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.	Applicant(s)
08/146,206	Carter et al.
Examiner	**Group Art Unit**
Patrick Nolan	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Of the above, claim(s) _____ is/are withdrawn from consideration.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

DIS 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	**Group**
17 Nov 1993	~~1806~~ 1816

U.S. PATENT DOCUMENTS

Examiner Initials		Document Number	Date	Name	Class	Subclass	Filing Date
PN	81	5,225,539 B 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 3-30	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	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.

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
Reg. No. 28,616
(for 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

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
(for 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

D

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 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;

(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
Janet Hasak
Reg. No. 28,616
(for 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

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	
	28
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 resdues.

(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

PTOL-413 (REV. 2 -93)

ORIGINAL FOR INSERTION IN RIGHT HAND FLAP OF FILE WRAPPER

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

#30

10/7/97

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

1 DNA Way
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

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 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
```

4

(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

RECEIVED
SEP 1 1997
MATRIX CUSTOMER SERVICE CENTER

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	

FOREIGN PATENT DOCUMENTS

Examiner Initials		Document Number	Date	Country	Class	Subclass	Translation Yes	Translation No
TDS	104	323,806 A1	12.07.89	EPO				
	105	338,745 A1	25.10.89	EPO				
	106	365,209 A2	25.04.90	EPO				
	107	365,997 A2	02.05.90	EPO				
	108	432,249 B1	25.09.96	EPO				
	109	682,040 A1	15.11.95	EPO				
	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				

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

TDS	115	Amit et al., "Three-Dimensional Structure of an Antigen-Antibody Complex at 2.8 A Resolution" Science 233:747-753 (Aug 1986)
	116	Amzel et al., "The Three Dimensional Structure of a Combining Region-Ligand Complex of Immunglobulin NEW at 3.5-A Resolution" Proc. Natl. Acad. Sci. USA 71(4):1427-1430 (Apr 1974)
	117	Baselga et al., "Phase II Study of Weekly Intravenous Recombinant Humanized Anti-p185/HER2 Monoclonal Antibody in Patients With HER2/neu-Overexpressing Metastatic Breast Cancer" J. Clin. Oncol. 14(3):737-744 (1996)
	118	Beverley & Callard, "Distinctive functional charcteristics of human "T" lymphocytes defined by E rosetting or a monoclonal anti-T cell antibody" European Journal of Immunology 11:329-334 (1981)
	119	Bird et al., "Single-chain antigen-binding proteins" Science 242:423-426 (Oct 1988)
	120	Brennan et al., "Preparation of bispecific antibodies by chemical recombination of monoclonal immunoglobulin G_1 fragments" Science 229:81-83 (July 1985)
	121	Bruccoleri et al., "Structure of antibody hypervariable loops reproduced by a conformational search algorithm" Nature 335:564-568 (Oct 1988)
	122	Caron et al., "Biological and Immunological Features of Humanized M195 (Anti-CD33) Monoclonal Antibodies" Cancer Research 52:6761-6767 (Dec 1992)
	123	Chothia & Lesk, "The relation between the divergence of sequence and structure in proteins" EMBO Journal 5(4):823-826 (1986)

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

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.)

TK	124	Co & Queen, "Humanized antibodies for therapy" Nature 351:501-502 (Jun 1991)
	125	Co et al., "Chimeric and Humanized Antibodies with Specificity for the CD33 Antigen" J. of Immunology 148(4):1149-1154 (Feb 1992)
	126	Co et al., "Humanized Anti-Lewis Y Antibodies: In Vitro Properties and Pharmacokinetics in Rhesus Monkeys" Cancer Research 56:1118-1125 (Mar 1996)
	127	Colman et al., "Crystal and Molecular Structure of the Dimer of Variable Domains of the Bence-Jones Protein ROY" J. Mol. Biol. 116:73-79 (1977)
	128	Colman et al., "Three-dimensional structure of a complex of antibody with influenza virus neuraminidase" Nature 326:358-363 (Mar 1987)
	129	Cook et al., "A map of the human immunoglbulin V_H locus completed by analysis of the telometric region of chromosome 14q" Nature Genetics 7:162-168 (Jun 1994)
	130	Darsley & Rees, "Nucleotide sequences of five anti-lysozyme monoclonal antibodies" EMBO Journal 4(2):393-398 (1985)
	131	Davies & Metzger, "Structural Basis of Antibody Function" Ann. Rev. Immunol. 1:87-117 (1983)
	132	Davies et al., "Antibody-Antigen Complexes" Journal of Biological Chemistry 263(22):10541-10544 (Aug. 1988)
	133	Eigenbrot et al., "X-Ray Structures of Fragments From Binding and Nonbinding Versions of a Humanized Anti-CD18 Antibody: Structural Indications of the Key Role of V_H Residues 59 to 65" Proteins 18:49-62 (1994)
	134	Eigenbrot et al., "X-ray structures of the antigen-binding domains from three variants of humanized anti-p185HER2 antibody 4D5 and comparison with molecular modeling" J. Mol. Biol. 229:969-995 (1993)
	135	Ellison et al., "The nucleotide sequence of a human immunoglobulin $C_{\gamma 1}$ gene" Nucleic Acids Research 10(13):4071-4079 (1982)
	136	Emery & Adair, "Humanised monoclonal antibodies for therapeutic applications" Exp. Opin. Invest. Drugs 3(3):241-251 (1994)
	137	Epp et al., "Crystal and Molecular Structure of a Dimer Composed of the Variable Portions of the Bence-Jones Protein REI" European Journal of Biochemistry 45:513-524 (1974)
	138	Fanger et al., "Bispecific antibodies and targeted cellular cytotoxicity" Immunology Today 12(2):51-54 (1991)
	139	Fanger et al., "Cytotoxicity mediated by human Fc receptors for IgG" Immunology Today 10(3):92-99 (1989)
	140	Feldmann et al., "A Hypothetical Space-Filling Model of the V-Regions of the Galactan-Binding Myeloma Immunoglobulin J539" Molecular Immunology 18(8):683-698 (1981)
	141	Fendley et al., "The Extracellular Domain of HER2/neu Is a Potential Immunogen for Active Specific Immunotherapy of Breast Cancer" J. Biol. Resp. Mod. 9:449-455 (1990)
	142	Glennie et al., "Preparation and Performance of Bispecific $F(ab'\gamma)_2$ Antibody Containing Thioether-Linked Fab'γ Fragments" J. Immunol. 139(7):2367-2375 (October 1, 1987)
	143	Gonzalez et al., "Humanization of Murine 6G425:An Anti-IL8 Monoclonal Antibody Which Blocks Binding of IL8 to Human Neutrophils" 1996 Keystone Symposia on Exploring and Exploiting Antibody and Ig Superfamily Combining Sites (Poster) pps. 1-21 (February 1996)

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.

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

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

TDS	144	Gussow & Seemann, "Humanization of Monoclonal Antibodies" Meth. Enzymology, Academic Press, Inc. Vol. 203:99-121 (1991)
	145	Hieter et al., "Cloned human and mouse kappa immunoglobulin constant and J region genes conserve homology in functional segments" Cell 22(Part 1):197-207 (1980)
	146	Houghton, A., "Building a better monoclonal antibody" Immunology Today 9(9):265-267 (1988)
	147	Huston et al., "Protein engineering of antibody binding sites: Recovery of specific activity in an anti-digoxin single-chain Fv analogue produced in Escherichia coli" Proc. Natl. Acad. Sci. USA 85:5879-5883 (Aug 1988)
	148	Isaacs et al., "Humanised Monoclonal Antibody Therapy for Rheumatoid Arthritis" Lancet 340:748-752 (September 26, 1992)
	149	Johnson et al., "Biological and Molecular Modeling Studies Comparing Murine Monoclonal Antibodies with Their Engineered Chimeric and Humanized Counterparts" J. Cell. Biochem. Suppl 0 (13 Part A) (18th Ann. UCLA Symp on Mol. & Cell. Biol., Park City, UT 1/17-22/89) pps. 87 (1989)
	150	Kabat E., "Origins of Antibody Complementarity and Specificity - Hypervariable Regions and the Minigenen Hypothesis" J. of Immunology 125(3):961-969 (Sep 1980)
	151	Kabat et al. Sequences of Proteins of Immunological Interest, U.S. Dept. of Health and Human Services, NIH, 5th edition Vol. 1:103-108, 324-331 (1991)
	152	~~Kabat et al., "Sequences of Proteins of Immunological Interest, 4th Edition" pps. iii-xxvii, 41-76,~~ 160-175 (1987) Dupl.
TDS	153	Kindt & Capra The Antibody Enigma, New York:Plenum Press pps. 79-86 (1984)
	154	Lesk & Chothia, "Evolution of Proteins Formed by β-Sheets" J. Mol. Biol. 160:325-342 (1982)
	155	Lesk & Chothia, "The response of protein structures to amino-acid sequence changes" Phil. Trans. R. Soc. Lond. A 317:345-356 (1986)
✓	156	Mariuzza et al., "The Structure Basis of Antigen-Antibody Recognition" Ann. Rev. Biophys. Biophys. Chem. 16:139-159 (1987)
	157	Nadler et al., "Immunogenicity of Humanized and Human Monoclonal Antibodies" Clin. Pharmacology & Therapeutics pps. 180 (Feb 1994)
	158	Nelson, H., "Targeted Cellular Immunotherapy with Bifunctional Antibodies" Cancer Cells 3:163-172 (1991)
	159	Neuberger et al., "Antibody Engineering" Proceedings 8th Intl. Biotech. Symp., Paris II:792-799 (1988)
	160	Newmark, P., "Making Chimeric Antibodies Even More Human" Bio/Technology 6:468 (May 1988)
	161	Nishimura et al., "Human c-erbB-2 Proto-Oncogene Product as a Target for Bispecific-Antibody-Directed Adoptive Tumor Immunotherapy" Int. J. Cancer 50:800-804 (1992)
	162	Nitta et al., "Preliminary trial of specific targeting therapy against malignant glioma" Lancet 335(8686):368-371 (Feb 17, 1990)
	163	Nitta, T. et al., "Bispecific F(ab')$_2$ monomer prepared with anti-CD3 and anti-tumor monoclonal antibodies is most potent in induction of cytolysis of human T cells" European Journal of Immunology 19:1437-1441 (1989)

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

*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

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

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

164	Nolan et al., "Bifunctional antibodies: concept, production and applications" Biochimica et Biophysica Acta 1040:1-11 (1990)
165	O'Connor et al., "Calcium Dependence of an Anti-Protein C Humanized Antibody Involves Framework Residues" (manuscript)
166	Orlandi et al., "Cloning Immunoglobulin Variable Domains for Expression by the Polymerase Chain Reaction" Proc. Natl. Acad. Sci. USA 86:3833-3837 (May 1989)
167	Orlandi et al., "Cloning of cDNA Corresponding to Heavy and Light Chain Immunoglobulin Variable Domains" Protein and Pharmaceutical Engineering pps. 90 (1989)
168	Ostberg & Queen, "Human and humanized monoclonal antibodies: preclinical studies and clinical experience" Biochem. Soc. Transactions pps. 1038-1043 (1995)
169	Padlan et al., "Model-building Studies of Antigen-binding Sites:The Hapten-binding Site of MOPC-315" Cold Springs Harbor Symposia On Quantitative Biology XLI:627-637 (1977)
170	Padlan, E., "Anatomy of the Antibody Molecule" Molecular Immunology 31(3):169-217 (1994)
171	Padlan, E., "Evaluation of the Structural Variation Among Light Chain Variable Domains" Molecular Immunology 16:287-296 (1979)
172	Palm & Hilschmann, "Primary structure of a crystalline monoclonal immunoglobulin K-type L-chain, subgroup I (Bence-Jones preotin Rei); isolation & characterization of the tryptic peptides:..." Hoppes-Seyler's Z. Physiol. Chem. 356:167-191 (Feb 1975)
173	Palm & Hilschmann, "The primary structure of a crystalline, monoclonal immunoglobulin-L-chain of the x-type, subgroup I (Bence-Jones Protein Rei): a contribution to the elucidation of the three-dimensional structure of the immunoglobulins" Hoppe-Seyler's Z. Physiol. Chem. 354:1651-1654 (Dec 1973)
174	Panka et al., "Variable region framework differences result in decreased or increased affinity of variant anti-digoxin antibodies" Proc. Natl. Acad. Sci. USA 85:3080-3084 (May 1988)
175	~~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~~
176	Presta et al., "Humanization of an Antibody Directed Against IgE" J. Immunol. 151(5):2623-2632 (September 1, 1993)
177	Preval & Fougereau, "Specific Interaction between V_H and V_L Regions of Human Monoclonal Immunoglobulins" J. Mol. Biol. 102:657-678 (1976)
178	Queen et al., "Construction of Humanized Antibodies and Testing in Primates" J. Cell. Biochem. Suppl. 15 (Part E) (20th Ann. Mtg. Keystone Symp. Denver, CO Mar 10-16, 1991) pps. 137 (1991)
179	Queen et al., "Humanised antibodies to the IL-2 receptor" Protein Eng. Antibody Mol. Prophyl. Ther. Appl. Man, Clark, M., Nottingham, UK:Academic Titles pps. 159-70 (1993)
180	Rhodes & Birch, "Large-Scale Production of Proteins from Mammalian Cells" Bio/Technology 6:518, 521, 523 (May 1988)
181	~~Riechmann, "Declaration" from EP Opposition to EP Patent No. 451,261 B1 (Oct 23, 1996)~~
182	Riechmann, "Humanizing of Recombinant Antibodies" (Intl. Symp. on Clin. Appl. of Monoclonal Antibodies, Guildford, England) pps. 33-34 (Sep 1987)
183	Riechmann & Winter, "Recombinant Antibodies" (U. of London Royal Postgraduate Medical School, Wolfson Institute, Abstract) (May 1987)

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

*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

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

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

184	Riechmann et al. Alignment of VL Sequences (1988)
185	Roberts & Rees, "Generation of an antibody with enhanced affinity and specificity for its antigen by protein engineering" Nature 328:731-734 (Aug 1987)
186	Rostapshov et al., "Effective method for obtaining long nucleotide chains on partially complementary templates" FEBS Letters 249(2):379-382 (Jun 1989)
187	Schneider et al., "The Anti-Idiotypic Response by Cynomolgus Mondkeys to Humanized Anti-Tac Is Primarily Directed to Complementarity-Determining Regions H1, H2, and L3" J. of Immunology 150:3086-3090 (Apr 1993)
188	Sedlacek et al., "Monoclonal Antibodies in Tumor Therapy", Karger pps. 119-126, 133-179 (1988)
189	Shields et al., "Inhibition of Allergic Reactions with Antibodies to IgE" International Archives of Allergy and Immunology 107(1-3):308-312 (May 1995)
190	Sims et al., "A Humanized CD18 Antibody Can Block Function Without Cell Destruction" The Journal of Immunology 151(4):2296-2308 (Aug 1993)
191	Smith-Gill et al., "A Three-dimensional Model of an Anti-lysozyme Antibody" Mol. Biol. 194:713-724 (1987)
192	Songsivilai et al., "Bispecific antibody: a tool for diagnosis and treatment of disease" Clin. Exp. Immunol. 79:315-321 (1990)
193	Stanford, "A Predictive Method for Determining Possible Three-dimensional Foldings of Immunoglobulin Backbones Around Antibody Combining Sites" Theor. Biol. 88:421-439 (1981)
194	Stickney et al., "Bifunctional Antibody: ZCE/CHA ^{111}Indium BLEDTA-IV Clinical Imaging in Colorectal Carcinoma" Antibody, Immuno Radiopharm 2:1-13 (1989)
195	Tighe et al., "Delayed Allograft Rejection in Primates Treated with Anti-IL-2 Receptor Monoclonal antibody Campath-6" Transplantation 45(1):226-228 (Jan 1988)
196	Verhoeyen & Riechmann, "Engineering of Antibodies" BioEssays 8(2):74-78 (Feb/Mar 1988)
197	Verhoeyen et al., "Grafting Hypervariable Regions in Antibodies" Protein Structure, Folding, and Design 2 (Proc. DuPont-UCLA Symp. Streamboat Springs, CO, Apr 4-11, 1987), Dale L. Oxender, New York:Alan R. Liss, Inc. pps. 501-502 (1987)
198	Verhoeyen et al., "Humanising Mouse Antibodies: A Protein Engineering Approach" Soc. for Analytical Cytology (XIIth Intl. Mtg. for the Soc. for Analytical Cytology, Cambridge, UK) pps. 22 and slide presented at mtg
199	Verhoeyen et al., "Re-shaped human anti-PLAP antibodies" Monoclonal Antibodies Applications in clinical oncology, Epenetos, 1st edition, Chapman & Hall Medical pps. 37-43 (1991)
200	Ward et al., "Expression and Secretion of Repertoires of VH Domains in Escherchia Coli: Isolation of Antigen Binding Activites" Progress in Immunology (7th Intl. Congress Immunol. Berlin, W. Germany), F. Melchers Vol. VII:1144-1151 (1989)
201	Ward, E.S. et al., "Binding activities of a repertoire of single immunoglobulin variable domains secreted from Escherichia coli" Nature 341:544-546 (1989)
202	Werther et al., "Humanization of an Anti-Lymphocyte Function-Associated Antigen (LFA)-1 Monoclonal Antibody and Reengineering of the Humanized Antibody for Binding to Rhesus LFA-1" J. of Immunology pps. 4986-4995 (1996)
203	Whittle et al., "Construction and Expression of A CDR-Grafted Anti-TNF Antibody" J. Cell Biochem. Suppl. Q (Symp. on Protein and Pharm. Eng. Mol. and Cell. Biol. Park City, Utah)13 Part A:96 (1989)

Examiner	Date Considered
M. T. DAVIS	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.

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

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

Thus	204	Winter & Neuberger, "Restructuring Enzymes and Antibodies" Investigation and Exploitation of Antibody Combining Sites, Eric Reid, Plenum Press pps. 139-140 (1985)
	205	Winter et al., "Protein Engineering by Site Directed Mutagenesis" Chemical Synthesis in Molecular Biology, H. Blocker et al., VCH pps. 189-197 (1987)
	206	Winter G., "Antibody Engineering" Phil. Trans. R. Soc. Lond. B 324:99-109 (1989)
4	207	Woodle et al., "Humanized OKT3 Antibodies: Successful Transfer of Immune Modulating Properties and Idiotype Expression" J. of Immunology 148(9):2756-2763 (May 1992)

Examiner M. T. DAVIS Date Considered 12/07/01

*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.

#.3 (Supp 10/7/97 OFFICIAL PLEASE ENTER P.N. 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: 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: _____
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

Duplicate of DS received 04/17/95

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 and Presta	
		Filing Date 17 Nov 1993	Group ~~1806~~ 1816

U.S. PATENT DOCUMENTS

Examiner Initials		Document Number	Date	Name	Class	Subclass	Filing Date
TDS PN	1	4,816,567	~~28.03.89~~ 3-28-89	Cabilly et al.			

FOREIGN PATENT DOCUMENTS

Examiner Initials		Document Number	Date	Country	Class	Subclass	Translation Yes	Translation No
TDS PN	2	0 239 400	~~9-30-87~~ ~~30.09.87~~	EPO				
	3	0 620 276	~~10-19-94~~ ~~19.10.94~~	EPO				
	4	WO 89/01783	~~3-9-89~~ ~~09.03.89~~	PCT				
	5	WO 89/06692	~~7-27-89~~ ~~27.07.89~~	PCT				
	6	WO 90/07861	~~7-26-90~~ ~~26.07.90~~	PCT				
	7	WO 91/09967	~~7-11-91~~ ~~11.07.91~~	PCT				
	8	WO 92/22653	~~12-23-92~~ ~~23.12.92~~	PCT				
PN	9	WO 93/02191	~~2-4-93~~ ~~04.02.93~~	PCT				

MAILED DEC 26 1995 GROUP 180

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

TDS PN	10	Amzel and Poljak, "Three-dimensional structure of immunoglobulins" Ann. Rev. Biochem. 48:961-967 (1979)
	11	Bindon et al., "Human monoclonal IgG isotypes differ in complement activating function at the level of C4 as well as C1q" Journal of Experimental Medicine 168(1):127-142 (July 1988)
	12	Boulianne, G. L. et al., "Production of functional chimaeric mouse/human antibody" Nature 312(5995):643-646 (December 1984)
	13	Brown et al., "Anti-Tac-H, a humanized antibody to the interleukin 2 receptor, prolongs primate cardiac allograft survival" Proc. Natl. Acad. Sci. USA 88:2663-2667 (1991)
	14	Bruccoleri, "Structure of antibody hypervariable loops reproduced by a conformational search algorithm" Nature (erratum to article in Nature 335(6190):564-568 and) 336:266 (1988)
	15	Bruggemann, M. et al., "Comparison of the effector functions of human immunoglobulins using a matched set of chimeric antibodies" Journal of Experimental Medicine 166:1351-1361 (1987)
	16	Burgess et al., "Possible Dissociation of the Heparin-binding and Mitogenic Activities of Heparin-binding (Acidic Fibroblast) Growth Factor-1 from Its Receptor-binding Activities by Site-directed Mutagenesis of a Single Lysine Residue" Journal of Cell Biology 111:2129-2138 (1990)
	17	Carter et al., "Humanization of an anti-p185^{HER2} antibody for human cancer therapy" Proc. Natl. Acad. Sci. 89:4285-4289 (1992)
	18	Cheetham, J., "Reshaping the antibody combining site by CDR replacement-tailoting or tinkering to fit?" Protein Engineering 2(3):170-172 (1988)
	19	Chothia and Lesk, "Canonical Structures for the Hypervariable Regions" J. Mol. Biol. 196:901-917 (1987)
PN	20	Chothia et al., "The predicted structure of immunoglobulin D1.3 and its comparison with the crystal structure" Science 233:755-758 (Aug. 15, 1986)

Examiner	Date Considered
Patrick Nolan	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.

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

LIST OF DISCLOSURES CITED BY APPLICANT

(Use several sheets if necessary)

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

21	Chothia, C. et al., "Conformations of immunoglobulin hypervariable regions" Nature 342(6252):877-883 (1989)
22	Chothia, Cyrus, "Domain association in immunoglobulin molecules: The packing of variable domains" J. Mol. Biol. 186:651-663 (1985)
23	Clark et al., "The improved lytic function and in vivo efficacy of monovalent monoclonal CD3 antibodies" European Journal of Immunology 19:381-388 (1989)
24	Co et al., "Humanized antibodies for antiviral therapy" Proc. Natl. Acad. Sci. USA 88:2869-2873 (1991)
25	Coussens et al., "Tyrosine Kinase Receptor with Extensive Homology to EGF Receptor Shares Chromosomal Location with neu Oncogene" Science 230:1132-1139 (1985)
26	Daugherty, BL et al., "Polymerase chain reaction facilitates the cloning, CDR-grafting, and rapid expression of a murine monoclonal antibody directed against the CD18 component of leukocyte integrins" Nucleic Acids Research 19(9):2471-2476 (May 11, 1991)
27	Davies, D. R. et al., "Antibody-Antigen Complexes" Ann. Rev. Biochem. 59:439-473 (1990)
28	Epp et al., "The molecular structure of a dimer composed of the variable portions of the Bence-Jones protein REI refined at 2.0-A resolution" Biochemistry 14(22):4943-4952 (1975)
29	Fendly et al., "Characterization of murine monoclonal antibodies reactive to either the human epidermal growth factor receptor or HER2/neu gene product" Cancer Research 50:1550-1558 (1990)
30	Furey et al., "Structure of a novel Bence-Jones protein (Rhe) fragment at 1.6 A resolution" J. Mol. Biol. 167(3):661-692 (July 5, 1983)
31	Gorman, SD et al., "Reshaping a therapeutic CD4 antibody" Proc. Natl. Acad. Sci. USA 88(10):4181-4185 (May 15, 1991)
32	Gregory et al., "The solution conformations of the subclasses of human IgG deduced from sedimentation and small angle X-ray scattering studies" Molecular Immunology 24(8):821-829 (August 1987)
33	Hale et al., "Remission induction in non-hodgkin lymphoma with reshaped human monoclonal antibody campath-1H" Lancet 1:1394-1399 (1988)
34	Harris and Emery, "Therapeutic antibodies - the coming of age" Tibtech 11:42-44 (February 1993)
35	Huber et al., "Crystallographic structure studies of an IgG molecule and an Fc fragment" Nature 264:415-420 (December 2, 1976)
36	Hudziak et al., "p185^{HER2} Monoclonal Antibody Has Antiproliferative Effects In Vitro and Sensitizes Human Breast Tumor Cells to Tumor Necrosis Factor" Molecular & Cellular Biology 9(3):1165-1172 (1989)
37	Jaffers, G. J. et al., "Monoclonal antibody therapy. Anti-idiotypic and non-anti-idiotypic antibodies to OKT3 arising despite intense immunosuppression" Transplantation 41(5):572-578 (May 1986)
38	Jones, P. T. et al., "Replacing the complementarity-determining regions in a human antibody with those from a mouse" Nature 321(6069):522-525 (1986)
39	Junghans et al., "Anti-Tac-H, a humanized antibody to the interleukin 2 receptor with new features for immunotherapy in malignant and immune disorders" Cancer Research 50(5):1495-1502 (Mar 1, 1990)
40	Kabat et al. Sequences of Proteins of Immunological Interest, Bethesda, MD:National Institutes of Health pps. iii-xxvii, 41-176 (1987)

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.

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 and Presta	MAILED DEC 20 1995
		Filing Date 17 Nov 1993	Group ~~1806~~ 1816

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

PN	41	King et al., "Amplification of a Novel v-erbB-Related Gene in a Human Mammary Carcinoma" Science 229:974-976 (1985)
	42	Lazar et al., "Transforming Growth Factor α: Mutation of Aspartic Acid 47 and Leucine 48 Results in Different Biological Activities" Molecular & Cellular Biology 8(3):1247-1252 (1988)
	43	Love et al, "Recombinant antibodies possessing novel effector functions" Methods in Enzymology 178:515-527 (1989)
	44	Lupu et al., "Direct interaction of a ligand for the erbB2 oncogene product with the EGF receptor and $p185^{erbB2}$" Science 249:1552-1555 (1990)
	45	Margni RA and Binaghi RA, "Nonprecipitating asymmetric antibodies" Ann. Rev. Immunol. 6:535-554 (1988)
	46	Margolies et al., "Diversity of light chain variable region sequences among rabbit antibodies elicited by the same antigens." Proc. Natl. Acad. Sci. USA 72:2180-84 (Jun 1975)
	47	Marquart et al., "Crystallographic refinement and atomic models of the intact immunoglobulin molecule Kol and its antigen-binding fragment at 3.0 A and 1.0 A resolution" J. Mol. Biol. 141(4):369-391 (Aug 25, 1980)
	48	Mian, IS et al., "Structure, function and properties of antibody binding sites" J. Mol. Biol. 217(1):133-151 (Jan 5, 1991)
	49	Miller, R. et al., "Monoclonal antibody therapeutic trials in seven patients with T-cell lymphoma" Blood 62:988-995 (1983)
	50	Morrison, S. L. et al., "Chimeric human antibody molecules: mouse antigen-binding domains with human constant region domains" Proc. Natl. Acad. Sci. USA 81(21):6851-6855 (Nov. 1984)
	51	Neuberger et al., "Recombinant antibodies possessing novel effector functions" Nature 312(5995):604-608 (December 1984)
	52	Neuberger, M. S. et al., "A hapten-specific chimaeric IgE antibody with human physiological effector function" Nature 314(6008):268-270 (March 1985)
	53	Novotny and Haber, "Structural invariants of antigen binding: comparison of immunoglobulin V_L-V_H and V_L-V_L domain dimers" Proc. Natl. Acad. Sci. USA 82(14):4592-4596 (July 1985)
	54	Pluckthun, Andreas, "Antibody engineering: advances from the use of escherichia coli expression systems" Biotechnology 9:545-51 (1991)
	55	Queen, M. et al., "A humanized antibody that binds to the interleukin 2 receptor" Proc. Natl. Acad. Sci. USA 86:10029-10033 (1989)
	56	Riechmann, L. et al., "Reshaping human antibodies for therapy" Nature 332:323-327 (1988)
	57	Roitt et al. Immunology (Gower Medical Publishing Ltd., London, England) pps. 5.5 (1985)
	58	Saul et al., "Preliminary refinement and structural analysis of the Fab fragment from human immonoglobulin new at 2.0 A resolution" Journal of Biological Chemistry 253(2):585-597 (January 25, 1978)
	59	Schroff, R. et al., "Human anti-murine immunoglobulin responses in patients receiving monoclonal antibody therapy" Cancer Research 45:879-885 (1985)
PN	60	Segal et al., "The three-dimensional structure of a phosphorylcholine-binding mouse immunoglobulin Fab and the nature of the antigen binding site" Proc. Natl. Acad. Sci. USA 71(11):4298-4302 (Nov 1974)

Examiner	Date Considered
Patrick J. Nolan	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.

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

LIST OF DISCLOSURES CITED BY APPLICANT

(Use several sheets if necessary)

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

	61	Shalaby et al., "Development of humanized bispecific antibodies reactive with cytotoxic lymphocytes and tumor cells overexpressing the HER2 protooncogene" Journal of Experimental Medicine 175(1):217-225 (Jan 1, 1992)
	62	Shepard and Lewis, "Resistance of tumor cells to tumor necrosis factor" J. Clin. Immunol. 8(5):333-395 (1988)
	63	Sheriff et al., "Three-dimensional structure of an antibody-antigen complex" Proc. Natl. Acad. Sci. USA 84(22):8075-8079 (Nov. 1987)
	64	Sherman et al., "Haloperidol binding to monoclonal antibodies" Journal of Biological Chemistry 263:4064-4074 (1988)
	65	Silverton et al., "Three-dimensional structure of an intact human immunoglobulin" Proc. Natl. Acad. Sci. USA 74:5140-5144 (1977)
	66	Slamon et al., "Human Breast Cancer: Correlation of Relapse and Survival with Amplification of the HER-2/neu Oncogene" Science 235:177-182 (1987)
	67	Slamon et al., "Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer" Science 244:707-712 (1989)
	68	Snow and Amzel, "Calculating three-dimensional changes in protein structure due to amino-acid substitutions: the variable region of immunoglobulins" Protein: Structure, Function, and Genetics, Alan R. Liss, Inc. Vol. 1:267-279 (1986)
	69	Sox et al., "Attachment of carbohydrate to the variable region of myeloma immunoglubulin light chains" Proc. Natl. Acad. Sci. USA 66:975-82 (July 1970)
	70	Spiegelberg et al., "Localization of the carbohydrate within the variable region of light and heavy chains of human γG myeloma proteins" Biochemistry 9:4217-23 (Oct 1970)
	71	Takeda et al., "Construction of chimaeric processed immunoglobulin genes containing mouse variable and human constant region sequences" Nature 314(6010):452-454 (April 1985)
	72	Tao et al., "Role of Carbohydrate in the Structure and Effector Functions Mediated by the H uman IgG Constant Region" J. Immunol. 143(8):2595-2601 (1989)
	73	Tramontano et al., "Framework residue 71 is a major determinant of the position and conformation of the second hypervariable region in the VH domains of immunoglobulins" J-Mol-Biol 215(1):175-182 (Sep 5, 1990)
	74	Verhoeyen, M. et al., "Reshaping human antibodies: grafting an antilysozyme activity" Science 239(4847):1534-1536 (Mar 25, 1988)
	75	Waldmann, T., "Monoclonal antibodies in diagnosis and therapy" Science 252:1657-1662 (1991)
	76	Wallick et al., "Glycosylation of a VH residue of a monoclonal antibody against alpha (1----6) dextran increases its affinity for antigen" Journal of Experimental Medicine 168(3):1099-1109 (Sep 1988)
	77	Winter and Milstein, "Man-made antibodies" Nature 349(6307):293-299 (Jan 24, 1991)
	78	Yamamoto et al., "Similarity of protein encoded by the human c-erb-B-2 gene to epidermal growth factor receptor" Nature 319:230-34 (1986)

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

RECEIVED
OCT - 7 1997
MAIL RM CUSTOMER SERVICE CENTER

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
Reg. No. 40,378

One DNA Way
So. San Francisco, CA 94080-4990
Phone: (415) 225-1994
Fax: (415) 952-9881

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

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

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.
(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:

(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	33
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		

TDavis 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
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

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

ORIGINAL FOR INSERTION IN RIGHT HAND FLAP OF FILE WRAPPER

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
Reg. No.40,378

1 DNA Way
So. San Francisco, CA 94080-4990
Phone: (415) 225-1994
Fax: (415) 952-9881

#39 Amdt H (Rule 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.

40 30
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

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

---

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: _____

Wendy M. Lee
Reg. No. 40,378

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

#40 Sodium 09/03/98

PATENT
Docket P709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

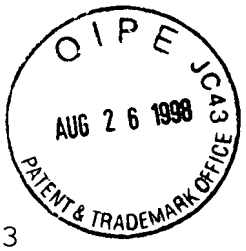
In re Application of

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

Steven Shak, M.D.

Current Addresses:

Home:
1133 Cambridge Road
Burlingame, CA 94010
Tel. No.: (650) 375-8122
Fax No.: (650) 548-1589
E-mail: StevenS18@aol.com

Work:
Genentech, Inc.
460 Pt. San Bruno Blvd.
S. San Francisco, CA 94080
Tel. No.: (650) 225-2476
Fax No.: (650) 225-5335
E-mail: shak@gene.com

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.

9. SHAK S, Goldstein IM: The leukotriene B_4 ω-hydroxylase in human polymorphonuclear leukocytes is a membrane-associated, NADPH-dependent cytochrome P-450 enzyme. TRANSACTIONS OF THE ASSOCIATION OF AMERICAN PHYSICIANS. 48:352-360, 1985.

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.

11. Davitz MA, Hereld D, SHAK S, Krakow JL, Englund PT, Nussenzweig V: A glycan-phosphatidylinositol-specific phospholipase D in human serum. SCIENCE. 238:81-4, 1987.

12. SHAK S, Davitz MA, Wolinsky ML, Nussenzweig V, Turner MJ, Gurnett A: Partial characterization of the cross reacting determinant, a carbohydrate epitope shared by decay accelerating factor (DAF) and the variant surface glycoprotein (VSG) of the african Trypanosoma brucei. THE JOURNAL OF IMMUNOLOGY. 140:2046-2050, 1988.

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.

15. Hubbard RC, McElvaney NG, Birrer P, SHAK S, Robinson WW, Jolley C, Wu M, Chernick MS, Crystal RG: A preliminary study of aerosolized recombinant human deoxyribonuclease I in the treatment of cystic fibrosis. THE NEW ENGLAND JOURNAL OF MEDICINE. 326:812-815, 1992.

16. Ramsey BW, Astley SJ, Aitken ML, Burke W, Colin AA, Dorkin HL, Eisenberg JD, Gibson RL, Harwood IR, Schidlow DV, WilmottRW, Wohl ME, Myerson LJ, SHAK S, Fuchs H, Smith AL: Efficacy and safety of short-term administration of aerosolized recombinant human deoxyribonuclease in patients with cystic fibrosis. AMERICAN REVIEW OF RESPIRATORY DISEASE. 148:145-151, 1993.

17. Ranasinha C, Assoufi B, SHAK S, Christiansen D, Fuchs H, Empey D, Geddes D, Hodson M: Efficacy and safety of short-term administration of aerosolised recombinant human DNase I in adults with stable stage cystic fibrosis. THE LANCET. 342:199-202, 1993.

18. Chamow SM, Kogan TP, Venuti M, Gadek T, Harris RJ, Peers DH, Mordenti J, SHAK S, Ashkenazi A: Modification of CD4 immunoadhesin with monomethoxypoly(ethylene glycol) aldehyde via reductive alkylation. BIOCONJUGATE CHEMISTRY. 5:133-140, 1994.

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.

20. SHAK S: Aerosolized recombinant human DNase I for the treatment of cystic fibrosis. CHEST 107:65S-70S, 1995.

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.

22. Puchelle E, Zahm JM, de Bentzmann S, Grosskopf C, SHAK S, Mougel D, Polu JM: Effects of rhDNase on purulent airway secretions in chronic bronchitis. EUROPEAN RESPIRATORY JOURNAL. 9:765-9, 1996.

23. Macanovic M, Sinicropi D, SHAK S, Baughman S, Thiru S, Lachmann PJ: The treatment of systemic lupus erythematosus (SLE) in NZB/W F1 hybrid mice; studies with recombinant murine DNase and with dexamethasone. CLINICAL AND EXPERIMENTAL IMMUNOLOGY. 106:243-252, 1996.

24. Ulmer JS, Herzka A, Toy KJ, Baker DL, Dodge AH, Sinicropi D, SHAK S, Lazarus RA: Engineering Actin Resistant Human DNase I for Treatment of Cystic Fibrosis. PROCEEDINGS NATIONAL ACADEMY OF SCIENCE, USA. 93:8225-8229, 1996.

#40 09/02/98
Groom

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
	#41

DATE MAILED:

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.
Ann Savelli
Type or print name of person signing certification
Ann S
Signature Date

YOU SHOULD RECEIVE 5 PAGES, INCLUDING THIS COVER SHEET. IF YOU DO NOT RECEIVE ALL THE PAGES, PLEASE CALL 650-225-7039

Comments:

42/I

Patent Docket P0709P1

11/6/98

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:

I,1 SUB J1

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.

I2 SUB J3

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

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

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

#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
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 1/15/99
Signature Date

YOU SHOULD RECEIVE 15 PAGE(S), INCLUDING THIS COVER SHEET. IF YOU DO NOT RECEIVE ALL THE PAGES, PLEASE CALL 650-225-7039

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
Reg. No. 40,378

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

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 → Framework Region (FR)

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.

J7

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.

J8

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

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

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./

/CONTACT: Dr. David Bloxham, Chief Executive of Celltech Therapeutics Ltd., or Peter Allen, Finance Director of Celltech Group plc, 0-1753-534655; or Jon Coles of Brunswick, 0-171-404-5959; or Rich Tammero of Noonan/Russo Communications, Inc., 212-696-4455 ext. 222, e-mail: news@noonanrusso.com/
08:52 EDT

0624 09/26/96 08:52 EDT HT
:TICKER: CEL.GB
:SUBJECT: BIOT PTNT ENGL USA

Received by NewsEDGE/LAN: 9/26/96 6:50 AM

09/146206 #45 attached

SERIAL NUMBER	PATENT DATE	PATENT NUMBER
07/290975		US 4,897,255

SERIAL NUMBER	FILING DATE	CLASS	SUBCLASS	GROUP ART UNIT	EXAMINER
07/290,975	12/28/88	435		185	

APPLICANTS

CARY L. QUEEN, PALO ALTO, CA; HAROLD E. SELICK, BELMONT, CA.

CONTINUING DATA********
VERIFIED

REC'D 28 DEC 1989
WIPO PCT

FOREIGN/PCT APPLICATIONS********
VERIFIED

PRIORITY DOCUMENT

FOREIGN FILING LICENSE GRANTED 01/24/89 ***** SMALL ENTITY *****

Foreign priority claimed	35 USC 119 conditions met	AS FILED	STATE OR COUNTRY	SHEETS DRWGS.	TOTAL CLAIMS	INDEP. CLAIMS	FILING FEE RECEIVED	ATTORNEY'S DOCKET NO.
☐yes ☐no	☐yes ☐no			10	26	8	$ 396.00	113233

Verified and Acknowledged Examiner Initials

ADDRESS

WILLIAM M. SMITH
TOWNSEND AND TOWNSEND
STEUART STREET TOWER, ONE MARKET PLAZA
SAN FRANCISCO, CA 94105

TITLE

NOVEL IL-2 RECEPTOR-SPECIFIC HUMAN IMMUNOGLOBULINS

This is to certify that annexed hereto is a true copy from the records of the United States Patent and Trademark Office of the application as originally filed which is identified above.

By authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

C. A. Lane

Certifying Officer

Date 1 JAN 1990

07/290975

PATENT APPLICATION SERIAL NO.

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

G 11411 01/04/89 290975 20-1430 110 201 394.00CH

PTO-1556
(5/87)

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
Steven W. Parmelee, Reg. No. 31,990
James M. Heslin, Reg. No. 29,541

SEND CORRESPONDENCE TO:	DIRECT TELEPHONE CALLS TO (name, registration number, and telephone number)
William M. Smith TOWNSEND and TOWNSEND Steuart Street Tower, One Market Plaza San Francisco, CA 94105	William M. Smith, Reg. 30,223 ☐ (415) 543-9600 or ☒ (415) 326-2400

201	FULL NAME OF INVENTOR	Last Name: Queen	First Name: Cary	Middle Name or Initial: L.	
	RESIDENCE & CITIZENSHIP	City: Palo Alto	State or Foreign Country: California	Country of Citizenship: USA	
	POST OFFICE ADDRESS	Post Office Address: 1300 Oak Creek Dr.	City: Palo Alto	State or Country: California	Zip Code: 94304
202	FULL NAME OF INVENTOR	Last Name: Selick	First Name: Harold	Middle Name or Initial: Edwin	
	RESIDENCE & CITIZENSHIP	City: Belmont	State or Foreign Country: California	Country of Citizenship: USA	
	POST OFFICE ADDRESS	Post Office Address: 1673 Sunnyslope Ave.	City: Belmont	State or Country: California	Zip Code: 94002
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
	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
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 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 _____

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 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

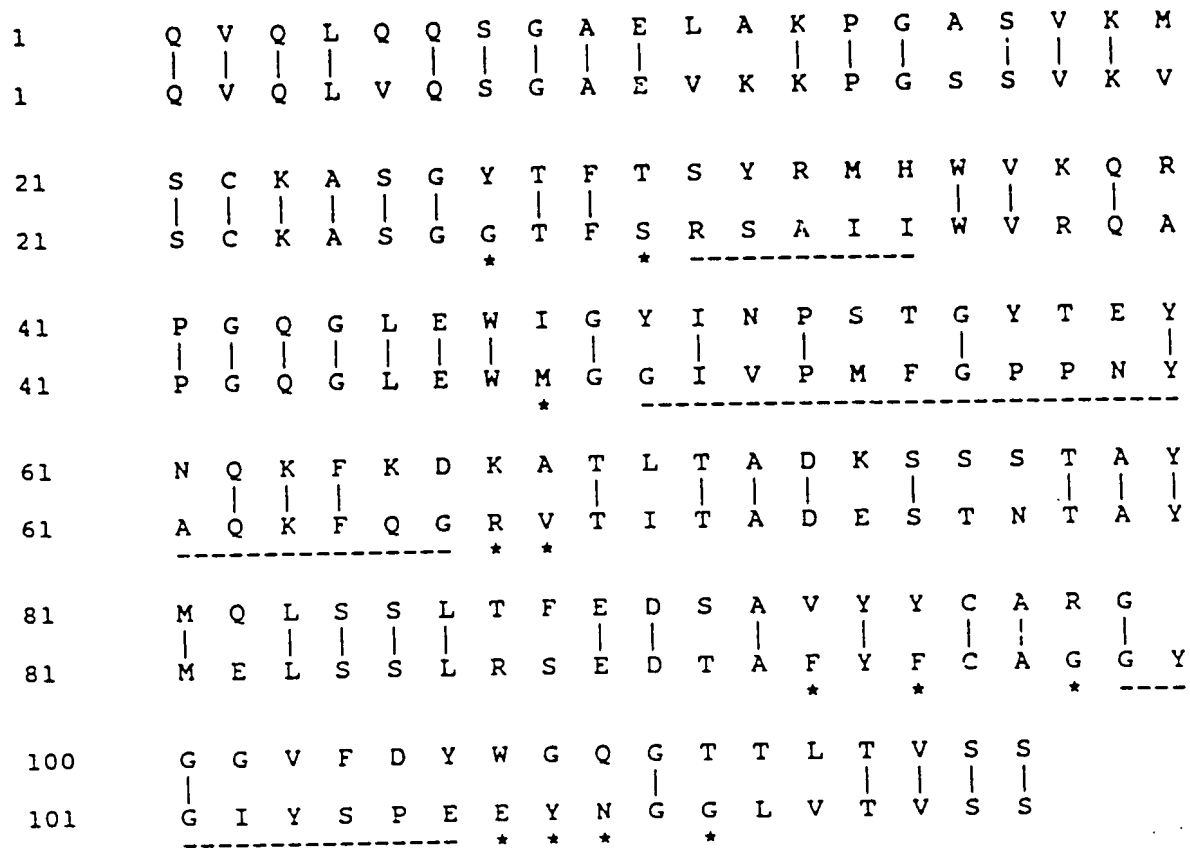


FIGURE 2

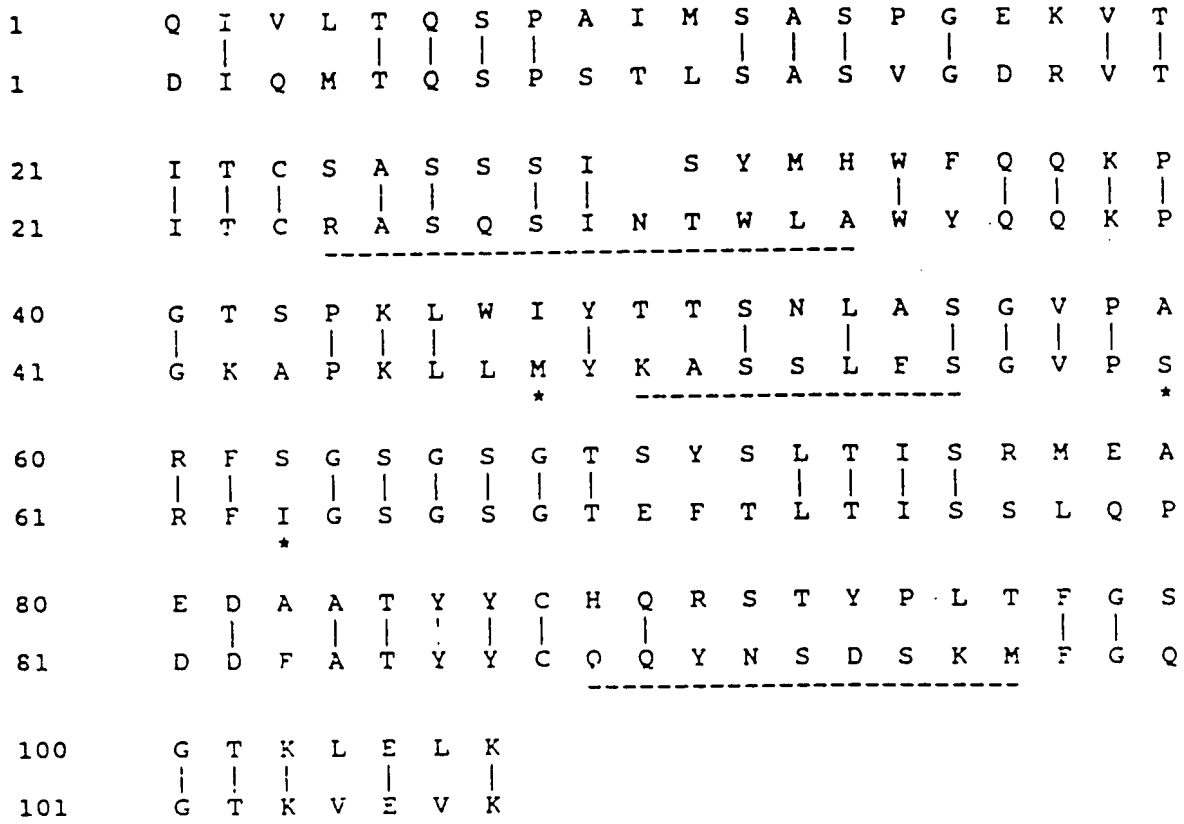


FIGURE 3

```
        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
CAGTAGTCAAAGACCCCCCCCCCTCTTGCACAGTAATAGACTGCGGTGTCCTCAGATCTC
AGGCTGCT

B

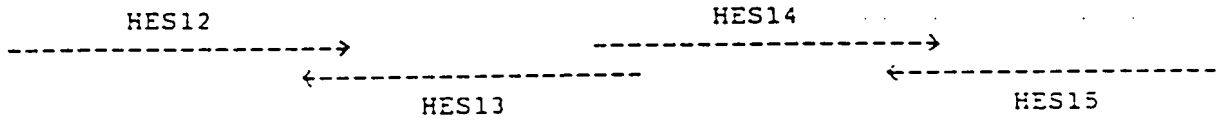


FIGURE 6

A

JFD1 CAAATCTAGATGGAGACCGATACCCTCCTGCTATGGGTCCTCCTGCTATGGGTCCCAGGA
TCAACCGGAGATATTCAGATGACCCAGTCTCCATCTACCCTCTCTGCTAGCGTCGGGGAT

JFD2 ATAAATTAGAAGCTTGGGAGCTTTGCCTGGCTTCTGCTGGTACCAGTGCATGTAACTTAT
ACTTGAGCTGGCAGAGCAGGTTATGGTGACCCTATCCCCGACGCTAGCAGAGAG

JFD3 GCTCCCAAGCTTCTAATTTATACCACATCCAACCTGGCTTCTGGAGTCCCTGCTCGCTTC
AGTGGCAGTGGATCTGGGACCGAGTTCACCCTCACAATCAGCTCTCTGCAGCCAGATGAT
TTC

JFD4 TATATCTAGAAAAGTGTACTTACGTTTGACCTCCACCTTGGTCCCCTGACCGAACGTGAG
TGGGTAAGTACTCCTTTGATGGCAGTAATAAGTGGCGAAATCATCTGGCTGCAGAGAGCT
GA

B

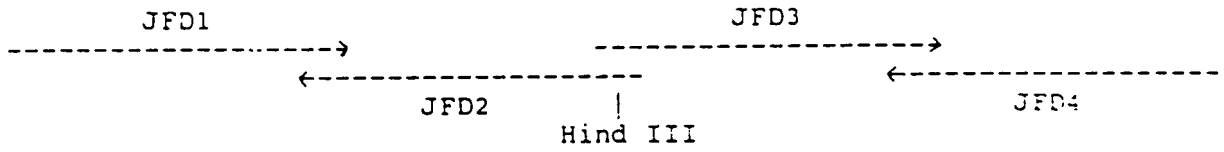


FIGURE 7

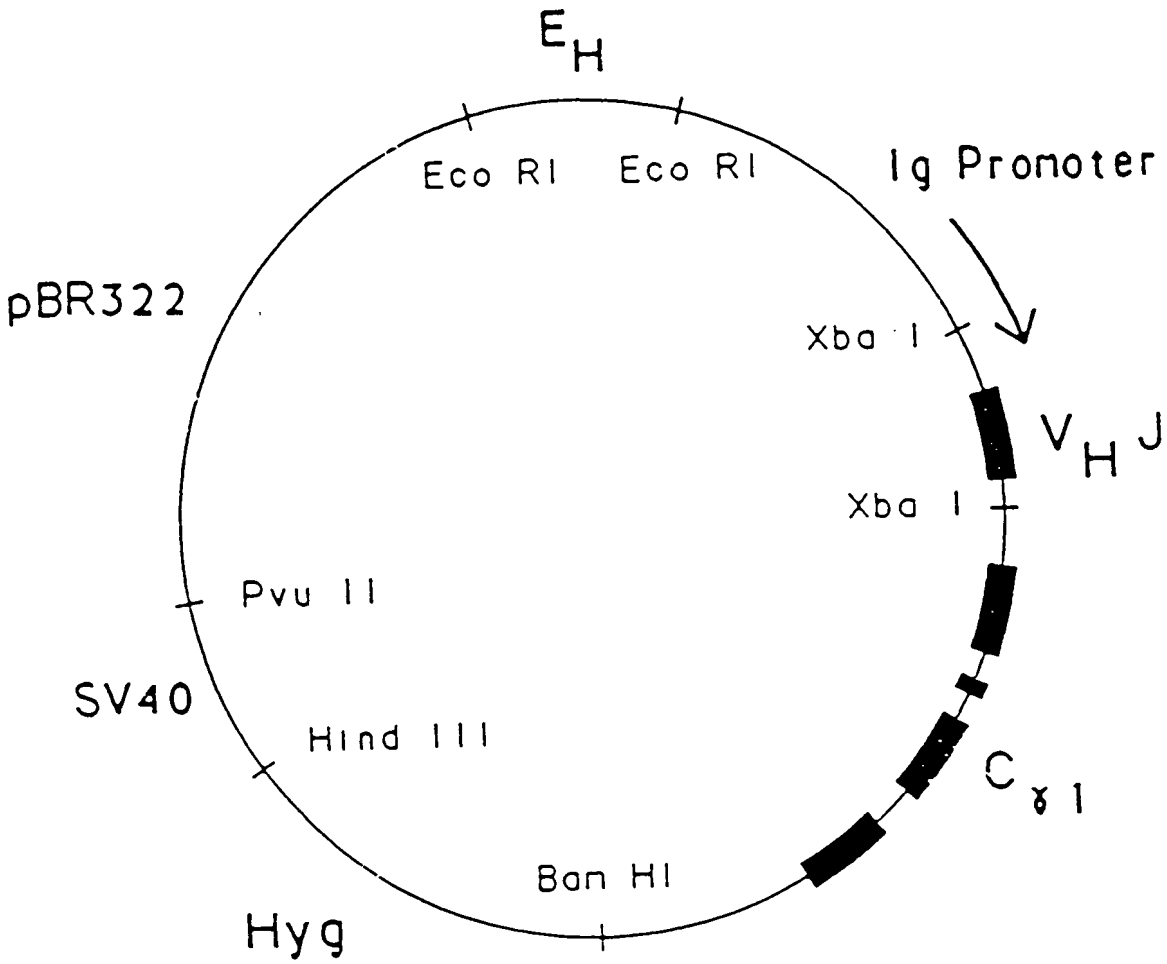


FIGURE 8

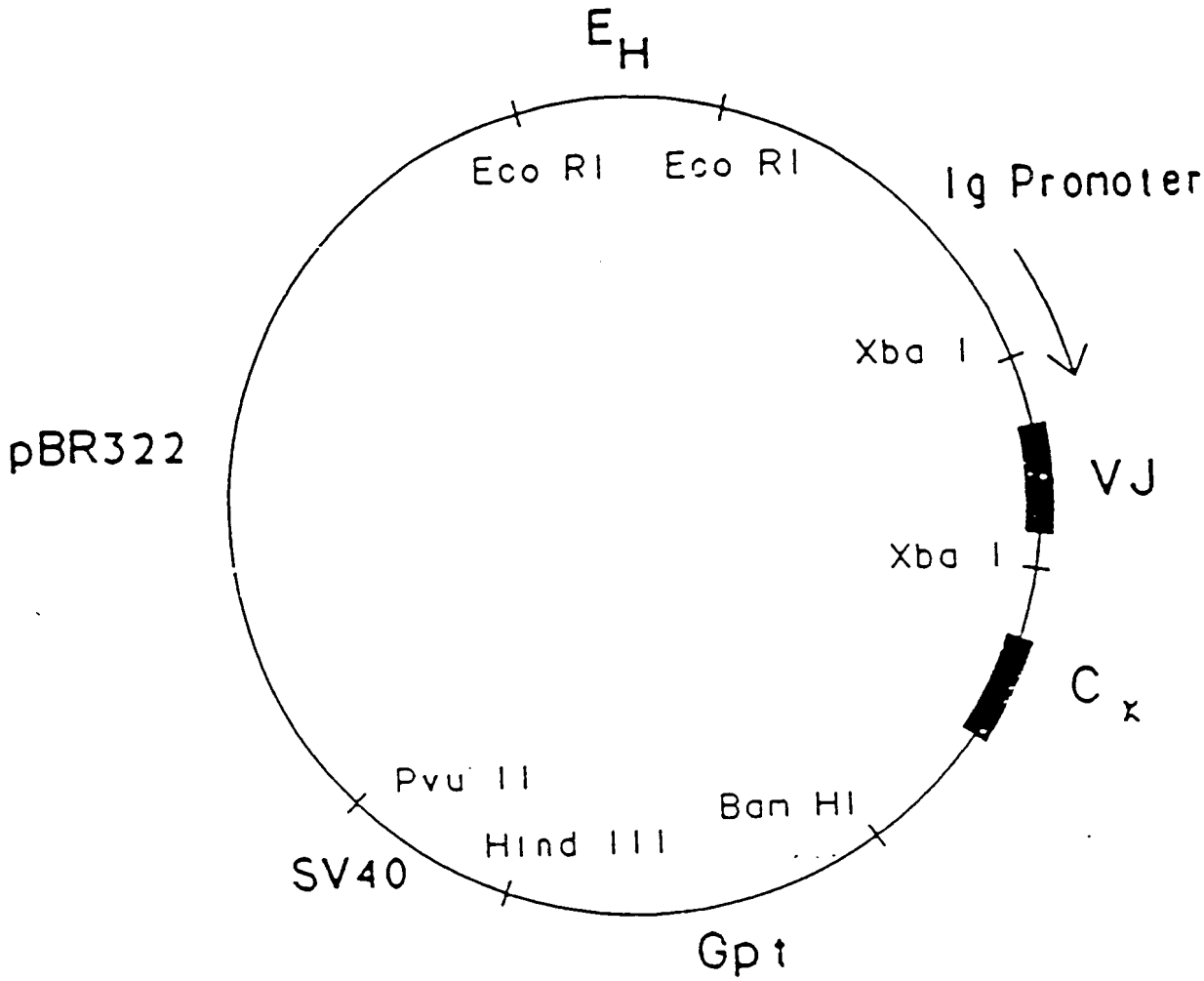


FIGURE 9

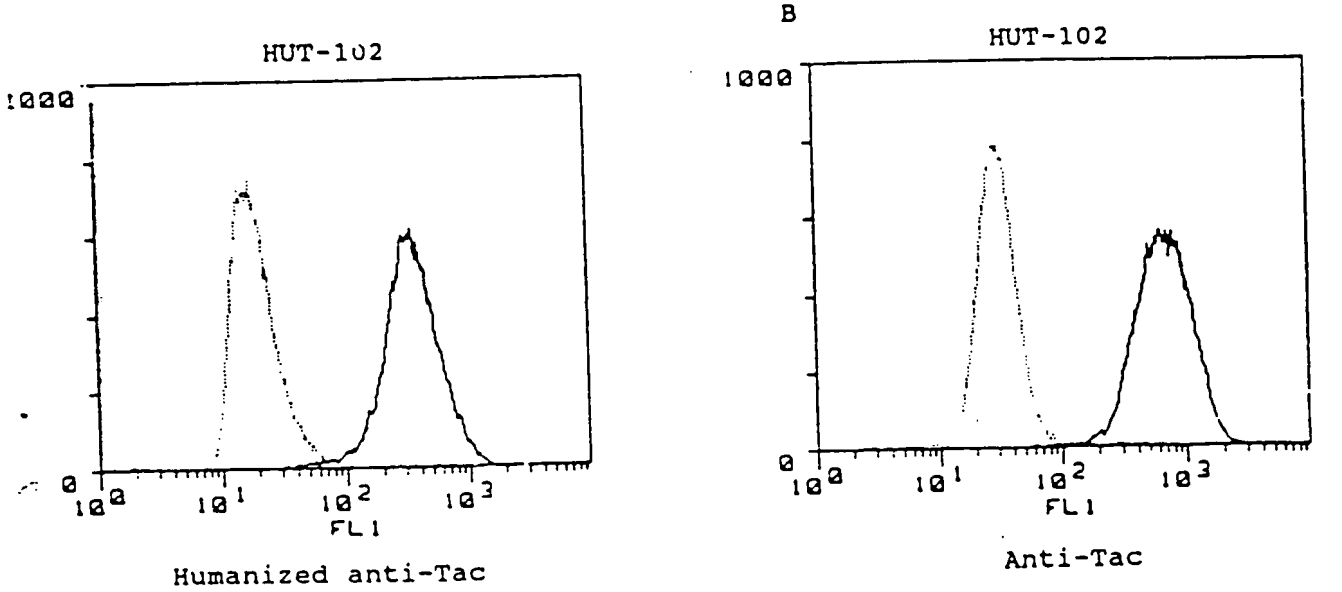
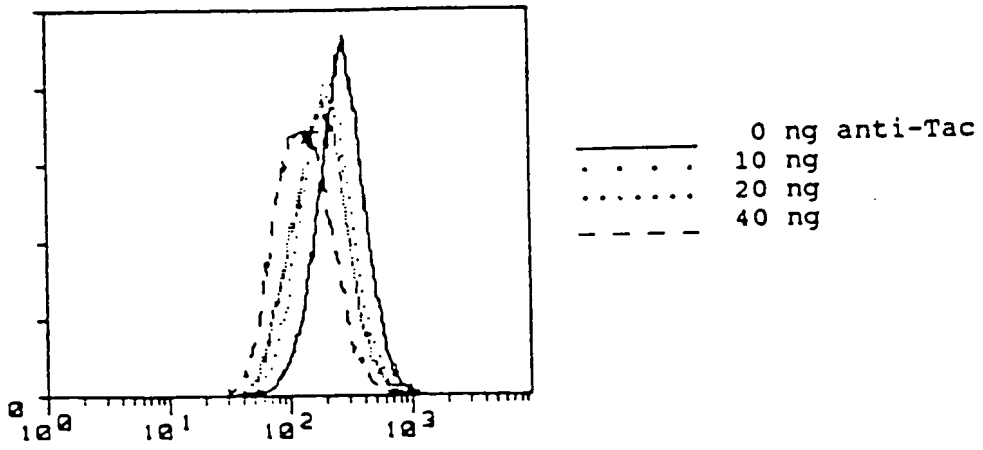
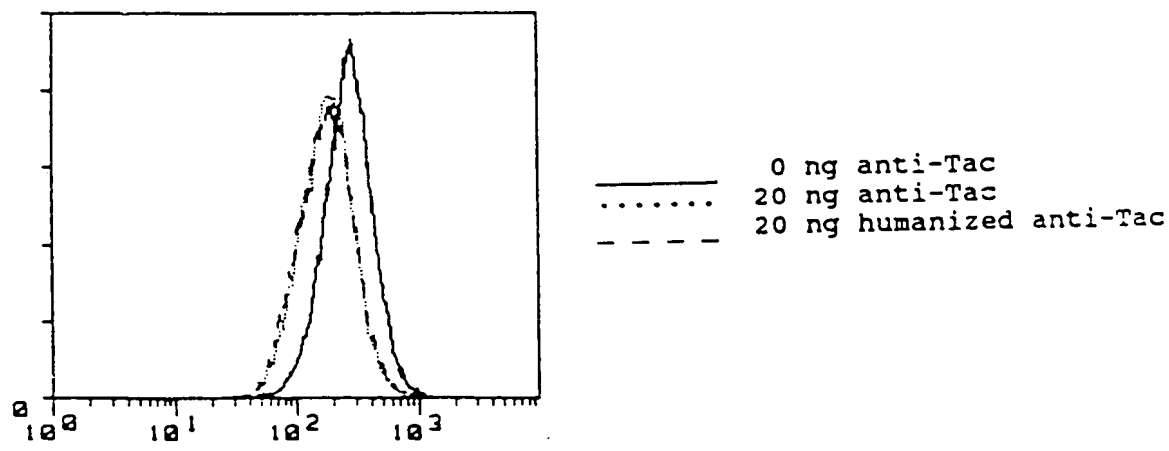


FIGURE 10

A



B



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
WIPO PCT

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

ADDRESS
WILLIAM M. SMITH
TOWNSEND AND TOWNSEND
STEUART STREET TOWER
ONE MARKET PLAZA
SAN FRANCISCO, CA 94105

TITLE
DESIGNING IMPROVED HUMANIZED IMMUNOGLOBULINS

U.S. DEPT. OF COMM. Pat. & TM Office — PTO-436L (rev. 10-78)

This is to certify that annexed hereto is a true copy from the records of the United States Patent and Trademark Office of the application as originally filed which is identified above.

By authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

H. L. Jackson

Certifying Officer

Date EB 1990

PATENT APPLICATION SERIAL NO. 07/310252

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

S 00016 02/17 89 310252 20 1430 [illegible] 201 [illegible]

PTO-1556
(5/87)

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)
William M. Smith TOWNSEND and TOWNSEND Steuart Street Tower, One Market Plaza San Francisco, CA 94105	William M. Smith, Reg. 30,223 ☐ (415) 543-9600 or ☒ (415) 326-2400

201	FULL NAME OF INVENTOR	Last Name: Queen	First Name: Cary		Middle Name or Initial: L.
	RESIDENCE & CITIZENSHIP	City: Palo Alto	State or Foreign Country: California		Country of Citizenship: USA
	POST OFFICE ADDRESS	Post Office Address: 1300 Oak Creek Dr.	City: Palo Alto	State or Country: California	Zip Code: 94304
202	FULL NAME OF INVENTOR	Last Name: Selick	First Name: Harold		Middle Name or Initial: Edwin
	RESIDENCE & CITIZENSHIP	City: Belmont	State or Foreign Country: California		Country of Citizenship: USA
	POST OFFICE ADDRESS	Post Office Address: 1673 Sunnyslope Ave.	City: Belmont	State or Country: California	Zip Code: 94002
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

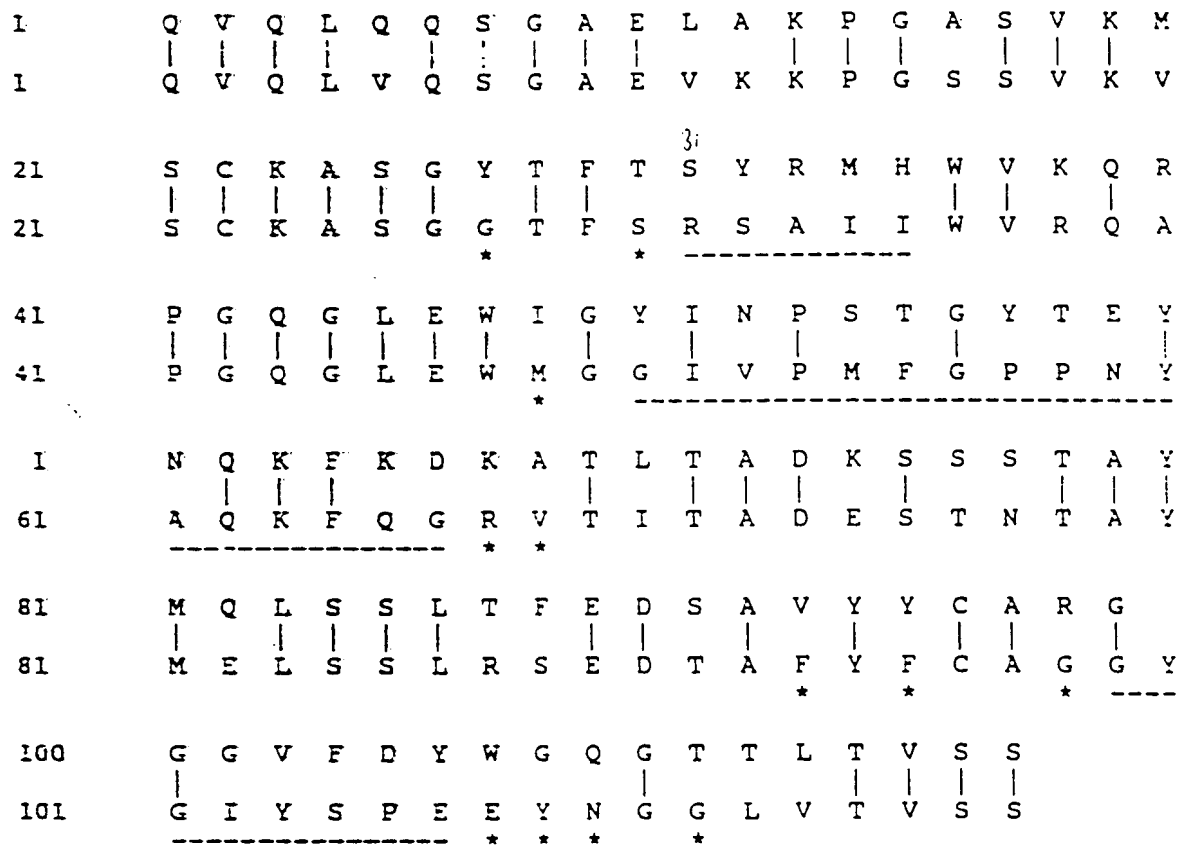


FIGURE 2

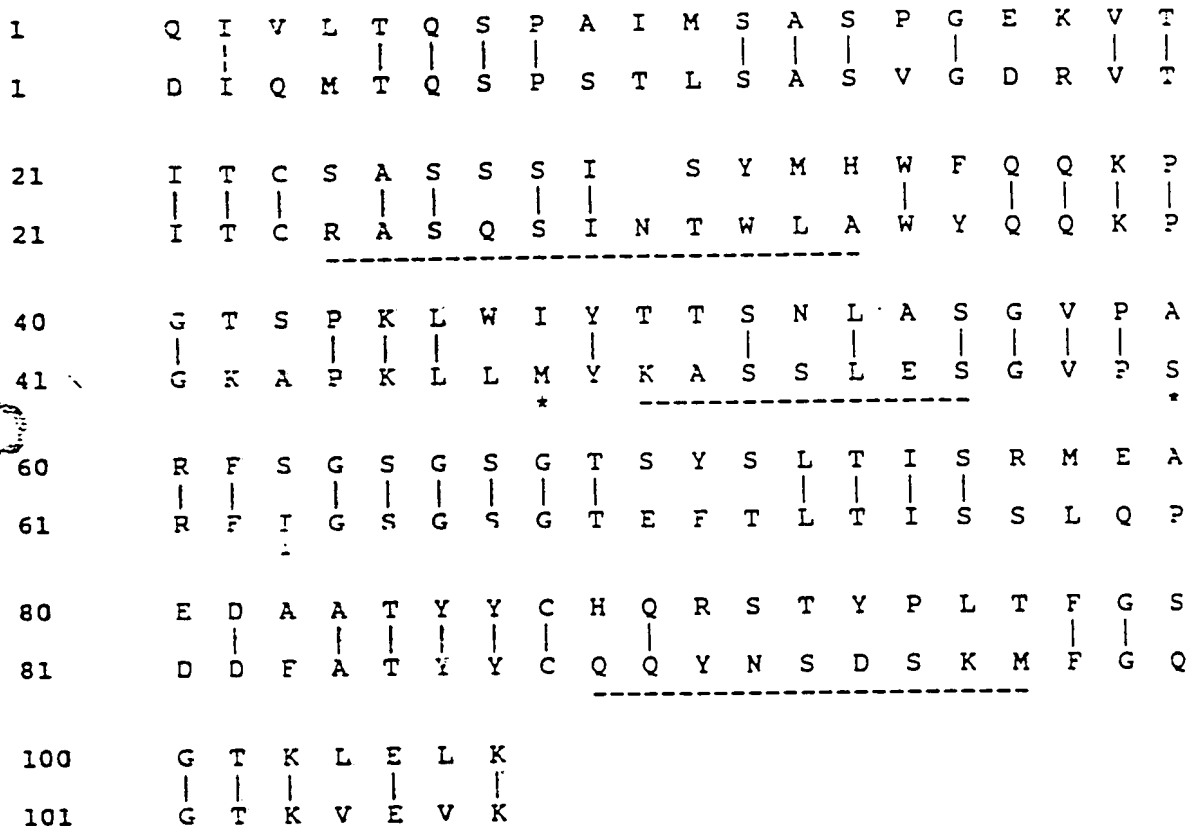


FIGURE 3

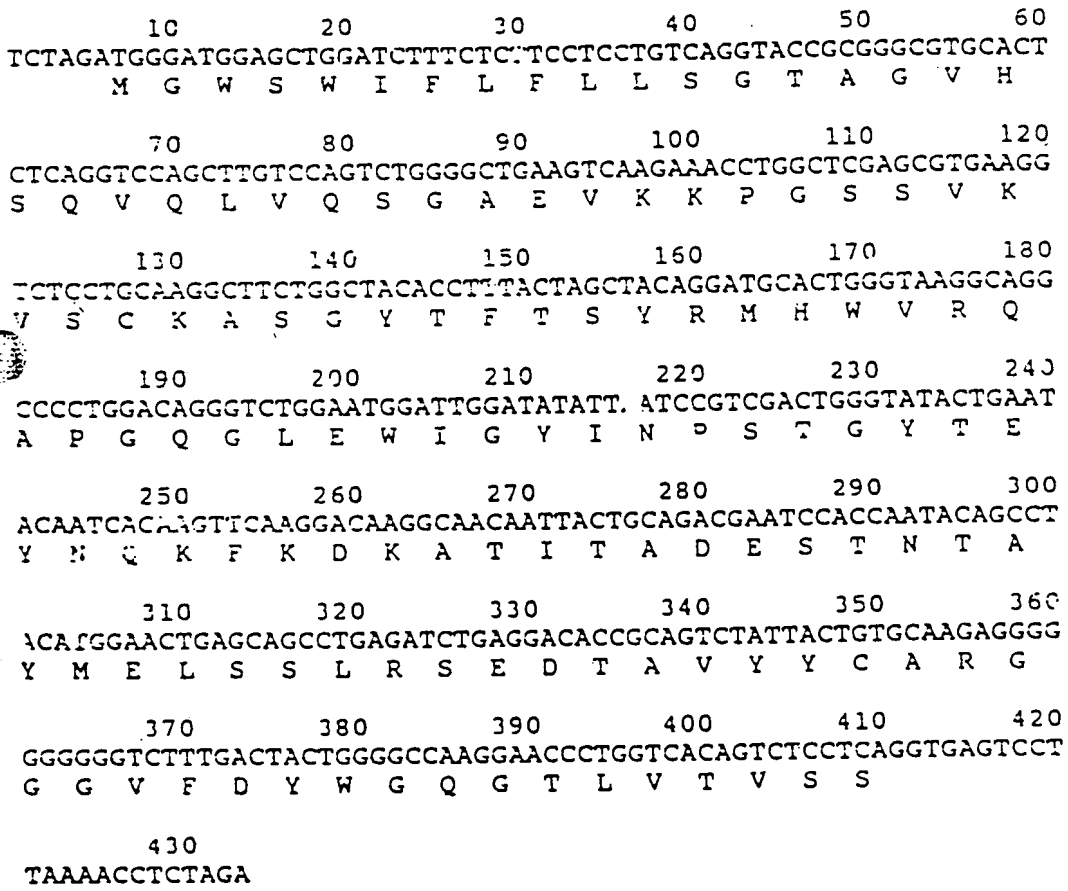


FIGURE 4

```
        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
CAGTAGTCAAAGACCCCCCCCCCTCTTGCACAGTAATAGACTGCGGTGTCCTCAGATCTC
AGGCTGCT

B

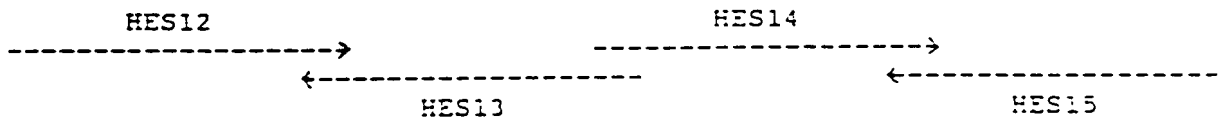


FIGURE 6

A

JFD1 CAAATCTAGATGGAGACCGATACCCTCCTGCTATGGGTCCTCCTGCTATGGGTCCCAGGA
TCAACCGGAGATATTCAGATGACCCAGTCTCCATCTACCCTCTCTGCTAGCGTCGGGGAT

JFD2 ATAAATTAGAAGCTTGGGAGCTTTGCCTGGCTTCTGCTGGTACCAGTGCATGTAACTTAT
ACTTGAGCTGGCAGAGCAGGTTATGGTGACCCTATCCCCGACGCTAGCAGAGAG

JFD3 GCTCCCAAGCTTCTAATTTATACCACATCCAACCTGGCTTCTGGAGTCCCTGCTCGCTTC
AGTGGCAGTGGATCTGGGACCGAGTTCACCCTCACAATCAGCTCTCTGCAGCCAGATGAT
TTC

JFD4 TATATCTAGAAAAGTGTACTTACGTTTGACCTCCACCTTGGTCCCCTGACCGAACGTGAG
TGGGTAAGTACTCCTTTGATGGCAGTAATAAGTGGCGAAATCATCTGGCTGCAGAGAGCT
GA

B

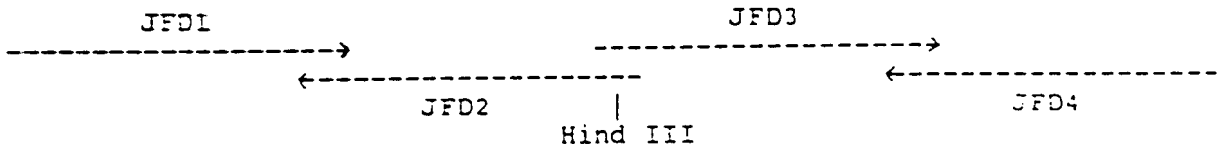


FIGURE 7

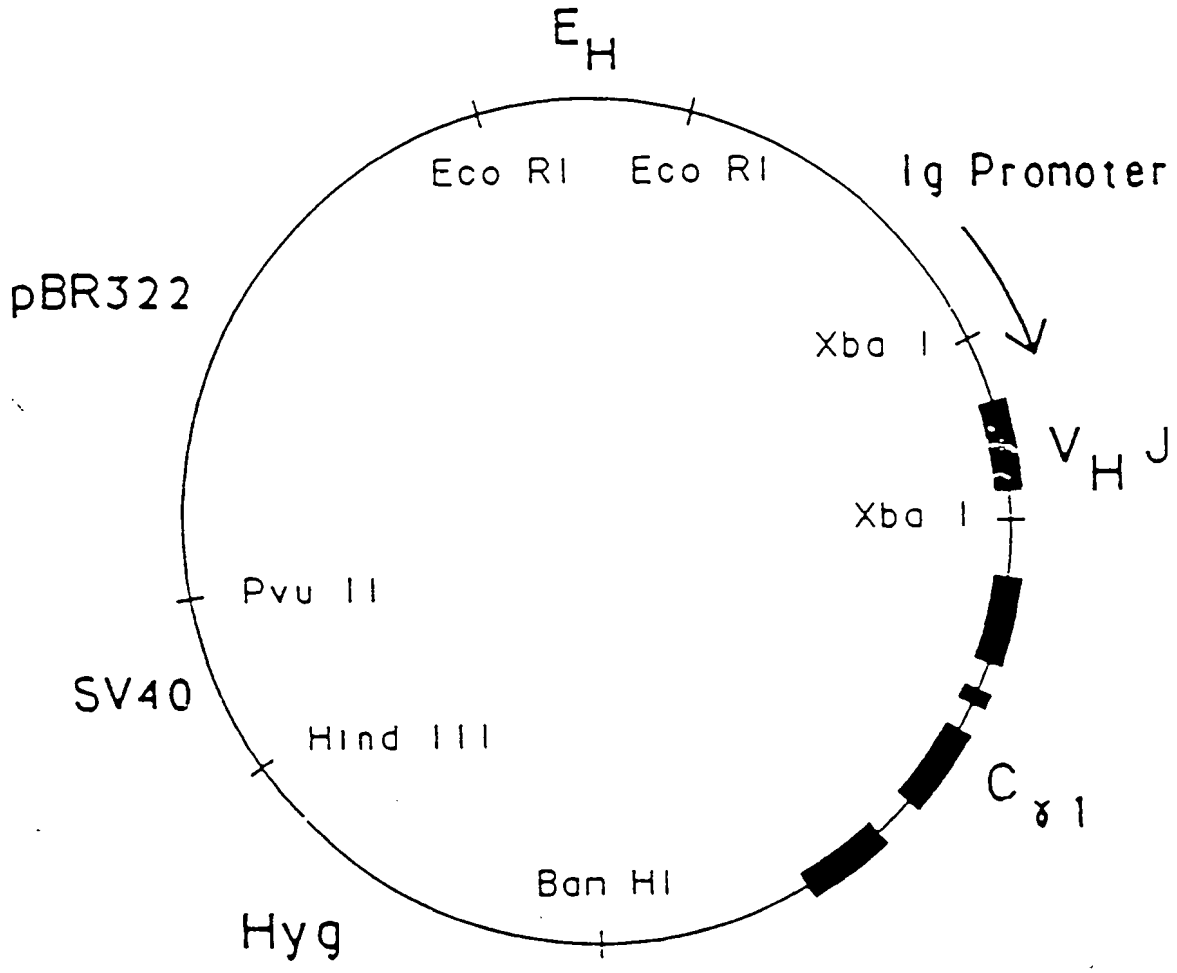


FIGURE 8

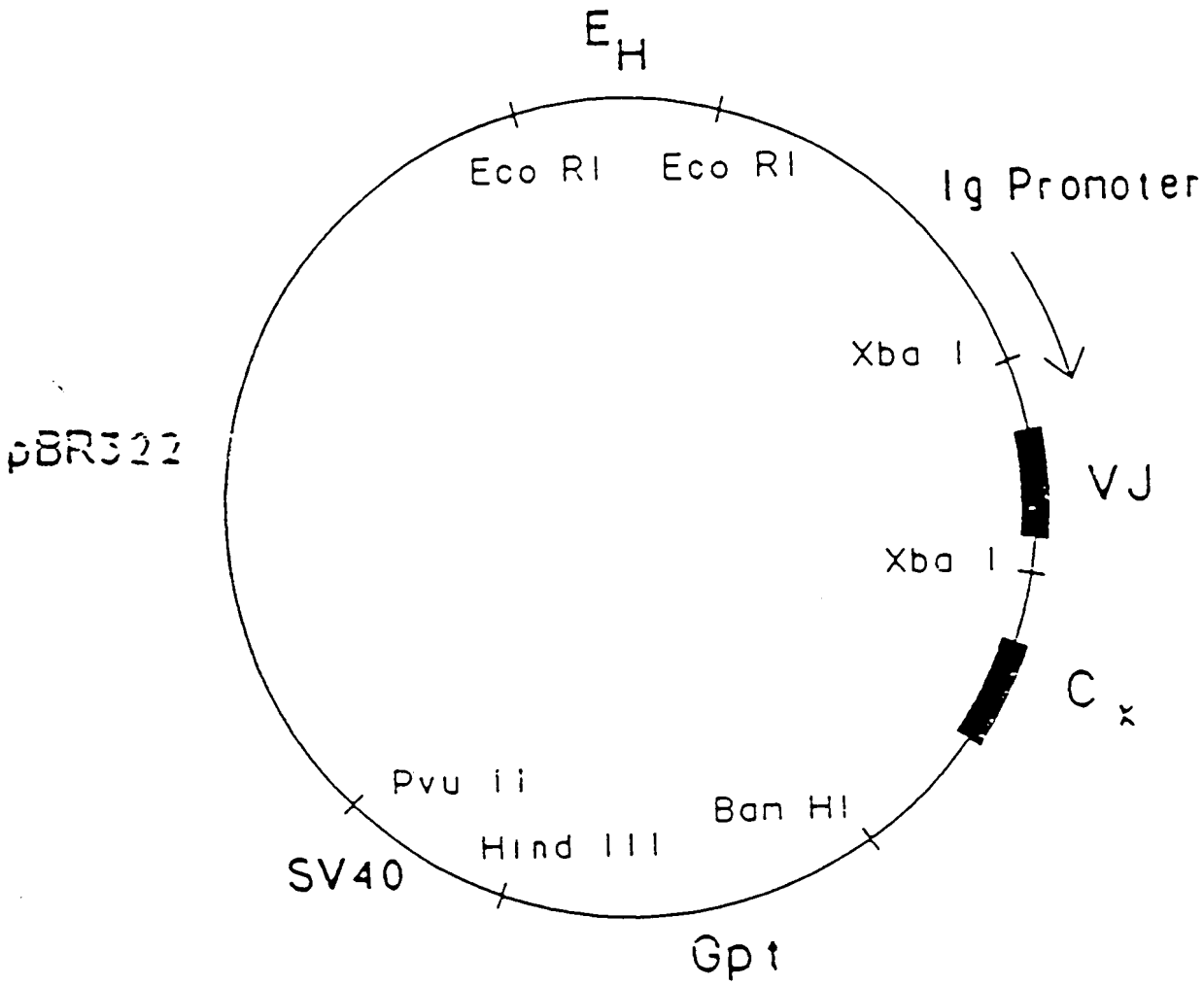


FIGURE 9

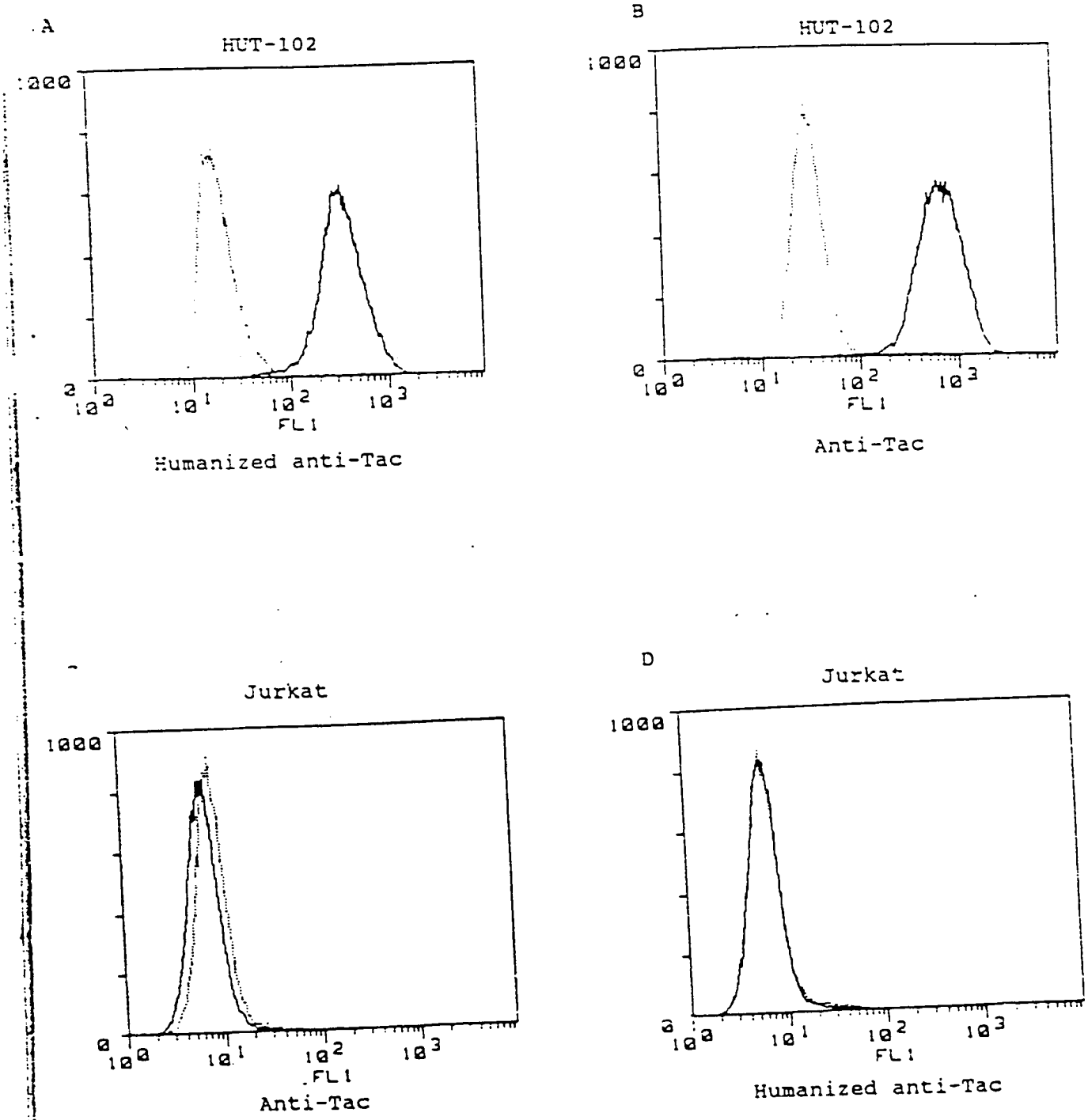
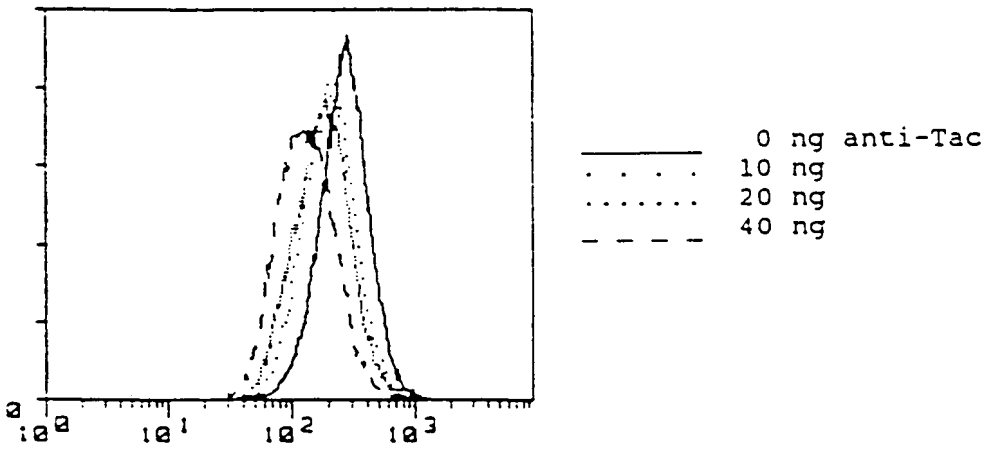
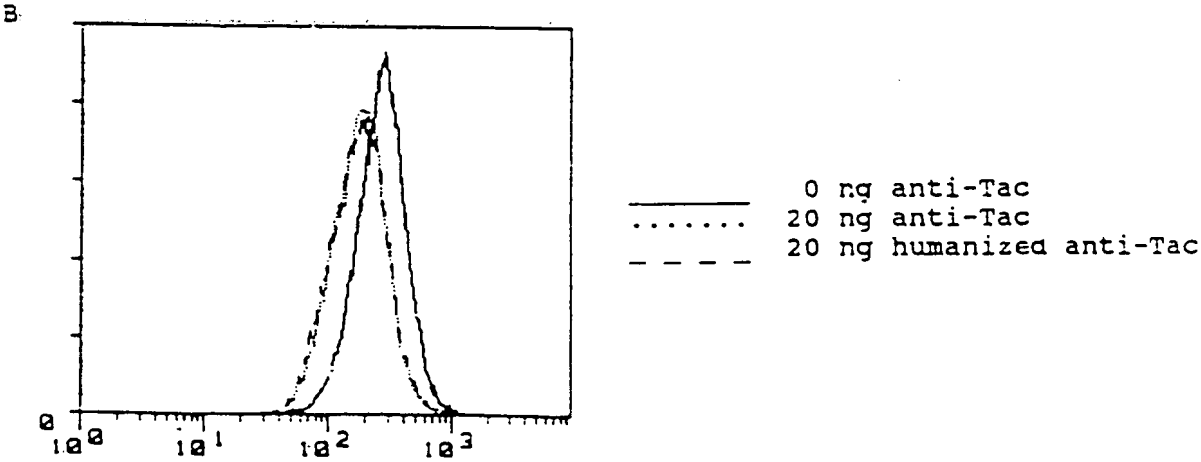


FIGURE 10

A



B



#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.

JULIE REEVES
PATENT EXAMINER

Examiner Note: You must sign and stamp this form unless it is an attachment to a signed Office action.

4/49

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
Signature *4/9/99* *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

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

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

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
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

HM22/1124

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 P 709P1 VS

DEA/FCE-1994

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

GB 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
Fax: (650) 952-9881

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
```

```
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 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
```

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:

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