depend. The undersigned pointed out that, due to the use of the "comprising" language, claims 43, 72 and 115 clearly encompass humanized antibody variable domains or antibodies with one or more Framework Region (FR) substitutions, wherein at least one of those FR substitutions is set forth in the group of sites in the claims. Hence, claims 45, 74 and 117 are further limiting and need not be cancelled. The Examiner then asserted that, without an upper limit on the number of FR substitutions, independent claims 43, 72 and 115 could read on a prior art antibody with an intact murine variable domain. Applicants respectfully submit, in this regard, that given that these claims are directed to a "humanized" antibody variable domain or antibody, it is apparent that the claims cannot encompass antibodies with intact murine variable domains. This is apparent from page 2, lines 29-34 and page 10, lines 27-31.

Respectfully submitted,
GENENTECH, INC.

Date: August 30, 1999

By: _____
Wendy M. Lee
Reg. No. 40,378

1 DNA Way
So. San Francisco, CA 94080-4990
Phone: (650) 225-1994
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UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
08/146,206	11/17/93	CARTER P	709P1

HM22/1124

GENENTECH, INC.
1 DNA WAY
SOUTH SAN FRANCISCO CA 94080-4990

EXAMINER	
BURKE, J	

ART UNIT	PAPER NUMBER
1642	11

DATE MAILED: 11/24/99

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

08/146,206

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

08/146,206 11/17/93 BEYER DEA/FCE-1994 P 709P1 VS

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
		HM22/1124	

GENENTECH, INC.
1 DNA WAY
SOUTH SAN FRANCISCO CA 94080-4990

BURKE, J

EXAMINER	
1642	
ART UNIT	PAPER NUMBER
	11/24/99 55

DATE MAILED:

Please find below a communication from the EXAMINER in charge of this application

Commissioner of Patents

1. Please see attachment.

2. Any inquiry concerning this communication should be directed to Examiner Julie E. Burke, née Reeves, Ph.D, Art Unit 1642, whose telephone number is (703) 308-7553.

JBurke

JULIE BURKE
PRIMARY EXAMINER

Attachment

~~DETAILED ACTION~~

98

1. Since this application is eligible for the transitional procedure of 37 CFR 1.129(a), and the fee set forth in 37 CFR 1.17(r) has been timely paid, the finality of the previous Office action is hereby withdrawn pursuant to 37 CFR 1.129(a). Applicant's second submission after final filed on 8/26/98 has been entered.

2. The amendment to claim 113, filed 8/30/97 as Amendment L, Paper no 54 is not in compliance with 37 CFR 1.121 because more than five words are included in the amendment to the claim.

3. The application is not in compliance with the Sequence Requirements for the reasons set forth on the attached raw sequence listing error report. In brief, the application contains a new paper copy of the sequence listing containing 30 sequences, which was added by amendment G filed 10/7/97. The computer readable form of the sequences filed on the same day has only 26 sequences. Therefore the statements on page 3 of Paper no 32 filed 10/7/97 that the paper copy and computer readable form are the same is not sufficient. Additionally, it is not clear which new sequences have been added to the application, whether these sequences are new matter or whether the new sequences have unique SEQ ID NO:s.

4. Since the above-mentioned reply appears to be *bona fide*, and (1) in order to allow applicant the opportunity to amend the claims as they intend and (2) to complete the application with regards to Sequence Requirements, applicant is given a TIME PERIOD of **ONE (1) MONTH** or **THIRTY (30) DAYS**, from the mailing date of this notice, whichever is longer,

within which to supply the omission or correction in order to avoid abandonment.

EXTENSIONS OF THIS TIME LIMIT MAY BE GRANTED UNDER 37 CFR 1.136(a).

5. In an interest to complete the record of which papers have been entered in to the application, the following section is enclosed.

6. Claims 1-8, 10-12, 15 and 22-42 have been canceled and claims 43-114 added by Amendment H filed 9/26/98 as paper no 39 along with the Shak Declaration under 1.132.

7. Claims 43, 72, 104-106 and 112 have been amended by Amendment I, filed 11/6/98 as paper no 42.

8. Claims 43-44, 72-73, 104-106, 113-114 have been amended and claims 115-128 added by Amendment J filed 1/15/99 as Paper no 44.

9. Claims 43 and 72 have been amended By amendment K filed 7/16/99 as paper no 51.

10. Claims 106-112 have been canceled, claims 114 and 128 amended by amendment L field 8/30/99 as paper no 54. Please note in view of the noncompliance with 37 CFR 1.121, the amendment to claim 113 has not been entered.

11. Claims 43-105, 113-128 are pending and under examination.

12. It is noted that the Restriction Requirement set forth in Paper no 48 mailed 3/29/99 has been withdrawn in view of the arguments set forth in Paper no 49 filed 4/9/99.

13. Once the application is in compliance with the Sequence Requirements and the claims are amended as applicant's intended, the claims will be examined for their merits.

14. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Julie E. Burke, née Reeves, Ph.D, whose telephone number is (703) 308-7553. The examiner can normally be reached on Monday through Friday from 8:00 am to 5:30 pm, with alternate Fridays off. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Paula Hutzell, can be reached on (703) 308-4310. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

15. Papers related to this application may be submitted to Group 1600 by facsimile transmission. Papers should be faxed to Group 1600 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). The CM1 Fax Center telephone number is (703) 305-7401.

Respectfully,

JBurke

Julie E. Burke, née Reeves, Ph.D.

Primary Patent Examiner

(703) 308-7553

JULIE BURKE
PRIMARY EXAMINER

GP 1642 BOX SEQ WD

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1642 Examiner: J. Burke CERTIFICATE OF MAILING I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on December 22, 1999 Ann Savelli Ann Savelli

SUPPLEMENTAL AMENDMENT AND RESPONSE TO OFFICE COMMUNICATION

56 | m KD 1-11-00

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Responsive to the communication dated November 24, 1999, please amend the present application as follows:

IN THE SPECIFICATION:

On page 9, line 16, please replace "(I)" with --(•)--.

On page 9, line 16, please replace "(n)" with --(○)--.

On page 9, line 17, please replace "(I)" with --(□)--.

On page 62, line 3, please replace "12301 Parklawn Drive, Rockville, MD" with --10801 University Blvd., Manassas, VA--.

On page 84, line 3, please replace "(Rockville, MD)" with --(Manassas, VA)--.

Please replace the existing sequence listing in the specification with the attached sequence listing (pages 90-105).

IN THE CLAIMS:

Please amend claim 113 as follows:

113. (Twice Amended) A humanized variant of a non-human parent antibody which binds an antigen with better affinity than the parent antibody and comprises a consensus human variable domain 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, wherein the humanized variant binds antigen up to about 3-fold more tightly than the parent antibody binds antigen.

REMARKS

In the above communication, the Examiner states that the amendment to claim 113 filed 8/30/99 (Paper # 54) was not in compliance with 37 CFR 1.121. Accordingly, claim 113 is amended herein in a manner which complies with 37 CFR 1.121. Comments in paragraph 2 on page 2 of the 8/30/99 amendment with respect to the amendment of claim 113 are incorporated herein.

The Examiner further states in the above communication that the substitute sequence listing filed 10/7/97 is not in compliance with the sequence requirements. Applicants submit that their records indicate that the content of the CRF of the sequence listing filed 10/7/97 was indeed the same as the paper copy of that sequence listing filed 10/7/97. Nevertheless, a replacement sequence listing (paper copy and CRF) are filed herewith. In accordance with 37 CFR §§ 1.821 (f) and (g), the undersigned hereby states (a) that the content of the paper and computer readable sequence listings submitted herewith is the same; and (b) that this submission includes no new matter.

With respect to the attached sequence listing, Applicants point out that due to the nonprejudicial cancellation of claim 41 (which referred to SEQ ID NO's 27-30) in the 8/24/98 amendment, SEQ ID NO's 27-30 have been removed from the sequence listing filed herewith.

For the Examiner's convenience, Applicants will summarize here the differences between the presently-filed sequence listing, and the originally-filed (11/17/93) sequence listing:

1. SEQ ID NO:4 was corrected 10/7/97 to correspond to the HUV_HIII sequence in Fig. 1B.
2. SEQ ID NO:19 was corrected 6/2/94 to correspond to the muxCD3 sequence in Fig. 5.
3. SEQ ID NO:23 was amended 6/2/94 to correspond to the pH52-8.0 sequence in Fig. 6A.
4. SEQ ID NO:26 was added 9/2/97 for the huxCD3v1 sequence in Fig. 5.

Corrections to the specification have been made hereinabove as follows: The symbols from Fig. 3 have been corrected on page 9; and the ATCC address has been updated on pages 62 and 84. Applicants submit that no new matter is added by these amendments.

Further prosecution on the merits is anxiously awaited. Should the Examiner have any questions concerning this submission, she is invited to call the undersigned at the number noted below.

Respectfully submitted,
GENENTECH, INC.

Date: December 22, 1999

By: WM Lee
Wendy M. Lee
Reg. No. 40,378

1 DNA Way
So. San Francisco, CA 94080-4990
Phone: (650) 225-1994
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3

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Carter, Paul J.
Presta, Leonard G.

(ii) TITLE OF INVENTION: Method for Making Humanized Antibodies

(iii) NUMBER OF SEQUENCES: 26

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Genentech, Inc.
(B) STREET: 1 DNA Way
(C) CITY: South San Francisco
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 94080

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 3.5 inch, 1.44 Mb floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: WinPatin (Genentech)

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: 08/146206
(B) FILING DATE: 17-Nov-1993
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 07/715272
(B) FILING DATE: 14-JUN-1991

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Lee, Wendy M.
(B) REGISTRATION NUMBER: 40,378
(C) REFERENCE/DOCKET NUMBER: P0709P1

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 650/225-1994
(B) TELEFAX: 650/952-9881

(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
95 100 105

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

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
95 100 105

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                 100                 105

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                 100                 105

Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
           110                 115                 120
```

(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                 100                 105

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
95 100 105

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

(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

(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
95 100 105

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
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
95 100 105

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
95 100 105

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

Ser Ala Val Tyr Tyr Cys Ala Arg Ser Gly Tyr Tyr Gly Asp Ser
95 100 105

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
95 100 105

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
95 100 105

Ser Gly Leu Tyr Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val
110 115 120

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
95 100 105

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

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
95 100 105

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

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

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
95 100 105

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
95 100 105

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

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
95 100 105

Asp Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val
110 115 120

Ser Ser
122

J. Burke

RAW SEQUENCE LISTING
PATENT APPLICATION *US/08/146,206C*

INPUT SET: S34518.raw

This Raw Listing contains the General Information Section and up to the first 5 pages

SEQUENCE LISTING

(1) General Information:

(i) APPLICANT: Carter, Paul J.
Presta, Leonard G.

(ii) TITLE OF INVENTION: Method for Making Humanized Antibodies

(iii) NUMBER OF SEQUENCES: 26

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Genentech, Inc.
(B) STREET: 1 DNA Way
(C) CITY: South San Francisco
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 94080

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 3.5 inch, 1.44 Mb floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: WinPatin (Genentech)

(vi) CURRENT APPLICATION DATA:
--> **(A) APPLICATION NUMBER: 08/146206**
(B) FILING DATE: 17-Nov-1993
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 07/715272
(B) FILING DATE: 14-JUN-1991

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Lee, Wendy M.
(B) REGISTRATION NUMBER: 40,378
(C) REFERENCE/DOCKET NUMBER: P0709P1

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 650/225-1994
(B) TELEFAX: 650/952-9881

(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                 100                 105

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
```

```
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                 100                 105

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                 100                 105

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
```

```
153
154    Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
155                     20                  25                  30
156
157    Asp Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
158                     35                  40                  45
159
160    Glu Trp Val Ala Val Ile Ser Glu Asn Gly Ser Asp Thr Tyr Tyr
161                     50                  55                  60
162
163    Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser
164                     65                  70                  75
165
166    Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
167                     80                  85                  90
168
169    Thr Ala Val Tyr Tyr Cys Ala Arg Asp Arg Gly Gly Ala Val Ser
170                     95                 100                 105
171
172    Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
173                    110                 115                 120
174
175   (2) INFORMATION FOR SEQ ID NO:5:
176
177      (i) SEQUENCE CHARACTERISTICS:
178          (A) LENGTH: 109 amino acids
179          (B) TYPE: Amino Acid
180          (D) TOPOLOGY: Linear
181
182     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
183
184    Asp Ile Val Met Thr Gln Ser His Lys Phe Met Ser Thr Ser Val
185      1               5                  10                  15
186
187    Gly Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asp Val Asn
188                     20                  25                  30
189
190    Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly His Ser Pro Lys
191                     35                  40                  45
192
193    Leu Leu Ile Tyr Ser Ala Ser Phe Arg Tyr Thr Gly Val Pro Asp
194                     50                  55                  60
195
196    Arg Phe Thr Gly Asn Arg Ser Gly Thr Asp Phe Thr Phe Thr Ile
197                     65                  70                  75
198
199    Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln
200                     80                  85                  90
201
202    His Tyr Thr Thr Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu
203                     95                 100                 105
204
205    Ile Lys Arg Ala
```

206 109
207
208 (2) INFORMATION FOR SEQ ID NO:6:
209
210 (i) SEQUENCE CHARACTERISTICS:
211 (A) LENGTH: 120 amino acids
212 (B) TYPE: Amino Acid
213 (D) TOPOLOGY: Linear
214
215 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
216
217 Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly
218 1 5 10 15
219
220 Ala Ser Leu Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys
221 20 25 30
222
223 Asp Thr Tyr Ile His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu
224 35 40 45
225
226 Glu Trp Ile Gly Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr
227 50 55 60
228
229 Asp Pro Lys Phe Gln Asp Lys Ala Thr Ile Thr Ala Asp Thr Ser
230 65 70 75
231
232 Ser Asn Thr Ala Tyr Leu Gln Val Ser Arg Leu Thr Ser Glu Asp
233 80 85 90
234
235 Thr Ala Val Tyr Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr
236 95 100 105
237
238 Ala Met Asp Tyr Trp Gly Gln Gly Ala Ser Val Thr Val Ser Ser
239 110 115 120
240
241 (2) INFORMATION FOR SEQ ID NO:7:
242
243 (i) SEQUENCE CHARACTERISTICS:
244 (A) LENGTH: 27 base pairs
245 (B) TYPE: Nucleic Acid
246 (C) STRANDEDNESS: Single
247 (D) TOPOLOGY: Linear
248
249 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:
250

SEQUENCE VERIFICATION REPORT
PATENT APPLICATION *US/08/146,206C*

DATE: 01/20/2000
TIME: 01:04:05

INPUT SET: S34518.raw

Line	Error	Original Text
27	Wrong application Serial Number	(A) APPLICATION NUMBER: 08/146206

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
08/146,206	11/17/93	CARTER	P 709P1

HM22/1025

GENENTECH, INC.
1 DNA WAY
SOUTH SAN FRANCISCO CA 94080-4990

EXAMINER	
DAVIS,M	
ART UNIT	**PAPER NUMBER**
1642	

DATE MAILED: 10/25/00

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary

Application No.	Applicant(s)
09/146,206	
Examiner	Group Art Unit
	1642

—The MAILING DATE of this communication appears on the cover sheet beneath the correspondence address—

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 (MONTH(S)) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, such period shall, by default, expire SIX (6) MONTHS from the mailing date of this communication .
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).

Status

☒ Responsive to communication(s) filed on 8/30/99.

☐ This action is **FINAL.**

☐ Since this application is 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. 1 1; 453 O.G. 213.

Disposition of Claims

☒ Claim(s) 43-105, 113-128 is/are pending in the application.

Of the above claim(s)_____ is/are withdrawn from consideration.

☐ Claim(s)_____ is/are allowed.

☒ Claim(s) 43-105, 113-128 is/are rejected.

☐ Claim(s)_____ is/are objected to.

☐ Claim(s)_____ are subject to restriction or election requirement.

Application Papers

☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The proposed drawing correction, filed on_____ is ☐ approved ☐ disapproved.

☐ The drawing(s) filed on_____ is/are objected to by the Examiner.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119 (a)-(d)

☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 11 9(a)-(d).

☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been

☐ received.

☐ received in Application No. (Series Code/Serial Number)_____.

☐ received in this national stage application from the International Bureau (PCT Rule 1 7.2(a)).

*Certified copies not received:_____.

Attachment(s)

☐ Information Disclosure Statement(s), PTO-1449, Paper No(s)._____

☒ Notice of Reference(s) Cited, PTO-892

☐ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Interview Summary, PTO-413

☐ Notice of Informal Patent Application, PTO-152

☐ Other_____

Office Action Summary

Effective February 7, 1998, the Group Art Unit location has been changed, and the examiner of the application has been changed. To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Minh-Tam Davis, Group Art Unit 1642.

Since this application is eligible for the transitional procedure of 37 CFR 1.129(a), and the fee set forth in 37 CFR 1.17(r) has been timely paid, the finality of the previous office action has been withdrawn pursuant to 37 CFR 1.129(a). Applicant's amendment filed on 08/26/98 has been entered.

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Applicant cancels claims 106-112, and adds new claims 115-128, which are related to claims 43-105, and are not new matter.

Accordingly, claims 43-105, 113-128 are being examined.

The following are the remaining rejections.

REJECTION UNDER 35 USC 112 FIRST PARAGRAPH, SCOPE, NEW REJECTION

Claims 43-105, 113-128 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for humanized antibody muMAb4D5, and an anti-CD3 antibody, or variable domains thereof, comprising CDR amino acids which bind specifically to p185, or CD3, does not reasonably provide enablement for any humanized antibody , or variable

domain thereof, comprising CDR amino acids which binds non-specifically to any antigen, wherein the framework region amino acids are substituted 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, or of 24H, 73H, 76H, 78H and 93H, for treating any chronic diseases. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Claims 43-105, 113-128 are drawn to a humanized antibody , or variable domain thereof, comprising CDR amino acids which bind an antigen, or which bind $p185^{HER2}$. The framework region amino acids of said antibody or variable domain are substituted 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, or of 24H, 73H, 76H, 78H and 93H. Claim 105 is further drawn to a humanized antibody which lacks immunogenicity upon repeated administration for treating a chronic disease, and wherein its non-human CDR amino acids bind an antigen.

The specification discloses examples of humanized antibody muMAb4D5, anti-CD3, and anti-CD18 antibody, or variable domain thereof, comprising CDR amino acids which bind specifically to p185, CD3, and CD18, respectively. The substituted framework residues for the heavy chain of antibody muMAb4D5 are amino acids number 71, 73, 78, 93, and for the light chains are amino acid number 66 (table 3, and p.68). Only one humanized antibody, huMab4D5-8,

with all of the above five substitutions in the framework region binds to p185 3-fold more tightly than the murine counterpart. The humanized antibodies, huMab4D5-2 and huMab4D5-3, with one and four substitutions in the framework region, respectively, are, however, at least 10-fold less potent than the murine counterpart, having a K_d of 4.7nM and 4.4nM, respectively, as compared to a K_d value of 0.30nM of the murine counterpart. The substituted framework residues for the heavy chain of antibody anti-CD3 are amino acids number 75 and 76. Although the specification discloses that humanized anti-CD3 antibody enhances the cytotoxic effects of cytotoxic T cells 4-fold against tumor cells expressing $p185^{HER2}$, there is no disclosure in the specification concerning the binding affinity of the humanized anti-CD3 or anti-CD18 as compared to the murine counterpart. The claims however encompass any humanized antibody, without any specificity, binding to $p185^{HER2}$ or any antigen, with just any one of 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, of 24H, 73H, 76H, 78H and 93H. The claims further encompass any humanized antibody for treating any chronic disease.

One cannot extrapolate from humanizing one antibody, which binds to $p185^{HER2}$ 3-fold more tightly than the murine counterpart, to humanizing any antibody, wherein its affinity would be up to 3-fold or at least 3-fold more tightly than the murine counterpart, or wherein its affinity would be still intact for therapeutic purposes. In addition, one cannot extrapolate from humanizing an anti-p185 antibody by substitution at all five framework amino acids number H71, H73, H78, H93 and L66 in an anti-p185 antibody, or from humanizing an anti-CD3 antibody by

substitution at both framework amino acids number H75 and H76 in an anti-CD3 antibody, with humanizing any antibody by substitution at only any one amino acid 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, or of 24H, 73H, 76H, 78H and 93H. Patent '101 teach that different antibodies require different combinations of different substitutions in the light chain and heavy chain (table 1). Even the specification discloses that only one variant, huMab4D5-8, wherein all five framework amino acids number H71, H73, H78, H93 and L66 are substituted, binds to p185 3-fold more tightly than the murine counterpart. Other variants, with only one or even four substitutions have much less binding affinity than the murine counterpart(table 3). Thus it is unpredictable that substitution at only one framework amino acid in any antibody, or any kind of combination of framework amino acid substitutions would result in a humanized antibody that binds to its antigen 3-fold more tightly than its murine counterpart, or retains adequate affinity for therapeutic purposes. The specification does not disclose whether subtitution at only one of the claimed amino acid positions would produce a humanized antibody that has 3-fold more in affinity as the murine counterpart, or retains adequate affinity for therapeutic purposes. The specification does not disclose which combination of what substituted framework amino acids, other than H71, H73, H78, H93 and L66 for anti-p185 antibody, and H75 and H76 in anti-CD3 antibody would produce a humanized antibody that has 3-fold more in affinity as the murine counterpart, or retains adequate affinity for therapeutic purposes. It is well known in the art that not any substitution at any amino acids would produce a humanized

antibody having an affinity similar to the murine counterpart, unless it is tested by binding assays. The specification provides insufficient guidance with regard to the issues raised above and provides no working examples which would provide guidance to one skilled in the art and no evidence has been provided which would allow one of skill in the art to make the claimed humanized antibodies with a reasonable expectation of success. In view of the above, one of skill in the art would be forced into undue experimentation to practice the claimed invention.

Moreover, a humanized antibody that does not have a specificity for a particular antigen is of little practical use for treating a chronic disease, because said antibody would not target to the target tissues. In addition, although the specification discloses that murine anti-$p185^{HER2}$ antibody has been suceessfully used in treating tumor cell growth in culture (p.5), $p185^{HER2}$ and CD-3 are not specific for any tissues responsible for chronic disease, e.g. chronic headache, chronic lung inflammation, or chronic kidney disease. The specification does not disclose how to treat any chronic disease using the claimed humanized antibody. In the absence of a teaching of a method of treating any chronic disease, using the claimed humanized antibody, one of skill in the art would be forced into undue experimentation to practice the claimed invention.

REJECTION UNDER 35 USC 102, NEW REJECTION

1. New claims 115-117, 123, 127 are rejected under 35 USC 102(e) or 102(b) pertaining to anticipation by PN=5,530,101 or Queen et al, 1989, PNAS, USA, 86: 10029-10033.

Claims 115-117, 123, 127 are drawn to a humanized antibody or its heavy chain variable domain comprising non-human CDR amino acids, and a framework region amino acid wherein amino acid position 93H is substituted, utilizing the numbering system of Kabat, and wherein the substituted residue is the residue found in the corresponding location of the non-human antibody.

PN=5,530,101, teach humanized anti-Tac antibody, wherein amino acid 93 is substituted in heavy chain, using the aligned Kabat Eu sequence to provide the framework for the humanized antibody (column 45).

Queen et al, PNAS, teach humanized anti-Tac antibody, wherein amino acid 93 is substituted in heavy chain, using the aligned Kabat Eu sequence to provide the framework for the humanized antibody (figure 2).

Since anti-Tac antibody is a mouse antibody, its inherent heavy chain variable domain would comprise non-human CDR amino acids. Thus the humanized antibody and its heavy chain variable domain taught by patent '101 or Queen et al is the same as the claimed invention.

2. Claims 43, 44, 48, 55, 67, 71, 105, 115-117, 120, 127 are rejected under 35 USC 102(e) pertaining to anticipation by PN=5,530,101.

It is noted that PN=5,530,101 is filed on Sept, 1990, which is within a year before the claimed filing date of 06/14/91.

Claims 43, 44, 48, 55, 67, 71, 105, 115-117, 120, 127 are drawn to a humanized antibody or its heavy chain variable domain comprising non-human CDR amino acids, and a framework region amino acid wherein amino acid position 38L, 67L, 69H, 73H or 93H is substituted,

utilizing the numbering system of Kabat, and wherein the substituted residue is the residue found in the corresponding location of the non-human antibody. Claim 105 is further drawn to said humanized antibody which lacks immunogenicity compared to a non-human parent antibody upon repeated administration to a human patient.

PN=5,530,101 teaches humanized antibodies, wherein amino acid 38 or 67 are substituted in light chain (table 1, antibody Fd79 and M195, respectively), and amino acid 69, 73 or 93 is substituted in heavy chain (table 1, antibody CMV5, mik-beta-1, and Fd138-80, respectively), using the aligned Kabat Eu sequence to provide the framework for the humanized antibody. The humanized antibodies in table 1 would comprise non-human CDR amino acids (Summary). Patent '101 further teaches that the humanized antibodies will be substantially non-immunogenic in humans (Abstract). Thus the humanized antibody taught by patent '101 and its variable domain is the same as the claimed invention.

REJECTION UNDER 35 USC 102

1. Claim 128 is rejected under 35 USC 102(e) as being anticipated by PN=5,530,101, for the same reasons set forth in paper No.27 for the rejection of previous claims 23-24.

Applicant amends the claim 128 to read that the humanized antibody binds the antigen up to about 3-fold more tightly than the parent antibody. The language "up to" 3-fold reads on anything below 3-fold. Thus the structure and binding affinity of the claimed humanized antibody is the same as that of the humanized antibody taught by '101.

2. Claim 113 is rejected under 35 USC 102(e) as being anticipated by PN=5,693,762, for the same reasons set forth in paper No.27 for the rejection of previous claims 22-25, 38 and 39.

Applicant argues that the "consensus sequence" in '762 is the most homologous sequence from a single human immunoglobulin, and is thus different from the consensus sequence of the claimed invention.

Applicant's arguments set forth in paper No. 39 have been considered but are not deemed to be persuasive for the following reasons:

Although '762 uses the most homologous sequence from a single human immunoglobulin as an example, '762 also teach that as a principle, a framework is used from either a human immunoglobulin which is unusually homologous to the donor immunoglobulin, **or** a consensus framework from many human antibodies is used (column 13, first paragraph, lines 4-7). Thus the consensus sequence taught by '762 is the same as the claimed consensus sequence, as defined by the specification, i.e. the most frequently occurring amino acids, based on immunoglobulin of a particular species (p.14).

REJECTION UNDER 35 USC 103

Claims 113, 115-118, 123, 127-128 are rejected under 35 USC 103 as being unpatentable over US PN=5,693,762 in view of Kabat et al, for the same reasons set forth in paper No:27, for the rejection of previous claims 26-36 and 40-41.

Applicant argues as follows:

The rejection is made using hindsight reconstruction of the present invention. Patent '762 actually teaches away from the invention. The term "consensus framework" from '762 patent was not intended to refer to a sequence representing the most frequently occurring amino acids in the present invention. Furthermore, Kabat et al do not use the term "consensus", but rather "occurrences of most common amino acid". Thus there is no motivation to combine "consensus framework" from '762 patent with "occurrences of most common amino acid", especially the term "consensus framework" from '762 patent was not intended to refer to a sequence representing the most frequently occurring amino acids. Moreover, the present invention produces humanized antibodies with unexpected results, such as 1) lack of significant immunogenecity, as disclosed in the Declaration by Dr. Shak, 2) higher increase in binding affinity as compared to that of humanized antibodies known in the art, and 3) the same consensus sequence could be used to generate many different strong affinity humanized antibodies.

Applicant's arguments set forth in paper No. 39 have been considered but are not deemed to be persuasive for the following reasons:

Although '762 uses the most homologous sequence from a single human immunoglobulin as an example, '762 also teach that **as a principle**, a framework is used from either a human immunoglobulin which is unusually homologous to the donor immunoglobulin, **or** use a consensus framework **from many human antibodies** is used (column 13, first paragraph, lines 4-7). Thus the consensus sequence taught by '762 is the same as the claimed consensus sequence, as defined by the specification, i.e. the most frequently occurring amino acids, based on immunoglobulin of a

particular species (p.14). It is only Applicant's interpretation that the term "consensus framework" from '762 patent was not intended to refer to a sequence representing the most frequently occurring amino acids in the present invention. Furthermore, although Kabat et al do not use the term "consensus", but rather "occurrences of most common amino acid", one of ordinary skill in the art would readily understand that " a consensus sequence" from many antibodies is a sequence that occurs most frequently.

In addition, .In re Kerkhoven (205 USPQ 1069, CCPA 1980) summarizes:

"It is prima facie obvious to combine two compositions each of which is taught by prior art to be useful for same purpose in order to form third composition that is to be used for very same purpose: idea of combining them flows logically from their having been individually taught in prior art."

Applicant asserts that the claimed humanized antibodies are not obvious in view of the cited references because the cited prior art does not suggest such a combination. However, the instant situation is amenable to the type of analysis set forth in In re Kerkhoven,205 USPQ 1069 (CCPA 1980) wherein the court held that it is prima facie obvious to combine two compositions each of which is taught by the prior art to be useful for the same purpose in order to for a third composition that is to be used for the very same purpose since the idea of combining them flows logically from their having been individually taught in the prior art. Applying the same logic to the instant claims, given the teaching of the prior art that as a principle, a framework is used from either a human immunoglobulin which is unusually

homologous to the donor immunoglobulin, **or** a consensus framework from many human antibodies is used, and the structures of sequences that are most commonly occurred among many antibodies, it would have been obvious to humanize antibodies as taught by patent '762, using the most commonly occurred sequences taught by Kabat et al, because the idea of doing so would have logically followed from their having been individually taught in the prior art, and because patent '762 teaches the use of "consensus sequence", for the same purpose of producing humanized monoclonal antibodies for therapeutic purposes. One of ordinary skill in the art would have motivated to make humanized antibodies using the methods taught by '762 and the sequences taught by Kabat et al with a reasonable expectation of success. In addition, the arguments that the claimed invention is unexpected are not applicable, because the claims are broad, and drawn to any antibodies, and not specifically the claimed antibodies, wherein their specific target antigens, and their binding properties are not disclosed in the claims.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Minh-Tam B. Davis whose telephone number is (703) 305-2008. The examiner can normally be reached on Monday-Friday from 9:30am to 3:30pm, except on Wesnesday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Tony Caputa, can be reached on (703) 308-3995. The fax phone number for this Group is (703) 308-4227.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0916.

Minh-Tam B. Davis

October 13, 2000

SUSAN UNGAR, PH.D
PRIMARY EXAMINER

Notice of References Cited

Application No.	Applicant(s)		
08/146,206	Carter et al.		
Examiner	Group Art Unit	Page 1 of 1	
M. T. Davis	1642		

U.S. PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	NAME	CLASS	SUBCLASS
	A					
	B					
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FOREIGN PATENT DOCUMENTS

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	N						
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NON-PATENT DOCUMENTS

*		DOCUMENT (Including Author, Title, Source, and Pertinent Pages)	DATE
	U	~~Queen et al. PNAS, USA, 86: 10029 - 10033~~ Dupl.	~~1989~~
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* A copy of this reference is not being funished with this Office action.
(See Manual of Patent Examining Procedure, Section 707.05(a).)

Part of Paper No. 57

1642

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1642 Examiner: M. Davis **CERTIFICATE OF MAILING** I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on April 25, 2001 Wendy M. Lee

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AMENDMENT UNDER 37 C.F.R. §1.111

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Responsive to the Office Action dated 10/25/00, reconsideration of the present application is respectfully requested in view of the following amendments and remarks. A request for a 3 month extension of time and the requisite fee accompany this amendment.

IN THE CLAIMS:

Please amend claims 113 and 114 as follows:

Sub 01

113. (Amended) A humanized variant of a non-human parent antibody which binds an antigen and comprises a consensus human variable domain 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

N'

respect to one another.

114. (Amended) The humanized variant of claim 128 which binds the antigen about 3-fold more tightly than the parent antibody binds antigen.

REMARKS

Claims 43-105 and 113-128 are in the application. Claims 113 and 114 have been amended. Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "Version with Markings to Show Changes Made".

Claim 113 no longer requires that the humanized variant bind antigen with better affinity than the parent antibody, up to about 3-fold tighter binding than the parent antibody. Hence, claim 114 has been amended herein to depend on claim 128, which claim requires that the humanized variant bind antigen more tightly than the parent antibody.

Prosecution History of the Present Application

Applicants first wish to express their concern about the undue prejudice to them due to the repeated transfer of this case from patent examiner to patent examiner, and to explain that this is a case which has thrice previously been indicated to be in condition for allowance.

The case was originally with Examiner Adams, then was transferred to Examiner Nolan. In the 8/13/98 interview, Examiner Nolan indicated that unexpected results would overcome the 103 rejection based on Queen Patent 5,693,762 (hereinafter "the '762 patent"). An amendment was filed 8/24/98 presenting the unexpected results. Shortly thereafter, the case was transferred to the present Examiner. Pending claims 43-114 were discussed in an interview on 10/16/98 between the undersigned, the present Examiner and Examiner Feisee at which time the only outstanding issue in the case related to the clarity of the terms "binding of CDR" and "significant immunogenicity". An amendment was filed 11/6/98 addressing those issues. The case was then transferred to Examiner Reeves, who issued a restriction requirement 3/29/99 at that late stage in prosecution. In an 8/23/99 interview, Examiners Reeves/Burke and Feisee indicated that the case would be in order for allowance with the filing of a terminal disclaimer for claim 111 and addition of an upper limit to affinity in claims 113 and 128. Claims 113 and 128 were amended as suggested by the Examiners and claim 111 was canceled to avoid the

obviousness-type double patenting rejection (see 8/30/99 amendment). Now the case has been transferred yet again to the present Examiner and prosecution has been re-opened on a case that was indicated to be in condition for allowance three times previously.

To the extent that any issues remain following entry of this amendment, Applicants specifically request an interview with the present Examiner and her supervisor to discuss this case so as to ensure speedy resolution of the issues and allowance of the application. It is noted that this is a pre-GATT case and two 129(a) responses have previously been filed.

Section 112, first paragraph, Scope, New Rejection

Claims 43-105 and 113-128 are rejected under 35 USC Section 112, first paragraph on the basis that the specification, while being enabling for humanized antibody muMAb4D5 and an anti-CD3 antibody, or variable domains thereof, "does not reasonably provide enablement for any humanized antibody, or variable domain thereof, comprising CDR amino acids which binds non-specifically to any antigen, wherein the framework region amino acids are substituted 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, or of 24H, 73H, 76H, 78H and 93H, for treating any chronic disease."

The Examiner contends that the specification discloses examples of humanized muMAb4D5, anti-CD3 and anti-CD18 antibodies or variable domains thereof; that the substituted FR residues for muMAb4D5 are 71H, 73H, 78H, 93H and 66L; and that only one humanized antibody (huMAb4D5-8) with all the above five substitutions binds to p185 3-fold more tightly than the murine counterpart. The Examiner further contends that the substituted framework residues for the heavy chain of antibody anti-CD3 are FR residues 75 and 76, and that there is no disclosure concerning the binding affinity of the humanized anti-CD3 or anti-CD18 as compared to the murine counterpart. The Examiner contends that one cannot extrapolate from humanizing one antibody, which binds to $p185^{HER2}$ 3-fold more tightly than the murine counterpart, to humanizing any antibody,

wherein its affinity would be up to 3-fold or at least 3-fold tighter than the murine counterpart, or wherein its affinity would still be intact for therapeutic purposes. The Examiner further argues that one cannot extrapolate from humanizing an anti-p185 antibody by substitution of all five FR residues at positions 71H, 73H, 78H, 93H and 66L in an anti-p185 antibody, or from humanizing an anti-CD3 antibody by substitution at both framework residues 75H and 76H, with humanizing any antibody by substitution at only one amino acid residue 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, or of 24H, 73H, 76H, 78H and 93H. The Examiner opines that the specification does not disclose whether substitution at only one of the claimed amino acid positions would produce a humanized antibody that has 3-fold more affinity, or which combination of what substituted FR residues (other than 71H, 73H, 78H, 93H and 66L for an anti-p185 antibody or 75H and 76H in an anti-CD3 antibody) would produce a humanized antibody that has 3-fold more affinity than the murine counterpart, or retains adequate affinity for therapeutic purposes. The Examiner contends that a humanized antibody that does not have specificity for a particular antigen is of little practical use for treating a chronic disease and that the specification does not disclose how to treat any chronic disease using the claimed humanized antibody.

Applicants submit that claims 43-105 and 113-128 are enabled by the present application.

First, Applicants point out that none of the claims (other than claim 114) require that the humanized antibody bind antigen about 3-fold more tightly than the parent antibody binds antigen, as the Office Action seems to imply. The independent claims herein merely recite that the humanized antibody variable domain comprises CDR residues which bind an antigen (claims 43, 104 and 115); the antibody comprising the humanized antibody variable domain binds $p185^{HER2}$ (claim 72); the humanized antibody comprises CDR residues which bind an antigen (claim 105); the humanized variant bind antigen (claim 113 herein); or the humanized variant bind

antigen more tightly than the parent antibody - up to about 3-fold more tightly than the parent antibody (claim 128).

Second, Applicants submit that the claims herein encompass the humanized variable domain or antibody having at least one of the FR substitutions specified, but optionally having further FR substitution(s) in order to improve affinity to a level at which an antibody comprising the variable domain is able to bind antigen.

Finally, Applicants wish to clarify some issues concerning the Office's characterization of the working examples. First, it is noted that Example 1 actually describes several humanized anti-p185^{HER2} variants with FR substitution(s) as set forth in the claims herein: huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7, huMAb4D5-8 (Table 3 on page 72). Thus, it is clear that this example teaches humanized variants which do not include substitution of all of FR residues 71H, 73H, 78H, 93H and 66L. Each of these FR substitution variants bound antigen with better affinity than the initial antibody (huMAb4D5-1) comprising non-human CDR amino acid residues, but lacking any FR substitution(s). Two of the humanized anti-p185^{HER2} variants surprisingly bound antigen better than the murine parent antibody muMAb4D5, *i.e.* huMAb4D5-6 and huMAb4D5-8. With regard to Example 3 concerning the humanized anti-CD3 variants, aside from the 75H and 76H FR substitutions noted by the Office, this Example further teaches the following FR substitutions: L71, 71H, 73H and 78H. See, *e.g.*, Fig. 5 which aligns the murine anti-CD3 "muxCD3" sequences, the humanized variant "huxCD3v1" sequences, and the human sequences, "huκI" and "huIII".

The specification clearly teaches how to make humanized antibody variable domains and antibodies comprising such domains, and identifies FR residues that can be substituted to improve the binding affinity of an antibody comprising the humanized variable domain. See, *e.g.* pages 12-13, 20-26 and 28-29; Example 1 on pages 63-74; Example 3 on pages 79-88; and Example 4 on page 89. The specification teaches FR substitution(s)

individually or in combination. Based on the disclosure of the present application, one is able to make an antibody comprising a humanized antibody variable domain which binds antigen. The Office has provided no evidence that the humanized antibody variable domains or humanized antibodies comprising the FR substitution(s) claimed herein would not be functional, beyond speculating that the affinity might not be about 3-fold better than the parent antibody (and, as noted above, the claims other than claim 114 do not require this improvement in affinity). Hence, Applicants submit that the presently claimed variable domains and antibodies are enabled by the specification.

Reconsideration and withdrawal of the enablement rejection is respectfully requested in view of the above.

Section 102 - Claims 115-117, 123 and 127

Claims 115-117, 123 and 127 are rejected under 35 USC Section 102(e) or 102(b) as anticipated by US Patent No. 5,530,101 (hereinafter "the '101 patent") or Queen *et al. PNAS (USA)* 86:10029-10033 (1989) (hereinafter "Queen *et al.*"). The Examiner contends that the '101 patent and Queen *et al.* teach a humanized anti-Tac antibody wherein amino acid 93 is substituted in the heavy chain, using the aligned Kabat Eu sequence to provide the framework for the humanized antibody.

Applicants point out that - as explained earlier in prosecution - the substituted 93 FR residue in the cited references is not 93H "utilizing the numbering system set forth in Kabat" (see page 13, line 33 through to line 22 on page 14 of the present application) as required by claims 115-117, 123 and 127 of the present application. In particular, as noted on page 6 of the amendment hand carried to the Office on 10/7/97, residue no. 93 in the heavy chain of the anti-Tac antibody in the cited references, is actually 89H utilizing the numbering system set forth in Kabat. The cited references use a sequential numbering system, rather than the Kabat numbering system claimed herein.

Reconsideration of the 102(e) and 102(b) rejections based on the '101

patent and Queen *et al.* is respectfully requested in view of the above.

Section 102 - Claims 43, 44, 48, 55, 67, 71, 105, 115-117, 120 and 127

Claims 43, 44, 48, 55, 67, 71, 105, 115-117, 120 and 127 are rejected under 35 USC Section 102(e) as being anticipated by the '101 patent. The Examiner urges that FR substitutions 38L, 67L, 69H, 73H and 93H are taught by the '101 patent. Specifically, the Examiner contends that amino acids 38 or 67 are substituted in the light chain of the Fd79 and M195 antibodies, respectively, and amino acids 69, 73 or 93 are substituted in the heavy chains of the CMV5, mik-β1 and Fd138-80 antibodies, respectively. The '101 patent is further alleged to teach (in the abstract thereof) that the humanized antibodies therein will be substantially non-immunogenic in humans.

Applicants submit that the presently claimed FR 38L, 67L, 69H and 93H substitutions are different from those in the '101 patent to which the Examiner refers, since the numbering of the presently claimed FR substitutions utilizes the numbering system set forth in Kabat, whereas the '101 patent uses sequential numbering for the residues. In particular, VL residue 38 of Fd79 is a CDR residue, as opposed to a FR residue (note Table 1 in column 43 of the '101 patent which states that residue 38 is in "Category 1" and therefore is a CDR residue; see lines 66-67 in column 13 of the '101 patent); VL residue 67 of M195 is FR residue 63L utilizing the numbering system set forth in Kabat (see page 8 of Applicants' 10/7/97 amendment); VH residue 69 of CMV5 is 68H utilizing the numbering system set forth in Kabat (see page 9 of the 10/7/97 amendment); and VH residue 93 of Fd138-80 is FR residue 89H utilizing the numbering system set forth in Kabat (see page 7 of the 10/7/97 amendment).

As to the FR 73H substitution (utilizing the numbering system set forth in Kabat) claimed herein, Applicants submit that the disclosure of the humanized mik-β1 antibody is too late to qualify as Section 102 prior art to claim 115 which recites that substitution. See page 11, first full paragraph of Applicants' 1/15/99 amendment.

Finally, as to the recitation in claim 105 herein that the humanized antibody "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", Applicants have shown that antibodies humanized according to one preferred embodiment of the present invention possess this property. See the Shak Declaration filed 8/24/99. The '101 patent merely states that the humanized antibodies will be "substantially non-immunogenic" in humans, but fails to disclose that the humanized antibodies lack substantial immunogenicity upon repeated administration to a human patient in order to treat a chronic disease in that patient.

Reconsideration and withdrawal of the Section 102(e) rejection is respectfully requested in view of the above.

Section 102(e) - Claim 128

Claim 128 is rejected under 35 USC Section 102(e) as being anticipated by the '101 patent. The Examiner states that the language "up to" 3-fold reads on anything below 3-fold.

Claim 128 pertains to a humanized antibody which binds antigen more tightly than the parent antibody (up to about 3-fold more tightly). The Queen patents state that the humanized antibodies therein bind the target antigen with the same affinity, or bind less tightly, than the parent antibody. See pages 21-22 of Applicants' amendment filed 8/24/98. While humanized M195 was later discovered to bind antigen up to about 3-fold more tightly than the parent antibody bound antigen (see paragraph 2 on page 2 of the 8/30/99 amendment), this property of the humanized M195 antibody is not described in the '101 patent (see lines 28-29 in column 60 of the '101 patent).

Reconsideration and withdrawal of the Section 102(e) rejection of claim 128 is respectfully requested.

Section 102(e) - Claim 113

Claim 113 is rejected under 35 USC Section 102(e) as being anticipated

by US Patent 5,693,762 ("the '762 patent") for the same reasons set forth in paper No. 27 for the rejection of previous claims 22-25, 38 and 39.

The Examiner contends that the '762 patent teaches "as a principle, a framework is used from either a human immunoglobulin which is unusually homologous to the donor immunoglobulin, or a consensus framework from many human antibodies is used".

Applicants submit that this disclosure in the '762 patent simply fails to anticipate the presently claimed "consensus human variable domain" in claim 113 as defined by the present specification. See the discussion of the '762 patent on pages 13-14 of the 8/24/98 amendment. The Examiner states on page 11 of the above Office Action that it 'is only Applicant's interpretation that the term "consensus framework" from '762 patent was not intended to refer to a sequence representing the most frequently occurring amino acids in the present invention'. Applicants respectfully disagree. Indeed the Office initially suggested the alternative interpretation for the term "consensus framework" as it was used by Queen *et al.* See page 4 of the Office Action dated 12/23/96 in which Examiner Nolan stated:

> "Regarding the consensus sequence, the combination of references teach the human framework regions having a significantly high degree of sequence homology (conservative regions). Queen et al. in particular point to Kabat as demonstrating that this was known in the art well in advance of applicant's filing date, see reference 38, cited by Queen et al." (Emphasis added).

The Queen *PNAS* paper to which Examiner Nolan referred, was concerned with using a human framework region from a human immunoglobulin which was unusually homologous to the donor immunoglobulin, and failed to mention a consensus human variable domain as that expression is used in the present application. Hence, the Office has previously used the expression "consensus sequence" to describe the highly homologous approach taught by Queen *et al.*

Nothwithstanding this, Applicants note that in order to anticipate a claimed invention, the reference alone much teach each and every element of the claim. Even if it were the case that the "consensus framework" in the '762 patent was intended to refer to an amino acid sequence which comprises the most frequently occurring amino acid residues at each location in all human immunoglobulins (see page 14, lines 29-31 of the present application), which is denied, the Office has not shown that the '762 patent unambiguously disclosed the selection invention recited in claim 113 herein pertaining to a "consensus human variable domain of a human heavy chain immunoglobulin subgroup". The Office has combined the '762 patent with Kabat *et al.* (see Section 103 discussion below) in an attempt to show that this particular consensus sequence had been disclosed previously. Hence, Applicants submit that claim 113 is novel over the '762 patent. Applicants will demonstrate in the following section how the invention set forth in claim 113 is also nonobvious over the '762 patent, due to the unexpected results attributable thereto.

Reconsideration and withdrawal of the Section 102 rejection based on the '762 patent is respectfully requested in view of the above.

Section 103

Claims 113, 115-118, 123 and 127-128 are rejected under 35 USC Section 103 as being unpatentable over the '762 patent in view of Kabat *et al.*

First, it is noted that the Examiner relies on the rejection based on the '762 patent in view of Kabat *et al.* for the same reasons as set forth in paper no. 27 (Applicants assume paper no. 34 - Examiner Nolan's Office Action dated 12/23/97 is intended). Examiner Nolan previously indicated that the unexpected results would overcome the 103 rejection based on the '762 patent combined with Kabat *et al.* (see Paper no. 37; 8/13/98 Interview Summary).

Applicants rely on the unexpected results attributable to the consensus human variable domain of a human heavy chain immunoglobulin subgroup as demonstrating that the presently claimed antibodies are not obvious over

the '762 patent combined with Kabat *et al.* See pages 18-23 of the 8/24/98 amendment and the Shak declaration attached thereto.

The Examiner urges that "the arguments that the claimed invention is unexpected are not applicable, because the claims are broad, and drawn to any antibodies, and not specifically the claimed antibodies, wherein their specific target antigens, and their binding properties are not disclosed in the claims."

Applicants submit that the Examiner's basis for ignoring the evidence of unexpected results is legally flawed - at least with respect to (1) the lack of significant immunogenicity of the claimed humanized antibodies upon repeated administration to a human patient, *e.g.* to treat a chronic disease in that patient and (2) the ability to make many strong affinity antibodies, thus avoiding tailoring each human framework to each non-human antibody to be humanized. Those unexpected results provide objective evidence of non-obviousness. *Specialty Composites v. Cabot Corp.*, 845 F. 2d 981, 6 USPQ 2d 1601 (Fed. Cir. 1988).

As to unexpected result (1), Applicants have demonstrated that antibodies humanized using a consensus human variable domain of a human heavy chain immunoglobulin subgroup as set forth in claim 113 herein lack significant immunogenicity upon repeated administration to a human patient in order to treat a chronic disease in that patient. This was shown in the Shak Declaration for humanized anti-HER2, anti-IgE, anti-VEGF and anti-CD11a antibodies. See pages 18-21 of the 8/24/98 amendment and the Shak Declaration attached thereto. Hence, this unexpected property is not linked to certain antibodies or specific target antigens, but is generally applicable and the claims are commensurate in scope with the unexpected result relied upon.

Turning now to unexpected result (2), Applicants have shown that a consensus human variable domain of a human heavy chain immunoglobulin subgroup as set forth in claim 113 can be used to generate many different strong affinity humanized antibodies, including anti-HER2, anti-CD3,

anti-CD18, anti-IgE, anti-CD11a and anti-VEGF humanized antibodies (see pages 22-23 of the 8/24/98 amendment). Again, this further unexpected property is not dependent on the antibody or target antigen, and hence should be considered with respect to the non-obviousness of the presently claimed antibodies.

Hence, Applicants submit that claim 113 directed to a humanized variant comprising a consensus human variable domain of a human heavy chain immunoglobulin subgroup is non-obvious over the cited art, because of unexpected results (1) and (2) noted above.

As to the other rejected claims, Applicants point out that claim 115 recites FR substitutions at one or more of positions 24H, 73H, 76H, 78H and 93H, utilizing the numbering system set forth in Kabat. The Office has not shown how the cited art disclosed or suggested substitution of FR residues 24H, 76H, 78H and 93H, utilizing the numbering system set forth in Kabat; and, as noted above, the disclosure concerning substitution of 73H in the mik-β1 antibody is too late to qualify as Section 102 prior art to the invention set forth in claim 115 herein. With regard to claim 117, the Office fails to teach a humanized antibody with FR substitution(s) limited to positions 24H, 73H, 76H, 78H and 93H, utilizing the numbering system set forth in Kabat. As to claim 118, the Office has not demonstrated how the art would have taught combining the listed FR substitution(s) in claim 115 with a consensus human variable domain. With regard to claim 123, as noted previously, substituted 93 FR residue in Queen's anti-Tac or Fd138-80 antibodies is not the same as FR substitution 93H "utilizing the numbering system set forth in Kabat." Finally, with respect to claim 128, as noted above, the Queen patents state that the humanized antibodies therein bind the target antigen with the same affinity, or bind <u>less tightly</u>, than the parent antibody. See pages 21-22 of Applicants' amendment filed 8/24/98. While humanized M195 was later discovered to bind antigen up to about 3-fold more tightly than the parent antibody bound antigen (see paragraph 2 on page 2 of the 8/30/99 amendment), this property of the humanized M195 antibody is not described in the '101 patent (see lines 28-29 in column 60 of the '101

patent). The ability to bind antigen more tightly than the parent antibody was a further unexpected result observed with respect to certain humanized antibodies of the present application.

Reconsideration and withdrawal of the Section 103 rejection of claims 113, 115-118, 123 and 127-128 is respectfully requested in view of the above.

Respectfully submitted,

GENENTECH, INC.

Date: April 25, 2001

By:
Wendy M. Lee
Reg. No. 40,378
Telephone: (650) 225-1994

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Claims 113 and 114 have been amended as follows:

113. (Three Times Amended) A humanized variant of a non-human parent antibody which binds an antigen [with better affinity than the parent antibody] and comprises a consensus human variable domain 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 [, wherein the humanized variant binds antigen up to about 3-fold more tightly than the parent antibody binds antigen].

114. (Amended) The humanized variant of claim [113] 128 which binds the antigen about 3-fold more tightly than the parent antibody binds antigen.

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1642 Examiner: M. Davis **CERTIFICATE OF MAILING** I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on April 25, 2001 Wendy M. Lee

PETITION AND FEE FOR THREE MONTH EXTENSION OF TIME
(37 CFR 1.136(a))

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Applicants petition the Commissioner of Patents and Trademarks to extend the time for response to the Office Action dated October 25, 2000 for three months from January 25, 2001 to April 25, 2001. The extended time for response does not exceed the statutory period.

Please charge Deposit Account No. 07-0630 in the amount of $890.00 to cover the cost of the extension. Any deficiency or overpayment should be charged or credited to this deposit account. A duplicate of this sheet is enclosed.

Respectfully submitted,
GENENTECH, INC.

Date: April 25, 2001

By: _____
Wendy M. Lee
Reg. No. 40,378
Telephone No. (650) 225-1994

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09157
PATENT TRADEMARK OFFICE

Interview Summary	Application No. 08/146,206	Applicant(s) Carter et al
	Examiner Minh-Tam Davis	Group Art Unit 1642

All participants (applicant, applicant's representative, PTO personnel):

(1) *Minh-Tam Davis* (3) _____

(2) *Ewndy Lee* (4) _____

Date of Interview *Apr 26, 2001*

Type: a)☒ Telephonic b)☐ Video Conference
c)☐ Personal [copy is given to 1)☐ applicant 2)☐ applicant's representative]

Exhibit shown or demonstration conducted: d)☐ Yes e)☒ No. If yes, brief description:

Claim(s) discussed: _____

Identification of prior art discussed:

Agreement with respect to the claims f)☐ was reached. g)☐ was not reached. h)☐ N/A.

Substance of Interview including description of the general nature of what was agreed to if an agreement was reached, or any other comments:

Applicant requests an iinterview if the case is not ready for allowance following entry of the amendment to be filed today.

(A fuller description, if necessary, and a copy of the amendments which the examiner agreed would render the claims allowable, if available, must be attached. Also, where no copy of the amendments that would render the claims allowable is available, a summary thereof must be attached.)

i)☒ It is not necessary for applicant to provide a separate record of the substance of the interview (if box is checked).

Unless the paragraph above has been checked, THE FORMAL WRITTEN REPLY TO THE LAST OFFICE ACTION MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP section 713.04). If a reply to the last Office action has already been filed, APPLICANT IS GIVEN ONE MONTH FROM THIS INTERVIEW DATE TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW. See Summary of Record of Interview requirements on reverse side or on attached

Examiner Note: You must sign this form unless it is an Attachment to a signed Office action.

7/13/01

#6
7/13/01

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1642 Examiner: M. Davis **Certificate of Facsimile Transmission Under 37 CFR § 1.8** In accordance with CFR § 1.6(d), this correspondence addressed to Examiner Minh-Tam Davis, The Patent and Trademark Office, Washington, DC 20231 is being transmitted to facsimile No. (703) 308-4426 on July 13, 2001 Wendy M. Lee

AMENDMENT TRANSMITTAL

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Transmitted herewith is an amendment in the above-identified application.

The fee has been calculated as shown below.

	Claims Remaining After Amendment		Highest No. Previously Paid For	Present Extra	Rate	Additional Fees
Total	82	-	86	0	18	$0.00
Independent	8	-	9	0	80	$0.00
__ 0Multiple dependent claim(s), if any					270	$0.00
				Total Fee Calculation		$0.00

X
X

No additional fee is required.
The reference O'Connor *et al. Protein Engineering* 11(4):321-328 (1998) is attached.

The Commissioner is hereby authorized to charge any additional fees required under 37 CFR 1.16 and 1.17, or credit overpayment to Deposit Account No. 07-0630.

Respectfully submitted,
GENENTECH, INC.

Date: July 13, 2001

By: _____
Wendy M. Lee
Reg. No. 40,378
Telephone No. (650) 225-1994

09157
PATENT TRADEMARK OFFICE

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Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE 9/13/01

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1642 Examiner: M. Davis **Certificate of Facsimile Transmission Under 37 CFR § 1.8** In accordance with CFR § 1.6(d), this correspondence addressed to Examiner Minh-Tam Davis, The Patent and Trademark Office, Washington, DC 20231 is being transmitted to facsimile No. (703) 308-4426 on July 13, 2001 Wendy M. Lee

SUPPLEMENTAL AMENDMENT

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

IN THE CLAIMS:

Please amend claims 113 and 128 as indicated below:

64 113. (Three times amended) 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.

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128. (Twice Amended) 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 a Framework Region (FR) 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 humanized variant binds the antigen more tightly than and up to about 3-fold more tightly than the parent antibody binds antigen.

Please add the following claims:

--129. 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.

130. The humanized variable domain of claim 129 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.

131. The humanized variable domain of claim 129 wherein no human Framework Region (FR) residue other than those set forth in the group has been substituted.--

REMARKS

Applicants wish to thank Examiners Davis and Caputa for granting an interview to the representatives of Applicants on July 3, 2001. It is noted that the interview was terminated before its completion due to a fire alarm and evacuation of the building. The response herein reflects points raised by the Office during the interview. To the extent that issues remain in the case following entry of this and the previous amendment, Applicants respectfully request a further interview given the protracted prosecution of the case as discussed in the interview.

The pending claims

In the above-noted interview Examiner Caputa asked how the framework in claim 113 differed from the "consensus framework from many human antibodies" as in column 13 of the cited Queen '762 patent. In the interests of expediting prosecution, Applicants have amended claim 113 herein to recite the language found on page 14, lines 29-31 of the present application. The differences between the disclosure of the '762 patent and the invention set forth in claim 113 will be discussed below.

As discussed in the interview, claim 128 is amended herein to emphasize that the humanized antibody of this claim is one with better affinity than the non-human parent. This amendment obviates the §102 rejection over the disclosure of the '101 patent.

Claims 129-131 have been added herein. Claim 129 represents a combination of claims 43 and 115 and includes the FR substitution language from claim 128. Claims 130-131 employ language from claims 44 and 45, respectively.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "**Version with markings to show changes made.**" Applicants submit that the amendments do not introduce new matter and therefore should be entered. Following entry of this amendment, claims 43-105 and 113-131 will be pending in the present application.

As was pointed out in the interview, the present application contains three different types of independent claims: (1) claims 43, 72, 104, 105 and 115 encompassing humanized antibody variable domains or antibodies comprising FR substitution(s) including one or more FR substitutions from a specified selection of FR positions; (2) claim 128 directed to a humanized variant which binds the antigen more tightly than the parent antibody binds antigen (up to about 3-fold more tightly); and (3) claim 113 directed to a humanized antibody comprising non-human CDR and FR residue(s) incorporated into 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.

Section 102

A comprehensive reply to the outstanding Section 102 rejections can be found in the amendment dated April 25, 2001. As discussed in the interview, it is believed that the Section 102 rejections should be withdrawn.

With respect to claims 43, 72, 104, 105 and 115, Applicants pointed out that Queen used sequential numbering, rather than Kabat numbering, for the FR residues, such that the 93H, 38H, 67L and 69H FR substitutions according to Kabat herein were not disclosed by Queen. As to the 73H FR substitution claimed herein, Applicants will submit shortly a swearing behind declaration showing completion of the invention of a humanized variable domain or antibody comprising that FR substitution, prior to cited Queen patent.

As to claim 128, Applicants pointed out that Queen did not describe humanized antibodies with improved affinity - affinity was either about the same or worse than the rodent antibody. The amendment herein clarifies that claim 128 pertains to antibodies with better affinity than the non-human parent antibody.

Finally, Applicants submit that recitation of "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" in claim 113 renders the humanized antibody therein novel over the cited Queen '762 patent. The Section 103 rejection will be addressed below.

Withdrawal of the outstanding Section 102 rejections is respectfully requested.

Section 112, first paragraph, scope

A full and complete response to the outstanding rejection of claims 43-105 and 113-128 may be found in the communication to the Office dated April 25, 2001.

In the outstanding Office Action, the Examiner maintains that each of the claims presented is not enabled by the disclosure. The basis for the assertion of the Examiner is that she believes the practice of the invention as reflected in each of the claims presented would constitute undue experimentation. Based on the points raised by the Examiner in the July 3 interview and the outstanding Office Action, Applicants believe this conclusion is based on misunderstandings of the law governing enablement, particularly as it pertains to the issue of undue experimentation, and a mischaracterization of the claims at issue and the disclosure. Moreover, Applicants will summarize hereinbelow relevant evidence which demonstrates the reproducibility of the methods disclosed in the present application for generating useful humanized antibody variable domains and antibodies encompassed by the claims herein.

Enablement must be measured in relation to the claims, the disclosure and what is known to a person skilled in the art. See, *United States v. Telectronics, Inc.*, 857 F.2d 778, 785, 8 USPQ2d 1217, 1223 (Fed. Cir. 1988) ("The test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation."). Undue experimentation, in turn, is a conclusion based on a number of discrete factual determinations. *In re Wands*, 858

F.2d 731, 737 (wherein the court listed eight factors that must be considered as a group when determining an issue of undue experimentation). In the present rejection, the only factors that apparently have been considered by the Examiner are the breadth of the claims and unpredictability in the art.

With respect to the scope of the claims, it is respectfully submitted that the Examiner has not accurately construed the claim scope, either in the rejections set forth in the outstanding Office Action or as characterized during the interview of July 3.

First, as has been noted in previous communications, only one claim (claim 114) specifically requires a three-fold increase in affinity of the humanized antibody relative to the non-human parent antibody. Claim 128, as amended, requires a binding affinity greater than the parent antibody, up to about three times the parent antibody affinity. Claims 43 to 105, 113 and 115 to 127 each contain no reference to minimum binding affinity relative to the parent antibody. Assertions that it would not have been possible to produce a humanized antibody subject to these claims having a three-fold increase in binding affinity are simply irrelevant to all but one claim.

Second, a requirement in each claim presented is that the variable domain retain the functional capacity to bind the antigen bound by the parent antibody. Thus, claims are not directed to single amino acid substitutions in an abstract sense that result in polypeptides that are inoperative as antibody binding domains. Instead, each of the claims presented requires the resulting humanized antibody variable domain or antibody to retain the antibody binding specificity of the parent antibody, and certain of the claims require the binding affinity to be greater than the parent antibody. Omitting the antibody binding limitation present in each claim improperly changes the scope of the claim.

Third, each of the independent claims is further limited in respect of one or more specific and objectively defined physical attributes of the resulting humanized antibody variable domain or antibody. For example, claim 43 identifies -- and thereby limits the claimed invention to -- a finite number of species of antibody binding domains which comprise amino acid substitutions in said binding domain selected from a finite range of substitutions in the framework region. If this physical characteristic of the humanized antibody variable domain is not present, it is outside the scope of this claim. Similarly, the claims do not encompass alterations of a human antibody variable domain that do not result in antibodies that bind to the antigen bound by the parent antibody.

Thus, it is respectfully submitted that the specific physical and functional characteristics of the claimed antibody variable regions must be given weight in determining the scope of the claims. The failure of the Examiner to do so has resulted in an improper characterization of the claimed invention, which is fundamental to the determination of enablement.

The second issue upon which the Examiner has not given sufficient weight are the extensive teachings in the disclosure, in view of what was known in the art as of the time of filing of the present application. The present disclosure provides more than ample direction to a person skilled in the art to rely upon in producing the variable domains and antibodies falling within the scope of the present claims. In particular, the present disclosure provides specific guidance to a person skilled in the art to produce, alter and select variants falling within the scope of the claims without the exercise of undue experimentation.

For example, the disclosure at pages 10-16, 20-29 and in the working examples recites a summary of the process to be used to produce the claimed humanized antibody domains and antibodies. As noted therein, steps for identifying and producing the variant sequences are described,

as are a variety of physical attributes of the resulting variants that are to be selected for through the process described therein (*e.g.*, the substituted FR residue interacts with a CDR, non-covalently binds antigen directly or participates in the V_L-V_H interface). A person reasonably skilled in this field would face no difficulty in taking any parent antibody having a particular binding specificity and, following the explicit and comprehensive teachings of the present disclosure, construct and select humanized antibody domains and antibodies as defined in the claims.

The third basis of the Examiner's rejection appears to be the belief that the claims cannot be practiced without undue experimentation. Undue experimentation is a conclusion that must be reached after considering a number of discrete factors. Two of these, claim scope and the teachings of the disclosure, have been addressed above and in the earlier response to the outstanding Office Action. In addition, the Examiner appears have relied on an assumption that there is an abnormally high level of unpredictability in the field of the invention. In particular, the Examiner is apparently asserting that there is such an inherent degree of unpredictability in the art that no claim to a humanized antibody could ever issue if it were not limited to a specifically defined amino acid sequence associated with a specific antibody specificity. This is an inaccurate characterization of the level of unpredictability in the field of the invention at the time the present application was filed, and is used in an improper manner by the Examiner in light of law governing lack of enablement due to undue experimentation.

Unpredictability in the art, standing alone, is not a conclusion that can support a rejection on the basis of lack of enablement. *In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988). Instead, it is a factor whose significance must be assessed in making the legal determination of whether practice of the claimed invention would involve undue experimentation. Moreover, the fact that an art has unpredictability

associated with it does not condemn any claim that goes beyond a specific working example. As §2164.03 of the MPEP provides:

> The "predictability or lack thereof" in the art refers to the ability of one skilled in the art to extrapolate the disclosed or known results to the claimed invention. If one skilled in the art can readily anticipate the effect of a change within the subject matter to which the claimed invention pertains, then there is predictability in the art. On the other hand, if one skilled in the art cannot readily anticipate the effect of a change within the subject matter to which that claimed invention pertains, then there is lack of predictability in the art.

In the present case, neither the Examiner's characterization of unpredictability nor the assessment of the significance of unpredictability in light of the present disclosure is accurate.

As to the former issue, and as noted in the earlier response to the outstanding Office Action, the number of examples of successful modifications (*i.e.*, modifications resulting in functional humanized antibody binding domains) made according to the teachings of the present disclosure far exceeds the number suggested by the Examiner. For example, for one target antigen (HER2), eight successful variants were constructed using the procedures of the present invention. Each of these variants preserved binding affinity of a nature to make it a useful humanized binding domain.

Examiner Davis explained in the interview her opinion that variants (*e.g.* huMAb4D5-2 and huMAb4D5-3) without all 5 FR substitutions of the huMAb4D5-8 variant were not able to bind antigen with appropriate affinity.

With respect to the huMAb4D5-2 variant in Table 3, it was acknowledged that the variant with the single FR substitution did not appear to have growth inhibitory activity in the SK-BR-3 assay used. However, the

undersigned explained that even the 4.7nM Kd of this variant rendered it useful, *e.g.*, for diagnostic uses (see pages 55-57), as an immunotoxin (see pages 58-59), and/or for killing cells *in vivo* via Antibody Dependent Cellular Cytotoxicity (ADCC, see pages 59-60). Indeed, the affinity of the huMAb4D5-2 variant significantly surpasses the affinity of the murine and humanized anti-gD antibodies in column 45 of the cited Queen '762 patent, for instance. There is nothing in the art to indicate that 4.7nM is not a useful Kd. The other variant relied on by the Examiner as supporting her view that the claims were not enabled (huMAb4D5-3 in Table 3 with 4.4nM Kd) would also have the above-noted uses in addition to its ability to inhibit the proliferation of breast cancer cells as assessed by the SK-BR-3 assay. Hence, it was emphasized that the antibodies of the present invention need not have superior binding affinities in order to be useful.

Examiner Caputa asked what evidence was available to demonstrate that the teachings of the present application could be applied to other useful humanized antibodies.

Applicants are able to demonstrate that humanized antibody variants that bind at least seven distinct antigens have been made based on the teachings in the above patent application. For each antigen, several humanized antibody variants with the claimed FR substitution(s) could be made. In particular:

1. Example 1 on pages 63-74 describes several humanized variants which bound HER2 comprising the presently claimed FR substitution(s). Each of those variants was able to bind HER2 antigen (see Table 3 on page 72).

2. Example 3 on pages 79-88 describes eight humanized anti-CD3 antibody variants ($BsF(ab')_2v1$ as well as variants v6-12) which comprised the presently claimed FR substitutions. That example describes the $BsF(ab')_2v1$ variant (see huxCD3v1 in Fig. 5) and the other variants which were useful for retargeting the cytotoxic activity of human CD3+

CTL against HER2 overexpressing breast cancer cells (see, page 79, first paragraph, and Shalaby *et al. J. Exp. Med.* 175:217-225 (1992), of record). The FR substitutions in the BsF(ab')$_2$v1 variant (71L, 71H, 73H and 78H) were those which (a) non-covalently bound antigen directly; (b) interacted with a CDR; or (c) participated in the V_L-V_H interface, such FR substitutions being described and enabled by the present specification. Example 3 describes how the affinity of the humanized antibody BsF(ab')$_2$v1 was further improved by incorporating additional rodent CDR amino acid residues in the humanized antibody to generate BsF(ab')$_2$v9. In addition, that example describes variants with further FR substitutions at positions 75H and/or 76H.

3. Example 4 on page 89 describes yet a further example of the presently claimed humanized antibody variable domains/antibodies. The humanized anti-CD18 antibody included the presently claimed FR substitutions that (a) non-covalently bound antigen directly; (b) interacted with a CDR; or (c) participated in the V_L-V_H interface, and were identified using molecular modeling as taught in the present application.

4. Presta *et al. Cancer Research* 57:4593-4599 (1997) (of record) describes nine humanized anti-VEGF variants that were generated following the enabling disclosure of the present application.

5. Various humanized anti-Protein C variants are described in O'Connor *et al. Protein Engineering* 11(4):321-328 (1998) (copy attached), those variants being enabled by the present application.

6. Humanized antibody variants which bind the IgE antigen covered by certain claims herein have also been made (see Presta *et al. J. Immunol.* 151(5): 2623-2632 (1993) (of record)).

7. Werther *et al. J. Immunol.* 157(11): 4986-4995 (1996) (of record) is concerned with the humanization of anti-LFA-1 antibodies and describes several humanized antibody variants encompassed by the present claims.

These facts suggest that the "unpredictability" in the art is in fact much lower than suggested by the Examiner. When this actual level of unpredictability is then considered in view of the claim scope and the breadth of the disclosure, it becomes clear that unpredictability in the present application is not a factor that can support an assertion of undue experimentation. Indeed, through the teachings of the present disclosure, the moderate degree of unpredictability that exists in the art does not operate as a barrier to practice of the claimed invention, particularly in light of the teachings of the disclosure as to how to produce, identify and select variants falling within the scope of the claims.

As a consequence, it is respectfully submitted that the basis of the Examiner's belief that there is a lack of enablement due to undue experimentation is misplaced and should be withdrawn. Moreover, it is respectfully submitted that unless the Examiner can provide specific evidence demonstrating that the procedures disclosed in the present application will not yield success in producing humanized antibody variable domains as claimed, to counter the evidence provided in the specification and the specific responses, the maintenance of this rejection is improper. *In re Wright*, 999 F.2d 1557, 1562 (Fed. Cir. 1993); *In re Marzocchi*, 439 F.2d 220, 224 (CCPA 1971). Accordingly, Applicants respectfully request that the Examiner reconsider and withdraw the rejections based on lack of enablement.

Section 103 rejection

Claims 113, 115-118, 123 and 127-128 are rejected under Section 103 as being unpatentable over the Queen '762 patent in view of Kabat *et al.* Applicants responded to the rejection in the amendment dated April 25, 2001 and that response is supplemented hereinbelow.

At the outset, it is noted that the 103 rejection as to 115-118, 123, 127-128 should fall with the withdrawal of the Section 102 rejections of these claims, since the Office has not advanced any reason why one would substitute the presently recited FR residues, or why one would

have thought it would be possible to make a humanized antibody with improved affinity compared to the rodent antibody based on the cited art.

With regard to claim 113, now reciting "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", Applicants pointed out that it is believed that a *prima facie* case for obviousness of this invention has not been established; and even if it had, unexpected results provide objective evidence as to the patentability of the presently claimed invention.

Applicants' representatives explained in the interview that the term "consensus framework from many human antibodies" was used in the Queen patent synonymously with "a framework from a particular human immunoglobulin that is unusually homologous to the donor immunoglobulin to be humanized" - the position also taken by a former Patent Examiner (see page 10 of the amendment dated April 25, 2001). This is abundantly clear from a reading of the relied upon reference to a "consensus framework from many human antibodies" in the '762 patent. Immediately after this phrase in column 13, first full paragraph, the '762 patent states "For example, comparison of the sequence of a mouse heavy (or light) chain variable region against human heavy (or light) variable regions in a data bank (for example, the National Biomedical Research Foundation Protein Identification Resource) shows that the extent of homology to different human regions varies greatly, typically from about 40% to about 60-70%. By choosing as the acceptor immunoglobulin one of the human heavy (respectively light) chain variable regions that is most homologous to the heavy (respectively light) chain variable region of the donor immunoglobulin, fewer amino acids will be changed in going from the donor immunoglobulin to the humanized immunoglobulin. Thus, it is clear from the '762 patent that what it intended by the "consensus framework from many human antibodies" was indeed the "most homologous" human framework region as selected in the quoted paragraph of the '762 patent above. Thus, Applicants submit that the rejection based on the

combination of the '762 patent and Kabat *et al.* has been made with the benefit of hindsight of the present invention, which is impermissible.

Aside from the lack of teaching or motivation in the '762 patent to use 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, the '762 patent teaches away from this approach. Indeed, Queen taught the importance of selecting an unusually homologous human framework in order to avoid distorting the CDRs (column 13, lines 19-27). Applicants have shown previously how antibodies humanized with the human variable domain in claim 113 lack the unusually high homology to the non-human variable domain (paragraph bridging pages 17-18 of the amendment filed August 24, 1998), but nonetheless bind antigen extremely well. For instance, Applicants referenced the humanized anti-CD18 antibody with only 53% homology between the rodent and human framework sequences; humanized anti-IgE antibody with only 58% homology; humanized anti-CD11a with only 57% homology etc. These homologies were much lower that the homologies considered by Queen to be critical to avoid distorting the CDRs and for retaining affinity. The present application goes beyond the Queen method and discloses the benefits of using 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 for humanizing many different antibodies. This was not possible based on Queen's work which required that the human variable domain be tailored to each new rodent variable domain sequence to be humanized.

Applicants believe that the above arguments make out a strong case for patentability of the presently claimed invention over the cited combination of the '762 patent and Kabat *et al.* Moreover, Applicants are able to demonstrate that the presently claimed invention is patentable over the cited art due to the unexpected results attributable thereto. In particular, Applicants have demonstrated through the Shak declaration that antibodies directed against four different antigens humanized with

the presently claimed human variable domain in claim 113 display the unexpected property of lack of significant immunogenicity upon repeated administration to a human patient. This was not predictable in view of art such as Isaacs *et al. The Lancet* 340:748-752 (1992) (of record) in which 3/4 patients developed inhibitory antiglobulins upon repeated administration of the prior art humanized antibody thereto.

The Examiner had indicated that the unexpected results are not applicable because "the claims are broad, and drawn to any antibodies, and not specifically the claimed antibodies, wherein their specific target antigens, and their binding properties are not disclosed in the claims". Applicants submit that the Shak declaration filed demonstrates that the unexpected result applies regardless of the antigen or binding properties of the antibodies; the unexpected result was shown for humanized anti-HER2, anti-IgE, anti-CD11a and anti-VEGF antibodies. Hence, Applicants submit that the unexpected results are commensurate in scope with the invention recited in claim 113.

Thus, Applicants submit that the presently claimed invention is patentable over the cited art.

Applicants believe that this application is now in order for allowance and look forward to early notification to that effect.

Respectfully submitted,

GENENTECH, INC.

Date: July 13, 2001

By: _____

Wendy M. Lee
Reg. No. 40,378
Telephone: (650) 225-1994

Part / #61

7/13/01

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the claims:
Please amend claims 113 and 128 as follows:

113. (Three times amended) A humanized variant of a non-human parent antibody which binds an antigen and comprises a [consensus] 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.

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128. (Twice Amended) 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 a Framework Region (FR) 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 humanized variant binds the antigen more tightly than and up to about 3-fold more tightly than the parent antibody binds antigen.

Please add the following claims:

129. 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.

130. The humanized variable domain of claim 129 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.

131. The humanized variable domain of claim 129 wherein no human Framework Region (FR) residue other than those set forth in the group has been substituted.

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#62

FACSIMILE TRANSMITTAL

1 DNA WAY
South San Francisco, CA 94080
(650) 225-1994
Facsimile: **(650) 952-9881**

DATE: July 30, 2001

Please deliver the following Amendment to:

NAME: Examiner Minh-Tam Davis
U.S. Patent and Trademark office
Washington, DC 20231

Fax No.:(703) 308-4426

FROM: Wendy M. Lee
Registration No.: 40,378

RE: U.S. Serial No.: 08/146,206
Our Docket No.: P0709P1

Number of Pages including this cover sheet - 13

Certificate of Facsimile Transmission Under 37 CFR § 1.8

In accordance with CFR § 1.6(d), this Amendment and Zenapax product information is addressed to Examiner Minh-Tam Davis, The Patent and Trademark Office, Washington, DC 20231 and is being transmitted to facsimile No. (703) 308-4426.

If you do not receive all pages, please notify Wendy Lee at (650) 225-1994.

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1642 Examiner: Minh-Tam Davis

Certificate of Facsimile Transmission Under 37 CFR § 1.8

In accordance with CFR § 1.6(d), this correspondence addressed to Examiner Minh-Tam Davis, The Patent and Trademark Office, Washington, DC 20231 is being transmitted to facsimile No. (703) 308-4426 on

July 30, 2001

Wendy M. Lee

SUPPLEMENTAL AMENDMENT

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

IN THE CLAIMS:

Please amend claim 128 as follows:

128. (Three Times Amended) 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.

REMARKS

Applicants confirm having discussed the above application with Examiner Davis in the telephonic interview of July 25, 2001. In that interview, Examiner Davis indicated that on reconsideration the Section 112, first paragraph rejection would be withdrawn except with respect to claim 128. The Examiner considers the claim to antibodies with improved affinity to be enabled only for variants with FR substitutions at all the positions

for the exemplified better-binding variants. Applicants strongly disagree with the rejection of claim 128 for all the reasons previously elaborated. Nonetheless, in order to expedite allowance of the case, claim 128 is amended herein to recite the FR substitutions in the heavy chain variable region of the huMAb4D5-8 and huMAb4D5-6 variants which bound antigen more tightly than the parent antibody. Support for the claim language can be found on page 72, for instance. Due to the recitation of the FR substitutions, the functional language concerning the improved binding has been removed from the claim. The Examiner indicated that such an amendment would address the maintained Section 112 rejection.

The Examiner further stated in the above interview that the previous Section 103 rejection of claims 113, 115-118, 123 and 127-128 would be maintained unless Applicants could demonstrate the unexpected results through a side-by-side comparison of the antibody described in the cited Queen prior art and the antibodies of the present application. Applicants are now able to provide that comparison. In particular, Applicants attach the Physicians' Desk Reference ® (PDR) entry for the humanized anti-Tac antibody (ZENAPAX®) of the Queen prior art. Applicants note that the other humanized antibodies added to the Queen patents by way of CIP applications are not prior art to Applicants' invention set forth in the rejected claims herein.

As noted in section entitled "PRECAUTIONS" in the PDR entry for ZENAPAX®, when patients received multiple doses of that humanized antibody, anti-idiotype antibodies to ZENAPAX® were detected in patients with an overall incidence of 8.4%. The presently disclosed antibodies produce unexpectedly lower immunogenicity compared to that of the Queen antibody. This is demonstrated in the Shak declaration previously submitted which explained that patients receiving multiple doses of the humanized anti-HER2 antibody (HERCEPTIN®) exemplified in the present application only developed an antibody response 0.1% of the time (1 of the 885 patients evaluated; see paragraph 4 of the Shak declaration); and 0% of the patients treated with an anti-IgE antibody humanized according to the

teachings of the above patent specification developed a HAHA response (paragraph 7 of the Shak declaration). The total lack of an immune response in the patients treated with the humanized anti-IgE antibody is particularly unexpected, given that the allergic rhinitis and asthma patients treated therewith were hyper-sensitive to foreign antigens. Likewise, no significant immunogenicity upon repeated administration was observed for the anti-VEGF and anti-CD11a antibodies humanized according to the teachings in the present application. Paragraphs 8 and 9 of the Shak declaration. Applicants submit that this side-by-side comparison shows that the antibodies of the present application possess unexpected properties with respect to minimal or no immunogenicity upon repeated administration to human patients.

Reconsideration and withdrawal of the Section 103 rejection is respectfully requested in view of the above.

Applicants believe that this case is now in order for allowance and look forward to early notification of same.

Respectfully submitted,
GENENTECH, INC.

Date: July 30, 2001

By: _____
Wendy M. Lee
Reg. No. 40,378
Telephone: (650) 225-1994

Serial No.: 08/146,206

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the claims:

Please amend claim 128 as follows:

128. (Three Times Amended) 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 [a] Framework Region (FR) substitutions at heavy chain positions 71H, 73H, 78H and 93H, utilizing the numbering system set forth in Kabat [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 humanized variant binds the antigen more tightly than and up to about 3-fold more tightly than the parent antibody binds antigen].

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
09/146,206			

EXAMINER	
ART UNIT	PAPER NUMBER

DATE MAILED: 8/29/01

INTERVIEW SUMMARY

All participants (applicant, applicant's representative, PTO personnel):

(1) Minh-Tam Davis (3) Wendy Lee

(2) Anthony Caputa (4)

Date of Interview 08/29/01

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. ☐ was not reached.

Claim(s) 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: Applicant representative discusses prior art of record & pending claims

(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.)

1. ☐ It is not necessary for applicant to provide a separate record of the substance of the interview.

Unless the paragraph above has 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. (See MPEP Section 713.04). If a response to the last Office action has are ready been filed, APPLICANT IS GIVEN ONE MONTH FROM THIS INTERVIEW DATE TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW.

2. ☐ 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. Applicant is not relieved from providing a separate record of the interview unless box 1 above is also checked.

Examiner Note: You must sign this form unless it is an attachment to another form.

FORM **PTOL-413** (REV.1-96)

M Tam Davis

Manual of Patent Examining Procedure, Section 713.04 Substance of Interview must Be Made of Record

A complete written statement as to the substance of any face-to-face or telephone interview with regard to an application must be made of record in the application, whether or not an agreement with the examiner was reached at the interview.

§1.133 Interviews

* * * * *

(b) In every instance where reconsideration is requested in view of an interview with an examiner, a complete written statement of the reasons presented at the interview as warranting favorable action must be filed by the applicant. An interview does not remove the necessity for response to Office action as specified in §§ 1.111,1.135. (35 U.S.C.132)

§ 1.2. Business to be transacted in writing. All business with the Patent or Trademark Office should be transacted in writing. The personal attendance of applicants or their attorneys or agents at the Patent and Trademark Office is unnecessary. The action of the Patent and Trademark Office will be based exclusively on the written record in the Office. No attention will be paid to any alleged oral promise, stipulation, or understanding in relation to which there is disagreement or doubt.

The action of the Patent and Trademark Office cannot be based exclusively on the written record in the Office if that record is itself incomplete through the failure to record the substance of interviews.

It is the responsibility of the applicant or the attorney or agent to make the substance of an interview of record in the application file, unless the examiner indicates he or she will do so. It is the examiner's responsibility to see that such a record is made and to correct material inaccuracies which bear directly on the question of patentability.

Examiners must complete a two-sheet carbon interleaf Interview Summary Form for each interview held after January 1, 1978 where a matter of substance has been discussed during the interview by checking the appropriate boxes and filling in the blanks in neat handwritten form using a ball point pen. Discussions regarding only procedural matters, directed solely to restriction requirements for which interview recordation is otherwise provided for in Section 812.01 of the Manual of Patent Examining Procedure, or pointing out typographical errors or unreadable script in Office actions or the like, are excluded from the interview recordation procedures below.

The Interview Summary Form shall be given an appropriate paper number, placed in the right hand portion of the file, and listed on the "Contents" list on the file wrapper. The docket and serial register cards need not be updated to reflect interviews. In a personal interview, the duplicate copy of the Form is removed and given to the applicant (or attorney or agent) at the conclusion of the interview. In the case of a telephonic interview, the copy is mailed to the applicant's correspondence address either with or prior to the next official communication. If additional correspondence from the examiner is not likely before an allowance or if other circumstances dictate, the Form should be mailed promptly after the telephonic interview rather than with the next official communication.

The Form provides for recordation of the following information:

- Serial Number of the application
- Name of applicant
- Name of examiner
- Date of interview
- Type of interview (personal or telephonic)
- Name of participant(s)) (applicant, attorney or agent, etc.)
- An indication whether or not an exhibit was shown or a demonstration conducted
- An identification of the claims discussed
- An identification of the specific prior art discussed
- An indication whether an agreement was reached and if so, a description of the general nature of the agreement (may be by attachment of a copy of amendments or claims agreed as being allowable). (Agreements as to allowability are tentative and do not restrict further action by the examiner to the contrary.)
- The signature of the examiner who conducted the interview
- Names of other Patent and Trademark Office personnel present.

The Form also contains a statement reminding the applicant of his responsibility to record the substance of the interview.

It is desireable that the examiner orally remind the applicant of his obligation to record the substance of the interview in each case unless both applicant and examiner agree that the examiner will record same. Where the examiner agrees to record the substance of the interview, or when it is adequately recorded on the Form or in an attachment to the Form, the examiner should check a box at the bottom of the Form informing the applicant that he need not supplement the Form by submitting a separate record of the substance of the interview.

It should be noted, however, that the Interview Summary Form will not normally be considered a complete and proper recordation of the interview unless it includes, or is supplemented by the applicant or the examiner to include, all of the applicable items required below concerning the substance of the interview:

A complete and proper recordation of the substance of any interview should include at least the following applicable items:

1) A brief description of the nature of any exhibit shown or any demonstration conducted,
2) an identification of the claims discussed,
3) an identification of specific prior art discussed,
4) an identification of the principal proposed amendments of a substantive nature discussed, unless these are already described on the Interview Summary Form completed by the examiner,
5) a brief identification of the general thrust of the principal arguments presented to the examiner. The identification of arguments need not be lengthy or elaborate. A verbatim or highly detailed description of the arguments is not required. The identification of the arguments is sufficient if the general nature or thrust of the principal arguments made to the examiner can be understood in the context of the application file. Of course, the applicant may desire to emphasize and fully describe those arguments which he feels were or might be persuasive to the examiner,
6) a general indication of any other pertinent matters discussed, and
7) if appropriate, the general results or outcome of the interview unless already described in the Interview Stimmary Form completed by the examiner.

Examiners are expected to carefully review the applicant's record of the substance of an interview. If the record is not complete or accurate, the examiner will give the applicant one month from the date of the notifying letter or the remainder of any period for response, whichever is longer, to complete the response and thereby avoid abandonment of the application (37 CFR 1.135(c)).

Examiner to Check for Accuracy

Applicant's summary of what took place at the interview should be carefully checked to determine the accuracy of any argument or statement attributed to the examiner during the interview. If there is an inaccuracy and it bears directly on the question of patentability, it should be pointed out in the next Office letter. If the claims are allowable for other reasons of record, the examiner should send a letter setting forth his or her version of the statement attributed to him. If the record is complete and accurate, the examiner should place the indication "Interview record OK" on the paper recording the substance of the interview along with the date and the examiner's initials.

1642

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1642 Examiner: Minh-Tam Davis CERTIFICATE OF MAILING I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on August 30, 2001 Anna Kan Anna Kan

#6 KO 10/10/01

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Applicants submit herewith patents, publications or other information (attached hereto and listed on the attached revised Form PTO-1449) of which they are aware, which they believe may be material to the examination of this application and in respect of which there may be a duty to disclose in accordance with 37 CFR §1.56.

This Information Disclosure Statement is filed in accordance with the provisions of:

[] **37 CFR §1.97(b)**
- within three months of the filing date of the application other than a continued prosecution application under 37 CFR §1.53(d); **or**
- within three months of the date of entry of the national stage of a PCT application as set forth in 37 CFR§1.491, **or**
- before the mailing of the first Office action on the merits; **or**
- before the mailing of the first Office action after the filing of a request for a continued examination under 37 CFR §1.114.

[X] **37 CFR §1.97(c)**
- by the applicant after the period specified in 37 CFR §1.97(b), but prior to the mailing date of any of a final action under 37 CFR §1.113, or a notice of allowance under 37 CFR §1.311, or an action that otherwise closes prosecution in the application, and is accompanied by either the fee set forth in 37 CFR §1.17(p) **or** a statement as specified in 37 CFR §1.97(e), as checked below.

[] **37 CFR §1.97(d)**
- after the period specified in CFR §1.97(c), and is accompanied by the fee set

09/06/2001 MGEBREN1 00000111 070330 08146206
01 FC:122 180.00 OP

forth in 37 CFR §1.17(p) **and** a statement as specified in 37 CFR §1.97(e), as checked below.

[If either of boxes 37 CFR §1.97(c) or 37 CFR §1.97(d) is checked above, the following statement under 37 CFR §1.97(e) may need to be completed.]

[] **37 CFR §1.97(e)**Each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of this information disclosure statement.

[] **37 CFR §1.704(d)** Each item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application and the communication was not received by any individual designated in §1.56(c) more than thirty days prior to the filing of this information disclosure statement. Therefore, in accordance with the provisions of 37 CFR §1.704(d), the filing of this information disclosure statement will not be considered a failure to engage in reasonable efforts to conclude prosecution under 37 CFR §1.704.

[X] The U.S. Patent and Trademark Office is hereby authorized to charge Deposit Account No. 07-0630 in the amount of $180.00 to cover the cost of this Information Disclosure Statement under 37 CFR §1.17(p). Any deficiency or overpayment should be charged or credited to this deposit account.

A list of the patent(s) or publication(s) is set forth on the attached revised Form PTO-1449 (Modified).

A copy of the items on PTO-1449 is supplied herewith.

Those patent(s) or publication(s) which are marked with an asterisk (*) in the attached PTO-1449 form are not supplied because they were previously cited by or submitted to the Office in a prior application Serial No. 07/715,272, filed 14 June 1991 and relied upon in this application for an earlier filing date under 35 USC §120.

A concise explanation of relevance of the items listed on PTO-1449 is:

[X] not given

[] given for each listed item

[] given for only non-English language listed item(s) [Required]

[] in the form of an English language copy of a Search Report from a foreign patent office, issued in a counterpart application, which refers to the relevant portions of the references.

In accordance with 37 CFR §1.97(g), the filing of this information disclosure statement shall not be construed as a representation that a search has been made.

In accordance with 37 CFR §1.97(h), the filing of this information disclosure statement shall not be construed to be an admission that the information cited in the statement is, or is considered to be, material to patentability as defined in 37 CFR § 1.56(b).

In the event that the Office determines a fee to be due where none is specifically authorized in this paper, the U.S. Patent and Trademark Office is hereby authorized to charge Deposit Account No. 07-0630 in the amount of $180.00 to cover the cost of this Information Disclosure Statement under 37 CFR §1.17(p).

Respectfully submitted,

GENENTECH, INC.

Date: August 30, 2001

By: _____

By: Steven X. Cui Reg. No.. 44,637
for Wendy M. Lee Reg. No. 40,378
Telephone No. (650) 225-1994

09157

PATENT TRADEMARK OFFICE

9/4/01

File History Report

□ Paper number(s) _____ is/are missing from the United States Patent and Trademark Office's original copy of the file history.

☑ The following page(s) 1 of paper number 64 is/are missing from the United States Patent and Trademark Office's original copy of the file history.

□ The following checked item(s) of paper number _____ is/are missing from the United States Patent and Trademark Office's original copy of the file history.

□ PTO-892 Form □ PTO-1449 Form

□ PTO-948 Form □ Other_____

to this declaration which represent excerpts from our laboratory notebooks with dates obscured.

4. Exhibit A provides the amino acid sequences of humanized 4D5 (anti-HER2) antibody variable domain sequences. A humanized antibody (Hu4D5 Fab) comprising the Hum4D5a V_L and Hum4D5a V_H sequences from Exhibit A (the variable domain sequences of the variant called "huMAb4D5-5" in the above application) was recombinantly produced and found to bind the HER2 antigen as evidenced by the laboratory notebook entries in Exhibit B attached hereto. Hu4D5 Fab comprised a heavy chain variable domain comprising non-human CDR amino acid residues which bound antigen incorporated into a human antibody variable domain, and further comprised a FR amino acid substitution at site 73H. The experimental work in Exhibits A and B was completed prior to September 28, 1990.

We declare further that all statements made herein of our 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 willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: _____

_____
Paul J. Carter

Date: Sept. 4, 2001

Leonard G. Presta
Leonard G. Presta

#63

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FACSIMILE TRANSMITTAL

DATE: October 2, 2001

Please deliver the following Supplemental Amendment, Vincenti et al. reference, and Declaration under 37 CFR §1.131 with attached Exhibits A and B to:

NAME: Examiner Minh-Tam Davis - Group 1642
U.S. Patent and Trademark office
Washington, DC 20231

Fax No.: (703) 308-4426

FROM: Wendy M. Lee
Registration No.: 40,378

RE: U.S. Serial No.: 08/146,206
Our Docket No.: P0709P1

Number of Pages including this cover sheet - 20

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Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1642 Examiner: Minh-Tam Davis Certificate of Facsimile Transmission Under 37 CFR § 1.8 In accordance with CFR § 1.8(d), this correspondence addressed to Examiner Minh-Tam Davis at the Patent and Trademark Office, Washington, DC 20231 is being transmitted to facsimile No. (703) 308-4426 October 2, 2001 Wendy M. Lee

#63 10-10-01

SUPPLEMENTAL SUBMISSION

Official Fax 10/04/01

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

The undersigned confirms having discussed the present application with Examiners Caputa and Davis in the interview on August 29, 2001. Based on and responsive to that discussion, Applicants wish to provide the following additional observations and information.

Status of Previous Rejections

During the most recent interviews, Examiner Davis indicated that the Section 112 and 102 rejections would likely be withdrawn, but that certain of the claims may continue to be rejected under Section 103. The following comments address the 103 rejection.

Additional Information on ZENAPAX®

Examiner Caputa requested that evidence be presented to demonstrate that ZENAPAX® - for which Applicants provided the side-by-side comparison in the July 30, 2001 amendment - was the same as the antibody in the cited Queen references. To confirm that ZENAPAX® (Daclizumab) is the humanized anti-IL2 receptor antibody described in the cited Queen patents and Queen, *PNAS* (1989) paper, Applicants direct the Examiner's attention to the attached copy of Vincenti *et al. N. Engl. J. Med.* 338:161-165 (1998). Vincenti *et al.* refers to Daclizumab (the generic

name for the ZENAPAX® antibody - see PDR entry attached to the July 30, 2001 amendment) and states in column 2 on page 161 that it is a molecularly engineered human IgG1 incorporating the antigen-binding regions of the parent, murine monoclonal antibody. There, Vincenti cross-references the Queen *et al. PNAS* (1989) paper (ref. no. 14 in Vincenti *et al.*) as describing Daclizumab. Hence, Applicants submit that ZENAPAX®/Daclizumab is the humanized anti-IL2 receptor antibody described in the cited Queen references.

Rejection of Claim 113 under 35 USC 103 based on Queen in view of Kabat

The Office Action dated October 25, 2000 (hereinafter, "Action") includes a rejection of claims 113, 115-118, 123, and 127-128 made under 35 USC 103 as being obvious over Queen in view of Kabat. Applicants submit this response to supplement and clarify their previous remarks.

Applicants have previously explained why the Action's conclusions of obviousness made against claim 113 are formed through improper use of hindsight in interpreting the words of the disclosure of Queen. Applicants have also pointed out functional attributes of the humanized antibodies of claim 113 of the present invention that reflect unexpected results, thus providing a distinct and separate basis for overcoming the rejection imposed under §103. Through this supplemental amendment, Applicants respond to points made by Examiner in the Action, and as suggested in personal and telephonic interviews conducted earlier this year. On the basis of each of these points, Applicants respectfully submit that the Examiner has not presented and cannot sustain a *prima facie* showing of obviousness of the claimed inventions. In particular, the Queen disclosure fails to disclose the requisite motivation to combine it with Kabat to set forth a *prima facie* case of obviousness of claim 113.

It is well established that in order for a combination of references to render an invention obvious, there must be a clear motivation in the references that their teachings can be combined. *In re Avery*, 518 F.2d 1228 (1975, CCPA). The mere fact that references address issues within the same field of the invention does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. *ACS Hospital Systems Inc. v. Montefiore Hospital*, 732 F.2d 1572 (Fed. Cir. 1984). In fact, "[t]he references, viewed by themselves and not in retrospect must suggest doing what applicant has done" *In re Skoll*, 523 F.2d 1392 (1975 CCPA). Furthermore, the Federal Circuit and the PTO have made it clear that where a modification must be made to the

prior art to reject or invalidate a claim under 35 USC §103, there must be a showing of proper motivation to do so. In order to establish obviousness, there must be suggestion or motivation in the references. *In re Gordon*, 733 F.2d 900 (Fed. Cir. 1984).

The Action asserts that combining the references to provide the advantages of the present invention would be obvious. However, it identifies nothing within the applied references that would suggest combining those references to arrive at the claimed invention. Rather, the Action improperly cites the findings of *In re Kerkhoven*, 626 F.2d 846 (C.C.P.A. 1980) to support the conclusion of obviousness. Specifically, the Action states that combining the references "would have logically flowed from their having been individually taught in the prior art, and because patent '762 teaches the use of 'consensus sequence', for the same purpose of producing humanized monoclonal antibodies for therapeutic purposes." Applicants contend, however, that the use of *Kerkhoven* in the present case to support a finding of obviousness is improper as the facts of that case are distinguishable from those at hand.

In *Kerkhoven*, the Appellant's claimed a process for producing a detergent containing a mixture of anionic and nonionic detergent materials. In that method, the Appellant's combined two compositions, each taught by the prior art to be useful for the same purpose, in order to form a third composition that was also useful for the same purpose. The patent examiner rejected the method as obvious in light of the prior art under *35 U.S.C. §103*. The Court of Patent Appeals affirmed the rejection and stated that the idea of combining two compositions taught by the prior art to be useful for the same purpose in order to form a third composition to be used for same purpose as the individual components is *prima facie* obvious. *Id* at 850.

The holding in *Kerkhoven* cannot be applied to the instant situation. Most significantly, the disclosure of Queen does not teach the usefulness of a sequence "comprising the most frequently occurring amino acid residues at each location in all human immunoglobulins of a human heavy chain immunoglobulin subgroup" for the purpose of humanizing antibodies, which concept is disclosed and claimed in the present application. In contrast, the Queen patent merely refers to using a "consensus framework from many human antibodies" for humanizing antibodies (column 13, line 7). One of skill in the art interpreting the phrase "many human antibodies" as recited in Queen would construe the phrase to refer to an arbitrarily selected group of human antibodies, with the specification

guiding that such an arbitrarily selected group should consist of sequences that are "unusually homologous to the donor immunoglobulin to be humanized" (column 13, line 6).

There is no specific teaching, suggestion or motivation found in the Queen disclosure that would direct a person of ordinary skill to select sets of consensus sequences that correspond to what is disclosed and claimed in the present application. Specifically, in contrast to Queen, the term "consensus" is used in the present application to refer to the relationship among a well-defined group of human antibody subgroups. See, page 14, lines 29 to 35 and page 15, lines 1-25 of disclosure.

The lack of any specific teaching or motivation in Queen is not cured by the disclosure of Kabat. The Action's analysis of Kabat does not provide any suggestion that the frequency of occurrence of amino acid residues in the immunoglobulin chains can be exploited or used for any particular purpose related to humanizing antibodies.

Indeed, nothing in the '762 patent or in Kabat teaches that 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 is useful for producing humanized monoclonal antibodies for therapeutic purposes. Therefore, regardless of what usefulness may be ascribed to the "consensus framework from many human antibodies" taught in the '762 patent, the sequences taught by Kabat could not have been, and were not, identified in the cited art as being useful for producing humanized monoclonal antibodies for therapeutic purposes. Because the prior art had not equated the potential use of the "consensus framework from many human antibodies" taught in the '762 patent with the potential use of the sequences taught by Kabat, the cited art does not provide motivation to substitute the sequences identified by Kabat for the sequences referred to in the '762 patent.

In summary, in *Kerkhoven*, both components had been taught by the prior art to be useful for the same purpose, and, in addition, the resulting component was also useful for the same purpose. However, in the instant situation only one of the prior art components, namely the "consensus framework from many human antibodies" as recited in the '762 patent, had been referred to for "producing humanized monoclonal antibodies for therapeutic purposes." Therefore, *Kerkhoven* does not control the facts of the present application, and a *prima facie* case of

obviousness on the basis of Queen in view of Kabat is improper because there is no suggestion or motivation to combine the cited references.

Applicants respectfully request that the rejection of claim 113 on the basis of Queen in view of Kabat be withdrawn.

Rejection of Claims 115-118, 123 and 127-128 under 35 USC 103 on the basis of Queen in view of Kabat

Claims 115-118, 123 and 127-128 have also been rejected under 35 USC 103 on the basis of Queen in view of Kabat Since the rationale for this rejection and the facts that control its disposition are distinct from those related to claim 113, Applicants are separately addressing the basis of the rejection of these claims.

Each of the rejected claims recite substitutions at specific FR positions. Applicants have explained that the Queen '762 patent relied on in the Section 103 rejection did not describe a humanized antibody having these specific FR substitution(s), except for antibodies comprising a 73H FR substitution as claimed herein. With respect to the 73H substitution, Applicants provide herewith a swearing behind declaration showing a completion of that invention by the inventors of the present application prior to September 28, 1990 - the 2nd Queen CIP filing date, after which time the disclosure concerning the 73H substitution was added.

The Office has not advanced any reasons why substituting the specifically identified FR positions recited in the claims would have been obvious in view of Queen. The previous 103 rejection was based on the sequential numbering of the FR residues, rather than the Kabat numbering as presently claimed - see the April 25, 2001 amendment which clarifies this distinction at pages 8 and 13. In this regard, Examiner Caputa asked that Applicants emphasize the selection invention claimed herein by contrasting the specifically recited FR substitutions to the disclosure in the Queen patent. Aside from the specific FR substitutions for the exemplified humanized antibodies, Queen refers to FR substitutions in Categories 2-4 (columns 13-15 of the '762 patent). Thus, according to Queen, any one of the approximately 80 V_L FR residues or approximately 87 V_H FR residues can be substituted according to those criteria. This would not provide a specific teaching as to the selection invention set forth in claims herein which recite specific FR positions to be substituted.

In considering the appropriateness of the rejection of these claims on the basis of Queen in view of Kabat, the Examiner's attention is directed to the Federal Circuit decision of *In re Baird*, 16 F.3d 380. In *Baird* the court held that a reference, which discloses a generic formula that encompasses a species claimed by applicant did not render the species obvious because there was no motivation provided to select the particular species that applicant claimed. Moreover, the vast number of species encompassed by the reference's generic disclosure, and the fact that the preferred species of the reference were different from the applicant's species led the court to conclude that the reference did not fairly suggest the selection of the particular species claimed by applicants.

Baird controls the question of non-obviousness of claims 115-118, 123 and 127 in the present situation. As Applicants have previously indicated, the Queen disclosure reveals a genus that encompasses a vast number of species. According to Queen, any one of the approximately 80 V_L FR residues or approximately 87 V_H FR residues can be substituted according to their criteria. This would not provide a specific teaching as to the selection invention set forth in claims herein which recite specifically identified substitutions in FR positions. Further, as explained at the interview, the present case is entitled to a 1991 filing date and, as such, represents one of the early disclosures concerning humanized antibodies. Applicants submit that this should be taken into account when reconsidering the patentability of the present invention over the prior art.

For these reasons, Applicants respectfully request that the rejection of claims 115-118, 123 and 127-128 be withdrawn.

Conclusions

In light of the above and previous amendments and remarks, Applicants respectfully submit that all pending claims as currently presented are in condition for allowance.

Applicants believe that is application is now in condition for allowance, and look forward to early notification that effect. If however, there are outstanding issues, the Examiner is invited to call the undersigned to discuss those.

Respectfully submitted,
GENENTECH, INC.

Date: October 2, 2001

By: _____
Wendy M. Lee
Reg. No. 40,378
Telephone: (650) 225-1994

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: Method for Making Humanized Antibodies	Group Art Unit: 1642 Examiner: Minh-Tam Davis

DECLARATION UNDER 37 CFR §1.131

Assistant Commissioner of Patents
Washington, D.C. 20231

10/04/01

Sir:

We, Paul J. Carter and Leonard G. Presta, do hereby declare and say as follows:

1. We are inventors of the subject matter of the above-identified patent application. All work described hereinafter was performed by us or on our behalf in the Unites States of America.

2. Prior to September 28, 1990, we conceived of and reduced to practice 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 site 73H, utilizing the numbering system set forth in Kabat, as well as an antibody comprising that humanized variable domain.

3. Evidence of the reduction to practice of the claimed invention is set forth in the exhibits attached

to this declaration which represent excerpts from our laboratory notebooks with dates obscured.

4. Exhibit A provides the amino acid sequences of humanized 4D5 (anti-HER2) antibody variable domain sequences. A humanized antibody (Hu4D5 Fab) comprising the Hum4D5a V_L and Hum4D5a V_H sequences from Exhibit A (the variable domain sequences of the variant called "huMAb4D5-5" in the above application) was recombinantly produced and found to bind the HER2 antigen as evidenced by the laboratory notebook entries in Exhibit B attached hereto. Hu4D5 Fab comprised a heavy chain variable domain comprising non-human CDR amino acid residues which bound antigen incorporated into a human antibody variable domain, and further comprised a FR amino acid substitution at site 73H. The experimental work in Exhibits A and B was completed prior to September 28, 1990.

We declare further that all statements made herein of our 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 willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 9/5/01 Paul J. Carter

Date: _____ _____
Leonard G. Presta

Project No.:_____

22 Book No._____ TITLE Humanized 4D5

From Page No.__

Dennis and Paul,
here are the human consensus sequences and the proposed humanized 4D5 sequences.

```
##################################################################################
VARIABLE LIGHT
HuLys has 83% similarity to human kappa subgroup I even when the
CDR sequences are included. The sequences inside the CDR boxes
(Chothia/Lesk definition) can be taken from HuLys or humkapI --
those outside the boxes should be taken from humkapI.

(Kabat/Wu CDR in capital letters; Chothia/Lesk CDR in boxes)

                1                                  10
humkapI  asp ile gln met thr gln ser pro ser ser leu ser ala ser val gly
HuLys

                        20                                  30
humkapI  asp arg val thr ile thr cys ARG ALA|SER GLN ASP ILE SER SER TYR
HuLys                                       |   GLY ASN     HIS ASN

                                        40
humkapI  LEU ASN trp tyr gln gln lys pro gly lys ala pro lys leu leu ile
HuLys        ALA

                    50                                  60
humkapI  tyr|ALA ALA SER|SER LEU GLU SER gly val pro ser arg phe ser gly
HuLys       |TYR THR THR|THR     ALA ASP

                                70                                  80
humkapI  ser gly ser gly thr asp phe thr leu thr ile ser ser leu gln pro
HuLys                                   phe

                                        90
humkapI  glu asp phe ala thr tyr tyr cys GLN GLN|TYR ASN SER LEU PRO TYR
HuLys            ile                    HIS|PHE TRP     THR     ARG

                        100
humkapI  THR phe gly gln gly thr lys val glu ile lys arg thr
HuLys
##################################################################################
VARIABLE HEAVY
The sequences inside the CDR boxes (Chothia/Lesk definition) can be taken
from KOL or humIII -- those outside the boxes should be taken from humIII.

(Kabat/Wu CDR in capital letters; Chothia/Lesk CDR between bars)

                1                                  10
humiii   glu val gln leu val glu ser gly gly gly leu val gln pro gly gly
kol                         gln                 val                 arg

                        20                                  30
humiii   ser leu arg leu ser cys ala ala ser|GLY PHE THR PHE SER ASP TYR
kol                                 ser ser|     ILE     SER

                                        40
humiii   ALA MET|SER trp val arg gln ala pro gly lys gly leu glu trp val
kol             |TYR

                    50      52 |52a 53                        60
humiii   ala VAL ILE SER|GLU ASN GLY SER|ASP THR TYR TYR ALA ASP SER VAL
kol          ILE     TRP|ASP ASP        |    GLN HIS
```

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Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by Leonard Presta	EXHIBIT A

TITLE Humanized 4D5 Project No._____ Book No._____ 23

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```
                                                    70
humiii  LYS GLY arg phe thr ile ser arg asp asp ser lys asn thr leu tyr
kol                                      asn                     phe

         60      62 62a 62b 62c  63                              90
humiii  leu gln met asn ser leu arg ala glu asp thr ala val tyr tyr cys
kol              asp             pro             gly         phe

                                                100  a   b   c   d   e   f   g   h
humiii  ala arg ASP ARG GLY GLY ALA VAL SER TYR CLY PHE PHE GLY TYR GLY
kol                 GLY     HIS GLY PHE CYS SER SER ALA SER CYS PHE GLY

         i   j   k  101                                          110
humiii  GLY PHE PHE ASP VAL trp gly gln gly thr leu val thr val ser ser
kol     PRO --- ---     TYR                     pro
```

The following are proposed humanized 4D5 sequences; changes in Hum4D5b and Hum4D5c from Hum4D5a are followed by an asterisk

```
          1                                        10
humkapI asp ile gln met thr gln ser pro ser ser leu ser ala ser val gly
Hum4D5a
Hum4D5b
Hum4D5c

                      20                                          30
humkapI asp arg val thr ile thr cys ARG ALA SER GLN ASP ILE SER SER TYR
Hum4D5a                                                   VAL ASN THR ALA
Hum4D5b                                                   VAL ASN THR ALA
Hum4D5c                                                   VAL ASN THR ALA

                                  40
humkapI LEU ASN trp tyr gln gln lys pro gly lys ala pro lys leu leu ile
Hum4D5a VAL ALA
Hum4D5b VAL ALA
Hum4D5c VAL ALA

          50                                                  60
humkapI tyr ALA ALA SER SER LEU GLU SER gly val pro ser arg phe ser gly
Hum4D5a     SER     PHE
Hum4D5b     SER     PHE
Hum4D5c     SER     PHE     TYR*

                            70                                        80
humkapI ser gly ser gly thr asp phe thr leu thr ile ser ser leu gln pro
Hum4D5a     arg
Hum4D5b     gly*
Hum4D5c     arg

                                              90
humkapI glu asp phe ala thr tyr tyr cys GLN GLN TYR ASN SER LEU PRO TYR
Hum4D5a                                       HIS TYR THR THR     PRO
Hum4D5b                                       HIS TYR THR THR     PRO
Hum4D5c                                       HIS TYR THR THR     PRO

                      100
humkapI THR phe gly gln gly thr lys val glu ile lys arg thr
Hum4D5a
Hum4D5b
Hum4D5c
```

VL

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Witnessed & Understood by me, Date | Invented by | Date

Recorded by

Project No.

24 Book No. TITLE Humanized 4D5

From Page No. 23

V_H

```
                1                                                  10
humiii   glu val gln leu val glu ser gly gly gly leu val gln pro gly gly
Hum4D5a
Hum4D5b
Hum4D5c

                        20                                                  30
humiii   ser leu arg leu ser cys ala ala ser|GLY PHE THR PHE SER ASP TYR
Hum4D5a                                     |     ASN ILE LYS      THR
Hum4D5b                                     |     ASN ILE LYS      THR
Hum4D5c                                     |     ASN ILE LYS      THR

                                        40
humiii   ALA MET|SER trp val arg gln ala pro gly lys gly leu glu trp val
Hum4D5a  TYR ILE|HIS
Hum4D5b  TYR ILE|HIS
Hum4D5c  TYR ILE|HIS

              50      52|52a  53                                    60
humiii   ala VAL ILE SER|GLU ASN GLY SER|ASP THR TYR TYR ALA ASP SER VAL
Hum4D5a      ARG     TYR|PRO THR ASN GLY|TYR     ARG
Hum4D5b      ARG     TYR|PRO THR ASN GLY|TYR     ARG
Hum4D5c      ARG     TYR|PRO THR ASN GLY|TYR     ARG

                                    70
humiii   LYS GLY arg phe thr ile ser arg asp asp ser lys asn thr leu tyr
Hum4D5a                           ala     thr                     ala
Hum4D5b                           ala     thr                     leu*
Hum4D5c                           ala     thr                     ala

          80      82 82a 82b 82c  83                                  90
humiii   leu gln met asn ser leu arg ala glu asp thr ala val tyr tyr cys
Hum4D5a
Hum4D5b
Hum4D5c

                                        100  a   b   c  101|
humiii   ala arg ASP|ARG GLY GLY ALA VAL SER TYR GLY ASP|VAL trp gly gln
Hum4D5a  ser     TRP|GLY GLY ASP GLY PHE TYR ALA MET ASP|
Hum4D5b  ser     TRP|GLY GLY ASP GLY PHE TYR ALA MET ASP|
Hum4D5c  ser     TRP|GLY GLY ASP GLY PHE TYR ALA MET ASP|TYR*

humiii   gly thr leu val thr val ser ser
Hum4D5a
Hum4D5b
Hum4D5c
```

To Page No. 25

Witnessed & Understood by me, Date

Invented by Leonard Presta Date

Recorded by Leonard Presta

Her2 binding

TITLE #313 Activity of Hu4D5 Fab

Project No.

Book No.

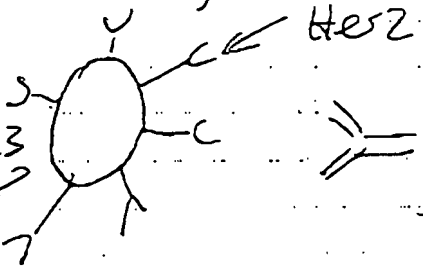
63

From Page No.

AIM: To investigate the activity of Hu4D5 for inhibiting the binding of 4D5 to the cell line SKBR3 which over-expresses Her2

PROTOCOL

Assay performed ~~per~~ by Jay Sarup + Kathy King



[125I]-4D5 (0.1nM).

+ Varying amounts of Hu4D5 Fab (2/2A shockate)

V_{assay} = 400λ

		$\bar{x}$	$\bar{x}$ corrected for non-specific binding (447cpm)	
Total counts	11665, 11567, 11603	11612	11365	
Test buffer	10862, 11473	11168	10921	
(200mM TRIS-HCl, pH8.0; 80mM Glycine)				0.1nM
Hu4D5 (200λ)		2482	2235	$[^{125}I]$-4D5
Hu4D5 (100λ)		4052	3805	

Test buffer	12746, 13147	12947	12700	0.1nM
Hu4D5 (200λ)	9044, 9583	9314	9067	$[^{125}I]$-6E9
Hu4D5 (100λ)	10938, 9918	10428	10181	

another mab which binds her2

Interpretation

- Hu4D5 specifically inhib the interaction of 4D5 with the her2 expressing cell line SKBR3, but does not interfere with the interaction between this cell line and the antibody 6E9
- Orders of Magnitude estimate of Kd (10-100 fold) above 4D5 From cell (opposite) - estimate that sample contains ~1.6ng/λ Hu4D5 Fab

To Page No.

Witnessed & Understood by me, | Date | Invented by Ann Cooke | Date , 1

TI | Recorded by

EXHIBIT B

INTERLEUKIN-2-RECEPTOR BLOCKADE WITH DACLIZUMAB TO PREVENT ACUTE REJECTION IN RENAL TRANSPLANTATION

FLAVIO VINCENTI, M.D., ROBERT KIRKMAN, M.D., SUSAN LIGHT, M.D., GINNY BUMGARDNER, M.D., PH.D., MARK PESCOVITZ, M.D., PHILIP HALLORAN, M.D., PH.D., JOHN NEYLAN, M.D., ALAN WILKINSON, M.D., HENRIK EKBERG, M.D., PH.D., ROBERT GASTON, M.D., LARS BACKMAN, M.D., PH.D., AND JAMES BURDICK, M.D., FOR THE DACLIZUMAB TRIPLE THERAPY STUDY GROUP*

ABSTRACT

Background Monoclonal antibodies that block the high-affinity interleukin-2 receptor expressed on alloantigen-reactive T lymphocytes may cause selective immunosuppression. Daclizumab is a genetically engineered human IgG1 monoclonal antibody that binds specifically to the α chain of the interleukin-2 receptor and may thus reduce the risk of rejection after renal transplantation.

Methods We administered daclizumab (1.0 mg per kilogram of body weight) or placebo intravenously before transplantation and once every other week afterward, for a total of five doses, to 260 patients receiving first cadaveric kidney grafts and immunosuppressive therapy with cyclosporine, azathioprine, and prednisone. The patients were followed at regular intervals for 12 months. The primary end point was the incidence of biopsy-confirmed acute rejection within six months after transplantation.

Results Of the 126 patients given daclizumab, 28 (22 percent) had biopsy-confirmed episodes of acute rejection, as compared with 47 of the 134 patients (35 percent) who received placebo ($P=0.03$). Graft survival at 12 months was 95 percent in the daclizumab-treated patients, as compared with 90 percent in the patients given placebo ($P=0.08$). The patients given daclizumab did not have any adverse reactions to the drug, and at six months, there were no significant differences between the two groups with respect to infectious complications or cancers. The serum half-life of daclizumab was 20 days, and its administration resulted in prolonged saturation of interleukin-2α receptors on circulating lymphocytes.

Conclusions Daclizumab reduces the frequency of acute rejection in kidney-transplant recipients. (N Engl J Med 1998;338:161-5.)

ACUTE rejection is a strong risk factor for chronic rejection in recipients of renal grafts from cadaveric donors.[1] This fact has prompted the development of new immunosuppressive agents designed to reduce the incidence and severity of acute rejection.[2-6] All these agents, however, achieve reductions in the frequency and severity of acute rejection at the price of generalized immunosuppression, with its attendant risks of opportunistic infection and cancer.

One potential target for more specific immunosuppressive therapy with monoclonal antibodies is the interleukin-2 receptor.[7] The high-affinity interleukin-2 receptor is composed of three noncovalently bound chains: a 55-kd α chain (also referred to as CD25 or Tac), a 75-kd β chain, and a 64-kd γ chain.[7] This receptor is present on nearly all activated T cells but not on resting T cells. The interaction of interleukin-2 with this high-affinity receptor is required for the clonal expansion and continued viability of activated T cells. A variety of rodent monoclonal antibodies directed against the α chain of the receptor have been used in animals and humans to achieve selective immunosuppression by targeting only T-cell clones responding to the allograft.[8-13] Daclizumab, a molecularly engineered human IgG1 incorporating the antigen-binding regions of the parent murine monoclonal antibody, offers the potential for greater therapeutic use of interleukin-2–receptor blockade.[14-17] We compared the efficacy of daclizumab with placebo for the prevention of acute rejection in renal-transplant recipients.

METHODS

Study Design

We performed a randomized, double-blind, placebo-controlled trial at 11 transplantation centers in the United States, 3 in Canada, and 3 in Sweden. Adults receiving first renal allografts from cadaveric donors were eligible for the study. Patients were excluded if they were receiving multiple organ transplants or had a positive crossmatch for T-cell lymphocytes. The protocol was approved by the institutional review board or ethics committee at each participating center, and all patients gave written informed consent.

Immunosuppressive Treatment

All patients received cyclosporine, azathioprine, and prednisone. The first dose of cyclosporine was given during the period from 12 hours before to 24 hours after transplantation.

Daclizumab (Zenapax, Hoffmann-LaRoche) or placebo was

From the University of California, San Francisco (F.V.); Brigham and Women's Hospital, Boston (R.K.); Hoffmann-LaRoche, Nutley, N.J. (S.L.); Ohio State University, Columbus (G.B.); Indiana University, Indianapolis (M.P.); the University of Alberta, Edmonton, Alta., Canada (P.H.); Emory University, Atlanta (J.N.); the University of California, Los Angeles (A.W.); Malmö University Hospital, Malmö, Sweden (H.E.); the University of Alabama, Birmingham (R.G.); Sahlgrenska Hospital, Gothenburg, Sweden (L.B.); and Johns Hopkins University, Baltimore (J.B.). Address reprint requests to Dr. Vincenti at the Transplant Service, University of California, San Francisco, 505 Parnassus Ave., Rm. M884, Box 0116, San Francisco, CA 94143-0116.

*Other members of the Daclizumab Triple Therapy Study Group are listed in the Appendix.

administered intravenously over a period of 15 minutes. Each patient received five doses of either daclizumab (1 mg per kilogram of body weight, to a maximum of 100 mg per dose) or placebo (0.2 mg of polysorbate 80 per milliliter in 67 mM phosphate buffer). The first dose was administered within 24 hours before transplantation, with subsequent doses given two, four, six, and eight weeks after transplantation.

Primary and Secondary End Points

The primary end point of the study was the incidence of biopsy-confirmed acute rejection within the first six months after transplantation. All patients with an unexplained rise in the serum creatinine concentration or one or more symptoms of acute rejection (fever, pain over the graft, or a decrease in urinary volume) were required to undergo a renal biopsy within 24 hours after the initiation of antirejection therapy, which consisted initially of intravenous methylprednisolone (7 mg per kilogram per day) for three days. The histologic diagnosis of rejection was based on the presence of acute tubulitis or vasculitis and was made by the pathologist at each institution. Patients were considered to have presumptive rejection if they received a course of antirejection therapy in the absence of histologic confirmation of rejection. The diagnosis of any subsequent episodes of rejection in patients presenting with renal dysfunction was based on clinical criteria, such as the absence of evidence of nephrotoxicity or of urinary tract obstruction or infection, with a biopsy for confirmation performed at the investigator's discretion.

Secondary end points included patient survival and graft survival at one year, the time to the first episode of acute rejection, the number of acute rejection episodes per patient, the need for antilymphocyte therapy (OKT3 or polyclonal antithymocyte globulin) because of glucocorticoid-resistant rejection (defined as the absence of a response to intravenous methylprednisolone pulse therapy), graft function (as indicated by the serum creatinine concentration and glomerular filtration rate), and the cumulative dose of prednisone in the first six months after transplantation.

Pharmacokinetic Measurements

Blood samples were collected immediately before and after (for trough and peak concentrations, respectively) the first and fifth infusions of daclizumab or placebo and on days 70 and 84 after transplantation. A sandwich enzyme-linked immunosorbent assay was used to measure daclizumab in serum.[18]

In 20 consecutive patients at one U.S. center (University of California, San Francisco), lymphocyte analysis was performed to determine the saturation of the interleukin-2–receptor α chain, with the use of methods reported previously.[17]

Glomerular Filtration Rate

The glomerular filtration rate was measured in all patients with functioning grafts six months after transplantation. Measurements were based on iohexol, radioisotope, or inulin clearance.

Statistical Analysis

Differences in categorical variables between the two groups were determined with the use of the Mantel–Haenszel test (with stratification according to center). Differences in the time to the first biopsy-confirmed episode of rejection were determined with the use of the log-rank test (with stratification according to center). The log-rank test was also used to analyze the time to graft failure (or death with a functioning graft) because of the small number of events reported. Kaplan–Meier estimates of the probability of patient survival and graft survival and the cumulative probability of biopsy-confirmed rejection were plotted over time. Differences in the number of presumptive or biopsy-confirmed rejection episodes per patient in the first six months were analyzed with a normal regression model. The serum creatinine concentrations, glomerular filtration rates, and cumulative doses of prednisone administered during the first six months after transplantation in the two groups were compared with the use of the Wilcoxon rank-sum test. Logistic-regression analysis was used to determine the effects of various factors on the probability of biopsy-confirmed rejection. Proportional-hazards analysis was used to determine the effects of various factors on the time to biopsy-confirmed rejection. The results of lymphocyte and interleukin-2–receptor assays were compared with the use of Student's t-test. All statistical tests were two-sided.

All patients randomly assigned to a treatment group were included in the primary analyses of efficacy and safety, according to the intention-to-treat principle. Values are reported as means ±SD.

RESULTS

A total of 260 patients were enrolled in the study: 134 patients were assigned to the placebo group, and 126 to the daclizumab group. The two groups were similar with respect to age, sex, race, cause of end-stage renal disease, presence or absence of panel-reactive anti-HLA antibodies, number of HLA-DR mismatches between donor and recipient, and duration of cold ischemia for the graft (Table 1).

All patients received at least one dose of the study drug, and 107 of the patients in the placebo group (80 percent) and 107 of those in the daclizumab group (85 percent) received all five doses. Graft function was delayed in 39 patients in the placebo group (29 percent) and 27 patients in the daclizumab group (21 percent). The early use of prophylactic antilymphocyte therapy for delayed graft function led to the discontinuation of the study drug in nine patients in the placebo group (7 percent) and nine in the daclizumab group (7 percent).

Efficacy

Daclizumab prophylaxis resulted in a significant reduction in the incidence of biopsy-documented acute rejection during the first six months after transplantation (22 percent, vs. 35 percent in the placebo group; $P=0.03$; odds ratio, 0.5; 95 percent confidence interval, 0.3 to 0.9) (Table 2). The proportion of patients with presumptive or biopsy-confirmed acute rejection and the number of rejection episodes per patient were also lower in the daclizumab group, and the time to the first rejection was longer. There was a trend toward a reduction in the number of patients with two or more rejection episodes and the number receiving antilymphocyte preparations for severe rejection in the daclizumab group. The beneficial effect of daclizumab was not influenced by delayed graft function, initial use of other antilymphocyte therapies, or exclusion of patients who did not receive all five infusions of the study drug (data not shown).

The patient-survival rates at one year were 98 percent in the daclizumab group and 96 percent in the placebo group (Table 3). The graft-survival rates in the daclizumab and placebo groups were 95 and 90 percent, respectively. None of the patients in the daclizumab group but three of those in the placebo group died of infections: one each of aspergillosis,

TABLE 1. BASE-LINE CHARACTERISTICS OF RENAL-ALLOGRAFT RECIPIENTS.*

CHARACTERISTIC	PLACEBO (N=134)	DACLIZUMAB (N=126)
Age — yr	47±13	47±13
Sex — no. of patients (%)		
Male	81 (60)	74 (59)
Female	53 (40)	52 (41)
Race or ethnic group — no. of patients (%)		
White	81 (60)	84 (67)
Black	27 (20)	24 (19)
Other	26 (19)	18 (14)
Cause of renal failure — no. of patients (%)		
Glomerulonephritis	40 (30)	33 (26)
Diabetes mellitus	29 (22)	32 (25)
Hereditary or polycystic kidney disease	20 (15)	24 (19)
Hypertension	19 (14)	18 (14)
Other	26 (19)	19 (15)
Panel-reactive serum antibodies — no. of patients (%)†		
0–10%	121 (90)	113 (89)
11–49%	10 (7)	12 (10)
50–100%	3 (2)	1 (1)
No. of HLA-DR mismatches — no. of patients (%)‡		
0	22 (16)	19 (15)
1	62 (46)	49 (39)
2	40 (30)	50 (40)
Graft cold-ischemia time — hr	21±9	22±8

*Plus–minus values are means ±SD. Percentages may not sum to 100 because of rounding.

†Panel-reactive antibodies are anti-HLA antibodies that have a cytotoxic effect on lymphocytes obtained from a panel of donors from the general population.

‡Data were missing for some patients.

TABLE 2. ACUTE REJECTION EPISODES IN THE FIRST SIX MONTHS AFTER RENAL TRANSPLANTATION IN THE PLACEBO AND DACLIZUMAB GROUPS.

REJECTION	PLACEBO (N=134)	DACLIZUMAB (N=126)	P VALUE
One or more biopsy-confirmed episodes — no. of patients (%)	47 (35)	28 (22)	0.03
One or more biopsy-confirmed or presumptive episodes — no. of patients (%)	52 (39)	32 (25)	0.04
Two or more biopsy-confirmed or presumptive episodes — no. of patients (%)	18 (13)	9 (7)	0.08
Mean no. of episodes/patient	0.6	0.3	0.01
Time to first episode — days*	30±27	75±59	0.008
Episode requiring antilymphocyte therapy — no. of patients (%)†	19 (14)	10 (8)	0.09

*Plus–minus values are means ±SD.

†Antilymphocyte therapy consisted of OKT3 or polyclonal antithymocyte globulin.

TABLE 3. CAUSES OF DEATH AND RENAL-GRAFT FAILURE AT ONE YEAR IN THE PLACEBO AND DACLIZUMAB GROUPS.

CAUSE	PLACEBO (N=134)	DACLIZUMAB (N=126)
	no. of patients (%)	
Death	5 (4)	3 (2)
Infection or lymphoma	3 (2)	1 (1)
Cardiovascular cause	1 (1)	0
Pulmonary embolism	1 (1)	0
Intracerebral bleeding	0	1 (1)
Suicide	0	1 (1)
Graft failure	13 (10)	6 (5)
Death	5 (4)	3 (2)
Rejection	3 (2)	1 (1)
Technical cause	4 (3)	2 (2)
Primary nonfunction	1 (1)	0

coccidioidomycosis, and pseudomonas sepsis. One patient in the daclizumab group died of lymphoma.

The mean serum creatinine concentrations six months after transplantation were the same in the two groups (1.7±0.7 mg per deciliter [150±60 μmol per liter]). The mean glomerular filtration rate was 55±23 ml per minute in the daclizumab group and 52±22 ml per minute in the placebo group. The average daily doses of prednisone and cyclosporine did not differ between the groups at any time during the study, nor was there a difference in the mean trough whole-blood cyclosporine concentrations at any time.

Adverse Events

The administration of daclizumab was not associated with any immediate side effects. There was no significant difference in reported adverse events between the two groups (Table 4). One patient in the placebo group and two patients in the daclizumab group had lymphoma during the first year after transplantation.

Pharmacokinetic Data

Pharmacokinetic data were available for 92 patients in the daclizumab group. The mean serum half-life of daclizumab was 20 days.

Circulating Peripheral-Blood Lymphocytes and Interleukin-2 α-Chain Receptor

There were no differences in absolute lymphocyte numbers between the placebo and daclizumab groups before transplantation or for six months afterward. Circulating CD3+ cell concentrations and T-cell subgroups were not measured, because they were not affected by daclizumab therapy in an earlier study.[17] There was a significant decrease in the percentage of circulating lymphocytes that stained with anti-

TABLE 4. ADVERSE EVENTS AT SIX MONTHS IN THE PLACEBO AND DACLIZUMAB GROUPS.

ADVERSE EVENTS	PLACEBO (N = 134)	DACLIZUMAB (N = 126)
	no. of patients (%)	
Serious event*	13 (10)	6 (5)
Fever	16 (12)	11 (9)
Sepsis and bacteremia	9 (7)	4 (3)
Pneumonia	4 (3)	3 (2)
Fungal infection	27 (20)	21 (17)
Fungemia	2 (1)	0
Local infection	25 (19)	21 (17)
Local infection†	70 (52)	59 (47)
Cellulitis and wound infection	4 (3)	7 (6)
Urinary tract infection	44 (33)	34 (27)
Other	38 (28)	36 (29)
Any viral infection†	32 (24)	29 (23)
Viremia	12 (9)	12 (10)
Local infection	21 (16)	20 (16)
Cytomegalovirus infection	14 (10)	15 (12)
Viremia	10 (7)	12 (10)
Tissue infection	4 (3)	3 (2)

*Serious adverse events were defined as complications other than death or rejection that prolonged or required hospitalization and were possibly or probably related to the study drug.

†Some patients had more than one type of infection.

CD25 antibody starting 10 hours after transplantation and lasting up to four months in the daclizumab group (data not shown). Similarly, there was a significant decrease in the percentage of circulating lymphocytes that stained with the fluorescein-conjugated antibody 7g7, which binds to an interleukin-2 α-chain–receptor epitope distinct from the epitope recognized by daclizumab and reflects total interleukin-2α–receptor expression (data not shown).

DISCUSSION

We found that the patients receiving daclizumab in addition to maintenance therapy with three immunosuppressive agents had a lower frequency of biopsy-confirmed acute rejection in the first six months after transplantation than the patients receiving placebo with the three immunosuppressive agents. In addition, the time to the first episode of acute rejection was significantly prolonged, and the mean number of episodes per patient significantly reduced in the daclizumab group. These results were obtained without a concomitant increase in infectious complications or cancers. The efficacy of daclizumab is probably related to its selective target, the α-chain component of the high-affinity interleukin-2 receptor, which is present almost exclusively on activated T cells. Use of the drug thus spares other immunocompetent cells.[7]

Only 10 percent of daclizumab is composed of murine sequences, which are from the antigen-binding regions of the parent antibody. These sequences are inserted into human immunoglobulin with the use of molecular biologic techniques.[14] Our study highlights the advantages of this type of antibody, including its prolonged serum half-life, approaching that of human IgG, and the absence of functional immunogenicity associated with its use.[15,16,19,20]

The exact mechanism or mechanisms of action of daclizumab are not known. A likely mechanism is that it binds to circulating lymphocytes with interleukin-2 α-chain receptors but does not activate the receptors, and the cells therefore have no free interleukin-2 α-chain receptors available for activation by interleukin-2. In addition, the decline in the percentage of circulating lymphocytes expressing CD25 (measured by staining with 7g7 antibody) without an accompanying decrease in the absolute number of lymphocytes suggests that the expression of interleukin-2 receptors is down-regulated or the shedding of the daclizumab-bound interleukin-2 α chain is increased.

In conclusion, when added to therapy with cyclosporine, azathioprine, and prednisone, daclizumab reduces the frequency of acute rejection and improves short-term graft survival in renal-transplant recipients.

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APPENDIX

In addition to the authors, the following investigators participated in the Daclizumab Triple Therapy Study Group: *Victoria General Hospital, Halifax, N.S., Canada* — B. Kibert; *Huddinge Hospital, Huddinge, Sweden* — G. Tyden; *University of Minnesota, Minneapolis* — A. Matas; *Beth Israel Deaconess Medical Center, Boston* — M. Shapiro; *Tampa General Hospital, Tampa, Fla.* — G. Chan; *Vancouver General Hospital, Vancouver, B.C., Canada* — P. Keown; *University of California, San Francisco* — M. Lantz; *University of Alberta, Edmonton, Alta., Canada* — K. Solez; and *Hoffmann–LaRoche, Nutley, N.J.* — A. Lin, I. Patel, K. Nieforth, A. Wolitzky, and J. Hakimi.

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Interview Summary

Application No.	Applicant(s)
08/146,206	Carter et al
Examiner	**Group Art Unit**
MINH TAM DAVIS	1642

All participants (applicant, applicant's representative, PTO personnel):

(1) *MINH TAM DAVIS* (3) _____

(2) *Wendy Lee* (4) _____

Date of Interview *Dec 11, 2001*

Type: a)☒ Telephonic b)☐ Video Conference
c)☐ Personal [copy is given to 1)☐ applicant 2)☐ applicant's representative]

Exhibit shown or demonstration conducted: d)☐ Yes e)☒ No. If yes, brief description:

Claim(s) discussed: _____

Identification of prior art discussed:

Agreement with respect to the claims f)☐ was reached. g)☐ was not reached. h)☐ N/A.

Substance of Interview including description of the general nature of what was agreed to if an agreement was reached, or any other comments:

Pending claims 43-105, 113-131 are allowable.

(A fuller description, if necessary, and a copy of the amendments which the examiner agreed would render the claims allowable, if available, must be attached. Also, where no copy of the amendments that would render the claims allowable is available, a summary thereof must be attached.)

i)☒ It is not necessary for applicant to provide a separate record of the substance of the interview (if box is checked).

Unless the paragraph above has been checked, THE FORMAL WRITTEN REPLY TO THE LAST OFFICE ACTION MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP section 713.04). If a reply to the last Office action has already been filed, APPLICANT IS GIVEN ONE MONTH FROM THIS INTERVIEW DATE TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW. See Summary of Record of Interview requirements on reverse side or on attached

Examiner Note: You must sign this form unless it is an Attachment to a signed Office action.

M-T DAVIS Re-Rec'd

1600

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PATENT APPLICATION: US/08/146,206C TIME: 13:58:59

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SEQUENCE LISTING

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8 Presta, Leonard G.
10 (ii) TITLE OF INVENTION: Method for Making Humanized Antibodies
12 (iii) NUMBER OF SEQUENCES: 26
14 (iv) CORRESPONDENCE ADDRESS:
15 (A) ADDRESSEE: Genentech, Inc.
16 (B) STREET: 1 DNA Way
17 (C) CITY: South San Francisco
18 (D) STATE: California
19 (E) COUNTRY: USA
20 (F) ZIP: 94080
22 (v) COMPUTER READABLE FORM:
23 (A) MEDIUM TYPE: 3.5 inch, 1.44 Mb floppy disk
24 (B) COMPUTER: IBM PC compatible
25 (C) OPERATING SYSTEM: PC-DOS/MS-DOS
26 (D) SOFTWARE: WinPatin (Genentech)
28 (vi) CURRENT APPLICATION DATA:
C--> 29 **(A) APPLICATION NUMBER: US/08/146,206C**
C--> 30 **(B) FILING DATE: 17-Nov-1993**
31 (C) CLASSIFICATION:
33 (vii) PRIOR APPLICATION DATA:
34 (A) APPLICATION NUMBER: 07/715272
35 (B) FILING DATE: 14-JUN-1991
37 (viii) ATTORNEY/AGENT INFORMATION:
38 (A) NAME: Lee, Wendy M.
39 (B) REGISTRATION NUMBER: 40,378
40 (C) REFERENCE/DOCKET NUMBER: P0709P1
42 (ix) TELECOMMUNICATION INFORMATION:
43 (A) TELEPHONE: 650/225-1994
44 (B) TELEFAX: 650/952-9881
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(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

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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                 100                 105
Ala Met Asp Tyr Trp Gly Gln Gly Ala Ser Val Thr Val Ser Ser
           110                 115                 120
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(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

(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

RAW SEQUENCE LISTING DATE: 12/11/2001
PATENT APPLICATION: US/08/146,206C TIME: 13:58:59

Input Set : A:\p0709pl.txt
Output Set: N:\CRF3\12112001\H146206C.raw

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288           (D) TOPOLOGY: Linear
290      (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:
293   TGAGGAGACG GTGACCGTGG TCCCTTGGCC CCAG 34
295  (2) INFORMATION FOR SEQ ID NO: 11:
297       (i) SEQUENCE CHARACTERISTICS:
298            (A) LENGTH: 36 base pairs
299            (B) TYPE: Nucleic Acid
300            (C) STRANDEDNESS: Single
301            (D) TOPOLOGY: Linear
303      (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:
306   GTAGATAAAT CCTCTAACAC AGCCTATCTG CAAATG 36
308  (2) INFORMATION FOR SEQ ID NO: 12:
310       (i) SEQUENCE CHARACTERISTICS:
311            (A) LENGTH: 36 base pairs
312            (B) TYPE: Nucleic Acid
313            (C) STRANDEDNESS: Single
314            (D) TOPOLOGY: Linear
316      (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:
319   GTAGATAAAT CCAAATCTAC AGCCTATCTG CAAATG 36
321  (2) INFORMATION FOR SEQ ID NO: 13:
323       (i) SEQUENCE CHARACTERISTICS:
324            (A) LENGTH: 36 base pairs
325            (B) TYPE: Nucleic Acid
326            (C) STRANDEDNESS: Single
327            (D) TOPOLOGY: Linear
329      (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:
332   GTAGATAAAT CCTCTTCTAC AGCCTATCTG CAAATG 36
334  (2) INFORMATION FOR SEQ ID NO: 14:
336       (i) SEQUENCE CHARACTERISTICS:
337            (A) LENGTH: 68 base pairs
338            (B) TYPE: Nucleic Acid
339            (C) STRANDEDNESS: Single
340            (D) TOPOLOGY: Linear
342      (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:
345   CTTATAAAGG TGTTTCCACC TATAACCAGA AATTCAAGGA TCGTTTCACG 50
347   ATATCCGTAG ATAAATCC 68
349  (2) INFORMATION FOR SEQ ID NO: 15:
351       (i) SEQUENCE CHARACTERISTICS:
352            (A) LENGTH: 30 base pairs
353            (B) TYPE: Nucleic Acid
354            (C) STRANDEDNESS: Single
355            (D) TOPOLOGY: Linear
357      (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:
360   CTATACCTCC CGTCTGCATT CTGGAGTCCC 30
362  (2) INFORMATION FOR SEQ ID NO: 16:
364       (i) SEQUENCE CHARACTERISTICS:
365            (A) LENGTH: 107 amino acids
366            (B) TYPE: Amino Acid
367            (D) TOPOLOGY: Linear
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VERIFICATION SUMMARY DATE: 12/11/2001
PATENT APPLICATION: US/08/146,206C TIME: 13:59:00

Input Set : A:\p0709pl.txt
Output Set: N:\CRF3\12112001\H146206C.raw

L:3 M:244 W: Invalid beginning of sequence listing, Data=[SEQUENCE LISTING], Duplicate Sequence Listing Title!
L:29 M:220 C: Keyword misspelled or invalid format, [(A) APPLICATION NUMBER:]
L:30 M:220 C: Keyword misspelled or invalid format, [(B) FILING DATE:]

Genentech, Inc.

Anna S. Kan
Legal Department

(650) 225-2830
Fax (650) 952-9881
kan@gene.com

TO: Examiner Minh-Tam Davis
From: Wendy Lee

This is the priority document for 08/146,206.

12/12/2001

Genentech Legal Department

1 DNA Way
South San Francisco, CA 94080
650-225-2830
Fax: 650-952-9881/9882

FAX TRANSMISSION COVER SHEET

Date: *December 12, 2001*

To: *Examiner Minh-Tam Davis*
Group Art 1642

Fax: *(703) 746-7145*

Re: *U.S. Ser. No 09/146,206*
filed August 1, 1995
Attorney Docket No.: P0709P1

Sender: *Anna Kan for Wendy Lee*

YOU SHOULD RECEIVE 12 PAGE(S), INCLUDING THIS COVER SHEET. IF YOU DO NOT RECEIVE ALL THE PAGES, PLEASE CALL 650-225-2830.

Dear Examiner Davis,

Pursuant to your request, attached are courtesy copies of the IDS Transmittals and PTO-1449 Forms filed on August 1, 1995 and February 1, 1999. We understand that you have the cited references but, if not, let us know and we will be happy to provide further copies.

Kindly send us initialed copies of the PTO-1449 Forms for the IDSs filed on the following dates. The reference nos. are noted below in parentheses.

09/02/97 (refs. 100-207)
08/24/98 (refs. 215-224)
02/01/99 (refs. 225-262)
03/09/99 (ref. 263)
08/30/01 (ref. 264-265)

Very truly yours,
Genentech, Inc.

Anna Kan

Anna Kan for Wendy Lee

WL/LS

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1642 Examiner: J. Reeves CERTIFICATE OF HAND DELIVERY I hereby certify that this correspondence is being hand delivered in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on February 1, 1999 R. H. Mitchell

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

Official

12/13/01

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Applicants submit herewith patents, publications or other information (attached hereto and listed on the attached Form PTO-1449) of which they are aware, which they believe may be material to the examination of this application and in respect of which there may be a duty to disclose in accordance with 37 CFR §1.56.

This Information Disclosure Statement:

(a) ☐ accompanies the new patent application submitted herewith. 37 CFR §1.97(a).

(b) ☐ is filed within three months after the filing date of the application or within three months after the date of entry of the national stage of a PCT application as set forth in 37 CFR§1.491.

(c) ☐ as far as is known to the undersigned, is filed before the mailing date of a first Office action on the merits.

(d) ☐ is filed after the first Office Action and more than three months after the application's filing date or PCT national stage date of entry filing but, as far as is known to the undersigned, prior to the mailing date of either a final rejection or a notice of allowance, whichever occurs first, and is accompanied by either the fee ($240) set forth in 37 CFR §1.17(p) or a statement as specified in 37 CFR §1.97(e), as checked below. Should any fee be due, the U.S. Patent and Trademark Office is hereby authorized to charge Deposit Account No. 07-0630 in the amount of $240.00 to cover the cost of this Information Disclosure Statement. Any deficiency or overpayment

should be charged or credited to this deposit account. A duplicate of this sheet is enclosed.

(e) 0 is filed after the mailing date of either a final rejection or a notice of allowance, whichever occurred first, and is accompanied by the fee ($130) set forth in 37 CFR §1.17(i) and a statement as specified in 37 CFR §1.97(e), as checked below. This document is to be considered as a petition requesting consideration of the information disclosure statement. The U.S. Patent and Trademark Office is hereby authorized to charge Deposit Account No. 07-0630 in the amount of $130.00 to cover the cost of this Information Disclosure Statement. Any deficiency or overpayment should be charged or credited to this deposit account. A duplicate of this sheet is enclosed.

(f) (x) is filed after the mailing date of a final rejection, but a request to withdraw the finality thereof under 37 CFR § 1.129(a) was submitted on August 24, 1998. The U.S. Patent and Trademark Office is hereby authorized to charge Deposit Account No. 07-0630 to cover the cost of this Information Disclosure Statement in the event that any fees are due. A duplicate of this sheet is enclosed.

(If either of boxes (d) or (e) is checked above, the following statement under 37 CFR §1.97(e) may need to be completed.) The undersigned states that:

0 Each item of information contained in the information disclosure statement was cited in a communication mailed from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of this information disclosure statement.

0 No item of information contained in this information disclosure statement was cited in a communication mailed from a foreign patent office in a counterpart foreign application and, to the knowledge of the undersigned after making reasonable inquiry, was known to any individual designated in 37 CFR §1.56(c) more than three months prior to the filing of this information disclosure statement.

A list of the patent(s) or publication(s) is set forth on the attached Form PTO-1449 (Modified).

A copy of the items on PTO-1449 is supplied herewith:

(x) each 0 none 0 only those listed below:

A concise explanation of relevance of the items listed on PTO-1449 is:

(x) not given

0 given for each listed item

0 given for only non-English language listed item(s) (Required)

0 In the form of an English language copy of a Search Report from a foreign patent

office, issued in a counterpart application, which refers to the relevant portions of the references.

The Examiner is reminded that a "concise explanation of the relevance" of the submitted prior art "may be nothing more than identification of the particular figure or paragraph of the patent or publication which has some relation to the claimed invention," MPEP §609.

While the information and references disclosed in this Information Disclosure Statement may be "material" pursuant to 37 CFR §1.56, it is not intended to constitute an admission that any patent, publication or other information referred to therein is "prior art" for this invention unless specifically designated as such.

In accordance with 37 CFR §1.97(g), the filing of this Information Disclosure Statement shall not be construed to mean that a search has been made or that no other material information as defined in 37 CFR §1.56(a) exists. It is submitted that the Information Disclosure Statement is in compliance with 37 CFR §1.98 and MPEP §609 and the Examiner is respectfully requested to consider the listed references.

Respectfully submitted,
GENENTECH, INC.

Date: January 29, 1999 By: _____
Wendy M. Lee
Reg. No. 40,378

1 DNA Way
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Phone: (650) 225-1994
Fax: (650) 952-9881

In re Application of: Paul J. Carter et al.
Serial No.: 08/146,206
Filed On: 17 November 1993
Mailed On: 1 August 1995

Docket No.: 709P1
By: Wendy M. Lee
Reg. No.:

The following has been received in the U.S. Patent Office on the date stamped:

___ Amendment/Response
___ Extension of Time Request (dup)
___ Communication/Transmittal Letter (dup)
___ Notice of Appeal (dup)
___ Issue Fee Transmittal Form
X Information Disclosure Statement (Supplemental)
X Form 1449 with 3 References
X Certificate of Mailing
___ Certificate of Express Mailing
Express Mail Label No.:
X Other: Limited Recognition

___ U.S. Patent Application
___ Rule 60 ___ Rule 62
___ Declaration/Power of Atty
___ Assignment
___ Recordation Form/Fee
___ Drawings: __ Sheets
___ Informal ___ Formal
___ Sequence Listing & Diskette
___ PCT Patent Application

In re Application of: Paul J. Carter et al.
Serial No.: 08/146,206
Filed On: 17 November 1993
Mailed On: 1 August 1995

Docket No.: 709P1
By: Wendy M. Lee
Reg. No.:

The following has been received in the U.S. Patent Office on the date stamped:

___ Amendment/Response
___ Extension of Time Request (dup)
___ Communication/Transmittal Letter (dup)
___ Notice of Appeal (dup)
___ Issue Fee Transmittal Form
X Information Disclosure Statement (Supplemental)
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___ Drawings: __ Sheets
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___ PCT Patent Application

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PATENT DOCKET 709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

Paul J. Carter et al.

Serial No. 08/146,206

Filed: 17 November 1993

For: METHOD FOR MAKING HUMANIZED ANTIBODIES

Group Art Unit: 1806

Examiner: D. Adams

CERTIFICATE OF MAILING

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

8/1/95

(Date of Deposit)

Duane Alexander Vick

Name of Depositing Party

Duane Alexander Vick

Signature of Depositing Party

8/1/95

Date of Signature

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

Official
TDis
12/13/01

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

Sir:

Applicants submit herewith patents, publications or other information (attached hereto and listed on the attached Form PTO-1449) of which they are aware, which they believe may be material to the examination of this application and in respect of which there may be a duty to disclose in accordance with 37 CFR §1.56.

This Information Disclosure Statement:

(a) [] accompanies the new patent application submitted herewith. 37 CFR §1.97(a).

(b) [] is filed within three months after the filing date of the application or within three months after the date of entry of the national stage of a PCT application as set forth in 37 CFR§1.491.

(c) [] as far as is known to the undersigned, is filed before the mailing date of a first Office action on the merits.

(d) [X] is filed after the first Office Action and more than three months after the application's filing date or PCT national stage date of entry filing but, as far as is known to the undersigned, prior to the mailing date of either a final rejection or a notice of allowance, whichever occurs first, and is accompanied by either the fee ($210) set forth in 37 CFR §1.17(p) or a certification as specified in 37 CFR §1.97(e), as checked below. Should any fee be due, the U.S. Patent and Trademark Office is hereby authorized to charge Deposit Account No. 07-0630 in the amount of $210.00 to cover the cost of this

Information Disclosure Statement. Any deficiency or overpayment should be charged or credited to this deposit account. **A duplicate of this sheet is enclosed**.

(e) [] is filed after the mailing date of either a final rejection or a notice of allowance, whichever occurred first, and is accompanied by the fee ($130) set forth in 37 CFR §1.17(i)(1) and a certification as specified in 37 CFR §1.97(e), as checked below. **This document is to be considered as a petition requesting consideration of the information disclosure statement.** The U.S. Patent and Trademark Office is hereby authorized to charge Deposit Account No. 07-0630 in the amount of $130.00 to cover the cost of this Information Disclosure Statement. Any deficiency or overpayment should be charged or credited to this deposit account. **A duplicate of this sheet is enclosed**.

[If either of boxes (d) or (e) is checked above, the following "certification" under 37 CFR §1.97(e) may need to be completed.] The undersigned certifies that:

[] Each item of information contained in the information disclosure statement was cited in a communication mailed from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of this information disclosure statement.

[] No item of information contained in this information disclosure statement was cited in a communication mailed from a foreign patent office in a counterpart foreign application or, to the knowledge of the undersigned after making reasonable inquiry, was known to any individual designated in 37 CFR §1.56(c) more than three months prior to the filing of this information disclosure statement.

A list of the patent(s) or publication(s) is set forth on the attached Form PTO-1449 (Modified).

A copy of the items on PTO-1449 is supplied herewith:

[X] each [] none [] only those listed below:

Those patent(s) or publication(s) which are marked with an asterisk (*) in the attached PTO-1449 form are not supplied because they were previously cited by or submitted to the Office in a prior application Serial No. , filed and relied upon in this application for an earlier filing date under 35 USC §120.

A concise explanation of relevance of the items listed on PTO-1449 is:

[X] not given

[] given for each listed item

[] given for only non-English language listed item(s) [Required]

[] in the form of an English language copy of a Search Report from a foreign patent office, issued in a counterpart application, which refers to the relevant portions of the references.

The Examiner is reminded that a "concise explanation of the relevance" of the submitted prior art "may be nothing more than identification of the particular figure or paragraph of the patent or publication which has some relation to the claimed invention," MPEP §609.

While the information and references disclosed in this Information Disclosure Statement may be "material" pursuant to 37 CFR §1.56, it is not intended to constitute an admission that any patent, publication or other information referred to therein is "prior art" for this invention unless specifically designated as such.

In accordance with 37 CFR §1.97(g), the filing of this Information Disclosure Statement shall not be construed to mean that a search has been made or that no other material information as defined in 37 CFR §1.56(a) exists. It is submitted that the Information Disclosure Statement is in compliance with 37 CFR §1.98 and MPEP §609 and the Examiner is respectfully requested to consider the listed references.

A copy of a document pursuant to 37 C.F.R. § 10.9(b) is attached as proof of the authorization of the undersigned to prosecute the above-mentioned application. The original of this document is on file in the Office of Enrollment and Discipline.

Respectfully submitted,
GENENTECH, INC.

Date: August 1, 1995

By: _____
Wendy M. Lee

460 Pt. San Bruno Blvd.
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Fax: (415) 952-9881

In re Application of: Paul J. Carter et al.
Serial No.: 08/146,206
Filed On: November 17, 1993
Hand Delivered On: ___ February 1999

Docket No.: P0709P1
By: Wendy M. Lee
Reg. No.: 40,378

The following has been received in the U.S. Patent Office on the date stamped:

_x_ Information Disclosure Statement
_x_ Form 1449 with 38 References
_x_ Communication with Exhibit A and two priority documents
_x_ Certificate of Hand Delivery

In re Application of: Paul J. Carter et al.
Serial No.: 08/146,206
Filed On: November 17, 1993
Hand Delivered On: ___ February 1999

Docket No.: P0709P1
By: Wendy M. Lee
Reg. No.: 40,378

The following has been received in the U.S. Patent Office on the date stamped:

_x_ Information Disclosure Statement
_x_ Form 1449 with 38 References
_x_ Communication with Exhibit A and two priority documents
_x_ Certificate of Hand Delivery

99 FEB -1 PM 12:21

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FORM PTO-1449	U.S. Dept. of Commerce Patent and Trademark Office	Atty Docket No. P0709P1	Serial No. 08/146,206
LIST OF DISCLOSURES CITED BY APPLICANT (Use several sheets if necessary)		Applicant Carter et al.	
		Filing Date 17 Nov 1993	Group 1806

U.S. PATENT DOCUMENTS

Examiner Initials		Document Number	Date	Name	Class	Subclass	Filing Date
	225	5,714,350	03.02.98	Co et al.			13.01.95
	226	5,821,337	13.10.98	Carter et al.			

FOREIGN PATENT DOCUMENTS

Examiner Initials		Document Number	Date	Country	Class	Subclass	Translation Yes	Translation No
	227	0 460 167 B1	11.12.91	EPO				
	228	0 519 596 A1	23.12.92	EPO				
	229	0 592 106 A1	13.04.94	EPO				
	230	120,694	03.10.84	EPO				
	231	125,023 A1	14.11.84	EPO				
	232	368,684	16.05.90	EPO				
	233	94/11509	26.05.94	PCT				
	234	WO 89/09622		PCT				
	235	WO 92/11383	09.07.92	PCT				
	236	2 188941	14.10.87	UNITED KINGDOM				

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

	237	"Biosym Technologies" in New Products, Chemical Design Automation 3" (December 1988)
	238	"Polygen Corporation" in New Products, Chemical Design Automation 3" (November 1988)
	239	Adair et al., "Humanization of the murine anti-human CD3 monoclonal antibody OKT3" Hum. Antibod. Hybridomas 5:41-47 (1994)
	240	Chothia et al., "Principles of protein-protein recognition" Nature 256:705-708 (1975)
	241	Chothia et al., "Transmission of conformational change in insulin" Nature 302:500-505 (1983)
	242	Corti et al., "Idiotope Determining Regions of a Mouse Monoclonal Antibody and Its Humanized Versions" J. Mol. Biol. 235:53-60 (1994)
	243	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)
	244	Couto et al., "Humanization of KC4G3, an Anti-Human Carcinoma Antibody" Hybridoma 13:215-219 (1994)
	245	Ellis et al., "Engineered Anti-CD38 Monoclonal Antibodies for Immunotherapy of Multiple Myeloma" The Journal of Immunology pps. 925-937 (1995)
	246	Hieter et al., "Evolution of Human Immunoglobulin K J Region Genes" The Journal of Biological Chemistry 257:1516-1522 (1982)
	247	Lesk, Arthur M., "How Different Amino Acid Sequences Determine Similar Protein Structures: The Structure and Evolutionary Dynamics of the Globins" J. Mol. Biol. 136:225-270 (1980)

Examiner	Date Considered

*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.

FORM PTO-1449	U.S. Dept. of Commerce Patent and Trademark Office	Atty Docket No. P0709P1	Serial No. 08/146,206
LIST OF DISCLOSURES CITED BY APPLICANT (Use several sheets if necessary)		Applicant Carter et al.	
		Filing Date 17 Nov 1993	Group 1806

		OTHER DISCLOSURES (Including Author, Title, Date, Pertinent Pages, etc.)
	248	Matsumura et al., "Hydrophobic stabilization in T4 lysozyme determined directly by multiple substitutions of Ile 3" Nature 334:406-410 (1988)
	249	Morrison, S. L., "Transfectomas Provide Novel Chimeric Antibodies" Science 229:1202-1207 (September 20, 1985)
	250	Nakatani et al., "Humanization of mouse anti-human IL-2 receptor antibody 8-B10" Protein Engineering 7:435-443 (1994)
	251	Ohtomo et al., "Humanization of Mouse ONS-M21 Antibody with the Aid of Hybrid Variable Regions" Molecular Immunology 32:407-416 (1995)
	252	Padlan et al., "Model-Building Studies of Antigen-Binding Sites: The Hapten-Binding Site of MOPC-315" C.S. Harbor Symp. Quant. Biol. 41:627-637 (1977)
	253	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)
	254	Sha et al., "A Heavy-Chain Grafted Antibody that Recognizes the Tumor-Associated TAG72 Antigen" Cancer Biotherapy 9:341-349 (1994)
	255	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)
	256	Tramontano, "Structural Determinants of the Conformations of Medium-Sized Loops in Proteins" Proteins 6:382-394 (1989)
	257	Uchiyama et al., "A Monoclonal Antibody (ANTI-Tac) Reactive with Activated and Functionally Mature Human T Cells" Journal of Immunology 126:1393-1397 (1981)
	258	Vincenti et al., "Interleukin-2-Receptor Blockade with Daclizumab to Prevent Acute Rejection in Renal Transplantation" New Engl. J. Med. 338:161-165 (1998)
	259	Vitetta et al., "Redesigning Nature's Poisons to Create Anti-Tumor Reagents" Science 238:1098-1104 (1987)
	260	Waldmann et al., "Interleukin 2 Receptor (Tac Antigen) Expression in HTLV-1-associated Adult T-Cell Leukemia" Cancer Research 45:4559s-4562s (1985)
	261	Waldmann, Thomas A., "The Structure, Function, and Expression of Interleukin-2 Receptors on Normal and Malignant Lymphocytes" Science 232:727-732 (1986)
	262	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)

Examiner	Date Considered

*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.

USCOMM-DC 80-398.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231
www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

7590 12/18/2001

GENENTECH, INC.
1 DNA WAY
SOUTH SAN FRANCISCO, CA 940804990

EXAMINER
DAVIS, MINH TAM B

ART UNIT	CLASS-SUBCLASS
1642	530-387300

DATE MAILED: 12/18/2001

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
08/146,206	11/17/1993	PAUL J. CARTER	709P1	3992

TITLE OF INVENTION: METHOD FOR MAKING HUMANIZED ANTIBODIES

TOTAL CLAIMS	APPLN. TYPE	SMALL ENTITY	ISSUE FEE	PUBLICATION FEE	TOTAL FEE(S) DUE	DATE DUE
82	nonprovisional	NO	$1280	$0	$1280	03/18/2002

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT.

PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151.

HOW TO REPLY TO THIS NOTICE:

I. Review the SMALL ENTITY status shown above. If the SMALL ENTITY is shown as YES, verify your current SMALL ENTITY status:

A. If the status is changed, pay the PUBLICATION FEE (if required) and twice the amount of the ISSUE FEE shown above and notify the United States Patent and Trademark Office of the change in status, or

B. If the status is the same, pay the TOTAL FEE(S) DUE shown above.

If the SMALL ENTITY is shown as NO:

A. Pay TOTAL FEE(S) DUE shown above, or

B. If applicant claimed SMALL ENTITY status before, or is now claiming SMALL ENTITY status, check the box below and enclose the PUBLICATION FEE and 1/2 the ISSUE FEE shown above.

☐ Applicant claims SMALL ENTITY status. See 37 CFR 1.27.

II. PART B - FEE(S) TRANSMITTAL should be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). Even if the fee(s) have already been paid, Part B - Fee(s) Transmittal should be completed and returned. If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Box ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PTOL-85 (REV. 07-01) Approved for use through 01/31/2004.

PART B - FEE(S) TRANSMITTAL

Complete and mail this form, together with applicable fee(s), to:

Box ISSUE FEE
Assistant Commissioner for Patents
Washington, D.C. 20231

MAILING INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 4 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Legibly mark-up with any corrections or use Block 1)

7590 12/18/2001

GENENTECH, INC.
1 DNA WAY
SOUTH SAN FRANCISCO, CA 940804990

Note: The certificate of mailing below can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing.

Certificate of Mailing

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Box Issue Fee address above on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
08/146,206	11/17/1993	PAUL J. CARTER	709P1	3992

TITLE OF INVENTION: METHOD FOR MAKING HUMANIZED ANTIBODIES

TOTAL CLAIMS	APPLN. TYPE	SMALL ENTITY	ISSUE FEE	PUBLICATION FEE	TOTAL FEE(S) DUE	DATE DUE
82	nonprovisional	NO	$1280	$0	$1280	03/18/2002

EXAMINER	ART UNIT	CLASS-SUBCLASS
DAVIS, MINH TAM B	1642	530-387300

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363). Use of PTO form(s) and Customer Number are recommended, but not required.

❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47) attached.

2. For printing on the patent front page, list (1) the names of up to 3 registered patent attorneys or agents OR, alternatively, (2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 _____

2 _____

3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. Inclusion of assignee data is only appropriate when an assignment has been previously submitted to the USPTO or is being submitted under separate cover. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) ❑ individual ❑ corporation or other private group entity ❑ government

4a. The following fee(s) are enclosed:

❑ Issue Fee

❑ Publication Fee

❑ Advance Order - # of Copies _____

4b. Payment of Fee(s):

❑ A check in the amount of the fee(s) is enclosed.

❑ Payment by credit card. Form PTO-2038 is attached.

❑ The Commissioner is hereby authorized by charge the required fee(s), or credit any overpayment, to Deposit Account Number _____(enclose an extra copy of this form).

The COMMISSIONER OF PATENTS AND TRADEMARKS is requested to apply the Issue Fee and Publication Fee (if any) to the application identified above.

(Authorized Signature) (Date)

NOTE; The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending on the needs of the individual case. Any comments on the amount of time required to complete this form should be sent to the Chief Information Officer, United States Patent and Trademark Office, Washington, D.C. 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND FEES AND THIS FORM TO: Box Issue Fee, Assistant Commissioner for Patents, Washington, D.C. 20231

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.

TRANSMIT THIS FORM WITH FEE(S)

PTOL-85 (REV. 07-01) Approved for use through 01/31/2004. OMB 0651-0033 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
08/146,206	11/17/1993	PAUL J. CARTER	709P1	3992

7590 12/18/2001

GENENTECH, INC.
1 DNA WAY
SOUTH SAN FRANCISCO, CA940804990

EXAMINER	
DAVIS, MINH TAM B	
ART UNIT	**PAPER NUMBER**
1642	

DATE MAILED: 12/18/2001

Determination of Patent Term Extension or Adjustment under 35 U.S.C. 154 (b)
(application filed prior to June 8, 1995)

This patent application was filed prior to June 8, 1995, thus no Patent Term Extension or Adjustment applies.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) system. (http://pair.uspto.gov)

PTOL-85 (REV. 07-01) Approved for use through 01/31/2004.

Notice of Allowability

Application No.	Applicant(s)
08/146,206	Carter et al
Examiner	**Art Unit**
MINH TAM DAVIS	1642

--The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance and Issue Fee Due or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *interview on 12/11/01*.

2. ☒ The allowed claim(s) is/are *43-105, 113-128, renumbered as 1-82*.

3. ☐ The drawings filed on _____ are acceptable as formal drawings.

4. ☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

   a) ☐ All b) ☐ Some* c) ☐ None of the:

   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____.
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   *Certified copies not received: _____.

5. ☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application. **THIS THREE-MONTH PERIOD IS NOT EXTENDABLE FOR SUBMITTING NEW FORMAL DRAWINGS, OR A SUBSTITUTE OATH OR DECLARATION.** ~~**This three-month period for complying with the REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL is extendable under 37 CFR 1.136(a).**~~

6. ☐ Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient. A SUBSTITUTE OATH OR DECLARATION IS REQUIRED.

7. ☒ Applicant MUST submit NEW FORMAL DRAWINGS

   (a) ☒ including changes required by the Notice of Draftsperson's Patent Drawing Review (PTO-948) attached

   1) ☐ hereto or 2) ☒ to Paper No. *12*.

   (b) ☐ including changes required by the proposed drawing correction filed _____, which has been approved by the examiner.

   (c) ☐ including changes required by the attached Examiner's Amendment/Comment or in the Office action of Paper No. _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings. The drawings should be filed as a separate paper with a transmittal letter addressed to the Official Draftsperson.

8. ☐ Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Any reply to this letter should include, in the upper right hand corner, the APPLICATION NUMBER (SERIES CODE/SERIAL NUMBER). If applicant has received a Notice of Allowance and Issue Fee Due, the ISSUE BATCH NUMBER and DATE of the NOTICE OF ALLOWANCE should also be included.

Attachment(s)

1 ☐ Notice of References Cited (PTO-892)
2 ☐ Notice of Informal Patent Application (PTO-152)
3 ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
4 ☐ Interview Summary (PTO-413), Paper No. _____.
5 ☒ Information Disclosure Statement(s) (PTO-1449), Paper No(s). *10 sheets*
6 ☐ Examiner's Amendment/Comment
7 ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
8 ☐ Examiner's Statement of Reasons for Allowance
9 ☐ Other

EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with Wendy Lee on 12/13/01.

The application has been amended as follows:

In the claims:

Claim 114. Delete "about", and replace it with --- up to ---

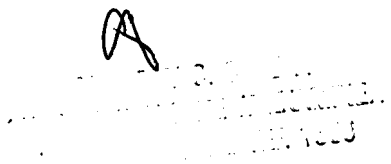
Delete "tightly", and replace it with --- in the binding affinity ---

Any inquiry concerning this communication or earlier communications from the examiner should be directed to MINH-TAM DAVIS whose telephone number is 703-305-2008. The examiner can normally be reached on 9:30AM-4:00PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, ANTHONY CAPUTA can be reached on 703-308-3995. The fax phone numbers for the organization where this application or proceeding is assigned are 703-308-4426 for regular communications and 703-308-4426 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 703-308-0916.

MINH TAM DAVIS
December 14, 2001



Attachment #64

FORM PTO-1449

U.S. Dept. of Commerce
Patent and Trademark Office

LIST OF DISCLOSURES CITED BY APPLICANT

(Use several sheets if necessary)

Atty Docket No.	Serial No.
P0709P1	08/146,206
Applicant: Carter et al.	
Filing Date: 17 Nov 1993	Group: 1642

U.S. PATENT DOCUMENTS

Examiner Initials		Document Number	Date	Name	Class	Subclass	Filing Date
MTS	264	5,834,598	10/11/98	Lowman et al.	530	399	

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

MTS	265	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 April 22-27, 1990) 199(1-2):BIOT 60 (Apr 1990)

Examiner: M.T. DAVIS

Date Considered: 12/05/01

*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.

USCOMM-DC 80-398.

Attachment to Paper #47

Sheet 1 of 1

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

LIST OF DISCLOSURES CITED BY APPLICANT

(Use several sheets if necessary)

Atty Docket No.	Serial No.
P0709P1	08/146,206
Applicant: Carter et al.	
Filing Date: 17 Nov 1993	Group: 1642

U.S. PATENT DOCUMENTS

Examiner Initials		Document Number	Date	Name	Class	Subclass	Filing Date
MJR	263	5,859,205	12.01.99	Adair et al.	530	387.3	07.09.94

Examiner: Julie Reeves, PhD Date Considered: 3/25/99

*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.

M. T. Davis 12/05/01

USCOMM-DC 80-398.

#46 Attach

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

LIST OF DISCLOSURES CITED BY APPLICANT

(Use several sheets if necessary)

Atty Docket No.	Serial No.
P0709P1	08/146,206
Applicant	
Carter et al.	
Filing Date	**Group**
17 Nov 1993	1806

U.S. PATENT DOCUMENTS

Examiner Initials		Document Number	Date	Name	Class	Subclass	Filing Date
TDS	225	5,714,350	03.02.98	Co et al.	435	69.6	13.01.95
	226	5,821,337	13.10.98	Carter et al.	530	387.3	

FOREIGN PATENT DOCUMENTS

Examiner Initials		Document Number	Date	Country	Class	Subclass	Translation Yes	Translation No
TDS	227	0 460 167 B1	11.12.91	EPO				
	228	0 519 596 A1	23.12.92	EPO				
	229	0 592 106 A1	13.04.94	EPO				
	230	120,694	03.10.84	EPO				
	231	125,023 A1	14.11.84	EPO				
	232	368,684	16.05.90	EPO				
	233	94/11509	26.05.94	PCT				
	234	WO 89/09622	10/1989	PCT				
	235	WO 92/11383	09.07.92	PCT				
	236	2 188941	14.10.87	UNITED KINGDOM				

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

Davis	237	"Biosym Technologies" in New Products, Chemical Design Automation 3" (December 1988)
	238	"Polygen Corporation" in New Products, Chemical Design Automation 3" (November 1988)
	239	Adair et al., "Humanization of the murine anti-human CD3 monoclonal antibody OKT3" Hum. Antibod. Hybridomas 5:41-47 (1994)
	240	Chothia et al., "Principles of protein-protein recognition" Nature 256:705-708 (1975)
	241	Chothia et al., "Transmission of conformational change in insulin" Nature 302:500-505 (1983)
	242	Corti et al., "Idiotope Determining Regions of a Mouse Monoclonal Antibody and Its Humanized Versions" J. Mol. Biol. 235:53-60 (1994)
	243	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)
	244	Couto et al., "Humanization of KC4G3, an Anti-Human Carcinoma Antibody" Hybridoma 13:215-219 (1994)
	245	Ellis et al., "Engineered Anti-CD38 Monoclonal Antibodies for Immunotherapy of Multiple Myeloma" The Journal of Immunology pps. 925-937 (1995)
	246	Hieter et al., "Evolution of Human Immunoglobulin K J Region Genes" The Journal of Biological Chemistry 257:1516-1522 (1982)
TDS	247	Lesk, Arthur M., "How Different Amino Acid Sequences Determine Similar Protein Structures: The Structure and Evolutionary Dynamics of the Globins" J. Mol. Biol. 136:225-270 (1980)

Examiner	Date Considered
M. T. DAVIS	12/05/01

*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.

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

Atty Docket No.	Serial No.
P0709P1	08/146,206
Applicant: Carter et al.	
Filing Date: 17 Nov 1993	Group: 1806

LIST OF DISCLOSURES CITED BY APPLICANT

(Use several sheets if necessary)

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

	248	Matsumura et al., "Hydrophobic stabilization in T4 lysozyme determined directly by multiple substitutions of Ile 3" Nature 334:406-410 (1988)
	249	Morrison, S. L., "Transfectomas Provide Novel Chimeric Antibodies" Science 229:1202-1207 (September 20, 1985)
	250	Nakatani et al., "Humanization of mouse anti-human IL-2 receptor antibody B-B10" Protein Engineering 7:435-443 (1994)
	251	Ohtomo et al., "Humanization of Mouse ONS-M21 Antibody with the Aid of Hybrid Variable Regions" Molecular Immunology 32:407-416 (1995)
	252	~~Padlan et al., "Model-Building Studies of Antigen-Binding Sites: The Hapten-Binding Site of MOPC-315" C.S. Harbor Symp. Quant. Biol. 41:627-637 (1977)~~ Dupl.
	253	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)
	254	Sha et al., "A Heavy-Chain Grafted Antibody that Recognizes the Tumor-Associated TAG72 Antigen" Cancer Biotherapy 9:341-349 (1994)
	255	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)
	256	Tramontano, "Structural Determinants of the Conformations of Medium-Sized Loops in Proteins" Proteins 6:382-394 (1989)
	257	Uchiyama et al., "A Monoclonal Antibody (ANTI-Tac) Reactive with Activated and Functionally Mature Human T Cells" Journal of Immunology 126:1393-1397 (1981)
	258	Vincenti et al., "Interleukin-2-Receptor Blockade with Daclizumab to Prevent Acute Rejection in Renal Transplantation" New Engl. J. Med. 338:161-165 (1998)
	259	Vitetta et al., "Redesigning Nature's Poisons to Create Anti-Tumor Reagents" Science 238:1098-1104 (1987)
	260	Waldmann et al., "Interleukin 2 Receptor (Tac Antigen) Expression in HTLV-1-associated Adult T-Cell Leukemia" Cancer Research 45:4559s-4562s (1985)
	261	Waldmann, Thomas A., "The Structure, Function, and Expression of Interleukin-2 Receptors on Normal and Malignant Lymphocytes" Science 232:727-732 (1986)
✓	262	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)

Examiner: M.T. Davis | Date Considered: 12/06/01

*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.

USCOMM-DC 80-398.

copy. Attachment # 15 (17)

Sheet 1 of 1

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

Atty Docket No.	Serial No.
P0709P1	08/146,206
Applicant: Carter et al.	
Filing Date: 17 Nov 1993	Group: 1806

LIST OF DISCLOSURES CITED BY APPLICANT

(Use several sheets if necessary)

FOREIGN PATENT DOCUMENTS

Examiner Initials		Document Number	Date	Country	Class	Subclass	Translation Yes	Translation No
TDB	79	WO 92/04381	19.03.92	PCT				
	80	WO 92/05274	02.04.92	PCT				
	81	WO 92/15683	17.09.92	PCT				

Examiner M. T. DAVIS Date Considered 12/05/01

*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.

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

Atty Docket No.	Serial No.
P0709P1	08/146,206
Applicant: Carter et al.	
Filing Date: 17 Nov 1993	Group: 1806

LIST OF DISCLOSURES CITED BY APPLICANT

(Use several sheets if necessary)

U.S. PATENT DOCUMENTS

Examiner Initials		Document Number	Date	Name	Class	Subclass	Filing Date
PN	81	5,225,539 7-6-93	~~06.07.93~~	Winter, G.	530/387.3	~~C07K~~	~~15/28~~ ~~25.10.91~~ 10/25/91
PN	82	5,530,101 6-25-96	~~25.06.96~~	Queen et al.	530/387.3	~~A61K~~	39/395 ~~19.12.90~~ 2/19/90

FOREIGN PATENT DOCUMENTS

Examiner Initials		Document Number	Date	Country	Class	Subclass	Translation Yes	Translation No
PN	83	85058/91 3-30-92	~~30.03.92~~	AUSTRALIA	C07K	15/12		
	84	328,404 A1 8-16-89	~~16.08.89~~	EPO	A61K	39/395		
	85	451,216 B1 1-24-96	~~24.01.96~~	EPO	C12P	21/08		
	86	WO 91/09966 7-11-91	~~11.07.91~~	PCT	C12P	21/08		
	87	WO 91/09968 7-11-91	~~11.07.91~~	PCT	C12P	21/08		
PN	88	WO 92/11018 9-7-92	~~07.09.92~~	PCT	A61K	35/14		

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

PN	89	Carter et al., "High level escherichia coli expression and production of a bivalent humanized antibody fragment" Bio/Technology 10:163-167 (1992)
	90	Foote et al., "Antibody Framework Residues Affecting the Conformation of the Hypervariable Loops" J. Mol. Biol. 224:487-499 (1992)
	91	Foote, J., "Humanized Antibodies" Nova acta Leopoldina 61(269):103-110 (1989)
	92	Kabat et al., "Sequences of Proteins of Immunological Interest", Bethesda, MD:National Institute of Health pps. 14-32 (1983)
	93	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)
	94	Maeda et al., "Construction of Reshaped Human Antibodies with HIV-neutralizing Activity" Hum. Antibod. Hybridomas 2:124-134 (July 1991)
	95	Riechmann et al, "Expression of an Antibody Fv Fragment in Myeloma Cells" J. Mol. Biol. 203:825-828 (1988)
	96	Routledge et al., "A Humanized Monovalent CD3 Antibody which Can Activate Homologous Complement" European Journal of Immunology 21:2717-2725 (1991)
	97	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 (December 15, 1991)
PN	98	Tempest et al., "Reshaping a Human Monoclonal Antibody to Inhibit Human Respiratory Syncytial Virus Infection In Vivo" Bio/Technology 9:266-271 (March 1991)

Examiner: Patrick J. Nolan — Date Considered: 12-16-96

*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.

M.T. Davis 12/05/01

USCOMM-DC 80-398.

#40 Attach

FORM PTO-1449

U.S. Dept. of Commerce
Patent and Trademark Office

Attorney Docket No.	Serial No.
P0709P1	08/146,206
Applicant: Carter et al.	
Filing Date: 17 Nov 1993	Group: 1806

LIST OF DISCLOSURES CITED BY APPLICANT

(Use several sheets if necessary)

U.S. PATENT DOCUMENTS

Examiner Initials		Document Number	Date	Name	Class	Subclass	Filing Date
	*215	~~07/934,373~~		~~Carter et al.~~			~~21.08.92~~
	*216	~~08/437,642~~		~~Carter et al.~~			~~09.05.95~~
	*217	~~09/115,800~~		~~Carter et al.~~			~~15.07.98~~
MT	218	5,677,171	14.10.97	Hudziak et al.	435	7.23	05.08.94
✓	219	5,772,997	30.06.98	Hudziak et al.	424	130.1	23.05.95

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

MT	220	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)
	221	Casale et al., "Use of an anti-IgE humanized monoclonal antibody in ragweed-induced allergic rhinitis" J. Allergy Clin. Immunol. 100:110-121 (1997)
	222	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)
	223	Mathieson et al., "Monoclonal-Antibody Therapy in Systemic Vasculitis" New England J. of Medicine pps. 250-254 (July 1990)
✓	224	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)

Examiner	Date Considered
M.T. DAVIS	12/05/01

*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.

USCOMM-DC 80-398.

[redacted] Attachment [redacted]

Sheet 1 of 1

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

LIST OF DISCLOSURES CITED BY APPLICANT

(Use several sheets if necessary)

Atty Docket No.	Serial No.
P0709P1	08/146,206
Applicant: Carter et al.	
Filing Date: 17 Nov 1993	Group: ~~1806~~ 1816

FOREIGN PATENT DOCUMENTS

Examiner Initials		Document Number	Date	Country	Class	Subclass	Translation Yes	Translation No
TM PN	208	403,156 A1	19.12.90	EPO				
	209	438,310 A2	24.07.91	EPO				
	210	438,312 A2	24.07.91	EPO				
	211	440,351 A2	07.08.91	EPO				
	212	WO 91/07492	30.05.91	PCT				
PN	213	WO 92/16562*	01.10.92	PCT				
	214	WO 94/12214	09.06.94	PCT				

Examiner: Patricia A-Nol Date Considered: 12/22/97

*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.

M. T. Davis 12/05/01

USCOMM-DC 80-398.

03-19-02

95

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1642 Examiner: Minh-Tam Davis **Date of Mailing of PTOL 85 entitled "Notice of Allowance and Issue Fee Due" December 18, 2001** CERTIFICATE OF MAILING I hereby certify that this correspondence 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 below and is addressed to: U.S. Patent and Trademark Office, Washington, D.C. 20231-9999 Express Mail Label No. EL 889 330 529 US March 18, 2002 Wendy M. Lee

#69MM

TRANSMITTAL OF NEW DRAWINGS TO CORRECT INFORMALITIES WITHIN THREE MONTH PERIOD OF RESPONSE SET IN NOTICE OF ALLOWABILITY (PTOL 37)

BOX ISSUE FEE
Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

1. To correct the informalities in the drawings as noted in the Draftsman's objections on PTO-948 applicant submits herewith new drawings for this application. Number of sheets of drawings submitted: 9.

2. The three month period of response set in the Notice of Allowability (PTOL 37) expires on March 18, 2002 and this submission is on or before this expiry date.

Respectfully submitted,
GENENTECH, INC.

Date: March 18, 2002 By: _____
Wendy M. Lee
Reg. No. 40,378
Telephone No. (650) 225-1994

09157
PATENT TRADEMARK OFFICE

FIG. 1A

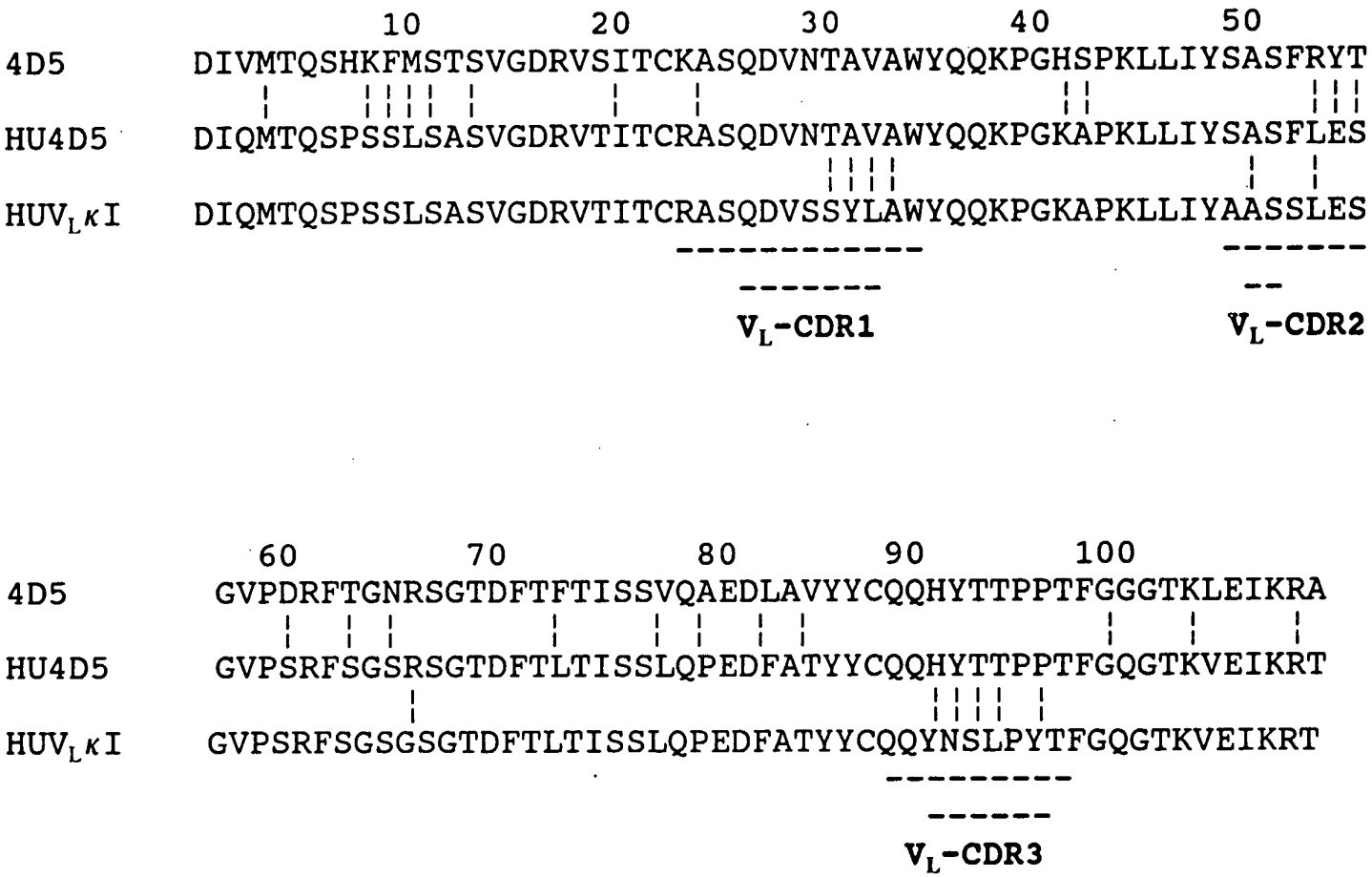
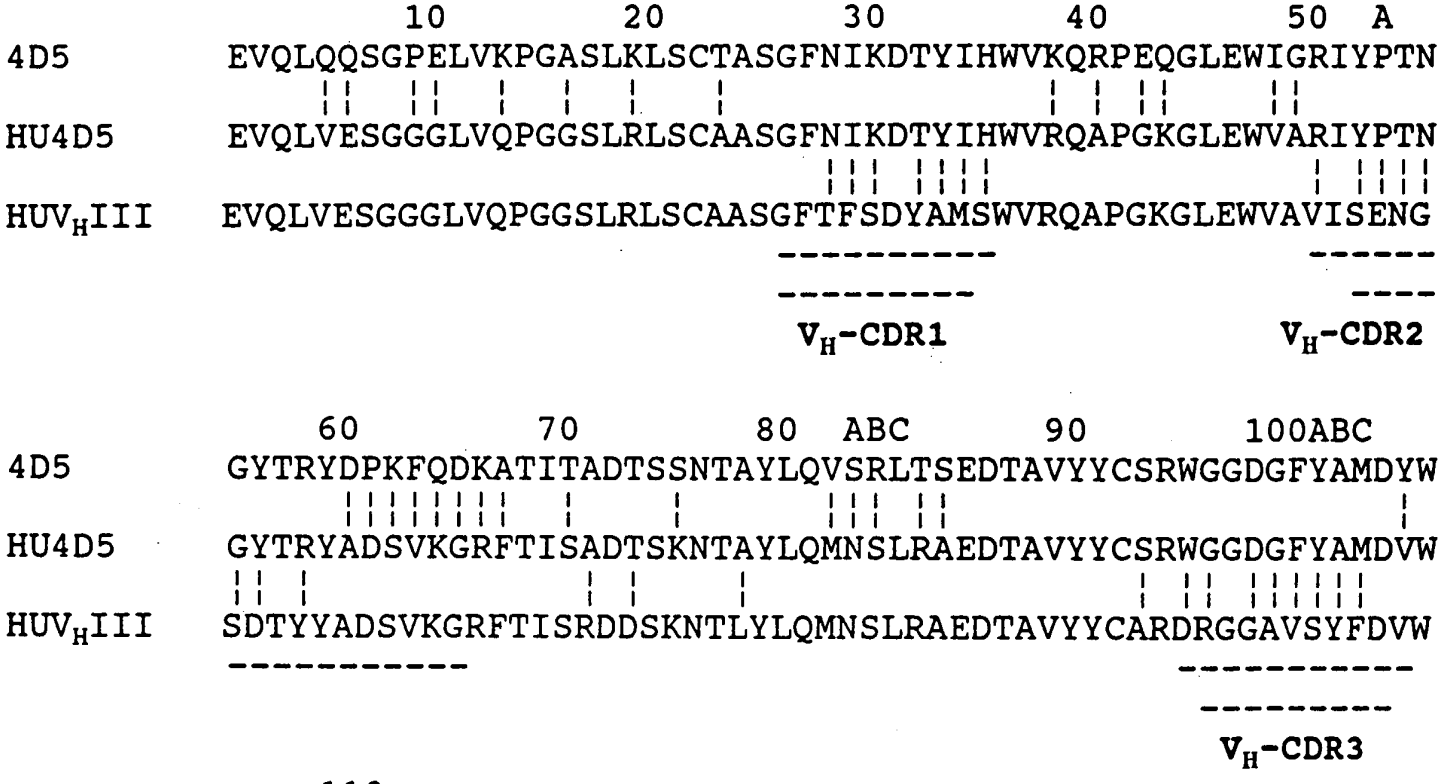
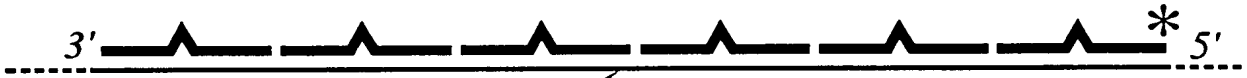


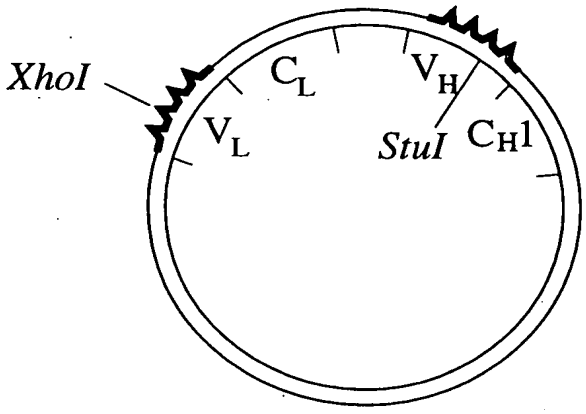
FIG. 1B



Anneal huV$_L$ or huV$_H$ oligomers to pAK1 template



1. Ligate
2. Isolate assembled oligomers
3. Anneal to pAK1 template (*XhoI* $^-$, *StuI* $^+$)
4. Extend and ligate



1. Transform *E. coli*
2. Isolate phagemid pool
3. Enrich for huV$_L$ and huV$_H$(*Xho I* $^+$, *StuI* $^-$)
4. Sequence verify

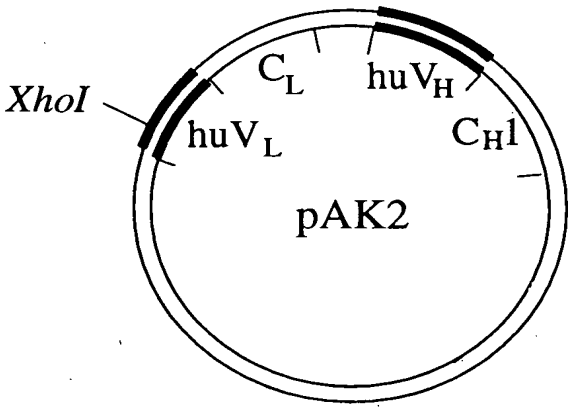


FIG. 2

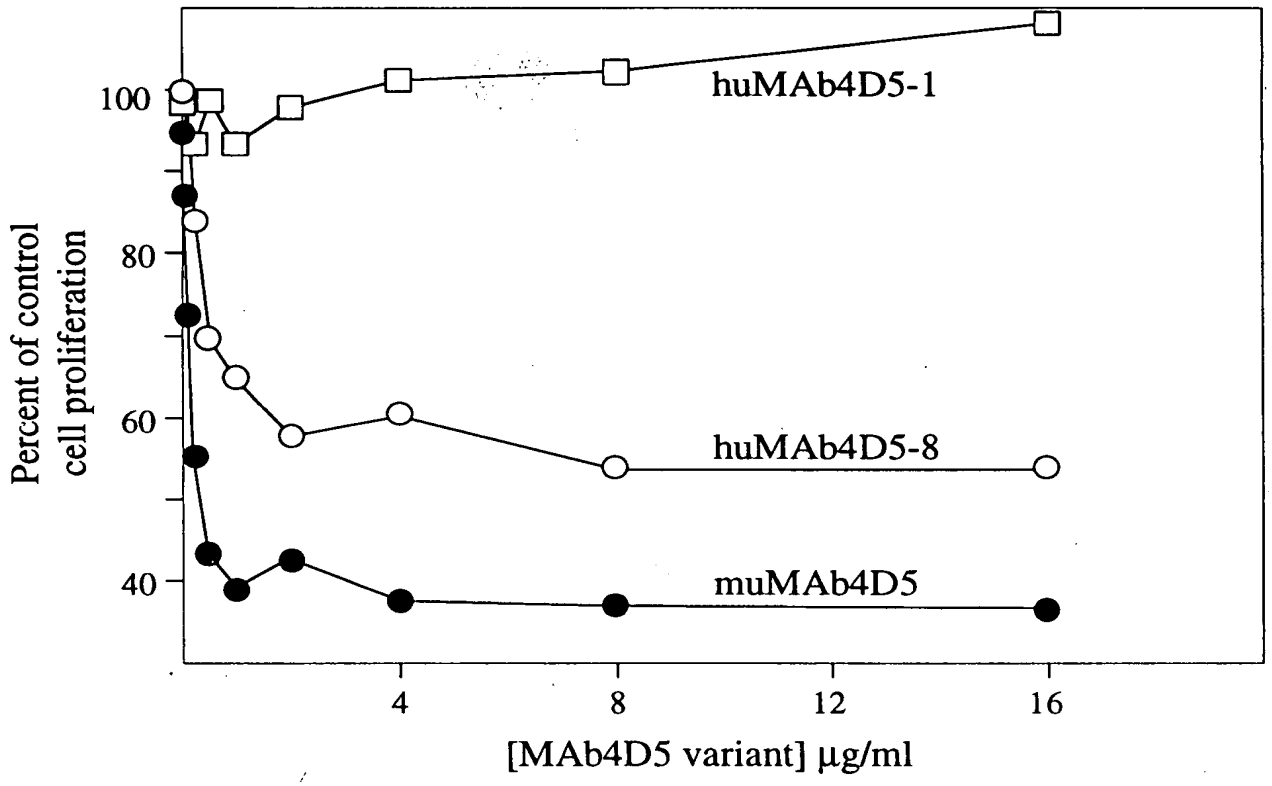

Percent of control cell proliferation
100
80
60
40
huMAb4D5-1
huMAb4D5-8
muMAb4D5
4
8
12
16
[MAb4D5 variant] µg/ml

FIG. 3

FIG. 4

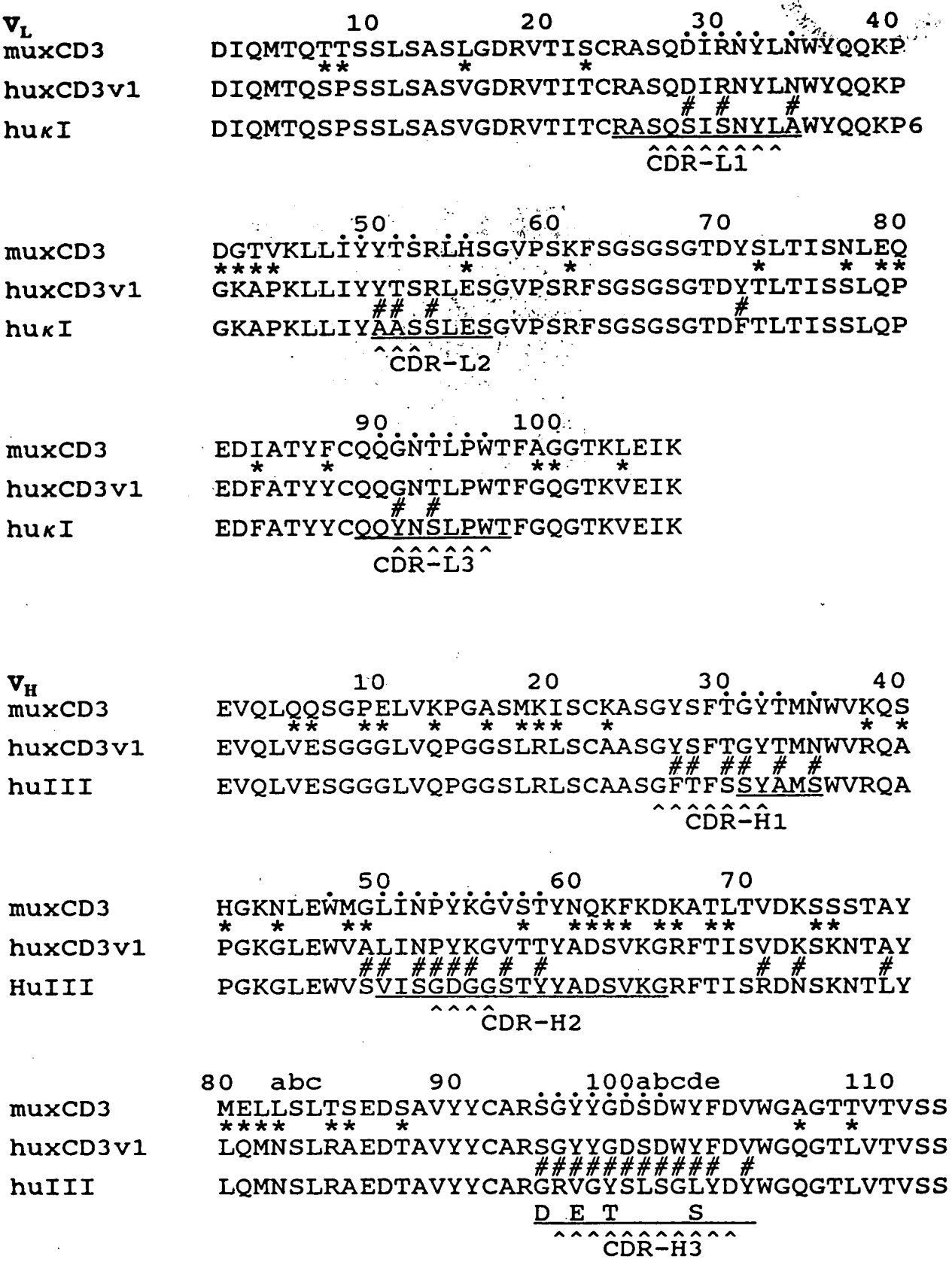

V_L
muxCD3 DIQMTQTTSSLSASLGDRVTISCRASQDIRNYLNWYQQKP
huxCD3v1 DIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKP
huκI DIQMTQSPSSLSASVGDRVTITCRASQSISNYLAWYQQKP
CDR-L1
muxCD3 DGTVKLLIYYTSRLHSGVPSKFSGSGSGTDYSLTISNLEQ
huxCD3v1 GKAPKLLIYYTSRLESGVPSRFSGSGSGTDYTLTISSLQP
huκI GKAPKLLIYAASSLESGVPSRFSGSGSGTDFTLTISSLQP
CDR-L2
muxCD3 EDIATYFCQQGNTLPWTFAGGTKLEIK
huxCD3v1 EDFATYYCQQGNTLPWTFGQGTKVEIK
huκI EDFATYYCQQYNSLPWTFGQGTKVEIK
CDR-L3
V_H
muxCD3 EVQLQQSGPELVKPGASMKISCKASGYSFTGYTMNWVKQS
huxCD3v1 EVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQA
huIII EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQA
CDR-H1
muxCD3 HGKNLEWMGLINPYKGVSTYNQKFKDKATLTVDKSSSTAY
huxCD3v1 PGKGLEWVALINPYKGVTTYADSVKGRFTISVDKSKNTAY
HuIII PGKGLEWVSVISGDGGSTYYADSVKGRFTISRDNSKNTLY
CDR-H2
muxCD3 MELLSLTSEDSAVYYCARSGYYGDSDWYFDVWGAGTTVTVSS
huxCD3v1 LQMNSLRAEDTAVYYCARSGYYGDSDWYFDVWGQGTLVTVSS
huIII LQMNSLRAEDTAVYYCARGRVGYSLSGLYDYWGQGTLVTVSS
D E T S
CDR-H3

FIG. 5

FIG. 6A-1

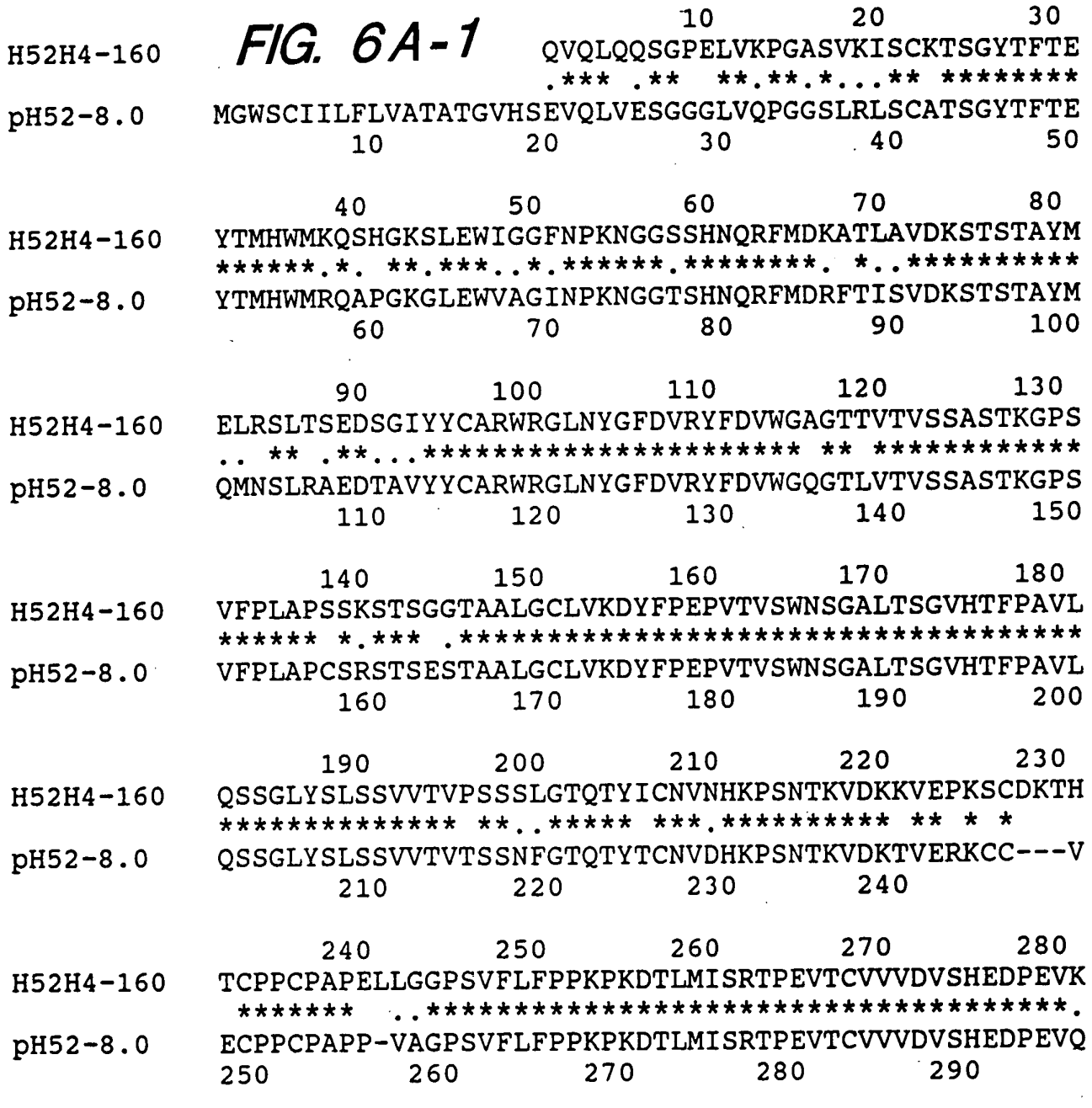

H52H4-160 QVQLQQSGPELVKPGASVKISCKTSGYTFTE
pH52-8.0 MGWSCIILFLVATATGVHSEVQLVESGGGLVQPGGSLRLSCATSGYTFTE
H52H4-160 YTMHWMKQSHGKSLEWIGGFNPKNGGSSHNQRFMDKATLAVDKSTSTAYM
pH52-8.0 YTMHWMRQAPGKGLEWVAGINPKNGGTSHNQRFMDRFTISVDKSTSTAYM
H52H4-160 ELRSLTSEDSGIYYCARWRGLNYGFDVRYFDVWGAGTTVTVSSASTKGPS
pH52-8.0 QMNSLRAEDTAVYYCARWRGLNYGFDVRYFDVWGQGTLVTVSSASTKGPS
H52H4-160 VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
pH52-8.0 VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
H52H4-160 QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH
pH52-8.0 QSSGLYSLSSVVTVTSSNFGTQTYTCNVDHKPSNTKVDKTVERKCC---V
H52H4-160 TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK
pH52-8.0 ECPPCPAPP-VAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQ

FIG. 6A-2

```
                290        300        310        320        330
H52H4-160       FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS
                *******.*************.***.********.***************
pH52-8.0        FNWYVDGMEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVS
                 300        310        320        330        340

                340        350        360        370        380
H52H4-160       NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP
                **.***********.************************************
pH52-8.0        NKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP
                 350        360        370        380        390

                390        400        410        420        430
H52H4-160       SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
                **********************.****************************
pH52-8.0        SDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFS
                 400        410        420        430        440

                440        450
H52H4-160       CSVMHEALHNHYTQKSLSLSPGK
                ***********************
pH52-8.0        CSVMHEALHNHYTQKSLSLSPGK
                 450        460
```

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
```

03-19-02

PART B - FEE(S) TRANSMITTAL

Complete and mail this form, together with applicable fee(s), to: **Box ISSUE FEE**
Assistant Commissioner for Patents
Washington, D.C. 20231

MAILING INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 4 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Legibly mark-up with any corrections or use Block 1)

7590 12/18/2001

GENENTECH, INC.
1 DNA WAY
SOUTH SAN FRANCISCO, CA 940804990

Note: The certificate of mailing below can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing.

Certificate of Mailing

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Box Issue Fee address above, on the date indicated below.

Wendy Maria Lee	(Typed or printed name)
[signature]	(Signature)
3/18/02	(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
08/146,206	11/17/1993	PAUL J. CARTER	7059.1	3992

TITLE OF INVENTION: METHOD FOR MAKING HUMANIZED ANTIBODIES

TOTAL CLAIMS	APPLN. TYPE	SMALL ENTITY	ISSUE FEE	PUBLICATION FEE	TOTAL FEE(S) DUE	DATE DUE
82	nonprovisional	NO	$1280	$0	$1280	03/18/2002

EXAMINER	ART UNIT	CLASS-SUBCLASS
DAVIS, MINH TAM B	1642	530-387300

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363). Use of PTO form(s) and Customer Number are recommended, but not required.

☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47) attached.

2. For printing on the patent front page, list (1) the names of up to 3 registered patent attorneys or agents OR, alternatively, (2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 Wendy M. Lee
2 _____
3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. Inclusion of assignee data is only appropriate when an assignment has been previously submitted to the USPTO or is being submitted under separate cover. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE: Genentech, Inc.

(B) RESIDENCE: (CITY and STATE OR COUNTRY): South San Francisco, California

Please check the appropriate assignee category or categories (will not be printed on the patent) ☐ individual ☒ corporation or other private group entity ☐ government

4a. The following fee(s) are enclosed:

☒ Issue Fee
☐ Publication Fee
☒ Advance Order - # of Copies 4

4b. Payment of Fee(s):

☐ A check in the amount of the fee(s) is enclosed.
☐ Payment by credit card. Form PTO-2038 is attached.
☒ The Commissioner is hereby authorized by charge the required fee(s), or credit any overpayment, to Deposit Account Number 07-0030 (enclose an extra copy of this form).

The COMMISSIONER OF PATENTS AND TRADEMARKS is requested to apply the Issue Fee and Publication Fee (if any) to the application identified above.

(Authorized Signature) [signature] (Date) 3/18/02

Wendy M. Lee - Reg. No. 40,378

NOTE: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

03/20/2002 SDENBER 00000176 070030 08146206
01 FC:142 1280.00 CH
02 FC:1461 12.00 CH

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending on the needs of the individual case. Any comments on the amount of time required to complete this form should be sent to the Chief Information Officer, United States Patent and Trademark Office, Washington, D.C. 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND FEES AND THIS FORM TO: Box Issue Fee, Assistant Commissioner for Patents, Washington, D.C. 20231

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.

TRANSMIT THIS FORM WITH FEE(S)

C7C
#70
DGO
R
9-25-02

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. U.S. Patent No.: 6,407,213 B1 Issued: June 18, 2002 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	CERTIFICATE OF MAILING I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on August 12, 2002 Wendy M. Lee

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 CFR 1.322

Certificate

AUG 27 2002

of Correction

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Enclosed is a Certificate of Correction for the above-referenced patent. Because the mistake occurred in the printing of the patent, it is not believed that any fee is required. However, if this is not the case, the Commissioner is hereby authorized to charge the required fee to Deposit Account No. 07-0630. Acceptance of this Certificate of Correction is respectfully requested.

Respectfully submitted,
GENENTECH, INC.

Date: August 12, 2002

By: _____
Wendy M. Lee
Reg. No. 40,378
Telephone No. (650) 225-1994

UNITED ST[illegible] AND TRADEMA[illegible] OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : U.S. 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:

In column 88, claim 65, line 63, please delete "63" and insert therefor --79--.

MAILING ADDRESS OF SENDER:

Wendy M. Lee

09157

PATENT TRADEMARK OFFICE

PATENT NO. U.S. 6,407,213

Form PTO1050(amended)

NOTICE RE: CERTIFICATES OF CORRECTION

DATE : 9-25-02

Paper No.: 11

TO : Supervisor, Art Unit 1642

SUBJECT : Certificate of Correction Request in Patent No.: 6,407,213

A response to the following question is requested with respect to the accompanying request for a certific of correction.

With respect to the change(s) requested, correcting Office and/or Applicant's errors, should the patent read as shown in the certificate of correction? No new matter should be introduced, nor should the scope or meaning of the claims be changed.

See red tags.

D. G. Olson

PLEASE COMPLETE THIS FORM AND
RETURN WITH FILE, WITHIN 7 DAYS,
TO CERTIFICATES OF CORRECTION BRANCH - PK 3-915/922
PALM LOCATION 7580 - TEL. NO. 305-8309

THANK YOU FOR YOUR ASSISTANCE!

Note your decision, regarding the changes requested in the Request for Certificate of Correction, placing a check mark (+) in the box that reflects your decision, which corresponds to the question checke above.

☒ YES ☐ NO ☐ Comments below

☐ Comments: Please enter the corrections Ex: Middle-Thai DAVIS

ANTHONY C. CAPUTA
SUPERVISORY PATENT EXAMINER
TECHNOLOGY

Supervisor

Art Unit

PTOL-306 (REV. 2/02) U.S. DEPARTMENT OF COMMERCE Patent and Trademark O

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

DAC

#45

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent of: Paul J. Carter *et al.* -- § 156

Patent No.: 6,407,213

Issued: June 18, 2002

Application No: 08/146,206

For: METHOD FOR MAKING HUMANIZED ANTIBODIES – Application for § 156 Patent Term Extension

Docket No: 22338-80060

Assignee: Genentech, Inc.

Unit: OPLA

CERTIFICATE OF MAILING - 37 C.F.R. § 1.10
EXPRESS MAIL LABEL NO. ER 736919973 US

I hereby certify this correspondence is being deposited with the U.S. Postal Service with sufficient postage as "Express Mail – Post Office to Addressee" addressed to: Mail Stop Patent Ext., Commissioner for Patents, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Signature	David Devereux	Aug. 25, 2006
	Printed Name	Date

Mail Stop Patent Ext.
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

APPLICATION FOR EXTENSION OF PATENT TERM UNDER 35 U.S.C. § 156

Dear Sir:

Applicant, Genentech, Inc., hereby submits this application for extension of the term of United States Letters Patent 6,407,213 under 35 U.S.C. § 156 by providing the following information in accordance with the requirements specified in 37 C.F.R. § 1.740.

Applicant represents that it is the assignee of the entire interest in and to United States Letters Patent No. 6,407,213, granted to Paul J. Carter and Leonard G. Presta (Carter *et al.*) by virtue of an assignment of such patent to Genentech, Inc., recorded June 28, 1994, at Reel 7035, Frame 0272.

1. Identification of the Approved Product [§ 1.740(a)(1)]

The name of the approved product is LUCENTIS™. The name of the active ingredient of LUCENTIS™ is ranibizumab. Ranibizumab is a recombinant humanized monoclonal IgG_1 antibody antigen-binding fragment (Fab) based on a humanized framework with complementarity-determining regions (CDRs) derived from a murine monoclonal antibody that binds to human Vascular Endothelial Growth Factor (VEGF).

2. Federal Statute Governing Regulatory Approval of the Approved Product [§ 1.740(a)(2)]

The approved product was subject to regulatory review under, *inter alia*, the Public Health Service Act (42 U.S.C. § 201 *et seq.*) and the Federal Food, Drug and Cosmetic Act (21 U.S.C. § 355 *et seq.*).

3. Date of Approval for Commercial Marketing [§ 1.740(a)(3)]

LUCENTIS™ was approved for commercial marketing or use under § 351 of the Public Heath Service Act on **June 30, 2006**.

4. Identification of Active Ingredient and Certifications Related to Commercial Marketing of Approved Product [§ 1.740(a)(4)]

(a) The active ingredient of LUCENTIS™ is ranibizumab. Ranibizumab is a humanized monoclonal IgG_1 antibody antigen-binding fragment produced by an *E. coli* expression system. It contains human framework regions (FRs) and the complementarity-determining regions (CDRs) derived from a murine antibody that binds to VEGF.

(b) Applicant certifies that ranibizumab had not been approved for commercial marketing or use under the Federal Food, Drug and Cosmetic Act, the Public Health Service Act or the Virus-Serum-Toxin Act prior to the approval granted on June 30, 2006 to the present Applicant.

(c) Ranibizumab has been approved for the treatment of patients with neovascular (wet) age-related macular degeneration. *See* LUCENTIS™ product label, provided as Attachment A.

(d) LUCENTIS™ was approved for commercial marketing pursuant to § 351 of the Public Health Service Act (42 U.S.C. § 262) under Genentech's existing Department of Health and Human Services (DHHS) U.S. License No. 1048. *See* LUCENTIS™ approval letter, provided as Attachment B.

5. Statement Regarding Timeliness of Submission of Patent Term Extension Request [§ 1.740(a)(5)]

Applicant certifies that this application for patent term extension is being timely submitted within the sixty (60) day period permitted for submission specified in 35 U.S.C. § 156(d)(1) and 37 C.F.R. § 1.720(f). The last date on which this application may be submitted is August 28, 2006.

6. Complete Identification of the Patent for Which Extension Is Being Sought [§ 1.740(a)(6)]

The complete identification of the patent for which an extension is being sought is as follows:

(a) Names of the inventors: Paul J. Carter and Leonard G. Presta.

(b) Patent Number: 6,407,213 ("the '213 patent")

(c) Date of Issue: June 18, 2002

(d) Date of Expiration: June 18, 2019

7. Copy of the Patent for Which an Extension is Being Sought [§ 1.740(a)(7)]

A copy of U.S. Patent No. 6,407,213 is provided as Attachment C to the present application.

8. Copies of Disclaimers, Certificates of Correction, Receipt of Maintenance Fee Payment, or Reexamination Certificate [§ 1.740(a)(8)]

(a) U.S. Patent No. 6,407,213 is not subject to a terminal disclaimer.

(b) A Certificate of Correction was issued for U.S. Patent No. 6,407,213 on December 3, 2002. A copy of the Certificate of Correction is provided in Attachment D to the present application.

(c) The first maintenance fee for U.S. Patent No. 6,407,213 has been paid and there are no maintenance fees currently due, as provided in Attachment E.

(d) U.S. Patent No. 6,407,213 has not been the subject of a reexamination proceeding.

9. Statement Regarding Patent Claims Relative to Approved Product [§ 1.740(a)(9)]

The statements below are made solely to comply with the requirements of 37 C.F.R. § 1.740(a)(9). Applicant notes that, as the M.P.E.P. acknowledges, § 1.740(a)(9) does not require an applicant to show whether or how the listed claims would be infringed, and that this question cannot be answered without specific knowledge concerning acts performed by third parties. As such, these comments are not an assertion or an admission of Applicant as to the scope of the listed claims, or whether or how any of the listed claims would be infringed, literally or under the doctrine of equivalents, by the manufacture, use, sale, offer for sale or the importation of any product.

(a) At least claims 1-2, 4-5, 25, 29, 62-64, 66-67, 69, 71-73, 75-78, and 80-81 of U.S. Patent No. 6,407,213 claim the active pharmaceutical ingredient in the approved product or a method that may be used to make or use that ingredient.

(b) Pursuant to M.P.E.P. § 2753 and 37 C.F.R. § 1.740(a)(9), the following explanation is provided which shows how at least one of the above-listed claims of the '213 patent claim the approved product.

(1) Description of the approved product

The approved product is described in Section 11 of the approved label for LUCENTIS™ as follows, a copy of which is provided as Attachment A.

> LUCENTIS™ (ranibizumab injection) is a recombinant humanized IgG1 kappa isotype monoclonal antibody fragment designed for intraocular use. Ranibizumab binds to and inhibits the biologic activity of human vascular endothelial growth factor A (VEGF-A). Ranibizumab has a molecular weight of approximately 48 kilodaltons and is produced by an *E. coli* expression system in a nutrient medium containing the antibiotic tetracycline. Tetracycline is not detectable in the final product.
>
> LUCENTIS™ is a sterile, colorless to pale yellow solution in a single-use glass vial. LUCENTIS™ is supplied as a preservative-free, sterile solution in a single-use glass vial designed to deliver 0.05 mL of 10 mg/mL LUCENTIS™ aqueous solution with 10 mM histidine HCL, 10% α, α-trehalose dihydrate, 0.01% polysorbate 20, pH 5.5.

Ranibizumab is further characterized in a scientific reference, Chen *et al.* published in 1999 in the Journal of Molecular Biology (JMB) entitled "Selection and Analysis of an Optimized Anti-VEGF Antibody: Crystal Structure of an Affinity-matured Fab in Complex with Antigen."[1] The Chen *et al.* article discusses the lineage of the ranibizumab antibody fragment. In this respect, the article states that "[a] murine monoclonal antibody, A.4.6.1, was found to block VEGF-dependent cell proliferation *in vitro* and to antagonize tumor growth *in vivo.* [Citation omitted]. The murine mAb was previously humanized in Fab form to yield a variant known as Fab-12." [Citation omitted] *See* p. 866, left col., ¶1. The abstract explains that the authors affinity-matured Fab-12 and obtained Fab fragment Y0317, now known as ranibizumab. According to the article, ranibizumab was derived from the humanization and affinity-maturation of a non-human, murine monoclonal antibody that binds to VEGF. The Chen *et al.* article also describes the humanized structure of ranibizumab. *See, e.g.,* Figure 1.

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[1] 293:865-881 (1999) (Attachment F)

WO 98/45331 (Figures 1A, 1B, 10A, 10B, provided as Attachment G) also provides sequence data for the heavy and light chain variable domains of Y0317, together with the heavy and light chain variable domains of murine A.4.6.1, the heavy and light chain variable domains of humanized variant Fab-12, and the Kabat human consensus framework, humIII. WO 98/45331 confirms that, in addition to non-human CDRs derived from the sequence of the murine antibody, ranibizumab comprises framework substitutions in the variable domains at positions 4 and 46 in the light chain (V_L) and positions 49, 69, 71, 73, 76, 78, and 94 in the heavy chain (V_H).[2]

(2) Explanation Regarding Claim 29 of the '213 Patent Relative to Ranibizumab

As explained below, the active pharmaceutical ingredient of the approved product, ranibizumab, is a humanized Fab fragment that is covered by at least claim 29.

Claim 29 of the '213 patent reads as follows:

> 29. An antibody comprising the humanized variable domain of claim 1.

Claim 29 depends from claim 1, which reads as follows:

> 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.

The term "antibody," as defined in the '213 patent specification includes, in addition to full-length antibodies, antibody fragments such as Fab, Fab', $F(ab)_2$ and Fv so long as those fragments retain the desired biological activity, *i.e.*, binding to VEGF (*See, e.g.,* '213 at col. 8, lines 11-17; col. 24, lines 13-18). As recited in the '213 specification – "FAb fragments with specificity for the antigen are specifically encompassed within the term 'antibody' as it is defined, discussed, and claimed herein." '213 at col. 24, lines 13-18. Ranibizumab, being

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[2] Compare sequence data for the heavy and light chain variable domains of Y0317 (Figs. 10A-10B), A.4.6.1 (Figs. 1A-1B) and humIII (Figs. 1A-1B) as set forth in WO 98/45331, provided as Attachment G.

a Fab fragment that binds VEGF, falls within the scope of the term "antibody" as used in Claims 1 and 29.

The amino acid sequences of the V_L and V_H domains of ranibizumab include human framework substitutions at positions 4L, 46L, 49H, 69H, 71H, 73H, 76H, 78H and 94H.[3] Of these, substitutions at positions 4L and 69H are among those recited in the Markush group of claim 1. Figures 1A-1B of WO 98/45331, provided as Attachment G, show the heavy and light chain variable domains of sequences of the same import antibody ("A4.6.1") used to design ranibizumab on the lines above the variable domains of the Fab-12 sequence and the Kabat consensus sequences ("humIII").[4] The A4.6.1 antibody is a murine monoclonal antibody; its sequence is therefore "non-human." *See, e.g.,* Chen *et al.* Figures 10A-10B of WO 98/45331, provided as Attachment G (and Figure 1 of Chen *et al.*), show the variable domains of the Y0317 sequence. When the heavy and light chain variable domains of A4.6.1, Y0317 and humIII are aligned, the framework substitutions noted above are apparent utilizing the Kabat numbering system.

In each of the V_L and V_H domains of ranibizumab, "substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species" (*i.e.*, the murine antibody A4.6.1). *See, e.g.,* '213 at col. 2, lines 27-31. Ranibizumab is therefore "humanized" within the meaning of claims 1 and 29 of the '213 patent.

As also required in claim 1, ranibizumab includes non-human amino acid residues in its CDRs. The CDRs in ranibizumab are also functional to "bind an antigen" – here, the VEGF protein. *See* LUCENTIS™ label, provided as Attachment A.

Ranibizumab thus meets the limitations of dependent claim 29.

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[3] *See* WO 98/45331 at Figures 1A-1B (humIII) and 10A-10B (Y0317).

[4] The residues in a human Ig sequence that are substituted with residues from an "import antibody" are identified according to standard numbering conventions published by Kabat. *See* '213 at col. 10, line 45 through col. 11, line 26. The Kabat sequences represent consensus amino acid sequences for various human antibodies in each subclass. *See id.*

10. Relevant Dates Under 35 U.S.C. § 156 for Determination of Applicable Regulatory Review Period [§ 1.740(a)(10)]

(a) Patent Issue Date

U.S. Patent No. 6,407,213 was issued on June 18, 2002.

(b) IND Effective Date [35 U.S.C. § 156(g)(1)(B)(i); 37 C.F.R. § 1.740(a)(10)(i)(A)]

The date that an exemption under § 505(i) of the Federal Food, Drug and Cosmetic Act became effective (*i.e.*, the date that an investigational new drug application ("IND") became effective) for LUCENTIS ™ (referred to as "Humanized Monoclonal Antibody Fragment (rhuFab V2)(E. coli, Genentech) to Vascular Endothelial Growth Factor (VEGF), Intravitreal) was October 7, 1999. The IND was assigned number BB-IND # 8633. A copy of the letter from the FDA reflecting the effective date of the IND is provided in Attachment H. The application date for this IND was October 6, 1999.

(c) BLA Submission Date [35 U.S.C. § 156(g)(1)(B)(i); 37 C.F.R. § 1.740(a)(10)(i)(B)]

The BLA was submitted by Genentech to the FDA on December 29, 2005. The BLA was assigned number BL# 125156/0. A copy of the letter from the FDA acknowledging receipt of the BLA and reflecting the BLA submission date is provided in Attachment I.

(d) BLA Issue Date [35 U.S.C. § 156(g)(1)(B)(ii); 37 C.F.R. § 1.740(a)(10)(i)(C)]

The FDA approved biologic license application 125156/0 authorizing the marketing of LUCENTIS ™ on June 30, 2006. LUCENTIS ™ was approved under Department of Health and Human Services (DHHS) U.S. License No. 1048. A copy of the approval letter from the FDA is provided as Attachment B.

11. Summary of Significant Events During Regulatory Review Period [§ 1.740(a)(11)]

Pursuant to 37 C.F.R. § 1.740(a)(11), the following provides a brief description of the activities of Genentech, Inc. before the FDA in relation to the regulatory review of LUCENTIS™. The brief description lists the significant events that occurred during the regulatory review period for the approved product. In several instances, communications to or from the FDA are referenced. Pursuant to 37 C.F.R. § 1.740(a)(11), 21 C.F.R. § 60.20(a), and M.P.E.P. § 2753, copies of all such communications are not provided in this application, but can be obtained from records maintained by the FDA.

- On October 6, 1999, Genentech submitted to FDA (See Attachment H) an investigational new drug application for a recombinant humanized monoclonal antibody fragment (rhuFab V2, now known as Ranibizumab) against Vascular Endothelial Growth Factor (VEGF). The antibody was developed as a potential new therapeutic in treating patients with the exudative (wet or neovascular) form of age-related macular degeneration (AMD).

- On October 7, 1999 FDA made BB-IND #8633 effective via a communication mailed to Genentech on October 13, 1999 (*See* Attachment H). According to the FDA, initiation of trials could begin 30 days after October 7, 1999.

- The first human clinical trial (Phase I) was initiated on February 8, 2000 followed by Phase II human trials and Phase III human trials, some of which remain ongoing at the time of this application.

- On February 5, 2002, representatives of Genentech and the FDA (CBER and CDER) participated in a Type C meeting to discuss the proposed clinical development plan for ranibizumab in AMD.

- On October 31, 2002 representatives of Genentech and FDA (CBER and CDER) participated in an Type B End-of-Phase II meeting.

- Beginning in approximately March 2003, and continuing at the time of this application, Phase III studies have been conducted. The three Phase III trials forming the basis of the Biologics License Application (BLA), FVF2598g, FVF2587g, and FVF3192g are studies of two year duration with primary endpoints of one year. FVF2587g and FVF3192g, along with extension study FVF3426g and safety study FVF3689g, remain ongoing at the time of this application.

- On September 21, 2005 representatives of Genentech and CDER participated in a Type B Pre-BLA submission meeting to discuss information requirements for the BLA.

- Genentech submitted a BLA for ranibizumab for the treatment of patients with wet AMD on December 29, 2005 (*See* Attachment I).

- FDA acknowledged receipt of the BLA for ranibizumab via a communication mailed to Genentech dated January 27, 2006. The letter indicated that FDA had assigned the Submission Tracking Number (STN) of BL #125156/0 to the BLA (*See* Attachment I).

- By way of a communication mailed to Genentech on March 14, 2006 FDA made Genentech aware that the BLA for ranibizumab was filed on February 28, 2006 and that FDA had assigned a user fee goal date of June 30, 2006 (*See* Attachment J).

- On June 30, 2006 FDA approved BLA 125156/0, issuing marketing authorization for LUCENTIS™ (*See* Attachment B).

12. Statement Concerning Eligibility for and Duration of Extension Sought Under 35 U.S.C. § 156 [37 C.F.R. § 1.740(a)(12)]

(a) In the opinion of the Applicant, U.S. Patent No. 6,407,213 is eligible for an extension under § 156 because:

(i) one or more claims of the '213 patent claim the approved product or a method of making or using the approved product;

(ii) the term of the '213 patent has not been previously extended on the basis of § 156;

(iii) the '213 patent has not expired;

(iv) no other patent has been extended pursuant to § 156 on the basis of the regulatory review process associated with the approved product, LUCENTIS™;

(v) there is an eligible period of regulatory review by which the patent may be extended pursuant to § 156;

(vi) the applicant for marketing approval exercised due diligence within the meaning of § 156(d)(3) during the period of regulatory review;

(vii) the present application has been submitted within the 60-day period following the approval date of the approved product, pursuant to § 156(c); and

(viii) this application otherwise complies with all requirements of 35 U.S.C. § 156 and applicable rules and procedures.

(b) The period by which the term of the '213 patent is requested by Applicant to be extended is **378 days.**

(c) The requested period of extension of term for the '213 patent corresponds to the regulatory review period that is eligible for extension pursuant to § 156, based on the facts and circumstances of the regulatory review associated with the approved product LUCENTIS™ and the issuance of the '213 patent. The period was determined as follows.

(i) The relevant dates for calculating the regulatory review period, based on the events discussed in the section above, are the following.

Exemption under FDCA § 505(i) became effective	October 7, 1999
Patent was granted	June 18, 2002
Biologics License Application (BLA) under PHSA § 351 was filed	December 29, 2005
BLA was approved	June 30, 2006

(ii) The '213 patent was granted during the period specified in § 156(g)(1)(B)(i) (*i.e.*, the period from the date of the grant of the exemption under § 505(i) of the FDCA until the date of submission of the BLA). Pursuant to § 156(b) and (c)(2), the calculated regulatory review period therefore includes a component of time between when the patent was granted and when the BLA was submitted (1/2 of 1289 days or 644 days).

(iii) The patent was granted prior to the start of the period specified in § 156(g)(1)(B)(ii) (*i.e.*, the period from the date of submission of the BLA until the date of approval). The regulatory review period under § 156(b) therefore includes a component equal to the total number of days in that period that are after the BLA was submitted (184 days).

(iv) The period determined according to § 156(b), (c)(2), and (g)(1) for the approved product (*i.e.*, the number of days following the date of issue of the patent between the dates of submission and of approval of the BLA for LUCENTIS™) is 828 days.

(v) The '213 patent will expire on June 18, 2019.

(vi) The date of approval of the approved product is June 30, 2006.

(vii) The date that is fourteen years from the date of approval of the approved product is June 30, 2020.

(viii) The period measured from the date the patent expires (*i.e.*, June 18, 2019) until the end of the fourteen-year period specified in §156 (c)(3) (*i.e.*, June 30, 2020) is approximately 1 year and 13 days or 378 days.

(ix) The number of days in the regulatory review period determined pursuant to § 156(g)(1)(B)(ii) (i.e., 828 days) exceeds the number of days that the

patent may be extended pursuant to §156(c)(3). As such, the period by which the patent may be extended is limited by the fourteen-year rule of §156(c)(3) to **378 days**.

(x) The '213 patent issued after the effective date of Public Law No. 98-417. As such, the two- or three-year limit of 35 U.S.C. § 156(g)(6)(C) does not apply.

13. Statement Pursuant to 37 C.F.R. § 1.740(a)(13)

Pursuant to 37 C.F.R. § 1.740(a)(13), Applicant acknowledges its duty to disclose to the Director of the PTO and to the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension sought, particularly as that duty is defined in 37 C.F.R. § 1.765.

14. Applicable Fee [§ 1.740(a)(14)]

Our check in payment of the fee prescribed in 37 C.F.R. § 1.20(j) for a patent term extension application under 35 U.S.C. § 156 accompanies this application. Please deduct any additional required fees from, or credit any overpayments to our deposit account no. 18-1260.

15. Name and Address for Correspondence [§ 1.740(a)(14)]

Please direct all inquiries, questions, and communications regarding this application for term extension to:

Jeffrey P. Kushan
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, D.C. 20005
Phone: 202-736-8914
Fax: 202-736-8111
email: jkushan@sidley.com

The correspondence address for U.S. Patent No. 6,407,213 is unchanged for all other purposes. A Power of Attorney granted to the undersigned by the patent assignee, a copy of which is included with this application as Attachment K, accompanies this communication.

Two additional copies of this application are enclosed, in compliance with 37 C.F.R. § 1.740(b). Applicant also provides herewith two further copies of the application for the convenience of the Office, pursuant to M.P.E.P. § 2763.

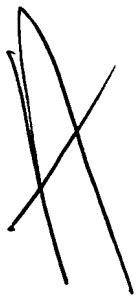
Sincerely,

Jeffrey P. Kushan
Attorney for Applicant
Registration No. 43,401

Sidley Austin LLP
1501 K Street, N.W.
Washington, D.C. 20005

Dated: August 25, 2006

INDEX OF ATTACHMENTS



HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use LUCENTIS safely and effectively. See full prescribing information for LUCENTIS.

LUCENTIS™ (ranibizumab injection)

Initial U.S. Approval: 2006

----------INDICATIONS AND USAGE----------

LUCENTIS is indicated for the treatment of patients with neovascular (wet) age-related macular degeneration (1).

----------DOSAGE AND ADMINISTRATION----------

- FOR OPHTHALMIC INTRAVITREAL INJECTION ONLY (2.1)
- LUCENTIS 0.5 mg (0.05 mL) is recommended to be administered by intravitreal injection once a month (2.2).
- Although less effective, treatment may be reduced to one injection every three months after the first four injections if monthly injections are not feasible. Compared to continued monthly dosing, dosing every 3 months will lead to an approximate 5-letter (1-line) loss of visual acuity benefit, on average, over the following 9 months. Patients should be evaluated regularly (2.2).

----------DOSAGE FORMS AND STRENGTHS----------

- 10 mg/mL single-use vial (3)

----------CONTRAINDICATIONS----------

- Ocular or periocular infections (4.1)
- Hypersensitivity (4.2)

----------WARNINGS AND PRECAUTIONS----------

- Endophthalmitis and retinal detachments may occur following intravitreal injections. Patients should be monitored during the week following the injection (5.1).
- Increases in intraocular pressure have been noted within 60 minutes of intravitreal injection (5.2).

----------ADVERSE REACTIONS----------

The most common adverse reactions (reported ≥ 6% higher in LUCENTIS-treated subjects than control subjects) are conjunctival hemorrhage, eye pain, vitreous floaters, increased intraocular pressure, and intraocular inflammation (6.2).

To report SUSPECTED ADVERSE REACTIONS, contact Genentech at 1-888-835-2555 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

See Section 17 for PATIENT COUNSELING INFORMATION.

FULL PRESCRIBING INFORMATION: CONTENTS*

* Sections or subsections omitted from the Full Prescribing Information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

LUCENTIS is indicated for the treatment of patients with neovascular (wet) age-related macular degeneration.

2 DOSAGE AND ADMINISTRATION

2.1 General Dosing Information

FOR OPHTHALMIC INTRAVITREAL INJECTION ONLY.

2.2 Dosing

LUCENTIS 0.5 mg (0.05 mL) is recommended to be administered by intravitreal injection once a month.

Although less effective, treatment may be reduced to one injection every three months after the first four injections if monthly injections are not feasible. Compared to continued monthly dosing, dosing every 3 months will lead to an approximate 5-letter (1-line) loss of visual acuity benefit, on average, over the following 9 months. Patients should be evaluated regularly [*see Clinical Studies (14.2)*].

2.3 Preparation for Administration

Using aseptic technique, all (0.2 mL) of the LUCENTIS vial contents are withdrawn through a 5-micron 19-gauge filter needle attached to a 1-cc tuberculin syringe. The filter needle should be discarded after withdrawal of the vial contents and should not be used for intravitreal injection. The filter needle should be replaced with a sterile 30-gauge × 1/2-inch needle for the intravitreal injection. The contents should be expelled until the plunger tip is aligned with the line that marks 0.05 mL on the syringe.

2.4 Administration

The intravitreal injection procedure should be carried out under controlled aseptic conditions, which include the use of sterile gloves, a sterile drape, and a sterile eyelid speculum (or equivalent). Adequate anesthesia and a broad-spectrum microbicide should be given prior to the injection.

Following the intravitreal injection, patients should be monitored for elevation in intraocular pressure and for endophthalmitis. Monitoring may consist of a check for perfusion of the optic nerve head immediately after the injection, tonometry within 30 minutes following the injection, and biomicroscopy between two and seven days following the injection. Patients should be instructed to report any symptoms suggestive of endophthalmitis without delay.

Each vial should only be used for the treatment of a single eye. If the contralateral eye requires treatment, a new vial should be used and the sterile field, syringe, gloves, drapes, eyelid speculum, filter, and injection needles should be changed before LUCENTIS is administered to the other eye.

No special dosage modification is required for any of the populations that have been studied (e.g., gender, elderly).

2.5 Stability and Storage

LUCENTIS should be refrigerated at 2°-8°C (36°-46°F). DO NOT FREEZE. Do not use beyond the date stamped on the label. LUCENTIS vials should be protected from light. Store in the original carton until time of use.

3 DOSAGE FORMS AND STRENGTHS

Single-use glass vial designed to deliver 0.05 mL of 10 mg/mL.

4 CONTRAINDICATIONS

4.1 Ocular or Periocular Infections

LUCENTIS is contraindicated in patients with ocular or periocular infections.

4.2 Hypersensitivity

LUCENTIS is contraindicated in patients with known hypersensitivity to ranibizumab or any of the excipients in LUCENTIS.

5 WARNINGS AND PRECAUTIONS

5.1 Endophthalmitis and Retinal Detachments

Intravitreal injections, including those with LUCENTIS, have been associated with endophthalmitis and retinal detachments. Proper aseptic injection technique should always be used when administering LUCENTIS. In addition, patients should be monitored during the week following the injection to permit early treatment should an infection occur [*see Dosage and Administration (2.3, 2.4) and Patient Counseling Information (17)*].

5.2 Increases in Intraocular Pressure

Increases in intraocular pressure have been noted within 60 minutes of intravitreal injection with LUCENTIS. Therefore, intraocular pressure as well as the perfusion of the optic nerve head should be monitored and managed appropriately [*see Dosage and Administration (2.4)*].

5.3 Thromboembolic Events

Although there was a low rate (<4%) of arterial thromboembolic events observed in the LUCENTIS clinical trials, there is a theoretical risk of arterial thromboembolic events following intravitreal use of inhibitors of VEGF [*see Adverse Reactions (6.3)*].

6 ADVERSE REACTIONS

6.1 Injection Procedure

Serious adverse events related to the injection procedure have occurred in < 0.1% of intravitreal injections, including endophthalmitis [*see Warnings and Precautions (5.1)*], rhegmatogenous retinal detachments, and iatrogenic traumatic cataracts.

6.2 Clinical Trials Experience – Ocular Events

Other serious ocular adverse events observed among LUCENTIS-treated patients occurring in <2% of patients

included intraocular inflammation and increased intraocular pressure [*see Warnings and Precautions (5.1, 5.2)*].

The available safety data include exposure to LUCENTIS in 874 patients with neovascular age-related macular degeneration in three double-masked, controlled studies with dosage regimens of 0.3 mg (375 patients) or 0.5 mg (379 patients) administered monthly by intravitreal injection (Studies 1 and 2) [*see Clinical Studies (14.1)*] and dosage regimens of 0.3 mg (59 patients) or 0.5 mg (61 patients) administered once a month for 3 consecutive doses followed by a dose administered once every 3 months (Study 3) [*see Clinical Studies (14.2)*].

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in one clinical trial of a drug cannot be directly compared with rates in the clinical trials of the same or another drug and may not reflect the rates observed in practice.

Table 1 shows the most frequently reported ocular adverse events that were reported with LUCENTIS treatment. The ranges represent the maximum and minimum rates across all three studies for control, and across all three studies and both dose groups for LUCENTIS.

Table 1

Adverse Event	LUCENTIS	Control
Conjunctival hemorrhage	77%-43%	66%-29%
Eye pain	37%-17%	33%-11%
Vitreous floaters	32%-3%	10%-3%
Retinal hemorrhage	26%-15%	56%-37%
Intraocular pressure increased	24%-8%	7%-3%
Vitreous detachment	22%-7%	18%-13%
Intraocular inflammation	18%-5%	11%-3%
Eye irritation	19%-4%	20%-6%
Cataract	16%-5%	16%-6%
Foreign body sensation in eyes	19%-6%	14%-6%
Lacrimation increased	17%-3%	16%-0%
Eye pruritis	13%-0%	12%-3%
Visual disturbance	14%-0%	9%-2%
Blepharitis	13%-3%	9%-4%
Subretinal fibrosis	13%-0%	19%-10%
Ocular hyperemia	10%-5%	10%-1%
Maculopathy	10%-3%	11%-3%
Visual acuity blurred/decreased	17%-4%	24%-10%
Detachment of the retinal pigment epithelium	11%-1%	15%-3%
Dry eye	10%-3%	8%-5%
Ocular discomfort	8%-0%	5%-0%
Conjunctival hyperemia	9%-0%	7%-0%
Posterior capsule opacification	8%-0%	5%-0%
Retinal exudates	9%-1%	11%-3%

6.3 Clinical Trials Experience – Non-Ocular Events

Table 2 shows the most frequently reported non-ocular adverse events with LUCENTIS treatment. The ranges represent the maximum and minimum rates across all three studies for control, and across all three studies and both dose groups for LUCENTIS.

Table 2

Adverse Event	LUCENTIS	Control
Hypertension/elevated blood pressure	23%-5%	23%-8%
Nasopharyngitis	16%-5%	13%-5%
Arthralgia	11%-3%	9%-0%
Headache	15%-2%	10%-3%
Bronchitis	10%-3%	8%-2%
Cough	10%-3%	7%-2%
Anemia	8%-3%	8%-0%
Nausea	9%-2%	6%-4%
Sinusitis	8%-2%	6%-4%
Upper respiratory tract infection	15%-2%	10%-4%
Back pain	10%-1%	9%-0%
Urinary tract infection	9%-4%	8%-5%
Influenza	10%-2%	5%-1%
Arthritis	8%-0%	8%-2%
Dizziness	8%-2%	10%-2%
Constipation	7%-3%	8%-2%

The rate of arterial thromboembolic events in the three studies in the first year was 2.1% of patients (18 out of 874) in the combined group of patients treated with 0.3 mg or 0.5 mg LUCENTIS compared with 1.1% of patients (5 out of 441) in the control arms of the studies. In the second year of Study 1, the rate of arterial thromboembolic events was 3.0% of patients (14 out of 466) in the combined group of patients treated with 0.3 mg or 0.5 mg LUCENTIS compared with 3.2% of patients (7 out of 216) in the control arm [*see Warnings and Precautions (5.3)*].

6.4 Immunogenicity

The pre-treatment incidence of immunoreactivity to LUCENTIS was 0%-3% across treatment groups. After monthly dosing with LUCENTIS for 12 to 24 months, low titers of antibodies to LUCENTIS were detected in approximately 1%-6% of patients. The immunogenicity data reflect the percentage of patients whose test results were considered positive for antibodies to LUCENTIS in an electrochemiluminescence assay and are highly dependent on the sensitivity and specificity of the assay. The clinical significance of immunoreactivity to LUCENTIS is unclear at this time, although some patients with the highest levels of immunoreactivity were noted to have iritis or vitritis.

7 DRUG INTERACTIONS

Drug interaction studies have not been conducted with LUCENTIS.

LUCENTIS intravitreal injection has been used adjunctively with verteporfin photodynamic therapy (PDT). Twelve of 105 (11%) patients developed serious intraocular inflammation; in 10 of the 12 patients, this occurred when LUCENTIS was administered 7 days (± 2 days) after verteporfin PDT.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy
Pregnancy Category C. Animal reproduction studies have not been conducted with ranibizumab. It is also not known whether ranibizumab can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. LUCENTIS should be given to a pregnant woman only if clearly needed.

8.3 Nursing Mothers
It is not known whether ranibizumab is excreted in human milk. Because many drugs are excreted in human milk, and because the potential for absorption and harm to infant growth and development exists, caution should be exercised when LUCENTIS is administered to a nursing woman.

8.4 Pediatric Use
The safety and effectiveness of LUCENTIS in pediatric patients has not been established.

8.5 Geriatric Use
In the controlled clinical studies, approximately 94% (822/879) of the patients randomized to treatment with LUCENTIS were ≥ 65 years of age and approximately 68% (601/879) were ≥ 75 years of age. No notable difference in treatment effect was seen with increasing age in any of the studies. Age did not have a significant effect on systemic exposure in a population pharmacokinetic analysis after correcting for creatinine clearance.

8.6 Patients with Renal Impairment
No formal studies have been conducted to examine the pharmacokinetics of ranibizumab in patients with renal impairment. Sixty-eight percent of patients (136 of 200) in the population pharmacokinetic analysis had renal impairment (46.5% mild, 20% moderate, and 1.5% severe). Reduction in ranibizumab clearance is minimal in patients with renal impairment and is considered clinically insignificant. Dose adjustment is not expected to be needed for patients with renal impairment.

8.7 Patients with Hepatic Dysfunction
No formal studies have been conducted to examine the pharmacokinetics of ranibizumab in patients with hepatic impairment. Dose adjustment is not expected to be needed for patients with hepatic dysfunction.

10 OVERDOSAGE
Planned initial single doses of ranibizumab injection 1.0 mg were associated with clinically significant intraocular inflammation in 2 of 2 patients injected. With an escalating regimen of doses beginning with initial doses of ranibizumab injection 0.3 mg, doses as high as 2.0 mg were tolerated in 15 of 20 patients.

11 DESCRIPTION
LUCENTIS™ (ranibizumab injection) is a recombinant humanized IgG1 kappa isotype monoclonal antibody fragment designed for intraocular use. Ranibizumab binds to and inhibits the biologic activity of human vascular endothelial growth factor A (VEGF-A). Ranibizumab has a molecular weight of approximately 48 kilodaltons and is produced by an *E. coli* expression system in a nutrient medium containing the antibiotic tetracycline. Tetracycline is not detectable in the final product.

LUCENTIS is a sterile, colorless to pale yellow solution in a single-use glass vial. LUCENTIS is supplied as a preservative-free, sterile solution in a single-use glass vial designed to deliver 0.05 mL of 10 mg/mL LUCENTIS aqueous solution with 10 mM histidine HCl, 10% α, α-trehalose dihydrate, 0.01% polysorbate 20, pH 5.5.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action
Ranibizumab binds to the receptor binding site of active forms of VEGF-A, including the biologically active, cleaved form of this molecule, $VEGF_{110}$. VEGF-A has been shown to cause neovascularization and leakage in models of ocular angiogenesis and is thought to contribute to the progression of the neovascular form of age-related macular degeneration (AMD). The binding of ranibizumab to VEGF-A prevents the interaction of VEGF-A with its receptors (VEGFR1 and VEGFR2) on the surface of endothelial cells, reducing endothelial cell proliferation, vascular leakage, and new blood vessel formation.

12.2 Pharmacodynamics
Neovascular AMD is associated with foveal retinal thickening as assessed by optical coherence tomography (OCT) and leakage from CNV as assessed by fluorescein angiography.

In Study 3, foveal retinal thickness was assessed by OCT in 118/184 patients. OCT measurements were collected at baseline, Months 1, 2, 3, 5, 8, and 12. In patients treated with LUCENTIS, foveal retinal thickness decreased, on average, more than the sham group from baseline through Month 12. Retinal thickness decreased by Month 1 and decreased further at Month 3, on average. Foveal retinal thickness data did not provide information useful in influencing treatment decisions [*see Clinical Studies (14.2)*].

In patients treated with LUCENTIS, the area of vascular leakage, on average, decreased by Month 3 as assessed by fluorescein angiography. The area of vascular leakage for an individual patient was not correlated with visual acuity.

12.3 Pharmacokinetics
In animal studies, following intravitreal injection, ranibizumab was cleared from the vitreous with a half-life of approximately 3 days. After reaching a maximum at approximately 1 day,

the serum concentration of ranibizumab declined in parallel with the vitreous concentration. In these animal studies, systemic exposure of ranibizumab is more than 2000-fold lower than in the vitreous.

In patients with neovascular AMD, following monthly intravitreal administration, maximum ranibizumab serum concentrations were low (0.3 ng/mL to 2.36 ng/mL). These levels were below the concentration of ranibizumab (11 ng/mL to 27 ng/mL) thought to be necessary to inhibit the biological activity of VEGF-A by 50%, as measured in an in vitro cellular proliferation assay. The maximum observed serum concentration was dose proportional over the dose range of 0.05 to 1.0 mg/eye. Based on a population pharmacokinetic analysis, maximum serum concentrations of 1.5 ng/mL are predicted to be reached at approximately 1 day after monthly intravitreal administration of LUCENTIS 0.5 mg/eye. Based on the disappearance of ranibizumab from serum, the estimated average vitreous elimination half-life was approximately 9 days. Steady-state minimum concentration is predicted to be 0.22 ng/mL with a monthly dosing regimen. In humans, serum ranibizumab concentrations are predicted to be approximately 90,000-fold lower than vitreal concentrations.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

No carcinogenicity or mutagenicity data are available for ranibizumab injection in animals or humans.

No studies on the effects of ranibizumab on fertility have been conducted.

14 CLINICAL STUDIES

The safety and efficacy of LUCENTIS were assessed in three randomized, double-masked, sham- or active-controlled studies in patients with neovascular AMD. A total of 1323 patients (LUCENTIS 879, Control 444) were enrolled in the three studies.

14.1 Study 1 and Study 2

In Study 1, patients with minimally classic or occult (without classic) CNV lesions received monthly LUCENTIS 0.3 mg or 0.5 mg intravitreal injections or monthly sham injections. Data are available through Month 24. Patients treated with LUCENTIS in Study 1 received a mean of 22 total treatments out of a possible 24 from Day 0 to Month 24.

In Study 2, patients with predominantly classic CNV lesions received one of the following: 1) monthly LUCENTIS 0.3 mg intravitreal injections and sham PDT; 2) monthly LUCENTIS 0.5 mg intravitreal injections and sham PDT; or 3) sham intravitreal injections and active verteporfin PDT. Sham PDT (or active verteporfin PDT) was given with the initial LUCENTIS (or sham) intravitreal injection and every 3 months thereafter if fluorescein angiography showed persistence or recurrence of leakage. Data are available through Month 12. Patients treated with LUCENTIS in Study 2 received a mean of 12 total treatments out of a possible 13 from Day 0 through Month 12.

In both studies, the primary efficacy endpoint was the proportion of patients who maintained vision, defined as losing fewer than 15 letters of visual acuity at 12 months compared with baseline. Almost all LUCENTIS-treated patients (approximately 95%) maintained their visual acuity. 34%-40% of LUCENTIS-treated patients experienced a clinically significant improvement in vision, defined as gaining 15 or more letters at 12 months. The size of the lesion did not significantly affect the results. Detailed results are shown in the tables below.

Table 3
Outcomes at Month 12 and Month 24 in Study 1

Outcome Measure	Month	Sham n = 238	LUCENTIS 0.5 mg n = 240	Estimated Difference (95% CI)[a]
Loss of < 15 letters in visual acuity (%)[b]	Month 12	62%	95%	32% (26%, 39%)
	Month 24	53%	90%	37% (29%, 44%)
Gain of ≥ 15 letters in visual acuity (%)[b]	Month 12	5%	34%	29% (22%,-35%)
	Month 24	4%	33%	29% (23%, 35%)
Mean change in visual acuity (letters) (SD)[b]	Month 12	-10.5 (16.6)	+7.2 (14.4)	17.5 (14.8, 20.2)
	Month 24	-14.9 (18.7)	+6.6 (16.5)	21.1 (18.1, 24.2)

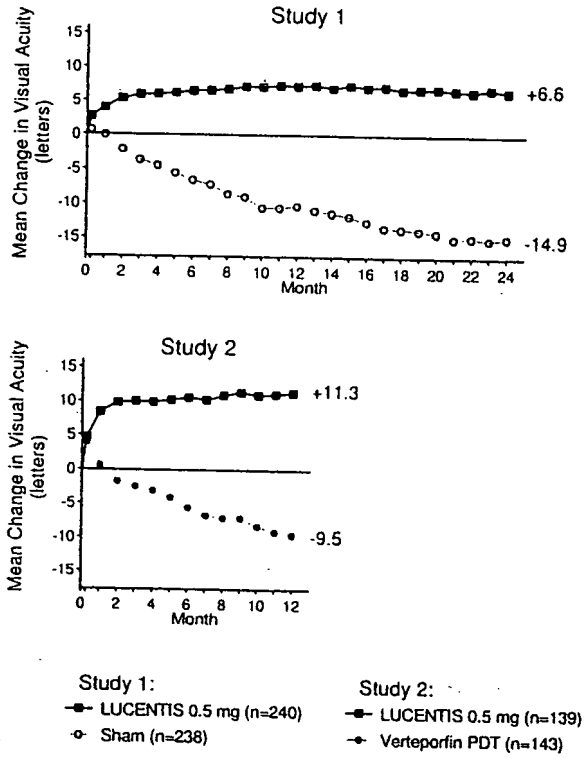
[a] Adjusted estimate based on the stratified model.
[b] $p<0.01$.

Table 4
Outcomes at Month 12 in Study 2

Outcome Measure	Verteporfin PDT n = 143	LUCENTIS 0.5 mg n = 140	Estimated Difference (95% CI)[a]
Loss of < 15 letters in visual acuity (%)[b]	64%	96%	33% (25%, 41%)
Gain of ≥ 15 letters in visual acuity (%)[b]	6%	40%	35% (26%, 44%)
Mean change in visual acuity (letters) (SD)[b]	-9.5 (16.4)	+11.3 (14.6)	21.1 (17.5, 24.6)

[a] Adjusted estimate based on the stratified model.
[b] $p<0.01$.

Figure 1
Mean Change in Visual Acuity from Baseline to Month 24 in Study 1 and to Month 12 in Study 2



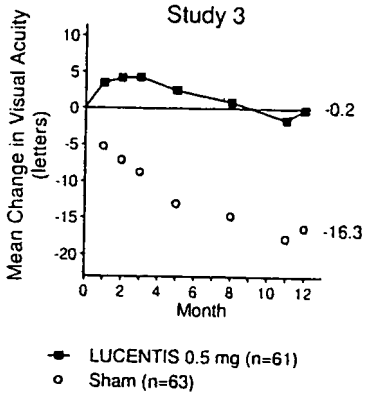
Patients in the group treated with LUCENTIS had minimal observable CNV lesion growth, on average. At Month 12, the mean change in the total area of the CNV lesion was 0.1-0.3 DA for LUCENTIS versus 2.3-2.6 DA for the control arms.

The use of LUCENTIS beyond 24 months has not been studied.

14.2 Study 3
Study 3 was a randomized, double-masked, sham-controlled, two-year study designed to assess the safety and efficacy of LUCENTIS in patients with neovascular AMD (with or without a classic CNV component). Data are available through Month 12. Patients received LUCENTIS 0.3 mg or 0.5 mg intravitreal injections or sham injections once a month for 3 consecutive doses, followed by a dose administered once every 3 months. A total of 184 patients were enrolled in this study (LUCENTIS 0.3 mg, 60; LUCENTIS 0.5 mg, 61; sham, 63); 171 (93%) completed 12 months of this study. Patients treated with LUCENTIS in Study 3 received a mean of 6 total treatments out of possible 6 from Day 0 through Month 12.

In Study 3, the primary efficacy endpoint was mean change in visual acuity at 12 months compared with baseline (see Figure 2). After an initial increase in visual acuity (following monthly dosing), on average, patients dosed once every three months with LUCENTIS lost visual acuity, returning to baseline at Month 12. In Study 3, almost all LUCENTIS-treated patients (90%) maintained their visual acuity at Month 12.

Figure 2
Mean Change in Visual Acuity from Baseline to Month 12 in Study 3



16 HOW SUPPLIED/STORAGE AND HANDLING
Each LUCENTIS carton, NDC 50242-080-01, contains one 2-cc glass vial of ranibizumab, one 5-micron, 19-gauge × 1-1/2-inch filter needle for withdrawal of the vial contents, one 30-gauge × 1/2-inch injection needle for the intravitreal injection, and one package insert [*see Dosage and*

Administration (2.4)]. VIALS ARE FOR SINGLE EYE USE ONLY.

17 PATIENT COUNSELING INFORMATION

In the days following LUCENTIS administration, patients are at risk of developing endophthalmitis. If the eye becomes red, sensitive to light, painful, or develops a change in vision, the patient should seek immediate care from an ophthalmologist [*see Warnings and Precautions (5.1)*].

LUCENTIS™ [ranibizumab injection]

Manufactured by:
Genentech, Inc.
1 DNA Way
South San Francisco, CA 94080-4990

8277700
LL1404
4833801
FDA Approval Date:
June 2006

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville, MD 20852

BLA 125156

Genentech, Inc.
Attention: Robert L. Garnick, Ph.D.
Senior Vice President, Regulatory Affairs, Quality & Compliance
1 DNA Way
South San Francisco, California 94080-4990

Dear Dr. Garnick:

We have approved your biologics' license application for Lucentis (ranibizumab injection) effective this date. You are hereby authorized to introduce or deliver for introduction into interstate commerce, ranibizumab injection under your existing Department of Health and Human Services U.S. License No. 1048. Lucentis (ranibizumab injection) is indicated for the treatment of patients with neovascular (wet) age-related macular degeneration.

Under this license, you are approved to manufacture ranibizumab drug substance at Genentech, Inc., South San Francisco, California; fill the final formulated product at (b) (4) (b) (4); and label and package filled vials at Genentech, Inc., South San Francisco, California. You may label your product with the proprietary name Lucentis and market it in 10 mg/mL single use glass vials.

We acknowledge receipt of your submissions dated December 29, 2005, and January 31, February 10, 17, 21, and 24, March 17, 23, and 31, April 10, and 28, May 5, 10, 25 (2), 26 (2), and 31, and June 1, 5 (2), 6, 9, 13, 16, 23, 26, 27, 28 (3), and 29, 2006.

The final printed labeling (FPL) must be identical in content to the enclosed labeling text for the package insert, submitted June 28, 2006; the immediate vial container submitted March 31, 2006; and the carton labels submitted June 5, 2006. The statement "No U.S. standard of potency" should be added with the next printing of carton labels. Marketing this product with FPL that is not identical in content to the approved labeling text may render the product misbranded and an unapproved new drug.

The dating period for formulated drug product shall be 18 months from the date of manufacture when stored at 2°-8°C (36°-46°F). The date of manufacture shall be defined as the date of final sterile filtration of the formulated drug product. The dating period for ranibizumab drug substance shall be (b) (4) when stored at -20 °C.

You currently are not required to submit samples of future lots of Lucentis to the Center for Drug Evaluation and Research (CDER) for release by the Director, CDER, under 21 CFR 610.2. We will continue to monitor compliance with 21 CFR 610.1 requiring completion of tests for conformity with standards applicable to each product prior to release of each lot.

You must submit information to your biologics license application for our review and written approval under 21 CFR 601.12 for any changes in the manufacturing, testing, packaging or labeling of Lucentis, or in the manufacturing facilities.

All applications for new active ingredients, new dosage forms, new indications, new routes of administration, and new dosing regimens are required to contain an assessment of the safety and effectiveness of the product in pediatric patients unless this requirement is waived or deferred. We are waiving the pediatric study requirement for this application.

The following are Postmarketing Studies that are subject to reporting requirements of 21 CFR 601.70:

1. Submit the final Clinical Study Report from Study FVF3689g by June 30, 2008.

2. Provide safety and efficacy data from a 2-year adequate and well-controlled clinical trial of a mutually acceptable design exploring multiple dosing frequencies of Lucentis.

   Date of submission of protocol: November 14, 2008.

   Date of start of study: September 21, 2009.

   Date of final clinical study report: April 1, 2013.

3. To detect and characterize immune responses to ranibizumab:

   a. Develop and validate a confirmatory assay capable of detecting both IgG and IgM isotype responses.

   b. Develop and validate an assay to detect neutralizing anti-ranibizumab antibodies.

   The assay methodology and validation reports: September 28, 2007.

4. To characterize further the immune response to ranibizumab, serum samples collected in studies FVF2587g, FVF2598g, FVF3192g will be assayed using the validated methods described above in Postmarketing Commitment #3. The data obtained will be analyzed to discover and evaluate any association between immunoreactivity and dosing frequency as well as any potential impact of immunoreactivity on efficacy or safety outcomes.

   The need for an additional clinical study will be determined based on the results from the analysis described above.

   Date of submission of protocol and statistical analysis plan: February 28, 2007.

   Date of submission of final study report: September 30, 2008.

The following are Postmarketing Studies that are not subject to reporting requirements of 21 CFR 601.70:

5. To revise release specifications, shelf-life specifications and in-process limits for ranibizumab drug substance and drug product after [redacted] commercial manufacturing runs to reflect increased manufacturing experience.

   These revisions to the Quality control system, the corresponding data from the [redacted] commercial manufacturing runs and the analysis plan used to create the revisions will be submitted as a supplement on or before June 30, 2008.

6. To perform additional Lucentis stability studies at 40°C using Ion Exchange Chromatography (IEC) to demonstrate that the corrective actions taken at [redacted] to address the atypical accelerated stability profile observed in the Lucentis 2005 qualification campaign have been sufficient.

   Specifically, a one time stability study consisting of [redacted] Lucentis Drug Product launch lots are placed at 40°C and tested by IEC at [redacted] months. These [redacted] Lucentis Drug Product lots are derived from the following:

   - [redacted] of these Lucentis Drug Product lots are manufactured from distinct lots of [redacted].
   - At least [redacted] these [redacted] lots are aliquoted and used to manufacture [redacted] Lucentis drug product lots.

   Data will be submitted as a supplement on or before March 31, 2007.

We request that you submit clinical protocols to your IND, with a cross-reference letter to this biologics license application. Submit nonclinical and chemistry, manufacturing, and controls protocols and all study final reports to this application. Please use the following designators to label prominently all submissions, including supplements, relating to these postmarketing study commitments as appropriate:

- **Postmarketing Study Protocol**
- **Postmarketing Study Final Report**
- **Postmarketing Study Correspondence**
- **Annual Report on Postmarketing Studies**

For each postmarketing study subject to the reporting requirements of 21 CFR 601.70, you must describe the status in an annual report on postmarketing studies for this product. The status report for each study should include:

- information to identify and describe the postmarketing commitment,
- the original schedule for the commitment,
- the status of the commitment (i.e. pending, ongoing, delayed, terminated, or submitted),

- an explanation of the status including, for clinical studies, the patient accrual rate (i.e. number enrolled to date and the total planned enrollment), and
- a revised schedule if the study schedule has changed and an explanation of the basis for the revision.

As described in 21 CFR 601.70(e), we may publicly disclose information regarding these postmarketing studies on our Web site (http://www.fda.gov/cder/pmc/default.htm). Please refer to the April 2001 Draft Guidance for Industry: Reports on the Status of Postmarketing Studies – Implementation of Section 130 of the Food and Drug Administration Modernization Act of 1997 (see http://www.fda.gov/cber/gdlns/post040401.htm) for further information.

You must submit adverse experience reports under the adverse experience reporting requirements for licensed biological products (21 CFR 600.80). You should submit postmarketing adverse experience reports to the Central Document Room, Center for Drug Evaluation and Research, Food and Drug Administration, 5901-B Ammendale Road, Beltsville, MD 20705-1266. Prominently identify all adverse experience reports as described in 21 CFR 600.80.

The MedWatch-to-Manufacturer Program provides manufacturers with copies of serious adverse event reports that are received directly by the FDA. New molecular entities and important new biologics qualify for inclusion for three years after approval. Your firm is eligible to receive copies of reports for this product. To participate in the program, please see the enrollment instructions and program description details at www.fda.gov/medwatch/report/mmp.htm.

You must submit distribution reports under the distribution reporting requirements for licensed biological products (21 CFR 600.81).

You must submit reports of biological product deviations under 21 CFR 600.14. You should promptly identify and investigate all manufacturing deviations, including those associated with processing, testing, packing, labeling, storage, holding and distribution. If the deviation involves a distributed product, may affect the safety, purity, or potency of the product, and meets the other criteria in the regulation, you must submit a report on Form FDA-3486 to the Division of Compliance Risk Management and Surveillance (HFD-330), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857. Biological product deviations sent by courier or overnight mail should be addressed to Food and Drug Administration, CDER, Office of Compliance, Division of Compliance Risk Management and Surveillance, HFD-330, Montrose Metro 2, 11919 Rockville Pike, Rockville, MD 20852.

Please submit all FPL at the time of use and include implementation information on FDA Form 356h. Please provide a PDF-format electronic copy as well as original paper copies (ten for circulars and five for other labels). In addition, you may wish to submit draft copies of the proposed introductory advertising and promotional labeling with a cover letter requesting advisory comments to the Food and Drug Administration, Center for Drug Evaluation and Research, Division of Drug Marketing, Advertising and Communication, 5901-B Ammendale Road, Beltsville, MD 20705-1266. Final printed advertising and promotional labeling should be submitted at the time of initial dissemination, accompanied by a FDA Form 2253.

All promotional claims must be consistent with and not contrary to approved labeling. You should not make a comparative promotional claim or claim of superiority over other products unless you have substantial evidence to support that claim.

Please refer to http://www.fda.gov/cder/biologics/default.htm for important information regarding therapeutic biological products, including the addresses for submissions.

If you have any questions, call Lori M. Gorski, Project Manager, at (301) 796-0722.

Sincerely,

Mark J. Goldberger, M.D., M.P.H.
Director
Office of Antimicrobial Products
Center for Drug Evaluation and Research

Enclosure

C

US006407213B1

(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

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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

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FIG. 1A

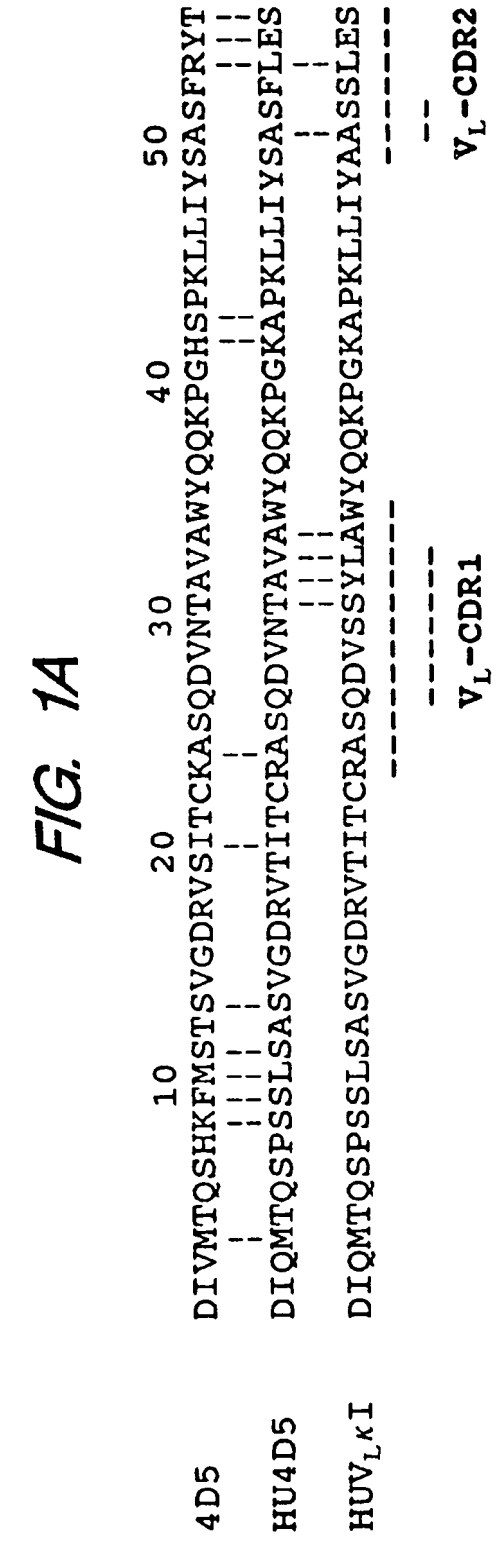
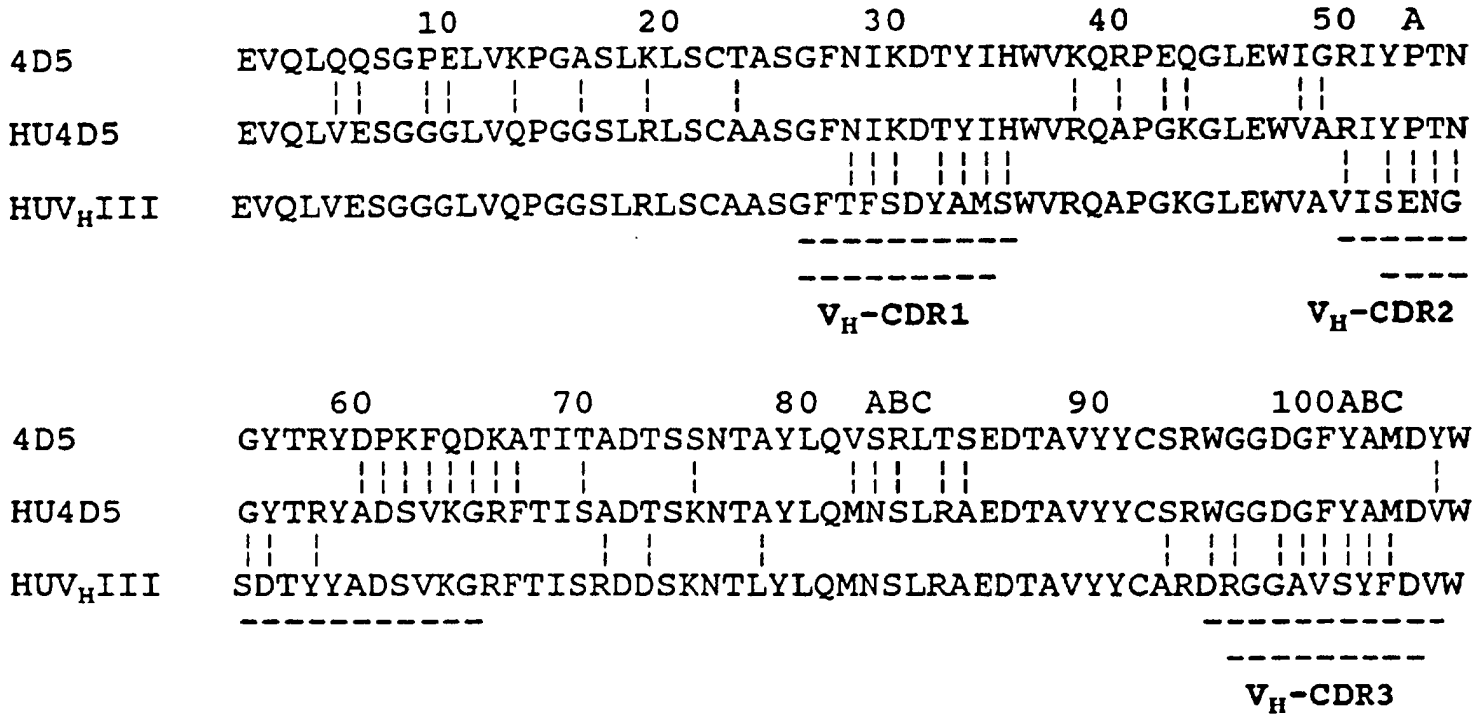

10 20 30 40 50
4D5 DIVMTQSHKFMSTSVGDRVSITCKASQDVNTAVAWYQQKPGHSPKLLIYSASFRYT
HU4D5 DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLES
HUVLκI DIQMTQSPSSLSASVGDRVTITCRASQDVSSYLAWYQQKPGKAPKLLIYAASSLES
VL-CDR1
VL-CDR2
60 70 80 90 100
4D5 GVPDRFTGNRSGTDFTFTISSVQAEDLAVYYCQQHYTTPPTFGGGTKLEIKRA
HU4D5 GVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRT
HUVLκI GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSLPYTFGQGTKVEIKRT
VL-CDR3

FIG. 1B



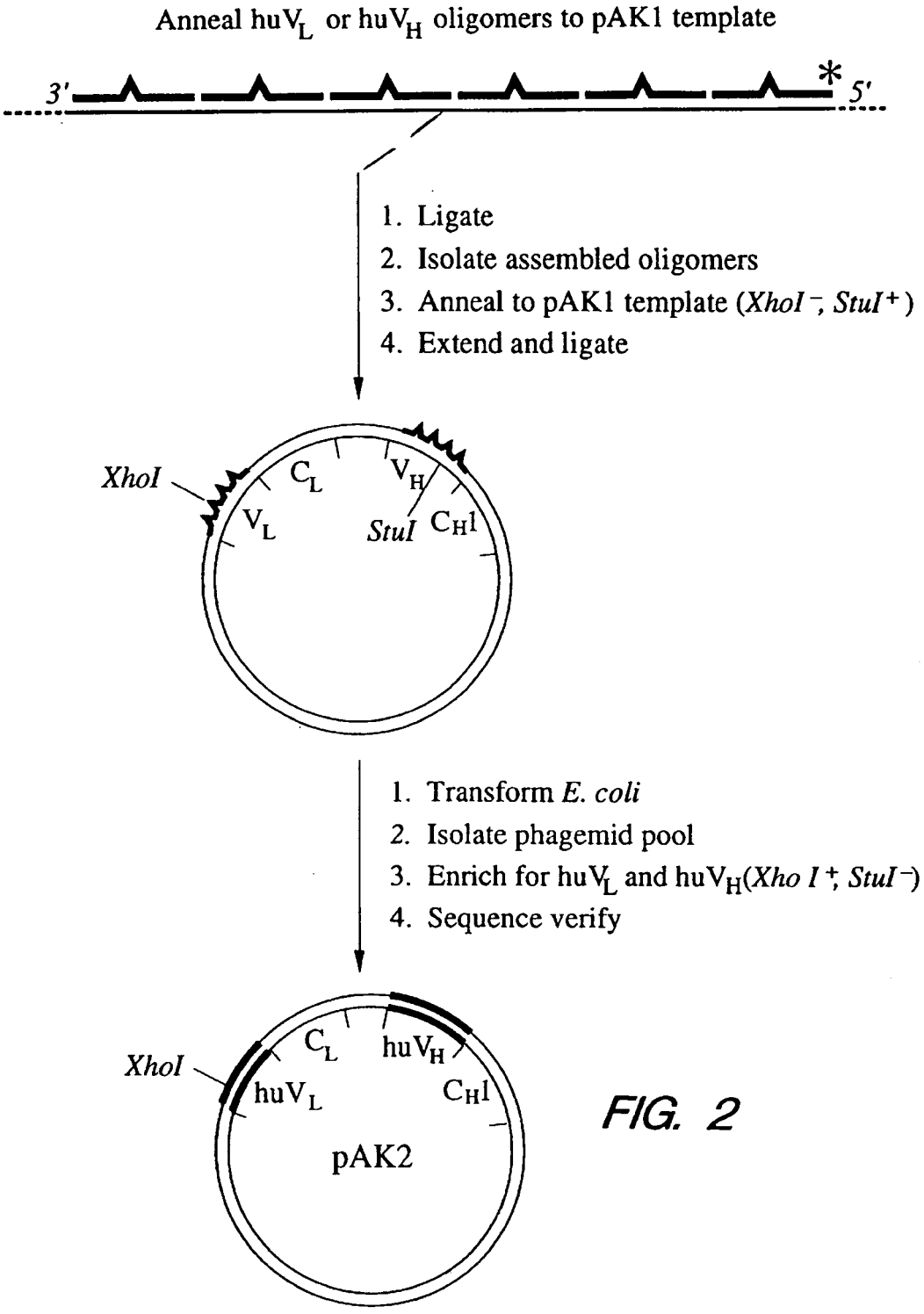

Anneal huV$_L$ or huV$_H$ oligomers to pAK1 template
3'
5'
1. Ligate
2. Isolate assembled oligomers
3. Anneal to pAK1 template (*XhoI*$^-$, *StuI*$^+$)
4. Extend and ligate
XhoI
C$_L$
V$_H$
V$_L$
StuI
C$_H$1
1. Transform *E. coli*
2. Isolate phagemid pool
3. Enrich for huV$_L$ and huV$_H$(*Xho I*$^+$, *StuI*$^-$)
4. Sequence verify
XhoI
C$_L$
huV$_H$
huV$_L$
C$_H$1
pAK2

FIG. 2

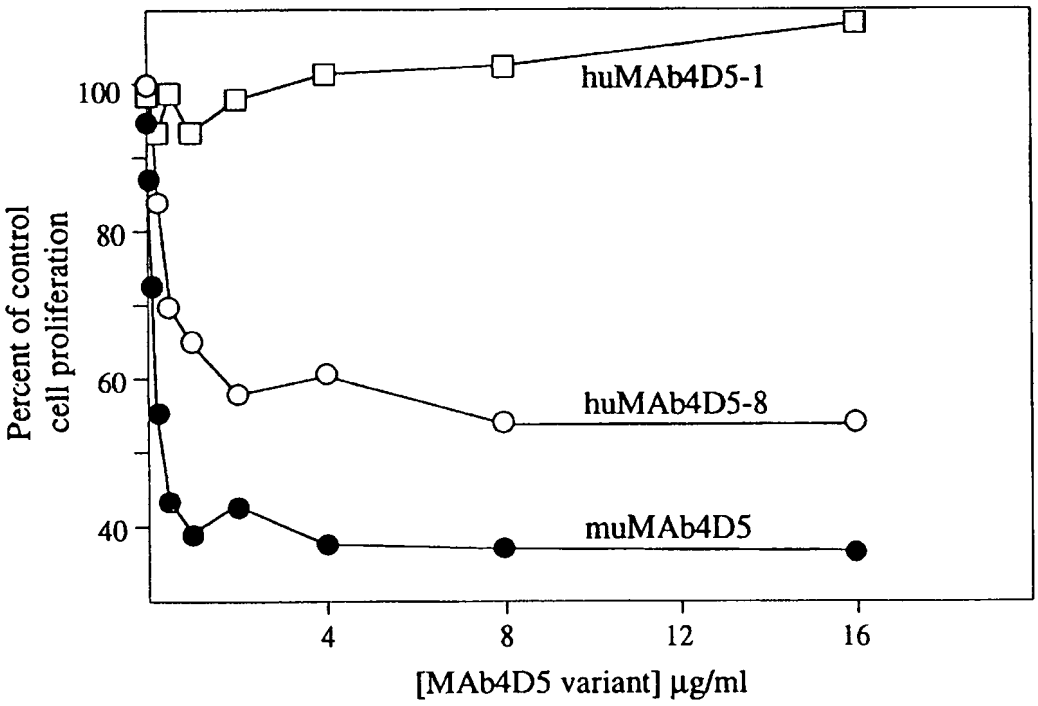

huMAb4D5-1
huMAb4D5-8
muMAb4D5
100
80
60
40
Percent of control cell proliferation
4
8
12
16
[MAb4D5 variant] µg/ml

FIG. 3

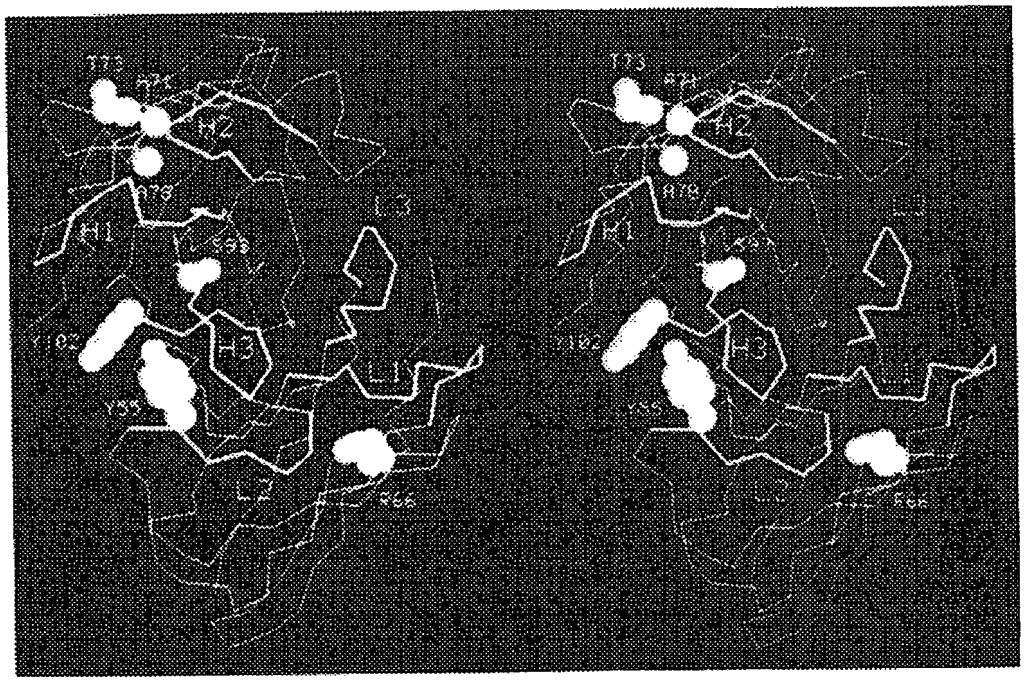


FIG. 4

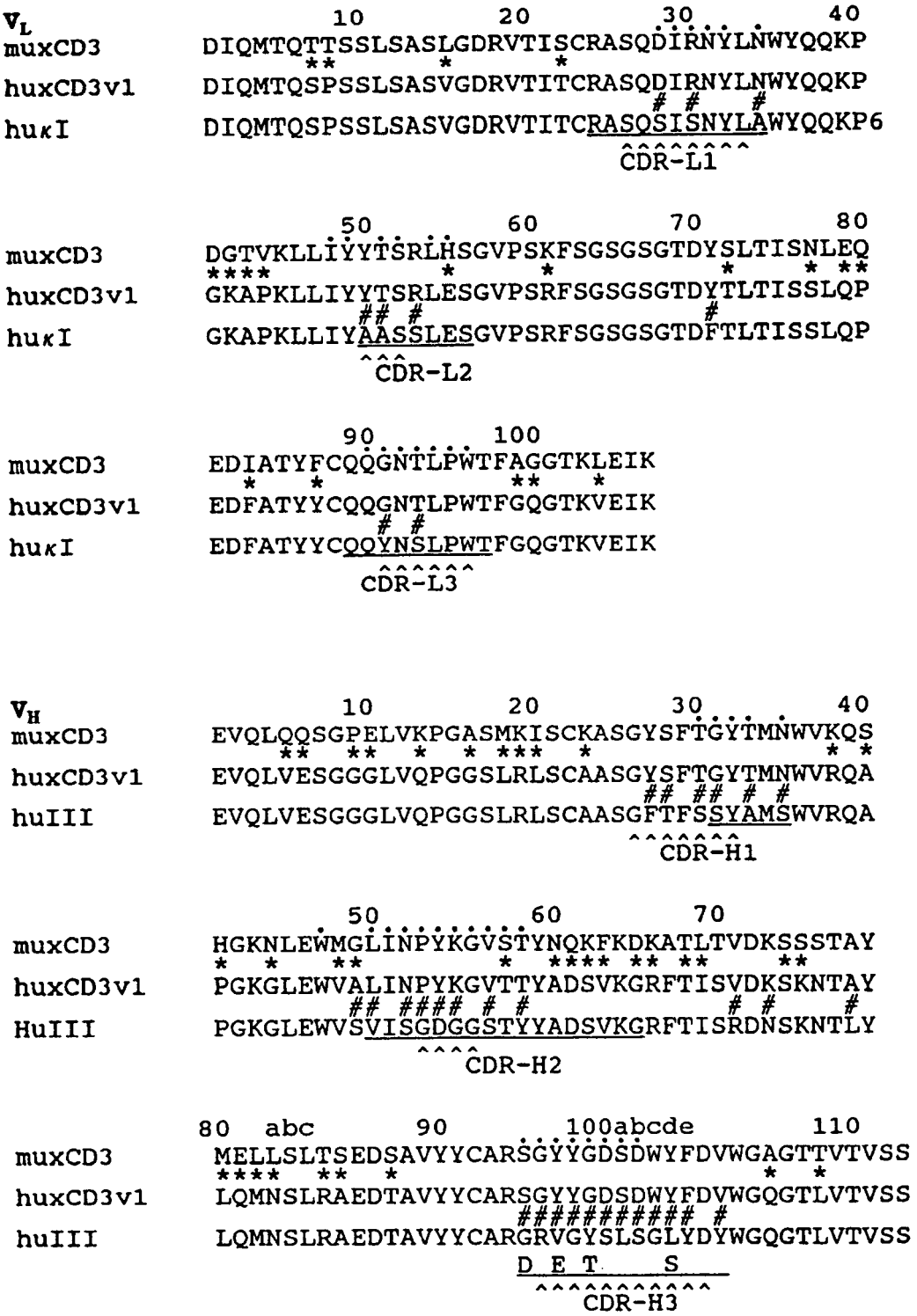

VL
muxCD3 DIQMTQTTSSLSASLGDRVTISCRASQDIRNYLNWYQQKP
huxCD3v1 DIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKP
huκI DIQMTQSPSSLSASVGDRVTITCRASQSISNYLAWYQQKP6
CDR-L1
muxCD3 DGTVKLLIYYTSRLHSGVPSKFSGSGSGTDYSLTISNLEQ
huxCD3v1 GKAPKLLIYYTSRLESGVPSRFSGSGSGTDYTLTISSLQP
huκI GKAPKLLIYAASSLESGVPSRFSGSGSGTDFTLTISSLQP
CDR-L2
muxCD3 EDIATYFCQQGNTLPWTFAGGTKLEIK
huxCD3v1 EDFATYYCQQGNTLPWTFGQGTKVEIK
huκI EDFATYYCQQYNSLPWTFGQGTKVEIK
CDR-L3
VH
muxCD3 EVQLQQSGPELVKPGASMKISCKASGYSFTGYTMNWVKQS
huxCD3v1 EVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQA
huIII EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQA
CDR-H1
muxCD3 HGKNLEWMGLINPYKGVSTYNQKFKDKATLTVDKSSSTAY
huxCD3v1 PGKGLEWVALINPYKGVTTYADSVKGRFTISVDKSKNTAY
HuIII PGKGLEWVSVISGDGGSTYYADSVKGRFTISRDNSKNTLY
CDR-H2
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

```
                290         300         310         320         330
H52H4-160       FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS
                *******.*************.***.********.***************
pH52-8.0        FNWYVDGMEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVS
                 300         310         320         330         340

                340         350         360         370         380
H52H4-160       NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP
                **.***********.*********************************
pH52-8.0        NKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP
                 350         360         370         380         390

                390         400         410         420         430
H52H4-160       SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
                **********************.****************************
pH52-8.0        SDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFS
                 400         410         420         430         440

                440         450
H52H4-160       CSVMHEALHNHYTQKSLSLSPGK
                ***********************
pH52-8.0        CSVMHEALHNHYTQKSLSLSPGK
                 450         460
```

FIG. 6B

```
                                    10                  20                  30
H52L6-158                           DVQMTQTTSSLSASLGDRVTINCRASQDINN
                                    *.*****. ******* ********** **********
pH52-9.0                MGWSCIILFLVATATGVHSDIQMTQSPSSLSASVGDRVTITCRASQDINN
                                10                  20                  30
                                    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:
DIQMTQSPSSLSASVGDRVTITCRASQD-
VNTAVAWYQQKPGKAPKLLIYSASFLES-
GVPSRFSGSRSGTDFTLTISSLQPEDFA-
TYYCQQHYTTPPTFGQGTKVEIKRT

2. SEQ. ID NO. 2, which is the heavy chain variable domain of a humanized version of muMAb4D5):
EVQLVESGGGLVQPGGSLRLSCAASGFNIK
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): DDIQMTQSPSSLSAS-
VGDRVTITCRASQDVSSYLAWYQQKPGKAPKLL
IYAASSLESGVPSRFSGSGSGTDFTLTISSLQP
EDFATYYCQQYNSLPYTFGQGTKVEIKRT, and

SEQ. ID NO. 4 (heavy chain): EVQLVESGGGLVQPG
GSLRLSCAASGFTFSDYAMSWVRQAPGKGL
EWVAVISENGGYTRYADSVKGRFTISADTSKNT
AYLQMNSLRAEDTAWYCSRWGGDGFYAMD
VWGQGTLVTVSS

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A shows the comparison of the V_L domain amino acid residues of muMAb4D5, huMAb4D5, and a consensus sequence (FIG. 1A, SEQ.ID NO. 5, SEQ. ID NO. 1 and SEQ. ID NO. 3, respectively). FIG. 1B shows the comparison between the V_H domain amino acid residues of the muMAb4D5, huMAb4D5, and a consensus sequence (FIG. 1B, SEQ. ID NO. 6, SEQ. ID NO. 2 and SEQ. ID NO. 4, respectively). Both FIGS. 1A and 1B 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. 1A

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. 6A 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. 6B 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 IgG4. 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_LK before (Å)	V_LK 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 1/10 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 II 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_H Y102. 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 ($K_d \leq 1$ 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 ($BsF(ab')_2v1$ by separate *E. coli* expression of each Fab' arm followed by directed chemical coupling in vitro. $BsF(ab')_2v1$ (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_l) 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, 5th* 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). $BsF(ab')_2v9$ binds much more efficiently to Jurkat cells than does our starting molecule, $BsF(ab')_2v1$, 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). $BsF(ab')_2v9$ 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, $BsF(ab')_2v1$ 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). $BsF(ab')_2v9$ binds to SK-BR-3 breast tumor cells with equal efficiency to $BsF(ab')_2v1$ 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. 6A and 6B 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

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PATENT NUMBER	FEE AMT	SUR-CHARGE	U.S. APPLICATION NUMBER	PATENT ISSUE DATE	APPL. FILING DATE	PAYMENT YEAR	SMALL ENTITY?	STAT	ATTY DKT NUMBER
6,407,213	$900.00	$0.00	08/146,206	06/18/02	11/17/93	04	NO	PAID	709P1

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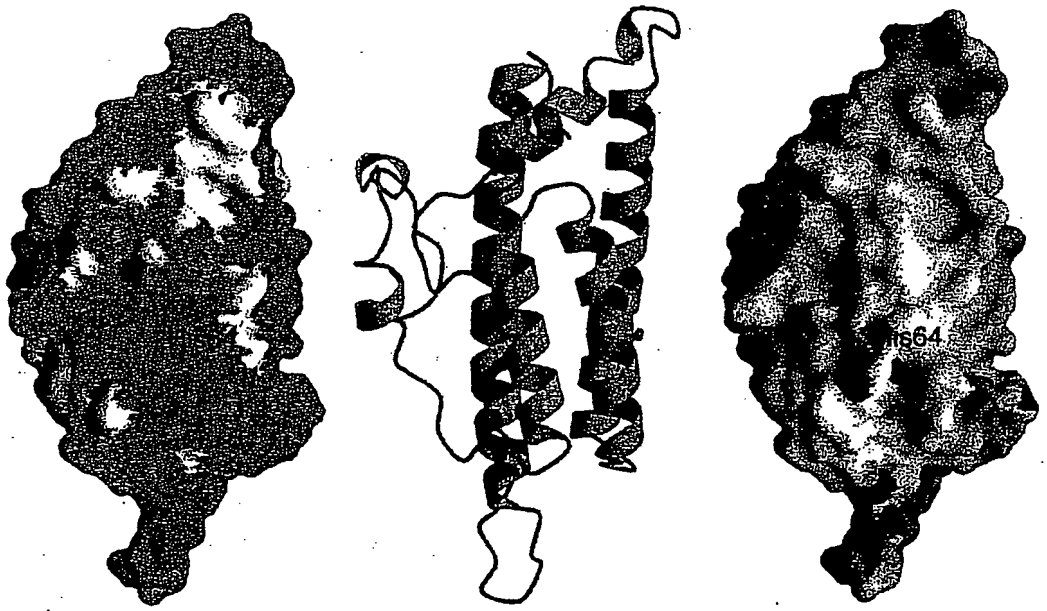

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JMB

Selection and Analysis of an Optimized Anti-VEGF Antibody: Crystal Structure of an Affinity-matured Fab in Complex with Antigen

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**Corresponding author*

The Fab portion of a humanized antibody (Fab-12; IgG form known as rhuMAb VEGF) to vascular endothelial growth factor (VEGF) has been affinity-matured through complementarity-determining region (CDR) mutation, followed by affinity selection using monovalent phage display. After stringent binding selections at 37 °C, with dissociation (off-rate) selection periods of several days, high affinity variants were isolated from CDR-H1, H2, and H3 libraries. Mutations were combined to obtain cumulatively tighter-binding variants. The final variant identified here, Y0317, contained six mutations from the parental antibody. *In vitro* cell-based assays show that four mutations yielded an improvement of about 100-fold in potency for inhibition of VEGF-dependent cell proliferation by this variant, consistent with the equilibrium binding constant determined from kinetics experiments at 37 °C. Using X-ray crystallography, we determined a high-resolution structure of the complex between VEGF and the affinity-matured Fab fragment. The overall features of the binding interface seen previously with wild-type are preserved, and many contact residues are maintained in precise alignment in the superimposed structures. However, locally, we see evidence for improved contacts between antibody and antigen, and two mutations result in increased van der Waals contact and improved hydrogen bonding. Site-directed mutants confirm that the most favorable improvements as judged by examination of the complex structure, in fact, have the greatest impact on free energy of binding. In general, the final antibody has improved affinity for several VEGF variants as compared with the parental antibody; however, some contact residues on VEGF differ in their contribution to the energetics of Fab binding. The results show that small changes even in a large protein-protein binding interface can have significant effects on the energetics of interaction.

Keywords: angiogenesis; humanized antibody-antigen complex; affinity maturation; phage display; X-ray crystallography

Introduction

Angiogenic factors (Folkman & Klagsbrun, 1987), which stimulate endothelial cells leading to new vascularization, have roles in such disease states as cancer, rheumatoid arthritis, and macular degeneration (reviewed by Ferrara, 1995; Folkman, 1995; Iruela-Arispe & Dvorak, 1997). Vascular endothelial growth factor (VEGF), a heparin-binding protein initially identified from pituitary cells (Ferrara & Henzel, 1989), is clearly a key angio-

Abbreviations used: CDR, complementarity-determining region; FR, framework region; HuVEC, human umbilical vein endothelial cell; $K_d^{25°}$, equilibrium dissociation constant determined at 25 °C; mAb, IgG form of monoclonal antibody; PBS, phosphate-buffered saline; SPR, surface plasmon resonance; VEGF, vascular endothelial growth factor; VEGF(109), receptor-binding fragment of VEGF with residues 8-109; VEGF(165), VEGF form with residues 1-165.

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0022-2836/99/440865–17 $30.00/0

genic factor in development as well as in certain disease states, including the growth of solid tumors (reviewed by Ferrara, 1999). A murine monoclonal antibody, A.4.6.1, was found to block VEGF-dependent cell proliferation *in vitro* and to antagonize tumor growth *in vivo* (Kim *et al.*, 1993). The murine mAb was previously humanized in Fab form to yield a variant known as Fab-12 (Presta *et al.*, 1997). Both chimeric and humanized antibodies retained high affinity binding to VEGF, with an apparent equilibrium dissociation constant, $K_d^{25°}$, of 0.9 to 3 nM (Presta *et al.*, 1997; Baca *et al.*, 1997; Muller *et al.*, 1998a). The corresponding full-length IgG form of this antibody, rhumAb VEGF, is being developed as a possible therapeutic agent for the treatment of human solid tumors (Mordenti *et al.*, 1999).

We became interested in obtaining higher affinity variants of Fab-12 in order to test whether affinity improvements of this antibody might improve its potency and efficacy. Phage display of randomized libraries of antibodies and other proteins has been extensively used to engineer proteins with improved affinity and specificity (Lowman *et al.*, 1991; reviewed by Kay & Hoess, 1996; Rader & Barbas, 1997; Griffiths & Duncan, 1998). In particular, a phage-based *in vitro* affinity maturation process has been successful in improving the binding affinity of antibodies previously identified from traditional monoclonal or naive-library sources (e.g. Hawkins *et al.*, 1992; Marks *et al.*, 1992; Barbas *et al.*, 1994; Yang *et al.*, 1995; Schier *et al.*, 1996; Thompson *et al.*, 1996).

In previous work, the humanized anti-VEGF antibody Fab-12 was adapted for improved monovalent phage display through selection of a CDR-L1 variant, designated Y0192 (Muller *et al.*, 1998a). To select target residues for randomization and affinity optimization, we also previously screened all CDR residues, as defined by a combination of the hypervariable (Kabat *et al.*, 1987) and structurally defined (Chothia & Lesk, 1987) CDR residues. Fab variants of Y0192 generated by alanine scanning were analyzed for side-chain contributions to antigen binding (Muller *et al.*, 1998a). In addition, a crystal structure of Fab-12 in complex with the receptor-binding domain of VEGF, VEGF(109), was determined (Muller *et al.*, 1998a). The results of these studies showed that the antigen binding site is almost entirely composed of residues from the heavy chain CDRs, CDR-H1, H2, and H3. Therefore, these CDRs appeared most likely to provide the opportunity for improved binding interactions with antigen.

Here, we describe the selection of an affinity-improved anti-VEGF antibody by phage display and off-rate selection. We show that the affinity-matured antibody binds VEGF with at least 20-fold improved affinity and inhibits VEGF-induced cell proliferation with enhanced potency in a cell-based assay. We also report the crystal structure of an affinity-optimized antibody in complex with VEGF, to our knowledge, representing the first reported structure of an *in vitro* affinity-matured antibody:antigen complex. The structure, together with mutational analysis, shows that subtle changes in the antibody-antigen interface account for improved affinity.

Results

Library design

We used the results of an alanine-scanning analysis, combined with a crystal structure of the wild-type Fab fragment in complex with VEGF (Muller *et al.*, 1998a), to design targeted libraries within the antibody CDRs for random mutagenesis and affinity selection. This strategy enabled us to construct theoretically complete libraries with a small number of residues randomized in each CDR. Although sites remote from the antigen-combining region or buried within the protein could modulate antigen binding affinity indirectly and have in fact been exploited for affinity improvement (Hawkins *et al.*, 1993), clearly residues shown to be important by alanine scanning are useful starting points for binding-affinity optimization (Lowman *et al.*, 1991; Lowman & Wells, 1993). Furthermore, we reasoned that by making mutations at residues of the antibody CDRs which were known to affect antigen binding and were located at or near points of contact in the bound complex, we could minimize the possibility of other indirect effects which might alter stability, immunogenicity, or other properties of the antibody.

Both Ala-scanning and crystallography (Muller *et al.*, 1998a) identified CDR-H3 as the predominant contact segment for VEGF, consistent with the general observation that CDR-H3 is often key to antigen binding (Chothia & Lesk, 1987). Within CDR-H3, residues Y95, P96, H97, Y98, Y99, S100b, H100c, W100d, Y100e, and F100f (numbering is as described by Kabat *et al.* (1987)), all showed effects on binding over a range of twofold to >150-fold when mutated to Ala, and Ala substitution at S100a caused a slight improvement in binding. However, H100c, Y100e, and F100f were found to have little or no direct contact with VEGF and presumed to have indirect effects on binding. On the other hand, Y95 and W100d have significant contacts with VEGF, and Ala substitutions resulted in no detectable binding to VEGF. Therefore, these residues were excluded from optimization. Inspection of the complex structure suggested that substitutions at P96 and Y98 could be disruptive to the antibody structure, while G100, where Ala mutation had little effect, might tolerate further substitutions. We therefore constructed a library (YC81) which fully randomized positions H97, Y99, G100, S100a, and S100b, within CDR-H3.

Significant effects of Ala substitution were also found in CDR-H2. Here, W50, I51, N52, T52a, Y53, T54, T58 alanine mutants all showed >twofold loss in binding affinity, with the greatest residue surface area buried at positions W50, I51, Y53, and

T58 (Muller *et al.*, 1998a). Indeed, W50 along with other aromatic side-chains was observed to form a deep pocket into which a loop of VEGF inserts in the complex, and was excluded from further optimization. Residue I51, on the other hand, showed no direct contact with VEGF and was also excluded. Residue T58 had multiple interactions within the interface, including contacts with VEGF and with the critical W50 of the CDR pocket. Although E56 showed no contact with VEGF and little effect (<twofold) upon alanine substitution, its side-chain lies at the periphery of the interface, near several hydrophobic residues of VEGF. We reasoned that these might be exploited for additional binding interactions. Two CDR-H2 libraries were constructed: YC266, randomizing positions T52a, Y53, T54, and E56; and YC103, randomizing positions N52, T52a, Y53, and T54.

In CDR-H1 G26, Y27, F29, N31, Y32, G33, M34, and N35 were implicated by alanine mutagenesis as important for binding VEGF; however, only N31, Y32, and G33 had significant direct contacts with VEGF. Since Ala substitution of G33 showed reduced binding, larger side-chains seemed less desirable; for this reason, this position was not randomized. Residues 27 (buried in the antibody structure) and T28 and T30 (which are mutually contacting) were included at the end of the H1 loop as possible indirect determinants of binding. Residues 27, 28, and 30-32 were randomized in a library designated YC265.

Framework residues, especially heavy chain residues 71 and 93, normally outside the region of contact with antigen, have also been found to affect antibody binding affinity (Tramontano *et al.*, 1990; Foote & Winter, 1992; Hawkins *et al.*, 1993; Xiang *et al.*, 1995), and sometimes participate in antigen contacts (reviewed by Nezlin, 1998). Therefore, an additional region of the anti-VEGF Fab, within FR-H3 and including position 71, was also targeted for randomization. Since the residue 71-76 region has contacts with CDR-H1 (at F29) and CDR-H2 (at I51 and T52a), these represented potential sites for affinity improvement through secondary effects on the interface residues. Residues L71, T73, and S76 were randomized in this FR-H3 library.

Phage selections

Fab libraries were constructed using a fusion to the g3p minor coat protein in a monovalent phage display (phagemid) vector (Bass *et al.*, 1990; Lowman *et al.*, 1991). For each library, stop codons were introduced by mutagenesis into the Y0192 phage template (Muller *et al.*, 1998a) at each residue position to be randomized. Each stop-codon construct was then used for construction of a fully randomized (using NNS codons) library as described in Materials and Methods. Phage were precipitated from overnight *Escherichia coli* shake-flask cultures and applied to VEGF-coated immunosorbant plates for binding selections. Cycles of selection followed by amplification were carried out essentially as described (Lowman, 1998).

We used an off-rate selection process (see Materials and Methods) similar to previously described procedures (Hawkins *et al.*, 1992; Yang *et al.*, 1995), modified by gradually increasing the selective pressure for binding to antigen during successive cycles of enrichment. The enrichment factor (ratio of displaying phage to non-displaying phage eluted *versus* applied) was used to monitor the stringency of selection at each step (Table 1). As a control, and to obtain a relative measure of affinity improvement, Y0192-phage were subjected to the same procedure at each cycle.

Fab-phage clones were sequenced from several phage-binding selection rounds that showed enrichment for Fab-phage over non-displaying phage. From round 6 of the CDR-H1 library selections, a dominant clone, Y0243-1 was found, having wild-type residues at Y27, T30, and Y32, and substitutions T28D and N31H (Table 2). Additional clones had related sequences, with N31H found in all selectants; Asp or Glu substituting for T28; and Thr, Ser, Gln, or Gly found at position T30.

Table 1. Enrichment factors from phage-displayed Fab libraries

Round	Wash time (hours)	CDR-H1 YC265	CDR-H2 YC266	CDR-H2 YC103	CDR-H3 YC81	FR-H3 YC101	Control Y0192
1	0	8.2	1.7	1.3	3.3	4	1.5
2	1	1.6	25	0.7	10	110	90
3	2	340	880	100	570	2300	22000
4	18	6800	880	5200	3700	600	2700
5	37[a]	210	900	920	1300	480	32
6	47[a]	130	80	100	3500	30	20
7	63[a]	1	1	>3	>25	1	>8

Libraries are designated by CDR region and oligonucleotide label (see the text for details). Library Fab-phage (ampicillin-resistant) were mixed with non-displaying control phage (chloramphenicol-resistant) in each starting pool, and subjected to VEGF binding selection, washing, and elution as described in the text.

The enrichment factor for each library is reported here as the ratio of Amp/Cam colony-forming units in the eluted pool, divided by the ratio of Amp/Cam colony-forming units in the starting pool. Starting phage concentrations were about 10^{12}/ml, except 10^{13}/ml in round 1. The wild-type Fab-phage, Y0192 was included at each round for comparison of enrichment under the particular conditions used.

[a] In some cases, the wash-step included incubation at 37 °C.

Table 2. Anti-VEGF Fab variants selected from a CDR-H1 library (HL-265)

Variant	n	Y 27	T 28	T 30	N 31	Y 32	I 34[a]	K_d(Y0192)/ K_d(variant)
Round 6 (HCl)								
Y0243-1	5	Y	D	T	H	Y	M	3.1
Y0243-2	1	Y	E	Q	H	Y	M	
Y0243-3	1	Y	E	T	H	Y	M	
Y0243-4	1	Y	D	G	H	Y	M	
Y0243-5	1	Y	D	S	H	Y	M	
Y0243-6	1	Y	E	S	H	Y	M	
Consensus:		Y	D	T	H	Y	M	3.1

All variants are in the background of Y0192 (Muller *et al.*, 1998a). n indicates the number of clones found with identical DNA sequence. The wild-type (Y0192) residue is shown at the top of each column, and the sequence position number is indicated according to Kabat *et al.* (1987).

[a] Position 34 was not randomized, but was changed to Met (as in Fab-12) in this library. The consensus reported here, equivalent to clone Y0243-1, represents the most abundant amino acid residue at each position (including clones with multiple representation ($n > 1$)). K_d(Y0192)/K_d (variant) indicates the fold increase in binding affinity *versus* the wild-type humanized antibody Y0192 (see Table 6).

Clones from two independently constructed CDR-H2 libraries were remarkable in that all sequenced library clones conserved wild-type residues at virtually all positions mutated, except at position Y53, where all clones contained a Trp substitution (Table 3).

Because of the strong enrichment observed from the CDR-H3 library, a number of clones were sequenced from rounds 5 and 7 (Table 4). Of 39 sequenced clones, 37 retained the wild-type residue S100b, and all contained the mutation H97Y. The remaining positions showed greater diversity, even after seven cycles of selection. The dominant clone at round 7, Y0238-3, contained the mutation S100aT (in addition to H97Y), with wild-type residues Y99 and G100. Other substitutions observed included Lys or Arg for Y99 (in 18 of 39 clones), G100N (11 of 39 clones), and a variety of substitutions including Arg, Glu, Gln, and Asn at S100a. In this library, the consensus sequence is represented by the dominant clone, Y0238-1 (Table 4).

Clones from round 6 of the FR-H3 library (Table 5) showed conservation of wild-type residue S76, with wild-type residues or various substitutions at the remaining positions: Val or Ile substituting for L71, and Val or Lys substitutions at T73.

Binding affinity of selected variants

For measurements of binding affinity, we made use of an amber stop codon placed between the genes for the Fab heavy chain and the g3p C-terminal domain, and expressed soluble Fab variants from *E. coli* shake-flask or fermentation cultures. Fab variants purified from protein-G affinity chromatography were characterized for binding affinity using an SPR-based assay on a BIAcore™-2000 instrument. The binding-kinetics assay has been described (Muller *et al.*, 1998a).

Association kinetics (k_{on}) for the wild-type antibody binding to immobilized VEGF are slow (Presta *et al.*, 1997; Baca *et al.*, 1997; Muller *et al.*, 1998a), and none of the variants tested had significantly improved on-rates. On the other hand, dissociation kinetics varied over a range of 10^{-4} s^{-1} to $\leqslant 4 \times 10^{-6}$ s^{-1} at 25 °C (Table 6). Based on measurements of instrumental drift, we could not accurately measure k_{off} (and consequently K_d)

Table 3. Anti-VEGF Fab variants selected from CDR-H2 libraries (HL-266, YC103)

Variant	n	N 52[a]	T 52a	Y 53	T 54	G 55[a,b]	E 56[a]	K_d(Y0192)/ K_d(variant)
Round 6 (HCl)								
HL266-A[b]	6	N	T	W	T	G	E	1.3
HL266-E	1	N	T	W	T	G	T	
HL266-I	1	N	T	W	T	G	Q	
YC103-A[b]	7	N	T	W	T	G	E	1.3
YC103-C	1	N	T	W	D	G	E	
Consensus		N	T	W	T	G	E	1.3

All variants are in the background of Y0192 (Muller *et al.*, 1998a). n indicates the number of clones found with identical DNA sequence. The wild-type (Y0192) residue is shown at the top of each column, and the sequence position number is indicated according to Kabat *et al.* (1987). The consensus reported here, equivalent to clones HL266A and YC103A, represents the most abundant amino acid at each position (including clones with multiple representation; i.e. $n > 1$). K_d(Y0192)/K_d(variant) indicates the fold increase in binding affinity *versus* the wild-type humanized antibody Y0192 (see Table 6).

[a] Constant positions were position 52 in the HL-266 library and position 56 in the YC103 library.

[b] Equivalent clones are assumed to have equal affinity.

Table 4. Anti-VEGF Fab variants selected from a CDR-H3 library (YC81)

Variant	*n*	H 97	Y 99	G 100	S 100a	S 100b	K_d(Y0192)/K_d(variant)
Round 5 (VEGF)							
Y0228-21	1	Y	R	N	A	S	
Y0228-22	1	Y	T	T	R	S	
Y0228-23	1	Y	E	G	S	S	
Y0228-24	1	Y	R	Q	R	G	
Y0228-26	1	Y	T	G	R	S	
Y0228-27	1	Y	T	N	T	S	
Y0228-28	1	Y	R	K	G	S	
Y0228-29	1	Y	T	G	S	S	
Y0228-30	1	Y	R	S	G	S	
Round 5 (HCl)							
Y0229-20	1	Y	T	N	R	S	
Y0229-21	1	Y	R	N	S	S	
Y0229-22	1	Y	K	E	S	S	
Y0229-23	1	Y	R	D	A	S	
Y0229-24	1	Y	R	Q	K	G	
Y0229-25	1	Y	K	G	G	S	
Y0229-26	1	Y	Y	G	A	S	
Y0229-27	1	Y	R	G	E	S	
Y0229-28	1	Y	R	S	T	S	
Y0238-10[a]	1	Y	R	N	T	S	3.8
Round 7 (HCl)							
Y0238-3	6	Y	Y	G	T	S	⩾9.4
Y0238-1	2	Y	R	G	T	S	7.3
Y0238-2	2	Y	I	N	K	S	
Y0238-10[a]	2	Y	R	N	T	S	3.8
Y0238-4	1	Y	Y	N	Q	S	
Y0238-5	1	Y	I	A	K	S	2.1
Y0238-6	1	Y	R	D	N	S	⩾5.4
Y0238-7	1	Y	W	G	T	S	
Y0238-8	1	Y	R	Q	N	S	
Y0238-9	1	Y	R	Q	S	S	
Y0238-11	1	Y	K	N	T	S	
Y0238-12	1	Y	I	E	R	S	
Consensus		Y	R	G	T	S	7.3

All variants are in the background of Y0192 (Muller *et al.*, 1998a). The clones are grouped according to the round of selection (5 or 7) and the type of elution (VEGF competition or HCl elution) used for recovery of bound phage. *n*, indicates the number of clones found with identical DNA sequence within each group. The wild-type (Fab-12, or Y0192) residue is shown at the top of each column, and the sequence position number is indicated according to Kabat *et al.* (1987). The consensus reported here, equivalent to clone Y0238-1, represents the most abundant amino acid at each position (including clones with multiple representation (n > 1)). K_d(Y0192)/K_d(variant) indicates the fold increase in binding affinity *versus* the wild-type humanized antibody Y0192 (see Table 6).

[a] One clone was identified at both rounds 5 and 7. Equivalent clones are assumed to have equal affinity.

under these conditions, but instead used the kinetics data to place an upper limit on K_d.

The phage-derived Fab variants tested showed a range of small (within experimental error of about twofold) to significant (>fivefold) improvements in binding affinity over the wild-type (parental phage) antibody Y0192 (Table 6). From the CDR-H1 library, the dominant clone (Y0243-1) showed threefold improved affinity. Variant Y0242-1, the dominant clone in each of three CDR-H2 libraries, showed an affinity equivalent to wild-type within experimental error, and two clones derived from the FR-H3 library (Y0244-1 and Y0244-4) were equivalent or slightly weaker in affinity. Small

Table 5. Anti-VEGF Fab variants selected from a FR-H3 library

Variant	*n*	L 71	T 73	S 76	K_d(Y0192)/K_d(variant)
Round 6 (HCl)					
Y0244-1	1	V	V	S	0.3
Y0244-2	1	L	K	S	
Y0244-3[a]	1	L	V	S	
Y0244-4	1	I	K	S	0.9

All variants are in the background of Y0192 (Muller *et al.*, 1998a). *n*, indicates the number of clones found with identical DNA sequence. The wild-type (Fab-12, or Y0192) residue is shown at the top of each column, and the sequence position number is indicated according to Kabat *et al.* (1987). K_d(Y0192)/K_d(variant) indicates the fold increase in binding affinity *versus* the wild-type humanized antibody Y0192 (see Table 6).

[a] One variant contained a spontaneous mutations, S74W.

Table 6. Binding kinetics of anti-VEGF Fab variants at 25 °C

Variant	$k_{on}/10^4$ (M^{-1} s^{-1})	$k_{off}/10^{-4}$ (s^{-1})	K_d (nM)	K_d(Y0192)/K_d(variant)
Y0192[a]	4.1	1.2	2.9	1
A. *Library-derived*				
Y0238-1	2.6	0.09	0.4	7.3
Y0238-3	1.3	≤0.04[b]	≤0.3[b]	≥9.4[b]
Y0238-5	0.57	0.08	1.4	2.1
Y0238-7	1.1	≤0.06[b]	≤0.5[b]	≥5.4[b]
Y0238-10	1.2	0.09	0.8	3.8
Y0242-1	3.8	0.86	2.3	1.3
Y0243-1	4.8	0.45	0.9	3.1
Y0244-1	3.0	2.7	9.0	0.3
Y0244-4	5.2	1.7	3.3	0.9
B. *Engineered*				
Y0268-1	4.0	0.15	0.38	7.6
Y0313-1	3.5	≤0.05[b]	≤0.15[b]	≥20[b]
Y0192(T28D)	6.8	1.4	2.0	1.4
Y0192(N31H)	4.8	0.37	0.8	3.6
Y0192(H97Y)	2.5	0.045	0.2	14
Y0192(S100aT)	6.8	1.0	1.5	1.9
Y0317	3.6	≤0.05[b]	≤0.14[b]	≥20[b]

Kinetic constants were determined from measurements using a BIAcore™-2000 instrument with a biosensor chip containing immobilized VEGF(109). Measurements were performed at 25 °C. Fab concentrations were calculated from quantitative amino acid analysis. The equilibrium dissociation constant, K_d, is calculated form the ratio of the rate constants, k_{off}/k_{on}. The relative affinity, reported as K_d(Y0192)/K_d(variant) indicates the fold increase in binding affinity versus the wild-type humanized antibody Y0192. Errors in K_d were approximately ±25%. Variant Y0242-1 corresponds to the point mutations Y53W in CDR-H2 of Fab Y0192; for descriptions of the other variants, see Tables 2, 3, 4, 5, and 8.

[a] Data for Y0192 is from Muller *et al.* (1998a,).

[b] In some cases, the dissociation rate constant observed was at or near the limit of detection; therefore, the reported k_{off} and K_d are upper limits, and the relative affinities are an upper limit.

improvements were seen in CDR-H3 variants Y0238-5 and Y0238-10. However, larger improvements (exceeding the limits of measurement (>fivefold to >ninefold)) were observed for the CDR-H3 variants Y0238-1, Y0238-3, and Y0238-7.

All tested variants (in fact all sequenced clones) from the CDR-H3 library contained the mutation H97Y. In the higher affinity group, Gly was conserved at position 100, while the lower affinity variant contained Ala (known to cause 1.8-fold reduction in Y0192 binding; Muller *et al.*, 1998a) or Asn (Table 4). The S100a position, while quite varied among sequenced clones, was changed to Thr in the higher affinity CDR-H3 variants, and Thr or Lys in the lower affinity ones. Substitutions at Y99, though mostly confined to basic or aromatic residues, apparently had little effect since Y0238-1 (representing the consensus CDR-H3 sequence with Y99R) was not significantly different in affinity from Y0238-3, which retained Y99.

Affinity improvements from combinations of CDR mutations

To improve affinity further, several combinations of the phage-selected CDR-H1, H2, and H3 mutations were made by site-directed mutagenesis (Table 7). Among these, the highest affinity was obtained with pY0313-1 (i.e. pY0192 with mutations CDR-H1 (T28D/N31H/I34M) and CDR-H3 (H97Y/S100aT); note I34M is a reversion to Fab-12 wild-type). From BIAcore™ kinetics measurements carried out at 25 °C, this Fab variant had ≥20-fold higher affinity than Y0192 (Table 6).

Addition of the Y53W mutation, which alone produced little or no improvement over Y0192, to Y0313-1 (producing variant Y0268-1) actually reduced binding affinity by >twofold (Table 6).

The final Fab version was constructed by removing the phage-expression enhancing mutations in CDR-L1 from pY0313-1 by site-directed mutagen-

Table 7. Anti-VEGF CDR combination variants

	CDR-L1					CDR-H1			CDR-H2	CDR-H3	
Y0192: Variant	R 24	N 26	E 27	Q 28	L 29	T 28	N 31	I 34	Y 53	H 97	S 100a
Y0313-1	-	-	-	-	-	D	H	M	-	Y	T
Y0268-1	-	-	-	-	-	D	H	M	W	Y	T
Y0317	S	S	Q	D	I	D	H	M	-	Y	T
Fab-12	S	S	Q	D	I	-	-	-	-	-	-

Substitutions are shown relative to Y0192. Fab-12 also contains T221 in the heavy chain. Dashes (-) indicate no substitution. Numbering is according to Kabat *et al.* (1987) for both the light chain (CDR-L1) and heavy chain (CDR-H1, H2, H3).

esis. The M4L substitution was identified during phage-humanization experiments (Baca *et al.*, 1997), and the Leu residue was retained so as to preclude possible oxidation of the Met side-chain. The first libraries were constructed from a Fab-12 phagemid derivative, pY0101, which contained a buried framework mutation, V_L(M4L), as well as a mutation (T221L) at the junction to g3p. Thus the final version, Y0317 (Table 7 and Figure 1) differs from Fab-12 by the following six mutations: V_L(M4L), V_H(T28D/N31H/H97Y/S100aT/T221L).

Each of the CDR mutations in H1 and H3 was tested for its effect on VEGF binding affinity by introducing the corresponding point mutation into the parental Y0192 Fab and measuring binding kinetics. The results (Table 6) show a 14-fold and 3.6-fold improvement with substitution of H97Y or N31H, respectively, into the parental Fab. However, T28D or S100aT had identical affinity to Y0192, within experimental error.

We compared Fab-12 and Y0317 Fab affinities in a solution binding assay, using VEGF competition with [^{125}I]VEGF for binding to Fab. The results showed Fab-12 having $K_d^{25°}$ = 433 pM and Y0317 Fab having $K_d^{25°}$ = 20 pM, a 22-fold improvement in binding affinity (Figure 2).

Because dissociation kinetics in surface plasmon resonance (SPR) experiments exceeded instrumental capabilities at 25 °C, and in order to assess binding affinity under more physiological conditions, we compared binding affinities of the original humanized antibody Fab-12 with the final variant Y0317 in kinetics experiments at 37 °C. k_{on} and k_{off} were faster for both antibodies than at 25 °C, and k_{off} was clearly measurable above background. Using either immobilized VEGF(109) or immobilized VEGF(165), Y0317 was 120-fold to 140-fold improved in affinity over Fab-12, with a $K_d^{37°}$ of 80-190 pM (Table 8).

VEGF Ala-scan of the Y0317 binding epitope

In order to understand how mutations in the Fab affected binding affinity to VEGF, we also tested VEGF variants for binding to the affinity-improved antibody. For these experiments, we made use of the full-length IgG forms of Fab-12 (known as rhuMab VEGF) and Y0317 (termed Y0317-IgG) produced in CHO cells (V. Chisholm, unpublished results). These VEGF variants were previously used for mapping the parental antibody's binding site on VEGF (Muller *et al.*, 1998a).

In this assay, carried out at 37 °C, VEGF competed with biotin-VEGF with an IC_{50} of 9 nM in binding rhuMab VEGF, compared with an IC_{50} of 1 nM for Y0317-IgG (Table 9). SPR measurements have shown similar affinity improvement of Y0317-IgG over rhuMAb VEGF (H. Lowman, unpublished results).

Alanine mutations of VEGF that affected rhuMab VEGF binding also affected Y0317-IgG. For example, M81A, G88A, and G92A all caused large (100 to >500-fold) losses in binding affinity. And smaller reductions (3 to 30-fold) in binding affinity for both antibodies were seen for I80A, K84A, I91A, E93A, and M94A.

However, significant differences in the magnitude of the effect were observed at certain sites, including Y45A, fourfold reduced in affinity for rhuMAb VEGF *versus* 26-fold for Y0317-IgG; Q89A, 19-fold *versus* sixfold; and M94A, 11-fold versus 25-fold. Most surprisingly, two mutations that led to loss of detectable binding affinity for rhumAb VEGF (>500-fold) had only modest effects (four- to ninefold) on binding to Y0317-IgG. These differences might suggest a shift in the binding epitope of the antibody, and this possibility was addressed with receptor-inhibition assays and structural analysis, both described below.

Inhibition of VEGF activity

Cell-proliferation assays have been described (Fairbrother *et al.*, 1998) for the measurement of VEGF mitogenic activity on human umbilical vein endothelial cells. Here, we compared the potency of Fab-12 and the affinity-improved variants Y0238-3 and Y0313-1.

The results (Figure 3) show both variants Y0238-3 and Y0313-1 inhibit VEGF activity more potently than Y0192 Fab. Comparing the Fab forms, variant Y0313-1 appeared at least 30-fold to 100-fold more potent than the wild-type Fab. In additional experiments, Y0317 activity was similar to that of Y0313-1 (data not shown). It should be noted that the amount of VEGF (0.2 nM) used in this assay is potentially limiting for determination of an accurate IC_{50} for the mutant. For example, if the bind-

Table 8. Binding kinetics of anti-VEGF Fab variants at 37 °C

Variant	Immobilized	$k_{on}/10^4$ (M^{-1} s^{-1})	$k_{off}/10^{-4}$ (s^{-1})	K_d (nM)	K_d(Fab-12)/K_d(variant)
Fab-12	VEGF(109)	5.1	6.6	13 ± 2.2	1
Y0317	VEGF(109)	5.4	0.059	0.11 ± 0.02	120
Fab-12	VEGF(165)	5.5	11	20 ± 3.8	1
Y0317	VEGF(165)	5.3	0.074	0.14 ± 0.05	140

Kinetic constants were determined by injecting Fab solutions onto a BIAcore™-2000 instrument with a biosensor chip containing approximately 190 RU of immobilized VEGF(109) or VEGF(165), as indicated. The equilibrium dissociation constant, K_d, is calculated from the ratio of the rate constants, k_{off}/k_{on}. The relative affinity, reported as K_d(Fab-12)/K_d(variant) indicates the fold increase in binding affinity *versus* the original humanized antibody (Fab-12; Presta *et al.*, 1997) under the specified conditions.

Light chain:

```
                1        10        20        30        40        50
Fab-12          DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYF
Y0192           DIQLTQSPSSLSASVGDRVTITCRANEQLSNYLNWYQQKPGKAPKVLIYF
Y0317.          DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYF

                1        10        20        30        40        50
                       60        70        80        90       100
Fab-12          TSSLHSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQ
Y0192           TSSLHSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQ
Y0317           TSSLHSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQ

                       60        70        80        90       100
                      110       120       130       140       150
Fab-12          GTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV
Y0192           GTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV
Y0317           GTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV

                      110       120       130       140       150
                      160       170       180       190       200
Fab-12          DNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG
Y0192           DNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG
Y0317           DNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG

                      160       170       180       190       200
                      210
Fab-12          LSSPVTKSFNRGEC
Y0192           LSSPVTKSFNRGEC
Y0317           LSSPVTKSFNRGEC

                      210
```

Figure 1 (*legend shown opposite*)

ing affinity (K_d) of the mutant is in fact <0.2 nM, then the IC_{50} in this experiment will appear higher than under conditions of lower VEGF concentration. The result therefore supports the conclusion that the affinity-improved variant is at least 30-fold improved in affinity for VEGF, and that it effectively blocks VEGF activity *in vitro*.

Structure of the complex

In order to compare the structure and binding site of the affinity-improved antibody with that of the parental antibody, we determined the complex structure by X-ray crystallography. Crystals of the complex between the receptor binding domain of VEGF (residues 8 to 109) and the affinity-matured Fab Y0317 were grown as described (see Materials and Methods) and diffracted to a maximum resolution of 2.4 Å. The structure was refined starting from the coordinates of the complex between VEGF and the parent of Fab Y0317, Fab-12 (Muller *et al.*, 1998a), and refined to an R-value of 19.9% (R_{free} = 27.4%) for the reflections between 20 Å and 2.4 Å resolution.

Heavy chain:

```
                1       10        20        30        40        50
Fab-12          EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGW
Y0192           EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGINWVRQAPGKGLEWVGW
Y0317           EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGW

                1       10        20        30        40        50
                       60        70        80        90       100
Fab-12          INTYTGEPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYP
Y0192           INTYTGEPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYP
Y0317           INTYTGEPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYP

                 a      60        70        80  abc   90     96
                      110       120       130       140       150
Fab-12          HYYGSSHWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC
Y0192           HYYGSSHWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC
Y0317           YYYGTSHWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC

                100abcdef 110       120       130       140
                     160       170       180       190       200
Fab-12          LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
Y0192           LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
Y0317           LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG

                     150       160       170       180       190
                     210       220       230
Fab-12          TQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
Y0192           TQTYICNVNHKPSNTKVDKKVEPKSCDKTHL
Y0317           TQTYICNVNHKPSNTKVDKKVEPKSCDKTHL

                     200       210       220
```

Figure 1. Sequence alignment of the original humanized antibody (Fab-12; Presta *et al.*, 1997), the phage-displayed antibody (Y0192; Muller *et al.*, 1998a) and the affinity-improved antibody (Y0317). Sequential numbering of each chain is shown above the sequences; numbering according to Kabat *et al.* (1987) is shown below. CDR regions are underlined. Positions at which Y0317 differs from Fab-12 are indicated with double underlining.

The final model consists of two Fab fragments bound to the symmetrical poles of the VEGF dimer. Only residues 14–107 of each VEGF monomer are well defined in the electron density, and therefore the six N-terminal and the two C-terminal residues of each monomer were omitted from the model. Each Fab light chain comprises residues 1 to 213, with the C-terminal residue disordered, whereas for each heavy chain residues 138 to 143 as well as the six C-terminal residues are absent from the model. As in the parental Fab complex, two out of 1050 residues, namely T51 in the V_L chain of each Fab fragment, are located in the "disallowed regions" (Laskowski *et al.*, 1993) of the Ramachandran plot; 85 % of all residues have their main-chain torsion angles in the "most favored"

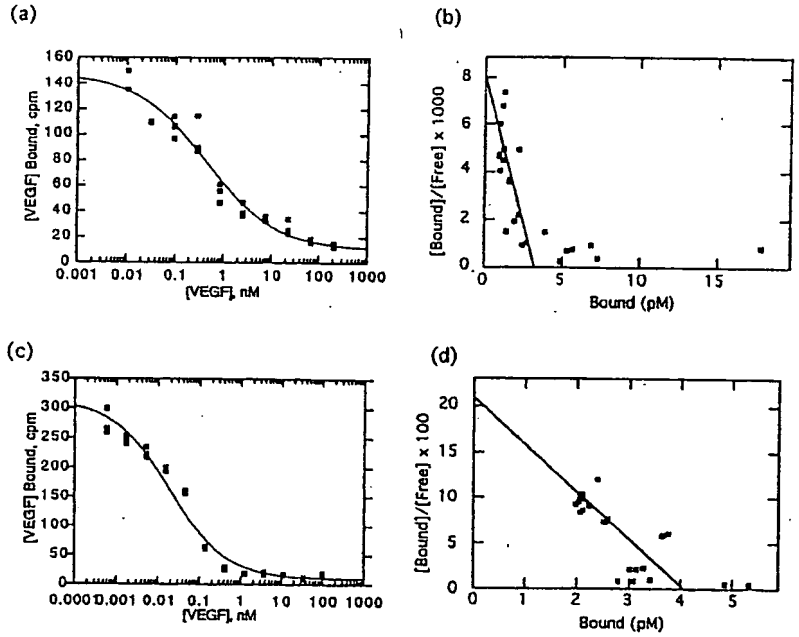

Figure 2. Radiolabeled VEGF binding assay. [^{125}I]VEGF was equilibrated (23 °C) with serial dilutions of unlabeled VEGF and (a) Fab-12 or (c) Y0317. Fabs were captured with an anti-Fab antibody-coated immunosorbant plate. Scatchard analysis (Munson & Rodbard, 1980) with a 1:1 binding model was used to calculate K_d of (b) 433 (±116) pM for Fab-12 and (d) 19.8(±4.3) pM for Y0317.

regions. The average *B*-factor of the model is 51.8 Å^2 and the mobility of the individual domains follows the pattern that was previously observed in the crystal structure of VEGF in complex with the Fab-12, with the constant domain dimer (C_L:C_H1) of one of the Fabs poorly ordered (Muller *et al.*, 1998a).

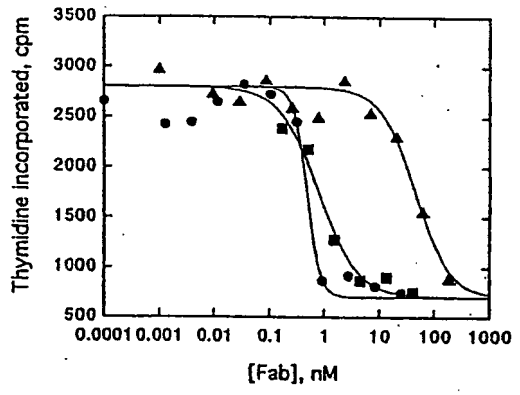

Figure 3. Human umbilical vein endothelial cell (HuVEC) assay of VEGF inhibition. Cells were cultured in the presence of 0.2 nM VEGF and serial dilutions of Fab Y0192 (triangles), Y0238-3 (squares), or Y0313-1 (circles). Cell proliferation was measured by incorporation of [^{3}H]thymidine. Curves show four-parameter fits to the data. Each point represents the mean of three treated wells.

Comparison of the final model with that of the parental Fab-VEGF complex (Muller *et al.*, 1998a) shows that the bound structures are very similar overall (Figure 4(a)) with Y0317 binding to the same site on VEGF as Fab-12 (Figure 4(b)). Side-chains show excellent overlap, and the main-chain structures show very little difference. The most prominent difference in contact residues is at H97Y (Figure 4(c); discussed below), where the tyrosine side-chain packs more favorably with VEGF and a buried water molecule from the parental Fab-VEGF complex is absent in the Y0317-Fab-VEGF complex.

Discussion

Antibody binding selections and affinity improvement

Here we made use of results from alanine-scanning and the previous structure of a humanized antibody-antigen complex to design Fab-phage libraries that randomized the three heavy-chain CDRs as well as one framework region (FR-H3) for improving the binding affinity of an anti-VEGF antibody. Affinity-improved Fab variants were obtained, with the largest effects seen in variants from the CDR-H3 library, although significant improvement was also obtained from mutation of CDR-H1. We therefore combined two mutations from H1 with two from H3, generating a further improved variant, Y0317. By making point mutations, we showed that the 20-fold (Figure 2)

Table 9. Alanine scan of VEGF by ELISA at 37 °C

VEGF(109) variant	IC_{50} (variant)/IC_{50} (VEGF)	
	Fab12-IgG	Y0317-IgG
VEGF(109)	1	1
F17A	1	1
Y21A	1	1
Y45A	4	26
K48A	2	1
Q79A	1	3
I80A	4	5
M81A	>500	930
R82A	>500	4
I83A	>500	9
K84A	3	10
H86A	1	1
Q87A	1	1
G88A	105	87
Q89A	19	6
H90A	1	1
I91A	2	6
G92A	>500	>900
E93A	4	7
M94A	11	25

ELISA assays were carried out using the full-length IgG form of Fab-12 or the IgG form of Y0317 and VEGF(109). Incubation of antibody with VEGF was at 37 °C for five hours. The IC_{50} for inhibition by each Ala mutant was evaluated using a four-parameter equation, and the relative affinities calculated as IC_{50}(mutant VEGF)/IC_{50}(wild-type VEGF). Under these conditions, Fab12-IgG and Y0317-IgG showed IC_{50} values of 9 nM and 1 nM, respectively.

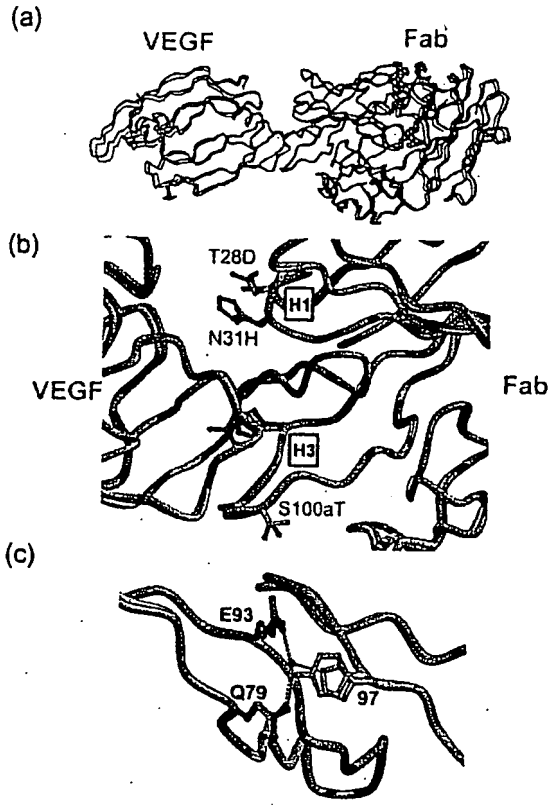

Figure 4. Structure of the affinity-improved Y0317 Fab in complex with VEGF. A superposition of the structure (Muller *et al.*, 1998a) of wild-type humanized antibody Fab-12 (gray) in complex with VEGF (gray) is shown with that of Fab Y0317 (green) in complex with VEGF (yellow). (a) Overall view of the complex, including one Fab molecule bound to one dimer of VEGF (a second Fab molecule is bound at left in the crystal) shows that the binding site for both antibody variants centers on the "80's loop" of VEGF. (b) A view of the four CDR changes between Fab-12 and Y0317 Fab shows that the new D28 and T100a side-chains do not directly contact antigen. However, H31 and Y97 form new contacts. (c) Interactions of H97 and an associated, buried water molecule in the Fab-12 complex, compared with those of Y97 in the Y0317 complex.

to >100-fold (Table 8) affinity improvement in Y0317 can be attributed to two CDR mutations: H97Y and N31H. In fact, H97Y alone improves binding affinty 14-fold.

Despite the relatively slow k_{on} and slow k_{off} of the parental antibody, binding selections described here yielded slower dissociation rates and improved equilibrium dissociation constants. Results of SPR measurements demonstrated that affinity is enhanced mainly through a slower dissociation rate (as opposed to faster association). These results are consistent with the idea of off-rate selection (Hawkins *et al.*, 1992) and with the progressively increased stringency in washing procedures used here (see Materials and Methods and Table 1). Previous binding-optimization efforts have also often yielded larger improvements in k_{off} than in k_{on} (see Lowman & Wells, 1993; Yang *et al.*, 1995; Schier *et al.*, 1996). This may suggest fundamental limitations to the improvements in k_{on} for a given binding site. Even if no conformational changes need occur between free and bound states, the on-rate is limited by the size of the binding interface and the translational and rotational diffusion rates of the binding components (reviewed by Delisi, 1983).

The association rate constants (k_{on}) for both the wild-type Y0192 and the final Y0317 antibodies are relatively slow (about 4×10^4 M^{-1} s^{-1} for both) compared to other antibodies of equal or weaker antigen binding affinity. In fact, the fastest k_{on} identified for any mutant was 6.8×10^4 M^{-1} s^{-1} (Table 6). Typically, k_{on} for antibodies binding to protein antigens, including affinity-matured antibodies, has fallen in the range of 3×10^4 to 1×10^6 M^{-1} s^{-1} (Karlsson *et al.*, 1991; Malmborg *et al.*, 1992; Barbas *et al.*, 1994; Yang *et al.*, 1995; Schier *et al.*, 1996; Wu *et al.*, 1998). In this particular protein-protein interaction, a likely explanation for the slow k_{on} is the high degree of flexibility associated with the binding site both on the Fab and on VEGF. In fact, crystallographic evidence suggests that the "80's loop" region is quite mobile (Muller *et al.*, 1997; Muller *et al.*, 1998b). We are pursuing other strategies to assess whether improvements to k_{on} can be obtained.

The contributions of point mutations in proteins to the free energy of binding or activation are often additive (Wells, 1990). This principle has been used to produce a variety of affinity-improved protein variants based on point or grouped mutations identified by phage display (Lowman & Wells, 1993; Yang *et al.*, 1995) or point-mutant screening (Wu *et al.*, 1998). Considering the initial library selectants Y0238-3 (>ninefold improved in affinity) and Y0243-1 (3.1-fold improved), we would have predicted an improvement of >27-fold for Y0313-1 or Y0317 (Table 7). In fact, a 22-fold improvement is observed (Figure 2) at 25 °C. Addition of the CDR-H1 mutation would be predicted to improve affinity slightly (1.3-fold), but in fact this mutation reduced affinity >twofold (Y0268-1 *versus* Y0313-1; Table 6). Certainly additivity does not always apply, particularly if interacting residues are involved (Wells, 1990). In this case, non-additivity probably results from steric interference between the new Trp in CDR-H2 and the new Tyr in CDR-H3.

To test the energetics of binding by the final Y0317 antibody to VEGF, we made use of a panel of alanine mutants that had been previously constructed for mapping the binding site of the original antibody (Muller *et al.*, 1998a). For these experiments, we made use of the full-length IgG forms of both antibodies. In view of the slow dissociation kinetics for both antibodies, ELISA assays were carried out at 37 °C with incubation for at least five hours to insure that equilibrium was reached. Under these conditions two dramatic differences appear in the Ala-scan of VEGF with respect to Y0317 *versus* Fab-12: both R82A and I83A have small effects on binding in Y0317, but result in large decreases in binding for Fab-12. The reasons for these differences are not clear, but R82 and I83 do have significant surface area (55 Å^2 and 32 Å^2, respectively) buried on binding to VEGF, and make contacts that include residues S100a of CDR-H3 and N52 of CDR-H2 in the wild-type antibody (Muller *et al.*, 1998a).

Structural analysis of the affinity-matured Fab

The structures of a number of antibodies derived from *in vivo* immunization and hybridoma techniques have been determined, in complex with their antigens (reviewed by Nezlin, 1998), and recently, crystallization and preliminary X-ray studies of a chain-shuffled anti-lysozyme scFv antibody in complex with antigen were reported (Küttner *et al.*, 1998). However, to our knowledge, the Y0317 Fab:VEGF structure is the first report of an *in vitro* affinity-matured Fab in complex with antigen. The structural basis of binding affinity improvement is therefore of interest

The Fab fragment of the affinity-matured anti-VEGF antibody Y0317 preserves the structure of the original humanized antibody, Fab-12. Superposition with Fab-12 results in an rmsd of only 0.38 Å for a total of 431 C^{α}-positions, demonstrating the absence of major structural changes between the two molecules. With a total of 1800 Å^2 of solvent-accessible surface buried in each VEGF-Fab interface, the contact area is about 50 Å^2 larger than in the Fab-12 complex. This small increase in buried surface area is mostly due to the exchange of H97 to a tyrosine residue. In the VEGF:Fab-12 complex, H97 buries a solvent-accessible area of 56 Å^2, while the larger tyrosine side-chain of the matured antibody accounts for 86 Å^2 of buried surface. The tyrosine side-chain also affects the hydrogen-bonding pattern and the number of ordered water molecules in the vicinity. In the parental antibody complex, a water molecule near H97 forms two hydrogen bonds to the side-chains of Q79 and E93 of VEGF (Figure 4(c)). In the complex with the affinity-matured Fab, this water molecule is replaced by the hydroxyl group of the newly introduced tyrosine side-chain at position 97. The H97Y mutation therefore not only increases the amount of buried surface area, but also introduces two additional hydrogen bonds between the ligand and Fab-0317 (Figure 4(c)). This is in good agreement with the observation that this single substitution improves VEGF binding affinity by 14-fold (Table 6). We therefore conclude that this single substiution is responsible for the majority of the improvement in binding affinity of Y0317 compared to the parent antibody.

In contrast, despite the availability of the crystal structures of both complexes, it remains uncertain what the structural basis is of the 3.6-fold enhanced binding caused by the N31H mutation. The side-chains of the asparagine and the histidine residues in this position adopt identical conformations in both crystal structures, and the amount of buried surface is not significantly increased in the VEGF:Fab-Y0317 complex. The only difference we can detect is a slight possible improvement in the hydrophobic interactions between the histidine side-chain and the phenyl group of VEGF residue F17, which has rotated slightly compared to the parent complex. It is unclear whether this could contribute to the increased affinity.

Neither of the remaining differences between Fab-12 and Fab-Y0317 has a significant effect on the binding affinity towards VEGF, and the structures show that these residues contribute only marginally to the interface. Some interactions are present between VEGF and the main-chain atoms of the serine and threonine residues in position 100a of the two Fabs, but the side-chains of these residues are not in contact with VEGF. Finally, no contact exist between VEGF and T28 (or D28) of the Fab fragments (the closest point on VEGF to this residue is more than 6 Å distant).

In summary, the analysis and comparison of the two crystal structures are in very good agreement with the results of the binding assays on the various single mutants of the Fab fragments. Although it is not possible to quantify the effects introduced by the amino acid exchanges solely based on the crystal structures, the detailed crystallographic

analysis supports and enables us to interpret the binding data.

Biological implications for antibody inhibition of VEGF

An inhibitory antibody of improved affinity may have improved potency or efficacy in treating diseases associated with VEGF expression. Preceding versions of the anti-VEGF antibody described here, including the murine A4.6.1 (Kim *et al.*, 1993), the humanized version Fab-12 (Presta *et al.*, 1997), as well as Y0192 (Muller *et al.*, 1998a), clearly demonstrated sufficient affinity to effect inhibition of VEGF activity. Here, we show that an affinity-improved variant, Fab Y0317, can inhibit endothelial cell proliferation *in vitro* with least 30-fold greater potency than the parental humanized Fab (Figure 3).

We have limited our optimization strategy to a subset of heavy-chain CDR residues implicated by alanine-scanning and crystallography (Muller *et al.*, 1998a). Furthermore, not all combinations of phage-derived mutations have been tested. One may therefore reasonably ask whether Y0317, with $K_d^{25°} = 20$ pM and $K_d^{37°} = 130$ pM, is the globally optimum variant for binding to this particular epitope (or others) on VEGF. Other affinity optimization efforts have resulted in protein-protein binding affinities in the low picomolar range, from $K_d = 6$ pM to 15 pM (see, e.g. Lowman & Wells, 1993; Schier *et al.*, 1996; Yang *et al.*, 1995). Indeed, we cannot exclude the possibility that higher affinity variants of the A4.6.1 antibody could be produced. However, it seems unlikely that further affinity improvement would greatly enhance biological potency or efficacy because for effective inhibition, the antibody must certainly occupy a significant fraction (perhaps >99%) of the available (VEGF) binding sites. Serum VEGF concentrations of about 20 pM in normals, and of >300 pM in patients with metastatic carcinoma, have been observed (Kraft *et al.*, 1999). Local or effective concentrations are likely higher. If we conservatively assume the effective concentration of VEGF *in vivo* to be about 400 pM, then 400 pM of even an infinite-affinity Fab would be required to block all sites.

Other factors may limit the improvement in potency of a full-length IgG resulting from an improvement in intrinsic binding affinity of the Fab for antigen. The full-length IgG form of the antibody may benefit from an avidity effect *in vivo*, especially since VEGF is known to associate with proteoglycans on the cell surface (Gitay-Goren *et al.*, 1992). Even in cell-based assays, the IgG form of Fab-12 is a more effective inhibitor than the Fab form (data not shown). Finally, the half-life for dissociation of the affinity-improved antibody is already significant, even on the time-scale of the half-life of clearance for IgG's (days to weeks). The effect of an improved association rate constant for antibody in this system is unknown.

The fact that point (Ala) mutations in the antibody binding site on VEGF sometimes have lesser effects on the binding of Y0317 than on the binding of Fab-12 may suggest that the optimized binding site is more tolerant than the parental one of variations in the antigen. Indeed, Y0317 showed greatly enhanced affinity for murine VEGF over that of Fab-12 (data not shown), though still >100-fold weaker than its affinity for human VEGF. This could provide an advantage against naturally arising VEGF variants.

Materials and Methods

Construction of phage libraries and mutagenesis

A variant of the Fab-12 antibody (a humanized form of murine antibody A4.6.1) was previously identified from phage-displayed Fab libraries for improved expression on phage particles (Muller *et al.*, 1998a). We made use of the plasmid pY0192, a phagemid construct with ampicillin (or carbenicillin) resistance, as the parental ("wild-type") construct for libraries described here. To prevent contamination by wild-type sequence (Lowman *et al.*, 1991; Lowman, 1998), templates with the TAA stop codon at each residue targeted for randomization were prepared from CJ236 *E. coli* cells (Kunkel *et al.*, 1991). Libraries are designated according to the mutagenic oligonucleotides used for their construction: YC265, TCC TGT GCA GCT TCT GGC NNS NNS TTC NNS NNS NNS GGT ATG AAC TGG GTC CG, randomizing residues 27-28, 30-32 in CDR-H1; YC266, GAA TGG GTT GGA TGG ATT AAC NNS NNS NNS GGT NNS CCG ACC TAT GCT GCG G, randomizing residues 52a-54, 56 in CDR-H2; YC103, GAA TGG GTT GGA TGG ATT NNS NNS NNS NNS GGT GAA CCG ACC TAT G, randomizing residues 52-54 in CDR-H2; YC81, C TGT GCA AAG TAC CCG NNS TAT NNS NNS NNS NNS CAC TGG TAT TTC GAC, randomizing residues 97, 99-100b in CDR-H3; and YC101, CGT TTC ACT TTT TCT NNS GAC NNS TCC AAA NNS ACA GCA TAC CTG CAG , randomizing residues 71, 73, and 76 in the "FR-H3" region. An additional library in CDR-H2 was designed to insert three new residues: YC90, GA TGG ATT AAC ACC TAT NNS NNS NNS ACC GGT GAA CCG ACC.

The products of random mutagenesis reactions were electroporated into XL1-Blue *E. coli* cells (Stratagene) and amplified by growing 15-16 hours with M13KO7 helper phage. The complexity of each library, ranging from 2×10^7 to 1.5×10^8 , was estimated based on plating of the initial transformation onto LB plates containing carbenicillin.

Site-directed mutagenesis for point mutations was carried out as above, using appropriate codons to produce the respective mutations, and sequences were confirmed by single-strand DNA sequencing using Sequenase™ (USB).

Phage binding selections

For each round of selection, approximately 10^9-10^{10} phage were screened for binding to plates (Nunc Maxisorp 96-well) coated with 2 μg/ml VEGF(109) in 50 mM carbonate buffer (pH 9.6) and blocked with 5% (w/v) instant milk in 50 mM carbonate buffer, (pH 9.6). Also included were phage prepared from a non-displaying

control phagemid (pCAT), which confers chloramphenicol resistance, as a means of measuring background and enrichment (Lowman & Wells, 1993). Bound phage were eluted with 0.1 M HCl and immediately neutralized with one-third volume of 1 M Tris (pH 8.0). The eluted phage were propagated by infecting XL1 cells for the next selection cycle as described (Lowman, 1998).

In the first cycle, the VEGF plate was incubated with Fab-phage, then was briefly washed to remove bound phage. In the second cycle, binding and washing were followed by a one hour dissociative incubation at room temperature with binding buffer, after which the plate was again washed prior to acid elution. This process was repeated in rounds 3, 4 and 5, except that 1 μM VEGF was included in the dissociative incubation, and the incubation time was increased to 2, 18, and 37 hours, respectively. During these selections, Y0192 phage showed enrichments ranging from 1.5-fold (at the lowest stringency) to 22,000-fold (using a two hour dissociation incubation). However, further increases in stringency (rounds 4-5) resulted in decreasing enrichments for the control phage, with higher enrichments observed for certain libraries, especially the two CDR-H2 libraries and the CDR-H3 library (Table 1).

In cycle 6, a 17 hour dissociative incubation at room temperature was followed by an additional 30 hour incubation at 37 °C (also including VEGF in the buffer). Under these conditions, Y0192-phage showed only slight binding enrichment (20-fold), whereas the CDR-H3 library phage were enriched by 3500-fold. Cycle 7 was carried out with a 63 hour dissociative incubation, after which only small enrichment factors were observed. However, some libraries were continued through eight cycles (with 120 hours of dissociative incubation in the presence of VEGF), after which Fab-phage were still recoverable by acid elution (data not shown).

Purification of Fab

For small-scale preparations, Y0317 Fab and mutants were prepared from *E. coli* shake-flasks as described (Muller *et al.*, 1998a).

For large-scale preparation, whole cell broth was obtained from a ten liter *E. coli* fermentation. The cells were lysed with a Manton-Gaulin homogenizer (two passes at 6000 psi; lysate temperature maintained at 15-25 °C with a heat exchanger). A 5% (v/v) solution of polyethylene imine (PEI), pH 6.0, was added to the lysate to give a final concentration of 0.25% (v/v). The lysate was mixed for 30 minutes at room temperature. The suspension was centrifuged, and the supernatant (containing the Fab) was processed further. The pH of the supernatant was adjusted to 6.0 with 6 M HCl, followed by dilution to a conductivity of 5 mS/cm with purified water. The conditioned supernatant was loaded onto a BakerBond ABx ion-exchange column. Following a wash with the column equilibration buffer, the Fab was eluted with an increasing sodium chloride gradient in the equilibration buffer. Fractions containing the Fab were identified by SDS-PAGE. The BakerBond ABx column fractions were pooled, pH adjusted to 5.5 with 1 M Mes and diluted to a conductivity of 5 mS/cm with purified water. The conditioned BakerBond ABx pool was loaded onto a SP Sepharose HP cation exchange column (Pharmacia). Once again, the Fab was eluted with a sodium chloride-containing gradient. Fractions containing the Fab were identified by SDS-PAGE. The level of purity of Fab (as determined by SDS-PAGE) after this two column purification was >95%.

BIAcore™ binding analysis

The VEGF-binding affinities of Fab fragments were calculated from association and dissociation rate constants measured using a BIAcore™ -2000 surface plasmon resonance system (BIAcore, Inc., Piscataway, NJ). A biosensor chip was activated for covalent coupling of VEGF using *N*-ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) according to the supplier's (BIAcore, Inc., Piscataway, NJ) instructions. VEGF(109) or VEGF(165) was buffer-exchanged into 20 mM sodium acetate, pH 4.8 and diluted to approximately 50 μg/ml. Aliquots of VEGF were injected at a flow rate of 2 μl/minute to achieve approximately 700-1400 response units (RU) of coupled protein. A solution of 1 M ethanolamine was injected as a blocking agent.

For kinetics measurements, twofold serial dilutions of Fab were injected in PBS/Tween buffer (0.05% Tween-20 in phosphate-buffered saline) at 25 °C or 37 °C at a flow rate of 10 μl/minute. Equilibrium dissociation constants, K_d values from SPR measurements were calculated as k_{off}/k_{on} (Tables 6 and 8).

Radiolabeled VEGF binding assay

Solution binding affinity of Fabs for VEGF was measured by equilibrating Fab with a minimal concentration of (^{125}I)-labeled VEGF(109) in the presence of a titration series of unlabeled VEGF, then capturing bound VEGF with an anti-Fab antibody-coated plate.

To establish conditions for the assay, microtiter plates (Dynex) were coated overnight with 5 μg/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23 °C). In a non-adsorbant plate (Nunc #269620), 100 pM or 26 pM [^{125}I]VEGF(109) was mixed with serial dilutions of Fab-12 or Fab Y0317, respectively. Fab-12 was incubated overnight; however, the Fab Y0317 incubation was continued for 65 hours to insure that equilibrium was reached. Thereafter, the mixtures were transferred to the capture plate for incubation at room temperature for one hour. The solution was then removed and the plate washed eight times with 0.1% Tween-20 in PBS. When the plates had dried, 150 μl/well of scintillant (MicroScint-20; Packard) was added, and the plates were counted on a Topcount gamma counter (Packard) for ten minutes. Concentrations of each Fab were chosen to give ≤20% of maximal binding.

For competitive binding assays, Dynex plates were coated and blocked as above, and serial threefold dilutions of unlabeled VEGF(109) were made in PBS/Tween buffer in a Nunc plate. [^{125}I]VEGF(109) was added, followed by addition of a fixed concentration of Fab-12 or Fab Y0317. The final concentrations of Fab-12, and Fab Y0317 were 100 pM and 10 pM, respectively. After incubation (as above), bound VEGF was captured and quantified as described above. The binding data was analyzed using a computer program to perform Scatchard analysis (Munson & Rodbard, 1980) for determination of the dissociation binding constants, K_d, for Fab-12 and Fab Y0317.

ELISA assay of VEGF Ala mutants

The binding affinities of VEGF Ala mutants for full-length Fab-12-IgG (known as rhuMAb VEGF) and Y0317-IgG, a full-length IgG form of the improved antibody expressed in CHO cells (V. Chisholm, unpublished results) were measured as previously described (Muller *et al.*, 1997; Muller *et al.*, 1998a) for the murine antibody A4.6.1, except that the temperature was increased to 37 °C, and the incubation time increased to five hours, to insure that equilibrium was reached with the high-affinity antibody.

Cell-based assay of VEGF inhibition

Several versions of the anti-VEGF antibody were tested for their ability to antagonize VEGF(165) induction of the growth of HuVECs (human umbilical vein endothelial cells). The 96-well plates were seeded with 1000 HuVECs per well and fasted in assay medium (F12:DMEM 50:50 supplemented with 1.5% (v/v) dia-filtered fetal bovine serum) for 24 hours.

The concentration of VEGF used for inducing the cells was determined by first titrating to identify the amount of VEGF that can stimulate 80% of maximal DNA synthesis. Fresh assay medium containing fixed amounts of VEGF (0.2 nM final concentration), and increasing concentrations of anti-VEGF Fab or mab were then added. After 40 hours of incubation, DNA synthesis was measured by incorporation of tritiated thymidine. Cells were pulsed with 0.5 μCi per well of [^{3}H]thymidine for 24 hours and harvested for counting, using a TopCount gamma counter.

Crystallization and refinement

The complex between the Fab fragment of affinity-matured, humanized antibody Y0317 Fab and the receptor binding fragment of VEGF (VEGF(109)) was purified and crystallized as described for the analogous complex with the parental humanized Fab-12 fragment (Muller *et al.*, 1998a). The resulting crystals had symmetry consistent with space group $P2_1$ with cell parameters $a = 89.1$ Å, $b = 66.4$ Å, $c = 138.7$ Å, and $\beta = 94.7°$, and were isomorphous with the crystals obtained with the parent complex. A data set was collected from a single frozen crystal at beam line 5.0.2 at the Advanced Light Source, Berkeley, and processed using programs MOSFLM and SCALA (CCP4, 1994). The final data set ($R_{merge} = 7.3\%$) is described in Table 10. Starting with the model of Brookhaven Protein Data Bank entry 1bj1 (Muller *et al.*, 1998a), the structure was refined using the programs X-PLOR (Brünger *et al.*, 1987) and REFMAC (CCP4, 1994). The free R-value was monitored using the identical set of reflections sequestered before refinement of parent complex. The differences in the primary structure between Fab-12 and Fab-Y0317 were modeled using the program O (Jones *et al.*, 1991). After correction for anisotropy and application of a bulk solvent correction, the R-value reached its final value of 19.9% for all reflections greater than 0.2σ (see Table 10; $R_{free} = 27.4\%$).

Table 10. Crystallographic data and refinement statistics

A. *Data collection*	Overall	Last shell
Resolution range (Å)	30-2.4	2.53-2.40
No. of observations	208,257	22,278
Unique reflections	61,742	8900
Completeness (%)	97.4	96.7
Mean $I/\sigma(I)$	13.6	2.7
R_{sym}	0.073	0.38
B. *Refinement*		
Resolution range (Å)	20-2.4	
No. of reflections	61,689	
No. of atoms	8577	
rmsd bond lengths (Å)	0.013	
rmsd angles (deg.)	1.9	
rmsd improper angles (deg.)	0.92	
rmsd B-factors for all bonded atoms, Å^2	3.5	
Number of main-chain torsion angles in disallowed regions of Ramachandran plot[a]	2	

[a] See Laskowski *et al.* (1993).

Protein Data Bank accession number

The coordinates for the VEGF:Y0317 Fab complex have been deposited in the Protein Data Bank, accession number 1cz8.

Acknowledgments

We thank Lyn Deguzman and Tom Zioncheck for providing ^{125}[I]VEGF; Alan Padua and Bill Henzel for quantitative amino acid analysis; James Bourell for mass spectrometry; Vanessa Chisholm and Lynne Krummen for construction of cell lines; and Manuel Baca, Napoleone Ferrara, Yves Muller, Leonard Presta, and James Wells for many helpful discussions.

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```
        1       10        20        30        40
A4.6.1  EIQLVQSGPELKQPGETVRISCKASGYTFTNYGMNWVKQA
         *   *  ** *   *** *  *               *
F(ab)-12 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQA
                                  *  ** * *
humIII  EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQA

                50 a      60        70        80
A4.6.1  PGKGLKWMGWINTYTGEPTYAADFKRRFTFSLETSASTAYL
          * *                             *  *
F(ab)-12 PGKGLEWVGWINTYTGEPTYAADFKRRFTFSLDTSKSTAYL
                ** **** ***  *** *   * * *  * *
humIII  PGKGLEWVSVISGDGGSTTYADSVKGRFTISRDNSKNTLYL

          abc    90                          110
A4.6.1  QISNLKNDDTATYFCAKYPHYYGSSHWYFDVWGAGTTVTVSS  (SEQ.ID NO:9)
         *** ***   * *                        *  *
F(ab)-12 QMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTLVTVSS  (SEQ.ID NO:7)
                       *                    *
humIII  QMNSLRAEDTAVYYCARG----------FDYWGQGTLVTVSS  (SEQ.ID NO:11)
```

FIG._1A

```
        1       10        20        30        40
A4.6.1  DIQMTQTTSSLSASLGDRVIISCSASQDISNYLNWYQQKP
          **      *    * *
F(ab)-12 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKP
                              *   *      *
humKI   DIQMTQSPSSLSASVGDRVTITCRASQSISNYLAWYQQKP

                50        60        70        80
A4.6.1  DGTVKVLIYFTSSLHSGVPSRFSGSGSGTDYSLTISNLEP
        ****                            **   * *
F(ab)-12 GKAPKVLIYFTSSLHSGVPSRFSGSGSGTDFTLTISSLQP
          *   **  *
humKI   GKAPKLLIYAASSLESGVPSRFSGSGSGTDFTLTISSLQP

                90       100
A4.6.1  EDIATYYCQQYSTVPWTFGGGTKLEIKR   (SEQ.ID NO:10)
          *                  *   *
F(ab)-12 EDFATYYCQQYSTVPWTFGQGTKVEIKR   (SEQ.ID NO:8)
                   ***
humKI   EDFATYYCQQYNSLPWTFGQGTKVEIKR   (SEQ.ID NO:12)
```

FIG._1B

```
                10          20          30          40
F(ab)-12    DIQMTQSPSS LSASVGDRVT ITCSASQDIS NYLNWYQQKP
Y0243-1     DIQLTQSPSS LSASVGDRVT ITCRANEQLS NYLNWYQQKP
Y0238-3     DIQLTQSPSS LSASVGDRVT ITCRANEQLS NYLNWYQQKP
Y0313-1     DIQLTQSPSS LSASVGDRVT ITCRANEQLS NYLNWYQQKP
Y0317       DIQLTQSPSS LSASVGDRVT ITCSASQDIS NYLNWYQQKP
                                   CDR-L1
                50          60          70          80
F(ab)-12    GKAPKVLIYF TSSLHSGVPS RFSGSGSGTD FTLTISSLQP
Y0243-1     GKAPKVLIYF TSSLHSGVPS RFSGSGSGTD FTLTISSLQP
Y0238-3     GKAPKVLIYF TSSLHSGVPS RFSGSGSGTD FTLTISSLQP
Y0313-1     GKAPKVLIYF TSSLHSGVPS RFSGSGSGTD FTLTISSLQP
Y0317       GKAPKVLIYF TSSLHSGVPS RFSGSGSGTD FTLTISSLQP
                       CDR-L2
                90          100         110
F(ab)-12    EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV    (SEQ.ID NO:8)
Y0243-1     EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV    (SEQ.ID NO:109)
Y0238-3     EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV    (SEQ.ID NO:111)
Y0313-1     EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV    (SEQ.ID NO:113)
Y0317       EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV    (SEQ.ID NO:115)
                       CDR-L3
```

FIG._10A

```
                10          20          30          40
F(ab)-12    EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGMNWVRQA
Y0243-1     EVQLVESGGG LVQPGGSLRL SCAASGYDFT HYGMNWVRQA
Y0238-3     EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGINWVRQA
Y0313-1     EVQLVESGGG LVQPGGSLRL SCAASGYDFT HYGMNWVRQA
Y0317       EVQLVESGGG LVQPGGSLRL SCAASGYDFT HYGMNWVRQA
                                   CDR-H1
                50          60          70          80
F(ab)-12    PGKGLEWVGW INTYTGEPTY AADFKRRFTF SLDTSKSTAY
Y0243-1     PGKGLEWVGW INTYTGEPTY AADFKRRFTF SLDTSKSTAY
Y0238-3     PGKGLEWVGW INTYTGEPTY AADFKRRFTF SLDTSKSTAY
Y0313-1     PGKGLEWVGW INTYTGEPTY AADFKRRFTF SLDTSKSTAY
Y0317       PGKGLEWVGW INTYTGEPTY AADFKRRFTF SLDTSKSTAY
                       CDR-H2                CDR-7
                90          100         110
F(ab)-12    LQMNSLRAED TAVYYCAKYP HYYGSSHWYF DVWGQGTL   (SEQ.ID NO:7)
Y0243-1     LQMNSLRAED TAVYYCAKYP HYYGSSHWYF DVWGQGTL   (SEQ.ID NO:110)
Y0238-3     LQMNSLRAED TAVYYCAKYP YYYGTSHWYF DVWGQGTL   (SEQ.ID NO:112)
Y0313-1     LQMNSLRAED TAVYYCAKYP YYYGTSHWYF DVWGQGTL   (SEQ.ID NO:114)
Y0317       LQMNSLRAED TAVYYCAKYP YYYGTSHWYF DVWGQGTL   (SEQ.ID NO:116)
                                   CDR-H3
```

FIG._10B

DEPARTMENT OF HEALTH & HUMAN SERVICES

rhuFab VEGF

Food and Drug Administration
1401 Rockville Pike
Rockville MD 20852-1448

Our Reference: BB-IND 8633

OCT 13 1999

21579

Genentech, Incorporated
Attention: Robert L. Garnick, Ph.D.
Vice President, Regulatory Affairs
1 DNA Way
South San Francisco, CA 94080-4990

Dear Dr. Garnick:

The Center for Biologics Evaluation and Research has received your **Investigational New Drug Application (IND)**. The following product name and BB-IND number have been assigned to this application. They serve only to identify it and do not imply that this Center either endorses or does not endorse your application.

BB-IND #: 8633

SPONSOR: Genentech, Incorporated

PRODUCT NAME: Humanized Monoclonal Antibody Fragment (rhuFab V2) (E. coli, Genentech) to Vascular Endothelial Growth Factor (VEGF), Intravitreal

DATE OF SUBMISSION: October 6, 1999

DATE OF RECEIPT: October 7, 1999

This BB-IND number should be used to identify all future correspondence and submissions, as well as telephone inquiries concerning this IND. Please provide an **original and two copies of every submission to this file**. Please include three originals of all illustrations which do not reproduce well.

It is understood that studies in humans will not be initiated until 30 days after the date of receipt shown above. If this office notifies you, verbally or in writing, of serious deficiencies that require correction before human studies can begin, it is understood that you will continue to withhold such studies until you are notified that the material you have submitted to correct the deficiencies is satisfactory. If such a clinical hold is placed on this file, you will be notified in writing of the reasons for placing the IND on hold.

10-18-99 P02:54 IN
10-18-99 P

You are responsible for compliance with applicable portions of the Public Health Service Act, the Federal Food, Drug, and Cosmetic Act, and the Code of Federal Regulations (CFR). A copy of 21 CFR Part 312, pertaining to INDs, is enclosed. Copies of other pertinent regulations are available from this Center upon request. The following points regarding obligations of an IND sponsor are included for your information only, and are not intended to be comprehensive.

Progress reports are required at intervals not exceeding one year and are due within 60 days of the anniversary of the date that the IND went into effect [21 CFR 312.33]. Any unexpected, fatal or immediately life-threatening reaction associated with use of this product must be reported to this Division by telephone or facsimile transmission no later than seven calendar days after initial receipt of the information, and all serious, unexpected adverse experiences must be reported, in writing, to this Division and to all study centers within fifteen calendar days after initial receipt of this information [21 CFR 312.32].

Charging for an investigational product in a clinical trial under an IND is not permitted without the prior written approval of the FDA.

Prior to use of each new lot of the investigational biologic in clinical trials, please submit the lot number, the results of all tests performed on the lot, and the specifications when established (i.e., the range of acceptable results).

If not included in your submission, please provide copies of the consent forms for each clinical study. A copy of the requirements for and elements of informed consent are enclosed. Also, please provide documentation of the institutional review board approval(s) for each clinical study.

All laboratory or animal studies intended to support the safety of this product should be conducted in compliance with the regulations for "Good Laboratory Practice for Nonclinical Laboratory Studies" (21 CFR Part 58, copies available upon request). If such studies have not been conducted in compliance with these regulations, please provide a statement describing in detail all differences between the practices used and those required in the regulations.

Item 7a of form FDA 1571 requests that either an "environmental assessment," or a "claim for categorical exclusion" from the requirements for environmental assessment, be included in the IND. If you did not include a response to this item with your application, please submit one. See the enclosed information sheet for additional information on how these requirements may be addressed.

Sponsors of INDs for products used to treat life-threatening or severely debilitating diseases are encouraged to consider the interim rule outlined in 21 CFR 312.80 through 312.88.

Telephone inquiries concerning this IND should be made directly to me at (301) 827-5101. Correspondence regarding this file should be addressed as follows:

Center for Biologics Evaluation and Research
Attn: Office of Therapeutics Research and Review
HFM-99, Room 200N
1401 Rockville Pike
Rockville, MD 20852-1448

If we have any comments after we have reviewed this submission, we will contact you.

Sincerely yours,

Kay Schneider

Kay Schneider, M.S.
Consumer Safety Officer
Division of Application Review and Policy
Office of Therapeutics
Research and Review
Center for Biologics
Evaluation and Research

Enclosures (3): 21 CFR Part 312
21 CFR 50.20, 50.25
Information sheet on 21 CFR 25.24

I

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville, MD 20852

JAN 27 2006

Genentech, Inc.
Attention: Robert L. Garnick, Ph.D.
Senior Vice President, Regulatory Affairs, Quality, and Compliance
1 DNA Way
South San Francisco, CA 94080-4990

Dear Dr. Garnick:

We have received your biologics license application (BLA) submitted under section 351 of the Public Health Service Act for the following biological product:

Our Submission Tracking Number (STN): BL #125156/0

Name of Biological Product: Lucentis™ (ranibizumab)

Indication: Treatment for patients with neovascular age-related macular degeneration

Date of Application: December 29, 2005

Date of Receipt: December 30, 2005

User Fee Goal Date: June 30, 2006

All applications for new active ingredients, new dosage forms, new indications, new routes of administration, and new dosing regimens are required to contain an assessment of the safety and effectiveness of the product in pediatric patients unless this requirement is waived or deferred. We note that you have not fulfilled the requirement. We are waiving the requirement for pediatric studies for this application.

If you have not already done so, promptly submit the *content of labeling* (21 CFR 601.14(b)) in electronic format as described at the following website:
http://www.fda.gov/oc/datacouncil/spl.html.

We will notify you within 60 days of the receipt date if the application is sufficiently complete to permit a substantive review.

We request that you submit all future correspondence, supporting data, or labeling relating to this application in triplicate, citing the above STN number. Please refer to http://www.fda.gov/cder/biologics/default.htm for important information regarding therapeutic biological products, including the addresses for submissions. Effective August 29, 2005, the new address for all submissions to this application is:

Food and Drug Administration
Center for Drug Evaluation and Research
Therapeutic Biological Products Document Room
5901-B Ammendale Road
Beltsville, MD 20705-1266

If you have any questions, please contact the Regulatory Project Manager, Lori Gorski, at (301) 796-0722.

Sincerely,

Maureen P. Dillon-Parker
Chief, Project Management Staff
Division of Anti-Infective and
Ophthalmology Products
Office of Antimicrobials
Center for Drug Evaluation and Research

J

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville, MD 20852

BLA 125156

MAR 14 2006

Genentech, Inc.
Attention: Robert L. Garnick, Ph.D.
Senior Vice President, Regulatory Affairs, Quality & Compliance
1 DNA Way
South San Francisco, California 94080-4990

Dear Dr. Garnick:

This letter is in regard to your biologics license application (BLA) submitted under section 351 of the Public Health Service Act.

We have completed an initial review of your application dated December 29, 2005, for Lucentis (ranibizumab injection) to determine its acceptability for filing. Under 21 CFR 601.2(a), your application was filed on February 28, 2006. The user fee goal date is June 30, 2006. This acknowledgment of filing does not mean that we have issued a license nor does it represent any evaluation of the adequacy of the data submitted.

At this time, we have not identified any potential review issues. Our filing review is only a preliminary review, and deficiencies may be identified during substantive review of your application. Following a review of the application, we shall advise you in writing of any action we have taken and request additional information if needed.

Please refer to http://www.fda.gov/cder/biologics/default.htm for important information regarding therapeutic biological products, including the addresses for submissions.

Please use the following address for any amendments to your application:

Food and Drug Administration
Center for Drug Evaluation and Research
Therapeutic Biological Products Document Room
5901-B Ammendale Road
Beltsville, MD 20705-1266

If you have any questions, call Lori M. Gorski, Project Manager, at (301) 796-0722.

Sincerely,

Maureen Dillon Parker
Chief, Project Management Staff
Division of Anti-Infective and Ophthalmology Products
Office of Antimicrobial Products
Center for Drug Evaluation and Research

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent of: Paul J. Carter *et al.*-- § 156	Docket No: 22338-80060
Patent No.: 6,407,213	Assignee: Genentech, Inc.
Issued: June 18, 2002	Unit: OPLA
Application No: 08/146,206	

For: METHOD FOR MAKING HUMANIZED ANTIBODIES – Application for § 156 Patent Term Extension

Mail Stop: **Patent Ext.**
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

POWER OF ATTORNEY BY ASSIGNEE

The assignee of the entire right, title, and interest in U.S. Patent No. 6,407,213 (granted on application serial no. 08/146,206), Genentech Inc., hereby appoints the practitioners associated with

CUSTOMER NUMBER 33694

as its attorneys and agents to prosecute the captioned patent application, and to transact all business in the U.S. 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 captioned patent/application by virtue of an assignment by the inventors to Genentech Inc. recorded at Reel 7035/ Frame 0272.

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

Respectfully submitted,

Genentech, Inc.

08/23/06

Jeffrey S. Kubinec
Associate General Counsel – Patent Law

Date

4-19-07

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1642 Examiner: Minh-Tam Davis CONFIRMATION NO: **CUSTOMER NO: 09157** **EXPRESS MAIL NUMBERS:** **EV 384 511 097 US** **EV 384 511 106 US** April 17, 2007 Anna Kan Anna Kan

RESPONSE TO NOTICE UNDER 37 CFR 1.251 -PATENT

Mail Stop RECONSTUCTION
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This is responsive to the Notice under 37 CFR 1.251 -Patent , mailed October 17, 2006. The copy of the papers listed in the Notice under 37 CFR 1.251 are a complete and accurate copy of the applicant's record of such papers, except for the following:

1. The PALM INTRANET record states that a Response After Non-Final Action was filed on 07/28/1997. The Response was received by the PTO on 06/27/1997. Please see the enclosed copy.
2. The PALM INTRANET record states that a Notice of Appeal was filed on 08/10/1998. The Notice of Appeal was received by the PTO on 06/26/1998. Please see the enclosed copy.
3. The PALM INTRANET record states that an Extension of Time was filed on 08/10/1998. The Extension of Time was received by the PTO on 06/26/1998. Please see the enclosed copy.
4. The PALM INTRANET record states that an Examiner Interview Summary Record was created on 11/01/2001. Applicants' papers show there was one on 12/11/2001, but not one on 11/01/2001. Please see the enclosed copy.

5. A Request for a Corrected Filing Receipt was mailed on 06/24/1994. Please see the enclosed copy. The PALM INTRANET does not list this.

6. A Request for a Corrected Filing Receipt was mailed on 04/10/1995. Please see the enclosed copy. The PALM INTRANET does not list this.

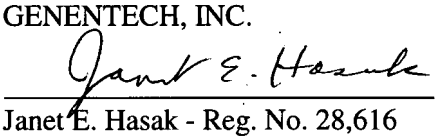
7. A Supplemental Information Disclosure Statement was filed on 10/07/1997. Please see the enclosed copy. The PALM INTRANET does not list this.

8.An Examiner Interview Summary Record was created on 07/16/1999. Please see the enclosed copy. The PALM INTRANET does not list this.

Respectfully submitted,

GENENTECH, INC.

Date: April 17, 2007

By: 

Janet E. Hasak - Reg. No. 28,616
for Wendy M. Lee - Reg. No. 40,378
Telephone: (650) 225-1994

PTO-2055-B (Rev. 10/03)
Approved for use through 07/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office; U. S. DEPARTMENT OF COMMERCE

In re Patent No.: 6,407,213 B1

Patentee: Carter et al

Patent Date: June 18 2002

Application No.: 08/146,206

Filing Date: November 17 1993

Direct to:

Mail Stop RECONSTRUCTION
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

NOTICE UNDER 37 CFR 1.251 - Patent

Statement (check the appropriate box):

☐ The copy submitted with this reply is a complete and accurate copy of applicant's record of all of the correspondence between the Office and the applicant for the above-identified application (except for U.S. patent documents), and applicant is not aware of any correspondence between the Office and applicant for the above-identified application that is not among applicant's records.

☒ The copy of the paper(s) listed in the notice under 37 CFR 1.251 is/are a complete and accurate copy of applicant's record of such paper(s). Except for the items listed in the Response to Notice under 37 CFR 1.251–Patent

☐ The papers produced by applicant are applicant's complete record of all of the correspondence between the Office and the applicant for the above-identified application (except for U.S. patent documents), and applicant is not aware of any correspondence between the Office and the applicant for the above-identified application that is not among applicant's records.

☐ Applicant does not possess any record of the correspondence between the Office and the applicant for the above-identified application.

April 17, 2007
Date

WMLee
Signature

Wendy Lee
Typed or printed name

A copy of this notice should be returned with the reply.

Burden Hour Statement: This collection of information is required by 37 CFR 1.251. The information is used by the public to reply to a request for copies of correspondence between the applicant and the USPTO in order to reconstruct an application file. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This form is estimated to take 60 minutes to complete. This time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.**

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1642 Examiner: Minh-Tam Davis Confirmation No: 3992 **Customer No: 09157** **EXPRESS MAIL NUMBERS:** **EV 384 511 097 US** **EV 384 511 106 US** April 17, 2007 Anna Kan Anna Kan

TRANSMITTAL LETTER

Mail Stop RECONSTRUCTION
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This is responsive to the Notice under 37 CFR 1.251 -Patent, mailed October 17, 2006. Transmitted herewith are the following documents:

1. Response to Notice Under 37 CFR 1.251 - Patent
2. Copy of the Notice Under 37 CFR 1.251 - Patent
3. Copy of the PALM INTRANET Listing
4. Copies of Correspondence between PTO and Applicant
5. Copies of References Cited in Information Disclosure Statements

In the event any additional fees are due in connection with the filing of these documents, the Commissioner is authorized to charge such fees to our Deposit Account No. 07-0630.

Respectfully submitted,
GENENTECH, INC.

Date: April 17, 2007

By: Janet E. Hasak

Janet E. Hasak - Reg. No. 28,616
for Wendy M. Lee - Reg. No. 40,378
Telephone: (650) 225-1994

4-19-07

COPY

In re Application of: Paul J. Carter et al.
Serial No.: 08/146,206
Filed On: November 17, 1993
Mailed On: April 17, 2007

Docket No.: P0709P1
By: Janet E. Hasak - Reg. 28,616
for Wendy M. Lee- Reg. 40,378

The following has been received in the U.S. Patent Office on the date stamped:

1. Response to Notice Under 37 CFR 1.251 - Patent
2. Copy of the Notice Under 37 CFR 1.251 - Patent and copy of the PALM INTRANET Listing
3. Copies of Correspondence between PTO and Applicant
4. Copies of References Cited in Information Disclosure Statements

Express Mail No. EV 384 511 097 US
EV 384 511 106 US

EV384511106US 4-19-07

EV384511097US

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1642 Examiner: Minh-Tam Davis Confirmation No: 3992 **Customer No: 09157** **EXPRESS MAIL NUMBERS:** **EV 384 511 097 US** **EV 384 511 106 US** April 17, 2007 Anna Kan Anna Kan

TRANSMITTAL LETTER

Mail Stop RECONSTRUCTION
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This is responsive to the Notice under 37 CFR 1.251 -Patent, mailed October 17, 2006. Transmitted herewith are the following documents:

1. Response to Notice Under 37 CFR 1.251 - Patent
2. Copy of the Notice Under 37 CFR 1.251 - Patent
3. Copy of the PALM INTRANET Listing
4. Copies of Correspondence between PTO and Applicant
5. Copies of References Cited in Information Disclosure Statements

In the event any additional fees are due in connection with the filing of these documents, the Commissioner is authorized to charge such fees to our Deposit Account No. 07-0630.

Respectfully submitted,
GENENTECH, INC.

Date: April 17, 2007

By: Janet E Hasak

Janet E. Hasak - Reg. No. 28,616
for Wendy M. Lee - Reg. No. 40,378
Telephone: (650) 225-1994

Day : Tuesday
Date: 10/17/2006

PALM INTRANET

Time: 12:35:09

Patent Number Information

Application Number: **08/146206**
Assignments
Filing or 371(c) Date: **11/17/1993**
Effective Date: **11/17/1993**
Application Received: **11/17/1993**
Patent Number: **6407213**
Issue Date: **06/18/2002**
Date of Abandonment: **00/00/0000**
Attorney Docket Number: **709P1**
Status: **150 / PATENTED CASE**
Confirmation Number: **3992**

Examiner Number: **73622 / DAVIS, MINH TAM**
Group Art Unit: **1642**
Class/Subclass: **530/387.300**
Lost Case: **YES**
Interference Number:
Unmatched Petition: **NO**
L&R Code: Secrecy Code:**1**
Third Level Review: **NO** Secrecy Order: **NO**
Status Date: **05/31/2002**
Oral Hearing: **NO**

Title of Invention: **METHOD FOR MAKING HUMANIZED ANTIBODIES**

Bar Code	PALM Location	Location Date	Charge to Loc	Charge to Name	Employee Name	Location
08146206	16M1	02/23/2006	16X1	DAVIS, MINH TAM	1600,OUTGOING MAIL	REM/00/A 89

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Day : Tuesday
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PALM INTRANET

Content Information for 08/146206

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Appln Info | **Contents** | Petition Info | Atty/Agent Info | Continuity/Reexam | Foreign Data

Date	Status	Code	Description
10/17/2006		M2512	MAIL RECONSTRUCTION NOTICE - PATENTED APPLIC
10/17/2006		2512	RECONSTRUCTION NOTICE UNDER 37 CFR 1.251 - PATI
08/29/2006		LFLOST	FILE MARKED LOST
10/30/2002		N423	POST ISSUE COMMUNICATION - CERTIFICATE OF COR
06/20/2002		CRFA	SEQUENCE MOVED TO PUBLIC DATABASE
06/18/2002		PGM/	RECORDATION OF PATENT GRANT MAILED
05/31/2002	150	WPIR	ISSUE NOTIFICATION MAILED
06/18/2002		PTAC	PATENT ISSUE DATE USED IN PTA CALCULATION
05/09/2002		R1021	RECEIPT INTO PUBS
05/04/2002		PILS	APPLICATION IS CONSIDERED READY FOR ISSUE
03/18/2002	95	N084	ISSUE FEE PAYMENT VERIFIED
03/18/2002		DRWF	WORKFLOW - DRAWINGS FINISHED
03/18/2002		DRWM	WORKFLOW - DRAWINGS MATCHED WITH FILE AT CO
05/02/2002		R1021	RECEIPT INTO PUBS
03/15/2002		CSRF	WORKFLOW - CUSTOMER SERVICE REQUEST - FINISH
03/26/2002		R1021	RECEIPT INTO PUBS
03/18/2002		DRWI	WORKFLOW - DRAWINGS RECEIVED AT CONTRACTOI
03/18/2002		DRWR	WORKFLOW - DRAWINGS SENT TO CONTRACTOR
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12/18/2001	92	MN/=.	MAIL NOTICE OF ALLOWANCE

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10/23/2000	40	CTNF	NON-FINAL REJECTION
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11/22/1999	40	CTMS	MISCELLANEOUS ACTION WITH SSP
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07/19/1999		FWDX	DATE FORWARDED TO EXAMINER
07/16/1999		SA..	SUPPLEMENTAL RESPONSE
05/07/1999		FWDX	DATE FORWARDED TO EXAMINER
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03/29/1999	41	MCTRS	MAIL RESTRICTION REQUIREMENT
03/26/1999	40	CTRS	REQUIREMENT FOR RESTRICTION / ELECTION

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11/06/1998		SA..	SUPPLEMENTAL RESPONSE
10/16/1998		EXIN	EXAMINER INTERVIEW SUMMARY RECORD (PTOL - 4
08/26/1998		M844	INFORMATION DISCLOSURE STATEMENT (IDS) FILED
08/26/1998		AF/D	AFFIDAVIT(S) (RULE 131 OR 132) OR EXHIBIT(S) RECEI
09/03/1998		FWDX	DATE FORWARDED TO EXAMINER
08/26/1998	71	R129	REQUEST UNDER RULE 129 TO REOPEN PROSECUTION
08/26/1998		MABN3	MAIL EXPRESS ABANDONMENT (DURING EXAMINATI
08/26/1998	168	ABN3	EXPRESS ABANDONMENT (DURING EXAMINATION)
08/10/1998	120	N/AP	NOTICE OF APPEAL FILED
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09/01/1997		M844	INFORMATION DISCLOSURE STATEMENT (IDS) FILED
10/10/1997		CRFL	CRF DISK HAS BEEN RECEIVED BY PREEXAM / GROUI
10/09/1997		CRFE	CRF IS GOOD TECHNICALLY / ENTERED INTO DATAB/
08/01/1997		FWDX	DATE FORWARDED TO EXAMINER
07/28/1997	71	A...	RESPONSE AFTER NON-FINAL ACTION
06/27/1997		XT/G	REQUEST FOR EXTENSION OF TIME - GRANTED
07/23/1997		EXIN	EXAMINER INTERVIEW SUMMARY RECORD (PTOL - 4
12/23/1996	41	MCTNF	MAIL NON-FINAL REJECTION
12/23/1996	40	CTNF	NON-FINAL REJECTION
12/03/1996		M844	INFORMATION DISCLOSURE STATEMENT (IDS) FILED
12/10/1996		FWDX	DATE FORWARDED TO EXAMINER

12/03/1996	71	R129	REQUEST UNDER RULE 129 TO REOPEN PROSECUTION
12/03/1996		MABN3	MAIL EXPRESS ABANDONMENT (DURING EXAMINATI
12/03/1996	168	ABN3	EXPRESS ABANDONMENT (DURING EXAMINATION)
08/30/1996		XT/G	REQUEST FOR EXTENSION OF TIME - GRANTED
08/30/1996		M844	INFORMATION DISCLOSURE STATEMENT (IDS) FILED
04/01/1996	120	N/AP	NOTICE OF APPEAL FILED
04/01/1996		XT/G	REQUEST FOR EXTENSION OF TIME - GRANTED
04/08/1996		EXIN	EXAMINER INTERVIEW SUMMARY RECORD (PTOL - 4
12/26/1995		M844	INFORMATION DISCLOSURE STATEMENT (IDS) FILED
10/27/1995	61	MCTFR	MAIL FINAL REJECTION (PTOL - 326)
10/26/1995	60	CTFR	FINAL REJECTION
08/03/1995		M844	INFORMATION DISCLOSURE STATEMENT (IDS) FILED
08/18/1995		FWDX	DATE FORWARDED TO EXAMINER
06/12/1995	71	A...	RESPONSE AFTER NON-FINAL ACTION
06/12/1995		XT/G	REQUEST FOR EXTENSION OF TIME - GRANTED
04/17/1995		M844	INFORMATION DISCLOSURE STATEMENT (IDS) FILED
12/09/1994	41	MCTNF	MAIL NON-FINAL REJECTION
12/06/1994	40	CTNF	NON-FINAL REJECTION
10/04/1994		FWDX	DATE FORWARDED TO EXAMINER
09/26/1994	71	ELC.	RESPONSE TO ELECTION / RESTRICTION FILED
08/26/1994	41	MCTRS	MAIL RESTRICTION REQUIREMENT
08/25/1994	40	CTRS	REQUIREMENT FOR RESTRICTION / ELECTION
06/15/1994		CRFE	CRF IS GOOD TECHNICALLY / ENTERED INTO DATABA
06/14/1994		CRFL	CRF DISK HAS BEEN RECEIVED BY PREEXAM / GROUP
06/06/1994		A.PE	PRELIMINARY AMENDMENT
11/17/1993		A.PE	PRELIMINARY AMENDMENT
05/24/1994	30	DOCK	CASE DOCKETED TO EXAMINER IN GAU
05/14/1994		FILM	APPLICATION CAPTURED ON MICROFILM
05/03/1994		COMP	APPLICATION IS NOW COMPLETE
05/09/1994		INCD	NOTICE MAILED--APPLICATION INCOMPLETE--FILING
04/15/1994		CRFD	CRF IS FLAWED TECHNICALLY / NOT ENTERED INTO I
04/07/1994		RTAD	RELEASED TO OIPE
04/04/1994		M903	NOTICE OF DO/EO ACCEPTANCE MAILED
03/31/1994		CRFL	CRF DISK HAS BEEN RECEIVED BY PREEXAM / GROUP
03/14/1994		DKTD	371 APPLICATION PREEXAMINATION DOCKETING
02/19/1994		IBPM	IB PAPER MATCH
12/02/1993		DKTD	371 APPLICATION PREEXAMINATION DOCKETING

12/02/1993		DYWD	APPLICANT DELAY WAIVED
12/02/1993		R331	DEMAND RECEIVED
11/17/1993		R371	RECEIPT OF 371 REQUEST

Appln Info | Contents | Petition Info | Atty/Agent Info | Continuity/Reexam | Foreign Data

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UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
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P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

JUN 19 2007

Office of Regulatory Policy
HFD-7
5600 Fishers Lane (Rockwall II Rm 1101)
Rockville, MD 20857

Attention: Beverly Friedman

The attached application for patent term extension of U.S. Patent No. 6,407,213, was filed on August 25, 2006, under 35 U.S.C. § 156. It is noted that patent term extension applications for the same regulatory review period for the human biological product, LUCENTIS® (ranibizumab), have been filed in U.S. Patent No. 7,060,269 (as indicated in a letter to FDA mailed on April 3, 2007) and U.S. Patent No. 6,884,879.

The assistance of your Office is requested in confirming that the product identified in the application, LUCENTIS® (ranibizumab), has been subject to a regulatory review period within the meaning of 35 U.S.C. § 156(g) before its first commercial marketing or use and that the application for patent term extension was filed within the sixty-day period after the product was approved. Since a determination has not been made whether the patent in question claims a product which has been subject to the Federal Food, Drug and Cosmetic Act, or a method of manufacturing or use of such a product, this communication is NOT to be considered as notice which may be made in the future pursuant to 35 U.S.C. § 156(d)(2)(A).

Our review of the application to date indicates that the subject patent would be eligible for extension of the patent term under 35 U.S.C. § 156.

Applicant is advised that despite the statement in compliance with 37 C.F.R. § 1.740(a)(14) regarding payment of the applicable fee by check for submission of a patent term extension application, no check was present and no record exists of the Office cashing the check. Therefore, in accordance with the express authorization provided in the same paragraph, the fee of $1,120 as perscribed in 37 C.F.R. § 1.20(j) is being charged to deposit account no. 18-1260.

Inquiries regarding this communication should be directed to the undersigned at (571) 272-7755 (telephone) or (571) 273-7755 (facsimile).

Mary C. Till
Legal Advisor
Office of Patent Legal Administration
Office of the Deputy Commissioner
for Patent Examination Policy

cc: Jeffrey P. Kushan
Sidley Austin LLP
1501 K Street, N.W.
Washington, DC 20005

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

NOV 2 1 2007

Re: Lucentis
Patent Nos. 6,407,213
6,884,879
Docket Nos. 2007E-0424
2007E-0425

The Honorable Jon Dudas
Under Secretary of Commerce for Intellectual Property
Director of the United States Patent and Trademark Office
Mail Stop Hatch-Waxman PTE
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Director Dudas:

This is in regard to the application for patent term extension for U.S. Patent Nos. 6,407,213 and 6,884,879 filed by Genentech, Inc. under 35 U.S.C. § 156. The human biological product claimed by these patents is Lucentis (ranibizumab), which was assigned biologic license application (BLA) No. 125156/0.

A review of the Food and Drug Administration's official records indicates that this product was subject to a regulatory review period before its commercial marketing or use, as required under 35 U.S.C. § 156(a)(4). Our records also indicate that it represents the first permitted commercial marketing or use of the product, as defined under 35 U.S.C. § 156(f)(1), and interpreted by the courts in *Glaxo Operations UK Ltd. v. Quigg*, 706 F. Supp. 1224 (E.D. Va. 1989), *aff'd*, 894 F. 2d 392 (Fed. Cir. 1990).

The BLA was approved on June 30, 2006, which makes the submission of the patent term extension applications on August 25, 2006, timely within the meaning of 35 U.S.C. § 156(d)(1).

Should you conclude that the subject patents are eligible for patent term extension, please advise us accordingly. As required by 35 U.S.C. § 156(d)(2)(A) we will then determine the applicable regulatory review period, publish the determination in the *Federal Register*, and notify you of our determination.

Please let me know if we can be of further assistance.

Sincerely yours,

Jane A. Axelrad

Jane A. Axelrad
Associate Director for Policy
Center for Drug Evaluation and Research

cc: Jeffrey P. Kushan
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, DC 20005

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

JAN - 8 2008

Office of Regulatory Policy
HFD - 7
5600 Fishers Lane (Rockwall II Rm. 1101)
Rockville, MD 20857

Attention: Beverly Friedman

Dear Ms. Axelrad:

Transmitted herewith is a copy of the application for patent term extension of U.S. Patent No. 6,407,213. The application was filed on August 25, 2006, under 35 U.S.C. § 156. It is noted that patent term extension applications for the same regulatory review period for the human biological product, LUCENTIS® (ranibizumab), have been filed in U.S. Patent Nos. 6,884,879 and 7,060,269.

The patent claims a product that was subject to regulatory review under the Federal Food, Drug and Cosmetic Act. Subject to final review, the subject patent is considered to be eligible for patent term extension. Thus, a determination by your office of the applicable regulatory review period is necessary. Accordingly, notice and a copy of the application are provided pursuant to 35 U.S.C. § 156(d)(2)(A).

Inquiries regarding this communication should be directed to the undersigned at (571)272-7755 (telephone) or (571) 273-7755 (facsimile).

Mary C. Till
Legal Advisor
Office of Patent Legal Administration
Office of the Deputy Commissioner
for Patent Examination Policy

cc: Jeffrey P. Kushan
Sidley Austin, LLP
1501 K Street, N.W.
Washington, DC 20005

RE: LUCENTIS® (ranibizumab)
FDA Docket No. 2007E-0424

DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration
Rockville MD 20857

APR 28 2008

Re: LUCENTIS - 6,407,213
Docket No.: 2007E-0424
LUCENTIS - 6,884,879
Docket No.: 2007E-0425
LUCENTIS - 7,060,269
Docket No.: 2007E-0146

The Honorable Jon Dudas
Undersecretary of Commerce for Intellectual Property
Director of the United States Patent and Trademark Office
Mail Stop Hatch-Waxman PTE
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Director Dudas:

This is in regard to the applications for patent term extension for U.S. Patent Nos. 6,407,213; 6,884,879; and 7,060,269, filed by Genentech, Inc., under 35 U.S.C. section 156 et seq. We have reviewed the dates contained in the application and have determined the regulatory review period for LUCENTIS (ranibizumab), the human biological product claimed by the patents.

The total length of the regulatory review period for LUCENTIS is 2,430 days. Of this time, 2,247 days occurred during the testing phase and 183 days occurred during the approval phase. These periods of time were derived from the following dates:

1. The date an exemption under subsection 505(i) of the Federal Food, Drug, and Cosmetic Act involving this biologic product became effective: November 6, 1999.

   The applicant claims October 7, 1999, as the date the investigational new drug application (IND) became effective. However, FDA records indicate that the IND effective date was November 6, 1999, which was thirty days after FDA receipt of the IND.

2. The date the application was initially submitted with respect to the human biological product under section 351 of the Public Health Service Act: December 30, 2005.

   The applicant claims December 29, 2005, as the date the biologics license application (BLA) for LUCENTIS (BLA 125156/0) was initially submitted. However, FDA records indicate that BLA 125156/0 was submitted on December 30, 2005.

3. The date the application was approved: June 30, 2006.

   FDA has verified the applicant's claim that BLA 125156/0 was approved on June 30, 2006.

This determination of the regulatory review period by FDA does not take into account the effective date of the patents, nor does it exclude one-half of the testing phase as required by 35 U.S.C. section 156(c)(2).

Please let me know if we can be of further assistance.

Sincerely yours,

Jane A. Axelrad

Jane A. Axelrad
Associate Director for Policy
Center for Drug Evaluation and Research

cc: Jeffrey P. Kushan
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, DC 20005

a person with Medicare could be identified because the sample is small enough to identify participants. CMS would make exceptions if the information is needed for one of the routine uses or if it's required by law.

POLICIES AND PRACTICES FOR STORING, RETRIEVING, ACCESSING, RETAINING, AND DISPOSING OF RECORDS IN THE SYSTEM:

STORAGE:

Records are stored on both tape cartridges (magnetic storage media) and in a DB2 relational database management environment (DASD data storage media).

RETRIEVABILITY:

Information is most frequently retrieved by HICN, provider number (facility, physician, IDs), service dates, and beneficiary state code.

SAFEGUARDS AND PROTECTIONS:

CMS has protections in place for authorized users to make sure they are properly using the data and there is no unauthorized use. Personnel having access to the system have been trained in the Privacy Act and information security requirements. Employees who maintain records in this system cannot use or disclose data until the recipient agrees to implement appropriate management, operational and technical safeguards that will protect the confidentiality, integrity, and availability of the information and information systems.

This system would follow all applicable Federal laws and regulations, and Federal, HHS, and CMS security and data privacy policies and standards. These laws and regulations include but are not limited to: the Privacy Act of 1974; the Federal Information Security Management Act of 2002 (when applicable); the Computer Fraud and Abuse Act of 1986; the Health Insurance Portability and Accountability Act of 1996; the E-Government Act of 2002, the Clinger-Cohen Act of 1996; the Medicare Modernization Act of 2003, and the corresponding implementing regulations. OMB Circular A–130, Management of Federal Resources, Appendix III, Security of Federal Automated Information Resources also applies. Federal, HHS, and CMS policies and standards include but are not limited to all pertinent National Institute of Standards and Technology publications, the HHS Information Systems Program Handbook, and the CMS Information Security Handbook.

RETENTION AND DISPOSAL:

Records are maintained with identifiers for all transactions after they are entered into the system for a period of 20 years. Records are housed in both active and archival files. All claims-related records are encompassed by the document preservation order and will be retained until notification is received from the Department of Justice.

SYSTEM MANAGER AND ADDRESS:

Director, Centers for Beneficiary Choices, CMS, Mail stop C5–19–07, 7500 Security Boulevard, Baltimore, Maryland 21244–1850.

NOTIFICATION PROCEDURE:

For purpose of notification, the subject individual should write to the system manager who will require the system name, and the retrieval selection criteria (e.g., HICN, facility/pharmacy number, service dates, etc.).

RECORD ACCESS PROCEDURE:

For purpose of access, use the same procedures outlined in Notification Procedures above. Requestors should also reasonably specify the record contents being sought. (These procedures are in accordance with Department regulation 45 CFR 5b.5 (a)(2).)

CONTESTING RECORD PROCEDURES:

The subject individual should contact the system manager named above, and reasonably identify the record and specify the information to be contested. State the corrective action sought and the reasons for the correction with supporting justification. (These procedures are in accordance with Department regulation 45 CFR 5b.7.)

RECORD SOURCE CATEGORIES:

Summary prescription drug claim information contained in this system is obtained from the Part D Sponsor daily and monthly drug event transaction reports, Medicare Beneficiary Database (09–70–0530), and other payer information to be provided by the TROOP Facilitator.

SYSTEMS EXEMPTED FROM CERTAIN PROVISIONS OF THE ACT:

None.

[FR Doc. E8–11949 Filed 5–28–08; 8:45 am]
BILLING CODE 4120–03–P

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

[Docket Nos. FDA–2007–E–0461 (formerly Docket No. 2007E–0424), FDA–2007–E–0165 (formerly Docket No. 2007E–0425), FDA–2007–E–0459 (formerly Docket No. 2007E–0146)]

Determination of Regulatory Review Period for Purposes of Patent Extension; LUCENTIS

AGENCY: Food and Drug Administration, HHS.

ACTION: Notice.

SUMMARY: The Food and Drug Administration (FDA) has determined the regulatory review period for LUCENTIS and is publishing this notice of that determination as required by law. FDA has made the determination because of the submission of applications to the Director of Patents and Trademarks, Department of Commerce, for the extension of patents which claim that human biological product.

ADDRESSES: Submit written or electronic comments and petitions to the Division of Dockets Management (HFA–305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. Submit electronic comments to *http://www.regulations.gov.*

FOR FURTHER INFORMATION CONTACT: Beverly Friedman, Center for Drug Evaluation and Research, Food and Drug Administration, 10903 New Hampshire Ave., Bldg. 51, rm. 6222, Silver Spring, MD, 20993–0002, 301–796–3602.

SUPPLEMENTARY INFORMATION: The Drug Price Competition and Patent Term Restoration Act of 1984 (Public Law 98–417) and the Generic Animal Drug and Patent Term Restoration Act (Public Law 100–670) generally provide that a patent may be extended for a period of up to 5 years so long as the patented item (human drug product, animal drug product, medical device, food additive, or color additive) was subject to regulatory review by FDA before the item was marketed. Under these acts, a product's regulatory review period forms the basis for determining the amount of extension an applicant may receive.

A regulatory review period consists of two periods of time: A testing phase and an approval phase. For human biological products, the testing phase begins when the exemption to permit the clinical investigations of the biological product becomes effective

and runs until the approval phase begins. The approval phase starts with the initial submission of an application to market the human biological product and continues until FDA grants permission to market the biological product. Although only a portion of a regulatory review period may count toward the actual amount of extension that the Director of Patents and Trademarks may award (for example, half the testing phase must be subtracted as well as any time that may have occurred before the patent was issued), FDA's determination of the length of a regulatory review period for a human biological product will include all of the testing phase and approval phase as specified in 35 U.S.C. 156(g)(1)(B).

FDA recently approved for marketing the human biologic product LUCENTIS (ranibizumab). LUCENTIS is indicated for the treatment of patients with neovascular (wet) age-related macular degeneration. Subsequent to this approval, the Patent and Trademark Office received patent term restoration applications for LUCENTIS (U.S. Patent Nos. 6,407,213; 6,884,879; and 7,060,269) from Genentech, Inc., and the Patent and Trademark Office requested FDA's assistance in determining this patent's eligibility for patent term restoration. In letters dated July 24, 2007, and November 21, 2007, FDA advised the Patent and Trademark Office that this human biological product had undergone a regulatory review period and that the approval of LUCENTIS represented the first permitted commercial marketing or use of the product. Shortly thereafter, the Patent and Trademark Office requested that FDA determine the product's regulatory review period.

FDA has determined that the applicable regulatory review period for LUCENTIS is 2,430 days. Of this time, 2,247 days occurred during the testing phase of the regulatory review period, while 183 days occurred during the approval phase. These periods of time were derived from the following dates:

1. *The date an exemption under section 505(i) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 355(i)) became effective*: November 6, 1999. The applicant claims October 7, 1999, as the date the investigational new drug application (IND) became effective. However, FDA records indicate that the IND effective date was November 6, 1999, which was 30 days after FDA receipt of the IND.

2. *The date the application was initially submitted with respect to the human biological product under section 351 of the Public Health Service Act (42 U.S.C. 262)*: December 30, 2005. The applicant claims December 29, 2005, as the date the biologics license application (BLA) for LUCENTIS (BLA 125156/0) was initially submitted. However, FDA records indicate that BLA 125156/0 was submitted on December 30, 2005.

3. *The date the application was approved*: June 30, 2006. FDA has verified the applicant's claim that BLA 125156/0 was approved on June 30, 2006.

This determination of the regulatory review period establishes the maximum potential length of a patent extension. However, the U.S. Patent and Trademark Office applies several statutory limitations in its calculations of the actual period for patent extension. In its applications for patent extension for U.S. Patent Nos. 6,407,213; 6,884,879; and 7,060,269, this applicant seeks 378 days; 307 days or 17 days, respectively, of patent term extension.

Anyone with knowledge that any of the dates as published are incorrect may submit to the Division of Dockets Management (see **ADDRESSES**) written or electronic comments and ask for a redetermination by July 28, 2008. Furthermore, any interested person may petition FDA for a determination regarding whether the applicant for extension acted with due diligence during the regulatory review period by November 25, 2008. To meet its burden, the petition must contain sufficient facts to merit an FDA investigation. (See H. Rept. 857, part 1, 98th Cong., 2d sess., pp. 41–42, 1984.) Petitions should be in the format specified in 21 CFR 10.30.

Comments and petitions should be submitted to the Division of Dockets Management. Three copies of any mailed information are to be submitted, except that individuals may submit one copy. Comments are to be identified with the docket number found in brackets in the heading of this document. Comments and petitions may be seen in the Division of Dockets Management between 9 a.m. and 4 p.m., Monday through Friday.

Please note that on January 15, 2008, the FDA Division of Dockets Management Web site transitioned to the Federal Dockets Management System (FDMS). FDMS is a Government-wide, electronic docket management system. Electronic comments or submissions will be accepted by FDA only through FDMS at *http://www.regulations.gov.*

Dated: May 8, 2008.

Jane A. Axelrad,

Associate Director for Policy, Center for Drug Evaluation and Research.

[FR Doc. E8–12007 Filed 5–28–08; 8:45 am]

BILLING CODE 4160–01–S

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

[Docket No. FDA–2007–M–0467] (formerly Docket No. 2007M–0408), [Docket No. FDA–2007–M–0481] (formerly Docket No. 2007M–0467), [Docket No. FDA–2007–M–0480] (formerly Docket No. 2007M–0409), [Docket No. FDA–2007–M–0472] (formerly Docket No. 2007M–0413), [Docket No. FDA–2007–M–0468] (formerly Docket No. 2007M–0446), [Docket No. FDA–2007–M–0494] (formerly Docket No. 2007M–0380), [Docket No. FDA–2007–M–0493] (formerly Docket No. 2007M–0411), [Docket No. FDA–2007–M–0492] (formerly Docket No. 2007M–0410), [Docket No. FDA–2007–M–0490] (formerly Docket No. 2007M–0415), [Docket No. FDA–2007–M–0491] (formerly Docket No. 2007M–0447]

Medical Devices; Availability of Safety and Effectiveness Summaries for Premarket Approval Applications

AGENCY: Food and Drug Administration, HHS.

ACTION: Notice.

SUMMARY: The Food and Drug Administration (FDA) is publishing a list of premarket approval applications (PMAs) that have been approved. This list is intended to inform the public of the availability of safety and effectiveness summaries of approved PMAs through the Internet and the agency's Division of Dockets Management.

ADDRESSES: Submit written requests for copies of summaries of safety and effectiveness data to the Division of Dockets Management (HFA–305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. Please cite the appropriate docket number as listed in Table 1 of this document when submitting a written request. See the **SUPPLEMENTARY INFORMATION** section for electronic access to the summaries of safety and effectiveness.

FOR FURTHER INFORMATION CONTACT: Samie Allen, Center for Devices and Radiological Health (HFZ–402), Food and Drug Administration, 9200 Corporate Blvd., Rockville, MD 20850, 240–276–4013.

SUPPLEMENTARY INFORMATION:

DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration
Rockville MD 20857

JAN 8 2009

Re: Lucentis
Docket Nos.: FDA-2007-E-0461
FDA-2007-E-0165
FDA-2007-E-0459

The Honorable Jon Dudas
Under Secretary of Commerce for Intellectual Property
Director of the United States Patent and Trademark Office
Mail Stop Hatch-Waxman PTE
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Director Dudas:

This is in regard to the patent term extension applications for U.S. Patent Nos. 6,407,213; 6,884,879; and 7,060,269 filed by Genentech, Inc., under 35 U.S.C. § 156. The patent claims Lucentis (ranibizumab), biologic license application (BLA) 125156/0.

In the May 29, 2008, issue of the Federal Register (73 Fed. Reg. 30949), the Food and Drug Administration published its determination of this product's regulatory review period, as required under 35 U.S.C. § 156(d)(2)(A). The notice provided that on or before November 25, 2008, 180 days after the publication of the determination, any interested person could file a petition with FDA under 35 U.S.C. § 156(d)(2)(B)(i) for a determination of whether the patent term extension applicant acted with due diligence during the regulatory review period.

The 180-day period for filing a due diligence petition pursuant to this notice has expired and FDA has received no such petition. Therefore, FDA considers the regulatory review period determination to be final.

Please let me know if we can provide further assistance.

Sincerely yours,

Jane A. Axelrad

Jane A. Axelrad
Associate Director for Policy
Center for Drug Evaluation and Research

cc: Jeffrey P. Kushan
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, DC 20005

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

MAR 26 2009

Jeffrey P. Kushan
Sidley Austin, LLP
1501 K Street, N.W.
Washington, DC 20005

In Re: Patent Term Extension
Application for
U.S. Patent No. 6,407,213

NOTICE OF FINAL DETERMINATION AND REQUIREMENT FOR ELECTION

A determination has been made that U.S. Patent No. 6,407,213, claims of which cover the human biologic drug product LUCENTIS® (ranibizumab), is eligible for patent term extension under 35 U.S.C. § 156. The period of extension has been determined to be 378 days.

A single request for reconsideration of this final determination as to the length of extension of the term of the patent may be made if filed within one month of the date of this notice. Extensions of time under 37 CFR § 1.136(a) are not applicable to this time period.

Applicant also has applied for patent term extension of U.S. Patent No. 6,884,879 and U.S. Patent No. 7,060,269 based on the regulatory review period for the human biologic drug product LUCENTIS® (ranibizumab).

When patent term extension applications are filed for extension of the terms of different patents based upon the same regulatory review period for a product, the certificate of extension is issued to the patent having the earliest date of issuance, unless applicant elects a different patent. In the absence of an election by applicant within ONE MONTH of the date of this notice, and in accordance with 37 CFR 1.785(b), the applications for patent term extension of U.S. Patent No. 6,884,879 and U.S. Patent No. 7,060,269 will be denied. Accordingly, the application for patent term extension of the patent having the earlier date of issuance will be granted, i.e., a certificate of extension will be issued to U.S. Patent No 6,407,213 for a period of 378 days.

In the absence of a request for reconsideration, and if U.S. Patent No. 6,407,213 is elected, the Director will issue to the applicant a certificate of extension, under seal, for a period of 378 days in U.S. Patent No. 6,407,213.

The period of extension, if calculated using the Food and Drug Administration determination of the length of the regulatory review period published in the Federal Register of May 29, 2008 (73 Fed. Reg. 30949), would be 828 days. Under 35 U.S.C. § 156(c):

Period of Extension	=	½ (Testing Phase) + Approval Phase
	=	½ (2,247 days - 956 days) + 183 days
	=	828 days (2.3 years)

Since the regulatory review period began November 6, 1999, before the patent issued (June 18, 2002), only that portion of the regulatory review period occurring after the date the patent issued has been considered in the above determination of the length of the extension period 35 U.S.C. § 156(c). (From November 6, 1999, to and including, June 18, 2002, is 956 days; this period is subtracted for the number of days occurring in the testing phase according to the FDA's determination of the length of the regulatory review period.) No determination of a lack of due diligence under 35 U.S.C. § 156(c)(1) was made.

However, the 14 year exception of 35 U.S.C. § 156(c)(3) operates to limit the term of the extension in the present situation because it provides that the period remaining in the term of the patent measured from the date of approval of the approved product plus any patent term extension cannot exceed fourteen years. The period of extension calculated above, 828 days, would extend the patent from June 18, 2019, to September 23, 2021, which is beyond the 14-year limit (the approval date is June 30, 2006, thus, the 14 year limit is June 30, 2020). The period of extension is thus limited to 378 days, by operation of 35 U.S.C. § 156(c)(3). Accordingly, the period of extension is the number of days to extend the term of the patent from its original expiration date, June 18, 2019, to and including, June 30, 2020, or 378 days.

The limitations of 35 U.S.C. 156(g)(6) do not operate to further reduce the period of extension determined above.

Upon issuance of the certificate of extension, the following information will be published in the Official Gazette:

U.S. Patent No.:	6,407,213
Granted:	June 18, 2002
Original Expiration Date[1]:	June 18, 2019
Applicant:	Paul J. Carter et al.
Owner of Record:	Genentech, Inc.
Title:	Method for Making Humanized Antibodies
Product Trade Name:	LUCENTIS® (ranibizumab)
Term Extended:	378 days
Expiration Date of Extension:	June 30, 2020

[1]Subject to the provisions of 35 U.S.C. § 41(b).

Any correspondence with respect to this matter should be addressed as follows:

By mail: Mail Stop Hatch-Waxman PTE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450.

By FAX: (571) 273-7755

Telephone inquiries related to this determination should be directed to the undersigned at (571) 272-7755.

Mary C. Till
Legal Advisor
Office of Patent Legal Administration
Office of the Deputy Commissioner
for Patent Examination Policy

cc: Office of Regulatory Policy
Food and Drug Administration
10903 New Hampshire Ave., Bldg. 51, Rm. 6222
Silver Spring, MD 20993-0002

Attention: Beverly Friedman

RE: LUCENTIS® (ranibizumab)
Docket No.: FDA-2007-E-0461

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

U.S. Patent No.	**6,407,213 – § 156**	Unit:	OPLA
Serial No.:	**08/ 146,206**		
Confirmation No.:	3992		
Filed:	25 August 2006		
First Inventor:	P.J. CARTER		
Patent Owner:	Genentech, Inc.		
For:	Method for making humanized antibodies Application for patent term extension under 35 U.S.C. § 156		

Mail Stop **Hatch-Waxman PTE**
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

ELECTION UNDER 37 C.F.R. § 1.785(b)

Sir:

This letter responds to the Notice of Final Determination and Requirement for Election mailed in the captioned application for patent term extension on 26 March 2009. The Notice observes that applicant filed applications to extend the terms of U.S. Patent Nos. 6,407,213, 6,884,879, and 7,060,269 based on the regulatory review period for LUCENTIS®. The Notice further states a requirement that applicant elect one of the patents to receive a term extension certificate within a period of one month of the date of the Notice. This election is filed within the stated period and is therefore timely.

Pursuant to § 1.785(b), **applicant elects U.S. Patent No. 6,407,213** to receive a certificate of extension under § 1.780 and 35 U.S.C. § 156(e)(1). Applicant requests that the Director proceed to issue a certificate of extension of U.S. Patent No. 6,407,213 based on the regulatory review period for LUCENTIS® for a period of 378 days, as indicated in the Notice of Final Determination and Requirement for Election issued in this application for patent term extension.

We believe that no fee is due in respect of this election. However, the Director is requested to debit any fee required for entry or consideration of this paper from our Deposit Account No. 18-1260.

Respectfully submitted,

/David L. Fitzgerald/

David L. Fitzgerald, Reg. No. 47,347
Attorney for Genentech, Inc.

24 April 2009

SIDLEY AUSTIN LLP
1501 K Street, NW
Washington, DC 20005

tel. (202) 736-8818
fax (202) 736-8711

Electronic Acknowledgement Receipt

EFS ID:	5212426
Application Number:	08146206
International Application Number:	
Confirmation Number:	3992
Title of Invention:	METHOD FOR MAKING HUMANIZED ANTIBODIES
First Named Inventor/Applicant Name:	PAUL J. CARTER
Customer Number:	33694
Filer:	David Laurence Fitzgerald
Filer Authorized By:	
Attorney Docket Number:	709P1
Receipt Date:	24-APR-2009
Filing Date:	17-NOV-1993
Time Stamp:	10:46:21
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Miscellaneous Incoming Letter	Lucentis_213_PTE_election.pdf	78045 b6baae5477e1a50f05bedfe33594cb57572ff209	no	2

Warnings:	
Information:	

Total Files Size (in bytes):	78045

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

NOV 18 2009

Jeffrey P. Kushan
Sidley Austin, LLP
1501 K Street, N.W.
Washington, DC 20005

In Re: Patent Term Extension
Application for
U.S. Patent No. 6,407,213

Dear Mr. Kushan :

A certificate under 35 U.S.C. § 156 is enclosed extending the term of U.S. Patent No. 6,407,213 for a period of 378 days. While a courtesy copy of this letter is being forwarded to the Food and Drug Administration (FDA), you should directly correspond with the FDA regarding any required changes to the patent expiration dates.

Inquiries regarding this communication should be directed to the undersigned by telephone at (571) 272-7755, or by e-mail at mary.till@uspto.gov.

Mary C. Till
Legal Advisor
Office of Patent Legal Administration
Office of the Deputy Commissioner
for Patent Examination Policy

cc: Office of Regulatory Policy
Food and Drug Administration
10903 New Hampshire Ave., Bldg. 51, Rm. 6222
Silver Spring, MD 20993-0002

Attention: Beverly Friedman

RE: LUCENTIS® (ranibizumab)
Docket No.: FDA-2007-E-0461

UNITED STATES PATENT AND TRADEMARK OFFICE

(12) CERTIFICATE EXTENDING PATENT TERM
UNDER 35 U.S.C. § 156

(68)	PATENT NO.	:	6,407,213
(45)	ISSUED	:	June 18, 2002
(75)	INVENTOR	:	Paul J. Carter et al.
(73)	PATENT OWNER	:	Genentech, Inc.
(95)	PRODUCT	:	LUCENTIS® (ranibizumab)

This is to certify that an application under 35 U.S.C. § 156 has been filed in the United States Patent and Trademark Office, requesting extension of the term of U.S. Patent No. 6,407,213 based upon the regulatory review of the product LUCENTIS® (ranibizumab) by the Food and Drug Administration. Since it appears that the requirements of the law have been met, this certificate extends the term of the patent for the period of

(94) 378 days

from June 18, 2019, the original expiration date of the patent, subject to the payment of maintenance fees as provided by law, with all rights pertaining thereto as provided by 35 U.S.C. § 156(b).

I have caused the seal of the United States Patent and Trademark Office to be affixed this 18th day of November 2009.

David J. Kappos
Under Secretary of Commerce for Intellectual Property and
Director of the United States Patent and Trademark Office

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
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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

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

Paper 1

Filed 2 February 2010

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Named Inventors:	John Robert Adair High Wycombe, United Kingdom Diljeet Singh Athwal London, United Kingdom John Spencer Emtage Marlow, United Kingdom
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/Sally Gardner Lane/
Administrative Patent Judge

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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.
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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):

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