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Docket No. 709

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

**NEW APPLICATION TRANSMITTAL**

SIR:

Transmitted herewith for filing is the patent application of Inventor(s): **PAUL J. CARTER ET AL.**

Title: **IMMUNOGLOBULIN VARIANTS**

**CERTIFICATION UNDER 37 CFR §1.10**

I hereby certify that this New Application and the documents referred to as enclosed herein are being deposited with the United States Postal Service on this date June 14, 1991, in an envelope bearing "Express Mail Post Office To Addressee" Mailing Label Number B59937585 addressed to: Patent Application, Honorable Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Carolyn R. Adler  
(Name of person mailing paper)

*Carolyn R. Adler*  
Signature

Enclosed are:

- The papers required for filing date under CFR §1.53(b):  
106 Pages of specification (including claims); 5 Sheets of drawings (   formal /   x informal)
- x Declaration/Oath/Power of Attorney
- Assignment of the invention to GENENTECH, INC.
- Fee Calculation

| CLAIMS AS FILED                     |              |              |           |                 |
|-------------------------------------|--------------|--------------|-----------|-----------------|
|                                     | Number Filed | Number Extra | Rate      | Basic Fee \$630 |
| Total Claims                        | 16 - 20 =    | *            | x \$20.00 | 630.            |
| Indep. Claims                       | 8 - 3 =      | * 5          | x \$60.00 | 300.            |
| Multiple dependent claim(s), if any |              |              | \$200.00  |                 |

\*If less than zero, enter "0".

7.    Recording Assignment [\$8.00] ..... \$  
**Total Fees Enclosed ..... \$930.00**

8. Payment of Fees

  x Charge Account No. 07-0630 in the amount of \$  . A duplicate of this transmittal is attached.

9.   x Authorization to Charge Additional Fees

The Commissioner is hereby authorized to charge any additional fees (or credit any overpayment) associated with this communication and which may be required under 37 CFR §1.16 or §1.17 to Account No. 07-0630. A duplicate sheet is attached.

10.    Information Disclosure Statement

11.   x Return Receipt Postcard

By: *Carolyn R. Adler*  
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Dated June 14, 1991

FIGURE 1A: V<sub>L</sub> DOMAIN

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|                     |  |    |    |                      |    |
|---------------------|--|----|----|----------------------|----|
|                     | 10   | 20 | 30 | 40                   | 50 |
| 4D5                 | DIVMTQSHKFMSTSVGDRVSITCKASQDVNTAVAWYQOKPGHSPKLLIYSASFRYT |    |    |                      |    |
|                     |  |    |    |                      |    |
| HU4D5               | DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQOKPGKAPKLLIYSASFLES |    |    |                      |    |
|                     |  |    |    |                      |    |
| HUV <sub>L</sub> κI | DIQMTQSPSSLSASVGDRVTITCRASQDVSSYLAWYQOKPGKAPKLLIYAASSLES |    |    |                      |    |
|                     |  |    |    |                      |    |
|                     | -----  |    |    | -----                |    |
|                     | V <sub>L</sub> -CDR1                                     |    |    | V <sub>L</sub> -CDR2 |    |

|                     |  |    |    |    |     |
|---------------------|--|----|----|----|-----|
|                     | 60   | 70 | 80 | 90 | 100 |
| 4D5                 | GVPDRFTGNRSGTDFFTISSVQAEDLAVYYCQQHYTTPPTFGGGTKLEIKRA |    |    |    |     |
|                     |  |    |    |    |     |
| HU4D5               | GVPSRFSGSRSGTDFTLTISLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRT |    |    |    |     |
|                     |  |    |    |    |     |
| HUV <sub>L</sub> κI | GVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQYNSLPYTFGQGTKVEIKRT |    |    |    |     |
|                     |  |    |    |    |     |
|                     | -----  |    |    |    |     |
|                     | V <sub>L</sub> -CDR3                                 |    |    |    |     |

FIGURE 1B: V<sub>H</sub> DOMAIN

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|                      |                       |                                     |                      |    |    |                      |
|----------------------|-----------------------|-------------------------------------|----------------------|----|----|----------------------|
|                      | 10                    | 20                                  | 30                   | 40 | 50 | A                    |
| 4D5                  | EVQLQQSGPELVKPGASLKL  | SCTASGFNIKDTYIHWVKQRPEQGLEWIGRIYPTN |                      |    |    |                      |
| HU4D5                | EVQLVESGGGLVQPGGSLRLS | CAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTN  |                      |    |    |                      |
| HUV <sub>H</sub> III | EVQLVESGGGLVQPGGSLRLS | CAASGFTFSDYAMSWVRQAPGKGLEWVAVISENG  |                      |    |    |                      |
|                      |                       |                                     | -----                |    |    | -----                |
|                      |                       |                                     | -----                |    |    | -----                |
|                      |                       |                                     | V <sub>H</sub> -CDR1 |    |    | V <sub>H</sub> -CDR2 |

|                      |                       |            |                            |                        |    |                      |
|----------------------|-----------------------|------------|----------------------------|------------------------|----|----------------------|
|                      | 60                    | 70         | 80                         | ABC                    | 90 | 100ABC               |
| 4D5                  | GYTRYDPKFQDKATITADTSS | NNTAYLQVSR | LTS                        | EDTAVYYCSRWGGDGFYAMDYW |    |                      |
| HU4D5                | GYTRYADSVKGRFTISADTS  | KNTAYLQMN  | SLRAEDTAVYYCSRWGGDGFYAMDVW |                        |    |                      |
| HUV <sub>H</sub> III | SDTYYADSVKGRFTISRDDS  | KNTLYLQMN  | SLRAEDTAVYYCARDRGGAVSYFDVW |                        |    |                      |
|                      |                       |            | -----                      |                        |    | -----                |
|                      |                       |            |                            |                        |    | -----                |
|                      |                       |            |                            |                        |    | V <sub>H</sub> -CDR3 |

|                      |            |
|----------------------|------------|
|                      | 110        |
| 4D5                  | GQGASVTVSS |
|                      |            |
| HU4D5                | GQGLVTVSS  |
| HUV <sub>H</sub> III | GQGLVTVSS  |

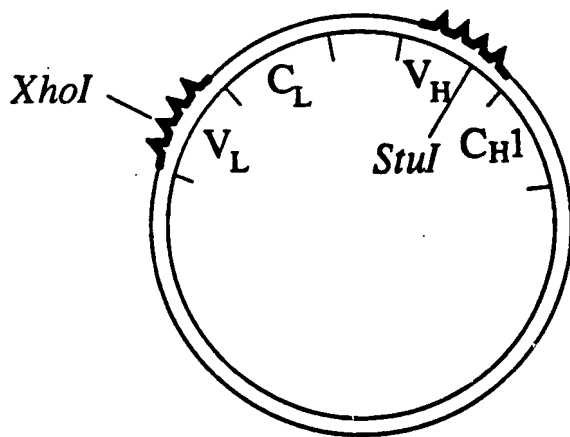
# FIGURE 2

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Anneal huV<sub>L</sub> or huV<sub>H</sub> oligomers to pAK1 template



1. Ligate
2. Isolate assembled oligomers
3. Anneal to pAK1 template (*Xho*I<sup>-</sup>, *Stu*I<sup>+</sup>)
4. Extend and ligate



1. Transform *E. coli*
2. Isolate phagemid pool
3. Enrich for huV<sub>L</sub> and huV<sub>H</sub> (*Xho*I<sup>+</sup>, *Stu*I<sup>-</sup>)
4. Sequence verify

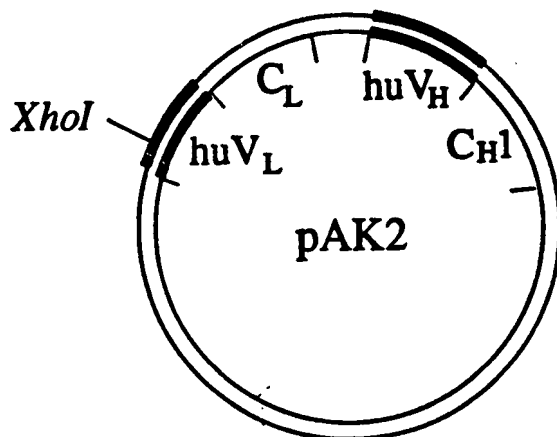
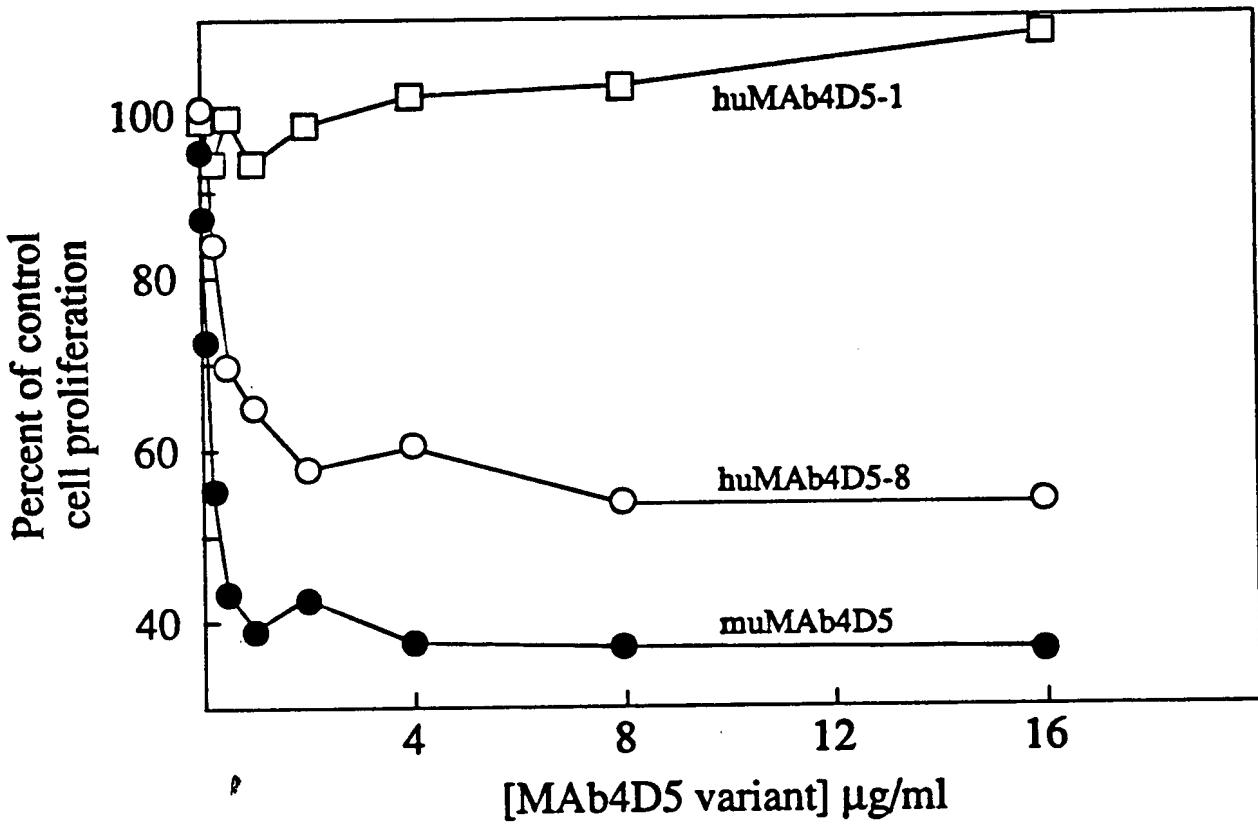


FIGURE 3



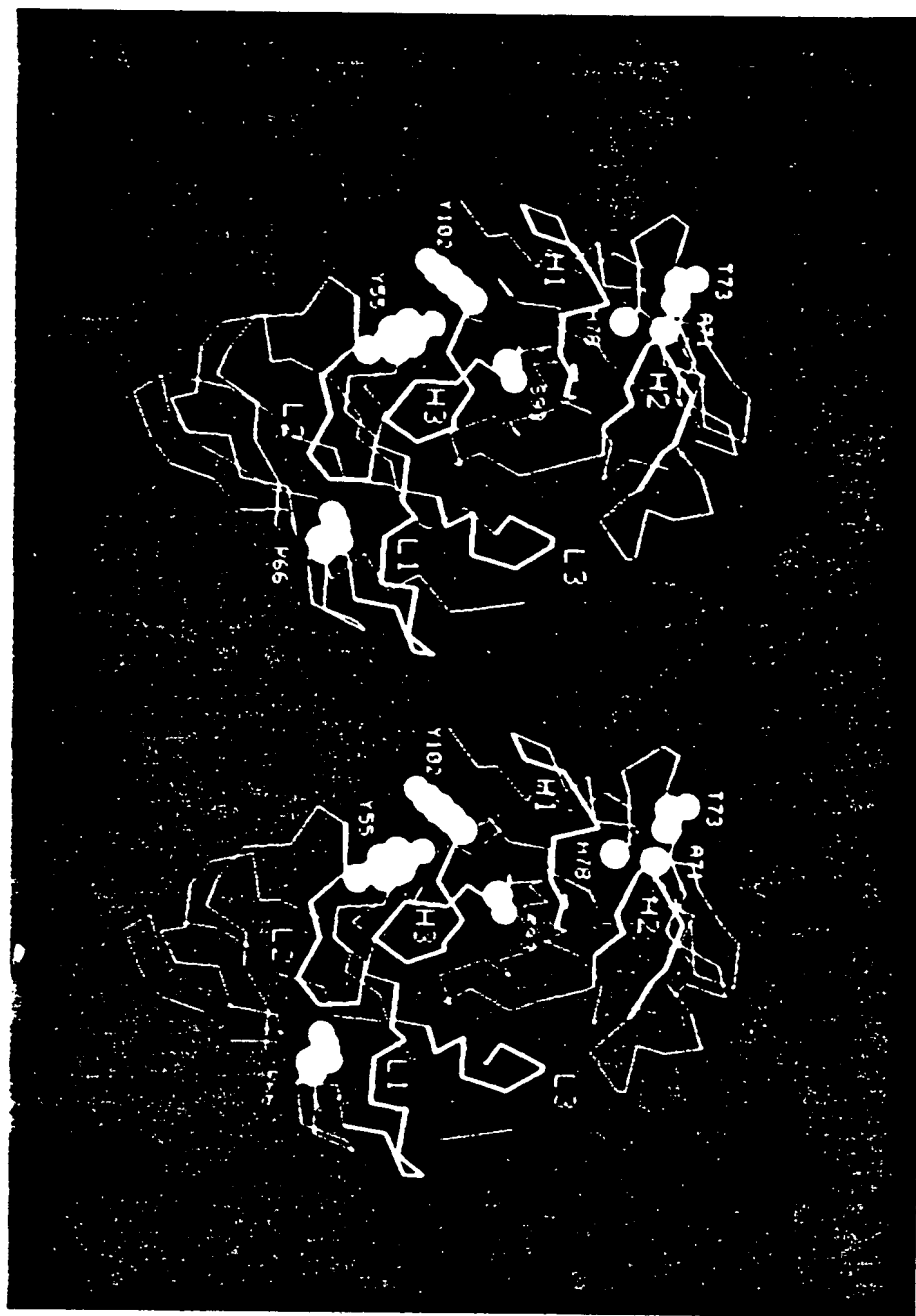


FIGURE 4



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501 IMMUNOGLOBULIN VARIANTS

Field of the Invention

This invention relates to methods for the preparation and use of variant antibodies and finds application particularly in the fields of immunology and cancer diagnosis and therapy.

Background of the Invention

Naturally occurring antibodies (immunoglobulins) comprise two heavy chains linked together by disulfide bonds and two light chains, one light chain being linked to each of the heavy chains by disulfide bonds. Each heavy chain has at one end a variable domain ( $V_H$ ) followed by a number of constant domains. Each light chain has a variable domain ( $V_L$ ) at one end and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light and heavy chain variable domains, see e.g. Chothia *et al.*, *J. Mol. Biol.* 186:651-663 (1985); Novotny and Haber, *Proc. Natl. Acad. Sci.*

USA 82:4592-4596 (1985).

5 The constant domains are not involved directly in binding the antibody to an antigen, but are involved in various effector functions, such as participation of the antibody in antibody-dependent cellular cytotoxicity. The variable domains of each pair of light and heavy chains are involved directly in binding the antibody to the antigen. The domains of natural light and heavy chains have the same general structure, and each domain comprises four framework (FR) regions, whose sequences are somewhat conserved, connected by three hyper-variable or complementarity determining regions (CDRs) (see Kabat, E. A. *et al.*, *Sequences of Proteins of Immunological Interest*, National Institutes of Health, Bethesda, MD, 10 (1987)). The four framework regions largely adopt a  $\beta$ -sheet conformation and the CDRs form loops connecting, and in some cases forming part of, the  $\beta$ -sheet structure. The CDRs in each chain are held in close proximity by the framework regions and, with the CDRs from the other chain, contribute to 15 the formation of the antigen binding site.

Widespread use has been made of monoclonal antibodies, particularly those derived from rodents including mice, however they are frequently antigenic in human clinical use. For example, a major limitation in 20 the clinical use of rodent monoclonal antibodies is an anti-globulin response during therapy (Miller, R. A. *et al.*, *Blood* 62:988-995 (1983); Schroff, R. W. *et al.*, *Cancer Res.* 45:879-885 (1985)).

25 The art has attempted to overcome this problem by constructing "chimeric" antibodies in which an animal antigen-binding variable domain is coupled to a human constant domain (Cabilly *et al.*, U.S. patent No. 4,816,567; Morrison, S. L. *et al.*, *Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984); Boulianne, G. L. *et al.*, *Nature* 312:643-646 (1984); Neuberger, M. S. *et al.*, *Nature* 314:268-270 (1985)). The term "chimeric" antibody is used herein to describe a polypeptide comprising at least the antigen binding 30 portion of an antibody molecule linked to at least part of another protein (typically an immunoglobulin constant domain).

The isotype of the human constant domain may be selected to tailor



the chimeric antibody for participation in antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (see e.g. Brüggemann, M. *et al.*, *J. Exp. Med.* 166:1351-1361 (1987); Riechmann, L. *et al.*, *Nature* 332:323-327 (1988); Love *et al.*, *Methods in Enzymology* 178:515-527 (1989); Bindon *et al.*, *J. Exp. Med.* 168:127-142 (1988).

In the typical embodiment, such chimeric antibodies contain about one third rodent (or other non-human species) sequence and thus are capable of eliciting a significant anti-globulin response in humans. For example, in the case of the murine anti-CD3 antibody, OKT3, much of the resulting anti-globulin response is directed against the variable region rather than the constant region (Jaffers, G. J. *et al.*, *Transplantation* 41:572-578 (1986)).

In a further effort to resolve the antigen binding functions of antibodies and to minimize the use of heterologous sequences in human antibodies, Winter and colleagues (Jones, P. T. *et al.*, *Nature* 321:522-525 (1986); Riechmann, L. *et al.*, *Nature* 332:323-327 (1988); Verhoeyen, M. *et al.*, *Science* 239:1534-1536 (1988)) have substituted rodent CDRs or CDR sequences for the corresponding segments of a human antibody. As used herein, the term "humanized" antibody is an embodiment of chimeric antibodies wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

The therapeutic promise of this approach is supported by the clinical efficacy of a humanized antibody specific for the CAMPATH-1 antigen with two non-Hodgkin lymphoma patients, one of whom had previously developed an anti-globulin response to the parental rat antibody (Riechmann, L. *et al.*, *Nature* 332:323-327 (1988); Hale, G. *et al.*, *Lancet* i:1394-1399 (1988)). A murine antibody to the interleukin 2 receptor has also recently been humanized (Queen, C. *et al.*, *Proc. Natl. Acad. Sci. USA* 86:10029-10033 (1989)) as a potential immunosuppressive reagent. Additional references related to humanization of antibodies include Co *et al.*, *Proc. Natl. Acad. Sci.*

USA 88:2869-2873 (1991); Gorman *et al.*, *Proc. Natl. Acad. Sci. USA* 88:4181-4185 (1991); Daugherty *et al.*, *Nucleic Acids Research* 19(9):2471-2476 (1991); Brown *et al.*, *Proc. Natl. Acad. Sci. USA* 88:2663-2667 (1991); Junghans *et al.*, *Cancer Research* 50:1495-1502 (1990).

5 In some cases, substituting CDRs from rodent antibodies for the human CDRs in human frameworks is sufficient to transfer high antigen binding affinity (Jones, P. T. *et al.*, *Nature* 321:522-525 (1986); Verhoeyen, M. *et al.*, *Science* 239:1534-1536 (1988)), whereas in other cases it has been necessary to additionally replace one (Riechmann, L. *et al.*, *Nature* 10 332:323-327 (1988)) or several (Queen, C. *et al.*, *Proc. Natl. Acad. Sci. USA* 86:10029-10033 (1989)) framework region (FR) residues. See also Co *et al.*, *supra*.

For a given antibody a small number of FR residues are anticipated to be important for antigen binding. Firstly for example, certain antibodies 15 have been shown to contain a few FR residues which directly contact antigen in crystal structures of antibody-antigen complexes (e.g., reviewed in Davies, D. R. *et al.*, *Ann. Rev. Biochem.* 59:439-473 (1990)). Secondly, a number of FR residues have been proposed by Chothia, Lesk and colleagues (Chothia, C. & Lesk, A. M., *J. Mol. Biol.* 196:901-917 (1987); Chothia, C. *et al.*, 20 *Nature* 342:877-883 (1989); Tramontano, A. *et al.*, *J. Mol. Biol.* 215:175-182 (1990)) as critically affecting the conformation of particular CDRs and thus their contribution to antigen binding. See also Margolies *et al.*, *Proc. Natl. Acad. Sci. USA* 72:2180-2184 (1975).

It is also known that, in a few instances, an antibody variable 25 domain (either V<sub>H</sub> or V<sub>L</sub>) may contain glycosylation sites, and that this glycosylation may improve or abolish antigen binding, Pluckthun, *Biotechnology* 9:545-51 (1991); Spiegelberg *et al.*, *Biochemistry* 9:4217-4223 (1970); Wallic *et al.*, *J. Exp. Med.* 168:1099-1109 (1988); Sox *et al.*, *Proc. Natl. Acad. Sci. USA* 66:975-982 (1970); Margni *et al.*, *Ann. Rev. Immunol.* 6:535-554 (1988). Ordinarily, however, glycosylation has no 30 influence on the antigen-binding properties of an antibody, Pluckthun, *supra*, (1991).

The three-dimensional structure of immunoglobulin chains has been studied, and crystal structures for intact immunoglobulins, for a variety of immunoglobulin fragments, and for antibody-antigen complexes have been published (see e.g., Saul *et al.*, *Journal of Biological Chemistry* 25:585-97 (1978); Sheriff *et al.*, *Proc. Natl. Acad. Sci. USA* 84:8075-79 (1987); Segal *et al.*, *Proc. Natl. Acad. Sci. USA* 71:4298-4302 (1974); Epp *et al.*, *Biochemistry* 14(22):4943-4952 (1975); Marquart *et al.*, *J. Mol. Biol.* 141:369-391 (1980); Furey *et al.*, *J. Mol. Biol.* 167:661-692 (1983); Snow and Amzel, *Protein: Structure, Function, and Genetics* 1:267-279, Alan R. Liss, Inc. pubs. (1986); Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987); Chothia *et al.*, *Nature* 342:877-883 (1989); Chothia *et al.*, *Science* 233:755-58 (1986); Huber *et al.*, *Nature* 264:415-420 (1976); Bruccoleri *et al.*, *Nature* 335:564-568 (1988) and *Nature* 336:266 (1988); Sherman *et al.*, *Journal of Biological Chemistry* 263:4064-4074 (1988); Amzel and Poljak, *Ann. Rev. Biochem.* 48:961-67 (1979); Silverton *et al.*, *Proc. Natl. Acad. Sci. USA* 74:5140-5144 (1977); and Gregory *et al.*, *Molecular Immunology* 24:821-829 (1987). It is known that the function of an antibody is dependent on its three dimensional structure, and that amino acid substitutions can change the three-dimensional structure of an antibody, Snow and Amzel, *supra*. It has previously been shown that the antigen binding affinity of a humanized antibody can be increased by mutagenesis based upon molecular modelling (Riechmann, L. *et al.*, *Nature* 332:323-327 (1988); Queen, C. *et al.*, *Proc. Natl. Acad. Sci. USA* 86:10029-10033 (1989)).

Humanizing an antibody with retention of high affinity for antigen and other desired biological activities is at present difficult to achieve using currently available procedures. Methods are needed for rationalizing the selection of sites for substitution in preparing such antibodies and thereby increasing the efficiency of antibody humanization.

The proto-oncogene *HER2* (human epidermal growth factor receptor 2) encodes a protein tyrosine kinase (p185<sup>HER2</sup>) that is related to and somewhat homologous to the human epidermal growth factor receptor

(see Coussens, L. *et al.*, *Science* 230:1132-1139 (1985); Yamamoto, T. *et al.*, *Nature* 319:230-234 (1986); King, C. R. *et al.*, *Science* 229:974-976 (1985)). *HER2* is also known in the field as *c-erbB-2*, and sometimes by the name of the rat homolog, *neu*. Amplification and/or overexpression of *HER2* is associated with multiple human malignancies and appears to be integrally involved in progression of 25-30% of human breast and ovarian cancers (Slamon, D. J. *et al.*, *Science* 235:177-182 (1987), Slamon, D. J. *et al.*, *Science* 244:707-712 (1989)). Furthermore, the extent of amplification is inversely correlated with the observed median patient survival time (Slamon, *supra*, *Science* 1989).

The murine monoclonal antibody known as muMAb4D5 (Fendly, B. M. *et al.*, *Cancer Res.* 50:1550-1558 (1990)), directed against the extracellular domain (ECD) of p185<sup>HER2</sup>, specifically inhibits the growth of tumor cell lines overexpressing p185<sup>HER2</sup> in monolayer culture or in soft agar (Hudziak, R. M. *et al.*, *Molec. Cell. Biol.* 9:1165-1172 (1989); Lupu, R. *et al.*, *Science* 249:1552-1555 (1990)). MuMAb4D5 also has the potential of enhancing tumor cell sensitivity to tumor necrosis factor, an important effector molecule in macrophage-mediated tumor cell cytotoxicity (Hudziak, *supra*, 1989; Shepard, H. M. and Lewis, G. D. *J. Clinical Immunology* 8:333-395 (1988)). Thus muMAb4D5 has potential for clinical intervention in and imaging of carcinomas in which p185<sup>HER2</sup> is overexpressed. The muMAb4D5 and its uses are described in copending U.S. patent applications 07/143,912 and 07/147,461, and in corresponding PCT application WO 89/06692 published 27 July 1989. This murine antibody was deposited with the ATCC and designated ATCC CRL 10463. However, this antibody may be immunogenic in humans.

It is therefore an object of this invention to provide methods for the preparation of antibodies which are less antigenic in humans than non-human antibodies but have desired antigen binding and other characteristics and activities.

It is a further object of this invention to provide methods for the efficient humanization of antibodies, i.e. selecting non-human amino acid

residues for importation into a human antibody background sequence in such a fashion as to retain or improve the affinity of the non-human donor antibody for a given antigen.

5 It is another object of this invention to provide humanized antibodies capable of binding p185<sup>HER2</sup>.

Other objects, features, and characteristics of the present invention will become apparent upon consideration of the following description and the appended claims.

10 Summary of the Invention

The objects of this invention are accomplished by a method for making a humanized antibody comprising amino acid sequence of an import, non-human antibody and a human antibody, comprising the steps of:

- 15 a. obtaining the amino acid sequences of at least a portion of an import antibody variable domain and of a consensus human variable domain;
- b. identifying Complementarity Determining Region (CDR) amino acid sequences in the import and the human variable domain sequences;
- 20 c. substituting an import CDR amino acid sequence for the corresponding human CDR amino acid sequence;
- d. aligning the amino acid sequences of a Framework Region (FR) of the import antibody and the corresponding FR of the consensus antibody;
- 25 e. identifying import antibody FR residues in the aligned FR sequences that are non-homologous to the corresponding consensus antibody residues;
- f. determining if the non-homologous import amino acid residue is reasonably expected to have at least one of the following effects:
- 30 1. non-covalently binds antigen directly,

2. interacts with a CDR; or
  3. participates in the  $V_L - V_H$  interface; and
- g. for any non-homologous import antibody amino acid residue which is reasonably expected to have at least one of these effects, substituting that residue for the corresponding amino acid residue in the consensus antibody FR sequence.

Optionally, the method of this invention comprises the additional steps of determining if any non-homologous residues identified in step (e) are exposed on the surface of the domain or buried within it, and if the residue is exposed but has none of the effects identified in step (f), retaining the consensus residue.

Additionally, in certain embodiments the method of this invention comprises the feature wherein the corresponding consensus antibody residues identified in step (e) above are selected from the group consisting of 4L, 35L, 36L, 38L, 43L, 44L, 46L, 58L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H (utilizing the numbering system set forth in Kabat, E. A. *et al.*, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)).

In certain embodiments, the method of this invention comprises the additional steps of searching either or both of the import, non-human and the consensus variable domain sequences for glycosylation sites, determining if the glycosylation is reasonably expected to be important for the desired antigen binding and biological activity of the antibody (i.e., determining if the glycosylation site binds to antigen or changes a side chain of an amino acid residue that binds to antigen, or if the glycosylation enhances or weakens antigen binding, or is important for maintaining antibody affinity). If the import sequence bears the glycosylation site, it is preferred to substitute that site for the corresponding residues in the consensus human sequence if the glycosylation site is reasonably expected to be important. If only the

consensus sequence, and not the import, bears the glycosylation site, it is preferred to eliminate that glycosylation site or substitute therefor the corresponding amino acid residues from the import sequence.

Another embodiment of this invention comprises aligning import antibody and the consensus antibody FR sequences, identifying import antibody FR residues which are non-homologous with the aligned consensus FR sequence, and for each such non-homologous import antibody FR residue, determining if the corresponding consensus antibody residue represents a residue which is highly conserved across all species at that site, and if it is so conserved, preparing a humanized antibody which comprises the consensus antibody amino acid residue at that site.

Certain alternate embodiments of the methods of this invention comprise obtaining the amino acid sequence of at least a portion of an import, non-human antibody variable domain having a CDR and a FR, obtaining the amino acid sequence of at least a portion of a consensus human antibody variable domain having a CDR and a FR, substituting the non-human CDR for the human CDR in the consensus human antibody variable domain, and then substituting an amino acid residue for the consensus amino acid residue at at least one of the following sites:

- a. (in the FR of the variable domain of the light chain) 4L, 35L, 36L, 38L, 43L, 44L, 58L, 46L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, or
- b. (in the FR of the variable domain of the heavy chain) 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H.

In preferred embodiments, the non-CDR residue substituted at the consensus FR site is the residue found at the corresponding location of the non-human antibody.

Optionally, this just-recited embodiment comprises the additional steps of following the method steps appearing at the beginning of this summary and determining whether a particular amino acid residue can

reasonably be expected to have undesirable effects.

This invention also relates to a humanized antibody comprising the CDR sequence of an import, non-human antibody and the FR sequence of a human antibody, wherein an amino acid residue within the human FR sequence located at any one of the sites 4L, 35L, 36L, 38L, 43L, 44L, 46L, 58L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H has been substituted by another residue. In preferred embodiments, the residue substituted at the human FR site is the residue found at the corresponding location of the non-human antibody from which the non-human CDR was obtained. In other embodiments, no human FR residue other than those set forth in this group has been substituted.

This invention also encompasses specific humanized antibody variable domains, and isolated polypeptides having homology with the following sequences.

1. SEQ. ID NO. 1, which is the light chain variable domain of a humanized version of muMAb4D5:

DIQMTQSPSSLSASVGDRVITTCRASQDVNTAVAWYQQKPGKAP  
KLLIYSASFLESGVPSRFSGSRSGTDFTLTSSLPEDFATYYCQQHY  
TTPPTFGQGKVEIKRT

2. SEQ. ID NO. 2, which is the heavy chain variable domain of a humanized version of muMAb4D5):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVROAPGKGLE  
WVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDT  
AVYYCSRWGGDGFYAMDVWGQGLVTVSS

In another aspect, this invention provides a consensus human antibody variable domain amino acid sequence for use in the preparation of humanized antibodies, methods for obtaining, using, and storing a computer representation of such a consensus sequence, and computers comprising the



sequence data of such a sequence. In one embodiment, the following consensus human antibody variable domain amino acid sequences are provided:

5 SEQ. ID NO. 3 (light chain):

DIQMTQSPSSLSASVGRVTITCRASQDVSSYLAWYQQKPGKAPK  
LLIYAASSLESQVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQYN  
SLPYTFGQGTKVEIKRT, and

10 SEQ. ID NO. 4 (heavy chain):

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYAMSWVRQAPGKG  
LEWVAVISENGGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAE  
DTAVYYCSRWGGDGFYAMDVWGQGLTVTVSS

15 Brief Description of the Drawings

FIGURE 1A shows the comparison of the  $V_L$  domain amino acid residues of muMAb4D5, huMAb4D5, and a consensus human sequence (Fig. 1A, SEQ.ID NO. 5, SEQ. ID NO. 1 and SEQ. ID NO. 3, respectively). FIGURE 1B shows the comparison between the  $V_H$  domain amino acid residues of the muMAb4d5, huMAb4D5, and a consensus human sequence (Fig. 1B, SEQ. ID NO. 6, SEQ. ID NO. 2 and SEQ. ID NO. 4, respectively). Both Figs 1A and 1B use the generally accepted numbering scheme from Kabat, E. A., *et al.*, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD (1987)). In both Fig. 1A and Fig. 1B, the CDR residues determined according to a standard sequence definition (as in Kabat, E. A. *et al.*, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)) are indicated by the first underlining beneath the sequences, and the CDR residues determined according to a structural definition (as in Chothia, C. & Lesk, A. M., *J. Mol. Biol.* 196:901-917 (1987)) are indicated by the second, lower underlines.

a The mismatches between <sup>sequences</sup> genes are shown by the vertical lines.

FIGURE 2 shows a scheme for humanization of muMAb4D5 V<sub>L</sub> and V<sub>H</sub> by gene conversion mutagenesis.

5 FIGURE 3 shows the inhibition of SK-BR-3 proliferation by MAb4D5 variants. Relative cell proliferation was determined as described (Hudziak, R. M. *et al.*, *Molec. Cell. Biol.* 9:1165-1172 (1989)) and data (average of triplicate determinations) are presented as a percentage of results with untreated cultures for muMAb4D5 (l), huMAb4D5-8 (n) and huMAb4D5-1 (l).

10 FIGURE 4 shows a stereo view of  $\alpha$ -carbon tracing for model of huMAb4D5-8 V<sub>L</sub> and V<sub>H</sub>. The CDR residues (Kabat, E. A. *et al.*, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)) are shown in bold and side chains of V<sub>H</sub> residues A71, T73, A78, S93, Y102 and V<sub>L</sub> residues Y55 plus R66 (see Table 1) are shown.

15 Detailed Description of the Invention

Definitions

20 In general, the following words or phrases have the indicated definitions when used in the description, examples, and claims:

25 The murine monoclonal antibody known as muMAb4D5 (Fendly, B. M. *et al.*, *Cancer Res.* 50:1550-1558 (1990)) is directed against the extracellular domain (ECD) of p185<sup>HER2</sup>. The muMAb4D5 and its uses are described in copending U.S. patent applications 07/143,912 and 07/147,461, and in corresponding PCT application WO 89/06692 published 27 July 1989. This murine antibody was deposited with the ATCC and designated ATCC CRL 10463. In this description and claims, the terms muMAb4D5, chMAb4D5 and huMAb4D5 represent murine, chimerized and humanized versions of the monoclonal antibody 4D5, respectively.

30 A humanized antibody for the purposes herein is an immunoglobulin amino acid sequence variant or fragment thereof which is capable of binding to a predetermined antigen and which comprises a FR region having

substantially the amino acid sequence of a human immunoglobulin and a CDR having substantially the amino acid sequence of a non-human immunoglobulin.

5 In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains (Fab) in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. Ordinarily, the antibody will contain both the light chain as well as at least the variable domain of a heavy chain. The antibody also may include the CH1, hinge, CH2, CH3, and CH4 regions of the heavy chain.

10 The humanized antibody will be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub> and IgG<sub>4</sub>. Usually the constant domain is a complement fixing constant domain where it is desired that the humanized antibody exhibit cytotoxic activity, and the class is typically IgG<sub>1</sub>. Where such cytotoxic activity is not desirable, the constant domain may be of the IgG<sub>2</sub> class. The humanized antibody may comprise sequences from more than one class or isotype, and selecting particular constant domains to optimize desired effector functions is within the ordinary skill in the art.

20 The FR and CDR regions of the humanized antibody need not correspond precisely to the parental sequences, e.g., the import CDR or the consensus FR may be mutagenized by substitution, insertion or deletion of a residue so that the CDR or FR residue at that site does not correspond to either the consensus or the import antibody. Such mutations, however, will not be extensive. Usually, at least 75% of the humanized antibody residues will correspond to those of the parental FR and CDR sequences, more often 30 90%, and most preferably greater than 95%.

In general, humanized antibodies prepared by the method of this invention are produced by a process of analysis of the parental sequences

and various conceptual humanized products using three dimensional models of the parental and humanized sequences. Three dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen.

Residues that influence antigen binding are defined to be residues that are substantially responsible for the antigen affinity or antigen specificity of a candidate immunoglobulin, in a positive or a negative sense. The object here is to select FR residues from the consensus and import sequence so that the desired immunoglobulin characteristic is achieved. Such desired characteristics include increases in affinity and greater specificity for the target antigen, although it is conceivable that in some circumstances the opposite effects might be desired. In general, the CDR residues are directly and most substantially involved in influencing antigen binding (although not all CDR residues are so involved and therefore need not be substituted into the consensus sequence). However, FR residues also have a significant effect and can exert their influence in at least three ways: They may noncovalently directly bind to antigen, they may interact with CDR residues and they may affect the interface between the heavy and light chains.

A residue that noncovalently directly binds to antigen is one that, by three dimensional analysis, is reasonably expected to noncovalently directly bind to antigen. Typically, it is necessary to impute the position of antigen from the spatial location of neighboring CDRs and the dimensions and structure of the target antigen. In general, only those humanized antibody residues that are capable of forming salt bridges, hydrogen bonds, or hydrophobic interactions are likely to be involved in non-covalent antigen binding, however residues which are separated spatially by 3.2 Angstroms or less may also non-covalently interact. Such residues typically are the

relatively larger amino acids, such as tyrosine, arginine, and lysine. Antigen-binding FR residues also typically will have side chains that are oriented into an envelope surrounding the solvent oriented face of a CDR which extends about 7 Angstroms into the solvent from the CDR domain and about 7 Angstroms on either side of the CDR domain, again as visualized by three dimensional modeling.

A residue that interacts with a CDR generally is a residue that either affects the conformation of the CDR polypeptide backbone or forms a noncovalent bond with a CDR residue side chain. Conformation-affecting residues ordinarily are those that change the spatial position of any CDR backbone atom (N, C $\alpha$ , C, O, C $\beta$ ) by more than about 0.2 Angstroms. Backbone atoms of CDR sequences are displaced for example by residues that interrupt or modify organized structures such as beta sheets, helices or loops. Residues that can exert a profound affect on the conformation of neighboring sequences include proline and glycine, both of which are capable of introducing bends into the backbone. Other residues that can displace backbone atoms are those that are capable of participating in salt bridges and hydrogen bonds.

A residue that interacts with a CDR side chain is one that is reasonably expected to form a noncovalent bond with a CDR side chain, generally either a salt bridge or hydrogen bond. Such residues are identified by three dimensional positioning of their side chains. A salt or ion bridge could be expected to form between two side chains positioned within about 2.5 - 3.2 Angstroms of one another that bear opposite charges, for example a lysinyl and a glutamyl pairing. A hydrogen bond could be expected to form between the side chains of residue pairs such as seryl or threonyl with aspartyl or glutamyl (or other hydrogen accepting residues). Such pairings are well known in the protein chemistry art and will be apparent to the artisan upon three dimensional modeling of the candidate immunoglobulin.

Immunoglobulin residues that affect the interface between heavy and light chain variable regions ("the V<sub>L</sub> - V<sub>H</sub> interface") are those that affect the proximity or orientation of the two chains with respect to one another.

Certain residues involved in interchain interactions are already known and include V<sub>L</sub> residues 34, 36, 38, 44, 46, 87, 89, 91, 96, and 98 and V<sub>H</sub> residues 35, 37, 39, 45, 47, 91, 93, 95, 100, and 103 (utilizing the nomenclature set forth in Kabat *et al.*, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)).

Additional residues are newly identified by the inventors herein, and include 43L, 85L, 43H and 60H. While these residues are indicated for IgG only, they are applicable across species. In the practice of this invention, import antibody residues that are reasonably expected to be involved in interchain interactions are selected for substitution into the consensus human sequence. It is believed that heretofore no humanized antibody has been prepared with an <sup>interchain</sup>~~intra-chain~~-affecting residue selected from an import antibody sequence.

Since it is not entirely possible to predict in advance what the exact impact of a given substitution will be it may be necessary to make the substitution and assay the candidate antibody for the desired characteristic. These steps, however, are *per se* routine and well within the ordinary skill of the art.

CDR and FR residues are determined according to a standard sequence definition (Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, National Institutes of Health, Bethesda MD (1987), hereby specifically incorporated by reference), and a structural definition (as in Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987), hereby specifically incorporated by reference). Where these two methods result in slightly different identifications of a CDR, the structural definition is preferred, but the residues identified by the alternate method are considered important FR residues for determination of which framework residues to import into a consensus sequence.

The terms "consensus sequence" and "consensus antibody" as used herein refers to an amino acid sequence which comprises the most frequently occurring amino acid residues at each location in all human immunoglobulins of any particular subclass. In preferred embodiments, the

consensus human variable domain sequences are derived from the most abundant subclasses in the sequence compilation of Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, National Institutes of Health, Bethesda MD (1987), namely V<sub>L</sub> κ subgroup I and V<sub>H</sub> group III. In such preferred embodiments, the V<sub>L</sub> consensus domain has the amino acid sequence:  
5 DIQMTQSPSSLSASVGDRVTITCRASQDVSSYLAWYQQKPGKAPKLLIYAAS  
SLESGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQYNSLPYTFGQGTKVEI  
KRT (SEQ. ID NO. 3);

the V<sub>H</sub> consensus domain has the amino acid sequence:  
10 EVQLVESGGGLVQPGGSLRLSCAASGFTFSYAMSWVRQAPGKGLEWVAVI  
SENGGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGD  
GFYAMDVWGQGLTVTVSS (SEQ. ID NO. 4).

While not wishing to be limited to any particular theories, it may be that these preferred embodiments are less likely to be immunogenic in an individual than less abundant subclasses. However, in other embodiments, the consensus sequence is derived from human constant domains, or from other subclasses of human immunoglobulin variable domains.

Identity or homology with respect to a specified amino acid sequence of this invention is defined herein as the percentage of amino acid residues in a candidate sequence that are identical with the specified residues, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent homology, and not considering any conservative substitutions as part of the sequence identity. None of N-terminal, C-terminal or internal extensions, deletions, or insertions into the specified sequence shall be construed as affecting homology. All sequence alignments called for in this invention are such maximal homology alignments.

Definition of Homology.

"Non-homologous" import antibody residues are those residues which are not identical to the amino acid residue at the analogous or corresponding location in a consensus sequence, after the import and consensus sequences are aligned.

The term "computer representation" refers to information which is

in a form that can be manipulated by a computer. The act of storing a computer representation refers to the act of placing the information in a form suitable for manipulation by a computer.

This invention is also directed to novel polypeptides, and in certain aspects, isolated novel humanized anti-p185<sup>HER2</sup> antibodies are provided. These novel anti-p185<sup>HER2</sup> antibodies are sometimes collectively referred to herein as huMAb4D5, and also sometimes as the light or heavy chain variable domains of huMAb4D5, and are defined herein to be any polypeptide sequence which possesses a biological property of a polypeptide comprising the following polypeptide sequence:

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP  
KLLIYSASFLESGVPSRFSGSRSGTDFTLTISLQPEDFATYYCQQHY  
TTPPTFGQGKVEIKRT (SEQ. ID NO. 1, which is the light chain  
variable domain of huMAb4D5); or

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLE  
WVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDT  
AVYYCSRWGGDGFYAMDVWGQGLTVTVSS (SEQ. ID NO. 2,  
which is the heavy chain variable domain of huMAb4D5).

"Biological property" for the purposes herein means an *in vivo* effector or antigenic function or activity that is directly or indirectly performed by huMAb4D5 (whether in its native or denatured conformation). Effector functions include receptor binding, any enzyme activity or enzyme modulatory activity, any carrier binding activity, any hormonal activity, any mitogenic or angiogenic activity, any cytotoxic activity, any activity in promoting or inhibiting adhesion of cells to extracellular matrix or cell surface molecules, or any structural role. However, effector functions do not include possession of an epitope or antigenic site that is capable of cross-reacting with antibodies raised against huMAb4D5. An antigenic function means possession of an epitope or antigenic site that is capable of cross-reacting with antibodies raised against the polypeptide sequence of huMAb4D5.



Biologically active huMAb4D5 is defined herein as a polypeptide that shares an effector function of huMAb4D5 and which may (but need not) in addition possess an antigenic function. A principal known effect or function of huMAb4D5 is its ability to bind to p185<sup>HER2</sup>.

5 Antigenically active huMAb4D5 is defined as a polypeptide that possesses an antigenic function of huMAb4D5 and which may (but need not) in addition possess an effector function.

10 In preferred embodiments, antigenically active huMAb4D5 is a polypeptide that binds with an affinity of at least about 10<sup>-9</sup> l/mole to an antibody capable of binding huMAb4D5. Ordinarily the polypeptide binds with an affinity of at least about 10<sup>-8</sup> l/mole. Isolated antibody capable of binding huMAb4D5 is an antibody which is identified and separated from a component of the natural environment in which it may be present. Most preferably, antigenically active huMAb4D5 is a polypeptide that binds to an antibody capable of binding huMAb4D5 in its native conformation. HuMAb4D5 in its native conformation is huMAb4D5 as recovered according to the methods described in Example 1 below, which has not been denatured by chaotropic agents, heat or other treatment that substantially modifies the three dimensional structure of huMAb4D5 as determined for example by migration on nonreducing, nondenaturing sizing gels. Antibody used in this determination is rabbit polyclonal antibody raised by formulating native huMAb4D5 in Freund's complete adjuvant, subcutaneously injecting the formulation, and boosting the immune response by intraperitoneal injection of the formulation until the titer of anti-huMAb4D5 antibody plateaus.

25 Ordinarily, biologically or antigenically active huMAb4D5 will have an amino acid sequence having at least 75% amino acid sequence identity with the huMAb4D5 amino acid sequence, more preferably at least 80%, more preferably at least 90%, and most preferably at least 95%. Identity or homology with respect to this sequence is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the huMAb4D5 residues, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent homology, and not considering

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any conservative substitutions as part of the sequence identity. None of N-terminal, C-terminal or internal extensions, deletions, or insertions into the huMAb4D5 sequence shall be construed as affecting homology.

Thus, the biologically active and antigenically active huMAb4D5 polypeptides that are the subject of certain embodiments of this invention include the sequence of the entire translated nucleotide sequence of huMAb4D5; mature huMAb4D5; fragments thereof having a consecutive sequence of at least 5, 10, 15, 20, 25, 30 or 40 amino acid residues from huMAb4D5; amino acid sequence variants of huMAb4D5 wherein an amino acid residue has been inserted N- or C-terminal to, or within, huMAb4D5 or its fragment as defined above; amino acid sequence variants of huMAb4D5 or its fragment as defined above wherein an amino acid residue of huMAb4D5 or its fragment as defined above has been substituted by another residue, including predetermined mutations by, e.g., site-directed or PCR mutagenesis; derivatives of huMAb4D5 or its fragments as defined above wherein huMAb4D5 or its fragments have been covalent modified, by substitution, chemical, enzymatic, or other appropriate means, with a moiety other than a naturally occurring amino acid; and glycosylation variants of huMAb4D5 (insertion of a glycosylation site or deletion of any glycosylation site by deletion, insertion or substitution of suitable residues). Such fragments and variants exclude any polypeptide heretofore identified, including muMAb4D5 or any known polypeptide fragment, which are anticipatory order 35 U.S.C.102 as well as polypeptides obvious thereover under 35 U.S.C. 103.

"Isolated" huMAb4D5 means huMAb4D5 which has been identified and separated and/or recovered from a component of its natural cell culture environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for huMAb4D5, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, huMAb4D5 will be purified (1) to greater than 95% by weight of protein as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a

degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated huMAb4D5 includes huMAb4D5 in situ within recombinant cells since at least one component of the huMAb4D5 natural environment will not be present. Ordinarily, however, isolated huMAb4D5 will be prepared by at least one purification step.

In accordance with this invention, huMAb4D5 nucleic acid is RNA or DNA containing greater than ten bases that encodes a biologically or antigenically active huMAb4D5, is complementary to nucleic acid sequence encoding such huMAb4D5, or hybridizes to nucleic acid sequence encoding such huMAb4D5 and remains stably bound to it under stringent conditions.

Preferably, the huMAb4D5 nucleic acid encodes a polypeptide sharing at least 75% sequence identity, more preferably at least 80%, still more preferably at least 85%, even more preferably at 90%, and most preferably 95%, with the huMAb4D5 amino acid sequence. Preferably, a nucleic acid molecule that hybridizes to the huMAb4D5 nucleic acid contains at least 20, more preferably 40, and most preferably 90 bases. Such hybridizing or complementary nucleic acid, however, is further defined as being novel under 35 U.S.C. 102 and unobvious under 35 U.S.C. 103 over any prior art nucleic acid.

Stringent conditions are those that (1) employ low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate/0.1% NaDodSO<sub>4</sub> at 50° C; (2) employ during hybridization a denaturing agent such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42° C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 g/ml), 0.1% SDS, and 10% dextran sulfate at 42 C, with washes at 42 C in 0.2 x SSC and 0.1% SDS.

The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, a ribosome binding site, and possibly, other as yet poorly understood sequences. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous and, in the case of a secretory leader, contiguous and in reading phase. However enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, then synthetic oligonucleotide adaptors or linkers are used in accord with conventional practice.

An "exogenous" element is defined herein to mean nucleic acid sequence that is foreign to the cell, or homologous to the cell but in a position within the host cell nucleic acid in which the element is ordinarily not found.

As used herein, the expressions "cell," "cell line," and "cell culture" are used interchangeably and all such designations include progeny. Thus, the words "transformants" and "transformed cells" include the primary subject cell and cultures derived therefrom without regard for the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Mutant progeny that have the same function or biological activity as screened for in the originally transformed cell are included. Where distinct designations are

intended, it will be clear from the context.

"Plasmids" are designated by a lower case p preceded and/or followed by capital letters and/or numbers. The starting plasmids herein are commercially available, are publicly available on an unrestricted basis, or can be constructed from such available plasmids in accord with published procedures. In addition, other equivalent plasmids are known in the art and will be apparent to the ordinary artisan.

"Restriction Enzyme Digestion" of DNA refers to catalytic cleavage of the DNA with an enzyme that acts only at certain locations in the DNA. Such enzymes are called restriction endonucleases, and the sites for which each is specific is called a restriction site. The various restriction enzymes used herein are commercially available and their reaction conditions, cofactors, and other requirements as established by the enzyme suppliers are used. Restriction enzymes commonly are designated by abbreviations composed of a capital letter followed by other letters representing the micro-organism from which each restriction enzyme originally was obtained and then a number designating the particular enzyme. In general, about 1  $\mu$ g of plasmid or DNA fragment is used with about 1-2 units of enzyme in about 20  $\mu$ l of buffer solution. Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer. Incubation of about 1 hour at 37°C is ordinarily used, but may vary in accordance with the supplier's instructions. After incubation, protein or polypeptide is removed by extraction with phenol and chloroform, and the digested nucleic acid is recovered from the aqueous fraction by precipitation with ethanol. Digestion with a restriction enzyme may be followed with bacterial alkaline phosphatase hydrolysis of the terminal 5' phosphates to prevent the two restriction cleaved ends of a DNA fragment from "circularizing" or forming a closed loop that would impede insertion of another DNA fragment at the restriction site. Unless otherwise stated, digestion of plasmids is not followed by 5' terminal dephosphorylation. Procedures and reagents for dephosphorylation are conventional as described in sections 1.56-1.61 of Sambrook *et al.* (Molecular Cloning: A Laboratory Manual New York: Cold

Spring Harbor Laboratory Press, 1989).

5 "Recovery" or "isolation" of a given fragment of DNA from a restriction digest means separation of the digest on polyacrylamide or agarose gel by electrophoresis, identification of the fragment of interest by comparison of its mobility versus that of marker DNA fragments of known molecular weight, removal of the gel section containing the desired fragment, and separation of the gel from DNA. This procedure is known generally. For example, see Lawn *et al.*, Nucleic Acids Res., 9: 6103-6114 (1981), and Goeddel *et al.*, Nucleic Acids Res. 8: 4057 (1980).

10 "Southern blot analysis" is a method by which the presence of DNA sequences in a restriction endonuclease digest of DNA or DNA-containing composition is confirmed by hybridization to a known, labeled oligonucleotide or DNA fragment. Southern analysis typically comprises electrophoretic separation of DNA digests on agarose gels, denaturation of the DNA after electrophoretic separation, and transfer of the DNA to nitrocellulose, nylon, 15 or another suitable membrane supports for analysis with a radiolabeled, biotinylated or enzyme-labeled probe as described in sections 9.37-9.52 of Sambrook *et al.*, *supra*.

20 "Northern analysis" is a method used to identify RNA sequences that hybridize to a known probe such as an oligonucleotide, DNA fragment, cDNA or fragment thereof, or RNA fragment. The probe is labeled with a radioisotope such as 32-P, or by biotinylation, or with an enzyme. The RNA to be analyzed is usually electrophoretically separated on an agarose or polyacrylamide gel, transferred to nitrocellulose, nylon, or other suitable 25 membrane, and hybridized with the probe, using standard techniques well known in the art such as those described in sections 7.39-7.52 of Sambrook *et al.*, *supra*.

30 "Ligation" refers to the process of forming phosphodiester bonds between two nucleic acid fragments. To ligate the DNA fragments together, the ends of the DNA fragments must be compatible with each other. In some cases, the ends will be directly compatible after endonuclease digestion. However, it may be necessary to first convert the staggered ends

commonly produced after endonuclease digestion to blunt ends to make them compatible for ligation. To blunt the ends, the DNA is treated in a suitable buffer for at least 15 minutes at 15°C with about 10 units of the Klenow fragment of DNA polymerase I or T4 DNA polymerase in the presence of the four deoxyribonucleotide triphosphates. The DNA is then purified by phenol-chloroform extraction and ethanol precipitation. The DNA fragments that are to be ligated together are put in solution in about equimolar amounts. The solution will also contain ATP, ligase buffer, and a ligase such as T4 DNA ligase at about 10 units per 0.5  $\mu$ g of DNA. If the DNA is to be ligated into a vector, the vector is first linearized by digestion with the appropriate restriction endonuclease(s). The linearized fragment is then treated with bacterial alkaline phosphatase, or calf intestinal phosphatase to prevent self-ligation during the ligation step.

"Preparation" of DNA from cells means isolating the plasmid DNA from a culture of the host cells. Commonly used methods for DNA preparation are the large and small scale plasmid preparations described in sections 1.25-1.33 of Sambrook *et al.*, *supra*. After preparation of the DNA, it can be purified by methods well known in the art such as that described in section 1.40 of Sambrook *et al.*, *supra*.

"Oligonucleotides" are short-length, single- or double-stranded polydeoxynucleotides that are chemically synthesized by known methods (such as phosphotriester, phosphite, or phosphoramidite chemistry, using solid phase techniques such as described in EP 266,032 published 4 May 1988, or via deoxynucleoside H-phosphonate intermediates as described by Froehler *et al.*, Nucl. Acids Res., 14: 5399-5407 [1986]). They are then purified on polyacrylamide gels.

The technique of "polymerase chain reaction," or "PCR," as used herein generally refers to a procedure wherein minute amounts of a specific piece of nucleic acid, RNA and/or DNA, are amplified as described in U.S. Pat. No. 4,683,195 issued 28 July 1987. Generally, sequence information from the ends of the region of interest or beyond needs to be available, such that oligonucleotide primers can be designed; these primers will be identical

or similar in sequence to opposite strands of the template to be amplified. The 5' terminal nucleotides of the two primers may coincide with the ends of the amplified material. PCR can be used to amplify specific RNA sequences, specific DNA sequences from total genomic DNA, and cDNA transcribed from total cellular RNA, bacteriophage or plasmid sequences, etc. See generally Mullis *et al.*, Cold Spring Harbor Symp. Quant. Biol., 51: 263 (1987); Erlich, ed., PCR Technology, (Stockton Press, NY, 1989). As used herein, PCR is considered to be one, but not the only, example of a nucleic acid polymerase reaction method for amplifying a nucleic acid test sample, comprising the use of a known nucleic acid (DNA or RNA) as a primer and utilizes a nucleic acid polymerase to amplify or generate a specific piece of nucleic acid or to amplify or generate a specific piece of nucleic acid which is complementary to a particular nucleic acid.

#### Suitable Methods for Practicing the Invention

Some aspects of this invention include obtaining an import, non-human antibody variable domain, humanizing the antibody sequence, and producing the humanized antibody. Methods for determining a desired humanized antibody sequence and for humanizing an antibody gene sequence are described below. A particularly preferred method of gene conversion from a non-human or consensus sequence into a humanized nucleic acid sequence is described in Example 1. Additionally, methods are given for obtaining and producing antibodies generally, which apply equally to native non-human antibodies as well as to humanized antibodies.

Generally, the antibodies and antibody variable domains of this invention are conventionally prepared in recombinant cell culture, as described in more detail below. Recombinant synthesis is preferred for reasons of safety and economy, but it is known to prepare peptides by chemical synthesis and to purify them from natural sources; such preparations are included within the definition of antibodies herein.



### Molecular Modeling

Where it is desired to prepare molecular models for the antibodies of this invention, one may utilize any of the commercially available modeling programs described in the literature cited in the Background above.

5 Generally, models for a particular antibody domains, for example non-human, import antibody variable  $V_H$  and  $V_L$  domains, are constructed separately from consensus coordinates based upon FAb structures which have similar sequences. Models of consensus human antibody sequences are similarly created.

10 For example, in modeling the muMAb4d5, the models were constructed based upon seven Fab structures from the Brookhaven protein data bank (entries 1FB4, 2RHE, 2MCP, 3FAB, 1FBJ, 2HFL and 1REI). The Fab fragment KOL (Marquart, M. *et al.*, *J. Mol. Biol.* 141:369-391 (1980)) was first chosen as a template for  $V_L$  and  $V_H$  domains and additional structures were then superimposed upon this structure using their main chain atom coordinates (INSIGHT program, Biosym Technologies). Similar programs and techniques are utilized for modeling the desired antibody.

15 The distance from the template  $C\alpha$  to the analogous  $C\alpha$  in each of the superimposed structures is calculated for each residue position. Generally, if all (or nearly all)  $C\alpha$ - $C\alpha$  distances for a given residue are  $\leq 1\text{\AA}$ , then that position is included in the consensus structure. In some cases the  $\beta$ -sheet framework residues will satisfy these criteria whereas the CDR loops may not. For each of these selected residues the average coordinates for individual N,  $C\alpha$ , C, O and  $C\beta$  atoms are calculated and then corrected for resultant deviations from non-standard bond geometry by 50 cycles of energy minimization using a commercially available program such as the DISCOVER program (Biosym Technologies) with the AMBER forcefield (Weiner, S. J. *et al.*, *J. Amer. Chem. Soc.* 106:765-784 (1984)), and the  $C\alpha$  coordinates are fixed. The side chains of highly conserved residues, such as

20 the disulfide-bridged cysteine residues, are then incorporated into the resultant consensus structure. Next the sequences of the particular antibody  $V_L$  and  $V_H$  domains are incorporated starting with the CDR residues and

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5 using the tabulations of CDR conformations from Chothia et al. (Chothia, C. et al., *Nature* 342:877-883 (1989)) as a guide. Side-chain conformations are chosen on the basis of Fab crystal structures, rotamer libraries (Ponder, J. W. & Richards, F. M., *J. Mol. Biol.* 193:775-791 (1987)) and packing considerations. Since V<sub>H</sub>-CDR3 typically cannot be assigned a definite backbone conformation from these criteria, models may be created from a search of similar sized loops using the INSIGHT program, derived using packing and solvent exposure considerations, or created using other routine and commercially available techniques. It is preferable to subject the model to 5000 cycles of energy minimization.

#### Methods for Obtaining a Humanized Antibody Sequence

15 In humanizing muMAb4D5, consensus human sequences are first derived, and then a molecular model is generated for these sequences using the methods described above. In certain embodiments of this invention, the consensus human sequences are derived from the most abundant subclasses in the sequence compilation of Kabat et al. (Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)), namely V<sub>L</sub> κ subgroup I and V<sub>H</sub> group III, and have the sequences indicated in the definitions above.

20 While these steps may be taken in different order, typically a structure for the candidate humanized antibody is created by transferring the CDRs from the non-human, import sequence into the consensus human structure. The humanized antibody may contain human replacements of the non-human import residues at positions within CDRs as defined by sequence variability (Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)) or as defined by structural variability (Chothia, C. & Lesk, A. M., *J. Mol. Biol.* 196:901-917 (1987)). For example, huMAb4D5 contains human replacements of the muMAb4D5 residues at three positions within CDRs as defined by sequence variability (Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987))

but not as defined by structural variability (Chothia, C. & Lesk, A. M., *J. Mol. Biol.* 196:901-917 (1987)): V<sub>L</sub>-CDR1 K24R, V<sub>L</sub>-CDR2 R54L and V<sub>L</sub>-CDR2 T56S.

5 Differences between the non-human import and the human consensus framework residues are individually investigated to determine their possible influence on CDR conformation and/or binding to antigen. Investigation of such possible influences is desirably performed through modeling, by examination of the characteristics of the amino acids at particular locations, or determined experimentally through evaluating the effects of substitution or mutagenesis of particular amino acids.

10 In certain preferred embodiments of this invention, a humanized antibody is made comprising amino acid sequence of an import, non-human antibody and a human antibody, utilizing the steps of:

- 15 a. obtaining the amino acid sequences of at least a portion of an import antibody variable domain and of a consensus human variable domain;
- b. identifying Complementarity Determining Region (CDR) amino acid sequences in the import and the human variable domain sequences;
- 20 c. substituting an import CDR amino acid sequence for the corresponding human CDR amino acid sequence;
- d. aligning the amino acid sequences of a Framework Region (FR) of the import antibody and the corresponding FR of the consensus antibody;
- 25 e. identifying import antibody FR residues in the aligned FR sequences that are non-homologous to the corresponding consensus antibody residues;
- f. determining if the non-homologous import amino acid residue is reasonably expected to have at least one of the following effects:
  - 30 1. non-covalently binds antigen directly,
  2. interacts with a CDR; or

3. participates in the  $V_L - V_H$  interface; and
- g. for any non-homologous import antibody amino acid residue which is reasonably expected to have at least one of these effects, substituting that residue for the corresponding amino acid residue in the consensus antibody FR sequence.

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Optionally, one determines if any non-homologous residues identified in step (e) are exposed on the surface of the domain or buried within it, and if the residue is exposed but has none of the effects identified in step (f), one may retain the consensus residue.

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Additionally, in certain embodiments the corresponding consensus antibody residues identified in step (e) above are selected from the group consisting of 4L, 35L, 36L, 38L, 43L, 44L, 46L, 58L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, 2H, 4H, 24H, 36H, 37H, 15 39H, 43H, 45H, 49H, 58H, 60H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H (utilizing the numbering system set forth in Kabat, E. A. *et al.*, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)).

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In preferred embodiments, the method of this invention comprises the additional steps of searching either or both of the import, non-human and the consensus variable domain sequences for glycosylation sites, determining if the glycosylation is reasonably expected to be important for the desired antigen binding and biological activity of the antibody (i.e., determining if the glycosylation site binds to antigen or changes a side chain of an amino acid residue that binds to antigen, or if the glycosylation enhances or weakens antigen binding, or is important for maintaining antibody affinity). If the import sequence bears the glycosylation site, it is preferred to substitute that site for the corresponding residues in the consensus human sequence if the glycosylation site is reasonably expected to be important. If only the 25 consensus sequence, and not the import, bears the glycosylation site, it is preferred to eliminate that glycosylation site or substitute therefor the corresponding amino acid residues from the import sequence.

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Another preferred embodiment of the methods of this invention comprises aligning import antibody and the consensus antibody FR sequences, identifying import antibody FR residues which are non-homologous with the aligned consensus FR sequence, and for each such non-homologous import antibody FR residue, determining if the corresponding consensus antibody residue represents a residue which is highly conserved across all species at that site, and if it is so conserved, preparing a humanized antibody which comprises the consensus antibody amino acid residue at that site.

In certain alternate embodiments, one need not utilize the modeling and evaluation steps described above, and may instead proceed with the steps of obtaining the amino acid sequence of at least a portion of an import, non-human antibody variable domain having a CDR and a FR, obtaining the amino acid sequence of at least a portion of a consensus human antibody variable domain having a CDR and a FR, substituting the non-human CDR for the human CDR in the consensus human antibody variable domain, and then substituting an amino acid residue for the consensus amino acid residue at at least one of the following sites:

- a. (in the FR of the variable domain of the light chain) 4L, 35L, 36L, 38L, 43L, 44L, 58L, 46L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, or
- b. (in the FR of the variable domain of the heavy chain) 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H.

Preferably, the non-CDR residue substituted at the consensus FR site is the residue found at the corresponding location of the non-human antibody. If desired, one may utilize the other method steps described above for determining whether a particular amino acid residue can reasonably be expected to have undesirable effects, and remedying those effects.

If after making a humanized antibody according to the steps above and testing its activity one is not satisfied with the humanized antibody, one

preferably reexamines the potential effects of the amino acids at the specific locations recited above. Additionally, it is desirable to reinvestigate any buried residues which are reasonably expected to affect the  $V_L - V_H$  interface but may not directly affect CDR conformation. It is also desirable to reevaluate the humanized antibody utilizing the steps of the methods claimed herein.

In certain embodiments of this invention, amino acid residues in the consensus human sequence are substituted for by other amino acid residues. In preferred embodiments, residues from a particular non-human import sequence are substituted, however there are circumstances where it is desired to evaluate the effects of other amino acids. For example, if after making a humanized antibody according to the steps above and testing its activity one is not satisfied with the humanized antibody, one may compare the sequences of other classes or subgroups of human antibodies, or classes or subgroups of antibodies from the particular non-human species, and determine which other amino acid side chains and amino acid residues are found at particular locations and substituting such other residues.

### Antibodies

Certain aspects of this invention are directed to natural antibodies and to monoclonal antibodies, as illustrated in the Examples below and by antibody hybridomas deposited with the ATCC (as described below). Thus, the references throughout this description to the use of monoclonal antibodies are intended to include the use of natural or native antibodies as well as humanized and chimeric antibodies. As used herein, the term "antibody" includes the antibody variable domain and other separable antibody domains unless specifically excluded.

In accordance with certain aspects of this invention, antibodies to be humanized (import antibodies) are isolated from continuous hybrid cell lines formed by the fusion of antigen-primed immune lymphocytes with myeloma cells.

In certain embodiments, the antibodies of this invention are

5 obtained by routine screening. Polyclonal antibodies to an antigen generally are raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the antigen and an adjuvant. It may be useful to conjugate the antigen or a fragment containing the target amino acid sequence to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride,  $\text{SOCl}_2$ , or  $\text{R}^1\text{N} = \text{C} = \text{NR}$ , where R and R<sup>1</sup> are different alkyl groups.

10 The route and schedule of the host animal or cultured antibody-producing cells therefrom are generally in keeping with established and conventional techniques for antibody stimulation and production. While mice are frequently employed as the test model, it is contemplated that any mammalian subject including human subjects or antibody-producing cells obtained therefrom can be manipulated according to the processes of this invention to serve as the basis for production of mammalian, including human, hybrid cell lines.

15 Animals are typically immunized against the immunogenic conjugates or derivatives by combining 1 mg or 1  $\mu\text{g}$  of conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with 1/5 to 1/10 the original amount of conjugate in Freund's complete adjuvant (or other suitable adjuvant) by subcutaneous injection at multiple sites. 7 to 14 days later animals are bled and the serum is assayed for antigen titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different protein and/or through a different cross-linking agent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are used to enhance the immune response.

After immunization, monoclonal antibodies are prepared by recovering immune lymphoid cells--typically spleen cells or lymphocytes from lymph node tissue--from immunized animals and immortalizing the cells in conventional fashion, e.g. by fusion with myeloma cells or by Epstein-Barr (EB)-virus transformation and screening for clones expressing the desired antibody. The hybridoma technique described originally by Kohler and Milstein, *Eur. J. Immunol.* 6:511 (1976) has been widely applied to produce hybrid cell lines that secrete high levels of monoclonal antibodies against many specific antigens.

It is possible to fuse cells of one species with another. However, it is preferable that the source of the immunized antibody producing cells and the myeloma be from the same species.

The hybrid cell lines can be maintained in culture *in vitro* in cell culture media. The cell lines of this invention can be selected and/or maintained in a composition comprising the continuous cell line in hypoxanthine-aminopterin thymidine (HAT) medium. In fact, once the hybridoma cell line is established, it can be maintained on a variety of nutritionally adequate media. Moreover, the hybrid cell lines can be stored and preserved in any number of conventional ways, including freezing and storage under liquid nitrogen. Frozen cell lines can be revived and cultured indefinitely with resumed synthesis and secretion of monoclonal antibody. The secreted antibody is recovered from tissue culture supernatant by conventional methods such as precipitation, ion exchange chromatography, affinity chromatography, or the like. The antibodies described herein are also recovered from hybridoma cell cultures by conventional methods for purification of IgG or IgM as the case may be that heretofore have been used to purify these immunoglobulins from pooled plasma, e.g. ethanol or polyethylene glycol precipitation procedures. The purified antibodies are sterile filtered, and optionally are conjugated to a detectable marker such as an enzyme or spin label for use in diagnostic assays of the antigen in test samples.

While routinely rodent monoclonal antibodies are used as the source



of the import antibody, the invention is not limited to any species. Additionally, techniques developed for the production of chimeric antibodies (Morrison *et al.*, *Proc. Natl. Acad. Sci.*, 81:6851 (1984); Neuberger *et al.*, *Nature* 312:604 (1984); Takeda *et al.*, *Nature* 314:452 (1985)) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity (such as ability to activate human complement and mediate ADCC) can be used; such antibodies are within the scope of this invention.

Techniques for creating recombinant DNA versions of the antigen-binding regions of antibody molecules (known as Fab fragments) which bypass the generation of monoclonal antibodies are encompassed within the practice of this invention. One extracts antibody-specific messenger RNA molecules from immune system cells taken from an immunized animal, transcribes these into complementary DNA (cDNA), and clones the cDNA into a bacterial expressions system. One example of such a technique suitable for the practice of this invention was developed by researchers at Scripps/Stratagene, and incorporates a proprietary bacteriophage lambda vector system which contains a leader sequence that causes the expressed Fab protein to migrate to the periplasmic space (between the bacterial cell membrane and the cell wall) or to be secreted. One can rapidly generate and screen great numbers of functional FAb fragments for those which bind the antigen. Such FAb fragments with specificity for the antigen are specifically encompassed within the term "antibody" as it is defined, discussed, and claimed herein.

#### Amino Acid Sequence Variants

Amino acid sequence variants of the antibodies and polypeptides of this invention (referred to in herein as the target polypeptide) are prepared by introducing appropriate nucleotide changes into the DNA encoding the target polypeptide, or by *in vitro* synthesis of the desired target polypeptide. Such variants include, for example, humanized variants of non-human antibodies, as well as deletions from, or insertions or substitutions of,

residues within particular amino acid sequences. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the target polypeptide, such as changing the number or position of glycosylation sites, altering any membrane anchoring characteristics, and/or altering the intra-cellular location of the target polypeptide by inserting, deleting, or otherwise affecting any leader sequence of the native target polypeptide.

In designing amino acid sequence variants of target polypeptides, the location of the mutation site and the nature of the mutation will depend on the target polypeptide characteristic(s) to be modified. The sites for mutation can be modified individually or in series, e.g., by (1) substituting first with conservative amino acid choices and then with more radical selections depending upon the results achieved, (2) deleting the target residue, or (3) inserting residues of the same or a different class adjacent to the located site, or combinations of options 1-3. In certain embodiments, these choices are guided by the methods for creating humanized sequences set forth above.

A useful method for identification of certain residues or regions of the target polypeptide that are preferred locations for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (Science, 244: 1081-1085 [1989]). Here, a residue or group of target residues are identified (e.g., charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with the surrounding aqueous environment in or outside the cell. Those domains demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at or for the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation *per se* need not be predetermined. For example, to optimize the performance of a mutation at

a given site, ala scanning or random mutagenesis may be conducted at the target codon or region and the expressed target polypeptide variants are screened for the optimal combination of desired activity.

5 There are two principal variables in the construction of amino acid sequence variants: the location of the mutation site and the nature of the mutation. In general, the location and nature of the mutation chosen will depend upon the target polypeptide characteristic to be modified.

10 Amino acid sequence deletions of antibodies are generally not preferred, as maintaining the generally configuration of an antibody is believed to be necessary for its activity. Any deletions will be selected so as to preserve the structure of the target antibody.

15 Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Intrasequence insertions (i.e., insertions within the target polypeptide sequence) may range generally from about 1 to 10 residues, more preferably 1 to 5, most preferably 1 to 3. Examples of terminal insertions include the target polypeptide with an N-terminal methionyl residue, an artifact of the direct expression of target polypeptide in bacterial recombinant cell culture, and fusion of a heterologous N-terminal signal sequence to the N-terminus of the target polypeptide molecule to facilitate the secretion of the mature target polypeptide from recombinant host cells. Such signal sequences generally will be obtained from, and thus homologous to, the intended host cell species. Suitable sequences include 20 STII or lpp for *E. coli*, alpha factor for yeast, and viral signals such as herpes gD for mammalian cells.

25 Other insertional variants of the target polypeptide include the fusion to the N- or C-terminus of the target polypeptide of immunogenic polypeptides, e.g., bacterial polypeptides such as beta-lactamase or an enzyme encoded by the *E. coli trp* locus, or yeast protein, and C-terminal fusions with proteins having a long half-life such as immunoglobulin constant regions (or other immunoglobulin regions), albumin, or ferritin, as described 30

in WO 89/02922 published 6 April 1989.

Another group of variants are amino acid substitution variants. These variants have at least one amino acid residue in the target polypeptide molecule removed and a different residue inserted in its place. The sites of greatest interest for substitutional mutagenesis include sites identified as the active site(s) of the target polypeptide, and sites where the amino acids found in the target polypeptide from various species are substantially different in terms of side-chain bulk, charge, and/or hydrophobicity. Other sites for substitution are described infra, considering the effect of the substitution of the antigen binding, affinity and other characteristics of a particular target antibody.

Other sites of interest are those in which particular residues of the target polypeptides obtained from various species are identical. These positions may be important for the biological activity of the target polypeptide. These sites, especially those falling within a sequence of at least three other identically conserved sites, are substituted in a relatively conservative manner. If such substitutions result in a change in biological activity, then other changes are introduced and the products screened until the desired effect is obtained.

Substantial modifications in function or immunological identity of the target polypeptide are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side chain properties:

- (1) hydrophobic: norleucine, met, ala, val, leu, ile;
- (2) neutral hydrophilic: cys, ser, thr;
- (3) acidic: asp, glu;
- (4) basic: asn, gln, his, lys, arg;
- (5) residues that influence chain orientation: gly, pro; and
- (6) aromatic: trp, tyr, phe.

5 Non-conservative substitutions will entail exchanging a member of one of these classes for another. Such substituted residues may be introduced into regions of the target polypeptide that are homologous with other antibodies of the same class or subclass, or, more preferably, into the non-homologous regions of the molecule.

Any cysteine residues not involved in maintaining the proper conformation of target polypeptide also may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking.

10 DNA encoding amino acid sequence variants of the target polypeptide is prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant  
15 version of the target polypeptide. A particularly preferred method of gene conversion mutagenesis is described below in Example 1. These techniques may utilize target polypeptide nucleic acid (DNA or RNA), or nucleic acid complementary to the target polypeptide nucleic acid.

20 Oligonucleotide-mediated mutagenesis is a preferred method for preparing substitution, deletion, and insertion variants of target polypeptide DNA. This technique is well known in the art as described by Adelman *et al.*, DNA, 2: 183 (1983). Briefly, the target polypeptide DNA is altered by hybridizing an oligonucleotide encoding the desired mutation to a DNA  
25 template, where the template is the single-stranded form of a plasmid or bacteriophage containing the unaltered or native DNA sequence of the target polypeptide. After hybridization, a DNA polymerase is used to synthesize an entire second complementary strand of the template that will thus incorporate the oligonucleotide primer, and will code for the selected  
30 alteration in the target polypeptide DNA.

Generally, oligonucleotides of at least 25 nucleotides in length are used. An optimal oligonucleotide will have 12 to 15 nucleotides that are

completely complementary to the template on either side of the nucleotide(s) coding for the mutation. This ensures that the oligonucleotide will hybridize properly to the single-stranded DNA template molecule. The oligonucleotides are readily synthesized using techniques known in the art such as that described by Crea *et al.* (Proc. Natl. Acad. Sci. USA, 75: 5765 [1978]).

Single-stranded DNA template may also be generated by denaturing double-stranded plasmid (or other) DNA using standard techniques.

For alteration of the native DNA sequence (to generate amino acid sequence variants, for example), the oligonucleotide is hybridized to the single-stranded template under suitable hybridization conditions. A DNA polymerizing enzyme, usually the Klenow fragment of DNA polymerase I, is then added to synthesize the complementary strand of the template using the oligonucleotide as a primer for synthesis. A heteroduplex molecule is thus formed such that one strand of DNA encodes the mutated form of the target polypeptide, and the other strand (the original template) encodes the native, unaltered sequence of the target polypeptide. This heteroduplex molecule is then transformed into a suitable host cell, usually a prokaryote such as *E. coli* JM101. After the cells are grown, they are plated onto agarose plates and screened using the oligonucleotide primer radiolabeled with 32-phosphate to identify the bacterial colonies that contain the mutated DNA. The mutated region is then removed and placed in an appropriate vector for protein production, generally an expression vector of the type typically employed for transformation of an appropriate host.

The method described immediately above may be modified such that a homoduplex molecule is created wherein both strands of the plasmid contain the mutation(s). The modifications are as follows: The single-stranded oligonucleotide is annealed to the single-stranded template as described above. A mixture of three deoxyribonucleotides, deoxyriboadenosine (dATP), deoxyriboguanosine (dGTP), and deoxyribothymidine (dTTP), is combined with a modified thio-deoxyribocytosine called dCTP-(aS) (which can be obtained from Amersham Corporation). This mixture is added to the

template-oligonucleotide complex. Upon addition of DNA polymerase to this mixture, a strand of DNA identical to the template except for the mutated bases is generated. In addition, this new strand of DNA will contain dCTP-(aS) instead of dCTP, which serves to protect it from restriction endonuclease digestion.

After the template strand of the double-stranded heteroduplex is nicked with an appropriate restriction enzyme, the template strand can be digested with ExoIII nuclease or another appropriate nuclease past the region that contains the site(s) to be mutagenized. The reaction is then stopped to leave a molecule that is only partially single-stranded. A complete double-stranded DNA homoduplex is then formed using DNA polymerase in the presence of all four deoxyribonucleotide triphosphates, ATP, and DNA ligase. This homoduplex molecule can then be transformed into a suitable host cell such as *E. coli* JM101, as described above.

DNA encoding target polypeptide variants with more than one amino acid to be substituted may be generated in one of several ways. If the amino acids are located close together in the polypeptide chain, they may be mutated simultaneously using one oligonucleotide that codes for all of the desired amino acid substitutions. If, however, the amino acids are located some distance from each other (separated by more than about ten amino acids), it is more difficult to generate a single oligonucleotide that encodes all of the desired changes. Instead, one of two alternative methods may be employed.

In the first method, a separate oligonucleotide is generated for each amino acid to be substituted. The oligonucleotides are then annealed to the single-stranded template DNA simultaneously, and the second strand of DNA that is synthesized from the template will encode all of the desired amino acid substitutions.

The alternative method involves two or more rounds of mutagenesis to produce the desired mutant. The first round is as described for the single mutants: wild-type DNA is used for the template, an oligonucleotide encoding the first desired amino acid substitution(s) is annealed to this template, and

the heteroduplex DNA molecule is then generated. The second round of mutagenesis utilizes the mutated DNA produced in the first round of mutagenesis as the template. Thus, this template already contains one or more mutations. The oligonucleotide encoding the additional desired amino acid substitution(s) is then annealed to this template, and the resulting strand of DNA now encodes mutations from both the first and second rounds of mutagenesis. This resultant DNA can be used as a template in a third round of mutagenesis, and so on.

PCR mutagenesis is also suitable for making amino acid variants of target polypeptide. While the following discussion refers to DNA, it is understood that the technique also finds application with RNA. The PCR technique generally refers to the following procedure (see Erlich, *supra*, the chapter by R. Higuchi, p. 61-70): When small amounts of template DNA are used as starting material in a PCR, primers that differ slightly in sequence from the corresponding region in a template DNA can be used to generate relatively large quantities of a specific DNA fragment that differs from the template sequence only at the positions where the primers differ from the template. For introduction of a mutation into a plasmid DNA, one of the primers is designed to overlap the position of the mutation and to contain the mutation; the sequence of the other primer must be identical to a stretch of sequence of the opposite strand of the plasmid, but this sequence can be located anywhere along the plasmid DNA. It is preferred, however, that the sequence of the second primer is located within 200 nucleotides from that of the first, such that in the end the entire amplified region of DNA bounded by the primers can be easily sequenced. PCR amplification using a primer pair like the one just described results in a population of DNA fragments that differ at the position of the mutation specified by the primer, and possibly at other positions, as template copying is somewhat error-prone.

If the ratio of template to product material is extremely low, the vast majority of product DNA fragments incorporate the desired mutation(s). This product material is used to replace the corresponding region in the plasmid that served as PCR template using standard DNA technology.



Mutations at separate positions can be introduced simultaneously by either using a mutant second primer, or performing a second PCR with different mutant primers and ligating the two resulting PCR fragments simultaneously to the vector fragment in a three (or more)-part ligation.

5 In a specific example of PCR mutagenesis, template plasmid DNA (1  $\mu$ g) is linearized by digestion with a restriction endonuclease that has a unique recognition site in the plasmid DNA outside of the region to be amplified. Of this material, 100 ng is added to a PCR mixture containing PCR buffer, which contains the four deoxynucleotide tri-phosphates and is  
10 included in the GeneAmp<sup>®</sup> kits (obtained from Perkin-Elmer Cetus, Norwalk, CT and Emeryville, CA), and 25 pmole of each oligonucleotide primer, to a final volume of 50  $\mu$ l. The reaction mixture is overlaid with 35  $\mu$ l mineral oil. The reaction is denatured for 5 minutes at 100°C, placed briefly on ice, and then 1  $\mu$ l *Thermus aquaticus* (Taq) DNA polymerase (5 units/ $\mu$ l,  
15 purchased from Perkin-Elmer Cetus, Norwalk, CT and Emeryville, CA) is added below the mineral oil layer. The reaction mixture is then inserted into a DNA Thermal Cycler (purchased from Perkin-Elmer Cetus) programmed as follows: 2 min. at 55°C, then 30 sec. at 72°C, then 19 cycles of the following: 30 sec. at 94°C, 30 sec. at 55°C, and 30 sec. at 72°C.

20 At the end of the program, the reaction vial is removed from the thermal cycler and the aqueous phase transferred to a new vial, extracted with phenol/chloroform (50:50:vol), and ethanol precipitated, and the DNA is recovered by standard procedures. This material is subsequently subjected to the appropriate treatments for insertion into a vector.

25 Another method for preparing variants, cassette mutagenesis, is based on the technique described by Wells *et al.* (Gene, 34: 315 [1985]). The starting material is the plasmid (or other vector) comprising the target polypeptide DNA to be mutated. The codon(s) in the target polypeptide DNA to be mutated are identified. There must be a unique restriction  
30 endonuclease site on each side of the identified mutation site(s). If no such restriction sites exist, they may be generated using the above-described oligonucleotide-mediated mutagenesis method to introduce them at

appropriate locations in the target polypeptide DNA. After the restriction sites have been introduced into the plasmid, the plasmid is cut at these sites to linearize it. A double-stranded oligonucleotide encoding the sequence of the DNA between the restriction sites but containing the desired mutation(s) is synthesized using standard procedures. The two strands are synthesized separately and then hybridized together using standard techniques. This double-stranded oligonucleotide is referred to as the cassette. This cassette is designed to have 3' and 5' ends that are compatible with the ends of the linearized plasmid, such that it can be directly ligated to the plasmid. This plasmid now contains the mutated target polypeptide DNA sequence.

#### Insertion of DNA into a Cloning Vehicle

The cDNA or genomic DNA encoding the target polypeptide is inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. Many vectors are available, and selection of the appropriate vector will depend on 1) whether it is to be used for DNA amplification or for DNA expression, 2) the size of the DNA to be inserted into the vector, and 3) the host cell to be transformed with the vector. Each vector contains various components depending on its function (amplification of DNA or expression of DNA) and the host cell for which it is compatible. The vector components generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence.

#### (a) Signal Sequence Component

In general, the signal sequence may be a component of the vector, or it may be a part of the target polypeptide DNA that is inserted into the vector.

The target polypeptides of this invention may be expressed not only directly, but also as a fusion with a heterologous polypeptide, preferably a

5 signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the vector, or it may be a part of the target polypeptide DNA that is inserted into the vector. Included within the scope of this invention are target polypeptides with any native signal sequence deleted and replaced with a heterologous signal sequence. The heterologous signal sequence selected should be one that is recognized and processed (i.e. cleaved by a signal peptidase) by the host cell. For prokaryotic host cells that do not recognize and process the native target polypeptide signal sequence, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. For yeast secretion the native target polypeptide signal sequence may be substituted by the yeast invertase, alpha factor, or acid phosphatase leaders. In mammalian cell expression the native signal sequence is satisfactory, although other mammalian signal sequences may be suitable.

(b) Origin of Replication Component

20 Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Generally, in cloning vectors this sequence is one that enables the vector to replicate independently of the host chromosomal DNA, and includes origins of replication or autonomously replicating sequences. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2 $\mu$  plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter).

30 Most expression vectors are "shuttle" vectors, i.e. they are capable

of replication in at least one class of organisms but can be transfected into another organism for expression. For example, a vector is cloned in *E. coli* and then the same vector is transfected into yeast or mammalian cells for expression even though it is not capable of replicating independently of the host cell chromosome.

DNA may also be amplified by insertion into the host genome. This is readily accomplished using *Bacillus* species as hosts, for example, by including in the vector a DNA sequence that is complementary to a sequence found in *Bacillus* genomic DNA. Transfection of *Bacillus* with this vector results in homologous recombination with the genome and insertion of the target polypeptide DNA. However, the recovery of genomic DNA encoding the target polypeptide is more complex than that of an exogenously replicated vector because restriction enzyme digestion is required to excise the target polypeptide DNA.

(c) Selection Gene Component

Expression and cloning vectors should contain a selection gene, also termed a selectable marker. This gene encodes a protein necessary for the survival or growth of transformed host cells grown in a selective culture medium. Host cells not transformed with the vector containing the selection gene will not survive in the culture medium. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g. ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, e.g. the gene encoding D-alanine racemase for *Bacilli*.

One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene express a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin (Southern *et al.*, J. Molec. Appl. Genet., 1: 327 [1982]), mycophenolic acid (Mulligan *et al.*, Science, 209: 1422 [1980]) or

hygromycin (Sugden *et al.*, Mol. Cell. Biol., 5: 410-413 [1985]). The three examples given above employ bacterial genes under eukaryotic control to convey resistance to the appropriate drug G418 or neomycin (geneticin), xgpt (mycophenolic acid), or hygromycin, respectively.

5 Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the target polypeptide nucleic acid, such as dihydrofolate reductase (DHFR) or thymidine kinase. The mammalian cell transformants are placed under selection pressure which only the transformants are uniquely adapted to survive by virtue of having taken up the marker. Selection pressure is  
10 imposed by culturing the transformants under conditions in which the concentration of selection agent in the medium is successively changed, thereby leading to amplification of both the selection gene and the DNA that encodes the target polypeptide. Amplification is the process by which genes  
15 in greater demand for the production of a protein critical for growth are reiterated in tandem within the chromosomes of successive generations of recombinant cells. Increased quantities of the target polypeptide are synthesized from the amplified DNA.

For example, cells transformed with the DHFR selection gene are  
20 first identified by culturing all of the transformants in a culture medium that contains methotrexate (Mtx), a competitive antagonist of DHFR. An appropriate host cell when wild-type DHFR is employed is the Chinese hamster ovary (CHO) cell line deficient in DHFR activity, prepared and propagated as described by Urlaub and Chasin, Proc. Natl. Acad. Sci. USA,  
25 77: 4216 [1980]. The transformed cells are then exposed to increased levels of methotrexate. This leads to the synthesis of multiple copies of the DHFR gene, and, concomitantly, multiple copies of other DNA comprising the expression vectors, such as the DNA encoding the target polypeptide. This amplification technique can be used with any otherwise suitable host, e.g.,  
30 ATCC No. CCL61 CHO-K1, notwithstanding the presence of endogenous DHFR if, for example, a mutant DHFR gene that is highly resistant to Mtx is employed (EP 1 17,060). Alternatively, host cells (particularly wild-type hosts

that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding the target polypeptide, wild-type DHFR protein, and another selectable marker such as aminoglycoside 3' phosphotransferase (APH) can be selected by cell growth in medium containing a selection agent for the selectable marker such as an aminoglycosidic antibiotic, e.g., kanamycin, neomycin, or G418. See U.S. Pat. No. 4,965,199.

A suitable selection gene for use in yeast is the *trp1* gene present in the yeast plasmid YRp7 (Stinchcomb *et al.*, Nature, 282: 39 [1979]; Kingsman *et al.*, Gene, 7: 141 [1979]; or Tschemper *et al.*, Gene, 10: 157 [1980]). The *trp1* gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1 (Jones, Genetics, 85: 12 [1977]). The presence of the *trp1* lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan. Similarly, *Leu2*-deficient yeast strains (ATCC 20,622 or 38,626) are complemented by known plasmids bearing the *Leu2* gene.

(d) Promoter Component

Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to the target polypeptide nucleic acid. Promoters are untranslated sequences located upstream (5') to the start codon of a structural gene (generally within about 100 to 1000 bp) that control the transcription and translation of a particular nucleic acid sequence, such as that encoding the target polypeptide, to which they are operably linked. Such promoters typically fall into two classes, inducible and constitutive. Inducible promoters are promoters that initiate increased levels of transcription from DNA under their control in response to some change in culture conditions, e.g. the presence or absence of a nutrient or a change in temperature. At this time a large number of promoters recognized by a variety of potential host cells are well known. These promoters are operably linked to DNA encoding the target polypeptide

by removing the promoter from the source DNA by restriction enzyme digestion and inserting the isolated promoter sequence into the vector. Both the native target polypeptide promoter sequence and many heterologous promoters may be used to direct amplification and/or expression of the target polypeptide DNA. However, heterologous promoters are preferred, as they generally permit greater transcription and higher yields of expressed target polypeptide as compared to the native target polypeptide promoter.

Promoters suitable for use with prokaryotic hosts include the  $\beta$ -lactamase and lactose promoter systems (Chang *et al.*, Nature, 275: 615 [1978]; and Goeddel *et al.*, Nature, 281: 544 [1979]), alkaline phosphatase, a tryptophan (trp) promoter system (Goeddel, Nucleic Acids Res., 8: 4057 [1980] and EP 36,776) and hybrid promoters such as the tac promoter (deBoer *et al.*, Proc. Natl. Acad. Sci. USA, 80: 21-25 [1983]). However, other known bacterial promoters are suitable. Their nucleotide sequences have been published, thereby enabling a skilled worker operably to ligate them to DNA encoding the target polypeptide (Siebenlist *et al.*, Cell, 20: 269 [1980]) using linkers or adaptors to supply any required restriction sites. Promoters for use in bacterial systems also generally will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding the target polypeptide.

Suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase (Hitzeman *et al.*, J. Biol. Chem., 255: 2073 [1980]) or other glycolytic enzymes (Hess *et al.*, J. Adv. Enzyme Reg., 7: 149 [1968]; and Holland, Biochemistry, 17: 4900 [1978]), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism,

metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in Hitzeman *et al.*, EP 73,657A. Yeast enhancers also are advantageously used with yeast promoters.

Promoter sequences are known for eukaryotes. Virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CXCAAT region where X may be any nucleotide. At the 3' end of most eukaryotic genes is an AATAAA sequence that may be the signal for addition of the poly A tail to the 3' end of the coding sequence. All of these sequences are suitably inserted into mammalian expression vectors.

Target polypeptide transcription from vectors in mammalian host cells is controlled by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g. the actin promoter or an immunoglobulin promoter, from heat-shock promoters, and from the promoter normally associated with the target polypeptide sequence, provided such promoters are compatible with the host cell systems.

The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. Fiers *et al.*, Nature, **273**:113 (1978); Mulligan and Berg, Science, **209**: 1422-1427 (1980); Pavlakis *et al.*, Proc. Natl. Acad. Sci. USA, **78**: 7398-7402 (1981). The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment. Greenaway *et al.*, Gene, **18**: 355-360 (1982). A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed



in U.S. 4,419,446. A modification of this system is described in U.S. 4,601,978. See also Gray *et al.*, Nature, 295: 503-508 (1982) on expressing cDNA encoding immune interferon in monkey cells; , Reyes *et al.*, Nature, 297: 598-601 (1982) on expression of human  $\beta$ -interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus, Canaani and Berg, Proc. Natl. Acad. Sci. USA, 79: 5166-5170 (1982) on expression of the human interferon  $\beta$ 1 gene in cultured mouse and rabbit cells, and Gorman *et al.*, Proc. Natl. Acad. Sci. USA, 79: 6777-6781 (1982) on expression of bacterial CAT sequences in CV-1 monkey kidney cells, chicken embryo fibroblasts, Chinese hamster ovary cells, HeLa cells, and mouse NIH-3T3 cells using the Rous sarcoma virus long terminal repeat as a promoter.

(e) Enhancer Element Component

Transcription of DNA encoding the target polypeptide of this invention by higher eukaryotes is often increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10-300 bp, that act on a promoter to increase its transcription. Enhancers are relatively orientation and position independent having been found 5' (Laimins *et al.*, Proc. Natl. Acad. Sci. USA, 78: 993 [1981]) and 3' (Lusky *et al.*, Mol. Cell Bio., 3: 1108 [1983]) to the transcription unit, within an intron (Banerji *et al.*, Cell, 33: 729 [1983]) as well as within the coding sequence itself (Osborne *et al.*, Mol. Cell Bio., 4: 1293 [1984]). Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin,  $\alpha$ -fetoprotein and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, Nature, 297: 17-18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a

position 5' or 3' to the target polypeptide DNA, but is preferably located at a site 5' from the promoter.

(f) Transcription Termination Component

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Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3' untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding the target polypeptide. The 3' untranslated regions also include transcription termination sites.

Construction of suitable vectors containing one or more of the above listed components the desired coding and control sequences employs standard ligation techniques. Isolated plasmids or DNA fragments are cleaved, tailored, and religated in the form desired to generate the plasmids required.

For analysis to confirm correct sequences in plasmids constructed, the ligation mixtures are used to transform *E. coli* K12 strain 294 (ATCC 31,446) and successful transformants selected by ampicillin or tetracycline resistance where appropriate. Plasmids from the transformants are prepared, analyzed by restriction endonuclease digestion, and/or sequenced by the method of Messing *et al.*, Nucleic Acids Res., **9**: 309 (1981) or by the method of Maxam *et al.*, Methods in Enzymology, **65**: 499 (1980).

Particularly useful in the practice of this invention are expression vectors that provide for the transient expression in mammalian cells of DNA encoding the target polypeptide. In general, transient expression involves the use of an expression vector that is able to replicate efficiently in a host cell, such that the host cell accumulates many copies of the expression vector and, in turn, synthesizes high levels of a desired polypeptide encoded by the

expression vector. Transient expression systems, comprising a suitable expression vector and a host cell, allow for the convenient positive identification of polypeptides encoded by cloned DNAs, as well as for the rapid screening of such polypeptides for desired biological or physiological properties. Thus, transient expression systems are particularly useful in the invention for purposes of identifying analogs and variants of the target polypeptide that have target polypeptide-like activity.

Other methods, vectors, and host cells suitable for adaptation to the synthesis of the target polypeptide in recombinant vertebrate cell culture are described in Gething *et al.*, Nature, 293: 620-625 [1981]; Mantei *et al.*, Nature, 281: 40-46 [1979]; Levinson *et al.*; EP 117,060; and EP 117,058. A particularly useful plasmid for mammalian cell culture expression of the target polypeptide is pRK5 (EP pub. no. 307,247) or pSVI6B (U.S. Ser. No. 07/441,574 filed 22 November 1989, the disclosure of which is incorporated herein by reference).

#### Selection and Transformation of Host Cells

Suitable host cells for cloning or expressing the vectors herein are the prokaryote, yeast, or higher eukaryote cells described above. Suitable prokaryotes include eubacteria, such as Gram-negative or Gram-positive organisms, for example, *E. coli*, *Bacilli* such as *B. subtilis*, *Pseudomonas* species such as *P. aeruginosa*, *Salmonella typhimurium*, or *Serratia marcescans*. One preferred *E. coli* cloning host is *E. coli* 294 (ATCC 31,446), although other strains such as *E. coli* B, *E. coli*  $\chi$ 1776 (ATCC 31,537), and *E. coli* W3110 (ATCC 27,325) are suitable. These examples are illustrative rather than limiting. Preferably the host cell should secrete minimal amounts of proteolytic enzymes. Alternatively, *in vitro* methods of cloning, e.g. PCR or other nucleic acid polymerase reactions, are suitable.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable hosts for target polypeptide-encoding vectors. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly

used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *Schizosaccharomyces pombe* [Beach and Nurse, Nature, 290: 140 (1981); EP 139,383 published May 2, 1985], *Kluyveromyces* hosts (U.S. 4,943,529) such as, e.g., *K. lactis* [Lourencourt *et al.*, J. Bacteriol., 737 (1983)], *K. fragilis*, *K. bulgaricus*, *K. thermotolerans*, and *K. marxianus*, *yarrowia* [EP 402,226], *Pichia pastoris* [EP 183,070; Sreekrishna *et al.*, J. Basic Microbiol., 28: 265-278 (1988)], *Candida*, *Trichoderma reesia* [EP 244,234], *Neurospora crassa* [Case *et al.*, Proc. Natl. Acad. Sci. USA, 76: 5259-5263 (1979)], and filamentous fungi such as, e.g., *Neurospora*, *Penicillium*, *Tolypocladium* [WO 91/00357 published 10 January 1991], and *Aspergillus* hosts such as *A. nidulans* [Ballance *et al.*, Biochem. Biophys. Res. Commun., 112: 284-289 (1983); Tilburn *et al.*, Gene, 26: 205-221 (1983); Yelton *et al.*, Proc. Natl. Acad. Sci. USA, 81: 1470-1474 (1984)] and *A. niger* [Kelly and Hynes, EMBO J., 4: 475-479 (1985)].

Suitable host cells for the expression of glycosylated target polypeptide are derived from multicellular organisms. Such host cells are capable of complex processing and glycosylation activities. In principle, any higher eukaryotic cell culture is workable, whether from vertebrate or invertebrate culture. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda* (caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosophila melanogaster* (fruitfly), and *Bombyx mori* host cells have been identified. See, e.g., Luckow *et al.*, Bio/Technology, 6: 47-55 (1988); Miller *et al.*, in Genetic Engineering, Setlow, J.K. *et al.*, eds., Vol. 8 (Plenum Publishing, 1986), pp. 277-279; and Maeda *et al.*, Nature, 315: 592-594 (1985). A variety of such viral strains are publicly available, e.g., the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells.

Plant cell cultures of cotton, corn, potato, soybean, petunia,

tomato, and tobacco can be utilized as hosts. Typically, plant cells are transfected by incubation with certain strains of the bacterium *Agrobacterium tumefaciens*, which has been previously manipulated to contain the target polypeptide DNA. During incubation of the plant cell culture with *A. tumefaciens*, the DNA encoding target polypeptide is transferred to the plant cell host such that it is transfected, and will, under appropriate conditions, express the target polypeptide DNA. In addition, regulatory and signal sequences compatible with plant cells are available, such as the nopaline synthase promoter and polyadenylation signal sequences. Depicker *et al.*, J. Mol. Appl. Gen., 1: 561 (1982). In addition, DNA segments isolated from the upstream region of the T-DNA 780 gene are capable of activating or increasing transcription levels of plant-expressible genes in recombinant DNA-containing plant tissue. See EP 321,196 published 21 June 1989.

However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure in recent years [Tissue Culture, Academic Press, Kruse and Patterson, editors (1973)]. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, J. Gen Virol., 36: 59 [1977]); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/DHFR (CHO, Urlaub and Chasin, Proc. Natl. Acad. Sci. USA, 77: 4216 [1980]); mouse sertoli cells (TM4, Mather, Biol. Reprod., 23: 243-251 [1980]); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather *et al.*, Annals N.Y. Acad. Sci., 383: 44-68 [1982]); MRC 5 cells; FS4 cells; and a human hepatoma cell line (Hep G2). Preferred host cells are human embryonic kidney 293 and Chinese hamster ovary cells.

Host cells are transfected and preferably transformed with the above-described expression or cloning vectors of this invention and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Transfection refers to the taking up of an expression vector by a host cell whether or not any coding sequences are in fact expressed. Numerous methods of transfection are known to the ordinarily skilled artisan, for example,  $\text{CaPO}_4$  and electroporation. Successful transfection is generally recognized when any indication of the operation of this vector occurs within the host cell.

Transformation means introducing DNA into an organism so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integrant. Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in section 1.82 of Sambrook *et al.*, *supra*, is generally used for prokaryotes or other cells that contain substantial cell-wall barriers. Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw *et al.*, Gene, **23**: 315 (1983) and WO 89/05859 published 29 June 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method described in sections 16.30-16.37 of Sambrook *et al.*, *supra*, is preferred. General aspects of mammalian cell host system transformations have been described by Axel in U.S. 4,399,216 issued 16 August 1983. Transformations into yeast are typically carried out according to the method of Van Solingen *et al.*, J. Bact., **130**: 946 (1977) and Hsiao *et al.*, Proc. Natl. Acad. Sci. (USA), **76**: 3829 (1979). However, other methods for introducing DNA into cells such as by nuclear injection, electroporation, or protoplast fusion may also be used.

#### Culturing the Host Cells

Prokaryotic cells used to produce the target polypeptide of this invention are cultured in suitable media as described generally in Sambrook *et al.*, *supra*.

5 The mammalian host cells used to produce the target polypeptide of this invention may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ([MEM], Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ([DMEM], Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham and Wallace, Meth. Enz., 58: 44  
10 (1979), Barnes and Sato, Anal. Biochem., 102: 255 (1980), U.S. 4,767,704; 4,657,866; 4,927,762; or 4,560,655; WO 90/03430; WO 87/00195; U.S. Pat. Re. 30,985; or copending U.S.S.N. 07/592,107 or 07/592,141, both filed in 3 October 1990, the disclosures of all of which are incorporated herein by reference, may be used as culture media for the host cells. Any of  
15 these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleosides (such as adenosine and thymidine), antibiotics (such as Gentamycin™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose  
20 or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

25 The host cells referred to in this disclosure encompass cells in *in vitro* culture as well as cells that are within a host animal.

30 It is further envisioned that the target polypeptides of this invention may be produced by homologous recombination, or with recombinant production methods utilizing control elements introduced into cells already containing DNA encoding the target polypeptide currently in use in the field. For example, a powerful promoter/enhancer element, a suppressor, or an

exogenous transcription modulatory element is inserted in the genome of the intended host cell in proximity and orientation sufficient to influence the transcription of DNA encoding the desired target polypeptide. The control element does not encode the target polypeptide of this invention, but the DNA is present in the host cell genome. One next screens for cells making the target polypeptide of this invention, or increased or decreased levels of expression, as desired.

#### Detecting Gene Amplification/Expression

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, northern blotting to quantitate the transcription of mRNA (Thomas, Proc. Natl. Acad. Sci. USA, 77: 5201-5205 [1980]), dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Various labels may be employed, most commonly radioisotopes, particularly <sup>32</sup>P. However, other techniques may also be employed, such as using biotin-modified nucleotides for introduction into a polynucleotide. The biotin then serves as the site for binding to avidin or antibodies, which may be labeled with a wide variety of labels, such as radionuclides, fluorescers, enzymes, or the like. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. With immunohistochemical staining techniques, a cell sample is prepared, typically by dehydration and fixation, followed by reaction with labeled antibodies specific for the gene product coupled, where the labels are



usually visually detectable, such as enzymatic labels, fluorescent labels, luminescent labels, and the like. A particularly sensitive staining technique suitable for use in the present invention is described by Hsu *et al.*, Am. J. Clin. Path., 75: 734-738 (1980).

5 Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared in any mammal. Conveniently, the antibodies may be prepared against a native target polypeptide or against a synthetic peptide based on the DNA sequences provided herein as described further in Section 4 below.

10 Purification of The Target polypeptide

The target polypeptide preferably is recovered from the culture medium as a secreted polypeptide, although it also may be recovered from host cell lysates when directly expressed without a secretory signal.

15 When the target polypeptide is expressed in a recombinant cell other than one of human origin, the target polypeptide is completely free of proteins or polypeptides of human origin. However, it is necessary to purify the target polypeptide from recombinant cell proteins or polypeptides to obtain preparations that are substantially homogeneous as to the target polypeptide. As a first step, the culture medium or lysate is centrifuged to  
20 remove particulate cell debris. The membrane and soluble protein fractions are then separated. The target polypeptide may then be purified from the soluble protein fraction and from the membrane fraction of the culture lysate, depending on whether the target polypeptide is membrane bound. The  
25 following procedures are exemplary of suitable purification procedures: fractionation on immunoaffinity or ion-exchange columns; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-75; and  
30 protein A Sepharose columns to remove contaminants such as IgG.

Target polypeptide variants in which residues have been deleted, inserted or substituted are recovered in the same fashion, taking account of

any substantial changes in properties occasioned by the variation. For example, preparation of a target polypeptide fusion with another protein or polypeptide, e.g. a bacterial or viral antigen, facilitates purification; an immunoaffinity column containing antibody to the antigen (or containing antigen, where the target polypeptide is an antibody) can be used to adsorb the fusion. Immunoaffinity columns such as a rabbit polyclonal anti-target polypeptide column can be employed to absorb the target polypeptide variant by binding it to at least one remaining immune epitope. A protease inhibitor such as phenyl methyl sulfonyl fluoride (PMSF) also may be useful to inhibit proteolytic degradation during purification, and antibiotics may be included to prevent the growth of adventitious contaminants. One skilled in the art will appreciate that purification methods suitable for native target polypeptide may require modification to account for changes in the character of the target polypeptide or its variants upon expression in recombinant cell culture.

#### Covalent Modifications of Target Polypeptides

Covalent modifications of target polypeptides are included within the scope of this invention. One type of covalent modification included within the scope of this invention is a target polypeptide fragment. Target polypeptide fragments having up to about 40 amino acid residues may be conveniently prepared by chemical synthesis, or by enzymatic or chemical cleavage of the full-length target polypeptide or variant target polypeptide. Other types of covalent modifications of the target polypeptide or fragments thereof are introduced into the molecule by reacting specific amino acid residues of the target polypeptide or fragments thereof with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues.

Cysteinyll residues most commonly are reacted with  $\alpha$ -haloacetates (and corresponding amines), such as chloroacetic acid or chloroacetamide, to give carboxymethyl or carboxyamidomethyl derivatives. Cysteinyll residues also are derivatized by reaction with bromotrifluoroacetone,  $\alpha$ -

bromo- $\beta$ -(5-imidazole)propionic acid, chloroacetyl phosphate, N-alkylmaleimides, 3-nitro-2-pyridyl disulfide, methyl 2-pyridyl disulfide, p-chloromercuribenzoate, 2-chloromercuri-4-nitrophenol, or chloro-7-nitrobenzo-2-oxa-1,3-diazole.

5 Histidyl residues are derivatized by reaction with diethylpyrocarbonate at pH 5.5-7.0 because this agent is relatively specific for the histidyl side chain. Para-bromophenacyl bromide also is useful; the reaction is preferably performed in 0.1M sodium cacodylate at pH 6.0.

10 Lysinyl and amino terminal residues are reacted with succinic or other carboxylic acid anhydrides. Derivatization with these agents has the effect of reversing the charge of the lysinyl residues. Other suitable reagents for derivatizing  $\alpha$ -amino-containing residues include imidoesters such as methyl picolinimate; pyridoxal phosphate; pyridoxal; chloroborohydride; trinitrobenzenesulfonic acid; O-methylisourea; 2,4-pentanedione; and  
15 transaminase-catalyzed reaction with glyoxylate.

Arginyl residues are modified by reaction with one or several conventional reagents, among them phenylglyoxal, 2,3-butanedione, 1,2-cyclohexanedione, and ninhydrin. Derivatization of arginine residues requires that the reaction be performed in alkaline conditions because of the high  $pK_a$   
20 of the guanidine functional group. Furthermore, these reagents may react with the groups of lysine as well as the arginine epsilon-amino group.

The specific modification of tyrosyl residues may be made, with particular interest in introducing spectral labels into tyrosyl residues by reaction with aromatic diazonium compounds or tetranitromethane. Most  
25 commonly, N-acetylimidazole and tetranitromethane are used to form O-acetyl tyrosyl species and 3-nitro derivatives, respectively. Tyrosyl residues are iodinated using  $^{125}I$  or  $^{131}I$  to prepare labeled proteins for use in radioimmunoassay, the chloramine T method described above being suitable.

30 Carboxyl side groups (aspartyl or glutamyl) are selectively modified by reaction with carbodiimides ( $R'-N=C=N-R'$ ), where R and R' are different alkyl groups, such as 1-cyclohexyl-3-(2-morpholinyl-4-ethyl) carbodiimide or 1-ethyl-3-(4-azonia-4,4-dimethylpentyl) carbodiimide. Furthermore, aspartyl

and glutamyl residues are converted to asparaginy and glutaminy residues by reaction with ammonium ions.

Derivatization with bifunctional agents is useful for crosslinking target polypeptide to a water-insoluble support matrix or surface for use in the method for purifying anti-target polypeptide antibodies, and vice versa. Commonly used crosslinking agents include, e.g., 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis(succinimidylpropionate), and bifunctional maleimides such as bis-N-maleimido-1,8-octane. Derivatizing agents such as methyl-3-[(p-azidophenyl)dithio]propioimidate yield photoactivatable intermediates that are capable of forming crosslinks in the presence of light. Alternatively, reactive water-insoluble matrices such as cyanogen bromide-activated carbohydrates and the reactive substrates described in U.S. 3,969,287; 3,691,016; 4,195,128; 4,247,642; 4,229,537; and 4,330,440 are employed for protein immobilization.

Glutaminy and asparaginy residues are frequently deamidated to the corresponding glutamyl and aspartyl residues, respectively. Alternatively, these residues are deamidated under mildly acidic conditions. Either form of these residues falls within the scope of this invention.

Other modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the  $\alpha$ -amino groups of lysine, arginine, and histidine side chains (T.E. Creighton, Proteins: Structure and Molecular Properties, W.H. Freeman & Co., San Francisco, pp. 79-86 [1983]), acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

Another type of covalent modification of the target polypeptide included within the scope of this invention comprises altering the native glycosylation pattern of the polypeptide. By altering is meant deleting one or more carbohydrate moieties found in the native target polypeptide, and/or adding one or more glycosylation sites that are not present in the native target polypeptide.

Glycosylation of polypeptides is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tri-peptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tri-peptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose, to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

Addition of glycosylation sites to the target polypeptide is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tri-peptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the native target polypeptide sequence (for O-linked glycosylation sites). For ease, the target polypeptide amino acid sequence is preferably altered through changes at the DNA level, particularly by mutating the DNA encoding the target polypeptide at preselected bases such that codons are generated that will translate into the desired amino acids. The DNA mutation(s) may be made using methods described above under the heading of "Amino Acid Sequence Variants of Target Polypeptide".

Another means of increasing the number of carbohydrate moieties on the target polypeptide is by chemical or enzymatic coupling of glycosides to the polypeptide. These procedures are advantageous in that they do not require production of the polypeptide in a host cell that has glycosylation capabilities for N- and O- linked glycosylation. Depending on the coupling mode used, the sugar(s) may be attached to (a) arginine and histidine, (b) free carboxyl groups, (c) free sulfhydryl groups such as those of cysteine, (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline, (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan,

or (f) the amide group of glutamine. These methods are described in WO 87/05330 published 11 September 1987, and in Aplin and Wriston (CRC Crit. Rev. Biochem., pp. 259-306 [1981]).

5 Removal of carbohydrate moieties present on the native target polypeptide may be accomplished chemically or enzymatically. Chemical deglycosylation requires exposure of the polypeptide to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the polypeptide  
10 intact. Chemical deglycosylation is described by Hakimuddin *et al.* (Arch. Biochem. Biophys., 259:52 [1987]) and by Edge *et al.* (Anal. Biochem., 118:131 [1981]). Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura *et al.* (Meth. Enzymol., 138:350 [1987]).  
15

Glycosylation at potential glycosylation sites may be prevented by the use of the compound tunicamycin as described by Duskin *et al.* (J. Biol. Chem., 257:3105 [1982]). Tunicamycin blocks the formation of protein-N-glycoside linkages.

20 Another type of covalent modification of the target polypeptide comprises linking the target polypeptide to various nonproteinaceous polymers, e.g. polyethylene glycol, polypropylene glycol or polyoxyalkylenes, in the manner set forth in U.S. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337.

25 The target polypeptide also may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-microcapsules and poly-[methylmethacrylate] microcapsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres,  
30 microemulsions, nano-particles and nanocapsules), or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences, 16th edition, Osol, A., Ed., (1980).

5 Target polypeptide preparations are also useful in generating antibodies, for screening for binding partners, as standards in assays for the target polypeptide (e.g. by labeling the target polypeptide for use as a standard in a radioimmunoassay, enzyme-linked immunoassay, or radioreceptor assay), in affinity purification techniques, and in competitive-type receptor binding assays when labeled with radioiodine, enzymes, fluorophores, spin labels, and the like.

10 Since it is often difficult to predict in advance the characteristics of a variant target polypeptide, it will be appreciated that some screening of the recovered variant will be needed to select the optimal variant. For example, a change in the immunological character of the target polypeptide molecule, such as affinity for a given antigen or antibody, is measured by a competitive-type immunoassay. The variant is assayed for changes in the suppression or enhancement of its activity by comparison to the activity  
15 observed for the target polypeptide in the same assay. Other potential modifications of protein or polypeptide properties such as redox or thermal stability, hydrophobicity, susceptibility to proteolytic degradation, stability in recombinant cell culture or in plasma, or the tendency to aggregate with carriers or into multimers are assayed by methods well known in the art.

#### 20 Diagnostic and Related Uses of the Antibodies

The antibodies of this invention are useful in diagnostic assays for antigen expression in specific cells or tissues. The antibodies are detectably labeled and/or are immobilized on an insoluble matrix.

25 The antibodies of this invention find further use for the affinity purification of the antigen from recombinant cell culture or natural sources.

Suitable diagnostic assays for the antigen and its antibodies depend on the particular antigen or antibody. Generally, such assays include competitive and sandwich assays, and steric inhibition assays. Competitive and sandwich methods employ a phase-separation step as an integral part of  
30 the method while steric inhibition assays are conducted in a single reaction mixture. Fundamentally, the same procedures are used for the assay of the

antigen and for substances that bind the antigen, although certain methods will be favored depending upon the molecular weight of the substance being assayed. Therefore, the substance to be tested is referred to herein as an analyte, irrespective of its status otherwise as an antigen or antibody, and proteins that bind to the analyte are denominated binding partners, whether they be antibodies, cell surface receptors, or antigens.

Analytical methods for the antigen or its antibodies all use one or more of the following reagents: labeled analyte analogue, immobilized analyte analogue, labeled binding partner, immobilized binding partner and steric conjugates. The labeled reagents also are known as "tracers."

The label used (and this is also useful to label antigen nucleic acid for use as a probe) is any detectable functionality that does not interfere with the binding of analyte and its binding partner. Numerous labels are known for use in immunoassay, examples including moieties that may be detected directly, such as fluorochrome, chemiluminescent, and radioactive labels, as well as moieties, such as enzymes, that must be reacted or derivatized to be detected. Examples of such labels include the radioisotopes  $^{32}\text{P}$ ,  $^{14}\text{C}$ ,  $^{125}\text{I}$ ,  $^3\text{H}$ , and  $^{131}\text{I}$ , fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luciferases, e.g., firefly luciferase and bacterial luciferase (U.S. Pat. No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase,  $\beta$ -galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like.

Conventional methods are available to bind these labels covalently to proteins or polypeptides. For instance, coupling agents such as dialdehydes, carbodiimides, dimaleimides, bis-imidates, bis-diazotized benzidine, and the like may be used to tag the antibodies with the above-



described fluorescent, chemiluminescent, and enzyme labels. See, for example, U.S. Pat. Nos. 3,940,475 (fluorimetry) and 3,645,090 (enzymes); Hunter *et al.*, Nature, 144: 945 (1962); David *et al.*, Biochemistry, 13: 1014-1021 (1974); Pain *et al.*, J. Immunol. Methods, 40: 219-230 (1981); and Nygren, J. Histochem. and Cytochem., 30: 407-412 (1982). Preferred labels herein are enzymes such as horseradish peroxidase and alkaline phosphatase.

The conjugation of such label, including the enzymes, to the antibody is a standard manipulative procedure for one of ordinary skill in immunoassay techniques. See, for example, O'Sullivan *et al.*, "Methods for the Preparation of Enzyme-antibody Conjugates for Use in Enzyme Immunoassay," in Methods in Enzymology, ed. J.J. Langone and H. Van Vunakis, Vol. 73 (Academic Press, New York, New York, 1981), pp. 147-166. Such bonding methods are suitable for use with the antibodies and polypeptides of this invention.

Immobilization of reagents is required for certain assay methods. Immobilization entails separating the binding partner from any analyte that remains free in solution. This conventionally is accomplished by either insolubilizing the binding partner or analyte analogue before the assay procedure, as by adsorption to a water-insoluble matrix or surface (Bennich *et al.*, U.S. 3,720,760), by covalent coupling (for example, using glutaraldehyde cross-linking), or by insolubilizing the partner or analogue afterward, e.g., by immunoprecipitation.

Other assay methods, known as competitive or sandwich assays, are well established and widely used in the commercial diagnostics industry.

Competitive assays rely on the ability of a tracer analogue to compete with the test sample analyte for a limited number of binding sites on a common binding partner. The binding partner generally is insolubilized before or after the competition and then the tracer and analyte bound to the binding partner are separated from the unbound tracer and analyte. This separation is accomplished by decanting (where the binding partner was preinsolubilized) or by centrifuging (where the binding partner was precipitated after the competitive reaction). The amount of test sample

analyte is inversely proportional to the amount of bound tracer as measured by the amount of marker substance. Dose-response curves with known amounts of analyte are prepared and compared with the test results to quantitatively determine the amount of analyte present in the test sample. These assays are called ELISA systems when enzymes are used as the detectable markers.

Another species of competitive assay, called a "homogeneous" assay, does not require a phase separation. Here, a conjugate of an enzyme with the analyte is prepared and used such that when anti-analyte binds to the analyte the presence of the anti-analyte modifies the enzyme activity. In this case, the antigen or its immunologically active fragments are conjugated with a bifunctional organic bridge to an enzyme such as peroxidase. Conjugates are selected for use with antibody so that binding of the antibody inhibits or potentiates the enzyme activity of the label. This method *per se* is widely practiced under the name of EMIT.

Steric conjugates are used in steric hindrance methods for homogeneous assay. These conjugates are synthesized by covalently linking a low-molecular-weight hapten to a small analyte so that antibody to hapten substantially is unable to bind the conjugate at the same time as anti-analyte. Under this assay procedure the analyte present in the test sample will bind anti-analyte, thereby allowing anti-hapten to bind the conjugate, resulting in a change in the character of the conjugate hapten, e.g., a change in fluorescence when the hapten is a fluorophore.

Sandwich assays particularly are useful for the determination of antigen or antibodies. In sequential sandwich assays an immobilized binding partner is used to adsorb test sample analyte, the test sample is removed as by washing, the bound analyte is used to adsorb labeled binding partner, and bound material is then separated from residual tracer. The amount of bound tracer is directly proportional to test sample analyte. In "simultaneous" sandwich assays the test sample is not separated before adding the labeled binding partner. A sequential sandwich assay using an anti-antigen monoclonal antibody as one antibody and a polyclonal anti-antigen antibody

as the other is useful in testing samples for particular antigen activity.

The foregoing are merely exemplary diagnostic assays for the import and humanized antibodies of this invention. Other methods now or hereafter developed for the determination of these analytes are included within the scope hereof, including the bioassays described above.

#### Immunotoxins

This invention is also directed to immunochemical derivatives of the antibodies of this invention such as immunotoxins (conjugates of the antibody and a cytotoxic moiety). Antibodies which carry the appropriate effector functions, such as with their constant domains, are also used to induce lysis through the natural complement process, and to interact with antibody dependent cytotoxic cells normally present.

For example, purified, sterile filtered antibodies are optionally conjugated to a cytotoxin such as ricin for use in AIDS therapy. US Patent Application Serial No. 07/350,895 illustrates methods for making and using immunotoxins for the treatment of HIV infection, and its teachings are specifically incorporated by reference herein. The methods of this invention, for example, are suitable for obtaining humanized antibodies for use as immunotoxins for use in AIDS therapy.

The cytotoxic moiety of the immunotoxin may be a cytotoxic drug or an enzymatically active toxin of bacterial, fungal, plant or animal origin, or an enzymatically active fragment of such a toxin. Enzymatically active toxins and fragments thereof used are diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. In another embodiment, the antibodies are conjugated to small molecule anticancer drugs such as cis-platin or 5FU. Conjugates of the monoclonal antibody and such cytotoxic moieties are made

5 using a variety of bifunctional protein coupling agents. Examples of such reagents are SPDP, IT , bifunctional derivatives of imidoesters such as dimethyl adipimidate HCl, active esters such as disuccinimidyl suberate, aldehydes such as glutaraldehyde, bis-azido compounds such as bis (p-azidobenzoyl) hexanediamine, bis-diazonium derivatives such as bis- (p-diazoniumbenzoyl)- -ethylenediamine, diisocyanates such as tolylene 2,6-diisocyanate and bis-active fluorine compounds such as 1,5-difluoro-2,4-dinitrobenzene. The lysing portion of a toxin may be joined to the Fab fragment of the antibodies.

10 Immunotoxins can be made in a variety of ways, as discussed herein. Commonly known crosslinking reagents can be used to yield stable conjugates.

15 Advantageously, monoclonal antibodies specifically binding the domain of the antigen which is exposed on the infected cell surface, are conjugated to ricin A chain. Most advantageously the ricin A chain is deglycosylated and produced through recombinant means. An advantageous method of making the ricin immunotoxin is described in Vitetta *et al.*, *Science* 238:1098 (1987) hereby incorporated by reference.

20 When used to kill infected human cells *in vitro* for diagnostic purposes, the conjugates will typically be added to the cell culture medium at a concentration of at least about 10 nM. The formulation and mode of administration for *in vitro* use are not critical. Aqueous formulations that are compatible with the culture or perfusion medium will normally be used. Cytotoxicity may be read by conventional techniques.

25 Cytotoxic radiopharmaceuticals for treating infected cells may be made by conjugating radioactive isotopes (e.g. I, Y, Pr) to the antibodies. Advantageously alpha particle-emitting isotopes are used. The term 'cytotoxic moiety' as used herein is intended to include such isotopes.

30 In a preferred embodiment, ricin A chain is deglycosylated or produced without oligosaccharides, to decrease its clearance by irrelevant clearance mechanisms (e.g., the liver). In another embodiment, whole ricin (A chain plus B chain) is conjugated to antibody if the galactose binding

property of B-chain can be blocked ("blocked ricin").

In a further embodiment toxin-conjugates are made with Fab or F(ab')<sub>2</sub> fragments. Because of their relatively small size these fragments can better penetrate tissue to reach infected cells.

5 In another embodiment, fusogenic liposomes are filled with a cytotoxic drug and the liposomes are coated with antibodies specifically binding the particular antigen.

#### Antibody Dependent Cellular Cytotoxicity

10 Certain aspects of this invention involve antibodies which are (a) directed against a particular antigen and (b) belong to a subclass or isotype that is capable of mediating the lysis of cells to which the antibody molecule binds. More specifically, these antibodies should belong to a subclass or isotype that, upon complexing with cell surface proteins, activates serum complement and/or mediates antibody dependent cellular cytotoxicity (ADCC) by activating effector cells such as natural killer cells or macrophages.

15 Biological activity of antibodies is known to be determined, to a large extent, by the constant domains or Fc region of the antibody molecule (Uananie and Benacerraf, *Textbook of Immunology*, 2nd Edition, Williams & Wilkins, p. 218 (1984)). This includes their ability to activate complement and to mediate antibody-dependent cellular cytotoxicity (ADCC) as effected by leukocytes. Antibodies of different classes and subclasses differ in this respect, as do antibodies from the same subclass but different species; according to the present invention, antibodies of those classes having the  
25 desired biological activity are prepared. Preparation of these antibodies involves the selection of antibody constant domains and their incorporation in the humanized antibody by known technique. For example, mouse immunoglobulins of the IgG3 and IgG2a class are capable of activating serum complement upon binding to the target cells which express the cognate  
30 antigen, and therefore humanized antibodies which incorporate IgG3 and IgG2a effector functions are desirable for certain therapeutic applications.

In general, mouse antibodies of the IgG2a and IgG3 subclass and

occasionally IgG1 can mediate ADCC, and antibodies of the IgG3, IgG2a, and IgM subclasses bind and activate serum complement. Complement activation generally requires the binding of at least two IgG molecules in close proximity on the target cell. However, the binding of only one IgM molecule activates serum complement.

The ability of any particular antibody to mediate lysis of the target cell by complement activation and/or ADCC can be assayed. The cells of interest are grown and labeled *in vitro*; the antibody is added to the cell culture in combination with either serum complement or immune cells which may be activated by the antigen antibody complexes. Cytolysis of the target cells is detected by the release of label from the lysed cells. In fact, antibodies can be screened using the patient's own serum as a source of complement and/or immune cells. The antibody that is capable of activating complement or mediating ADCC in the *in vitro* test can then be used therapeutically in that particular patient.

This invention specifically encompasses consensus Fc antibody domains prepared and used according to the teachings of this invention.

#### Therapeutic and Other Uses of the Antibodies

When used *in vivo* for therapy, the antibodies of the subject invention are administered to the patient in therapeutically effective amounts (i.e. amounts that have desired therapeutic effect). They will normally be administered parenterally. The dose and dosage regimen will depend upon the degree of the infection, the characteristics of the particular antibody or immunotoxin used, e.g., its therapeutic index, the patient, and the patient's history. Advantageously the antibody or immunotoxin is administered continuously over a period of 1-2 weeks, intravenously to treat cells in the vasculature and subcutaneously and intraperitoneally to treat regional lymph nodes. Optionally, the administration is made during the course of adjunct therapy such as combined cycles of radiation, chemotherapeutic treatment, or administration of tumor necrosis factor, interferon or other cytoprotective or immunomodulatory agent.

For parenteral administration the antibodies will be formulated in a unit dosage injectable form (solution, suspension, emulsion) in association with a pharmaceutically acceptable parenteral vehicle. Such vehicles are inherently nontoxic, and non-therapeutic. Examples of such vehicles are water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Nonaqueous vehicles such as fixed oils and ethyl oleate can also be used. Liposomes may be used as carriers. The vehicle may contain minor amounts of additives such as substances that enhance isotonicity and chemical stability, e.g., buffers and preservatives. The antibodies will typically be formulated in such vehicles at concentrations of about 1 mg/ml to 10 mg/ml.

Use of IgM antibodies may be preferred for certain applications, however IgG molecules by being smaller may be more able than IgM molecules to localize to certain types of infected cells.

There is evidence that complement activation *in vivo* leads to a variety of biological effects, including the induction of an inflammatory response and the activation of macrophages (Uananue and Benecerraf, *Textbook of Immunology*, 2nd Edition, Williams & Wilkins, p. 218 (1984)). The increased vasodilation accompanying inflammation may increase the ability of various agents to localize in infected cells. Therefore, antigen-antibody combinations of the type specified by this invention can be used therapeutically in many ways. Additionally, purified antigens (Hakomori, *Ann. Rev. Immunol.* 2:103 (1984)) or anti-idiotypic antibodies (Nepom *et al.*, *Proc. Natl. Acad. Sci.* 81:2864 (1985); Koprowski *et al.*, *Proc. Natl. Acad. Sci.* 81:216 (1984)) relating to such antigens could be used to induce an active immune response in human patients. Such a response includes the formation of antibodies capable of activating human complement and mediating ADCC and by such mechanisms cause infected cell destruction.

Optionally, the antibodies of this invention are useful in passively immunizing patients, as exemplified by the administration of humanized anti-HIV antibodies.

The antibody compositions used in therapy are formulated and dosages established in a fashion consistent with good medical practice taking into account the disorder to be treated, the condition of the individual patient, the site of delivery of the composition, the method of administration and other factors known to practitioners. The antibody compositions are prepared for administration according to the description of preparation of polypeptides for administration, *infra*.

#### Deposit of Materials

As described above, cultures of the muMAb4D5 have been deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, USA (ATCC).

This deposit was made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of viable cultures for 30 years from the date of the deposit. The organisms will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Genentech, Inc. and ATCC, which assures permanent and unrestricted availability of the progeny of the cultures to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 USC §122 and the Commissioner's rules pursuant thereto (including 37 CFR §1.12 with particular reference to 886 OG 638).

In respect of those designations in which a European patent is sought, a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample. (Rule 28(4) EPC)



The assignee of the present application has agreed that if the cultures on deposit should die or be lost or destroyed when cultivated under suitable conditions, they will be promptly replaced on notification with a viable specimen of the same culture. Availability of the deposited strain is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by the constructs deposited, since the deposited embodiments are intended to illustrate only certain aspects of the invention and any constructs that are functionally equivalent are within the scope of this invention. The deposit of material herein does not constitute an admission that the written description herein contained is inadequate to enable the practice of any aspect of the invention, including the best mode thereof, nor is it to be construed as limiting the scope of the claims to the specific illustrations that they represent. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

It is understood that the application of the teachings of the present invention to a specific problem or situation will be within the capabilities of one having ordinary skill in the art in light of the teachings contained herein. Examples of the products of the present invention and representative processes for their isolation, use, and manufacture appear below, but should not be construed to limit the invention. All literature citations herein are expressly incorporated by reference.

## EXAMPLES

### EXAMPLE 1. HUMANIZATION OF muMAb4D5

Here we report the chimerization of muMAb4D5 (chMAb4D5) and the rapid and simultaneous humanization of heavy ( $V_H$ ) and light ( $V_L$ ) chain variable region genes using a novel "gene conversion mutagenesis" strategy. Eight humanized variants (huMAb4D5) were constructed to probe the importance of several FR residues identified by our molecular modeling or previously proposed to be critical to the conformation of particular CDRs (see Chothia, C. & Lesk, A. M., *J. Mol. Biol.* **196**:901-917 (1987); Chothia, C. *et al.*, *Nature* **342**:877-883 (1989); Tramontano, A. *et al.*, *J. Mol. Biol.* **215**:175-182 (1990)). Efficient transient expression of humanized variants in non-myeloma cells allowed us to rapidly investigate the relationship between binding affinity for p185<sup>HER2</sup> ECD and anti-proliferative activity against p185<sup>HER2</sup> overexpressing carcinoma cells.

#### MATERIALS and METHODS

**Cloning of Variable Region Genes.** The muMAb4D5  $V_H$  and  $V_L$  genes were isolated by polymerase chain reaction (PCR) amplification of mRNA from the corresponding hybridoma (Fendly, B. M. *et al.*, *Cancer Res.* **50**:1550-1558 (1990)) as described by Orlandi *et al.* (Orlandi, R. *et al.*, *Proc. Natl. Acad. Sci. USA* **86**:3833-3837 (1989)). Amino terminal sequencing of muMAb4D5  $V_L$  and  $V_H$  was used to design the sense strand PCR primers, whereas the anti-sense PCR primers were based upon consensus sequences of murine framework residues (Orlandi, R. *et al.*, *Proc. Natl. Acad. Sci. USA* **86**:3833-3837 (1989); Kabat, E. A. *et al.*, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)) incorporating restriction sites for directional cloning shown by underlining and listed after the sequences:  $V_L$  sense, 5'-TCCGATATCCAGCTGACCCAGTCTCCA-3' (SEQ. ID NO. 7), *EcoRV*;  $V_L$  anti-sense, 5'-GTTTGATCTCCAGCTTGGTACCHSCDCCGAA-3' (SEQ. ID NO. 8), *Asp718*;  $V_H$  sense, 5'-AGGTSMARCTGCAGSAGTCWGG-3' (SEQ. ID NO. 9), *PstI* and  $V_H$  anti-sense, 5'-TGAGGAGACGGTGACCGTGGTCCCTTGCCCCAG-3' (SEQ. ID NO. 10), *BstEII*; where H = A or C or T, S = C or G, D = A or G or T, M = A or C, R = A or G and W = A or T. The PCR products were cloned into pUC119

(Vieira, J. & Messing, J., *Methods Enzymol.* 153:3-11 (1987)) and five clones for each variable domain sequenced by the dideoxy method (Sanger, F. *et al.*, *Proc. Natl. Acad. Sci. USA* 74:5463-5467 (1977)).

**Molecular Modelling.** Models for muMAb4D5 V<sub>H</sub> and V<sub>L</sub> domains were constructed separately from consensus coordinates based upon seven Fab structures from the Brookhaven protein data bank (entries 1FB4, 2RHE, 2MCP, 3FAB, 1FBJ, 2HFL and 1REI). The Fab fragment KOL (Marquart, M. *et al.*, *J. Mol. Biol.* 141:369-391 (1980)) was first chosen as a template for V<sub>L</sub> and V<sub>H</sub> domains and additional structures were then superimposed upon this structure using their main chain atom coordinates (INSIGHT program, Biosym Technologies). The distance from the template C $\alpha$  to the analogous C $\alpha$  in each of the superimposed structures was calculated for each residue position. If all (or nearly all) C $\alpha$ -C $\alpha$  distances for a given residue were  $\leq 1 \text{ \AA}$ , then that position was included in the consensus structure. In most cases the  $\beta$ -sheet framework residues satisfied these criteria whereas the CDR loops did not. For each of these selected residues the average coordinates for individual N, C $\alpha$ , C, O and C $\beta$  atoms were calculated and then corrected for resultant deviations from non-standard bond geometry by 50 cycles of energy minimization using the DISCOVER program (Biosym Technologies) with the AMBER forcefield (Weiner, S. J. *et al.*, *J. Amer. Chem. Soc.* 106:765-784 (1984)) and C $\alpha$  coordinates fixed. The side chains of highly conserved residues, such as the disulfide-bridged cysteine residues, were then incorporated into the resultant consensus structure. Next the sequences of muMAb4D5 V<sub>L</sub> and V<sub>H</sub> were incorporated starting with the CDR residues and using the tabulations of CDR conformations from Chothia *et al.* (Chothia, C. *et al.*, *Nature* 342:877-883 (1989)) as a guide. Side-chain conformations were chosen on the basis of Fab crystal structures, rotamer libraries (Ponder, J. W. & Richards, F. M., *J. Mol. Biol.* 193:775-791 (1987)) and packing considerations. Since V<sub>H</sub>-CDR3 could not be assigned a definite backbone conformation from these criteria, two models were created from a search of similar sized loops using the INSIGHT program. A third model was derived using packing and solvent exposure considerations. Each model

was then subjected to 5000 cycles of energy minimization.

In humanizing muMAb4D5, consensus human sequences were first derived from the most abundant subclasses in the sequence compilation of Kabat et al. (Kabat, E. A. *et al.*, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)), namely V<sub>L</sub>  $\kappa$  subgroup I and V<sub>H</sub> group III, and a molecular model generated for these sequences using the methods described above. A structure for huMAb4D5 was created by transferring the CDRs from the muMAb4D5 model into the consensus human structure. All huMAb4D5 variants contain human replacements of muMAb4D5 residues at three positions within CDRs as defined by sequence variability (Kabat, E. A. *et al.*, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)) but not as defined by structural variability (Chothia, C. & Lesk, A. M., *J. Mol. Biol.* 196:901-917 (1987)): V<sub>L</sub>-CDR1 K24R, V<sub>L</sub>-CDR2 R54L and V<sub>L</sub>-CDR2 T56S. Differences between muMAb4D5 and the human consensus framework residues (Fig. 1) were individually modeled to investigate their possible influence on CDR conformation and/or binding to the p185<sup>HER2</sup> ECD.

**Construction of Chimeric Genes.** Genes encoding chMAb4D5 light and heavy chains were separately assembled in previously described phagemid vectors containing the human cytomegalovirus enhancer and promoter, a 5' intron and SV40 polyadenylation signal (Gorman, C. M. *et al.*, *DNA & Prot. Engin. Tech.* 2:3-10 (1990)). Briefly, gene segments encoding muMAb4D5 V<sub>L</sub> (Fig. 1A) and REI human  $\kappa_1$  light chain C<sub>L</sub> (Palm, W. & Hilschmann, N., *Z. Physiol. Chem.* 356:167-191 (1975)) were precisely joined as were genes for muMAb4D5 V<sub>H</sub> (Fig. 1B) and human  $\gamma_1$  constant region (Capon, D. J. *et al.*, *Nature* 337:525-531 (1989)) by simple subcloning (Boyle, A., in *Current Protocols in Molecular Biology*, Chapter 3 (F. A. Ausubel *et al.*, eds., Greene Publishing & Wiley-Interscience, New York, 1990)) and site-directed mutagenesis (Carter, P., in *Mutagenesis: A Practical Approach*, Chapter 1 (IRL Press, Oxford, UK 1991)). The  $\gamma_1$  isotype was chosen as it has been found to be the preferred human isotype for

5 supporting ADCC and complement dependent cytotoxicity using matched sets of chimeric (Brüggemann, M. *et al.*, *J. Exp. Med.* 166:1351-1361 (1987)) or humanized antibodies (Riechmann, L. *et al.*, *Nature* 332:323-327 (1988)). The PCR-generated  $V_L$  and  $V_H$  fragments (Fig. 1) were subsequently mutagenized so that they faithfully represent the sequence of muMAb4D5 determined at the protein level:  $V_H$  Q1E,  $V_L$  V104L and T109A (variants are denoted by the amino acid residue and number followed by the replacement amino acid). The human  $\gamma 1$  constant regions are identical to those reported by Ellison *et al.* (Ellison, J. W. *et al.*, *Nucleic Acids Res.* 10 13:4071-4079 (1982)) except for the mutations E359D and M361L (Eu numbering, as in Kabat, E. A. *et al.*, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)) which we installed to convert the antibody from the naturally rare A allotype to the much more common non-A allotype (Tramontano, A. *et al.*, *J. Mol. Biol.* 15 215:175-182 (1990)). This was an attempt to reduce the risk of anti-allotype antibodies interfering with therapy.

**Construction of Humanized Genes.** Genes encoding chMAb4D5 light chain and heavy chain Fd fragment ( $V_H$  and  $C_H1$  domains) were subcloned together into pUC119 (Vieira, J. & Messing, J., *Methods Enzymol.* 20 153:3-11 (1987)) to create pAK1 and simultaneously humanized in a single step (Fig. 2). Briefly, sets of 6 contiguous oligonucleotides were designed to humanize  $V_H$  and  $V_L$  (Fig. 1). These oligonucleotides are 28 to 83 nucleotides in length, contain zero to 19 mismatches to the murine antibody template and are constrained to have 8 or 9 perfectly matched residues at each end to promote efficient annealing and ligation of adjacent oligonucleotides. The sets of  $V_H$  and  $V_L$  humanization oligonucleotides (5 pmol each) were phosphorylated with either ATP or  $\gamma$ - $^{32}$ P-ATP (Carter, P. 25 *Methods Enzymol.* 154:382-403 (1987)) and separately annealed with 3.7 pmol of pAK1 template in 40  $\mu$ l 10 mM Tris-HCl (pH 8.0) and 10 mM  $MgCl_2$  by cooling from 100  $^{\circ}$ C to room temperature over  $\sim$ 30 min. The annealed oligonucleotides were joined by incubation with T4 DNA ligase (12 units; New England Biolabs) in the presence of 2  $\mu$ l 5 mM ATP and 2  $\mu$ l 0.1 M DTT 30

for 10 min at 14 °C. After electrophoresis on a 6% acrylamide sequencing gel the assembled oligonucleotides were located by autoradiography and recovered by electroelution. The assembled oligonucleotides (~0.3 pmol each) were simultaneously annealed to 0.15 pmol single-stranded deoxyuridine-containing pAK1 prepared according to Kunkel et al. (Kunkel, T. A. et al., *Methods Enzymol.* **154**:367-382 (1987)) in 10 µl 40 mM Tris-HCl (pH 7.5) and 16 mM MgCl<sub>2</sub> as above. Heteroduplex DNA was constructed by extending the primers with T7 DNA polymerase and transformed into *E. coli* BMH 71-18 *mutL* as previously described (Carter, P., in *Mutagenesis: A Practical Approach*, Chapter 1 (IRL Press, Oxford, UK 1991)). The resultant phagemid DNA pool was enriched first for huV<sub>L</sub> by restriction purification using *XhoI* and then for huV<sub>H</sub> by restriction selection using *StuI* as described in Carter, P., in *Mutagenesis: A Practical Approach*, Chapter 1 (IRL Press, Oxford, UK 1991); and in Wells, J. A. et al., *Phil. Trans. R. Soc. Lond. A* **317**:415-423 (1986). Resultant clones containing both huV<sub>L</sub> and huV<sub>H</sub> genes were identified by nucleotide sequencing (Sanger, F. et al., *Proc. Natl. Acad. Sci. USA* **74**:5463-5467 (1977)) and designated pAK2. Additional humanized variants were generated by site-directed mutagenesis (Carter, P., in *Mutagenesis: A Practical Approach*, Chapter 1 (IRL Press, Oxford, UK 1991)). The muMAb4D5 V<sub>L</sub> and V<sub>H</sub> gene segments in the transient expression vectors described above were then precisely replaced with their humanized versions.

**Expression and Purification of MAb4D5 Variants.** Appropriate MAb4D5 light and heavy chain cDNA expression vectors were co-transfected into an adenovirus transformed human embryonic kidney cell line, 293 (Graham, F. L. et al., *J. Gen. Virol.* **36**:59-72 (1977)) using a high efficiency procedure (Gorman, C. M. et al., *DNA & Prot. Engin. Tech.* **2**:3-10 (1990); Gorman, C., in *DNA Cloning*, vol II, pp 143-190 (D. M. Glover, ed., IRL Press, Oxford, UK 1985)). Media were harvested daily for up to 5 days and the cells re-fed with serum free media. Antibodies were recovered from the media and affinity purified on protein A sepharose CL-4B (Pharmacia) as described by the manufacturer. The eluted antibody was buffer-exchanged

into phosphate-buffered saline by G25 gel filtration, concentrated by ultrafiltration (Centriprep-30 or Centricon-100, Amicon), sterile-filtered (Millex-GV, Millipore) and stored at 4 °C. The concentration of antibody was determined by using both total immunoglobulin and antigen binding ELISAs. The standard used was huMAb4D5-5, whose concentration had been determined by amino acid composition analysis.

**Cell Proliferation Assay.** The effect of MAb4D5 variants upon proliferation of the human mammary adenocarcinoma cell line, SK-BR-3, was investigated as previously described (Fendly, B. M. *et al.*, *Cancer Res.* 50:1550-1558 (1990)) using saturating MAb4D5 concentrations.

**Affinity Measurements.** The antigen binding affinity of MAb4D5 variants was determined using a secreted form of the p185<sup>HER2</sup> ECD prepared as described in Fendly, B. M. *et al.*, *J. Biol. Resp. Mod.* 9:449-455 (1990). Briefly, antibody and p185<sup>HER2</sup> ECD were incubated in solution until equilibrium was found to be reached. The concentration of free antibody was then determined by ELISA using immobilized p185<sup>HER2</sup> ECD and used to calculate affinity ( $K_d$ ) according to Friguet *et al.* (Friguet, B. *et al.*, *J. Immunol. Methods* 77:305-319 (1985)).

## RESULTS

**Humanization of muMAb4D5.** The muMAb4D5 V<sub>L</sub> and V<sub>H</sub> gene segments were first cloned by PCR and sequenced (Fig. 1). The variable genes were then simultaneously humanized by gene conversion mutagenesis using preassembled oligonucleotides (Fig. 2). A 311-mer oligonucleotide containing 39 mismatches to the template directed 24 simultaneous amino acid changes required to humanize muMAb4D5 V<sub>L</sub>. Humanization of muMAb4D5 V<sub>H</sub> required 32 amino acid changes which were installed with a 361-mer containing 59 mismatches to the muMAb4D5 template. Two out of 8 clones sequenced precisely encode huMAb4D5-5, although one of these clones contained a single nucleotide imperfection. The 6 other clones were essentially humanized but contained a small number of errors: < 3 nucleotide changes and < 1 single nucleotide deletion per kilobase.

Additional humanized variants (Table 1) were constructed by site-directed mutagenesis of huMAb4D5-5.

5 Expression levels of huMAb4D5 variants were in the range of 7 to 15  $\mu\text{g/ml}$  as judged by ELISA using immobilized p185<sup>HER2</sup> ECD. Successive harvests of five 10 cm plates allowed 200  $\mu\text{g}$  to 500 mg of each variant to be produced in a week. Antibodies affinity purified on protein A gave a single band on a Coomassie blue stained SDS polyacrylamide gel of mobility consistent with the expected  $M_r$  of  $\sim 150$  kDa. Electrophoresis under reducing conditions gave 2 bands consistent with the expected  $M_r$  of free heavy (48 kDa) and light (23 kDa) chains (not shown). Amino terminal sequence analysis (10-cycles) gave the mixed sequence expected (see Fig. 1) from an equimolar combination of light and heavy chains (not shown).

15 huMAb4D5 Variants. In general, the FR residues were chosen from consensus human sequences (Kabat, E. A. *et al.*, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)) and CDR residues from muMAb4D5. Additional variants were constructed by replacing selected human residues in huMAb4D5-1 with their muMAb4D5 counterparts. These are  $V_H$  residues 71, 73, 78, 93 plus 102 and  $V_L$  residues 55 plus 66 identified by our molecular modeling.  $V_H$  residue 71 has previously been proposed by others (Tramontano, A. *et al.*, *J. Mol. Biol.* 215:175-182 (1990)) to be critical to the conformation of  $V_H$ -CDR2. Amino acid sequence differences between huMAb4D5 variant molecules are shown in Table 1, together with their p185<sup>HER2</sup> ECD binding affinity and maximal anti-proliferative activities against SK-BR-3 cells. Very similar  $K_d$  values were obtained for binding of MAb4D5 variants to either SK-BR-3 cells (unpublished data) or to p185<sup>HER2</sup> ECD (Table 1). However,  $K_d$  estimates derived from binding of MAb4D5 variants to p185<sup>HER2</sup> ECD were more reproducible with smaller standard errors and consumed much smaller quantities of antibody than binding measurements with whole cells.

30 The most potent humanized variant designed by molecular modeling, huMAb4D5-8, contains 5 FR residues from muMAb4D5. This



antibody binds the p185<sup>HER2</sup> ECD 3-fold *more* tightly than does muMAb4D5 itself (Table 1) and has comparable anti-proliferative activity with SK-BR-3 cells (Fig. 3). In contrast, huMAb4D5-1 is the most humanized but least potent muMAb4D5 variant, created by simply installing the muMAb4D5 CDRs into the consensus human sequences. huMAb4D5-1 binds the p185<sup>HER2</sup> ECD 80-fold *less* tightly than does the murine antibody and has no detectable anti-proliferative activity at the highest antibody concentration investigated (16 µg/ml).

The anti-proliferative activity of huMAb4D5 variants against p185<sup>HER2</sup> overexpressing SK-BR-3 cells is not simply correlated with their binding affinity for the p185<sup>HER2</sup> ECD. For example, installation of three murine residues into the V<sub>H</sub> domain of huMAb4D5-2 (D73T, L78A and A93S) to create huMAb4D5-3 does not change the antigen binding affinity but does confer significant anti-proliferative activity (Table 1).

The importance of V<sub>H</sub> residue 71 (Tramontano, A. *et al.*, *J. Mol. Biol.* 215:175-182 (1990)) is supported by the observed 5-fold increase in affinity for p185<sup>HER2</sup> ECD on replacement of R71 in huMAb4D5-1 with the corresponding murine residue, alanine (huMAb4D5-2). In contrast, replacing V<sub>H</sub> L78 in huMAb4D5-4 with the murine residue, alanine (huMAb4D5-5), does not significantly change the affinity for the p185<sup>HER2</sup> ECD or change anti-proliferative activity, suggesting that residue 78 is not of critical functional significance to huMAb4D5 and its ability to interact properly with the extracellular domain of p185<sup>HER2</sup>.

V<sub>L</sub> residue 66 is usually a glycine in human and murine κ chain sequences (Kabat, E. A. *et al.*, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)) but an arginine occupies this position in the muMAb4D5 κ light chain. The side chain of residue 66 is likely to affect the conformation of V<sub>L</sub>-CDR1 and V<sub>L</sub>-CDR2 and the hairpin turn at 68-69 (Fig. 4). Consistent with the importance of this residue, the mutation V<sub>L</sub> G66R (huMAb4D5-3 → huMAb4D5-5) increases the affinity for the p185<sup>HER2</sup> ECD by 4-fold with a concomitant increase in anti-proliferative activity.

From molecular modeling it appears that the tyrosyl side chain of muMAB4D5 V<sub>L</sub> residue 55 may either stabilize the conformation of V<sub>H</sub>-CDR3 or provide an interaction at the V<sub>L</sub>-V<sub>H</sub> interface. The latter function may be dependent upon the presence of V<sub>H</sub> Y102. In the context of huMAB4D5-5 the mutations V<sub>L</sub> E55Y (huMAB4D5-6) and V<sub>H</sub> V102Y (huMAB4D5-7) individually increase the affinity for p185<sup>HER2</sup> ECD by 5-fold and 2-fold respectively, whereas together (huMAB4D5-8) they increase the affinity by 11-fold. This is consistent with either proposed role of V<sub>L</sub> Y55 and V<sub>H</sub> Y102.

**Secondary Immune Function of huMAB4D5-8.** MuMAB4D5 inhibits the growth of human breast tumor cells which overexpress p185<sup>HER2</sup> (Hudziak, R. M. *et al.*, *Molec. Cell. Biol.* 9:1165-1172 (1989)). The antibody, however, does not offer the possibility of direct tumor cytotoxic effects. This possibility does arise in huMAB4D5-8 as a result of its high affinity ( $K_D = 0.1 \mu\text{M}$ ) and its human IgG<sub>1</sub> subtype. Table 2 compares the ADCC mediated by huMAB4D5-8 with muMAB4D5 on a normal lung epithelial cell line, WI-38, which expresses a low level of p185<sup>HER2</sup> and on SK-BR-3, which expresses a high level of p185<sup>HER2</sup>. The results demonstrate that: (1) huMAB4D5 has a greatly enhanced ability to carry out ADCC as compared with its murine parent; and (2) that this activity may be selective for cell types which overexpress p185<sup>HER2</sup>.

## DISCUSSION

MuMAB4D5 is potentially useful for human therapy since it is cytostatic towards human breast and ovarian tumor lines overexpressing the *HER2*-encoded p185<sup>HER2</sup> receptor-like tyrosine kinase. Since both breast and ovarian carcinomas are chronic diseases it is anticipated that the optimal MAB4D5 variant molecule for therapy will have low immunogenicity and will be cytotoxic rather than solely cytostatic in effect. Humanization of muMAB4D5 should accomplish these goals. We have identified 5 different huMAB4D5 variants which bind tightly to p185<sup>HER2</sup> ECD ( $K_D \leq 1 \text{ nM}$ ) and which have significant anti-proliferative activity (Table 1). Furthermore

5 huMAb4D5-8 but not muMAb4D5 mediates ADCC against human tumor cell lines overexpressing p185<sup>HER2</sup> in the presence of human effector cells (Table 2) as anticipated for a human  $\gamma$ 1 isotype (Brüggemann, M. *et al.*, *J. Exp. Med.* 166:1351-1361 (1987); Riechmann, L. *et al.*, *Nature* 332:323-327 (1988)).

10 Rapid humanization of huMAb4D5 was facilitated by the gene conversion mutagenesis strategy developed here using long preassembled oligonucleotides. This method requires less than half the amount of synthetic DNA as does total gene synthesis and does not require convenient restriction sites in the target DNA. Our method appears to be simpler and more reliable than a variant protocol recently reported (Rostapshov, V. M. *et al.*, *FEBS Lett.* 249:379-382 (1989)). Transient expression of huMAb4D5 in human embryonic kidney 293 cells permitted the isolation of a few hundred micrograms of huMAb4D5 variants for rapid characterization by growth inhibition and antigen binding affinity assays. Furthermore, different combinations of light and heavy chain were readily tested by co-transfection of corresponding cDNA expression vectors.

20 The crucial role of molecular modeling in the humanization of muMAb4D5 is illustrated by the designed variant huMAb4D5-8 which binds the p185<sup>HER2</sup> ECD 250-fold more tightly than the simple CDR loop swap variant, huMAb4D5-1. It has previously been shown that the antigen binding affinity of a humanized antibody can be increased by mutagenesis based upon molecular modelling (Riechmann, L. *et al.*, *Nature* 332:323-327 (1988); Queen, C. *et al.*, *Proc. Natl. Acad. Sci. USA* 86:10029-10033 (1989)). Here we have extended this earlier work by others with a designed humanized antibody which binds its antigen 3-fold more tightly than the parent rodent antibody. While this result is gratifying, assessment of the success of the molecular modeling must await the outcome of X-ray structure determination. From analysis of huMAb4D5 variants (Table 1) it is apparent that their anti-proliferative activity is not a simple function of their binding affinity for p185<sup>HER2</sup> ECD. For example the huMAb4D5-8 variant binds p185<sup>HER2</sup> 3-fold more tightly than muMAb4D5 but the humanized variant is

slightly less potent in blocking the proliferation of SK-BR-3 cells. Additional huMAb4D5 variants are currently being constructed in an attempt to identify residues triggering the anti-proliferative activity and in an attempt to enhance this activity.

5           In addition to retaining tight receptor binding and the ability to inhibit cell growth, the huMAb4D5-8 also confers a secondary immune function (ADCC). This allows for direct cytotoxic activity of the humanized molecule in the presence of human effector cells. The apparent selectivity of the cytotoxic activity for cell types which overexpress p185<sup>HER2</sup> allows  
10           for the evolution of a straightforward clinic approach to those human cancers characterized by overexpression of the HER2 protooncogene.

Table 1. p185<sup>HER2</sup> ECD binding affinity and anti-proliferative activities of MAb4D5 variants

| MAb4D5 Variant | V <sub>H</sub> Residue* |     |     |     |     | V <sub>L</sub> Residue* |     |      | Relative cell proliferation† | nM |
|----------------|-------------------------|-----|-----|-----|-----|-------------------------|-----|------|------------------------------|----|
|                | 71                      | 73  | 78  | 93  | 102 | 55                      | 66  | FR3  |                              |    |
| huMAb4D5-1     | R                       | D   | L   | A   | V   | E                       | G   | 102  |                              |    |
| huMAb4D5-2     | Ala                     | D   | L   | A   | V   | E                       | G   | 4.7  | 101                          |    |
| huMAb4D5-3     | Ala                     | Thr | Ala | Ser | V   | E                       | G   | 4.4  | 66                           |    |
| huMAb4D5-4     | Ala                     | Thr | L   | Ser | V   | E                       | Arg | 0.82 | 56                           |    |
| huMAb4D5-5     | Ala                     | Thr | Ala | Ser | V   | E                       | Arg | 1.1  | 48                           |    |
| huMAb4D5-6     | Ala                     | Thr | Ala | Ser | V   | Tyr                     | Arg | 0.22 | 51                           |    |
| huMAb4D5-7     | Ala                     | Thr | Ala | Ser | Tyr | E                       | Arg | 0.62 | 53                           |    |
| huMAb4D5-8     | Ala                     | Thr | Ala | Ser | Tyr | Tyr                     | Arg | 0.10 | 54                           |    |
| muMAb4D5       | Ala                     | Thr | Ala | Ser | Tyr | Tyr                     | Arg | 0.30 | 37                           |    |

\* Human and murine residues are shown in one letter and three letter amino acid code respectively.

†  $K_D$  values for the p185<sup>HER2</sup> ECD were determined using the method of Friguet *et al.* (43) and the standard error of each estimate is  $\leq \pm 10\%$ .

‡ Proliferation of SK-BR-3 cells incubated for 96 hr with MAb4D5 variants shown as a percentage of the untreated control as described (Hudziak, R. M. *et al.*, *Molec. Cell. Biol.* 9:1165-1172 (1989)). Data represent the maximal anti-proliferative effect for each variant (see Fig. 3A) calculated as the mean of triplicate determinations at a MAb4D5 concentration

of 8  $\mu\text{g/ml}$ . Data are all taken from the same experiment with an estimated standard error of  $\leq \pm 15\%$ .

Table 2. Selectivity of antibody dependent tumor cell cytotoxicity mediated by huMAb4D5-8

| Effector:Target<br>ratio <sup>†</sup> | WI-38*   |            | SK-BR-3  |            |      |
|---------------------------------------|----------|------------|----------|------------|------|
|                                       | muMAb4D5 | huMAb4D5-8 | muMAb4D5 | huMAb4D5-8 |      |
| A.‡                                   | 25:1     | <1.0       | 9.3      | 7.5        | 40.6 |
|                                       | 12.5:1   | <1.0       | 11.1     | 4.7        | 36.8 |
|                                       | 6.25:1   | <1.0       | 8.9      | 0.9        | 35.2 |
|                                       | 3.13:1   | <1.0       | 8.5      | 4.6        | 19.6 |
| B.                                    | 25:1     | <1.0       | 3.1      | 6.1        | 33.4 |
|                                       | 12.5:1   | <1.0       | 1.7      | 5.5        | 26.2 |
|                                       | 6.25:1   | 1.3        | 2.2      | 2.0        | 21.0 |
|                                       | 3.13:1   | <1.0       | 0.8      | 2.4        | 13.4 |

\* Sensitivity to ADCC of two human cell lines (WI-38, normal lung epithelium; and SK-BR-3, human breast tumor cell line) are compared. WI-38 expresses a low level of p185<sup>HER2</sup> (0.6 pg per  $\mu$ g cell protein) and SK-BR-3 expresses a high level of p185<sup>HER2</sup> (64 pg p185<sup>HER2</sup> per  $\mu$ g cell protein), as determined by ELISA (Fendly *et al.*, *J. Biol. Resp. Mod.* 9:449-455 (1990)).

<sup>†</sup> ADCC assays were carried out as described in Brüggemann *et al.*, *J. Exp. Med.* 166:1351-1361 (1987). Effector to target ratios were of IL-2 activated human peripheral blood lymphocytes to either WI-38 fibroblasts or SK-BR-3 tumor cells in 96-well microtiter plates for 4 hours at 37 °C. Values given represent percent specific cell lysis as determined by <sup>51</sup>Cr release. Estimated standard error in these quadruplicate determinations was  $\leq \pm 10\%$ .

<sup>‡</sup> Monoclonal antibody concentrations used were 0.1  $\mu$ g/ml (A) and 0.1  $\mu$ g/ml (B).

**EXAMPLE 2. Schematic Method for Humanizing an Antibody Sequence**

5 This example illustrates one stepwise elaboration of the methods for creating a humanized sequence described above. It will be understood that not all of these steps are essential to the claimed invention, and that steps may be taken in different order.

- 10 1. ascertain a consensus human variable domain amino acid sequence and prepare from it a consensus structural model.
2. prepare model of import (the non-human domain to be humanized) variable domain sequences and note structural differences with respect to consensus human model.
- 15 3. identify CDR sequences in human and in import, both by using Kabat (*supra*, 1987) and crystal structure criteria. If there is any difference in CDR identity from the different criteria, use of crystal structure definition of the CDR, but retain the Kabat residues as important framework residues to import.
- 20 4. substitute import CDR sequences for human CDR sequences to obtain initial "humanized" sequence.
5. compare import non-CDR variable domain sequence to the humanized sequence and note divergences.
- 25 6. Proceed through the following analysis for each amino acid residue where the import diverges from the humanized.
  - a. If the humanized residue represents a residue which is generally highly conserved across all species, use the residue in the humanized sequence. If the residue is not conserved across all species, proceed with the analysis described in 6b.
  - 30 b. If the residue is not generally conserved across all species, ask if the residue is generally conserved in humans.
    - i. If the residue is generally conserved in humans but the import residue differs, examine the structural models of the



import and human sequences and determine if the import residue would be likely to affect the binding or biological activity of the CDRs by considering 1) could it bind antigen directly and 2) could it affect the conformation of the CDR. If the conclusion is that an affect on the CDRs is likely, substitute the import residue. If the conclusion is that a CDR affect is unlikely, leave the humanized residue unchanged.

ii. If the residue is also not generally conserved in humans, examine the structural models of the import and human sequences and determine if the import residue would be likely to affect the binding or biological activity of the CDRs be considering 1) could it bind antigen directly and 2) could it affect the conformation of the CDR. If the conclusion is that an affect on the CDRs is likely, substitute the import residue. If the conclusion is that a CDR affect is unlikely, proceed to the next step.

a) Examine the structural models of the import and human sequences and determine if the residue is exposed on the surface of the domain or is buried within. If the residue is exposed, use the residue in the humanized sequence. If the residue is buried, proceed to the next step.

(i) Examine the structural models of the import and human sequences and determine if the residue is likely to affect the  $V_L - V_H$  interface. Residues involved with the interface include: 34L, 36L, 38L, 43L, 33L, 36L, 85L, 87L, 89L, 91L, 96L, 98L, 35H, 37H, 39H, 43H, 45H, 47H, 60H, 91H, 93H, 95H, 100H, and 103H. If no effect is likely, use the residue in the humanized sequence. If some affect is likely, substitute the

import residue.

- 5
7. Search the import sequence, the consensus sequence and the humanized sequence for glycosylation sites outside the CDRs, and determine if this glycosylation site is likely to have any affect on antigen binding and/or biological activity. If no effect is likely, use the human sequence at that site; if some affect is likely, eliminate the glycosylation site or use the import sequence at that site.
- 10
8. After completing the above analysis, determine the planned humanized sequence and prepare and test a sample. If the sample does not bind well to the target antigen, examine the particular residues listed below, regardless of the question of residue identity between the import and humanized residues.
- 15
- a. Examine particular peripheral (non-CDR) variable domain residues that may, due to their position, possibly interact directly with a macromolecular antigen, including the following residues (where the \* indicates residues which have been found to interact with antigen based on crystal structures):
- 20
- i. Variable light domain: 36, 46, **49\***, 63-70
- ii. Variable heavy domain: 2, **47\***, 68, 70, 73-76.
- 25
- b. Examine particular variable domain residues which could interact with, or otherwise affect, the conformation of variable domain CDRs, including the following (not including CDR residues themselves, since it is assumed that, because the CDRs interact with one another, any residue in one CDR could potentially affect the conformation of another CDR residue) (L= LIGHT, H=HEAVY, residues appearing in **bold** are indicated to be structurally important according the Chothia *et al.*, Nature 342:877 (1989), and residues appearing in *italic* were altered during humanization by Queen *et al.* (PDL), Proc. Natl. Acad. Sci. USA 86:10029 (1989) and Proc. Natl. Acad. Sci. USA 88:2869 (1991).):
- 30
- i. Variable light domain:

- a) CDR-1 (residues 24L-34L): 2L, 4L, 66L-69L, 71L
- b) CDR-2 (residues 50L-56L): 35L, 46L, 47L, 48L, 49L, 58L, 62L, 64L-66L, 71L, 73L
- c) CDR-3 (residues 89L-97L): 2L, 4L, 36L, 98L, 37H, 45H, 47H, 58H, 60H

ii. Variable heavy domain:

- a) CDR-1 (residues 26H-35H): 2H, 4H, 24H, 36H, 71H, 73H, 76H, 78H, 92H, **94H**
- b) CDR-2 (residues 50H-55H): 49H, 69H, 69H, 71H, 73H, 78H
- c) CDR-3 (residues 95H-102H): examine all residues as possible interaction partners with this loop, because this loop varies in size and conformation much more than the other CDRs.

9. If after step 8 the humanized variable domain still is lacking in desired binding, repeat step 8. In addition, re-investigate any buried residues which might affect the  $V_L - V_H$  interface (but which would not directly affect CDR conformation). Additionally, evaluate the accessibility of non-CDR residues to solvent.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- 5 (i) APPLICANT: Carter, Paul J.  
Presta, Leonard G.
- (ii) TITLE OF INVENTION: Immunoglobulin Variants
- 10 (iii) NUMBER OF SEQUENCES: 10
- (iv) CORRESPONDENCE ADDRESS:  
15 (A) ADDRESSEE: Genentech, Inc.  
(B) STREET: 460 Point San Bruno Blvd  
(C) CITY: South San Francisco  
(D) STATE: California  
(E) COUNTRY: USA  
(F) ZIP: 94080
- 20 (v) COMPUTER READABLE FORM:  
(A) MEDIUM TYPE: 5.25 inch, 360 Kb floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
25 (D) SOFTWARE: patin (Genentech)
- (vi) CURRENT APPLICATION DATA:  
(A) APPLICATION NUMBER:  
(B) FILING DATE: 14-June-1991  
30 (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:  
(A) APPLICATION NUMBER:  
(B) FILING DATE:
- 35 (viii) ATTORNEY/AGENT INFORMATION:  
(A) NAME: Adler, Carolyn R.  
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(C) REFERENCE/DOCKET NUMBER: 709
- 40 (ix) TELECOMMUNICATION INFORMATION:  
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45 (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:

5  
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val  
1 5 10 15  
10  
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  
15  
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  
20  
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  
25 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:

40  
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  
45  
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  
 5  
 Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp  
                          80                      85                      90  
 10  
 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

15

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 109 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear  
 20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

25  
 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  
 30  
 Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys  
                          35                      40                      45  
 35  
 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  
 40  
 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  
 45  
 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:

5  
10  
15  
20  
25  
30  
35  
40  
45

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:

45

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

5 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

10 Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln  
80 85 90

15 His Tyr Thr Thr Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu  
95 100 105

Ile Lys Arg Ala  
109

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

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 120 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

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

30 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

35 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

40 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

45 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

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

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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

15 TCCGATATCC AGCTGACCCA GTCTCCA 27

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

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

25  
30 GTTTGATCTC CAGCTTGGTA CCXXCDDCGA A 31

35 (2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

40  
45 AGGTXAXCT GCAGXAGTCX GG 22

**(2) INFORMATION FOR SEQ ID NO:10:**

**(i) SEQUENCE CHARACTERISTICS:**

- (A) LENGTH: 34 bases**
- (B) TYPE: nucleic acid**
- (C) STRANDEDNESS: single**
- (D) TOPOLOGY: linear**

**(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:**

**TGAGGAGACG GTGACCGTGG TCCCTTGGCC CCAG 34**

CLAIMS

WE CLAIM:

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1. A method for making a humanized antibody comprising amino acid sequence 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 variable domain and of a consensus human variable domain;
  - b. identifying Complementarity Determining Region (CDR) amino acid sequences in the import and the human amino variable domain sequences;
  - c. substituting an import CDR amino acid sequence for the corresponding human CDR amino acid sequence;
  - d. aligning the amino acid sequences of a Framework Region (FR) of the import antibody and the corresponding FR of the consensus antibody;
  - e. identifying import antibody FR residues in the aligned FR sequences that are non-homologous to the corresponding consensus antibody residues;
  - f. determining if the non-homologous import amino acid residue is reasonably expected to have at least one of the following effects:
    1. non-covalently binds antigen directly,
    2. interacts with a CDR; or
    3. participates in the  $V_L - V_H$  interface; and
  - g. for any non-homologous import antibody amino acid residue which is reasonably expected to have at least one of these effects, substituting that residue for the corresponding amino acid residue in the consensus antibody FR sequence.
  
2. The method of claim 1, having an additional step of determining if

*Paul Carter et al*  
*07/7/15, 272*  
*6/14/91*

any such non-homologous residues are exposed on the surface of the domain or buried within it, and if the residue is exposed, retaining the consensus residue.

*Handwritten:*  
446  
221  
2011  
A large handwritten scribble with a line pointing to the text below.

5  
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3. The method of claim 1, having the additional steps of searching the import variable domain sequence for glycosylation sites, determining if any such glycosylation site is reasonably expected to affect the antigen binding or affinity of the antibody, and if so, substituting the glycosylation site into the consensus sequence.
4. The method of claim 1, having the additional steps of searching the consensus variable domain sequence for glycosylation sites which are not present at the corresponding amino acid in the import sequence, and if the glycosylation site is not present in the import sequence, substituting the import amino acid residues for the amino acid residues comprising the consensus glycosylation site.
5. The method of claim 1, having an additional step which comprises aligning import antibody and consensus antibody FR sequences, identifying import antibody FR residues which are non-homologous with the aligned consensus FR sequence, and for each such non-homologous import antibody FR residue, determining if the corresponding consensus antibody residue represents a residue which is highly conserved across all species at that site, and if it is so conserved, preparing a humanized antibody which comprises the consensus antibody amino acid residue at that site.
6. The method of claim 1, wherein the corresponding consensus antibody residues are selected from the group consisting of 4L, 35L, 36L, 38L, 43L, 44L, 46L, 58L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 68H, 69H, 70H, 73H, 74H, 75H,

76H, 78H, 91H, 92H, 93H, and 103H.

7.

A method comprising providing at least a portion of an import, non-human antibody variable domain amino acid sequence having a CDR and a FR, obtaining the amino acid sequence of at least a portion of a consensus human antibody variable domain having a CDR and a FR, substituting the non-human CDR for the human CDR in the consensus human antibody variable domain, and then substituting an amino acid residue for the consensus amino acid residue at at least one of the following sites:

4L, 35L, 36L, 38L, 43L, 44L, 46L, 58L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H.

8. The method of claim 7, wherein the substituted residue is the residue found at the corresponding location of the non-human antibody.

9. A humanized antibody variable domain having a non-human CDR incorporated into a human antibody variable domain, wherein the improvement comprises substituting an amino acid residue for the human residue at a site selected from the group consisting of:  
4L, 35L, 36L, 38L, 43L, 44L, 46L, 58L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H.

10. The humanized antibody variable domain of claim 9, 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.

11. The humanized antibody variable domain of claim 9, wherein no human FR residue other than those set forth in the group has been substituted.

12. A polypeptide comprising the amino acid sequence:  
DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP  
KLLIYSASFLESGVPSRFSGSRSGTDFTLTISSLPEDFATYYCQOHY  
TTPPTFGQGKVEIKRT

13. A polypeptide comprising the sequence:  
EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLE  
WVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDT  
AVYYCSRWGGDGFYAMDVWGQGLVTVSS

14. <sup>101</sup> A computer comprising the sequence data of the following amino acid sequence:

a. DIQMTQSPSSLSASVGDRVTITCRASQDVSSYLAWYQQ  
KPGKAPKLLIYAASSLESGVPSRFSGSGSGTDFTLTISSLO  
PEDFATYYCQQYNSLPYTFGQGKVEIKRT, or

b. EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYAMSWVR  
QAPGKGLEWVAVISENGGYTRYADSVKGRFTISADTSKN  
TAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDVWGQ  
TLVTVSS

15. <sup>101</sup> A computer representation of the following amino acid sequence:

a. DIQMTQSPSSLSASVGDRVTITCRASQDVSSYLAWYQQ  
KPGKAPKLLIYAASSLESGVPSRFSGSGSGTDFTLTISSLO  
PEDFATYYCQQYNSLPYTFGQGKVEIKRT, or

b. EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYAMSWVR  
QAPGKGLEWVAVISENGGYTRYADSVKGRFTISADTSKN  
TAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDVWGQ

TLVTVSS

16. <sup>101</sup> A method comprising storing a computer representation of the following amino acid sequence:

5 a. DIQMTQSPSSLSASVGDRVTITCRASQDVSSYLAWYQQ  
KPGKAPKLLIYAASSLESGVPSRFSGSGSGTDFTLTISLQ  
PEDFATYYCQQYNLPTFGQGTKVEIKRT, or

10 b. EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYAMSWVR  
QAPGKGLEWVAVISENGGYTRYADSVKGRFTISADTSKN  
TAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDVWGQG  
TLVTVSS



Abstract

5 Variant immunoglobulins, particularly humanized antibody polypeptides are provided, along with methods for their preparation and use. Consensus immunoglobulin sequences and structural models are also provided.



COMBINED DECLARATION FOR PATENT APPLICATION  
 AND POWER OF ATTORNEY

Docket No. 709

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 names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

**IMMUNOGLOBULIN VARIANTS**

the specification of which (check one)  is attached hereto or  was filed on \_\_\_\_\_ as Application Serial No. \_\_\_\_\_ and was amended on \_\_\_\_\_ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I hereby state that any Sequence Listing submitted with this application is submitted in paper copy and a computer-readable diskette, and that the content of the paper and computer readable copies are the same.

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, Section 1.56(a).

I hereby 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 have a filing date before that of the application on which priority is claimed:

| Prior Foreign Application(s) | Priority Claimed |    |
|------------------------------|------------------|----|
|                              | Yes              | No |
| Number                       |                  |    |
| Country                      |                  |    |
| Day/Month/Year Filed         |                  |    |

I hereby claim the benefit under Title 35, United States Code, §120 of any United States applications(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 Ser. No. | Filing Date | Status: Patented, Pending, Abandoned |
|----------------------|-------------|--------------------------------------|
|                      |             |                                      |

| Application Ser. No. | Filing Date | Status: Patented, Pending, Abandoned |
|----------------------|-------------|--------------------------------------|
|                      |             |                                      |

**POWER OF ATTORNEY:** As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

- |   |                                   |
|---|-----------------------------------|
| Carolyn R. Adler - Reg. No. <u>32,324</u> | Max D. Hensley - Reg. No. 27,043  |
| Robert H. Benson - Reg. No. 30,446        | Dennis G. Kleid - Reg. No. 32,037 |
| Walter E. Buting - Reg. No. 23,092        | Nancy Olseki - Reg. No. 34,688    |
| Ginger R. Dreger - Reg. No. 33,055        | Stephen Raines - Reg. No. 25,912  |
| Debbie Glaister - Reg. No. 33,888         | Daryl B. Winter - Reg. No. 32,637 |
| Janet E. Hasak - Reg. No. 28,616          |                                   |

Send correspondence to <sup>601</sup> Genentech, Inc.  
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<sup>702</sup> South San Francisco, CA 94080  
Telephone: (415) 266-2614

I hereby declare that all statements made herein of my own knowledge and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that willful false statements may jeopardize the validity of the application or any patent issued thereon.

The undersigned hereby authorizes the U.S. attorney or agent named herein to accept and follow instructions from his foreign patent agent as to any action to be taken in the Patent and Trademark Office regarding this application without direct communication between the U.S. attorney or agent and the undersigned. In the event of a change in the persons from whom instructions may be taken, the U.S. attorney or agent named herein will be so notified by the undersigned.

Full name of sole or first inventor

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Inventor's signature

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*40200*

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Full name of third joint inventor, if any

Third Inventor's signature

Date

Residence

Citizenship

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South San Francisco, CA 94080

BAR CODE LABEL



# U.S. PATENT APPLICATION

|                             |                         |              |                       |
|-----------------------------|-------------------------|--------------|-----------------------|
| SERIAL NUMBER<br>07/715,272 | FILING DATE<br>06/14/91 | CLASS<br>530 | GROUP ART UNIT<br>183 |
|-----------------------------|-------------------------|--------------|-----------------------|

APPLICANT

PAUL J. CARTER, SAN FRANCISCO, CA; LEONARD G. PRESTA, SAN FRANCISCO, CA.

\*\*CONTINUING DATA\*\*\*\*\*  
VERIFIED

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

FOREIGN FILING LICENSE GRANTED 08/03/91

|                        |                     |                    |                         |                                   |                            |
|------------------------|---------------------|--------------------|-------------------------|-----------------------------------|----------------------------|
| STATE OR COUNTRY<br>CA | SHEETS DRAWING<br>5 | TOTAL CLAIMS<br>16 | INDEPENDENT CLAIMS<br>8 | FILING FEE RECEIVED<br>\$1,050.00 | ATTORNEY DOCKET NO.<br>709 |
|------------------------|---------------------|--------------------|-------------------------|-----------------------------------|----------------------------|

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SOUTH SAN FRANCISCO, CA 94080

TITLE

IMMUNOGLOBULIN VARIANTS

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

Date

\_\_\_\_\_  
Certifying Officer

07 715272

PATENT APPLICATION SERIAL NO. \_\_\_\_\_

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

P 50192 06/27/91 07715272

07-0630 030 101

930.00CH

**PATENT APPLICATION FEE DETERMINATION RECORD**

Application or Docket Number

715272

**CLAIMS AS FILED - PART I**

(Column 1) (Column 2)

| FOR                              | NUMBER FILED    | NUMBER EXTRA |
|----------------------------------|-----------------|--------------|
| BASIC FEE                        |                 |              |
| TOTAL CLAIMS                     | 16 minus 20 = * |              |
| INDEPENDENT CLAIMS               | 8 minus 3 = *   | 5            |
| MULTIPLE DEPENDENT CLAIM PRESENT |                 |              |

**SMALL ENTITY**

OR

**OTHER THAN SMALL ENTITY**

| RATE         | FEE       |
|--------------|-----------|
|              | \$ 315.00 |
| x \$10 =     |           |
| x 30 =       |           |
| + 100 =      |           |
| <b>TOTAL</b> |           |

OR

OR

OR

OR

OR

| RATE         | FEE       |
|--------------|-----------|
|              | \$ 630.00 |
| x \$20 =     |           |
| x 60 =       | 300       |
| + 200 =      |           |
| <b>TOTAL</b> | 930       |

\* If the difference in column 1 is less than zero, enter "0" in column 2

**CLAIMS AS AMENDED - PART II**

(Column 1) (Column 2) (Column 3)

| AMENDMENT A |  | CLAIMS REMAINING AFTER AMENDMENT | HIGHEST NUMBER PREVIOUSLY PAID FOR | PRESENT EXTRA |
|-------------|--|----------------------------------|------------------------------------|---------------|
|             | Total  | *                                | Minus                              | ** =          |
|             | Independent                                    | *                                | Minus                              | *** =         |
|             | FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM |                                  |                                    |               |

**SMALL ENTITY**

OR

**OTHER THAN SMALL ENTITY**

| RATE                  | ADDITIONAL FEE |
|-----------------------|----------------|
| x \$10 =              |                |
| x 30 =                |                |
| + 100 =               |                |
| <b>TOTAL</b>          |                |
| <b>ADDITIONAL FEE</b> |                |

OR

OR

OR

OR

OR

| RATE                  | ADDITIONAL FEE |
|-----------------------|----------------|
| x \$20 =              |                |
| x 60 =                |                |
| + 200 =               |                |
| <b>TOTAL</b>          |                |
| <b>ADDITIONAL FEE</b> |                |

(Column 1) (Column 2) (Column 3)

| AMENDMENT B |  | CLAIMS REMAINING AFTER AMENDMENT | HIGHEST NUMBER PREVIOUSLY PAID FOR | PRESENT EXTRA |
|-------------|--|----------------------------------|------------------------------------|---------------|
|             | Total  | *                                | Minus                              | ** =          |
|             | Independent                                    | *                                | Minus                              | *** =         |
|             | FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM |                                  |                                    |               |

TOTAL  
ADDITIONAL FEE

OR

OR

OR

OR

OR

OR

OR

OR

OR

OR

(Column 1) (Column 2) (Column 3)

| AMENDMENT C |  | CLAIMS REMAINING AFTER AMENDMENT | HIGHEST NUMBER PREVIOUSLY PAID FOR | PRESENT EXTRA |
|-------------|--|----------------------------------|------------------------------------|---------------|
|             | Total  | *                                | Minus                              | ** =          |
|             | Independent                                    | *                                | Minus                              | *** =         |
|             | FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM |                                  |                                    |               |

TOTAL  
ADDITIONAL FEE

OR

OR

OR

OR

OR

OR

OR

OR

OR

OR

\* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

\*\* If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

\*\*\* If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

|   |   |  |
|---|---|--|
| Applicant's or agent's file reference<br><b>709P1</b>   | <b>FOR FURTHER ACTION</b> see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below. |  |
| International application No.<br><b>PCT/US 92/05126</b> | International filing date (day/month/year)<br><b>15/06/92</b>   | (Earliest) Priority Date (day/month/year)<br><b>14/06/91</b> |
| Applicant<br><b>GENENTECH, INC. et al.</b>              |   |  |

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

It is also accompanied by a copy of each prior art document cited in this report.

1.  Certain claims were found unsearchable (see Box I).

2.  Unity of invention is lacking (see Box II).

3.  The international application contains disclosure of a nucleotide and/or amino acid sequence listing and the international search was carried out on the basis of the sequence listing

filed with the international application.

furnished by the applicant separately from the international application,

but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.

Transcribed by this Authority

4. With regard to the title,  the text is approved as submitted by the applicant.

the text has been established by this Authority to read as follows:

**METHOD FOR MAKING HUMANIZED ANTIBODIES.**

5. With regard to the abstract,

the text is approved as submitted by the applicant.

the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is:

Figure No. 2  as suggested by the applicant.

None of the figures.

because the applicant failed to suggest a figure.

because this figure better characterizes the invention.

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: 17-18  
because they relate to subject matter not required to be searched by this Authority, namely:  
see PCT-Rule 39.1(iv)
2.  Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

**INTERNATIONAL SEARCH REPORT**

PCT/US 92/05126

International Application No

|   |   |                                     |
|---|---|-------------------------------------|
| <b>I. CLASSIFICATION OF SUBJECT MATTER</b> (If several classification symbols apply, indicate all) <sup>6</sup>   |   |                                     |
| According to International Patent Classification (IPC) or to both National Classification and IPC   |   |                                     |
| Int.Cl. 5 C12N15/13;<br>G06F15/00   | C12P21/08;  | C07K13/00; C12N5/10                 |
| <b>II. FIELDS SEARCHED</b>  |   |                                     |
| Minimum Documentation Searched <sup>7</sup>   |   |                                     |
| Classification System   | Classification Symbols  |                                     |
| Int.Cl. 5   | C07K ; C12N ; G06F  |                                     |
| Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>8</sup>  |   |                                     |
|   |   |                                     |
| <b>III. DOCUMENTS CONSIDERED TO BE RELEVANT<sup>9</sup></b>   |   |                                     |
| Category <sup>10</sup>  | Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>  | Relevant to Claim No. <sup>13</sup> |
| Y   | JOURNAL OF MOLECULAR BIOLOGY<br>vol. 215, 1990, ACADEMIC PRESS<br>pages 175 - 182<br>Tramontano, Anna; Chothia, Cyrus; Lesk, Arthur M. 'Framework residue 71 is a major determinant of the position and conformation of the second hypervariable region in the VH domains of immunoglobulins'<br>cited in the application<br>See the whole document, especially paragraph 7 | 1-12, 15                            |
| Y   | WO,A,9 007 861 (PROTEIN DESIGN LABS, INC.)<br>26 July 1990<br>See pages 1-6; 9-25   | 1-12, 15                            |
|   | ---   |                                     |
|   |   | -/--                                |
| <p><sup>10</sup> Special categories of cited documents :<br/>         "A" document defining the general state of the art which is not considered to be of particular relevance<br/>         "E" earlier document but published on or after the international filing date<br/>         "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)<br/>         "O" document referring to an oral disclosure, use, exhibition or other means<br/>         "P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention<br/>         "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step<br/>         "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.<br/>         "&amp;" document member of the same patent family</p> |   |                                     |
| <b>IV. CERTIFICATION</b>  |   |                                     |
| Date of the Actual Completion of the International Search   | Date of Mailing of this International Search Report   |                                     |
| 07 OCTOBER 1992   | 02. 11. 92  |                                     |
| International Searching Authority   | Signature of Authorized Officer   |                                     |
| EUROPEAN PATENT OFFICE  | NAUCHE S.A.   |                                     |



**ANNEX TO THE INTERNATIONAL SEARCH REPORT  
ON INTERNATIONAL PATENT APPLICATION NO. US 9205126  
SA 61838**

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on  
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 07/10/92

| Patent document cited in search report | Publication date | Patent family member(s)                         | Publication date                 |
|--|------------------|---|----------------------------------|
| WO-A-9007861                           | 26-07-90         | AU-A- 5153290<br>CA-A- 2006865<br>EP-A- 0451216 | 13-08-90<br>28-06-90<br>16-10-91 |
| -----                                  |                  |   |                                  |

EPO FORM P0079

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

## III. DOCUMENTS CONSIDERED TO BE RELEVANT

(CONTINUED FROM THE SECOND SHEET)

| Category ° | Citation of Document, with indication, where appropriate, of the relevant passages   | Relevant to Claim No. |
|------------|--|-----------------------|
| Y          | <p>NATURE.<br/> vol. 342, December 1989, LONDON GB<br/> pages 877 - 883<br/> Chothia, Cyrus; Lesk, Arthur M.;<br/> Tramontano, Anna; Levitt, Michael;<br/> Smith-Gill, Sandra J.; Air, Gillian;<br/> Sheriff, Steven; Padlan, 'Conformations of<br/> immunoglobulin hypervariable region'<br/> cited in the application<br/> See the whole document, especially<br/> 'Discussion'</p> <p style="text-align: center;">---</p> | 1-12, 15              |
| P, X       | <p>PROCEEDINGS OF THE NATIONAL ACADEMY OF<br/> SCIENCES OF USA.<br/> vol. 89, May 1992, WASHINGTON US<br/> pages 4285 - 4289<br/> Carter, Paul et al. 'Humanization of an<br/> anti-p185HER2 antibody for human cancer<br/> therapy.'<br/> see the whole document</p> <p style="text-align: center;">-----</p>   | 1-15                  |

## SCORE Placeholder Sheet for IFW Content

Application Number: **07715272**

Document Date: **06/14/1991**

The presence of this form in the IFW record indicates that the following document type was received in paper and is scanned and stored in the SCORE database.

- Drawings

Images of the original documents are scanned in gray scale or color and stored in SCORE. Bi-tonal images are also stored in IFW. Defects visible in both IFW and SCORE are indicative of defects in the original paper documents.

To access the documents in the SCORE database, refer to instructions developed by SIRA.

At the time of document entry (noted above):

- Examiners may access SCORE content via the eDAN interface.
- Other USPTO employees can bookmark the current SCORE URL (<http://es/ScoreAccessWeb/>).
- External customers may access SCORE content via the Public and Private PAIR interfaces.

Form Revision Date: December 8, 2006

|   |           |  |                 |                              |                    |                    |                    |                                   |                              |
|---|-----------|--|-----------------|------------------------------|--------------------|--------------------|--------------------|-----------------------------------|------------------------------|
| FILING DATE<br>08/14/91   |           | CLASS<br>539   | SUBCLASS<br>387 | GROUP ART UNIT<br>187<br>186 | EXAMINER<br>Feisee |                    |                    |                                   |                              |
| APPLICANT: <b>LEONARD G. PRESTA, SAN FRANCISCO, CA.</b>   |           |  |                 |                              |                    |                    |                    |                                   |                              |
| **CONTINUING DATA*****<br>VERIFIED<br>None  |           |  |                 |                              |                    |                    |                    |                                   |                              |
| **FOREIGN/PCT APPLICATIONS*****<br>VERIFIED<br>None   |           |  |                 |                              |                    |                    |                    |                                   |                              |
| FOREIGN FILING LICENSE GRANTED 08/03/91   |           |  |                 |                              |                    |                    |                    |                                   |                              |
| Foreign priority claimed<br>35 USC 119 conditions met   |           | <input type="checkbox"/> yes <input checked="" type="checkbox"/> no<br><input type="checkbox"/> yes <input checked="" type="checkbox"/> no | AS FILED<br>CA  | STATE OR COUNTRY<br>CA       | SHEETS DRWGS.<br>5 | TOTAL CLAIMS<br>16 | INDEP. CLAIMS<br>8 | FILING FEE RECEIVED<br>\$1,050.00 | ATTORNEY'S DOCKET NO.<br>709 |
| Verified and Acknowledged<br>EXAMINER'S INITIALS  |           | GENENTECH, INC.<br>ATTN: CAROLYN R. ADLER<br>460 POINT SAN BRUNO BLVD.<br>SOUTH SAN FRANCISCO, CA 94080                                    |                 |                              |                    |                    |                    |                                   |                              |
| TITLE: IMMUNOGLOBULIN VARIANTS  |           |  |                 |                              |                    |                    |                    |                                   |                              |
| U.S. DEPT. OF COMM. Pat. & TM Office - PTO-436L (rev. 10-78)  |           |  |                 |                              |                    |                    |                    |                                   |                              |
| PARTS OF APPLICATION FILED SEPARATELY   |           |  |                 |                              |                    |                    |                    |                                   |                              |
| NOTICE OF ALLOWANCE MAILED  |           | PREPARED FOR ISSUE   |                 | CLAIMS ALLOWED               |                    |                    |                    |                                   |                              |
|   |           | Assistant Examiner   | Docket Clerk    | Total Claims                 | Print Claim        |                    |                    |                                   |                              |
| ISSUE FEE   |           | Primary Examiner   |                 | DRAWING                      |                    |                    |                    |                                   |                              |
| Amount Due  | Date Paid |  |                 | Sheets                       | Figs. Drwg.        | Print Fig.         |                    |                                   |                              |
| Label Area  |           | ISSUE CLASSIFICATION   |                 | ISSUE BATCH NUMBER           |                    |                    |                    |                                   |                              |
|   |           | Class  | Subclass        |                              |                    |                    |                    |                                   |                              |
| WARNING: The information disclosed herein may be restricted. Unauthorized disclosure may be prohibited by the United States Code Title 35, Sections 122, 181 and 368. Possession outside the U.S. Patent & Trademark Office is restricted to authorized employees and contractors only. |           |  |                 |                              |                    |                    |                    |                                   |                              |

Form PTO-436  
Rev. 9/80

| SEARCHED |       |          |       |
|----------|-------|----------|-------|
| Class    | Sub.  | Date     | Exmr. |
| 530      | 387.3 | 9/29/02  | ur    |
| 530      | 350   | ↓        | ↓     |
| updated  |       | 4/29/03  | ur    |
| updated  |       | 10/15/03 | ur    |
| updated  |       | 1/10/04  | ur    |

4/6

| INTERFERENCE SEARCHED |      |      |       |
|-----------------------|------|------|-------|
| Class                 | Sub. | Date | Exmr. |
|                       |      |      |       |

- 33. Request for access 6-21-02
- 34. Request for access
- 35. Request for access 11-16-02
- 36. Request for access 11-10-02
- 37. Request for access 12/15/02
- 38. Request for access 5/21/03
- 39. Request for access 11/3/03
- 40. Request for access 11-19-03
- 41. Request for access 12/18/03
- 42. Request for access 3/9/04
- 43. Request for access 2-2-04
- 44. Request for access 3/1/04
- 45. Request for access 6-2-04
- 46. Request for access 9/16/04
- 47. Request for access 8-16-04
- 48. Request for access 11-10-04
- 49. Request for access 11-29-04
- 50. Request for access 1-18-05
- 51. Request for access 1-21-05
- 52. Request for access 4-29-05
- 53. Request for access 8-2-05
- 59. Request for access 8/2/06

**SEARCH NOTES**

|                    |          |    |
|--------------------|----------|----|
| Request for access | 10/15/05 | ur |
| updated            | 1/10/04  | ur |

Request for access 4-15-05  
Turn over please

| POSITION    | INIT.           | DATE     |
|-------------|-----------------|----------|
| CLASSIFIER  | 18              | 6-19-41  |
| EXAMINER    | 46              | 6/27/41  |
| TYPIST      |                 |          |
| VERIFIER    | V. J. [unclear] | 8-5-41   |
| CORPS CORR. |                 |          |
| SPEC. HAND  |                 |          |
| FILE MAINT. | J. [unclear]    | 11-21-41 |

INDEX OF CLAIMS

| Claim | Final | Original | Date |
|-------|-------|----------|------|
| 1     |       |          |      |
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SYMBOLS  
 \* Rejected  
 + Allowed  
 (Through general) Cancelled  
 N Restricted  
 + Non-elected  
 I Interference  
 A Appeal  
 O Objected

| Claim | Final | Original | Date |
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Patent Application US/07/715,272

#2

SEQUENCE LISTING

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(1) GENERAL INFORMATION:

- (i) APPLICANT: Carter, Paul J.  
Presta, Leonard G.
- (ii) TITLE OF INVENTION: Immunoglobulin Variants

(iii) NUMBER OF SEQUENCES: 10

- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: Genentech, Inc.
  - (B) STREET: 460 Point San Bruno Blvd
  - (C) CITY: South San Francisco
  - (D) STATE: California
  - (E) COUNTRY: USA
  - (F) ZIP: 94080

- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: 5.25 inch, 360 Kb floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: patin (Genentech)

- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER:
  - (B) FILING DATE: 14-June-1991
  - (C) CLASSIFICATION:

- (vii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER:
  - (B) FILING DATE:

- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Adler, Carolyn R.
  - (B) REGISTRATION NUMBER: 32,324
  - (C) REFERENCE/DOCKET NUMBER: 709

- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: 415/266-2614
  - (B) TELEFAX: 415/952-9881
  - (C) TELEX: 910/371-7168

(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:

## Patent Application US/07/715,272

54 Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val  
55 1 5 10 15  
56  
57 Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn  
58 20 25 30  
59  
60 Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys  
61 35 40 45  
62  
63 Leu Leu Ile Tyr Ser Ala Ser Phe Leu Glu Ser Gly Val Pro Ser  
64 50 55 60  
65  
66 Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile  
67 65 70 75  
68  
69 Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln  
70 80 85 90  
71  
72 His Tyr Thr Thr Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu  
73 95 100 105  
74  
75 Ile Lys Arg Thr  
76 109  
77

## (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:

86  
87 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly  
88 1 5 10 15  
89  
90 Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys  
91 20 25 30  
92  
93 Asp Thr Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu  
94 35 40 45  
95  
96 Glu Trp Val Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr  
97 50 55 60  
98  
99 Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser  
100 65 70 75  
101  
102 Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp  
103 80 85 90  
104  
105 Thr Ala Val Tyr Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr  
106 95 100 105



## Patent Application US/07/715,272

107  
 108 Ala Met Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser  
 109 110 115 120  
 110  
 111

## 112 (2) INFORMATION FOR SEQ ID NO:3:

113  
 114 (i) SEQUENCE CHARACTERISTICS:  
 115 (A) LENGTH: 109 amino acids  
 116 (B) TYPE: amino acid  
 117 (D) TOPOLOGY: linear  
 118

## 119 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

120  
 121 Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val  
 122 1 5 10 15  
 123  
 124 Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser  
 125 20 25 30  
 126  
 127 Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys  
 128 35 40 45  
 129  
 130 Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser  
 131 50 55 60  
 132  
 133 Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile  
 134 65 70 75  
 135  
 136 Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln  
 137 80 85 90  
 138  
 139 Tyr Asn Ser Leu Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu  
 140 95 100 105  
 141  
 142 Ile Lys Arg Thr  
 143 109  
 144

## 145 (2) INFORMATION FOR SEQ ID NO:4:

146  
 147 (i) SEQUENCE CHARACTERISTICS:  
 148 (A) LENGTH: 120 amino acids  
 149 (B) TYPE: amino acid  
 150 (D) TOPOLOGY: linear  
 151

## 152 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

153  
 154 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly  
 155 1 5 10 15  
 156  
 157 Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser  
 158 20 25 30  
 159

## Patent Application US/07/715,272

160 Asp Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu  
 161 35 40 45  
 162  
 163 Glu Trp Val Ala Val Ile Ser Glu Asn Gly Gly Tyr Thr Arg Tyr  
 164 50 55 60  
 165  
 166 Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser  
 167 65 70 75  
 168  
 169 Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp  
 170 80 85 90  
 171  
 172 Thr Ala Val Tyr Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr  
 173 95 100 105  
 174  
 175 Ala Met Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser  
 176 110 115 120  
 177  
 178

## (2) INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

- 182 (A) LENGTH: 109 amino acids  
 183 (B) TYPE: amino acid  
 184 (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

188 Asp Ile Val Met Thr Gln Ser His Lys Phe Met Ser Thr Ser Val  
 189 1 5 10 15  
 190  
 191 Gly Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asp Val Asn  
 192 20 25 30  
 193  
 194 Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly His Ser Pro Lys  
 195 35 40 45  
 196  
 197 Leu Leu Ile Tyr Ser Ala Ser Phe Arg Tyr Thr Gly Val Pro Asp  
 198 50 55 60  
 199  
 200 Arg Phe Thr Gly Asn Arg Ser Gly Thr Asp Phe Thr Phe Thr Ile  
 201 65 70 75  
 202  
 203 Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln  
 204 80 85 90  
 205  
 206 His Tyr Thr Thr Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu  
 207 95 100 105  
 208  
 209 Ile Lys Arg Ala  
 210 109

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

## Patent Application US/07/715,272

213  
214  
215  
216  
217  
218

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 120 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

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

220

221 Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly  
 222 1 5 10 15

223

224 Ala Ser Leu Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys  
 225 20 25 30

226

227 Asp Thr Tyr Ile His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu  
 228 35 40 45

229

230 Glu Trp Ile Gly Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr  
 231 50 55 60

232

233 Asp Pro Lys Phe Gln Asp Lys Ala Thr Ile Thr Ala Asp Thr Ser  
 234 65 70 75

235

236 Ser Asn Thr Ala Tyr Leu Gln Val Ser Arg Leu Thr Ser Glu Asp  
 237 80 85 90

238

239 Thr Ala Val Tyr Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr  
 240 95 100 105

241

242 Ala Met Asp Tyr Trp Gly Gln Gly Ala Ser Val Thr Val Ser Ser  
 243 110 115 120

244

245

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

247

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 27 bases  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

253

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

255

256

257 TCCGATATCC AGCTGACCCA GTCTCCA 27

258

259

260

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

262

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 31 bases  
 (B) TYPE: nucleic acid

265

Patent Application US/07/715,272

266 (C) STRANDEDNESS: single  
267 (D) TOPOLOGY: linear  
268

269 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:  
270

271  
272 GTTTGATCTC CAGCTTGGA ~~CCXX~~CCGA A 31  
273  
274  
275

*X's are not valid according to the rule.*

276 (2) INFORMATION FOR SEQ ID NO:9:  
277

278 (i) SEQUENCE CHARACTERISTICS:  
279 (A) LENGTH: 22 bases  
280 (B) TYPE: nucleic acid  
281 (C) STRANDEDNESS: single  
282 (D) TOPOLOGY: linear  
283

284 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:  
285

286  
287 AGG~~TX~~AXCT GCAG~~X~~AGT~~CX~~GG 22  
288  
289  
290

291 (2) INFORMATION FOR SEQ ID NO:10:  
292

293 (i) SEQUENCE CHARACTERISTICS:  
294 (A) LENGTH: 34 bases  
295 (B) TYPE: nucleic acid  
296 (C) STRANDEDNESS: single  
297 (D) TOPOLOGY: linear  
298

299 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:  
300

301  
302 TGAGGAGACG GTGACCGTGG TCCCTTGGCC CCAG 34

LINE ERROR

ORIGINAL TEXT

|     |                                      |   |
|-----|--------------------------------------|---|
| 272 | Wrong Nucleic Acid Designator        | GTTTGATCTC CAGCTTGGTA C <del>CCXX</del> CDCCGA A 31 |
| 269 | Entered and Calc. Seq. Length differ | (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:             |
| 287 | Wrong Nucleic Acid Designator        | AGGTX <del>Y</del> AXCT GCAGXAGTCX GG 22            |
| 284 | Entered and Calc. Seq. Length differ | (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:             |

*error due to*

PAGE: 1

SEQUENCE MISSING ITEM REPORT  
PATENT APPLICATION US/07/715,272

DATE: 06/25/91  
TIME: 10:32:20

MANDATORY IDENTIFIER THAT WAS NOT FOUND

PAGE: 1

SEQUENCE CORRECTION REPORT  
PATENT APPLICATION US/07/715,272

DATE: 06/25/91  
TIME: 10:32:20

LINE ORIGINAL TEXT

CORRECTED TEXT

Genentech, Inc.  
Attn: Carolyn R. Adler  
460 Point San Bruno Blvd.  
South San Francisco, CA 94080

Paul J. Carter  
07/715,272  
June 14, 1991

~~100~~  
#3

**NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS  
CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE  
DISCLOSURES**

Mailed: 7-3-91

This application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 CFR § 1.821(a)(1) and (a)(2). However, this application fails to comply with one or more of the requirements of 37 CFR §§ 1.821 through 1.825 as follows:

- 1. This application clearly fails to comply with the collective requirements of §§ 1.821 through 1.825. Applicant's attention is directed to these regulations, a copy of which is attached.
- 2. This application does not conform exclusively to the requirements of §§ 1.821 through 1.825. The non-conforming material should be deleted. § 1.821(b).
- 3. This application does not contain, as a separate part of the disclosure on paper copy, a "Sequence Listing." § 1.821(c).
- 4. This application does contain, as a separate part of the disclosure on paper copy, a "Sequence Listing." However, the "Sequence Listing" does not comply with the requirements of §§ 1.821 through 1.825 as follows:
  - a. The sequence data does not comply with the symbol and format requirements of paragraphs (b) through (p) of § 1.822. Specifically: \_\_\_\_\_
  - b. The "Sequence Listing" does not comply with the location and page requirements of paragraph (a) of § 1.823.
  - c. The "Sequence Listing" does not comply with the information requirements of paragraph (b) of § 1.823. Specifically: \_\_\_\_\_
  - d. Other: \_\_\_\_\_
- 5. The description and/or claims of the patent application mention a sequence that is set forth in the "Sequence Listing" but reference is not properly made to the sequence by use of a sequence identifier as required by § 1.821(d).
- 6. A copy of the "Sequence Listing" in computer readable form has not been submitted as required by § 1.821(e).
- 7. A copy of the "Sequence Listing" in computer readable form has been submitted. However, the computer readable form does not comply with the requirements of § 1.824. Specifically: See attachment
- 8. A statement that the content of the paper and computer readable copies are the same has not been submitted as required by § 1.821(f).
- 9. The amendment to or replacement of the paper and/or computer readable copies of the "Sequence Listing" does not comply with the requirements of § 1.825(a) through (c).
- 10. The computer readable form that has been filed with this application has been found to be damaged and/or unreadable. Applicant must provide a substitute copy of the data in computer readable form accompanied by a statement that the substitute data is identical to that originally § 1.825(d). Specifically: \_\_\_\_\_

Other: \_\_\_\_\_

**GIVEN ONE MONTH FROM THE DATE OF THIS LETTER WITHIN WHICH  
THE ABOVE REQUIREMENTS. Failure to comply with the above require-  
MENT of the application under 37 CFR 1.821(g). Extensions of  
filing a petition accompanied by the extension fee under the provisions of  
the response to, and any questions about, this notice to the undersigned. A  
be returned with your response.**





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 | CLASSIFICATION | ATTY DOCKET NO./TITLE |
|--------------------|-------------|-----------------------|----------------|-----------------------|
| 07/715,272         | 06/14/91    | CARTER                | P              | 709                   |

GENENTECH, INC.  
ATTN: CAROLYN R. ADLER  
460 POINT SAN BRUNO BLVD.  
SOUTH SAN FRANCISCO, CA 94080

000

DATE MAILED: 07/03/91

#3

NOTICE TO FILE MISSING PARTS OF APPLICATION  
FILING DATE GRANTED

A filing date has been granted to this application. However, the following parts are missing.

If all missing parts are filed within the period set below, the total amount owed by applicant as a

large entity,  small entity (verified statement filed), is \$ 120.00.

- The statutory basic filing fee is:  missing  insufficient. Applicant as a  large entity  small entity, must submit \$ \_\_\_\_\_ to complete the basic filing fee and MUST ALSO SUBMIT THE SURCHARGE AS INDICATED BELOW.
- Additional claim fees of \$ \_\_\_\_\_ as a  large entity  small entity, including any required multiple dependent claim fee, are required. Applicant must submit the additional claim fees or cancel the additional claims for which fees are due. NO SURCHARGE IS REQUIRED FOR THIS ITEM.
- The oath or declaration:
  - is missing.
  - does not cover items omitted at time of execution.

An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date is required. A SURCHARGE MUST ALSO BE SUBMITTED AS INDICATED BELOW.
- The oath or declaration does not identify the application to which it applies. An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date is required. A SURCHARGE MUST ALSO BE SUBMITTED AS INDICATED BELOW.
- The signature to the oath or declaration is:  missing;  a reproduction;  by a person other than the inventor or a person qualified under 37 CFR 1.42, 1.43, or 1.47. A properly signed oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date is required. A SURCHARGE MUST ALSO BE SUBMITTED AS INDICATED BELOW.
- The signature of the following joint inventor(s) is missing from the oath or declaration: \_\_\_\_\_  
An oath or declaration listing the names of all inventors and signed by the omitted inventor(s), identifying this application by the above Application Number and Receipt Date is required. A SURCHARGE MUST ALSO BE SUBMITTED AS INDICATED BELOW.
- The application was filed in a language other than English. Applicant must file a verified English translation of the application and a fee of \$30.00 under 37 CFR 1.17(k), unless this fee has already been paid. NO SURCHARGE IS RERQUIRED FOR THIS ITEM.
- A \$50.00 processing fee is required for returned checks. (37 CFR 1.21(m)).
- Your filing receipt was mailed in error because check was returned without payment.
- Other.

An Application Number and Filing Date have been assigned to this application. The missing parts and fees identified above in items 1 and 3-6 must be timely provided ALONG WITH THE PAYMENT OF A SURCHARGE of \$120.00 for large entities or \$60.00 for small entities who have filed a verified statement claiming such status. The surcharge is set forth in 37 CFR 1.16(e). Applicant is given ONE MONTH FROM THE DATE OF THIS LETTER, OR TWO MONTHS FROM THE FILING DATE of this application, WHICHEVER IS LATER, within which to file all missing parts and pay any fees required above to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

Direct the response to, and any questions about, this notice to ATTENTION: Application Division, Special Handling Unit.

**A copy of this notice MUST be returned with response.**

*C. M. Hayward*  
For: Manager, Application Division  
(703) 557-3087/1242



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 ATTY DOCKET NO./TITLE

07/715,272 06/14/91 CARTER

710

5

GENENTECH, INC.  
ATTN: CAROLYN R. ADLER  
460 POINT SAN BRUNO BLVD.  
SOUTH SAN FRANCISCO, CA 94080

000

DATE MAILED: 07/02/91

**NOTICE TO FILE MISSING PARTS OF APPLICATION  
FILING DATE GRANTED**

A filing date has been granted to this application. However, the following parts are missing.

If all missing parts are filed within the period set below, the total amount owed by applicant as a

large entity,  small entity (verified statement filed), is \$ 120.00.

1.  The statutory basic filing fee is:  missing  insufficient. Applicant as a  large entity  small entity, must submit \$ \_\_\_\_\_ to complete the basic filing fee and **MUST ALSO SUBMIT THE SURCHARGE AS INDICATED BELOW.**
2.  Additional claim fees of \$ \_\_\_\_\_ as a  large entity  small entity, including any required multiple dependent claim fee, are required. Applicant must submit the additional claim fees or cancel the additional claims for which fees are due. **NO SURCHARGE IS REQUIRED FOR THIS ITEM.**
3.  The oath or declaration:
  - is missing.
  - does not cover items omitted at time of execution.

An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date is required. **A SURCHARGE MUST ALSO BE SUBMITTED AS INDICATED BELOW.**
4.  The oath or declaration does not identify the application to which it applies. An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date is required. **A SURCHARGE MUST ALSO BE SUBMITTED AS INDICATED BELOW.**
5.  The signature to the oath or declaration is:  missing;  a reproduction;  by a person other than the inventor or a person qualified under 37 CFR 1.42, 1.43, or 1.47. A properly signed oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date is required. **A SURCHARGE MUST ALSO BE SUBMITTED AS INDICATED BELOW.**
6.  The signature of the following joint inventor(s) is missing from the oath or declaration: \_\_\_\_\_  
An oath or declaration listing the names of all inventors and signed by the omitted inventor(s), identifying this application by the above Application Number and Receipt Date is required. **A SURCHARGE MUST ALSO BE SUBMITTED AS INDICATED BELOW.**
7.  The application was filed in a language other than English. Applicant must file a verified English translation of the application and a fee of \$30.00 under 37 CFR 1.17(k), unless this fee has already been paid. **NO SURCHARGE IS REQUIRED FOR THIS ITEM.**
8.  A \$50.00 processing fee is required for returned checks. (37 CFR 1.21(m)).
9.  Your filing receipt was mailed in error because check was returned without payment.
10.  Other.

An Application Number and Filing Date have been assigned to this application. The missing parts and fees identified above in items 1 and 3-6 must be timely provided **ALONG WITH THE PAYMENT OF A SURCHARGE** of \$120.00 for large entities or \$60.00 for small entities who have filed a verified statement claiming such status. The surcharge is set forth in 37 CFR 1.16(e). Applicant is given **ONE MONTH FROM THE DATE OF THIS LETTER, OR TWO MONTHS FROM THE FILING DATE** of this application, **WHICHEVER IS LATER**, within which to file all missing parts and pay any fees required above to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

Direct the response to, and any questions about, this notice to **ATTENTION: Application Division, Special Handling Unit.**

**A copy of this notice MUST be returned with response.**

For: Manager, Application Division  
(703) 557- 5111

DS20157 07/17/91 715272  
FORM PTO-1533 (REV. 6-90)

138 of 389

07 0630 020 105

120.00001

Celltrion, Inc., Exhibit 1094



PATENT DOCKET 709

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

45

|                              |   |                               |
|------------------------------|---|-------------------------------|
| In re Application of         | ) | Group Art Unit:               |
| Paul J. Carter et al.        | ) | Examiner:                     |
| Serial No. 07/715,272        | ) |                               |
| Filed: 14 June 1991          | ) |                               |
| For: IMMUNOGLOBULIN VARIANTS | ) | 460 Point San Bruno Boulevard |
|                              | ) | South San Francisco, CA 94080 |
|                              | ) | (415) 266-2614                |
|                              | ) |                               |

TRANSMITTAL LETTER

Honorable Commissioner of Patents  
and Trademarks  
Washington, D.C. 20231  
Attn: Application Branch

Sir:

Transmitted herewith are the following documents:

1. Declaration duly executed.
2. Copy of PTO-1553.

The Commissioner is hereby authorized to deduct the appropriate surcharge fee of \$120 associated with this communication or credit any overpayment to Deposit Account No. 07-0630. A duplicate of this sheet is enclosed.

Respectfully submitted,  
GENENTECH, INC.

*Carolyn R. Adler*  
Carolyn R. Adler  
Reg. No. 32,324

RECEIVED

JUL 18 1991

APPLICATION DIVISION-401

9 July 1991

CERTIFICATE OF MAILING (37 CFR 1.8a)

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

*Carol Koehler*  
Carol Koehler

Date: 9 July 1991

75

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

Docket No. 709

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 names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

IMMUNOGLOBULIN VARIANTS

the specification of which (check one) \_\_\_ is attached hereto or x was filed on 14 June 1991 as Application Serial No. 07/715,272 and was amended on (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I hereby state that any Sequence Listing submitted with this application is submitted in paper copy and a computer-readable diskette, and that the content of the paper and computer readable copies are the same.

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, Section 1.56(a).

I hereby 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 have a filing date before that of the application on which priority is claimed:

Table with columns: Prior Foreign Application(s), Priority Claimed (Yes/No), Number, Country, Day/Month/Year Filed

I hereby claim the benefit under Title 35, United States Code, §120 of any United States applications(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:

Table with columns: Application Ser. No., Filing Date, Status: Patented, Pending, Abandoned

Table with columns: Application Ser. No., Filing Date, Status: Patented, Pending, Abandoned

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

- Carolyn R. Adler - Reg. No. 32,324 301
Robert H. Benson - Reg. No. 30,446
Walter E. Buting - Reg. No. 23,092
Ginger R. Dreger - Reg. No. 33,055
Debbie Glaister - Reg. No. 33,888
Janet E. Hasak - Reg. No. 27,043

- Max D. Hensley - Reg. No. 27,043
Dennis G. Kleid - Reg. No. 32,037 302
Nancy Olseki - Reg. No. 34,688
Stephen Raines - Reg. No. 25,912
Daryl B. Winter - Reg. No. 32,637

Send correspondence to <sup>601</sup> Genentech, Inc.  
<sup>602</sup> Attn: Carolyn R. Adler  
<sup>701</sup> 460 Point San Bruno Boulevard  
<sup>702</sup> South San Francisco, CA 94080  
Telephone: (415) 266-2614

I hereby declare that all statements made herein of my own knowledge and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that willful false statements may jeopardize the validity of the application or any patent issued thereon.

The undersigned hereby authorizes the U.S. attorney or agent named herein to accept and follow instructions from his foreign patent agent as to any action to be taken in the Patent and Trademark Office regarding this application without direct communication between the U.S. attorney or agent and the undersigned. In the event of a change in the persons from whom instructions may be taken, the U.S. attorney or agent named herein will be so notified by the undersigned.

Full name of sole or first inventor  
Paul J. Carter <sup>40100</sup> *Paul John Carter* <sup>18<sup>th</sup> June 1991</sup>  
Inventor's signature Date

Residence  
2074 18th Avenue  
San Francisco, CA 94116

Citizenship  
United Kingdom

Post Office Address  
460 Point San Bruno Boulevard  
South San Francisco, CA 94080

Full name of second joint inventor, if any  
Leonard G. Presta <sup>40200</sup>  
Second Inventor's signature Date  
*Leonard G. Presta* <sup>6-19-91</sup>

Residence  
1900 Gough Street, #206  
San Francisco, CA 94109

Citizenship  
United States of America

Post Office Address  
460 Point San Bruno Boulevard  
South San Francisco, CA 94080

Full name of third joint inventor, if any  
Third Inventor's signature Date

Residence

Citizenship

Post Office Address  
460 Point San Bruno Boulevard  
South San Francisco, CA 94080



Genentech, Inc. 1991  
Attn: Carolyn Adler  
460 Point San Bruno Blvd.  
South San Francisco, CA 94080

RECEIVED

JUL 08 1991

Genentech, Inc. Legal Dept.

Paul J. Carter  
07/715,272  
June 14, 1991

CALENDARED

3 Aug 91

**NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES**

Mailed:

This application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 CFR § 1.821(a)(1) and (a)(2). However, this application fails to comply with one or more of the requirements of 37 CFR §§ 1.821 through 1.825 as follows:

- 1. This application clearly fails to comply with the collective requirements of §§ 1.821 through 1.825. Applicant's attention is directed to these regulations, a copy of which is attached.
- 2. This application does not conform exclusively to the requirements of §§ 1.821 through 1.825. The non-conforming material should be deleted. § 1.821(b).
- 3. This application does not contain, as a separate part of the disclosure on paper copy, a "Sequence Listing." § 1.821(c).
- 4. This application does contain, as a separate part of the disclosure on paper copy, a "Sequence Listing." However, the "Sequence Listing" does not comply with the requirements of §§ 1.821 through 1.825 as follows:

- a. The sequence data does not comply with the symbol and format requirements of paragraphs (b) through (p) of § 1.822. Specifically: \_\_\_\_\_
- b. The "Sequence Listing" does not comply with the location and page requirements of paragraph (a) of § 1.823.
- c. The "Sequence Listing" does not comply with the information requirements of paragraph (b) of § 1.823. Specifically: \_\_\_\_\_
- d. Other: \_\_\_\_\_

- 5. The description and/or claims of the patent application mention a sequence that is set forth in the "Sequence Listing" but reference is not properly made to the sequence by use of a sequence identifier as required by § 1.821(d).
- 6. A copy of the "Sequence Listing" in computer readable form has not been submitted as required by § 1.821(e).

7. A copy of the "Sequence Listing" in computer readable form has been submitted. However, the computer readable form does not comply with the requirements of § 1.824. Specifically: See attachment

- 8. A statement that the content of the paper and computer readable copies are the same has not been submitted as required by § 1.821(f).
- 9. The amendment to or replacement of the paper and/or computer readable copies of the "Sequence Listing" does not comply with the requirements of § 1.825(a) through (c).
- 10. The computer readable form that has been filed with this application has been found to be damaged and/or unreadable. Applicant must provide a substitute copy of the data in computer readable form accompanied by a statement that the substitute data is identical to that originally filed. § 1.825(d). Specifically: \_\_\_\_\_
- 11. Other: \_\_\_\_\_

APPLICANT IS GIVEN ONE MONTH FROM THE DATE OF THIS LETTER WITHIN WHICH TO COMPLY WITH THE ABOVE REQUIREMENTS. Failure to comply with the above requirements will result in ABANDONMENT of the application under 37 CFR 1.821(g). Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR § 1.136. Direct the response to, and any questions about, this notice to the undersigned. A copy of this notice MUST be returned with your response.

Cynthia 389  
Patent Application Processing Division

Celltrion, Inc. Exhibit 1094



SEQUENCE LISTING

(1) GENERAL INFORMATION:

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

(ii) TITLE OF INVENTION: Immunoglobulin Variants

10 (iii) NUMBER OF SEQUENCES: 10

(iv) CORRESPONDENCE ADDRESS:

- (A) ADDRESSEE: Genentech, Inc.
- (B) STREET: 460 Point San Bruno Blvd
- (C) CITY: South San Francisco
- (D) STATE: California
- (E) COUNTRY: USA
- (F) ZIP: 94080

15 (v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: 5.25 inch, 360 Kb floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: patin (Genentech)

20 (vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER: 07/715,272
- (B) FILING DATE: 14-June-1991
- (C) CLASSIFICATION:

25 (vii) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER:
- (B) FILING DATE:

30 (viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: Adler, Carolyn R.
- (B) REGISTRATION NUMBER: 32,324
- (C) REFERENCE/DOCKET NUMBER: 709

35 (ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: 415/266-2614
- (B) TELEFAX: 415/952-9881
- (C) TELEX: 910/371-7168

40 (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:

45 Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val  
 55 1 5 10 15  
 Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn  
 60 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  
5 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  
10 Ile Lys Arg Thr  
109

(2) INFORMATION FOR SEQ ID NO:2:

15 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 120 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

20 (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  
25 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  
30 Glu Trp Val Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr  
50 55 60  
35 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  
40 Thr Ala Val Tyr Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr  
95 100 105  
45 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:

50 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 109 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

55 (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  
60 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
-5 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
10 Tyr Asn Ser Leu Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu
                    95                    100                    105
Ile Lys Arg Thr
                    109
15

```

(2) INFORMATION FOR SEQ ID NO:4:

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(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 120 amino acids
    (B) TYPE: amino acid
    (D) TOPOLOGY: linear
20

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```

25 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
30 Asp Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
                    35                40                45
35 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
40 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
45 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
55

```

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```

60 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  
 5 50 55 60  
 Arg Phe Thr Gly Asn Arg Ser Gly Thr Asp Phe Thr Phe Thr Ile  
 65 70 75  
 10 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  
 15 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 bases  
 (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 bases  
(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 bases  
(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 bases  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TGAGGAGACG GTGACCGTGG TCCCTTGGCC CCAG 34

APN  
a #7

800 630



PATENT DOCKET 709

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of )  
 )  
PAUL J. CARTER ET AL. )  
 )  
Serial No. 07/715,272 )  
 )  
Filed: June 14, 1991 )  
 )  
For: IMMUNOGLOBULIN VARIANTS )  
 )  
 )

Art Unit: to be assigned

Examiner: to be assigned

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231 on July 12, 1991  
(Date of Deposit)

LOUISE STRASBAUGH

Name of Depositing Party

RESPONSE AND PRELIMINARY AMENDMENT

Louise Strasbaugh

Signature of Depositing Party

July 12, 1991

Date of Signature

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

Sir:

This is responsive to the Notice to Comply with Requirements for Patent Applications Containing Nucleotide and/or Amino Acid Sequence Disclosures, mailed June 25, 1991. The inventors also take this opportunity to correct two minor grammatical errors in the application, and add no new matter.

Enclosed is an amended sequence listing submitted with a paper copy and a computer-readable diskette. The sequence listing has been corrected to conform exactly to the sequences as recited in the specification as originally filed. I hereby state that the content of this paper and computer readable copies are the same, and that this amendment corrects errors in the previous sequence listing submission without adding new matter.

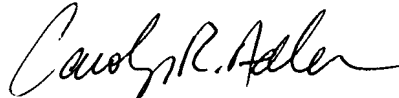
IN THE SPECIFICATION:

Please make the following amendments:

On page 12, line 1, delete genes and insert --sequences--.

On page 16, line 12, delete intrachain-affecting and insert --interchain--affecting.

Respectfully Submitted,  
GENENTECH, INC.



Carolyn R. Adler  
Reg. No. 32,324

July 12, 1991  
460 Point San Bruno Blvd  
South San Francisco, CA 94080

## Patent Application US/07/715,272A

## SEQUENCE LISTING

1  
2  
3 (1) GENERAL INFORMATION:  
4  
5 (i) APPLICANT: Carter, Paul J.  
6 Presta, Leonard G.  
7  
8 (ii) TITLE OF INVENTION: Immunoglobulin Variants  
9  
10 (iii) NUMBER OF SEQUENCES: 10  
11  
12 (iv) CORRESPONDENCE ADDRESS:  
13 (A) ADDRESSEE: Genentech, Inc.  
14 (B) STREET: 460 Point San Bruno Blvd  
15 (C) CITY: South San Francisco  
16 (D) STATE: California  
17 (E) COUNTRY: USA  
18 (F) ZIP: 94080  
19  
20 (v) COMPUTER READABLE FORM:  
21 (A) MEDIUM TYPE: 5.25 inch, 360 Kb floppy disk  
22 (B) COMPUTER: IBM PC compatible  
23 (C) OPERATING SYSTEM: PC-DOS/MS-DOS  
24 (D) SOFTWARE: patin (Genentech)  
25  
26 (vi) CURRENT APPLICATION DATA:  
27 (A) APPLICATION NUMBER: 07/715,272  
28 (B) FILING DATE: 14-June-1991  
29 (C) CLASSIFICATION:  
30  
31 (vii) PRIOR APPLICATION DATA:  
32 (A) APPLICATION NUMBER:  
33 (B) FILING DATE:  
34  
35 (viii) ATTORNEY/AGENT INFORMATION:  
36 (A) NAME: Adler, Carolyn R.  
37 (B) REGISTRATION NUMBER: 32,324  
38 (C) REFERENCE/DOCKET NUMBER: 709  
39  
40 (ix) TELECOMMUNICATION INFORMATION:  
41 (A) TELEPHONE: 415/266-2614  
42 (B) TELEFAX: 415/952-9881  
43 (C) TELEX: 910/371-7168  
44  
45 (2) INFORMATION FOR SEQ ID NO:1:  
46  
47 (i) SEQUENCE CHARACTERISTICS:  
48 (A) LENGTH: 109 amino acids  
49 (B) TYPE: amino acid  
50 (D) TOPOLOGY: linear  
51  
52 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:  
53

## Patent Application US/07/715,272A

54 Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val  
 55 1 5 10 15  
 56  
 57 Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn  
 58 20 25 30  
 59  
 60 Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys  
 61 35 40 45  
 62  
 63 Leu Leu Ile Tyr Ser Ala Ser Phe Leu Glu Ser Gly Val Pro Ser  
 64 50 55 60  
 65  
 66 Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile  
 67 65 70 75  
 68  
 69 Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln  
 70 80 85 90  
 71  
 72 His Tyr Thr Thr Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu  
 73 95 100 105  
 74  
 75 Ile Lys Arg Thr  
 76 109  
 77  
 78 (2) INFORMATION FOR SEQ ID NO:2:  
 79  
 80 (i) SEQUENCE CHARACTERISTICS:  
 81 (A) LENGTH: 120 amino acids  
 82 (B) TYPE: amino acid  
 83 (D) TOPOLOGY: linear  
 84  
 85 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:  
 86  
 87 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly  
 88 1 5 10 15  
 89  
 90 Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys  
 91 20 25 30  
 92  
 93 Asp Thr Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu  
 94 35 40 45  
 95  
 96 Glu Trp Val Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr  
 97 50 55 60  
 98  
 99 Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser  
 100 65 70 75  
 101  
 102 Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp  
 103 80 85 90  
 104  
 105 Thr Ala Val Tyr Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr  
 106 95 100 105

## Patent Application US/07/715,272A

107  
 108 Ala Met Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser  
 109 110 115 120  
 110  
 111

## 112 (2) INFORMATION FOR SEQ ID NO:3:

113  
 114 (i) SEQUENCE CHARACTERISTICS:  
 115 (A) LENGTH: 109 amino acids  
 116 (B) TYPE: amino acid  
 117 (D) TOPOLOGY: linear  
 118

## 119 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

120  
 121 Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val  
 122 1 5 10 15  
 123  
 124 Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser  
 125 20 25 30  
 126  
 127 Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys  
 128 35 40 45  
 129  
 130 Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser  
 131 50 55 60  
 132  
 133 Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile  
 134 65 70 75  
 135  
 136 Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln  
 137 80 85 90  
 138  
 139 Tyr Asn Ser Leu Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu  
 140 95 100 105  
 141  
 142 Ile Lys Arg Thr  
 143 109  
 144

## 145 (2) INFORMATION FOR SEQ ID NO:4:

146  
 147 (i) SEQUENCE CHARACTERISTICS:  
 148 (A) LENGTH: 120 amino acids  
 149 (B) TYPE: amino acid  
 150 (D) TOPOLOGY: linear  
 151

## 152 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

153  
 154 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly  
 155 1 5 10 15  
 156  
 157 Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser  
 158 20 25 30  
 159



## Patent Application US/07/715,272A

160 Asp Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu  
 161 35 40 45  
 162  
 163 Glu Trp Val Ala Val Ile Ser Glu Asn Gly Gly Tyr Thr Arg Tyr  
 164 50 55 60  
 165  
 166 Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser  
 167 65 70 75  
 168  
 169 Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp  
 170 80 85 90  
 171  
 172 Thr Ala Val Tyr Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr  
 173 95 100 105  
 174  
 175 Ala Met Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser  
 176 110 115 120  
 177  
 178

## 179 (2) INFORMATION FOR SEQ ID NO:5:

180

## 181 (i) SEQUENCE CHARACTERISTICS:

182 (A) LENGTH: 109 amino acids

183 (B) TYPE: amino acid

184 (D) TOPOLOGY: linear

185

## 186 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

187

188 Asp Ile Val Met Thr Gln Ser His Lys Phe Met Ser Thr Ser Val  
 189 1 5 10 15  
 190

191 Gly Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asp Val Asn  
 192 20 25 30  
 193

194 Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly His Ser Pro Lys  
 195 35 40 45  
 196

197 Leu Leu Ile Tyr Ser Ala Ser Phe Arg Tyr Thr Gly Val Pro Asp  
 198 50 55 60  
 199

200 Arg Phe Thr Gly Asn Arg Ser Gly Thr Asp Phe Thr Phe Thr Ile  
 201 65 70 75  
 202

203 Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln  
 204 80 85 90  
 205

206 His Tyr Thr Thr Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu  
 207 95 100 105  
 208

209 Ile Lys Arg Ala  
 210 109  
 211

## 212 (2) INFORMATION FOR SEQ ID NO:6:

## Patent Application US/07/715,272A

213

214 (i) SEQUENCE CHARACTERISTICS:

215 (A) LENGTH: 120 amino acids

216 (B) TYPE: amino acid

217 (D) TOPOLOGY: linear

218

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

220

221 Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly

222 1 5 10 15

223

224 Ala Ser Leu Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys

225 20 25 30

226

227 Asp Thr Tyr Ile His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu

228 35 40 45

229

230 Glu Trp Ile Gly Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr

231 50 55 60

232

233 Asp Pro Lys Phe Gln Asp Lys Ala Thr Ile Thr Ala Asp Thr Ser

234 65 70 75

235

236 Ser Asn Thr Ala Tyr Leu Gln Val Ser Arg Leu Thr Ser Glu Asp

237 80 85 90

238

239 Thr Ala Val Tyr Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr

240 95 100 105

241

242 Ala Met Asp Tyr Trp Gly Gln Gly Ala Ser Val Thr Val Ser Ser

243 110 115 120

244

245

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

247

248 (i) SEQUENCE CHARACTERISTICS:

249 (A) LENGTH: 27 bases

250 (B) TYPE: nucleic acid

251 (C) STRANDEDNESS: single

252 (D) TOPOLOGY: linear

253

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

255

256

257 TCCGATATCC AGCTGACCCA GTCTCCA 27

258

259

260

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

262

263 (i) SEQUENCE CHARACTERISTICS:

264 (A) LENGTH: 31 bases

265 (B) TYPE: nucleic acid

Patent Application US/07/715,272A

266 (C) STRANDEDNESS: single  
267 (D) TOPOLOGY: linear  
268  
269 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:  
270  
271  
272 GTTTGATCTC CAGCTTGTA CCHSCDCCGA A 31  
273  
274  
275  
276 (2) INFORMATION FOR SEQ ID NO:9:  
277  
278 (i) SEQUENCE CHARACTERISTICS:  
279 (A) LENGTH: 22 bases  
280 (B) TYPE: nucleic acid  
281 (C) STRANDEDNESS: single  
282 (D) TOPOLOGY: linear  
283  
284 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:  
285  
286  
287 AGGTSMARCT GCAGSAGTCW GG 22  
288  
289  
290  
291 (2) INFORMATION FOR SEQ ID NO:10:  
292  
293 (i) SEQUENCE CHARACTERISTICS:  
294 (A) LENGTH: 34 bases  
295 (B) TYPE: nucleic acid  
296 (C) STRANDEDNESS: single  
297 (D) TOPOLOGY: linear  
298  
299 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:  
300  
301  
302 TGAGGAGACG GTGACCGTGG TCCCTTGGCC CCAG 34  
303  
304

PAGE: 1

SEQUENCE VERIFICATION REPORT  
PATENT APPLICATION US/07/715,272A

DATE: 07/19/91  
TIME: 16:16:36

LINE ERROR

ORIGINAL TEXT

27 Wrong application Serial Number

*OK* (A) APPLICATION NUMBER: 07/715,272

SEQUENCE MISSING ITEM REPORT  
PATENT APPLICATION US/07/715,272A

DATE: 07/19/91  
TIME: 16:16:36

MANDATORY IDENTIFIER THAT WAS NOT FOUND

PAGE: 1

SEQUENCE CORRECTION REPORT  
PATENT APPLICATION US/07/715,272A

DATE: 07/19/91  
TIME: 16:16:36

LINE ORIGINAL TEXT

CORRECTED TEXT

18M Feisee

186

PATENT DOCKET 708

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#10  
5-18-92

In re Application of  
Paul J. Carter et al.  
Serial No. 07/715272  
Filed: June 14, 1991  
For: Immunoglobulin Variants



RECEIVED  
Group Art Unit:  
Examiner: MAY U 8 1992  
GROUP 180

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

INFORMATION DISCLOSURE STATEMENT

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

Sir:

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

- Chothia *et al.*, *J. Mol. Biol.* 186:651-663 (1985)
- Novotny and Haber, *Proc. Natl. Acad. Sci. USA* 82:4592-4596 (1985)
- Cabilly *et al.*, U.S. patent No. 4,816,567
- Morrison, S. L. *et al.*, *Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984)
- Boulianne, G. L. *et al.*, *Nature* 312:643-646 (1984)
- Neuberger, M. S. *et al.*, *Nature* 314:268-270 (1985)
- Brüggemann, M. *et al.*, *J. Exp. Med.* 166:1351-1361 (1987)
- Riechmann, L. *et al.*, *Nature* 332:323-327 (1988)
- Love *et al.*, *Methods in Enzymology* 178:515-527 (1989)
- Bindon *et al.*, *J. Exp. Med.* 168:127-142 (1988)
- Jones, P. T. *et al.*, *Nature* 321:522-525 (1986)
- Verhoeyen, M. *et al.*, *Science* 239:1534-1536 (1988)
- Hale, G. *et al.*, *Lancet* i:1394-1399 (1988)
- Queen, C. *et al.*, *Proc. Natl. Acad. Sci. USA* 86:10029-10033 (1989)
- Co *et al.*, *Proc. Natl. Acad. Sci. USA* 88:2869-2873 (1991)
- Gorman *et al.*, *Proc. Natl. Acad. Sci. USA* 88:4181-4185 (1991)
- Daugherty *et al.*, *Nucleic Acids Research* 19(9):2471-2476 (1991)

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

LOUISE STRASBAUGH  
Name of Depositing Party  
Louise Strasbaugh  
Signature of Depositing Party  
April 30, 1992  
Date of Signature

Brown *et al.*, *Proc. Natl. Acad. Sci. USA* **88**:2663-2667 (1991)  
Junghans *et al.*, *Cancer Research* **50**:1495-1502 (1990)  
Davies, D. R. *et al.*, *Ann. Rev. Biochem.* **59**:439-473 (1990)  
Chothia, C. & Lesk, A. M., *J. Mol. Biol.* **196**:901-917 (1987)  
Chothia, C. *et al.*, *Nature* **342**:877-883 (1989)  
Tramontano, A. *et al.*, *J. Mol. Biol.* **215**:175-182 (1990)  
Margolies *et al.*, *Proc. Natl. Acad. Sci. USA* **72**:2180-2184 (1975)  
Pluckthun, *Biotechnology* **9**:545-51 (1991)  
Spiegelberg *et al.*, *Biochemistry* **9**:4217-4223 (1970)  
Wallick *et al.*, *J. Exp. Med.* **168**:1099-1109 (1988)  
Sox *et al.*, *Proc. Natl. Acad. Sci. USA* **66**:975-982 (1970)  
Margni *et al.*, *Ann. Rev. Immunol.* **6**:535-554 (1988)  
Fendly, B. M. *et al.*, *Cancer Res.* **50**:1550-1558 (1990)  
Neuberger *et al.*, *Nature* **312**:604-608 (1984)  
Takeda *et al.*, *Nature* **314**:452-454 (1985)  
Snow and Amzel, *Protein: Structure, Function, and Genetics* **1**:267-279, Alan R. Liss, Inc. pubs. (1986)  
Cheetham, J., *Protein Engineering*, **2**(3): 170-172 (1988)  
WO 91/09967, pub. 07/11/91, Adair *et al.*

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

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

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

Respectfully submitted,  
GENENTECH, INC.



Carolyn R. Adler  
Reg. No. 32,324

Dated: April 30, 1992





UNITED STATES DEPARTMENT OF COMMERCE  
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

|               |             |                      |                     |
|---------------|-------------|----------------------|---------------------|
| SERIAL NUMBER | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. |
| 07/715,272    | 06/14/91    | CARTER               | P 709               |

EXAMINER  
FEISEL

GENENTECH, INC.  
ATTN: CAROLYN R. ADLER  
460 POINT SAN BRUNO BLVD.  
SOUTH SAN FRANCISCO, CA 94080

ART UNIT: 1806  
PAPER NUMBER: 9  
DATE MAILED: 05/12/92

This is a communication from the examiner in charge of your application.  
COMMISSIONER OF PATENTS AND TRADEMARKS

This application has been examined *revised*  Response to communication filed on 7/15/91  This action is made final.  
A shortened statutory period for response to this action is set to expire 0 month(s), 30 days from the date of this letter.  
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

- Notice of References Cited by Examiner, PTO-892.
- Notice re Patent Drawing, PTO-948.
- Notice of Art Cited by Applicant, PTO-1449.
- Notice of Informal Patent Application, Form PTO-152
- Information on How to Effect Drawing Changes, PTO-1474.
- 

Part II SUMMARY OF ACTION

- Claims 1-16 are pending in the application.  
Of the above, claims \_\_\_\_\_ are withdrawn from consideration.
- Claims \_\_\_\_\_ have been cancelled.
- Claims \_\_\_\_\_ are allowed.
- Claims \_\_\_\_\_ are rejected.
- Claims \_\_\_\_\_ are objected to.
- Claims 1-16 are subject to restriction or election requirement.
- This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.
- Formal drawings are required in response to this Office action.
- The corrected or substitute drawings have been received on \_\_\_\_\_ Under 37 C.F.R. 1.84 these drawings are  acceptable;  not acceptable (see explanation or Notice re Patent Drawing, PTO-948).
- The proposed additional or substitute sheet(s) of drawings, filed on \_\_\_\_\_, has (have) been  approved by the examiner;  disapproved by the examiner (see explanation).
- The proposed drawing correction, filed \_\_\_\_\_, has been  approved;  disapproved (see explanation).
- Acknowledgement is made of the claim for priority under U.S.C. 119. The certified copy has  been received  not been received  been filed in parent application, serial no. \_\_\_\_\_; filed on \_\_\_\_\_.
- Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.
- Other

Serial No. 715272

Art Unit 1806

Restriction to one of the following inventions is required under 35 U.S.C. 121:

I. Claims 1-13, drawn to a method of making an antibody and an antibody comprising a polypeptide, classified in Class 435, 530 subclass 69.1, 350.

II. Claims 14-16, drawn to computer representations, classified in Class 364, subclass 282.1+.

The inventions are distinct, each from the other because of the following reasons:

The two Groups are drawn to two different products, Group I being a biological molecule and Group II being a machine. These constitute two different statutory classes of invention and are therefore patentably distinct one from the other.

Because these inventions are distinct for the reasons given above and have acquired a separate status in the art as shown by their different classification and divergent subject matter, and because the searches for the individual Groups are not coextensive, restriction for examination purposes as indicated is proper.

Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 C.F.R. 1.48(b) if one or more of the currently

Serial No. 715272

Art Unit 1806

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 diligently-filed petition under 37 C.F.R. 1.48(b) and by the fee required under 37 C.F.R. 1.17(h).

5 A telephone call was made to Carolyn Adler, on 12/9/91, to request an oral election to the above restriction requirement, but did not result in an election being made and a written restriction was requested.

10 Applicant is advised that the response to this requirement to be complete must include an election of the invention to be examined even though the requirement be traversed. (37 C.F.R. 1.143).

15 Any inquiry concerning this communication or earlier communications from the examiner should be directed to Lila Feisee whose telephone number is (703) 308-2731.


Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.


20 Papers related to this application may be submitted to Group 180 by facsimile transmission. Papers should be faxed to Group 180 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

Serial No. 715272

Art Unit 1806

Center number is (703) 308-4227. The hours of operation of the  
Center are 8:45 am - 4:45 pm, Monday - Friday.

Feisee/lf   
May 11, 1992

  
JOHN J. DOLL  
SUPERVISORY PATENT EXAMINER  
GROUP 180

9

715272

## NOTICE OF DRAFTSMAN'S PATENT DRAWING REVIEW

**THE PTO DRAFTSMEN REVIEW ALL ORIGINALLY FILED DRAWINGS REGARDLESS OF WHETHER THEY WERE DESIGNATED AS INFORMAL OR FORMAL.**

The drawings filed \_\_\_\_\_

6/14/91

A.  are approved.

B.  are objected to under 37 CFR 1.84 for the reason(s) checked below. The examiner will require submission of new, corrected drawings at the appropriate time. Corrected drawings must be submitted according to the instructions listed on the back of this Notice.

1. Paper and ink. 37 CFR 1.84(a)

Sheet(s) \_\_\_\_\_ Poor.

2. Size of Sheet and Margins. 37 CFR 1.84(b)

Acceptable Paper Sizes and Margins

| Margin | Paper Size         |                    |                               |
|--------|--------------------|--------------------|-------------------------------|
|        | 8 1/2 by 14 inches | 8 1/2 by 13 inches | DIN size A4<br>21 by 29.7 cm. |
| Top    | 2 inches           | 1 inch             | 2.5 cm.                       |
| Left   | 1/4 inch           | 1/4 inch           | 2.5 cm.                       |
| Right  | 1/4 inch           | 1/4 inch           | 1.5 cm.                       |
| Bottom | 1/4 inch           | 1/4 inch           | 1.0 cm.                       |

Proper Size Paper Required.  
All Sheets Must be Same Size.  
Sheet(s) figs 1-4

Proper Margins Required.  
Sheet(s) \_\_\_\_\_

- TOP       RIGHT  
 LEFT       BOTTOM

3. Character of Lines. 37 CFR 1.84(c)

Lines Pale or Rough and Blurred.  
Fig(s) \_\_\_\_\_

Solid Black Shading Not Allowed.  
Fig(s) \_\_\_\_\_

4. Hatching and Shading. 37 CFR 1.84(d)

Shade Lines are Required.  
Fig(s) \_\_\_\_\_

Criss-Cross Hatching Not Allowed.  
Fig(s) \_\_\_\_\_

Double Line Hatching Not Allowed.  
Fig(s) \_\_\_\_\_

Parts in Section Must be Hatched.  
Fig(s) \_\_\_\_\_

5. Reference Characters. 37 CFR 1.84(f)

Reference Characters Poor or Incorrectly Sized.  
Fig(s) 2-4

Reference Characters Placed Incorrectly.  
Fig(s) \_\_\_\_\_

6. Views. 37 CFR 1.84(i) & (j)

Figures Must be Numbered Properly.  
\_\_\_\_\_

Figures Must Not be Connected.  
Fig(s) \_\_\_\_\_

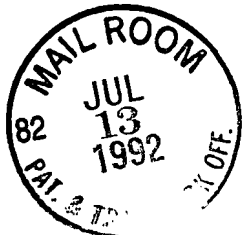
7.  Photographs Not Approved.  
fig 7

8.  Other.

Telephone inquiries concerning this review should be directed to the Chief Draftsman at telephone number (703) 557-6404.

\_\_\_\_\_  
Reviewing Draftsman

8/6  
\_\_\_\_\_  
Date



PATENT DOCKET 709

*See #12 S18 7/24/92*

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

|                              |   |                               |
|------------------------------|---|-------------------------------|
| In re Application of         | ) | Group Art Unit: 1806          |
| Paul J. Carter et al.        | ) | Examiner: L. Feisee           |
| Serial No. 07/715,272        | ) |                               |
| Filed: 14 June 1991          | ) |                               |
| For: Immunoglobulin Variants | ) | 460 Point San Bruno Boulevard |
|                              | ) | South San Francisco, CA 94080 |
|                              | ) | (415) 225-2614                |

Response

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

JUL 22 1992

Sir:

This is responseive to the Restriction Requirement mailed 12 May 1992. A request for a one-month extension of time to respond is submitted herewith, bringing the due date for this response to 11 July 1992. This response is timely filed.

The inventors hereby elect to prosecute Group 1, claims 1-13.

Respectfully submitted,  
GENENTECH, INC.

Carolyn R. Adler  
Reg. No. 32,324

10 July 1992

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on 10 July 1992.

Dated: 10 July 1992

  
Carolyn R. Adler

186

18C Fee

PATENT DOCKET 709

#1  
SM  
712452



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

|                              |   |                               |
|------------------------------|---|-------------------------------|
| In re Application of         | ) | Group Art Unit: 1806          |
|                              | ) |                               |
| Paul J. Carter et al.        | ) | Examiner: L. Feisee           |
|                              | ) |                               |
| Serial No. 07/715,272        | ) |                               |
|                              | ) |                               |
| Filed: 14 June 1991          | ) |                               |
|                              | ) |                               |
| For: Immunoglobulin Variants | ) |                               |
|                              | ) | 460 Point San Bruno Boulevard |
|                              | ) | South San Francisco, CA 94080 |
|                              | ) | (415) 225-2614                |

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

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

**JUL 22 1992**

Sir:

Applicant petitions the Commissioner of Patents and Trademarks to extend the time for response to the Office action dated 12 May 1992 for one month(s) from 11 June 1992 to 11 July 1992. The extended time for response does not exceed the statutory period.

Please charge Deposit Account Number 07-0630 in the amount of \$110 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.

Carolyn R. Adler  
Reg. No. 32,324

Date: 10 July 1992

CERTIFICATE OF MAILING (37 CFR 1.8a)

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

Carolyn R. Adler

Date: 10 July 1992

ONLINE SEARCH REQUEST FORM

\*\*\*\*\*  
USER ~~W. Feix~~ Feix SERIAL NUMBER 715272  
ART UNIT 1806 PHONE 2731 DATE 9/11/92

Please give a detailed statement of requirements. Describe as specifically as possible the subject matter to be searched. Define any terms that may have special meaning. Give examples or relevant citations, authors, or keywords, if known.

You may include a copy of the broadest and or relevant claim(s).

Please search  
Making Humanized Antibodies

by - CDR - Grafting - ~~and~~

~~by - CDR - Grafting - and~~

See claims 1-13  
especially!!!

138  
98  
17M

RECEIVED  
STIC  
BIOTECH/CHEMICAL  
LABORATORY  
02 SEP 10 11 30 AM  
U.S. PAT. & TM. OFF.



show files

9/15/92

Feisel  
715272

File 155: MEDLINE 1966-1992/NOV (9211W1)  
 File 5: BIOSIS PREVIEWS 69-92/OCT BA9407: BARRM4307  
 (C. BIOSIS 1992)  
 File 73: EMBASE (EXCERPTA MEDICA) 74-92/ISS37  
 (COPR. ESP BV/EM 1992)  
 File 399: CA SEARCH 1967-1992 UD=11710  
 (Copr. 1992 by the Amer. Chem. Soc.)

?ds

| Set | Items   | Description   |
|-----|---------|---|
| S1  | 16      | HUMANIZED() ANTIBODIES/TI   |
| S2  | 332298  | ANTIBODIES! FROM 155  |
| S3  | 2253    | IMMUNOGLOBULIN VARIABLE REGION! FROM 155  |
| S4  | 2253    | S2 AND S3   |
| S5  | 862     | HUMANIZ?  |
| S6  | 2005    | HUMANIS?  |
| S7  | 16      | S4 AND (HUMANIZ? OR HUMANIS?)   |
| S8  | 636823  | ANTIBOD? FROM 5,73,399  |
| S9  | 165469  | IMMUNOGLOBULIN  |
| S10 | 41830   | IG  |
| S11 | 113462  | VARIABLE  |
| S12 | 392448  | REGION  |
| S13 | 862     | (IMMUNOGLOBULIN OR IG) (W) VARIABLE (W) REGION  |
| S14 | 604     | CDR   |
| S15 | 67991   | COMPLEMENTARY   |
| S16 | 112646  | DETERMINING   |
| S17 | 63      | COMPLEMENTARY (W) DETERMINING   |
| S18 | 1904    | HYPERVARIABLE   |
| S19 | 392448  | REGION  |
| S20 | 747     | (COMPLEMENTARY (W) DETERMINING OR HYPERVARIABLE) (W) REGION   |
| S21 | 428778  | ANTIBODY  |
| S22 | 1469126 | RELATED   |
| S23 | 623755  | BINDING   |
| S24 | 544344  | SITE? ?   |
| S25 | 0       | ANTIBODY (W) RELATED (W) BINDING (W) SITE? ?  |
| S26 | 2161    | (IMMUNOGLOBULIN OR IG) () VARIABLE () REGION OR CDR OR (COMPLEMENTARY () DETERMINING OR HYPERVARIABLE) () REGION OR ANTIBODY () RELATED () BINDING () SITE? ? FROM 5,73,399 |
| S27 | 897     | 8 AND 26  |
| S28 | 18      | 27 AND (5 OR 6)   |
| S29 | 34      | 28 OR 7   |
| S30 | 21      | RD (unique items)   |
| S31 | 21      | Sort S30/ALL/PY;D   |

?t31/7/1-21

*synonym for CDR*

*synonyms for CDR*

31/7/1 (Item 1 from file: 5)  
 9568885 BIOSIS Number: 94073885  
 \*HUMANIZED\* OKT3 \*ANTIBODIES\* SUCCESSFUL TRANSFER OF IMMUNE MODULATING  
 PROPERTIES AND IDIOTYPE EXPRESSION  
 WOODLE E S; THISTLEWAITE J R; JOLLIFFE L K; ZIVIN R A; COLLINS A; ADAIR J  
 A; BODMER M; ATHWAL D; ALEGRE M-L; BLUESTONE J A  
 SECT. ORGAN TRANSPLANTATION, DEP. SURGERY, WASH. UNIV. SCH. MED., ONE  
 BARNES HOSP. PLAZA, QUEENY TOWER, SUITE 6107, ST. LOUIS, MO. 63110.  
 J IMMUNOL 148 (9). 1992. 2756-2763. CODEN: JOIMA  
 Full Journal Title: Journal of Immunology  
 Language: ENGLISH

. . \*Antibodies\* that possess the Ag-binding regions of OKT3 within the context of a human framework (Hu-OKT3 Ab) offer distinct advantages for optimizing anti-CD3 mAb therapy. First, manipulation of Ab genes to produce \*humanized\*. Ab that retain Ag-binding activity may circumvent antigenicity problems. Second, Ab gene engineering provides a means for modifying functional properties, including T cell activation and immune suppression. The purpose of this study was to determine the functional properties of Hu-OKT3 Ab and to compare the functional properties and idiotypes of Hu-OKT3 Ab to those of murine OKT3. Three Hu-OKT3 IgG4 aAb, a chimeric OKT3 \*antibody\* (cOKT3-1) (grafted sequences comprising all OKT3 VH and VL regions) and two complementarity determining region (\*CDR\*)-grafted \*antibodies\* , gOKT3-5 and gOKT3-6 (grafted sequences comprising only OKT3 VH and VL \*CDR\* and some framework amino acids, were analyzed. Initial studies demonstrated that the cOKT3 and gOKT3-5 Ab bound selectively to T cells and competitively inhibited OKT3-FITC binding with avidities similar to that of murine OKT3. binding avidity of the gOKT3-6 Ab was markedly less than that of the other Hu-OKT3 Ab. Serologic analysis suggested that cOKT3 and gOKT3-5 Ab possess idiotypes (combining sites) similar to murine OKT3. C cell activation potency of all three Hu-OKT3 Ab was assessed by proliferation, induction of activation marker expression (IL-2R and Leu 23), and lymphokine production (TNF-.alpha. and IFN-.gamma.). The cOKT3 and gOKT3-5 Ab demonstrated T cell activation potencies similar to murine OKT3 as assessed by each parameter. CD3 coating and modulation by these two Ab was effective but somewhat less potent than that observed with OKT3. Finally, cOKT3 and gOKT3-5 Ab both inhibited CTL activity comparably to murine OKT3. In conclusion, these studies indicate that gOKT3-5 and cOKT3 Ab possess immune modulating properties similar to murine OKT3 and thus offer attractive alternatives to murine OKT3 for in vivo therapy.

31/7/2 (Item 2 from file: 155)  
08124424 92262424

\*Humanization\* of an anti-p185HER2 antibody for human cancer therapy.  
Carter P; Presta L; Gorman CM; Ridgway JB; Henner D; Wong WL; Rowland AM;  
Kotts C; Carver ME; Shepard HM  
Department of Protein Engineering, Genentech Inc., South San Francisco,  
CA 94080.

Proc Natl Acad Sci U S A (UNITED STATES) May 15 1992, 89 (10) p4285-9,  
ISSN 0027-8424 Journal Code: PV3

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The murine monoclonal antibody mumAb4D5, directed against human epidermal growth factor receptor 2 (p185HER2), specifically inhibits proliferation of human tumor cells overexpressing p185HER2. However, the efficacy of mumAb4D5 in human cancer therapy is likely to be limited by a human anti-mouse antibody response and lack of effector functions. A "\*humanized\*" antibody, humAb4D5-1, containing only the antigen binding loops from mumAb4D5 and human variable region framework residues plus IgG1 constant domains was constructed. Light- and heavy-chain variable regions were simultaneously \*humanized\* in one step by "gene conversion mutagenesis" using 311-mer and 361-mer preassembled oligonucleotides, respectively. The humAb4D5-1 variant does not block the proliferation of human breast carcinoma SK-BR-3 cells, which overexpress p185HER2, despite tight antigen binding (Kd = 25 nM). One of seven additional \*humanized\* variants designed by molecular modeling (humAb4D5-8) binds the p185HER2 antigen 250-fold and 3-fold more tightly than humAb4D5-1 and mumAb4D5, respectively. In addition, humAb4D5-8 has potency comparable to the murine antibody in blocking SK-BR-3 cell proliferation. Furthermore, humAb4D5-8 is much more efficient in supporting antibody-dependent cellular cytotoxicity against SK-BR-3 cells than mumAb4D5, but it does not efficiently kill WI-38 cells, which express p185HER2 at lower levels.

31/7/3 (Item 3 from file: 155)

08081267 92219267

Antibody framework residues affecting the conformation of the hypervariable loops.

Foote J; Winter G

MRC Laboratory of Molecular Biology, Cambridge, England.

J Mol Biol (ENGLAND) Mar 20 1992, 224 (2) p487-99, ISSN 0022-2836

Journal Code: J6V

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Rodent monoclonal antibodies have been "\*humanized\*" or "reshaped" for therapy by transplanting the antigen-binding loops from their variable domains onto the beta-sheet framework regions of human antibodies. However, additional substitutions in the human framework regions are sometimes required for high affinity antigen binding. Here we describe antigen binding by a reshaped antibody derived from the mouse anti-lysozyme antibody D1.3, and several variants in which point mutations had been introduced into framework positions to improve its affinity. The affinities were determined from the relaxation kinetics of reactant mixtures using quenching of fluorescence that occurs upon formation of the antibody-antigen complex. The dissociation constant of lysozyme ranged from 3.7 nM (for D1.3) to 260 nM. Measurement of antibody-antigen association kinetics using stopped-flow showed that D1.3 and most of the reshaped antibodies had bimolecular rate constants of  $1.4 \times 10^6 \text{ s}^{-1} \text{ M}^{-1}$ , indicating that differences in equilibrium constant were predominantly due to different rates of dissociation of lysozyme from immune complexes. Mutations in a triad of heavy chain residues, 27, 29 and 71, contributed 0.9 kcal/mol in antigen binding free energy, and a Phe to Tyr substitution of light chain residue 71 contributed an additional 0.8 kcal/mol. The combined effect of all these mutations brought the affinity of the reshaped antibody to within a factor of 4 of D1.3. All of these substitutions were in the beta-sheet framework closely underlying the complementarity-determining regions, and do not participate in a direct interaction with antigen. The informed selection of residues in such positions may prove essential for the success of loop transplants in antibodies. Variation of these sites may also have a role in shaping the diversity of structures found in the primary repertoire, and in affinity maturation.

31/7/4 (Item 4 from file: 155)

08010135 92148135

Chimeric and "\*humanized\*" antibodies with specificity for the CD33 antigen.

Co MS; Avdalovic NM; Caron PC; Avdalovic MV; Scheinberg DA; Queen C

Protein Design Labs, Inc., Mountain View, CA 94043.

J Immunol (UNITED STATES) Feb 15 1992, 148 (4) p1149-54, ISSN 0022-1767 Journal Code: IFB

Contract/Grant No.: NIH CA55349

Languages: ENGLISH

Document type: JOURNAL ARTICLE

L and H chain cDNAs of M195, a murine mAb that binds to the CD33 Ag on normal and leukemic myeloid cells, were cloned. The cDNAs were used in the construction of mouse/human IgG1 and IgG3 chimeric antibodies. In addition, "\*humanized\*" antibodies were constructed which combined the complementarity-determining regions of the M195 antibody with human framework and constant regions. The human framework was chosen to maximize homology with the M195 V domain sequence. Moreover, a computer model of M195 was used to identify several framework amino acids that are likely to interact with the complementarity-determining regions, and these residues

were also retained in the \*humanized\* antibodies. Unexpectedly, the \*humanized\* IgG1 and IgG3 M195 antibodies, which have reshaped V regions, have higher apparent binding affinity for the CD33 Ag than the chimeric or mouse antibodies.

31/7/5 (Item 5 from file: 155)

07996790 92134790

Gene conversion of immunoglobulin variable regions in mutagenesis cassettes by replacement PCR mutagenesis.

Near RI

Cellular and Molecular Research Laboratory, Massachusetts General Hospital, Boston 02144.

Biotechniques (UNITED STATES) Jan 1992, 12 (1) p88-97, ISSN 0736-6205  
Journal Code: AN3

Contract/Grant No.: HL-19259

Languages: ENGLISH

Document type: JOURNAL ARTICLE

A technique, Replacement PCR Mutagenesis, was developed to replace one immunoglobulin variable region (V) in a M13 phage cassette with a different, homologous V. This allows the use of the same mutagenesis and subsequent expression vectors for many V regions or V segments. The method combines PCR of V fragments and in vitro mutagenesis. Primers homologous to 3' and 5' ends of both V regions initiate PCR synthesis of the V DNA fragment (donor) that will replace the V region (recipient) in M13. Donor V PCR DNA may originate from mRNA, cloned V genes or genomic templates. The donor V PCR DNA is denatured and annealed to the M13 cassette containing the recipient V to be supplanted. The second strand is synthesized, transfected into bacteria and mutant plaques selected by hybridization. Since restriction sites in primers are not required, altered primer-encoded amino acids are avoided. Further, the PCR donor piece can be of any length if it shares homology with the recipient gene. This allows construction and expression of complete gene replacements and chimeras. This method is also applicable to V "\*humanization\*" and studying sets of homologous genes containing polymorphic or evolutionary disparities. The potential uses of the technique are discussed.

31/7/6 (Item 6 from file: 5)

8779979 BIOSIS Number: 42004979

IMMUNOHISTOCHEMICAL CHARACTERIZATION OF THE \*CDR\*-GRAFTED \*HUMANIZED\* MONOCLONAL \*ANTIBODY\* BW 431-26 HUMAB PRECLINICAL STUDY

MASCHEK W; BOSSLET K

INST. NUCLEARMED., LINZ BEHRING RES. LABS, MARBURG, FRG.

EUROPEAN ASSOCIATION OF NUCLEAR MEDICINE CONGRESS, VIENNA, AUSTRIA, SEPTEMBER 1-5, 1991. EUR J NUCL MED 18 (8). 1991. 546. CODEN: EJNMD

Language: ENGLISH

31/7/7 (Item 7 from file: 5)

8563624 BIOSIS Number: 92028624

POLYMERASE CHAIN REACTION FACILITATES THE CLONING \*CDR\*-GRAFTING AND RAPID EXPRESSION OF A MURINE MONOCLONAL \*ANTIBODY\* DIRECTED AGAINST THE CD18 COMPONENT OF LEUKOCYTE INTEGRINS

DAUGHERTY B L; DEMARTINO J A; LAW M-F; KAWKA D W; SINGER I I; MARK G E

DEP. CELL. MOL. BIOL., MERCK SHARP DOHME RES. LAB., RAHWAY, N.J. 07065, USA.

NUCLEIC ACIDS RES 19 (9). 1991. 2471-2476. CODEN: NARHA

Full Journal Title: Nucleic Acids Research

Language: ENGLISH

Two novel approaches of recombinant PCR technology were employed to graft the complementarity determining regions from a murine monoclonal \*antibody\* (mAb) onto human \*antibody\* frameworks. One approach relied on the

availability of cloned human variable region templates, whereas the other strategy was dependent only on human variable region protein sequence data. The transient expression of recombinant \*humanized\* \*antibody\* was driven by the adenovirus major late promoter and was detected 48 hrs post-transfection into non-lymphoid mammalian cells. The application of these new approaches enables the expression of a recombinant \*humanized\* \*antibody\* just 6 weeks after initiating the cDNA cloning of the murine mAb.

31/7/8 (Item 8 from file: 155)  
08049594 92187594

\*Humanization\* of a mouse monoclonal antibody by CDR-grafting: the importance of framework residues on loop conformation.

Kettleborough CA; Saldanha J; Heath VJ; Morrison CJ; Bendig MM  
Medical Research Council Collaborative Centre, London, UK.

Protein Eng (ENGLAND) Oct 1991, 4 (7) p773-83, ISSN 0269-2139

Journal Code: PR1

Languages: ENGLISH

Document type: JOURNAL ARTICLE

A mouse monoclonal antibody (mAb 425) with therapeutic potential was ' \*humanized\* ' in two ways. Firstly the mouse variable regions from mAb 425 were spliced onto human constant regions to create a chimeric 425 antibody. Secondly, the mouse complementarity-determining regions (CDRs) from mAb 425 were grafted into human variable regions, which were then joined to human constant regions, to create a reshaped human 425 antibody. Using a molecular model of the mouse mAb 425 variable regions, framework residues (FRs) that might be critical for antigen-binding were identified. To test the importance of these residues, nine versions of the reshaped human 425 heavy chain variable (VH) regions and two versions of the reshaped human 425 light chain variable (VL) regions were designed and constructed. The recombinant DNAs coding for the chimeric and reshaped human light and heavy chains were co-expressed transiently in COS cells. In antigen-binding assays and competition-binding assays, the reshaped human antibodies were compared with mouse 425 antibody and to chimeric 425 antibody. The different versions of 425-reshaped human antibody showed a wide range of avidities for antigen, indicating that substitutions at certain positions in the human FRs significantly influenced binding to antigen. Why certain individual FR residues influence antigen-binding is discussed. One version of reshaped human 425 antibody bound to antigen with an avidity approaching that of the mouse 425 antibody.

31/7/9 (Item 9 from file: 155)  
07969093 92107093

\*Humanization\* of monoclonal antibodies.

Gussow D; Seemann G

Methods Enzymol (UNITED STATES) 1991, 203 p99-121, ISSN 0076-6879

Journal Code: MVA

Languages: ENGLISH

Document type: JOURNAL ARTICLE

31/7/10 (Item 10 from file: 155)  
07953750 92091750

Construction, expression and characterization of \*humanized\* antibodies directed against the human alpha/beta T cell receptor.

Shearman CW; Pollock D; White G; Hehir K; Moore GP; Kanzy EJ; Kurrle R  
Genzyme Corporation, Framingham, MA 01701.

J Immunol (UNITED STATES) Dec 15 1991, 147 (12) p4366-73, ISSN 0022-1767 Journal Code: IFB

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Completely \*humanized\* antibodies with specificity for the human alpha/beta TCR have been produced by genetic engineering. The L and H chain V region exons encoding the murine mAb BMA 031 CD regions and human EU framework regions were synthesized and replaced into previously isolated genomic fragments. These fragments were inserted into mammalian expression vectors containing the human kappa and gamma 1 C region exons. Two variants were constructed each containing selected BMA 031 amino acids within the human frameworks. The \*humanized\* genes were transfected into Sp2/0 hybridoma cells by electroporation and transfectomas secreting \*humanized\* antibody were isolated. Levels of antibody expression up to 7 pg/cell/24 h were obtained. The \*humanized\* antibody, BMA 031-EUCIV2, competed poorly with murine BMA 031 for binding to T cells. BMA 031-EUCIV3, however, bound specifically to T cells and competed effectively with both the murine BMA 031 antibody and a previously constructed chimeric BMA 031 antibody for binding to these cells. The relative affinity of BMA 031-EUCIV3 was about 2.5 times lower than BMA 031. The ability to promote antibody dependent cell-mediated cytotoxicity was significantly enhanced with the engineered antibodies as compared to murine BMA 031. \*Humanized\* BMA 031 is a clinically relevant, genetically engineered antibody with potential uses in transplantation, graft vs host disease, and autoimmunity.

31/7/11 (Item 11 from file: 155)  
07909485 92047485

Antigenicity of mouse monoclonal antibodies. A study on the variable region of the heavy chain.

Olsson PG; Hammarstrom L; Smith CI  
Department of Clinical Immunology, Karolinska Institute, Huddinge University Hospital, Sweden.

J Theor Biol (ENGLAND) Jul 7 1991, 151 (1) p111-22, ISSN 0022-5193

Journal Code: K8N

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Mouse monoclonal antibodies (Mabs) against human tumour antigens are currently used in therapy, but up to 50% of the patients receiving treatment form anti-Mab antibodies thus reducing the efficiency of the treatment. One attempt to minimize the immunogenicity of the mouse Mabs is to "\*humanize\*" them by replacing the constant part of the molecule with the human equivalent by genetic engineering. However, this does not reduce the immunogenicity of the variable part of the antibody. Some variable regions may be expected to be less antigenic than others. We therefore compared consensus sequences for the 11 mouse VH families with the human VH sequences published so far. Theoretical antigenicity predictions (hydrophilicity, flexibility, surface accessibility and relative antigenicity) were made and two families; VH I (J558) and VH XI (CP5 B5-3) were predicted to be immunogenic by all four methods. One family, VH X (MRL-DNA4), was not predicted to be immunogenic by any of the four methods. The residues predicted to form antigenic epitopes in the two families VH II (Q52) and VH III (36-60) are predicted not to be exposed on the surface of the antibody molecule and may therefore not be immunogenic.

31/7/12 (Item 12 from file: 5)  
7905670 BIOSIS Number: 40106670

CHIMERIC MOUSE-HUMAN AND \*CDR\*-GRAFTED \*ANTIBODIES\* TO HUMAN IL2 RECEPTOR  
WEIDLE U H; RUSSMANN E; LENZ H; KALUZA B

BOEHRINGER MANNHEIM GMBH, NONNENWALD 2, D-8122 PENZBERG, FRG.

MEETING ON MOLECULAR BIOLOGY AND THE IMMUNOPATHOGENESIS OF RHEUMATOID ARTHRITIS HELD AT THE 20TH ANNUAL MEETING OF THE KEYSTONE SYMPOSIA ON MOLECULAR AND CELLULAR BIOLOGY, LAKE TAHOE, CALIFORNIA, USA, MARCH 15-21, 1991. J CELL BIOCHEM SUPPL 15 (PART E). 1991. 186. CODEN: JCBSD

Language: ENGLISH

QH506. J67

31/7/13 (Item 13 from file: 155)  
07899816 92037816

A \*humanized\* monovalent CD3 antibody which can activate homologous complement.

Routledge EG; Lloyd I; Gorman SD; Clark M; Waldmann H  
Department of Pathology, Cambridge University.

Eur J Immunol (GERMANY) Nov 1991, 21 (11) p2717-25, ISSN 0014-2980

Journal Code: EN5

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The rat monoclonal antibody (mAb) YTH12.5, specific for the CD3 antigen complex on human T cells has been modified in order to improve its efficacy in human therapy. With the aim of rendering it less immunogenic, it has been \*humanized\* using the method of framework grafting. During this process sequence analysis of the YTH12.5 VL gene indicated that it was of the lambda subclass, however, it was markedly dissimilar from previously published rat and mouse V lambda gene sequences and may represent a new V lambda gene family. The \*humanization\* of this light chain represents the first successful reshaping of a lambda light chain V region. To improve the effector function of the antibody we have created a monovalent form (1 Fab, 1 Fc) using a novel method involving the introduction of an N-terminally truncated human IgG1 heavy chain gene into cells producing the \*humanized\* CD3 mAb. Comparison of the mono- and bivalent \*humanized\* mAb in a complement-mediated cell lysis assay revealed that the monovalent antibody mediated lysis of human T cell blasts whereas the bivalent form did not. The availability of a \*humanized\*, complement-fixing CD3 mAb may improve opportunities for human therapy, in the management of organ rejection, autoimmunity and the treatment of T cell lymphoma.

31/7/14 (Item 14 from file: 155)  
07768736 91287736

A possible procedure for reducing the immunogenicity of antibody variable domains while preserving their ligand-binding properties.

Padlan EA

Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892.

Mol Immunol Apr-May 1991, 28 (4-5) p489-98, ISSN 0161-5890

Journal Code: NG1

Languages: ENGLISH

Document type: JOURNAL ARTICLE

It is proposed to reduce the immunogenicity of allogeneic antibody variable domains, while preserving ligand-binding properties, by reducing their antigenicity through replacement of the exposed residues in the framework regions which differ from those usually found in host antibodies. The results of a comparison of representative murine antibody sequences with those of human origin suggest that the number of residues that need to be replaced to "\*humanize\*" those antibodies could be small.

31/7/15 (Item 15 from file: 155)  
07757287 91276287

Immunoglobulin complementarity-determining region grafting by recombinant polymerase chain reaction to generate \*humanised\* monoclonal antibodies.

Lewis AP; Crowe JS

Department of Cell Biology, Wellcome Research Laboratories, Beckenham, Kent, U.K.

Gene May 30 1991, 101 (2) p297-302, ISSN 0378-1119 Journal Code:

FOF

Languages: ENGLISH

*Get this*

*QR180.I52*

*Get this*

*QH 442.B43*

Document type: JOURNAL ARTICLE

We describe an approach to rapidly generate \*humanised\* monoclonal antibodies by grafting rodent complementarity-determining regions onto human immunoglobulin frameworks using recombinant polymerase chain reaction (PCR) methodology. The approach was applied to grafting a rat complementarity-determining region onto a human framework and amplifying the entire \*humanised\* heavy chain. The terminal oligodeoxyribonucleotide primers incorporated restriction sites to allow forced cloning into plasmid vectors for sequencing and expression. No nucleotide errors were introduced into the 1463-bp sequence even after sequential applications of PCR.

31/7/16 (Item 16 from file: 155)

07668893 91187893

\*Humanized\* antibodies for antiviral therapy.

Co MS; Deschamps M; Whitley RJ; Queen C  
Protein Design Labs, Inc., Mountain View, CA 94043.

Proc Natl Acad Sci U S A Apr 1 1991, 88 (7) p2869-73, ISSN 0027-8424

Journal Code: PV3

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Antibody therapy holds great promise for the treatment of cancer, autoimmune disorders, and viral infections. Murine monoclonal antibodies are relatively easy to produce but are severely restricted for therapeutic use by their immunogenicity in humans. Production of human monoclonal antibodies has been problematic. \*Humanized\* antibodies can be generated by introducing the six hypervariable regions from the heavy and light chains of a murine antibody into a human framework sequence and combining it with human constant regions. We \*humanized\*, with the aid of computer modeling, two murine monoclonal antibodies against herpes simplex virus gB and gD glycoproteins. The binding, virus neutralization, and cell protection results all indicate that both \*humanized\* antibodies have retained the binding activities and the biological properties of the murine monoclonal antibodies.

31/7/17 (Item 17 from file: 399)

117024688 CA: 117(3)24688r PATENT

Humanized complementarity-determining region (CDR)-grafted antibodies to intercellular adhesion molecule-1 (ICAM-1), methods of preparation and usage thereof

INVENTOR(AUTHOR): Adair, John Robert; Athwal, Diljeet Singh; Rothlein, Robert A.

LOCATION: UK,

ASSIGNEE: Celltech Ltd.; Boehringer Ingelheim Pharmaceuticals, Inc.

PATENT: PCT International ; WO 9116927 A1 DATE: 911114

APPLICATION: WO 91US2942 (910429) \*GB 909549 (900427)

PAGES: 81 pp. CODEN: PIXXD2 LANGUAGE: English CLASS: A61K-039/395A;

C07K-015/28B DESIGNATED COUNTRIES: AT; AU; BB; BG; BR; CA; CH; DE; DK; ES; FI; GB; HU; JP; KP; KR; LK; LU; MC; MG; MW; NL; NO; PL; RO; SD; SE; SU; US

DESIGNATED REGIONAL: AT; BE; BF; BJ; CF; CG; CH; CM; DE; DK; ES; FR; GA; GB; GR; IT; LU; ML; MR; NL; SE; SN; TD; TG

SECTION:

CA215003 Immunochemistry

CA201XXX Pharmacology

CA203XXX Biochemical Genetics

IDENTIFIERS: humanized antibody intercellular adhesion mol 1, inflammation inhibitor humanized antibody ICAM1, asthma inhibitor humanized antibody ICAM1, AIDS virus humanized antibody ICAM1, virucide humanized antibody ICAM1, diagnosis humanized antibody ICAM1

DESCRIPTORS:

Dermatitis...



acute, treatment of, with humanized antibody to intercellular adhesion mol.-1

Immunosuppressants...  
and humanized antibody to intercellular adhesion mol.-1, pharmaceutical compn. contg.

Rodent...  
anti-intercellular adhesion mol.-1 antibody variable region complementary detg. region of, in humanized antibody prodn.

Integrins, antigens LFA-1...  
antibody to, and humanized antibody to intercellular adhesion mol.-1, for inflammation treatment

Neoplasm inhibitors, metastasis...  
chimeric antibody to intercellular adhesion mol.-1, for hemopoietic cell tumors

Toxicity...  
cytokine-induced, inflammation of, treatment of, humanized antibody to intercellular adhesion mol.-1 for

Inflammation...  
diagnosis of, with chimeric antibody binding to cell expressing intercellular adhesion mol.-1

Deoxyribonucleic acids...  
for antibody heavy and light chains, in humanized antibody to intercellular adhesion mol.-1 prodn.

Deoxyribonucleic acid sequences...  
for monoclonal antibody R6-5-D6 heavy and light chain components for humanized antiintercellular adhesion mol.-1 antibody

Leukocyte...  
human immunodeficiency virus infection of, inhibition of, with humanized antibody to intercellular adhesion mol.-1

Bronchodilators, antiasthmatics... Inflammation inhibitors... Inflammation inhibitors, antirheumatics... Therapeutics... Virucides and Virustats...  
humanized antibody to intercellular adhesion mol.-1

Toxins...  
humanized antibody to intercellular adhesion mol.-1 derivatized with, for inhibition of intercellular adhesion mol.-1-expressing tumor cell

Diagnosis...  
humanized antibody to intercellular adhesion mol.-1 for

Inflammation inhibitors, antiarthritics...  
humanized antibody to intercellular adhesion mol.-1, for reaction arthritis

Glycoproteins, specific or class, ICAM-1 (intercellular adhesion mol. 1)...  
humanized recombinant antibody to

Antibodies...  
humanized recombinant, to intercellular adhesion mol.-1

Thyroid gland, disease, autoimmune thyroiditis...  
inflammation in, treatment of, with humanized antibody to intercellular adhesion mol.-1

Nervous system, central...  
inflammation of, treatment of, humanized antibody to intercellular adhesion mol.-1 for

Autoimmune disease... Blood vessel, disease, Raynaud's phenomenon...  
Brain, disease, stroke... Dialysis, hemo-... Encephalomyelitis...  
Intestine, disease, Crohn's... Intestine, disease, pseudomembranous enterocolitis... Intestine, disease, ulcerative colitis... Kidney, disease, acute glomerulonephritis... Leukapheresis... Lupus erythematosus...  
Multiple sclerosis... Psoriasis... Respiratory distress syndrome, adult...  
inflammation of, treatment of, with humanized antibody to intercellular adhesion mol.-1

Neoplasm, composition...  
intercellular adhesion mol.-1-expressing, diagnosis of, with humanized

antibody to intercellular adhesion mol.-1  
Mouse...  
monoclonal antibody R6-5-D6 of, in humanized antibody to intercellular adhesion mol.-1 prodn.  
Sepsis and Septicemia...  
multiple organ injury syndrome secondary to, inflammation of, treatment of, humanized antibody to intercellular adhesion mol.-1 for  
Protein sequences...  
of monoclonal antibody R6-5-D6 heavy and light chain components for humanized antiintercellular adhesion mol.-1 antibody  
Plasmid and Episome...  
pAL5, in grafted humanized antibody to intercellular adhesion mol.-1 prodn.  
Plasmid and Episome...  
pAL6, in grafted humanized antibody to intercellular adhesion mol.-1 prodn.  
Plasmid and Episome...  
pBJ1, in grafted humanized antibody to intercellular adhesion mol.-1 prodn.  
Kidney,transplant... Organ,transplant... Transplant and Transplantation...  
rejection of, inflammation of, treatment of, humanized antibody to intercellular adhesion mol.-1 for  
Antibodies,monoclonal...  
R6-5-D6, of mouse, in humanized antibody to intercellular adhesion mol.-1 prodn.  
Organ,disease, multiple organ failure...  
secondary to septicemia or trauma, treatment of, humanized antibody to intercellular adhesion mol.-1 for  
Temperature effects,biological...  
thermal injury, inflammation of, treatment of, humanized antibody to intercellular adhesion mol.-1 for  
Perfusion,re-...  
tissue injury from, treatment of, humanized antibody to intercellular adhesion mol.-1 for  
Lymphokines and Cytokines...  
toxicity induced by, inflammation of, treatment of, humanized antibody to intercellular adhesion mol.-1 for  
Neoplasm inhibitors...  
toxin-derivatized humanized antibody to intercellular adhesion mol.-1, for intercellular adhesion mol.-1-expressing tumor cell  
Leukocyte,granulocyte...  
transfusion-assocd. syndrome, treatment of, humanized antibody to intercellular adhesion mol.-1 for  
Allergy,delayed hypersensitivity...  
treatment of, humanized antibody to intercellular adhesion mol.-1 for  
Picornaviridae... Virus,animal, Coxsackie A... Virus,animal, human immunodeficiency... Virus,animal, human immunodeficiency 1... Virus,animal, Mengo... Virus,animal, rhino-...  
treatment of infection with, with humanized antibody to intercellular adhesion mol.-1  
Hematopoietic precursor cell...  
tumorous, metastasis of, inhibition of, chimeric antibody to intercellular adhesion mol.-1  
Genetic vectors...  
with DNA for antibody heavy and light chains, in humanized antibody to intercellular adhesion mol.-1 prodn.

CAS REGISTRY NUMBERS:

142007-78-1 142007-79-2 142007-80-5 142007-81-6 142007-82-7  
142007-83-8 142007-85-0 amino acid sequence of  
142007-84-9 amino acid sequence of, humanized antibody to intercellular

adhesion mol.-1 in relation to  
140876-28-4 140876-29-5 142007-86-1 142007-87-2 amino acid sequence of,  
humanized antibody to intercellular adhesion mol.-1 prodn. in relation  
to  
140857-88-1 142008-94-4 nucleotide sequence of, humanized antibody to  
intercellular adhesion mol.-1 prodn. in relation to  
140857-89-2 142008-93-3 nucleotide sequence of, humanized antibody to  
intercellular adhesion mol.01 prodn. in relation to  
Copyright 1992 by the American Chemical Society

31/7/18 (Item 18 from file: 155)  
07449972 90356972

Immunoglobulin V regions of a bactericidal anti-Neisseria meningitidis  
outer membrane protein monoclonal antibody.

Larrick JW; Coloma MJ; del Valle J; Fernandez ME; Fry KE;  
Gavilondo-Cowley JV

Genelabs Inc., Redwood City, California.

Scand J Immunol Aug 1990, 32 (2) p121-8, ISSN 0300-9475

Journal Code: UCW

Languages: ENGLISH

Document type: JOURNAL ARTICLE

C6 is a potentially therapeutic murine monoclonal antibody that  
recognizes the class 1 outer membrane protein of Neisseria meningitidis. C6  
specifically immunoblots this antigen and augments in vitro killing of N.  
meningitidis bacteria. We describe a general method of obtaining the heavy  
and light chain variable-region sequence from immunoglobulin-secreting  
cells. The method uses mixed polymerase chain reaction (PCR) primers  
designed from the 5' end of the framework 1 (FR1) sequences of the heavy  
and light chains, and 3'-end primers for constant-region conserved  
sequences. The method has been applied to the cloning and sequencing of the  
variable region of C6 to construct a \*humanized\* monoclonal antibody. Rapid  
amplification and sequencing of variable regions by this general method  
have multiple applications in the study of the immune response to  
infectious diseases.

31/7/19 (Item 19 from file: 155)  
07292738 90199738

Cloning of the genes for T84.66, an antibody that has a high specificity  
and affinity for carcinoembryonic antigen, and expression of chimeric  
human/mouse T84.66 genes in myeloma and Chinese hamster ovary cells.

Neumaier M; Shively L; Chen FS; Gaida FJ; Ilgen C; Paxton RJ; Shively JE;  
Riggs AD

Division of Biology, Beckman Research Institute of the City of Hope,  
Duarte, California 91010.

Cancer Res Apr 1 1990, 50 (7) p2128-34, ISSN 0008-5472

Journal Code: CNF

Contract/Grant No.: CA 43904

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Carcinoembryonic antigen (CEA) is one of the best characterized  
tumor-associated antigens and is extensively used in the in vitro  
immunodiagnosis of human colon adenocarcinomas. Among a number of anti-CEA  
monoclonal antibodies, the murine monoclonal antibody T84.66 shows the  
highest specificity and affinity for CEA and has been used successfully for  
in vivo tumor imaging in mice and humans. We report here the cloning and  
sequencing of the genes coding for monoclonal antibody T84.66 and the amino  
acid sequence of the variable regions for the heavy and light chains. We  
also report the construction of mouse/human chimeric IgG1 antibody genes  
using T84.66 variable region genes and human constant region genes. The  
resulting chimeric gene constructs were transfected into murine myeloma

cells (Sp2/0) by electroporation and into Chinese hamster ovary cells by lipofection. The chimeric antibodies obtained exhibited the same specificity and affinity for CEA as that of the T84.66 immunoglobulin produced by the murine hybridoma cell line. Antibody concentrations in culture medium supernatants were clonally variable but similar (15-480 ng/ml) for both Sp2/0 and Chinese hamster ovary transfectants; the average production by Chinese hamster ovary transfectants was only 3-5-fold less than Sp2/0 transfectants. Ascites production of Sp2/0 transfectants is sufficiently high (900 micrograms/ml) for initial in vivo studies with \*humanized\* T84.66.

31/7/20 (Item 20 from file: 155)  
07192290 90099290

A \*humanized\* antibody that binds to the interleukin 2 receptor.  
Queen C; Schneider WP; Selick HE; Payne PW; Landolfi NF; Duncan JF;  
Avdalic NM; Levitt M; Junghans RP; Waldmann TA  
Protein Design Labs, Palo Alto, CA 94304.  
Proc Natl Acad Sci U S A Dec 1989, 86 (24) p10029-33, ISSN 0027-8424  
Journal Code: PV3  
Languages: ENGLISH  
Document type: JOURNAL ARTICLE

*Get this.*

The anti-Tac monoclonal antibody is known to bind to the p55 chain of the human interleukin 2 receptor and to inhibit proliferation of T cells by blocking interleukin 2 binding. However, use of anti-Tac as an immunosuppressant drug would be impaired by the human immune response against this murine antibody. We have therefore constructed a "\*humanized\*" antibody by combining the complementarity-determining regions (CDRs) of the anti-Tac antibody with human framework and constant regions. The human framework regions were chosen to maximize homology with the anti-Tac antibody sequence. In addition, a computer model of murine anti-Tac was used to identify several amino acids which, while outside the CDRs, are likely to interact with the CDRs or antigen. These mouse amino acids were also retained in the \*humanized\* antibody. The \*humanized\* anti-Tac antibody has an affinity for p55 of  $3 \times 10^9$  M<sup>-1</sup>, about 1/3 that of murine anti-Tac.

31/7/21 (Item 21 from file: 155)  
06533056 88178056

Reshaping human antibodies: grafting an antilysozyme activity.  
Verhoeyen M; Milstein C; Winter G  
Medical Research Council Laboratory of Molecular Biology, Cambridge,  
England.  
Science Mar 25 1988, 239 (4847) p1534-6, ISSN 0036-8075  
Journal Code: UJ7  
Languages: ENGLISH  
Document type: JOURNAL ARTICLE

The production of therapeutic human monoclonal antibodies by hybridoma technology has proved difficult, and this has prompted the "\*humanizing\*" of mouse monoclonal antibodies by recombinant DNA techniques. It was shown previously that the binding site for a small hapten could be grafted from the heavy-chain variable domain of a mouse antibody to that of a human myeloma protein by transplanting the hypervariable loops. It is now shown that a large binding site for a protein antigen (lysozyme) can also be transplanted from mouse to human heavy chain. The success of such constructions may be facilitated by an induced-fit mechanism.

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File 351:Derwent World Patents Index Latest  
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\*\*FILE351: Formats 32,33,35,37 & 39 display the new 'Expanded' Patent Family table for UD=9216 and greater. For more info. type ?NEWS351

File 350:Derwent World Patents Index  
1963-1980, EQUIVALENTS THRU DW=9227

\*\*FILE350: Formats 32,33,35,37 & 39 display the new 'Expanded' Patent Family table for UD=9219 and greater. For more info. type ?NEWS350

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>>>"S4" does not exist

S2 0 S4

S3 1 HUMANIZ?

S4 26 HUMANIS?

S5 0 S4 AND (HUMANIZ? OR HUMANIS?)

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>>>File 399 is not open

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>>>File 399 is not open

>>>No valid files specified in FROM

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>>>Set "S28" does not exist

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S6 13936 ANTIBOD?

1 S3

26 S4

S7 22 ANTIBOD? AND (S3 OR S4)

?ss cdr or (ig or immunoglobulin)()variable()region or (complementary())determing

Processing

Processing

S8 31 CDR

S9 786 IG

S10 1576 IMMUNOGLOBULIN  
 S11 108404 VARIABLE  
 S12 108131 REGION  
 S13 4 (IG OR IMMUNOGLOBULIN) (W) VARIABLE (W) REGION  
 S14 23564 COMPLEMENTARY  
 S15 501 DETERMING  
 S16 0 COMPLEMENTARY (W) DETERMING  
 S17 23 HYPERVARIABLE  
 S18 108131 REGION  
 S19 12 (COMPLEMENTARY (W) DETERMING OR HYPERVARIABLE) (W) REGION  
 S20 11218 ANTIBODY  
 S21 43127 RELATED  
 S22 28329 BINDING  
 S23 29492 SITE? ?  
 S24 0 ANTIBODY (W) RELATED (W) BINDING (W) SITE? ?  
 S25 45 CDR OR (IG OR IMMUNOGLOBULIN) () VARIABLE () REGION OR  
 (COMPLEMENTARY () DETERMING OR HYPERVARIABLE) () REGION OR  
 ANTIBODY () RELATED () BINDING () SITE? ?

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22 7  
 45 25

~~S26 8 7 AND 25~~

?t26/7/1-8

26/7/1 (Item 1 from file: 351)  
 009040436 WPI Acc No: 92-167794/21  
 XRAM Acc No: C92-077239

New \*humanised\* \*antibody\* specific for interleukin-2 receptor - with complementarity determin. regions and framework from different immunoglobulin(s), is non immunogenic and used to treat T-cell

Patent Assignee: (PROT-) PROTEIN DESIGN LABS INC

Author (Inventor): QUEEN C L; SELICK H E

Number of Patents: 001

Number of Countries: 001

Patent Family:

| CC Number | Kind | Date   | Week |         |
|-----------|------|--------|------|---------|
| DD 296964 | A5   | 911219 | 9221 | (Basic) |

Priority Data (CC No Date): DD 337159 (900117)

Abstract (Basic): DD 296964 A

Compsn. comprises a practically pure human-type immunoglobulin (Ig) that reacts specifically with p55-Tac protein and/or inhibits binding of human interleukin-2 (IL-2) to its specific receptor.

Also new are (1) human-type Ig having 2 pairs of light chain/heavy chain dimers and able to react specifically with an epitope of human IL-2 receptor with affinity at least  $10^8$  M<sup>-1</sup>, in which the complementarity determining regions (\*CDR\*) and human-type framework regions are from different Ig molecules; (2) \*humanised\* Ig able to bind to IL-2 receptors with one or more \*CDR\* from anti-Tac \*antibody\* in a human framework, where the framework includes at least one amino acid (AA) from anti-Tac; (3) nucleic acid encoding a human Ig framework and murine \*CDR\* which, when expressed, produces an Ig specifically reactive with p55-Tac protein and can block binding of IL-2 to its receptor; (4) cells transformed with this nucleic acid.

USE/ADVANTAGES - These Ig are used to treat humans with T-cell related diseases (e.g. transplant rejection; T-cell leukaemia or autoimmune diseases such as diabetes, multiple sclerosis, etc.). They are specific for the IL-2 receptors; are engineered to be

non-immunising and can be produced by recombinant DNA method. The new Ig are admin. in usual parenteral formulation e.g. in doses of 150 mg for therapy or 0.5-2.5 mg for prophylaxis. Ig can also be used, opt. labelled, for diagnosis; T-cell typing; specific receptor isolation or vaccine prodn. 0/10

Derwent Class: B04; D16;

Int Pat Class: A61K-039/395; C12N-015/13

26/7/2 (Item 2 from file: 351)

009039793 WPI Acc No: 92-167155/20

XRAM Acc No: C92-076891

Prepn. of chimeric \*humanised\* \*antibodies\* - using a new polymerase chain reaction technique; PCR

Patent Assignee: (WELL ) WELLCOME FOUND LTD

Author (Inventor): CROWE J S; LEWIS A P

Number of Patents: 001

Number of Countries: 015

Patent Family:

| CC Number  | Kind | Date   | Week |         |
|------------|------|--------|------|---------|
| WO 9207075 | A1   | 920430 | 9220 | (Basic) |

Priority Data (CC No Date): GB 9022011 (901010)

Applications (CC,No,Date): WO 91GB1744 (911008)

Language: English

EP and/or WO Cited Patents: 4.Jnl.Ref; WO 9007861

Designated States

(National): JP; US

(Regional): AT; BE; CH; DE; DK; ES; FR; GB; GR; IT; LU; NL; SE

Abstract (Basic): WO 9207075 A

Prodn. of ds or ss DNA of formula: 5' F1-M-F2 3' encoding an \*antibody\* (Ab) chain or fragment in which at least one of the complementarily determining regions (CDRs) of the variable region is derived from a first mammalian Ab and the framework of the variable region is derived from a second different mammalian Ab, where M is DNA encoding a \*CDR\* of the second Ab and F1 and F2 resp. encode 5' and 3' sequences flanking M, by: (a) prepg. a ss or ds DNA template of formula: 5' f1-H-f2 3' where H is DNA encoding a \*CDR\* of a different specificity from M, and f1 and f2 are homologous to F1 and F2, resp.; (b) obtaining DNA oligonucleotide primers A, B, C and D, where: A comprises the sequence a1 with a 5' end corresp. to the 5' end of F1 and which is identical to the corresp. length of F1 and is oriented in a 5' to 3' direction towards H; B has of the sequence 5' b1-b2 3', where b1 comprises a sequence complementary to a corresp. length of M and has a 3' end complementary to the 5' end of M, and b2 is complementary to a sequence of corresp. length in F1 and has a 5' end which starts at the nucleotide complementary to the 3' end of F1, C has of the sequence 5' c1-c2 3' where c1 comprises a sequence identical to the corresp. length of M and has a 3' end corresp. to the 3' end of M, and c2 is identical to a sequence of corresp. length in F2 and has a 5' end which starts at the nucleotide corresp. to the 5' end of F2, and D comprises a sequence d1 which has a 5' end complementary to the 3' end of F2 and which is complementary to a corresp. length of F2 and is oriented in a 5' to 3' direction towards H, where b1 and c1 overlap by a sufficient length to permit annealing of their 5' ends under conditions which allow PCR to be performed; (c) performing, in any desired order, PCR reactions with primer pairs A, B and C, D on the template prepd. in (a), and (d) mixing the prods. of (c) and performing PCR using primers A and D.

USE/ADVANTAGE - The method allows the prepn. of chimeric, esp. \*humanised\* Abs. The resulting Ab retains the antigen binding

capability of the non-human Ab from which the \*CDR\*(s) are derived.

0/4

Derwent Class: B04; D16;

Int Pat Class: C12N-005/10; C12N-015/12; C12N-015/69; C12P-021/08

26/7/3 (Item 3 from file: 351)

008937440 WPI Acc No: 92-064709/08

XRAM Acc No: C92-029621

New multivalent anti-cytokine immunoglobulins - for treating disorders associated with elevated cytokine levels, e.g. septic and endotoxic shock, AIDS, allergies, etc.; ACQUIRE IMMUNE DEFICIENT SYNDROME

Patent Assignee: (CLLT ) CELLTECH LTD; (CELL-) CELLTECH LTD

Author (Inventor): ALLEN R A; MORGAN S A

Number of Patents: 002

Number of Countries: 035

Patent Family:

| CC Number  | Kind | Date   | Week |         |
|------------|------|--------|------|---------|
| WO 9201472 | A    | 920206 | 9208 | (Basic) |
| AU 9182381 | A    | 920218 | 9222 |         |

Priority Data (CC No Date): GB 9015908 (900719)

Applications (CC,No,Date): AU 9182381 (910719); WO 91GB1216 (910719)

Language: English

EP and/or WO Cited Patents: 2.Jnl.Ref; EP 347057; EP 355067; WO 9006371; WO 9007118; WO 9106305

Designated States

(National): AT; AU; BB; BG; BR; CA; CH; CS; DE; DK; ES; FI; GB; HU; JP; KR; ; KR; LK; LU; MC; MG; MW; NL; NO; PL; RO; SD; SE; SU; US

(Regional): AT; BE; CH; DE; DK; ES; FR; GB; GR; IT; LU; NL; OA; SE

Filing Details: AU9182381 Based on WO 9201472

Abstract (Basic): WO 9201472

New multivalent immunoglobulin (I) has at least 3 linked antigen-binding domains (ABD's) each being specific for a complementary site on a cytokine.

The combining interactions between ABD and cytokine sites are neutralising. (I) is specific for tumour necrosis factor (TNF) alpha or beta; an interleukin, an interferon or a colony-stimulating factor, and it contains 4-20 ABD.

ABD are all of class IgG (most pref.) or all of class IgM (but must be different from a native IgM molecule) and can be linked by covalent crosslinking (e.g. 2-iminothiolane/ maleimide system) or by non-covalent interaction (e.g. using an \*antibody\* reactive with sites on Ig other than those involved in antigen binding; or the biotin-avidin system). (I) are made by joining together appropriate immunoglobulin molecules or fragments esp \*CDR\*-grafted or \*humanised\* chimaeric Ig. USE/ADVANTAGE- (I) are used to treat or prevent diseases associated with elevated cytokine levels, e.g. immuno regulatory and inflammatory disease, sepsis, endotoxic or cardiovascular shock, AIDS, psoriasis, organ transplant rejection or excessive TNF generation induced cancer therapy etc., Compared with monomeric Ig, (I) have much greater neutralising activity. @(43pp)@

Derwent Class: B04; D16;

Int Pat Class: A61K-039/39; A61K-039/395; C07K-015/28; C12P-021/08

26/7/4 (Item 4 from file: 351)

008929605 WPI Acc No: 92-056874/07

Related WPI Accession(s): 91-222915

XRAM Acc No: C92-025713

New \*cdr\*-grafted anti carcinoembryonic antigen \*antibodies\* - useful in therapy and diagnosis of carcinoma



Patent Assignee: (CELL-) CELLTECH LTD

Author (Inventor): ADAIR J R; BODMER M W; MOUNTAIN A; OWENS R J

Number of Patents: 001

Patent Family:

| CC Number  | Kind | Date   | Week         |
|------------|------|--------|--------------|
| WO 9201059 | A    | 920123 | 9207 (Basic) |

Priority Data (CC No Date): WO 91GB1108 (910705); GB 9014932 (900705); WO 90GB2017 (901221)

Language: English

EP and/or WO Cited Patents: WO 8910140; WO 8901783; EP 323806; 6.Jnl.REF

Designated States

(National): AT; AU; BB; BG; BR; CA; CH; CS; DE; DK; ES; FI; GB; HU; JP; KR ; KR; LK; LU; MC; MG; MW; NL; NO; PL; RO; SD; SE; SU; US

(Regional): AT; BE; CH; DE; DK; ES; FR; GB; GR; IT; LU; NL; SE; OA

Abstract (Basic): WO 9201059

New \*humanised\* \*antibody\* molecule (HAM) is specific for carcino-embryonic antigen (CEA) and has an antigen binding site in which at least one of the complementarity determining regions (\*CDR\*'s) of the variable domain is derived from the mouse monoclonal \*antibody\* (Mab) A5B7. The remaining Ig-derived parts of HAM are of human origin.

HAM is a chimeric or \*CDR\*-grafted \*humanised\* \*antibody\*, prepd. by recombinant DNA techniques. It can be a complete \*antibody\* or an Fab, Fab', (Fab')<sub>2</sub> or Fv fragment, or a single-chain fragment. It may have a reporter or effector molecule attached to it.

USE/ADVANTAGE - HAM are useful in therapy or diagnosis (including imaging) of carcinomas which produce CEA, e.g., when coupled to a toxin such as ricin. @ (70pp Dwg.No.0/19)

Derwent Class: B04; D16;

Int Pat Class: A61K-039/39; C07K-015/28; C12N-015/13; C12P-021/08

26/7/5 (Item 5 from file: 351)

008849515 WPI Acc No: 91-353533/48

XRAM Acc No: C91-152448

New \*humanised\* \*CDR\*-grafted anti-ICAM \*antibodies\* - used to treat and prevent inflammation (e.g. psoriasis) tumours, viral infections and asthma and in diagnosis; INTER CELLULAR ADHESIVE MOLECULAR

Patent Assignee: (CELL-) CELLTECH LTD; (BOEH ) BOEHRINGER INGELHEIM PHA

Author (Inventor): ADAIR J R; ATHWAL D S; ROTHLEIN R A

Number of Patents: 002

Patent Family:

| CC Number  | Kind | Date   | Week         |
|------------|------|--------|--------------|
| WO 9116927 | A    | 911114 | 9148 (Basic) |
| AU 9179001 | A    | 911127 | 9210         |

Priority Data (CC No Date): GB 909549 (900427)

Applications (CC,No,Date): WO 91US2942 (910429)

Language: English

EP and/or WO Cited Patents: US 4816567; WO 8901783; 7.Jnl.REF

Designated States

(National): AT; AU; BB; BG; BR; CA; CH; DE; DK; ES; FI; GB; HU; JP; KR; KR ; LK; MC; MG; MW; NL; NO; PL; RO; SD; SE; SU; US

(Regional): AT; BE; CH; DE; DK; ES; FR; GB; GR; IT; LU; NL; SE; OA

Abstract (Basic): WO 9116927

A recombinant \*antibody\* molecule comprising antigen binding regions derived from the heavy and/or light chain variable regions of an anti-intracellular adhesion molecule-1 (anti-ICAM-1) \*antibody\* is claimed. The Ab is \*CDR\*-grafted and comprises several non-human residues. Also claimed are DNA encoding an Ab heavy or light chain, a vector comprising the DNA, host cells transformed with the vector and a method for producing the anti-ICAM-1 grafted Ab.

USE/ADVANTAGE - The Abs are used to treat - and prevent

inflammation in e.g. delayed type hypersensitivity, psoriasis, an autoimmune disease e.g. Reynaud's syndrome, autoimmune thyroiditis, EAE, multiple sclerosis, rheumatoid arthritis and lupus erythematosus, tissue or organ transplant or graft rejection. They are also used to treat and prevent tumours, viral infections (e.g. rhinoviruses of the major serotype within the genus Picornaviridae, group A coxsackievirus, a Mengo virus and HIV); asthma and non-specific defence system response, e.g. adult respiratory distress syndrome, CNS inflammatory disorder, multiple organ injury syndrome secondary to septicaemia or trauma, ulcerative colitis and Crohn's disease. Administration can be enteral, parenteral, topical, intranasal or by inhalation. The Abs are also used to diagnose an ICAM-1-expressing tumour cell and inflammation. @ (68pp Dwg.No.0/4

Derwent Class: B04; D16;  
Int Pat Class: A61K-039/39; C07K-015/28

26/7/6 (Item 6 from file: 351)  
008718897 WPI Acc No: 91-222916/30  
XRAM Acc No: C91-096865

CD3 specific \*humanised\* recombinant \*antibody\* - is chimeric or \*cdr\* grafted for immunotherapy and diagnosis; COMPLEMENTARY DETERMINE REGION

Patent Assignee: (CELL-) CELLTECH LTD

Author (Inventor): JOLLIFFE L K; ZIVIN R A; ADAIR J R; ATHWAL D S

Number of Patents: 003

Patent Family:

| CC Number  | Kind | Date   | Week |         |
|------------|------|--------|------|---------|
| WO 9109968 | A    | 910711 | 9130 | (Basic) |
| AU 9170330 | A    | 910724 | 9143 |         |
| GB 2246781 | A    | 920212 | 9207 |         |

Priority Data (CC No Date): WO 90GB2018 (901221); GB 8928874 (891221); GB 9117611 (910815)

Applications (CC, No, Date): GB 9017611 (901221)

Language: English

EP and/or WO Cited Patents: EP 403156; EP 328404

Designated States

(National): AT; AU; BB; BG; BR; CA; CH; DE; DK; ES; FI; GB; GR; HU; JP; KR ; LK; LU; MC; MG; MW; NL; NO; RO; SD; SE; SU; US

(Regional): AT; BE; CH; DE; DK; ES; FR; GB; GR; IT; LU; NL; SE; OA

Filing Details: GB2246781 Based on WO9109968 (E) (1251CH)

Abstract (Basic): WO 9109968

A recombinant \*antibody\* (RAM) comprising antigen binding regions derived from the heavy and or light chain variable regions of a donor anti-CD3 \*antibody\*. The \*antibody\* preferably has binding affinity similar to that of OKT3. The RAM comprises antigen binding regions from suitable anti-CD3 \*antibodies\* such as rodent e.g. mouse or rat anti-CD3 MAb. The RAM may comprise only the variable region (VH and/or VL) or one or more CDRs of such a MAb.

The RAM is preferably a \*humanised\* \*antibody\* molecule specific for CD3 having an antigen binding site where at least one of the CDRs of the variable domain and usually two more of the CDRs are derived from non human anti-CD3 \*antibody\*. The RAM may be a chimeric or \*CDR\* grafted \*antibody\*. Usually, the donor and acceptor \*antibodies\* are derived from different species. Typically the donor anti CD3 \*antibody\* is non-human (e.g. rodent) and the acceptor \*antibody\* is human. A \*CDR\* grafted \*antibody\* heavy chain comprising variable region with acceptor and donor CD3 binding comprising donor residues at one or more of positions 6, 37, 48 and 94. The \*CDR\* grafted light chain is also claimed.

DNA coding these \*antibodies\* and their production by recombinant DNA technology is claimed.

USE/ADVANTAGE - The \*antibodies\* may be used for treatment or diagnosis of human or veterinary conditions. The \*humanised\* \*antibodies\* do not have the immunologic complications associated with administration of non human \*antibodies\* to human subjects. @ (81pp Dwg.No.0/13)@

Derwent Class: B04; D16;

Int Pat Class: A61K-039/39; A61K-049/00; C07K-015/06; C12N-005/10; C12N-015/13; C12P-021/08

26/7/77 (Item 7 from file: 351)

008718896 WPI Acc No: 91-222915/30

Related WPI Accession(s): 92-056874

XRAM Acc No: C92-025713

New \*humanised\* \*antibodies\* comprising \*CDR\* grafted \*antibody\* - with heavy and light chains, for use in vivo therapy and diagnosis;  
COMPLEMENTARY DETERMINE REGION

Patent Assignee: (CLLT ) CELLTECH LTD; (CELL-) CELLTECH LTD

Author (Inventor): ADAIR J R; BODMER M W; MOUNTAIN A; OWENS R J; ATHWAL D S ; EMTAGE J S

Number of Patents: 005

Number of Countries: 035

Patent Family:

| CC Number   | Kind | Date   | Week |         |
|-------------|------|--------|------|---------|
| WO 9109967. | A    | 910711 | 9130 | (Basic) |
| AU 9169740  | A    | 910724 | 9143 |         |
| GB 2246570  | A    | 920205 | 9206 |         |
| WO 9201059  | A    | 920123 | 9207 |         |
| AU 9182005  | A    | 920204 | 9220 |         |

*June 21*

Priority Data (CC No Date): GB 8928874 (891221); WO 90GB20174 (901221); GB 9014932 (900705)

Applications (CC, No, Date): AU 9182005 (910705); WO 91GB1108 (910705); GB 9017612 (901221)

Language: English

EP and/or WO Cited Patents: EP 239400; EP 323806; EP 328404; EP 403156; 6.Jnl.Ref; WO 8901783; WO 8910140

Designated States

(National): AT; AU; BB; BG; BR; CH; DE; DK; FI; GB; HU; JP; KP; KR; LK; LU ; MC; MG; MW; NL; NO; RO; SD; SE; SU; US; CA; CS; ES; PL

(Regional): AT; BE; CH; DE; FR; GB; GR; IT; LU; NL; OA; SE; DK; ES

Filing Details: AU9182005 Based on WO 9201059

Abstract (Basic): WO 9109967

A \*CDR\* grafted \*antibody\* heavy chain is claimed having a variable region comprising acceptor frame-work and donor antigen binding regions in at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91. Preferably, the heavy chain framework also comprises donor residues at positions 6, 37, 48 and 94. Also claimed is a \*CDR\*-grafted \*antibody\* light chain having a variable region domain comprising acceptor framework and donor antigen binding regions comprising donor residues in at least one of positions 1 and/or 3 and preferably at positions 46 and/or 47. A \*CDR\* grafted \*antibody\* molecule is also claimed comprising at least one \*CDR\* grafted heavy chain and light chain. DNA encoding the \*CDR\* grafted heavy and light chains is also claimed. The heavy or light chains may have an effector or reporter molecule attached e.g. a macrocycle for chelating a metal atom or a toxin such as ricin. The \*CDR\* grafted \*antibodies\* preferably have non-human e.g. rodent donor and human acceptor frameworkers.

USE/ADVANTAGE - For use in treatment and diagnosis of human and veterinary conditions. @ (91pp Dwg.No.0/13

Derwent Class: B04; D16;  
Int Pat Class: A61K-039/39; A61K-039/395; C07K-015/06; C07K-015/28;  
C12N-005/10; C12N-015/13; C12P-021/08; C12R-001/91

26/7/8 (Item 8 from file: 351)  
008366799 WPI Acc No: 90-253800/33  
XRAM Acc No: C90-109897

Chimaeric immunoglobulin(s) blocking IL-2 binding to receptors -  
comprising human framework and murine complementary determining  
regions, less immunogenic than murine \*antibodies\*

Patent Assignee: (PROT-) PROTEIN DESIGN LABS INC; (PROT-) PROTEIN DESIGN  
LABS

Author (Inventor): QUEEN C L; SELICK H E

Number of Patents: 010

Number of Countries: 034

Patent Family:

| CC Number  | Kind | Date   | Week |         |
|------------|------|--------|------|---------|
| WO 9007861 | A    | 900726 | 9033 | (Basic) |
| PT 92758   | A    | 900629 | 9033 |         |
| CA 2006865 | A    | 900628 | 9037 |         |
| AU 9051532 | A    | 900813 | 9044 |         |
| ZA 8909956 | A    | 901031 | 9048 |         |
| CN 1043875 | A    | 900718 | 9115 |         |
| FI 9102436 | A    | 910520 | 9133 |         |
| NO 9102385 | A    | 910619 | 9142 |         |
| DK 9101191 | A    | 910619 | 9143 |         |
| JP 4502408 | W    | 920507 | 9225 |         |

Priority Data (CC No Date): US 290975 (881228); US 310252 (890213)

Applications (CC,No,Date): WO 89US5857 (891228); JP 90503677 (891228); ZA  
899956 (891228)

Language: English; German

EP and/or WO Cited Patents: 7.Jnl.Ref; EP 239400; GB 2188941; US 4816567;  
WO 8901783

Designated States

(National): AT; AU; BB; BG; BR; CH; DE; DK; FI; GB; HU; JP; KP; KR; LK; LU  
; MC; MG; MW; NL; NO; RO; SD; SE; SU

(Regional): AT; BE; CH; DE; ES; FR; GB; IT; LU; NL; OA; SE

Filing Details: JP04502408 Based on WO 9007861

Abstract (Basic): WO 9007861

Compsn. comprises a pure human-like immunoglobulin (Ig) which (a)  
reacts specifically with p55 Tac protein and/or (b) inhibits binding of  
human interleukin-2 (IL-2) to its receptor. Also new are (1) human-like  
Ig having 2 pairs of light/heavy chains and able to react specifically  
with an epitope of a human IL-2 receptor with affinity at least 10  
power 8 per mole, the chains including complementarily determg. regions  
(\*CDR\*'s) and human-like framework regions (FR's), the \*CDR\*'s being  
from different Ig molecules than FR's; (2) \*humanised\* Ig (hIg) which  
can bind to IL-2 receptors and contain at least one \*CDR\* from anti-Tac  
\*antibody\* in a human-like FR contg. at least one amino acid from the  
anti-Tac \*antibody\*; (3) nucleic acid encoding for human-like FR and at  
least one murine \*CDR\*, and (4) cells transfected with nucleic acid.

USE/ADVANTAGE - hIG are not significantly immunogenic in humans;  
are easily and economically produced, and have a longer half-life in  
vivo than mouse \*antibodies\*. They are useful (opt. when attached to a  
cytotoxic agent, for treatment of T-cell mediated disorders, e.g. graft  
or transplant rejection, and autoimmune diseases. LIG can also be used  
in vitro for T-cell typing; isolation of IL-2 receptor bearing cells,  
vaccine prodn., etc. @ (52pp Dwg.No.0/10)@

Abstract (EP): 9142 EP 451216

Compsn. comprises a pure human-like immunoglobulin (Ig) which (a) reacts specifically with p55 Tac protein and/or (b) inhibits binding of human interleukin-2 (IL-2) to its receptor. Also new are (1) human like Ig having 2 pairs of light/heavy chains and able to react specifically with an epitope of a human IL-2 receptor with affinity at least 10 power 8 per mole, the chains including complementarily determg. regions (\*CDR\*'s) and human-like framework regions (FR's) the \*CDR\*'s being from different Ig molecules than FR's. (2) \*humanised\* IG (hIg) which can bind to IL-2 receptors and contain at least one \*CDR\* from anti-Tac \*antibody\* in a human-like FR contg. at lesdt one amino acid from the anti-Tac \*antibody\*, (3) nucleic acid encoding for human-like FR and at least one murine \*CDR\*, and (4) cells transfected with nucleic acid.

USE/ADVANTAGE - hIG are not significantly immunogenic in humans, are easily and economically produced, and have a longer half-life in vivo than mouse \*antibodies\*. They are useful (opt. when attached to a cytotoxic agent, for treatment of T-cell mediated disorders, e.g. graft or transplant rejection, and autoimmune diseases, LIG can also be used in vitro for T-cell typing, isolation of IL-2 receptor bearing cells, vaccine prodn etc.

Derwent Class: B04; D16;

Int Pat Class: A61K-039/39; C07K-007/10; C07K-013/00; C07K-015/14;  
C12N-005/10; C12N-007/01; C12N-015/00; C12P-021/08

?

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COST = OFF.  
?set hi \*

HILIGHT set on as '\*'  
Hilight option is not available in file(s) 399.  
?show files

File 155: MEDLINE 1966-1992/NOV (9211W1)  
File 5: BIOSIS PREVIEWS 69-92/OCT BA9407: BARRM4307  
(C. BIOSIS 1992)  
File 73: EMBASE (EXCERPTA MEDICA) 74-92/ISS37  
(COPR. ESP BV/EM 1992)  
File 399: CA SEARCH 1967-1992 UD=11710  
(Copr. 1992 by the Amer. Chem. Soc.)

?ds

| Set | Items   | Description   |
|-----|---------|---|
| S1  | 16      | HUMANIZED() ANTIBODIES/TI   |
| S2  | 332298  | ANTIBODIES! FROM 155  |
| S3  | 2253    | IMMUNOGLOBULIN VARIABLE REGION! FROM 155  |
| S4  | 2253    | S2 AND S3   |
| S5  | 862     | HUMANIZ?  |
| S6  | 2005    | HUMANIS?  |
| S7  | 16      | S4 AND (HUMANIZ? OR HUMANIS?)   |
| S8  | 636823  | ANTIBOD? FROM 5,73,399  |
| S9  | 165469  | IMMUNOGLOBULIN  |
| S10 | 41830   | IG  |
| S11 | 113462  | VARIABLE  |
| S12 | 392448  | REGION  |
| S13 | 862     | (IMMUNOGLOBULIN OR IG) (W) VARIABLE (W) REGION  |
| S14 | 604     | CDR   |
| S15 | 67991   | COMPLEMENTARY   |
| S16 | 112646  | DETERMINING   |
| S17 | 63      | COMPLEMENTARY (W) DETERMINING   |
| S18 | 1904    | HYPERVARIABLE   |
| S19 | 392448  | REGION  |
| S20 | 747     | (COMPLEMENTARY (W) DETERMINING OR HYPERVARIABLE) (W) REGION   |
| S21 | 428778  | ANTIBODY  |
| S22 | 1469126 | RELATED   |
| S23 | 623755  | BINDING   |
| S24 | 544344  | SITE? ?   |
| S25 | 0       | ANTIBODY (W) RELATED (W) BINDING (W) SITE? ?  |
| S26 | 2161    | (IMMUNOGLOBULIN OR IG) () VARIABLE () REGION OR CDR OR (COMPLEMENTARY () DETERMINING OR HYPERVARIABLE) () REGION OR ANTIBODY () RELATED () BINDING () SITE? ? FROM 5,73,399 |
| S27 | 897     | S8 AND S26  |
| S28 | 18      | S27 AND (S5 OR S6)  |
| S29 | 34      | S28 OR S7   |
| S30 | 21      | RD (unique items)   |
| S31 | 21      | Sort S30/ALL/PY,D   |
| S32 | 3165    | COMPLEMENTARITY   |
| S33 | 2005813 | DETERMIN?   |
| S34 | 524927  | REGION  |
| S35 | 358     | COMPLEMENTARITY (W) DETERMIN? (W) REGION  |
| S36 | 12      | COMPLEMENTARITY () DETERMIN? () REGION AND (S5 OR S6) AND S8  |
| S37 | 28      | 7 OR 36   |
| S38 | 8       | (37 OR 29) NOT 29   |

*used complementary rather than complementarity in previous search.*

*Picked a few more references here.*

S39 6 RD (unique items)  
S40 6 Sort S39/ALL/PY,D  
?t40/7/1-6

40/7/1 (Item 1 from file: 5)  
9081780 BIOSIS Number: 93066780  
DEVELOPMENT OF \*HUMANIZED\* BISPECIFIC \*ANTIBODIES\* REACTIVE WITH  
CYTOTOXIC LYMPHOCYTES AND TUMOR CELLS OVEREXPRESSING THE HER2 PROTOONCOGENE  
SHALABY M R; SHEPARD H M; PRESTA L; RODRIGUES M L; BEVERLEY P C L;  
FELDMANN M; CARTER P  
DEP. CELL BIOL., GENENTECH, INC., 460 POINT SAN BRUNO BOULEVARD, SOUTH  
SAN FRANCISCO, CALIF. 94080.  
J EXP MED 175 (1). 1992. 217-226. CODEN: JEMEA  
Full Journal Title: Journal of Experimental Medicine  
Language: ENGLISH

The HER2 protooncogene encodes a 185-kD transmembrane phosphoglycoprotein, human epidermal growth factor receptor 2 (p185HER2), whose amplified expression on the cell surface can lead to malignant transformation. Overexpression of HER2/p185HER2 is strongly correlated with progression of human ovarian and breast carcinomas. Recent studies have shown that human T cells can be targeted with bispecific \*antibody\* to react against human tumor cells in vitro. We have developed a bispecific F(ab')<sub>2</sub> \*antibody\* molecule consisting of a \*humanized\* arm with a specificity to 185HER2 linked to another arm derived from a murine anti-CD3 monoclonal \*antibody\* that we have cloned from UCHT1 hybridoma. The antigen-binding loops for the anti-CD3 were installed in the context of human variable region framework residues, thus forming a fully \*humanized\* BsF(ab')<sub>2</sub> fragment. Additional variants were produced by replacement of amino acid residues located in light chain \*complementarity\* \*determining\* \*region\* 2 and heavy chain framework region 3 of the \*humanized\* anti-CD3 arm. Flow cytometry analysis showed that the bispecific F(ab')<sub>2</sub> molecules can bind specifically to cells overexpressing p185HER2 and to normal human peripheral blood mononuclear cells bearing the CD3 surface marker. In additional experiments, the presence of bispecific F(ab')<sub>2</sub> caused up to fourfold enhancement in the cytotoxic activities of human T cells against tumor cells overexpressing p185HER2 as determined by a 51Cr release assay. These bispecific molecules have a potential use as therapeutic agents for the treatment of cancer.

40/7/2 (Item 2 from file: 399)  
117068366 CA: 117(7)68366p PATENT  
Chimeric and complementarity-determining region-grafted  
anti-carcinoembryonic antigen antibodies and their production  
INVENTOR(AUTHOR): Adair, John Robert; Bodmer, Mark William; Mountain,  
Andrew; Owens, Raymond John  
LOCATION: UK,  
ASSIGNEE: Celltech Ltd.  
PATENT: PCT International ; WO 9201059 A1 DATE: 920123  
APPLICATION: WO 91GB1108 (910705) \*GB 9014932 (900705) \*WO 90GB2017  
(901221)  
PAGES: 70 pp. CODEN: PIXXD2 LANGUAGE: English CLASS: C12P-021/08A;  
A61K-039/395B; C12N-015/13B; C07K-015/28B DESIGNATED COUNTRIES: AT; AU; BB  
; BG; BR; CA; CH; CS; DE; DK; ES; FI; GB; HU; JP; KP; KR; LK; LU; MC; MG;  
MN; MW; NL; NO; PL; RO; SD; SE; SU; US DESIGNATED REGIONAL: AT; BE; BF; BJ  
; CF; CG; CH; CI; CM; DE; DK; ES; FR; GA; GB; GN; GR; IT; LU; ML; MR; NL;  
SE; SN; TD; TG  
SECTION:  
CA215003 Immunochemistry  
IDENTIFIERS: carcinoembryonic antigen humanized chimeric antibody,

complementarity detg region grafted antibody CEA, cloning DNA humanized antibody CEA  
DESCRIPTORS:  
Antibodies, monoclonal...  
A5B7 murine, to carcinoembryonic antigen, in humanized antibody prodn.  
Animal cell line...  
CHO L761 h, humanized anti-carcinoembryonic antigen antibody recombinant prodn. in  
Deoxyribonucleic acid sequences...  
for antibody variable regions in humanized anti-carcinoembryonic antigen antibody prodn.  
Genetic vectors... Molecular cloning...  
for humanized anti-carcinoembryonic antigen antibody prodn.  
Diagnosis... Therapeutics...  
humanized anti-carcinoembryonic antigen antibodies for  
Escherichia coli...  
humanized anti-carcinoembryonic antigen antibody fragment recombinant prodn. in  
Animal cell line, CHO-K1... Animal cell line, COS-1... Bacteria...  
humanized anti-carcinoembryonic antigen antibody recombinant prodn. in  
Mammal...  
humanized anti-carcinoembryonic antigen antibody recombinant prodn. in cells of  
Immunoglobulins, fusion products...  
humanized, prodn. of  
Antibodies...  
humanized, to carcinoembryonic antigen  
Immunoglobulins...  
in humanized anti-carcinoembryonic antigen antibody prodn.  
Protein sequences...  
of antibody variable regions in humanized anti-carcinoembryonic antigen antibody prodn.  
Plasmid and Episome...  
pAL43, for humanized anti-carcinoembryonic antigen antibody prodn.  
Plasmid and Episome...  
pAL44, for humanized anti-carcinoembryonic antigen antibody prodn.  
Plasmid and Episome...  
pAL45, for humanized anti-carcinoembryonic antigen antibody prodn.  
Plasmid and Episome...  
pAL46, for humanized anti-carcinoembryonic antigen antibody prodn.  
Plasmid and Episome...  
pAL53, for humanized anti-carcinoembryonic antigen antibody prodn.  
Plasmid and Episome...  
pAL54, for humanized anti-carcinoembryonic antigen antibody prodn.  
Genetic vectors...  
pEE6hCMV gpt, for humanized anti-carcinoembryonic antigen antibody prodn.  
Plasmid and Episome...  
pHMC19, for humanized anti-carcinoembryonic antigen antibody prodn.  
Plasmid and Episome...  
pHMC30, for humanized anti-carcinoembryonic antigen antibody prodn.  
Plasmid and Episome...  
pHMC31, for humanized anti-carcinoembryonic antigen antibody prodn.  
Plasmid and Episome...  
pHMC43, for humanized anti-carcinoembryonic antigen antibody prodn.  
Plasmid and Episome...  
pHMC44, for humanized anti-carcinoembryonic antigen antibody prodn.  
Genetic vectors...  
pMRR028, for humanized anti-carcinoembryonic antigen antibody fragment prodn.



Genetic vectors...

pMRR045, for humanized anti-carcinoembryonic antigen antibody fragment prodn.

CAS REGISTRY NUMBERS:

142661-53-8 142661-54-9 142661-55-0 142661-56-1 142661-57-2  
142661-58-3 amino acid sequence of, humanized anti-carcinoembryonic  
antigen antibody prodn. in relation to  
142662-69-9 142662-70-2 142662-71-3 142662-72-4 142662-81-5  
142662-82-6 nucleotide sequence of, humanized anti-carcinoembryonic  
antigen antibody prodn. in relation to

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40/7/3 (Item 3 from file: 5)  
8599131 BIOSIS Number: 92064131

IMMUNOGLOBULIN \*COMPLEMENTARITY\*--\*DETERMINING\* \*REGION\* GRAFTING BY  
RECOMBINANT POLYMERASE CHAIN REACTION TO GENERATE \*HUMANIZED\* MONOCLONAL  
\*ANTIBODIES\*

LEWIS A P; CROWE J S  
DEP. CELL BIOLOGY, WELLCOME RES. LAB., LANGLEY COURT, BECKENHAM, KENT,  
BR3 3BS UK.

GENE (AMST) 101 (2). 1991. 297-302. CODEN: GENED

Full Journal Title: GENE (Amsterdam)

Language: ENGLISH

We describe an approach to rapidly generate \*humanised\* monoclonal  
\*antibodies\* by grafting rodent complementarity-determining regions into  
human immunoglobulin frameworks using recombinant polymerase chain reaction  
(PCR) methodology. The approach was applied to grafting a rat  
\*complementarity\*--\*determining\* \*region\* onto a human framework and  
amplifying the entire \*humanised\* heavy chain. The terminal  
oligodeoxyribonucleotide primers incorporated restriction sites to allow  
forced cloning into plasmid vectors for sequencing and expression. No  
nucleotide errors were introduced into the 1463-bp sequence even after  
sequential applications of PCR.

40/7/4 (Item 4 from file: 5)  
7912269 BIOSIS Number: 40113269

CONSTRUCTION OF \*HUMANIZED\* \*ANTIBODIES\* AND TESTING IN PRIMATES  
QUEEN C; CO M S; DESCHAMPS M; WHITLEY R; BENJAMIN W; HAKIMI J  
PROTEIN DESIGN LAB. INC., 2375 GARCIA AVE., MOUNTAIN VIEW, CALIF. 94043.  
MEETING ON MONOCLONAL ANTIBODIES HELD AT THE 20TH ANNUAL MEETING OF THE  
KEYSTONE SYMPOSIA ON MOLECULAR AND CELLULAR BIOLOGY, DENVER, COLORADO, USA,  
MARCH 10-16, 1991. J CELL BIOCHEM SUPPL 15 (PART E) 1991: 137. CODEN:  
JCBSD

Language: ENGLISH

40/7/5 (Item 5 from file: 5)  
7400987 BIOSIS Number: 89052006

A \*HUMANIZED\* \*ANTIBODY\* THAT BINDS TO THE INTERLEUKIN 2 RECEPTOR  
QUEEN C; SCHNEIDER W P; SELICK H E; PAYNE P W; LANDOLFI N F; DUNCAN J F;  
AVDALOVIC N M; LEVITT M; JUNGHANS R P; WALDMANN T A  
PROTEIN DESIGN LABS., 3181 PORTER DRIVE, PALO ALTO, CALIF. 94304.  
PROC NATL ACAD SCI U S A 86 (24). 1989. 10029-10033. CODEN: PNASA  
Full Journal Title: Proceedings of the National Academy of Sciences of  
the United States of America

Language: ENGLISH

The anti-Tac monoclonal \*antibody\* is known to bind to the p55 chain of  
the human interleukin 2 receptor and to inhibit proliferation of T cells by  
blocking interleukin 2 binding. However, use of anti-Tac as an  
immunosuppressant drug would be impaired by the human immune response

against this murine \*antibody\*. We have therefore constructed a " \*humanized\*" \*antibody\* by combining the complementarity-determining regions (CDRs) of the anti-Tac \*antibody\* with human framework and constant regions. The human framework regions were chosen to maximize homology with the anti-Tac \*antibody\* sequence. In addition, a computer model of murine anti-Tac was used to identify several amino acids which, while outside the CDRs, are likely to interact with the CDRs or antigen. These mouse amino acids were also retained in the \*humanized\* \*antibody\*. The \*humanized\* anti-Tac \*antibody\* has an affinity for p55 of 3 .times. 10<sup>9</sup> M<sup>-1</sup>, about 1/3 that of murine anti-Tac.

40/7/6 (Item 6 from file: 399)

113170316 CA: 113(19)170316b PATENT

Recombinant antibodies to Campath-1 antigen, containing foreign complementarity determining region(s), and their use in immunosuppression and cancer therapy

INVENTOR(AUTHOR): Waldmann, Herman; Clark, Michael Ronald; Winter, Gregory Paul; Riechmann, Lutz

LOCATION: UK,

ASSIGNEE: Medical Research Council

PATENT: PCT International ; WO 8907452 A1 DATE: 890824

APPLICATION: WO 89GB113 (890210) \*GB 883228 (880212) \*GB 884464 (880225)

PAGES: 61 pp. CODEN: PIXXD2 LANGUAGE: English CLASS: A61K-039/395A;

C12N-015/00B DESIGNATED COUNTRIES: AU; DK; JP; US

SECTION:

CA215003 Immunochemistry

CA201XXX Pharmacology

CA203XXX Biochemical Genetics

IDENTIFIERS: chimeric antibody Campath 1 antigen, lymphoma neoplasm inhibitor Campath 1H antibody

DESCRIPTORS:

Rat...

complementarity detg. regions of, in recombinant antibody to Campath-1 antigen

Immunoglobulins,G2... Immunoglobulins,G3... Immunoglobulins,G4...

const. domains of human, in recombinant antibody contg. complementarity detg. regions to Campath-1 antigen

Lymphocyte...

depletion of, in human, by recombinant human antibody contg. foreign complementarity detg. regions to Campath-1 antigen

Gene and Genetic element,animal, synthetic...

for humanized light chain variable region, construction of, in prodn. of recombinant human antibody contg. rat complementarity detg. regions to Campath-1 antigen

Protein sequences...

of IgG2a YTH 34.5 HL heavy and light chain variable domains, of rat

Deoxyribonucleic acid sequences,IgG2a-specifying...

of rat

Antigens,CAMPATH-1...

recombinant antibodies to, foreign complementarity detg. regions in Immunosuppressants... Neoplasm inhibitors... Neoplasm inhibitors,lymphoma

...

recombinant antibody contg. foreign complementarity detg. regions to Campath-1 antigen as

Gene and Genetic element,animal...

recombinant, for anti-Campath-1 antigen antibody of human, sequences encoding rat complementary detg. regions in

Immunoglobulins,G2a...

recombinant human antibody to Campath-1 antigen contg. complementary detg. regions of rat

Leukemia, B-cell...

recombinant human antibody to Campath-1 antigen killing leukemia cells of

Antibodies...

recombinant, to Campath-1 antigen, foreign complementarity detg. regions in

Immunoglobulins, G1... Immunoglobulins, G... Immunoglobulins, M...

recombinant, to Campath-1 antigen, foreign complementary detg. regions in

CAS REGISTRY NUMBERS:

- 129711-40-6 amino acid sequence encoded by HuVLLYS gene
- 129711-41-7 amino acid sequence encoded by synthetic HuVLLYS.degree. gene
- 129711-01-9 129711-02-0 cloning and nucleotide sequence of, of human and rat
- 129711-19-9 129711-20-2 cloning and nucleotide sequence of, of rat
- 128096-06-0 128096-07-1 128096-08-2 128096-09-3 128096-10-6 128096-11-7 complementarity detg. region of rat YTH 34.5 HL, human recombinant antibody contg., Campath-1 antigen binding by
- 129711-56-4 heavy chain variable region of human contg. rat complementarity detg. regions, recombinant antibody contg., Campath-1 antigen binding by
- 129711-60-0 heavy chain variable region of rat YTH 34.5 HL, recombinant antibody contg., Campath-1 antigen binding by
- 129710-86-7P HuVLLYS gene, prepn. of, in prepn. of recombinant human antibody contg. rat complementarity detg. regions to Campath-1 antigen
- 129711-59-7 light chain variable region of human contg. rat complementarity detg. regions, recombinant antibody contg., Campath-1 antigen binding by
- 129711-61-1 light chain variable region of rat YTH 34.5 HL, recombinant antibody contg., Campath-1 antigen binding by
- 127859-21-6P 127859-23-8P 127859-24-9P 127859-26-1P 127859-62-5P 127859-70-5P 127859-72-7P 127859-79-4P 127859-82-9P 127859-92-1P 127859-93-2P 127859-94-3P 127859-99-8P 127860-01-9P 127860-02-0P 127860-03-1P 127860-04-2P 129924-57-8P 129924-59-0P prepn. of, in gene synthesis for recombinant human antibody contg. rat complementarity detg. regions to Campath-1 antigen
- 129711-57-5 129711-58-6 recombinant human antibody contg., Campath-1 antigen binding by
- 129710-91-4P synthetic gene HuVLLYS.degree., prepn. of, in prepn. of recombinant human antibody contg. rat complementary detg. regions to Campath-1 antigen

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?b351,350

15sep92 10:26:26 User209197 Session D127.2

SYSTEM:OS - DIALOG OneSearch

File 351:Derwent World Patents;Index Latest  
1981+;DW=9227,UA=9214,UM=9143

\*\*FILE351: Formats 32,33,35,37 & 39 display the new 'Expanded' Patent Family table for UD=9216 and greater. For more info. type ?NEWS351

File 350:Derwent World Patents Index  
1963-1980, EQUIVALENTS THRU DW=9227

\*\*FILE350: Formats 32,33,35,37 & 39 display the new 'Expanded' Patent Family table for UD=9219 and greater. For more info. type ?NEWS350

| Set | Items | Description |
|-----|-------|-------------|
| --- | ----- | -----       |

?ds

| Set           | Items        | Description   |
|---------------|--------------|---|
| S1            | 22           | ANTIBOD? AND (HUMANIS? OR HUMANIZ?)   |
| S2            | 8            | S1 AND (CDR OR (IG OR IMMUNOGLOBULIN) () VARIABLE() REGION OR HYPERVARIABLE() REGION) |
| S3            | 0            | S1 AND COMPLEMENTARITY() DETERMIN?() REGION   |
| S4            | 3            | S1 AND COMPLEMENT?() DETERMIN?() REGION   |
| <del>S5</del> | <del>1</del> | <del>(2 OR 4) NOT 2</del>   |

?t5/7/1

5/7/1 (Item 1 from file: 351)  
 007820291 WPI Acc No: 89-085403/11  
 XRAM Acc No: C89-037905

Recombinant \*humanised\* \*antibody\* specific for TAG-72 - having complementarity determining regions of variable domains from mouse \*antibody\* and the remainder from human immunoglobulin

Patent Assignee: (CELL-) CELLTECH LTD

Author (Inventor): BODMER M W; ADAIR J R; WHITTLE N R

Number of Patents: 001

Patent Family:

| CC Number  | Kind | Date   | Week         |
|------------|------|--------|--------------|
| WO 8901783 | A    | 890309 | 8911 (Basic) |

Priority Data (CC No Date): WO 88GB731 (880905); GB 8720833 (870904)

Language: English

EP and/or WO Cited Patents: No.SR.Pub; 4.Jnl.REF

Designated States

(National): AU; DK; FI; HU; JP; KR; NO; RO; SU; US

(Regional): AT; BE; CH; DE; FR; GB; IT; LU; NL; SE

Abstract (Basic): WO 8901783

A \*humanised\* \*antibody\* molecule (HAM) is claimed having specificity for the TAG-72 antigen and having an antigen binding site in which at least the \*complementary\* \*determining\* \*region\* (CDRs) of the variable domains are derived from the mouse monoclonal \*antibodies\* (MAb) B72.3 and the remaining immunoglobulin-derived parts of the HAM are derived from a human immunoglobulin.

USE/ADVANTAGE - \*Humanising\* the B72.3 MAb does not adversely affect its binding activity and this produces a HAM which is useful in both therapy and diagnosis of certain carcinomas, e.g. solid tumours expressing TAG-72. @(49pp Dwg.No.0/13)@

Derwent Class: B04; D16;

Int Pat Class: A61K-039/39; C12N-015/00; C12P-021/00

?s complement?()determin?(w)region? ?

Processing

Processing

Processing

27431 COMPLEMENT?

234285 DETERMIN?

124968 REGION? ?

S6 23 COMPLEMENT?() DETERMIN?(W) REGION? ?

?c 1 and 6

22 1

23 6

S7 10 1 AND 6

?

?c 7 not (2 or 4)

10 7  
8 2  
3 4  
S8 3 7 NOT (2 OR 4)

?t8/7/1-3

8/7/1 (Item 1 from file: 351)  
009004842 WPI Acc No: 92-132139/16  
XRAM Acc No: C92-061892

\*Humanisation\* of \*antibodies\* binding to human CD4 antigen - by mutation of framework-encoding regions of DNA encoding variable domain of rat or mouse \*antibody\* chain

Patent Assignee: (GORM/) GORMAN S D  
Author (Inventor): CLARK M R; COBBOLD S P; GORMAN S D; WALDMANN H  
Number of Patents: 001  
Number of Countries: 018  
Patent Family:

| CC Number  | Kind | Date   | Week |         |
|------------|------|--------|------|---------|
| WO 9205274 | A    | 920402 | 9216 | (Basic) |

Priority Data (CC No Date): GB 9020282 (900917)  
Applications (CC,No,Date): WO 91GB1578 (910916)  
Language: English  
EP and/or WO Cited Patents: 7.Jnl.Ref; EP 328404; EP 365209; EP 403156; WO 9007861; WO 9107492; WO 9109966; WO 9109967

Designated States

(National): AU; CA; JP; KR; US  
(Regional): AT; BE; CH; DE; DK; ES; FR; GB; GR; IT; LU; NL; SE  
Abstract (Basic): WO 9205274 A

\*Complementarity\* \*determining\* \*regions\* (CDRs) of the variable domain of the \*antibody\* chain are derived from a first mammalian species and the framework of the variable domain and any constant domains of the Ab chain are derived from a second different mammalian species; comprising (a) mutating the framework-encoding regions of DNA encoding a variable domain of the first mammalian Ab chain such that it encodes the framework derived from the second species; and (b) expressing the Ab chain using this mutated DNA.

The process specifically comprises: (i) determining nucleotide and predicted aminoacid sequence of a variable domain of a selected Ab chain of the first species; (ii) determining the Ab framework to which the framework of this domain is to be altered; (iii) mutating framework-encoding regions of DNA encoding this variable domain such that the mutated region encodes the framework determined in (ii); (iv) linking mutated DNA to DNA encoding a constant domain of the second species and cloning the DNA into an expression vector; and (v) introducing expression vector into a compatible host cell and culturing it to express Ab chain.

USE/ADVANTAGE - Altered Abs is prepd., used to \*humanise\* an Ab, typically a monoclonal Ab and, e.g. a rat or mouse Ab. The resulting Ab retains the antigen binding capabilities of the Ab from which it is derived. Reshaped CD4 Ab is used to induce tolerance against an antigen. Used to alleviate autoimmune diseases e.g. rheumatoid arthritis, and to prevent graft rejection. 0/13

Derwent Class: B04; D16;  
Int Pat Class: A61K-039/39; C12N-015/13; C12P-021/08

8/7/2 (Item 2 from file: 351)  
008712964 WPI Acc No: 91-216983/30

XRAM Acc No: C91-094177

Prodn. of \*humanised\* recombinant immunoglobulin - including polymerase chain reaction amplification of murine \*antibody\* light and heavy chain variable portions

Patent Assignee: (MERI ) MERCK & CO INC

Author (Inventor): LAW M F; MARK G E; WILLIAMSON A R

Number of Patents: 002

Patent Family:

| CC Number  | Kind | Date   | Week         |
|------------|------|--------|--------------|
| EP 438310  | A    | 910724 | 9130 (Basic) |
| CA 2034553 | A    | 910720 | 9139         |

Priority Data (CC No Date): US 627423 (901220); US 467700 (900119)

Applications (CC,No,Date): EP 91300362 (910117)

Language: English

EP and/or WO Cited Patents: EP 239400; WO 8901783; 1.Jnl.REF

Designated States

(Regional): CH; DE; FR; GB; IT; LI; NL

Abstract (Basic): EP 438310

Method for producing a \*humanised\* recombinant immunoglobulin comprises: (a) prepg. polymerase chain reaction (PCR) primers to amplify the variable portion of the light and heavy chain of a murine \*antibody\* which binds to a predefined antigen; (b) using the primers to amplify the variable portions of both heavy and light chains and sequencing the resulting nucleotide chains; (c) determining the murine \*complementary\* \*determining\* \*regions\* of the heavy and light chains; (d) selecting human variable heavy and light chain frameworks which show a high degree of amino acid similarity with the variable heavy and light chain framework of the murine immunoglobulin; (e) selecting human constant heavy and light chain frameworks; (f) grafting the murine \*complementary\* \*determining\* \*regions\* of (c) to the human framework regions of (e); (g) incorporating the complete DNA sequence for the \*humanised\* recombinant immunoglobulin into an appropriate expression vector; (h) transfecting host cells with the vector; (i) growing the transfected cells in an environment in which the \*humanised\* recombinant immunoglobulin is expressed; and (j) collecting the immunoglobulin.

A PCR method for the simultaneous synthesis and assembly of at least 4 deoxyoligonucleotides is also claimed.

USE/ADVANTAGE - The \*humanised\* recombinant immunoglobulins are weakly immunogenic or non-immunogenic when admin. to humans, and may be used as therapeutic agents. Recombinant human anti-CD18 \*antibodies\* or active fragments which bind to the CD18 antigen of leukocytes can be used to inhibit influx of the leukocytes into a site of inflammation or tissue liable to become inflamed following influx. @(78pp Dwg.No.0/38)@

Derwent Class: B04; D16;

Int Pat Class: C12N-015/13; C12P-021/08; C12Q-001/68

8/7/3 (Item 3 from file: 351)

007275804 WPI Acc No: 87-272811/39

XRAM Acc No: C87-115825

Recombinant altered \*antibodies\* - having \*complementarity\* \*determining\* \*regions\* replaced with those from \*antibody\* of different specificity

Patent Assignee: (WINT/) WINTER G P

Author (Inventor): WINTER G P

Number of Patents: 004

Patent Family:

| CC Number  | Kind | Date   | Week         |
|------------|------|--------|--------------|
| EP 239400  | A    | 870930 | 8739 (Basic) |
| GB 2188638 | A    | 871007 | 8740         |

JP 62296890 A 871224 8806  
GB 2188638 B 900523 9021

Priority Data (CC No Date): GB 867679 (860327); GB 877252 (870326)  
Applications (CC, No, Date): EP 87302620 (870326); JP 8773980 (870327)  
Language: English  
EP and/or WO Cited Patents: A3...8914; 3.Jnl.REF  
Designated States

(Regional): AT; BE; CH; DE; ES; FR; GB; GR; IT; LI; LU; NL; SE  
Abstract (Basic): EP 239400

An altered \*antibody\* in which at least parts of the \*complementary\* \*determining\* \*regions\* (CDRs) in the light or heavy chain variable domains have been replaced by analogous parts of CDRs from an \*antibody\* of different specificity is new.

The altered \*antibody\* can be produced by (a) prepg. a first replicable expression vector including a suitable promoter operably linked to a DNA sequence which encodes at least a variable domain of an Ig heavy or light chain, the variable domain comprising framework regions from a first \*antibody\* and CDRs comprising at least parts of the CDRs from a second \*antibody\* of different specificity, (b) if necessary, prepg. a second replicable expression vector including a suitable promoter operably linked to a DNA sequence which encodes at least the variable domain of a complementary Ig light or heavy chain, (c) transforming a cell line with the first or both prepd. vectors and (d) culturing the transformed cell line to produce the altered \*antibody\*.

USE/ADVANTAGE - The method is used for '\*humanising\*' non-human monoclonal \*antibodies\* (MAbs) e.g. CDRs from mouse MAb can be partially or totally grafted into the framework regions of a human MAb, which is then produced in quantity by a suitable cell line. Only the CDRs of the \*antibody\* will be foreign to the body and this should minimise side effects if used for human therapy. @(41pp Dwg.No.0/8)@

Derwent Class: B04; D16;

Int Pat Class: C12N-015/00; C12P-021/02; C07K-015/00; A61K-039/39;  
C12N-005/00; C12R-001/91

?ds

| Set            | Items | Description  |
|----------------|-------|--|
| S1             | 22    | ANTIBOD? AND (HUMANIS? OR HUMANIZ?)  |
| S2             | 8     | S1 AND (CDR OR (IG OR IMMUNOGLOBULIN) ( ) VARIABLE ( ) REGION OR HYPERVARIABLE ( ) REGION) |
| S3             | 0     | S1 AND COMPLEMENTARITY ( ) DETERMIN? ( ) REGION  |
| S4             | 3     | S1 AND COMPLEMENT? ( ) DETERMIN? ( ) REGION  |
| S5             | 1     | (2 OR 4) NOT 2   |
| S6             | 23    | COMPLEMENT? ( ) DETERMIN? (W) REGION? ?  |
| S7             | 10    | 1 AND 6  |
| S8             | 3     | 7 NOT (2 OR 4)   |
| S9             | 5     | S1 AND CDRS  |
| <del>S10</del> | 0     | <del>(9 OR 7 OR 2 OR 4) NOT (7 OR 2 OR 4)</del>  |

?



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Address: COMMISSIONER OF PATENTS AND TRADEMARKS  
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07/215-272 06/11/91

| SERIAL NUMBER                 | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. |
|-------------------------------|-------------|----------------------|---------------------|
| GENENTECH, INC.               |             |                      |                     |
| ALICE CAROLYN R. AMLER        |             |                      |                     |
| 160 POINT SAN BRUNO BLVD.     |             |                      |                     |
| SOUTH SAN FRANCISCO, CA 94080 |             |                      |                     |

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|----------|
| EXAMINER |
| 1800     |

|          |              |
|----------|--------------|
| ART UNIT | PAPER NUMBER |
|          | 13           |

DATE MAILED:

This is a communication from the examiner in charge of your application.  
COMMISSIONER OF PATENTS AND TRADEMARKS

This application has been examined  Responsive to communication filed on 7/13/92  This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s), 0 days from the date of this letter.  
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

**Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:**

- 1.  Notice of References Cited by Examiner, PTO-892.
- 2.  Notice re Patent Drawing, PTO-948.
- 3.  Notice of Art Cited by Applicant, PTO-1448. 3 pages
- 4.  Notice of Informal Patent Application, Form PTO-152.
- 5.  Information on How to Effect Drawing Changes, PTO-1474.
- 6.  \_\_\_\_\_

**Part II SUMMARY OF ACTION**

1.  Claims 1-16 are pending in the application.

Of the above, claims 14-16 are withdrawn from consideration.

2.  Claims \_\_\_\_\_ have been cancelled.

3.  Claims \_\_\_\_\_ are allowed.

4.  Claims 1-13 are rejected.

5.  Claims \_\_\_\_\_ are objected to.

6.  Claims \_\_\_\_\_ are subject to restriction or election requirement.

7.  This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.

8.  Formal drawings are required in response to this Office action.

9.  The corrected or substitute drawings have been received on \_\_\_\_\_ Under 37 C.F.R. 1.84 these drawings are  acceptable  not acceptable (see explanation or Notice re Patent Drawing, PTO-948).

10.  The proposed additional or substitute sheet(s) of drawings, filed on \_\_\_\_\_ has (have) been  approved by the examiner.  disapproved by the examiner (see explanation).

11.  The proposed drawing correction, filed on \_\_\_\_\_, has been  approved.  disapproved (see explanation).

12.  Acknowledgment is made of the claim for priority under U.S.C. 119. The certified copy has  been received  not been received  been filed in parent application, serial no. \_\_\_\_\_; filed on \_\_\_\_\_

13.  Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.

14.  Other



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Applicant's election of Group 1, in Paper No. 12, is acknowledged. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without  
5 traverse. See M.P.E.P. 818.03(a).

Claims 1-10 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claims 1, 3, 4, 5 and 7 are indefinite  
10 in the use of the language "import antibody" in that it is not clear what constitutes an important antibody, ie. what determines what is to be an import antibody. Claim 1 step a) is indefinite in that it is not clear what is meant by a "consensus  
human variable domain". Claim 1 step d) is indefinite in that it  
15 is not clear what is actually taking place when one aligns the amino acid sequences of the FR, ie. is this a physical or mental step? Claim 1 step e) is unclear in what type of homology is indicated, ie. are conservative amino acids considered as homologs or should their be identical amino acid residues at the indicated  
20 portion of the framework. Claim 1 step f), 3 is indefinite in the use of the language "participates" in that the nature of participation is unclear. Claim 1 step f) is indefinite in that it is not clear how one of ordinary skill can determine the effects which are listed in steps 1-3, ie. through antigen binding, through  
25 hybridization? Claim 1 step g) is indefinite in that it is not clear what effects are reasonably expected to occur. Claim 2 is indefinite in that the antecedent basis for "the domain" is unclear. Claim 3 is indefinite in that it is not clear when in the process of making the antibody one would search for the  
30 glycosylation sites. Claim 4 is indefinite for the same reason that claim 3 is indefinite. Claim 5 is indefinite in that it is believed that the claims up to this point were directed to making a

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"humanized antibody", and it is unclear how "preparing a humanized antibody" in claim 5 differs from the preparation of the antibody up to this point. Furthermore, it is not clear what is intended in the preparation of the antibody of claim 5. Claim 6 is vague in that it is not clear what the numbers are meant to designate. It is suggested that applicant clarify the nature of the numbers or point to a figure. Claim 7 is indefinite in that it is not clear what the method is drawn to. It is suggested that the language "a method of making a humanized antibody" be inserted within the claim.

The following is a quotation of the first paragraph of 35 U.S.C. § 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

The specification is objected to under 35 U.S.C. § 112, first paragraph, as failing to adequately describe the invention and failing to adequately teach how to make and or use the invention, ie. failing to provide an enabling disclosure. The following terms lack enablement in the specification:.

Claims 1 and 7 lack enablement in the language "at least a portion of an import variable domain". Applicant has only indicated specific residues which may be transferred, but they are claiming an antibody wherein a portion of the import antibody are to be transferred. There is no guidance in the specification which would enable one of skill in the art to make antibodies with

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transferred variable domains other than CDRs. Applicant is aware that a portion of the variable domain can be any one of the CDRs as well as the framework regions. However, this language also reads on small amino acid sequences which are incomplete regions of the variable region of the antibody. There is no support in the specification for linking the variable region of the antibody to any or all of the myriad "portions" which are encompassed within this language. One of skill in the art would neither expect nor predict the appropriate functioning of the antibody as broadly as is claimed. It is suggested that the specific portion of the human variable region which is described in the specification be recited within the claim or this language be removed completely in order to obviate this rejection.

Claim 1 step c) lacks enablement in that it is not clear how one would determine which amino acids are to be substituted. There is no specific recitation of what characteristics of the amino acids are necessary for deciding whether it is to be replaced or not. Without this description one of skill in the art would not be able to choose the appropriate amino acid residues without hindering the function of the antibody.

Claim 1 step f), lacks enablement in that the protocol for determining whether the amino acid residues in the import amino acid sequence are reasonably expected to interact with the antigen is not described anywhere in the specification. There is no

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explicit step which enables one of ordinary skill in the art to determine the effects which are recited. It would require undue experimentation of one of ordinary skill in the art to make the variations which may be made in order to test the effects of the mutant antibodies.

*W/draw*  
Claim 2 lacks enablement in that there is no description in the specification of how to determine which residues are exposed on the surface or which residues are buried within the domain, is this through computer modeling or through x-ray crystallography or other methods?

*Reason*  
Claim 3 lacks enablement in that there is no guidance in the specification on how one would determine which glycosylation site affects antigen binding, or what comprises "reasonable expectation".

15 Claims 6, 7 and 9 lack enablement in that it would appear that these amino acids are relevant to IgG and not to other isotypes. There is no indication that one of skill in the art would extrapolate the use of these amino acids to all or other isotypes of immunoglobulins. Furthermore, there is insufficient description and guidance in the specification with regards to the properties of  
20 these amino acids which would enable one of ordinary skill in the art to make humanized antibodies with other isotypes using these amino acid sequences.

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Applicant has not shown that antibodies which have been modified as that which is claimed are capable of functioning as that which is being disclosed, ie. maintaining the binding affinity of the parent antibody. Protein chemistry is probably one of the  
5 most unpredictable areas of biotechnology. For example, replacement of a single lysine residue at position 118 of acidic fibroblast growth factor by glutamic acid led to the substantial loss of heparin binding, receptor binding and biological activity of the protein. Burgess et. al. Journal of Cell biology, 111:  
10 2129-2138 (1990). In transforming growth factor alpha, replacement of aspartic acid at position 47 with alanine or asparagine did not affect biological activity while replacement with serine or glutamic acid sharply reduced the biological activity of the mitogen. Lazar et. al. Molecular and Cellular Biology, 8:1247-1252  
15 (1988). Similarly it has been shown that aglycosylation of antibodies reduces the resistance of the antibodies to proteolytic degradation, while CH2 deletions increase the binding affinity of the antibodies. See Tao et. al. The Journal of Immunology, Vol. 143, No. 8. 2595-2601 (1989) and Gillies et. al. Human Antibodies and Hybridomas, Vol 1, no. 1, 47-54 (1990). These references  
20 demonstrate that even a single amino acid substitution or what appears to be an inconsequential chemical modification, will often dramatically affect the biological activity and characteristic of a protein. Therefore, without sufficient guidance in the

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specification to support the use of the above terms and for the reasons mentioned above one of ordinary skill in the art would be forced into undue experimentation in order to practice the invention as is claimed.

5            Claims 1-11 are rejected under 35 U.S.C. § 112, first paragraph, for the reasons set forth in the objection to the specification.

35 U.S.C. § 101 reads as follows:

10            Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

15            Claims 1-4, 6-8 are rejected under 35 U.S.C. § 101 because the claimed invention is directed to non-statutory subject matter. The above claims are drawn to a method of preparing an antibody, however, there is no indication within the claims that actual physical steps are taking place. For example, there is no step  
20            which includes isolating an antibody, rather obtaining an amino acid sequence. All of the steps which are listed in the claims can be done on paper as mental steps or on a computer terminal.

            The specification is objected to under 35 U.S.C. § 112, first paragraph, and claims 9-13 are rejected under 35 U.S.C. § 112,  
25            first paragraph and 35 U.S.C. § 101 as the specification fails to adequately teach how to use the claimed monoclonal antibodies in the manner in which they are disclosed i.e. for the therapeutic

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purposes. Applicants claims are supported only by in vitro data showing the ability of muMab4D5, which is a humanized anti-p185 antibody which reacts with breast and ovarian cancers, to react with different cell lines (see page 88-90 of the specification).

5 Applicant has made no showing that these data correlate with utility for in vivo therapy in humans of the complex array of diseases encompassed by the claims. In general, effective treatment of human cancers has not been routinely achieved in the art using monoclonal antibodies. Further, in vitro data such as

10 that reported in the specification and animal model studies frequently do not correlate with clinical utility in in vivo trials in patients. Based on the evidence of record, the alleged utility of the claimed composition for the treatment of cancer would not be believable on its face to the person of skill in the art in view of

15 the contemporary knowledge in the art. Applicant has not provided any showing of therapeutic utility of the subject monoclonal antibodies which would lead one of skill in the art to believe that the antibodies are broadly applicable for the treatment of all types of autoimmune diseases. Applicant is required to provide

20 evidence commensurate with the scope of the claims, which would be convincing to those skilled in the art that the claimed compositions have utility for the treatment of malignant and autoimmune diseases in humans. See MPEP 608.01(p).

Waldmann, in a recent review of the literature pertaining to clinical applications of monoclonal antibodies for diagnosis and therapy of human disease, teaches that effective therapy using monoclonal antibodies has been elusive and indicates that hopes for antibody-based treatment methods engendered by in vitro studies have not correlated well with in vivo clinical trial results in patients with cancer. It does not appear that the exemplary material provided in the specification in support of the assertions that the claimed antibodies have therapeutic utility would be viewed by those skilled in the art as being predictive of their utility for treating humans. Applicant has not exemplified how to use the claimed antibodies in vivo and has not shown that the antibodies would be effective in vivo. It appears that undue experimentation would be required of one skilled in the art to practice the claimed invention for the single utility disclosed in the specification.

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--

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

(b) the invention was patented or described in a printed publication in this country or a



foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

5 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:

10 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  
15 negated by the manner in which the invention was made.

20 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.

25 Claims 1, 2, 5-10 are rejected under 35 U.S.C. § 102(b) as being anticipated by Queen et. al.. The above claims are drawn to a method of producing a humanized antibody wherein the amino acid sequences of an import antibody and a consensus antibody are compared, wherein the CDRs of the import antibody are substituted for the antibody of the consensus antibody, and wherein certain  
30 framework residues which are responsible for the binding of antigen, interaction with CDR, or participating in the V1-Vh interaction are also imported to the consensus antibody. In essence, residues of the framework region are also transferred with

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the CDRs in order to retain the antigen binding affinity of the parent antibody.

Queen et. al. describe the production of humanized antibodies wherein the murine antibody is compared to human antibodies and the most homologous human antibody is chosen as the acceptor molecule. The CDRs of the murine antibody are then substituted for the CDRs of the human antibody and certain framework residues are also changed. Queen et. al. describe computer modeling and sequence comparison in order to determine the amino acid residues which are to be substituted (see page 10031-10033). Although the steps of the methods are not in exactly the same order, all of the claimed elements are present with in the reference.

Claims 1,2 and 5-10 are rejected under 35 U.S.C. § 102(a) as being anticipated by Co et. al.. See above discussion.

Co et. al. show the production of humanized anti-HSV using the general concept of Queen et. al. (see Results and Table 1).

Claims 3 and 4 are rejected under 35 U.S.C. § 103 as being unpatentable over Queen et. al. or Co et. al. in view of Wallick et. al.

The above claims are drawn to a method of making a humanized antibody wherein the CDRs of an import antibody are transferred to a consensus human antibody along with certain residues of the framework. Furthermore, the claims require that the glycosylation

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sites, if any, of the import amino acid also be imported with the CDRs and framework regions if these sites have an affect on the binding of antigen.

Queen et. al. and Co et. al. both describe the production of  
5 humanized antibodies by transferring the CDRs and certain framework regions of the donor antibody to the human consensus antibody (see Queen et. al. pages 10031-10033 and Co et. al. page 2871). They further state that any residue which might have an affect on the antigen binding of the antibody should be changed substituted in  
10 order to maintain the binding affinity of the parent antibody (see page 10033 of Queen et. al. at the last paragraph on the page). They do not however, specifically discuss the glycosylation sites as potential targets for transfer. Wallick et. al. teach the importance of carbohydrate interaction with antigen for maintaining  
15 or increasing antigen binding affinity (see pages 1107-1108). It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to make humanized antibodies using the method of Queen et. al. or Co et. al. and further incorporating the concept taught by Wallick et. al.. One of  
20 ordinary skill in the art would have been motivated to combine the teachings of the two references in view of the teaching of Queen that retaining high antigen binding affinity is desirable in the production of humanized antibodies. Knowing the role of carbohydrates in antigen antibody interaction as was pointed out by

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Wallick et. al. one of ordinary skill would have had the means and the motivation to make humanized antibodies using both of the teachings of the primary and secondary references.

Claim 11 is rejected under 35 U.S.C. § 103 as being unpatentable over Queen et. al. or Co et. al. in view of Reichmann et. al.

The above claim is drawn to a humanized antibody wherein only one amino acid (listed in claim 9) in the framework and the CDRs have been substituted in the consensus antibody.

Queen et. al. and Co et. al. both teach the production of humanized antibodies by transferring the CDRs of a murine antibody along with specific residues of the framework region to the acceptor antibody molecule. They do not however teach only substituting one of the framework residues among those listed in claim 9. Queen et. al. introduce the general concept of a scaffold wherein certain amino acid residues of the framework must be present and certain are dispensable. Reichmann et. al. teach that a single amino acid substitution in an antibody is sufficient to retain the antigen binding specificity of the parent antibody (see final paragraph). It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to make only a single substitution in the antibody of Queen et. al. or Co et. al. in positions among those listed in claim 9. It would have been obvious to one of ordinary skill to complete the

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invention in light of the success of Reichmann et. al. in only  
mutating one amino acid of the framework. Knowing that each  
antibody varies slightly in the non-conserved region, and given the  
computer modelling protocol set forth by Queen et. al. one of  
5 ordinary skill would have been motivated to make a single mutation  
in the variable region with the expectation of obtaining a  
functional antibody.

Any inquiry concerning this communication or earlier  
communications from the examiner should be directed to Lila Feisee  
10 whose telephone number is (703) 308-2731.

Any inquiry of a general nature or relating to the status of  
this application should be directed to the Group receptionist whose  
telephone number is (703) 308-0196.

15 Feisee/lf *CP*  
September 29, 1992

*David L. Lacey*  
DAVID L. LACEY  
SUPERVISORY PATENT EXAMINER  
GROUP 180  
*9/20/92*

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

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| FORM PTO-892<br>(REV. 3-78) | U.S. DEPARTMENT OF COMMERCE<br>PATENT AND TRADEMARK OFFICE | SERIAL NO.                         | GROUP UNIT | ATTACHMENT<br>TO<br>PAPER<br>NUMBER |
|                             |  | 715272                             | 1806       | 13                                  |
| NOTICE OF REFERENCES CITED  |  | APPLICANT(S)<br>Paul Carter et al. |            |                                     |

| U.S. PATENT DOCUMENTS |              |      |      |       |           |                            |
|-----------------------|--------------|------|------|-------|-----------|----------------------------|
|                       | DOCUMENT NO. | DATE | NAME | CLASS | SUB-CLASS | FILING DATE IF APPROPRIATE |
| A                     |              |      |      |       |           |                            |
| B                     |              |      |      |       |           |                            |
| C                     |              |      |      |       |           |                            |
| D                     |              |      |      |       |           |                            |
| E                     |              |      |      |       |           |                            |
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| FOREIGN PATENT DOCUMENTS |              |      |         |      |       |           |  |
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|                          | DOCUMENT NO. | DATE | COUNTRY | NAME | CLASS | SUB-CLASS | PERTINENT<br>SHTS.   PP.<br>DWG.   SPEC. |
| L                        |              |      |         |      |       |           |  |
| M                        |              |      |         |      |       |           |  |
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| O                        |              |      |         |      |       |           |  |
| P                        |              |      |         |      |       |           |  |
| Q                        |              |      |         |      |       |           |  |

| OTHER REFERENCES (Including Author, Title, Date, Pertinent Pages, Etc.) |   |
|---|---|
| R   | Sagar et al. Molecular and Cellular Biology 8: 1247 1988  |
| S   | Burgess et al. The Journal of Cell Biology 111: 2129 1990 |
| T   | Tao et al. The Journal of Immunology 143(8) 2595 1989     |
| U   |   |

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| EXAMINER<br>P S | DATE<br>9/29/92 |
|-----------------|-----------------|

\* A copy of this reference is not being furnished with this office action.  
(See Manual of Patent Examining Procedure, section 707.05 (a))

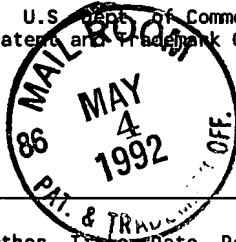
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Applicant  
Paul J. Carter et al.

#19 attach  
1806

Filing Date  
June 14, 1991

Group

| *Examiner Initials | OTHER PRIOR ART (Including Author, Title, Date, Pertinent Pages, Etc.)                  |
|--------------------|---|
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Examiner

Date Considered 9/29/92

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

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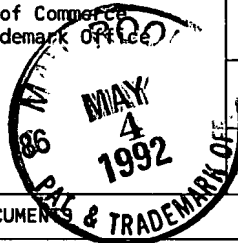
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Applicant  
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| AB                 |                 |         |                |       |          |             |
| AC                 |                 |         |                |       |          |             |
| AD                 |                 |         |                |       |          |             |
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| AJ                 |                 |         |                |       |          |             |
| AK                 |                 |         |                |       |          |             |

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|    | Document Number | Date | Country | Class | Subclass | Translation Yes | Translation No |
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| AL |                 |      |         |       |          |                 |                |
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| AN |                 |      |         |       |          |                 |                |
| AO |                 |      |         |       |          |                 |                |
| AP |                 |      |         |       |          |                 |                |

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

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| AX | Sox et al., <u>Proc. Natl. Acad. Sci. USA</u> 66:975-982 (1970)         |

Examiner

Date Considered

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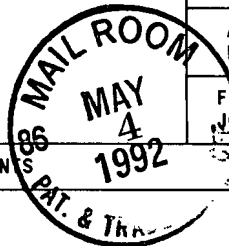
Atty Docket No. 709

Serial No. 07/715272

Applicant Paul J. Carter et al.

Filing Date June 14, 1991

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U.S. PATENT DOCUMENTS


| *Examiner Initials | Document Number | Date | Name | Class | Subclass | Filing Date |
|--------------------|-----------------|------|------|-------|----------|-------------|
| BA                 |                 |      |      |       |          |             |
| BB                 |                 |      |      |       |          |             |
| BC                 |                 |      |      |       |          |             |
| BD                 |                 |      |      |       |          |             |
| BE                 |                 |      |      |       |          |             |
| BF                 |                 |      |      |       |          |             |
| BG                 |                 |      |      |       |          |             |
| BH                 |                 |      |      |       |          |             |
| BI                 |                 |      |      |       |          |             |
| BJ                 |                 |      |      |       |          |             |
| BK                 |                 |      |      |       |          |             |

FOREIGN PATENT DOCUMENTS

|    | Document Number | Date    | Country | Class | Subclass | Translation Yes | Translation No |
|----|-----------------|---------|---------|-------|----------|-----------------|----------------|
| VA | WO 91/09967     | 7/11/91 | PCT     |       |          |                 |                |
| BL |                 |         |         |       |          |                 |                |
| BM |                 |         |         |       |          |                 |                |
| BN |                 |         |         |       |          |                 |                |
| BO |                 |         |         |       |          |                 |                |
| BP |                 |         |         |       |          |                 |                |

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

|    |    |  |
|----|----|--|
| UB | BR | Margni et al., <u>Ann. Rev. Immunol.</u> 6:535-554 (1988)  |
| UC | BS | Fendly, B. M. et al., <u>Cancer Res.</u> 50:1550-1558 (1990)   |
| UD | BT | Neuberger et al., <u>Nature</u> 312:604-608 (1984)   |
| UE | BU | Takeda et al., <u>Nature</u> 314:452-454 (1985)  |
| UF | BV | Snow and Amzel, <u>Protein: Structure, Function, and Genetics</u> 1:267-279, Alan R. Liss, Inc. pubs. (1986) |
| UG | BW | Cheetham, J., <u>Protein Engineering</u> , 2(3): 170-172 (1988)  |
|    | BX |  |

Examiner 

Date Considered 9/29/92

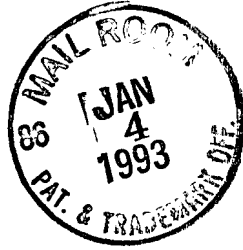
\*Examiner: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

180C 116 #14 of 1-25-93

PATENT DOCKET 709

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of  
Paul J. Carter et al.  
Serial No. 07/715,272  
Filed: 14 JUNE 1991  
For: IMMUNOGLOBULIN VARIANTS



Art Unit: 1806

Examiner: L. FEISEE

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JAN 21 1993  
GROUP 180

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

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

Sir:

The attached materials were received in connection with the prosecution of a foreign patent application corresponding to the captioned case. These materials contain at least two reference citations, the relevance of which is apparent from the communication from the foreign patent office that is also enclosed.

A PTO Form 1449 is submitted herewith to facilitate citation to the record of all references contained in these materials.

Respectfully submitted,  
GENENTECH, INC.  
*Carolyn R. Adler*  
Carolyn R. Adler  
Reg. No. 32,324

December 30, 1992  
460 Point San Bruno Boulevard  
South San Francisco, CA 94080  
415-225-2614

CERTIFICATE OF MAILING (37 CFR 1.8a)

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

*Louise Strasbaugh*  
Louise Strasbaugh

Date: December 30, 1992

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

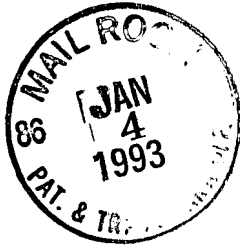
In re Application of

Paul J. Carter et al.

Serial No. 07/715,272

Filed: June 14, 1991

For: Immunoglobulin Variants



) Group Art Unit: 1806

) Examiner: L. FEISEE

) 460 Point San Bruno Boulevard  
) South San Francisco, CA 94080  
) (415) 225-2614  
)

**CERTIFICATION UNDER 37 C.F.R. § 1.97(e)**

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

Sir:

I hereby certify that each item of information contained in this information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of this statement. Pursuant to §1.97, this information disclosure has been filed in a timely fashion and no fees are required.

Respectfully submitted,

GENENTECH, INC.

Carolyn R. Adler  
Reg. No. 32,324

Dated: December 30, 1992

**CERTIFICATE OF MAILING (37 CFR 1.8a)**

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

Louise Strasbaugh

Date: December 30, 1992

AR. 1806



PATENT DOCKET 709

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

|                              |   |                               |     |
|------------------------------|---|-------------------------------|-----|
| In re Application of         | ) | Group Art Unit: 1806          | #16 |
| Paul J. Carter et al.        | ) | Examiner: L. Feisee           |     |
| Serial No. 07/715,272        | ) |                               |     |
| Filed: 14 June 1991          | ) |                               |     |
| For: Immunoglobulin Variants | ) | 460 Point San Bruno Boulevard |     |
|                              | ) | South San Francisco, CA 94080 |     |
|                              | ) | (415) 225-2614                |     |

Amendment and Response

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

Sir:

Responsive to the Office Action mailed 5 October 1992, please amend the claims as follows:

(Amended) A method for making at least a portion of a humanized antibody variable domain comprising amino-acid sequence of a non-human[, import] antibody which is desired to be humanized (import antibody) and a human antibody, comprising the steps of:

- a. obtaining the amino acid sequences of [at least a portion of] an import variable domain and of a consensus human variable domain;
- b. identifying Complementarity-Determining Region (CDR) amino acid sequences in the import and the human amino variable domain sequences;
- c. substituting an import CDR amino acid sequence for the corresponding human CDR amino acid sequence;
- d. aligning the amino acid sequences of a Framework Region (FR) of the import antibody and the corresponding FR of the consensus antibody;
- e. identifying import antibody FR residues in the aligned FR sequences that are non-homologous to the corresponding consensus antibody residues;
- f. determining if the non-homologous import amino acid residue is reasonably expected to have at least one of the following effects:
  1. non-covalently binds antigen directly,

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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 antibody amino acid residue which is [reasonably] expected to have at least one of these effects, substituting that residue for the corresponding amino acid residue in the consensus antibody FR sequence; and
- h. preparing a humanized antibody variable domain having amino acid sequences determined in steps a-g.
2. (Amended) The method of claim 1, having an additional step of determining if any such non-homologous residues are exposed on the surface of the consensus human antibody variable domain or buried within it, and if the residue is exposed, retaining the consensus residue.
3. (Amended) The method of claim 1, having the additional steps, which may be taken between any two steps in the method of claim 1, of searching the import antibody variable domain amino acid sequence for glycosylation sites, determining if any such glycosylation site is reasonably expected to affect the antigen binding ~~or~~ affinity of the antibody, and if so, substituting the glycosylation site into the consensus amino acid sequence.
4. (Amended) The method of claim 1, having the additional steps, which may be taken between any two steps in the method of claim 1, of searching the consensus variable domain amino acid sequence for glycosylation sites which are not present at the corresponding amino acid in the import antibody amino acid sequence, and if the glycosylation site is not present in the import sequence, substituting the import amino acid residues for the amino acid residues comprising the consensus glycosylation site.
5. (Amended) The method of claim 1, having an additional step which comprises aligning import antibody and consensus antibody FR amino acid sequences, identifying import antibody FR amino acid residues which are non-homologous with the aligned consensus FR sequence, and for each such non-homologous import antibody FR amino acid residue, determining if the corresponding consensus antibody amino acid residue represents a residue which is highly conserved across all species at that site, and if it is so conserved, preparing a humanized antibody which comprises the consensus antibody amino acid residue at that site.

7. (Amended) A method for making a humanized antibody comprising providing [at least a portion of] a non-human antibody variable domain amino acid sequence which is desired to be humanized (import antibody) having a CDR and a FR, obtaining the amino acid sequence of at least a portion of a consensus human antibody variable domain having a CDR and a FR, substituting the non-human CDR for the human CDR in the consensus human antibody variable domain, and then substituting an amino acid residue for the consensus amino acid residue at at least one of the following sites:

4L, 35L, 36L, 38L, 43L, 44L, 46L, 58L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H.

[Please add the following new claims 17-21:]

- 17. A method of using a consensus human antibody variable domain amino acid sequence in the preparation of a humanized antibody.-- *11/2/2nd no method steps.*
- 18. In a method for making a humanized antibody variable domain, the improvement consisting of using consensus human antibody variable domain amino acid sequence.--
- 19. A method for making an improved antibody, comprising amino acid sequence from a non-human (import) antibody and a human antibody, comprising the steps of:
- obtaining the amino acid sequences of at least a portion of an import antibody variable domain and of a consensus human antibody variable domain;
  - identifying Complementarity Determining Region (CDR) amino acid sequences in the import and the human amino variable domain sequences;
  - substituting an import CDR amino acid sequence for the corresponding human CDR amino acid sequence;
  - aligning the amino acid sequences of a Framework Region (FR) of the import antibody and the corresponding FR of the consensus antibody;
  - identifying import antibody FR residues in the aligned FR sequences that are non-homologous to the corresponding consensus antibody residues;
  - determining if the non-homologous import amino acid residue is reasonably expected to have at least one of the following effects:
    - non-covalently binds antigen directly,

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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;
- g. for any non-homologous import antibody amino acid residue which is reasonably expected to have at least one of these effects, substituting that residue for the corresponding amino acid residue in the consensus antibody FR sequence; and
  - h. preparing an improved, humanized antibody having amino acid sequences determined in steps a-g; and
  - i. evaluating the antigen binding or immunogenicity of the improved, humanized antibody with respect to the parental antibody.--

--20. A method comprising, following the identification of an antibody by the method of any one of claims 1, 7, or 17-19, the manufacture of the antibody.--

--21. A method comprising, following the identification of an antibody by the method of any one of claims 1, 7, or 17-19, the expression of nucleic acid encoding the antibody.--

#### Remarks

Claims 1-13, and 17-21 are presented herein for examination. Reconsideration of the outstanding rejections is respectfully requested for the reasons that follow. A request for a one-month extension of time to respond is submitted herewith, bringing the due date for this response to 5 February 1993. This response is timely filed.

#### Amendments

Claims 1, 3, 4, 5 and 7 have been amended to indicate that an import antibody is a non-human antibody which is desired to be humanized. Support for this language is found in the specification at page 6, line 27 to page 7, line 3.

Claim 1, step (f) has been amended to clarify that the word "participates" in the  $V_L - V_H$  interface means to affect the proximity or orientation of the  $V_L$  and  $V_H$  regions with respect to one another. Support for this amendment is found on page 15, lines 30-32. New step (h) has been added to claim 1, directed to the physical step of preparation of a humanized antibody variable domain. Support for this step appear throughout the specification.

Claims 3 and 4 have been amended to provide that the additional steps may be taken between

any two steps in the method of claim 1. Claims 2-7 have been amended to clarify that the residues or sequences referred to relate to amino acids.

New claims 17 - 21 have been added. These claims are alternate approaches to claiming the subject matter claimed in claim 1. Additional support for claims 20-21 is found in Example 1.

It is believed that these amendments introduce no new matter. The inventors respectfully request entry of these amendments.

The rejection under 35 U.S.C. § 112, second paragraph

Claims 1-10 were rejected under 35 U.S.C. § 112, second paragraph for indefiniteness. Claims 1, 3, 4, 5 and 7 were rejected for use of the term "import antibody". These claims have been amended to indicate that the import antibody is a non-human antibody which is desired to be humanized.

Claim 1 step (a) was rejected because of the term "consensus human variable domain". The terms "consensus sequence", "consensus antibody" and "consensus human variable domain" are defined at specification page 16, line 29 to page 17, line 17:

The terms "consensus sequence" and "consensus antibody" as used herein refers to an amino acid sequence which comprises the most frequently occurring amino acid residues at each location in all human immunoglobulins of any particular subclass. In preferred embodiments, the consensus human variable domain sequences are derived from the most abundant subclasses in the sequence compilation of Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, National Institutes of Health, Bethesda MD (1987), namely V<sub>L</sub> κ subgroup I and V<sub>H</sub> group III....

As described in the specification, a "consensus human variable domain" would have an amino acid sequence comprising, amino acid residue by residue, the most frequently occurring amino acid residue gathered from a group of human immunoglobulins. The identity of each amino acid residue making up the consensus sequence is determined separately, requiring merely routine tabulation of the amino acids present in each member of a particular immunoglobulin subclass. To expedite the routine tabulation of the most commonly occurring amino acids, workers in the field are referred to the Kabat *et al.* publication cited in the quoted material above, which presents such tabulations.

Claim 1 step (d) was rejected as indefinite as to whether the alignment of the amino acid sequences is a physical or mental step. This rejection is somewhat confusing. The inventors intend claim 1, step (d) to refer to a maximal homology alignment of representations of amino acid sequences, as described in the specification at page 17, lines 18-27. Preparing such a homology alignment typically combines physical and mental actions. This connotation for the phrase "alignment of sequences" is common in the art to which this invention pertains. Step (d) of claim 1 does not require



manipulation of the actual, tangible amino acids, merely manipulation of symbolic representations of the actual amino acids.

Claim 1 step (e) was rejected because of the term "homology"; the Examiner questioned whether conservative amino acids are to be considered as homologs. Identity or homology with respect to a specified amino acid sequence of this invention is defined on page 17, lines 18-27. At lines 22-23, the specification indicates that this invention does "not consider[ing] any conservative substitutions as part of the sequence identity". Conservative substitutions are therefore not considered as homologs.

Claim 1 step (f) was rejected for use of the language "participates". Step (f) of claim 1 refers to an amino acid residue which "participates in the in the V<sub>L</sub> - V<sub>H</sub> interface". This step has been amended to clarify that immunoglobulin residues which so participate are those that affect the proximity or orientation of the V<sub>L</sub> and V<sub>H</sub> regions with respect to one another.

Claim 1 step (f) was also rejected as indefinite as to how one of ordinary skill can determine the effects listed in steps 1-3. Steps 1-3 presently list the following effects an import amino acid residue might have:

1. non-covalently binds antigen directly,
2. interacts with a CDR; or
3. participates in the V<sub>L</sub> - V<sub>H</sub> interface by affecting the proximity or orientation of the V<sub>L</sub> and V<sub>H</sub> regions with respect to one another.

The specification discusses, at pages 13-16, the interactions of amino acid residues within an immunoglobulin and describes at least two methods for evaluating the role of any particular amino acid residue: three dimensional models and assays. As stated at page 14, lines 2-9:

"Three dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen."

The specification provides detailed information how to evaluate the three-dimensional models to determine the various potential effects of amino acid residue changes.

The specification also suggests an alternate method for evaluating the effect of an amino acid residue change. On page 16, lines 14-18, the specification teaches:

"Since it is not entirely possible to predict in advance what the exact impact of a given substitution will be it may be necessary to make the substitution and assay the candidate antibody for the desired characteristic. These steps, however, are *per se*

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routine and well within the ordinary skill of the art."

The inventors submit that methods for determining the effects of amino acid changes are known in the art, and that those skilled in the art would understand what is claimed in step (f).

Claim 1 step (g) was rejected as being indefinite as to what effects are reasonably expected to occur. The word "reasonably" has been deleted from the claim.

Claim 2 was rejected as lacking antecedent basis for "the domain". This claim has been amended to clarify that the intended domain is the consensus human antibody variable domain.

Claims 3 and 4 were rejected as indefinite for not specifying when in the process one would search for the glycosylation sites. These claims have been amended to indicate that one would search for glycosylation sites between any two steps in the method of claim 1.

Claim 5 was rejected as unclear in the use of the phrase "preparing a humanized antibody"; this phrase has now been added by amendment as the last step of claim 1. The phrase is intended to mean the physical making of a humanized antibody, methods for which are described in the specification, including *in vitro* mutagenesis and recombinant engineering. The Examiner also seems to be questioning how claim 5 differs from the previous claims. Claim 5 adds an additional step of determining if a particular amino acid residue in the consensus human variable domain--which differs from the import antibody amino acid residue at that site--also appears at that site in antibodies of other species at that particular site (is conserved). If the particular amino acid residue is conserved across species at that site, than that residue is retained in the humanized antibody, and not substituted by the import antibody amino acid residue at that site, and without requiring evaluation of the impact of such a change on the antibody's characteristics.

Claim 6 was rejected as vague for unclear use of numbers. These numbers refer to particular amino acids in the light (L) and heavy (H) chains of immunoglobulins. By convention, workers in this field generally utilize the immunoglobulin numbering system set forth in Kabat, E. A. *et al.*, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)), as described in the specification at page 8, lines 19-21. The Examiner's attention is drawn to Queen *et al.*, already of record in this case, especially at page 10032 column 1 first paragraph (and reference 38 therein) where antibody amino acid residues are referred to with numbers representing certain positions. It is submitted that workers in the field will understand clearly what is claimed in claim 5.

Claim 7 was rejected as indefinite as to what the method is drawn, and has been amended according to the Examiner's suggestion.

According to the CAFC, a decision as to whether a claim is invalid for indefiniteness "requires a determination whether those skilled in the art would understand what is claimed", Amgen v. Chugai,

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18 USPQ2d 1116, 1030 (CAFC 1991). The presently pending claims use terminology with clear meanings in the field, especially in light of the definitions provided in the specification. The wordings of the claims comply with the requirements of 35 USC § 112, and this rejection should be reconsidered and withdrawn.

The rejection under 35 U.S.C. § 112, first paragraph

Claims 1-11 were rejected under 35 U.S.C. § 112, first paragraph as lacking enablement.

Claims 1 and 7 were rejected as lacking enablement in the language "at least a portion of an import variable domain". These terms have been deleted from the claims.

Claim 1 step (c) was rejected for being unclear as to how one would determine which amino acids are to be substituted. This step recites "substituting an import CDR amino acid sequence for the corresponding human CDR amino acid sequence". The identification of the Complementarity Determining Region (CDR) amino acid sequence of the import and the human amino variable domain sequences is made in previous step (b). To accomplish step (c), therefore, one substitutes the amino acids identified in step (b).

Methods for identifying CDRs and distinguishing them from Framework Residues (FRs) are known in the art. As the specification describes on page two, antibody variable domains of natural light and heavy chains have the same general structure, and each domain comprises four framework (FR) regions, whose sequences are somewhat conserved, connected by three hyper-variable or complementarity determining regions (CDRs) (see Kabat, E. A. *et al.*, *Sequences of Proteins of Immunological Interest*, National Institutes of Health, Bethesda, MD, (1987)). The four framework regions largely adopt a  $\beta$ -sheet conformation and the CDRs form loops connecting, and in some cases forming part of, the  $\beta$ -sheet structure. The CDRs in each chain are held in close proximity by the framework regions and, with the CDRs from the other chain, contribute to the formation of the antigen binding site. The CDR may be identified following three-dimensional modeling of the antibody. The CDRs may also be identified based on comparison of the an antibody amino acid sequence with a known antibody.

Attached as Exhibit A for the Examiner's convenience are pages from the Introduction to Kabat, E. A. *et al.*, *Sequences of Proteins of Immunological Interest, Fifth Edition*, National Institutes of Health, Bethesda, MD, (1991). This work, along with the earlier Kabat compendiums referred to in the specification and other references, guide the practitioner in the numbering of antibody amino acid sequences, and the assignment of particular amino acids to one of the FR or CDR regions. The Examiner's attention is drawn the sections beginning on page xv, the section entitled "Variable Region Sequence" and especially to Table I, page xvi. Table I presents the amino acid residues associated

with FRs and CDRs of the variable domains of immunoglobulin light and heavy chains. See also Figure 1, page xviii, which shows a schematic view of an immunoglobulin; please note the mention in that figure description to the use of a maximum homology alignment to determine the proper numbering of the amino acids (as referred to in the response to the previous § 112 rejection). The inventors submit that the identification of immunoglobulin amino acid residues as belonging to a CDR or to the framework is routine in the art, requiring no undue experimentation.

The specification teaches, in detail, several ways to substitute amino acid residues, including mutagenesis and the construction of nucleic acid encoding the desired sequence. Alanine scanning mutagenesis is described at page 36, line 20 to page 37, line 3. Oligonucleotide-mediated mutagenesis, PCT mutagenesis and cassette mutagenesis are described in the specification at page 39, line 10 through page 44, line 10. The inventors submit that steps (b) and (c) of claim 1 are fully enabled by the specification.

Claim 1 step (f) was rejected as lacking enablement for determining which amino acid residues may be expected to interact with the antigen. At page 29, lines 4-10, the specification teaches that:

"Differences between the non-human import and the human consensus framework residues are individually investigated to determine their possible influence on CDR conformation and/or binding to antigen. Investigation of such possible influences is desirably performed through modeling, by examination of the characteristics of the amino acids at particular locations, or determined experimentally through evaluating the effects of substitution or mutagenesis of particular amino acids."

Techniques for molecular modeling are described on pages 27-28. Experimental evaluation of the role of particular amino acids will utilize assays tailored to the activities of the antibody to be humanized.

More detailed teaching on identifying residues that influence antigen binding is contained in the specification at page 14, line 10 through page 15, line 6, where it is stated:

"A residue that noncovalently directly binds to antigen is one that, by three dimensional analysis, is reasonably expected to noncovalently directly bind to antigen. Typically, it is necessary to impute the position of antigen from the spatial location of neighboring CDRs and the dimensions and structure of the target antigen. In general, only those humanized antibody residues that are capable of forming salt bridges, hydrogen bonds, or hydrophobic interactions are likely to be involved in non-covalent antigen binding, however residues which are separated spatially by 3.2 Angstroms or less may also non-covalently interact. Such residues typically are the relatively larger amino acids, such as tyrosine, arginine, and lysine. Antigen-binding FR residues also typically will

have side chains that are oriented into an envelope surrounding the solvent oriented face of a CDR which extends about 7 Angstroms into the solvent from the CDR domain and about 7 Angstroms on either side of the CDR domain, again as visualized by three dimensional modeling.

The inventors submit that determining whether a residue may be expected to influence antigen binding is routine in the art, in light of the detailed teachings of the specification.

Claim 2 was rejected as lacking enablement for determining which residues are exposed on the surface or buried within the domain. As indicated in the specification, for example at page 91, lines 18-21, the worker in this field would examine the structural models of the import and human sequences to determine if an amino acid residue is exposed on the surface of the domain or is buried within. Evaluation of structural models, preparation of which are described in the specification, to determine whether a residue is exposed or buried is routine and within the ordinary skill in the art.

Claim 3 was rejected as lacking enablement for how one would determine which glycosylation site affects antigen binding, or what comprises "reasonable expectation". The specification teaches, at page 8, lines 22-32, teaches that determining if the glycosylation is reasonably expected to be important for the desired antigen binding and biological activity of the antibody involves determining if the glycosylation site binds to antigen or changes a side chain of an amino acid residue that binds to antigen, or if the glycosylation enhances or weakens antigen binding, or is important for maintaining antibody affinity. As with other aspects of this invention, evaluation of the impact of glycosylation typically is performed by evaluation of molecular models, or experimental evaluation of a modified polypeptide. Such evaluation is routine within the field.

Claims 6, 7 and 9 were rejected as being enabled only with respect to IgG and not other antibody isotypes. The specification, at page 13 lines 14-22, states:

"The humanized antibody will be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub> and IgG<sub>4</sub>. Usually the constant domain is a complement fixing constant domain where it is desired that the humanized antibody exhibit cytotoxic activity, and the class is typically IgG<sub>1</sub>. Where such cytotoxic activity is not desirable, the constant domain may be of the IgG<sub>2</sub> class. The humanized antibody may comprise sequences from more than one class or isotype, and selecting particular constant domains to optimize desired effector functions is within the ordinary skill in the art."

The Examples presented in the specification involve the use of a IgG<sub>1</sub> constant domain. As noted in the specification, specific method steps and illustrative reagents for the use of IgG<sub>1</sub> are taught, as well

as their applicability to other antibody isotypes. The inventors respectfully reminds the Examiner that working examples are not a required component of a patent application. As stated in MPEP § 608.01(h), "There is no statutory requirement for the disclosure of a specific example." Thus, the absence of a working example describing particular embodiments of the invention cannot negative the patentability of the invention. The examples included in the specification, which illustrate the preparation of IgG<sub>1</sub> antibodies, are representative of the manner in which the invention may be practiced. From reading these examples and the detailed description of the invention, the ordinarily skilled artisan would immediately deduce the applicability of the methods described in the specification to other immunoglobulin isotypes.

The Examiner has not made a prima facie case for the § 112, first paragraph rejections, supplying no basis for her skepticism about the scope of the claims. The burden is on the Examiner to provide evidence to support rejections of this sort. "Mere broad generalizations and allegations are insufficient for holding of non-enablement," Ex parte Goeddel, 5 U.S.P.Q. 1449, 1450 (TTAB 1987).

If the Examiner is only prepared to allow claims to exemplified embodiments, what incentive exists for an inventor to disclose the invention to the public? Trade secret protection obviously would be superior to the following circumstances foreseen by the CCPA in In re Goffe, 191 USPO 429, 431 (CCPA 1976):

For all practical purposes, the board would limit appellant to claims involving the specific materials disclosed in the examples, so that a competitor seeking to avoid [literally] infringing the claims would merely have to follow the disclosure in the subsequently-issued patent to find a substitute. However, to provide effective incentives, claims must adequately protect inventors. To demand that the first to disclose shall limit his claims to what he has found will work or to materials which meet the guidelines specified for 'preferred' materials in a process such as the one herein involved would not serve the constitutional purpose of promoting progress in the useful arts. See In re Fuetterer, 50 CCPA 1453, 1462, 319 F.2d 259, 265, 138 USPO 217, 223 (1963).

For a similar case, see In re Strahilevitz, 212 USPO 561 (P.O.B.A. 1982), where the Board was reversed for rejecting as non-enabling an application that was devoid of even a single working example.

The first paragraph of 35 U.S.C. § 112 requires nothing more than objective enablement. Whether this is achieved by the use of illustrative examples or by broad terminology is of no importance, In re Marzocchi et al., 169 USPO 267 (CCPA 1971). Further, an assertion by the Examiner that the enabling disclosure is not commensurate with the protection being sought must be supported by reasons for doubting the truth or accuracy of any statement in the presumptively accurate supporting disclosure. It is also incumbent upon the Examiner to back up such assertions with acceptable evidence or reasoning to substantiate the doubts so expressed, In re Armbruster, 185

USPQ 152 (CCPA 1975), In re Strahilevitz, *op cit*.

See also In re Smith, *supra*, wherein the CCPA reversed an Office ruling that the description in the specification of two categories of prepolymers was not sufficient to support the broad claim for all polymers having a certain desired property. In this case, the court even acknowledged that the specification did not contain language that was precisely identical to the language of the claims. However, the tenor of the specification was that the applicant had made a generic invention rather than one limited to two categories of polymers.

In the present situation, the Examiner has provided no evidence to support the assertion that the invention is not enabled for the preparation of humanized antibodies. Broad claims should be allowed if there is adequate disclosure and where, as in the present situation, there is no pertinent art to prevent such claims. As stated in In re Sus and Schaefer, 134 USPQ 301, 304 (CCPA 1962) (emphasis added):

The public purpose on which the patent law rests required the granting of claims commensurate in scope with the invention disclosed. This requires as much the granting of broad claims on broad inventions as it does the granting of more specific claims on more specific inventions. It is neither contemplated by the public purpose of the patent laws nor required by the statute that an inventor shall be forced to accept claims narrower than his invention in order to secure allowance of his patent.

The inventors submit that in view of the detailed information provided in the specification as discussed above, the specification adequately teaches how to practice the claimed invention. The rejections under 35 USC § 112, first paragraph, should be reconsidered and withdrawn, as they are not statutorily based, are inconsistent with court and Patent Office decisions on the subject, and are contrary to public policy.

The rejection under 35 U.S.C. § 101

Claims 1-4, and 6-8 were rejected under 35 U.S.C. § 101 as being directed to non-statutory subject matter. It is believed that the amendments to the claims made above render moot this ground of rejection.

The rejection under 35 U.S.C. § 112, first paragraph and under 35 U.S.C. § 101

Claims 9-13 were rejected under 35 U.S.C. § 112, first paragraph and under 35 U.S.C. § 101 as lacking utility for the treatment of malignant and autoimmune diseases in humans. The inventors request clarification of this rejection, because none of claims 9-13 are directed to methods of treatment. These claims are directed to humanized antibody variable domains and the polypeptides

Withdraw

Withdraw

of recited sequences. These polypeptides are useful as probes, and in diagnostic assays, as described in the specification at pages 65-66, and need not rely on therapeutic utility.

The rejections under 35 U.S.C. § 102(b) and § 102(a)

Claims 1, 2, and 5-10 were rejected under 35 U.S.C. § 102(b) as being anticipated by Queen *et al.*, and under 35 U.S.C. § 102(a) as being anticipated by Co *et al.*. The inventors respectfully traverse these rejections.

To constitute anticipation, all material elements of a claim must be found in one prior art source. In re Marshall, 198 USPQ 344 (CCPA 1978); In re Kalm, 154 USPQ 10 (CCPA 1967). The inventors will show that neither Queen nor Coe contains all the material elements of these claims, particularly the limitation regarding the use of a consensus sequence.

The rejected claims are directed to the humanization an antibody, namely the combination of amino acid sequence from a non-human antibody desired to be humanized, and from a consensus human variable domain. Methods for preparing such a consensus sequence are fully described in the specification and are discussed above. The inventors believe that the use of a such a consensus sequence achieve a superior result, or a "better" humanized antibody.

The cited prior art utilizes a different approach, which approach had apparently been taken by all other workers in the field prior to the present invention. These workers did not prepare a consensus human antibody to combine with their non-human antibody. Instead, they selected only one human antibody for use, based on the similarity of that human antibody to their non-human antibody. Queen *et al.* state this objective explicitly, at page 10031, column 2 of their paper:

"In selecting a human antibody to provide the variable region framework for the humanized anti-Tac antibody, we reasoned that the more homologous the human antibody was to the original anti-Tac antibody, the less likely would combining the anti-Tac CDRs with the human framework be to introduce distortions into the CDRs."

Queen continues to describe selecting a human heavy chain V region which was 57% identical to their non-human antibody, after dismissing all other candidate as between 30-52% identical to their non-human. They selected the human light chain V region from the same human antibody for their use.

Co *et al.* are equally explicit describing their similar reasoning. At page 2871, column 1 they state:

"First, a human antibody variable region with maximal homology to the mouse antibody is selected to provide the framework sequence for humanization of the mouse antibody. Normally the heavy chain and light chain from the same human antibody are



chosen so as to reduce the possibility of incompatibility in the assembly of the two chains. Based on a sequence homology search against the NBRF protein sequence data base, the antibody Pom was chosen to provide the framework sequences for humanization of Fd79."

The approach of the present invention is quite distinct, in its use of a consensus human variable domain sequence. This consensus sequence might or might not have a high degree of homology with the non-human antibody. Neither Queen *et al.* or Coe *et al.* supply this teaching, and therefore do not anticipate the claimed invention. This rejection should be reconsidered and withdrawn.

#### The rejection under 35 U.S.C. § 103

Claims 3 and 4 were rejected under 35 U.S.C. § 103 as being obvious over Queen *et al.* or Coe *et al.* in view of Wallick *et al.*. Claim 11 was rejected under 35 U.S.C. § 103 as being unpatentable over Queen *et al.* or Coe *et al.* in view of Reichmann *et al.*

None of the cited references teaches or suggests the claimed invention, which involves the preparation humanized antibodies using a consensus human antibody variable domain. Such a method is not suggested in any of the prior references, and absent such a teaching there was no motivation to try the methods described in the present specification.

#### The Obviousness Rejections Do Not Meet the Test of Graham v. Deere

The proper context for determining the issue of obviousness is provided in the seminal decision of Graham v. John Deere, 383 U.S. 1, 148 U.S.P.Q. 459 (1966). In that case, the U.S. Supreme Court set forth the following considerations for deciding this issue:

- (1) The scope and the content of the prior art;
- (2) The difference between the prior art and the claims at issue;
- (3) The level of ordinary skill in the pertinent art; and
- (4) Secondary considerations such as commercial success, long-felt and unresolved needs, failure of others, etc.

##### **a. Scope and Content of the Prior Art.**

1. Queen *et al.* teach the humanization of an anti-Tac antibody. They do not teach the use of a human consensus variable domain to provide the framework for their non-human CDRs.

2. Co *et al.* teach the humanization of an anti-HSV antibody. They do not teach the use of a human consensus variable domain to provide the framework for their non-human CDRs.

3. Wallick *et al.* teach the importance of glycosylation for maintaining the affinity of a monoclonal antibody for its antigen. They do not teach methods for humanization of antibodies, nor teach the creation of a human antibody variable domain consensus sequence.

4. Reichmann *et al.* teach the humanization of an anti-CAMPATH-1 antibody. They do not teach the creation of a human antibody variable domain consensus sequence, or suggest that such might be desirable to provide the framework for their non-human antibody CDRs.

**b. The Differences Between the Prior Art and the Claims at Issue**

The Examiner has chosen various pieces of prior art and concludes that the combination of these references would have rendered the invention obvious.

The prior art shows that it was known as of the filing date to produce antibody fragments comprising sequence from a non-human antibody and from a human antibody. Prior to the present filing date, however, methods were not known which included the use of a consensus human variable domain for mounting the non-human CDRs. There would have been no impetus on the part of the skilled artisan at the filing date to attempt to produce such a consensus sequence or use it in antibody humanization, in view of the teachings of the prior art literature. The cited references do not teach or suggest the claimed invention, alone or in any combination, nor would there have been any reason from these references to practice the claimed methods. The absence of a suggestion of the claimed invention in the art of record precludes the Patent Office from satisfying its initial burden of showing prima facie obviousness.

**c. Level of Ordinary Skill in the Art.**

The Graham inquiries point to a conclusion of non-obviousness of the present claims regardless of the presumed level of skill in the art. However, absent evidence to the contrary, a person of ordinary skill in the art is presumed to be one who essentially follows conventional wisdom and does not undertake to innovate. As stated by the Federal Circuit in Standard Co. v. American Cyanamid Co., 227 U.S.P.Q. 293, 298 (Fed. Cir. 1985):

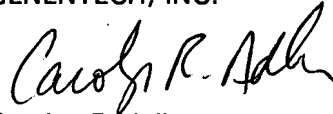
A person of ordinary skill in the art is also presumed to be one who thinks along the line of convention wisdom in the art and is not one who undertakes to innovate, whether by patient, and often expensive, systematic research or by extraordinary insights, it makes no difference which.

The inventors submit that one who followed the conventional wisdom would not have

extrapolated from the teachings of the cited references methods for using a consensus human antibody variable domain for humanizing a non-human antibody. Such an extension of the prior art teachings is based entirely upon hindsight analysis of the inventors' methods. The teachings of this invention should not be considered sufficient to support a conclusion of obviousness in this regard.

The inventors submit that in light of the foregoing amendments and remarks the subject matter defined by the pending claims is useful, enabled, and patentable over the references relied upon by the Examiner, which in no way teach or suggest the invention. The inventors believe the claims are now in condition for allowance and earnestly solicit a Notice to that effect. If the Examiner has any questions, she should feel free to contact the undersigned attorney at the telephone number indicated above.

Respectfully Submitted,  
GENENTECH, INC.



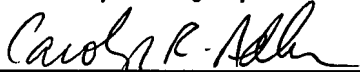
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29 January 1993

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Dated: 29 January 1993

  
Carolyn R. Adler



**SEQUENCES OF  
PROTEINS OF  
IMMUNOLOGICAL  
INTEREST**

**FIFTH EDITION**

Tabulation and Analysis of  
Amino Acid and Nucleic Acid Sequences of Precursors,  
V-Regions, C-Regions, J-Chain, T-Cell Receptors for Antigen,  
T-Cell Surface Antigens,  $\beta_2$ -Microglobulins,  
Major Histocompatibility Antigens, Thy-1, Complement,  
C-Reactive Protein, Thymopoietin, Integrins, Post-gamma Globulin,  
 $\alpha_2$ -Macroglobulins, and Other Related Proteins

1991

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## INTRODUCTION

Our earlier "Variable Regions of Immunoglobulin Chains" (1), the second edition "Sequences of Immunoglobulin Chains" (2) and the third edition "Sequences of Proteins of Immunological Interest" (3) have been further expanded in the Fourth Edition (4) and now in the Fifth Edition to include amino acid and nucleotide sequences of precursors, variable regions, constant regions, J-chains of immunoglobulins,  $\beta$ 2-microglobulins, antigens of the major histocompatibility complex (HLA, H-2, Ia, DR) as well as of Thy-1, complement, T-lymphocyte receptors for antigens, other T-cell antigens of the immunoglobulin superfamily, interleukins, integrins and various other proteins related to immune functions. The identification and sequencing of clones obtained using recombinant DNA techniques has yielded nucleotide sequences of signal, variable, and constant regions of immunoglobulins (5,6), and these nucleotide sequences have been translated into amino acid sequences. The latter have been included in the tables of amino acid sequences with those determined earlier directly by amino acid sequencing and are indicated by an apostrophe followed by CL after the name of the clone. We have continued to use the PROPHET Software Package of the National Center for Research Resources, National Institutes of Health (7,8) to tabulate the sequences.

In compiling the data for this Fifth Edition we have tried to be as up-to-date as possible and have included only sequences which have been published or which have been accepted for publication. Residues which have not been definitely determined have been excluded. It should be remembered that sequences are often published in review articles without detailed documentary evidence. These have often been revised. We have listed such revisions in the notes in many instances; others can readily be found by comparison with sequences in previous editions. We have compiled sequences determined directly as amino acids and have merged with them those translated from the nucleotide sequences thus making all comparable data available. When antibody activities were known, they have been listed after the amino acid and nucleotide sequence tables and are included in the indexes.

When doubts arise as to the validity of any residue in a sequence, the original reference should be examined to ascertain whether definitive evidence for the sequence has been provided. In earlier editions, we have sent the amino acid and nucleotide sequences as stored in the computer to the original authors for verification. If so verified, this was denoted by "checked by author" at the end of each reference and except for the earliest sequences, the date on which the checked sequence was returned to us is given. Whenever possible, nucleotide sequences from GenBank (9) have been used. Programs for converting a GenBank sequence to the codon format of our tables have been developed. The correctness of the table sequence has been verified by converting back into the linear form and comparing with GenBank. When this has been done the sequence is listed as "from GenBank". Recently we have developed newer programs that automatically process a GenBank entry completely - e.g.: extract the relevant feature, determine the appropriate table, and perform alignment. In such cases, the reference will end with "processed automatically from GenBank:" followed by a list of the GenBank accession numbers from which the data was obtained. Some nucleotide sequences were transmitted to us by electronic mail, and they are indicated by "received from authors through e-mail." If the sequences were entered by us from the literature and then checked with GenBank, this is indicated by "checked with GenBank". We have entered many nucleotide sequences which were not then available from GenBank. In general, we have not included stretches of sequence such as enhancers, switch regions and introns. Much information about such sequences may be found in references (10-13). We have also had access to the Protein Information Resource (14) and to the European Molecular Biology Laboratories Data Base (15).

It is also possible, by examining the numbers of sequences at the

end of each table and the summary tables, to evaluate the probability that a given amino acid at a given position may not be correct. This is most readily done for the framework residues of the V-region and for the C-region; in the complementarity-determining regions this is more difficult because of the high variability.

### AMINO ACID SEQUENCES

The first column in each table gives the residue number. Except for complement, T-cell surface antigens, integrins and miscellaneous proteins, the second column is a tabulation of invariant residues. Since exceptions to invariance are found, the frequency, if less than 1.0 and greater than or equal to 0.95, is indicated alongside the residue listed as invariant; when only a single sequence is available, this is not given. These rows are shaded in grey. Each sequence is tabulated in each subsequent column. Three dashes (---) indicate that no amino acid is present at that position and that the sequence continues. In all instances residues considered uncertain by the authors have not been included in the table. In some instances the symbol # is used to indicate that several amino acid residues were found in one position, and these residues are listed in the notes. The four columns at the end of each table give:

1. the number of residues sequenced at that position,
2. the number of different amino acids found at that position,
3. the number of times the most common amino acid occurred and that amino acid in parentheses, and
4. the variability.

These columns are included only in tables with more than five sequences. Miscellaneous tables have only columns corresponding to the first two above.

Variability is calculated (16) as:

$$\text{Variability} = \frac{\text{Number of different amino acids occurring at a given position}}{\text{Frequency of the most common amino acid at that position}}$$

An invariant position would have a variability of one; if 20 amino acids occurred with equal frequency, the variability would be 20 divided by 0.05 equals 400. If, for example, four different amino acids Ser, Asp, Pro, and Thr occurred at a given position, and of 100 sequences available at that position, Ser occurred 80 times, the variability would be  $4/0.8 = 5$ . When any of the amino acid residues, sequenced directly as amino acids, were not identified completely and are listed as Glx (or Asx), two values, separated by a comma, are given in the last three columns. The first value in each of these columns is calculated assuming that only one of the two possibilities, e.g., Glu or Gln (or Asp or Asn) occurred, while the second considers that both were present and maximizes variability. In the variability plots, the horizontal bars indicate the two values.

When two or more amino acids are most common and occur with equal frequency, they are tabulated as a note, and the symbol + is used in the next to last column. If no sequence data have been reported for any position, there are no entries in the last four columns. Variability is not calculated for insertions or if only a single sequence is known. When the translated sequence of a clone corresponds to a previously listed sequence of a plasmacytoma from

which it was prepared, variability computation is indicated by an antibody specificity constant if available; rabbit heavy chain domain of the rabbit sequence is given; usually the most ne included, especially Notes are of two t: the symbol #, and s;

### Signal Sequences

The signal (precursor) chains are listed light chains, for total of nine precursor sequencing of signal sequences from DNA acid residues in Genomic DNA clones the coding sequence -4, and in rare cases leader peptide to for positions -4 t

The signal amino antigens,  $\beta$ 2-microglobulin, complement proteins are listed

By conformational Leu-Leu-Leu-Trp-V $\alpha$  alpha helical conformations in the four amino terminus (20).

### Variable Region Sequences

The variable regions contain hypervariable (27-30) chains, labeled with hypervariable segments of light examination of segments of light chains aligned in These and the three were hypothesized regions or segments contact with variable high resolution x-ray been verified by all antibodies hypervariable region antibody combining the framework (1) framework segments complementarity-determining the three CDRs and Figures 3-47 have comments are given bibliography. The Table I.

which it was prepared, only one sequence is listed so that the variability computations are not affected, and a note is included. If a given sequence is associated with any antibody activity, this is indicated by an asterisk alongside the protein heading, and the antibody specificities are given in a separate list with binding constants if available. The notes list the a-allotypes for the rabbit heavy chain V-region and the b-allotypes for the constant domain of the rabbit kappa light chain. A key reference to the sequence is given; generally the most recent reference since it is usually the most nearly complete, but often several references are included, especially when revisions of a sequence have been made. Notes are of two types: general notes about a table indicated by the symbol #, and specific notes indicated by the sequence number.

#### Signal Sequences

The signal (precursor) amino acid sequences of immunoglobulin chains are listed as human, mouse, and miscellaneous for kappa light chains, for lambda light chains, and for heavy chains for a total of nine precursor tables. They were obtained either by direct sequencing of signal proteins (17-19) or by translating nucleotide sequences from DNA clones. Signal segments range from 17-29 amino acid residues in length and are thus numbered from -29 to -1. Genomic DNA clones contain introns of varying length that interrupt the coding sequence of the precursor within the codon for position -4, and in rare cases for position -6. Thus, the L-gene encodes the leader peptide to position -4 and the 5' end of the V-gene codes for positions -4 to -1.

The signal amino acid sequences of the T-cell receptors for antigens,  $\beta$ 2-microglobulins, major histocompatibility complex proteins, complement components, integrins, and other related proteins are listed in separate tables.

By conformational energy calculations, the core  $V_L$  hydrophobic Leu-Leu-Leu-Trp-Val-Leu-Leu-Leu (MOPC321, MOPC63) exists in an alpha helical conformation, terminated by chain reversal conformations in the four C-terminal residues Trp-Val-Pro-Gly; the four amino terminal residues are compatible with the alpha helix (20).

#### Variable Region Sequences

The variable regions (21) of immunoglobulins have been shown to contain hypervariable segments in their light (16,22-26) and heavy (27-30) chains, of which certain residues have been affinity labeled with haptenic determinants (31-44). Three hypervariable segments of light chain were delineated from a statistical examination of sequences of human  $V_L$ , human  $V_L$ , and mouse  $V_L$  light chains aligned for maximum sequence similarity (16,23,24,27). These and the three corresponding segments of the heavy chains (27) were hypothesized (16,27) to be the complementarity-determining regions or segments (CDR) containing the residues which make contact with various antigenic determinants, several years before high resolution x-ray structures were determined, and this has now been verified by X-ray diffraction studies at high resolution for all antibodies examined Figures 3-47. The proposed fourth hypervariable region (cf. 30) of heavy chains is not part of the antibody combining site (27). The rest of the V-region constitutes the framework (16,27,45-54). It is convenient to identify the framework segments (FR1, FR2, FR3, and FR4) and the complementarity-determining segments (CDR1, CDR2, and CDR3) with the three CDRs separating the four FRs. The CDRs in the stereo Figures 3-47 have solid circles for each residue. References and comments are given with each figure and are not listed in the bibliography. The residue numbers for these segments are given in Table I.



TABLE I

Amino Acid Residues Associated with Framework(FR) and Complementarity Determining Regions (CDR) of the Variable Domains of Immunoglobulin Light ( $V_L$ ) and Heavy ( $V_H$ ) Chains

| Segment          | Light Chain  | Heavy Chain  |
|------------------|--|--|
| FR1              | 1-23 (with an occasional residue at 0, and a deletion at 10 in $V_L$ chains) | 1-30 (with an occasional residue at 0)                                 |
| CDR1             | 24-34 (with possible insertions numbered as 27A,B,C,D,E,F)                   | 31-35 (with possible insertions numbered as 35A,B)                     |
| FR2 <sup>a</sup> | 35-49 <sup>a</sup>   | 36-49  |
| CDR2             | 50-56  | 50-65 (with possible insertions numbered as 52A,B,C) <sup>b</sup>      |
| FR3              | 57-88  | 66-94 (with possible insertions numbered as 82A,B,C)                   |
| CDR3             | 89-97 (with possible insertions numbered as 95A,B,C,D,E,F)                   | 95-102 (with possible insertions numbered as 100A,B,C,D,E,F,G,H,I,J,K) |
| FR4              | 98-107 (with a possible insertion numbered as 106A)                          | 103-113  |

<sup>a</sup> Five Basilea rabbits ( $\lambda$ ) immunized with type II pneumococci and which produced anti-type II pneumococcal polysaccharide had Met at position 48 and an insertion of four amino acid residues between positions 48 and 49; in four of the five the sequence was Glu, Leu, Lys, Ser and the fifth was Trp, Leu, Arg, Lys (53,54,63,64); the others were not sequenced at these positions (for references see table of rabbit  $\lambda$  amino acid sequences.)

<sup>b</sup> In the rabbit, Mage et al. (65) consider position 65 in  $V_H$  to be in FR3, since it is allotype related.

The V-genes for the and the J-minigenes kappa light chains. E by recombination and by the J-minigene. I occur at different positions residues may result of the inserted residue for better alignment the V-gene region. I times more frequently.

The V-genes for the and are followed by extensive variation ability to be read boundary between D and acid position. In addition sequences vary by a of D-J joining appears between V and D and and correlates with B cells (60). The has therefore been re evidence suggesting perhaps a minigene nucleotides. Light  $V_L$ - $J_L$  junction (62), probably results from in fetal and neonatal and 17/146 RNA sequences lower than in adults regulated both in T diversity but are te

In the tables of v horizontal lines for chain, MPC 11, has between position 1. have internal deletions

The V-genes for the light chains code to amino acid position 95, and the J-minigenes from position 97 to 107 for lambda and 108 for kappa light chains. Position 96 is usually the site of V-J joining by recombination and may be coded partly by the V-gene and partly by the J-minigene. Because the site of V-J recombination could occur at different positions within a codon, different amino acid residues may result at this position. We have changed the location of the inserted residues from 97A-F (2) to 95A-F, since it makes for better alignment by confining chains of different lengths to the V-gene region. In mouse  $V_k$  chains, J1 and J2 were used 5 to 10 times more frequently than J4 and J5 (55).

The V-genes for the heavy chains code up to amino acid position 94 and are followed by the D- and J-minigenes. Because of the extensive variation in the lengths of D-minigenes, and their ability to be read in different reading frames (56), the exact boundary between D and J is not always located at the same amino acid position. In addition, the lengths of the J encoded amino acid sequences vary by a few amino acid residues. Moreover, the process of D-J joining appears to involve insertions of extra nucleotides between V and D and between D and J, termed the N region (57-61) and correlates with the appearance of terminal deoxytransferase in B cells (60). The original numbering system for the heavy chains has therefore been retained. Wysocki et al. (61) have provided some evidence suggesting a non-random origin for the  $V_H$ - $D_H$  junction, perhaps a minigene, rather than random addition of the N nucleotides. Light chains do not appear to have N sequences at the  $V_L$ - $J_L$  junction (62), but show an additional residue 95A which probably results from  $V_L$ - $J_L$  joining. N sequences are generally rare in fetal and neonatal mouse  $V_H$ - $D$ - $J_H$  junctions (62), only 1/87 DNA and 17/146 RNA sequences contained N regions, an incidence much lower than in adults indicating that N insertion is developmentally regulated both in T and B cells. P elements also contribute to diversity but are templated (62a).

In the tables of V-regions, the FR and CDR are separated by horizontal lines for convenience in reading. One mouse kappa light chain, MPC 11, has an extra segment of 12 amino acid residues between position 1 and the signal sequence (66). Several chains have internal deletions.

Figure 1 (50) shows the domain structure for IgG1 protein EU. Numbering on the left half indicates the CDR for the light and heavy chains (50), while that on the right half gives the EU numbering (67).

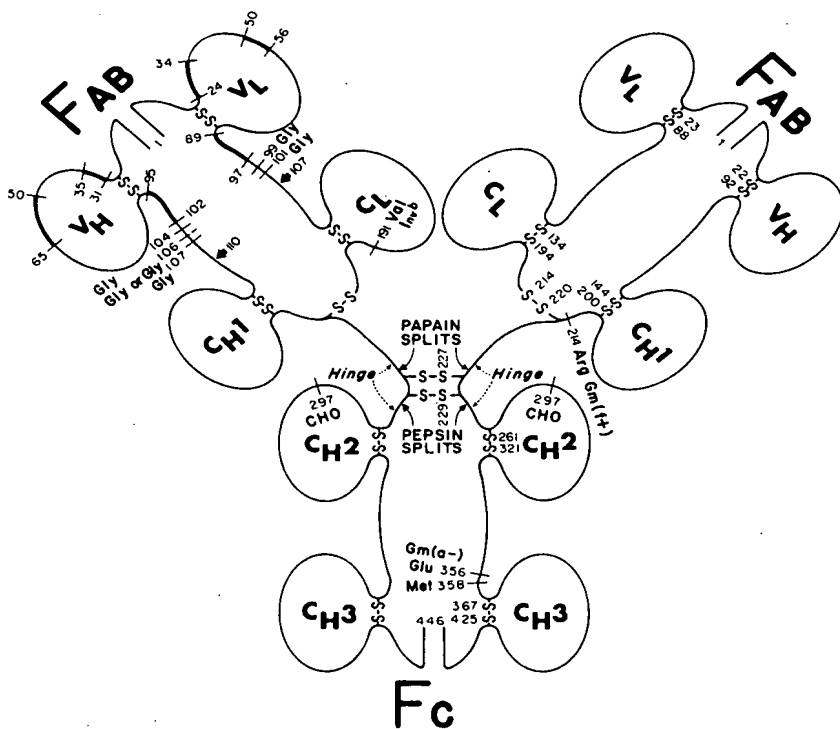


FIG. 1. Schematic view of four-chain structure of human IgG1 molecule. Numbers on right side: actual residue numbers in protein EU [Edelman et al. (67)]; Numbers of Fab fragment on left side aligned for maximum homology; light chains numbered as in Wu and Kabat (16) and heavy chains as in Kabat and Wu (27). Heavy chains of EU have residue 52A, three residues 82A,B,C, and lack residues termed 100A,B,C,D,E,F,G,H,I,J,K, and 35A,B. Thus residue 110 (end of variable region) is 114 in actual sequence. Hypervariable regions, complementarity-determining segments or regions (CDR): heavier lines.  $V_L$  and  $V_H$ : light and heavy chain variable region;  $C_{H1}$ ,  $C_{H2}$ ,  $C_{H3}$ : domains of constant region of heavy chain;  $C_L$ : constant region of light chain. Hinge region in which two heavy chains are linked by disulfide bonds is indicated approximately. Attachment of carbohydrate is at residue 297. Arrows at residues 107 and 110 denote transition from variable to constant regions. Sites of action of papain and pepsin and locations of a number of genetic factors are given. Modified from 50.

Critical understanding sites and the genetic antibody complementarity evaluation of a large and especially of the C and heavy chains of immunoglobulin to locate residues in determinants (68,69) at combining sites will depend on scope  $V_H$  and  $V_L$  chains must be resolved. The immunochemical data in addition to other methods resolution X-ray crystallography.

Through the generous cooperation been provided with the Fab molecules,  $V_H$  dimer. Drs. Eduardo Padlan and shown. Legends and key model.

Critical understanding of the architecture of antibody combining sites and the genetics of the generation of diversity and of antibody complementarity depends to a great extent on the evaluation of a large number of sequences of the variable regions and especially of the complementarity-determining segments of light and heavy chains of immunoglobulins of different species. Ability to locate residues in the site making contact with antigenic determinants (68,69) and to predict (70) the structures of antibody combining sites will depend heavily upon such sequences. The role and scope  $V_H$  and  $V_L$  chains in contributing to binding of the epitope must be resolved. This can be often accomplished by use of immunochemical data in defining antibody combining sites (68,70-73) in addition to other methodologies such as 2D-NMR (71,51) or high resolution X-ray crystallography.

Through the generous cooperation of X-ray crystallographers we have been provided with the  $\alpha$ -carbon coordinates of almost all available Fab molecules,  $V_H$  dimers and antigen-antibody complexes from which Drs. Eduardo Padlan and Chantal Abergel made the stereo models shown. Legends and key references for each are listed with the model.

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of Fab and antigen  $\alpha$ -carbon coordinates in the text where these and Chantal Abergel (stereodiagrams from I am indebted to them. Dr. the x-ray structure of I am indebted to Drs. Rose Mage the introduction and to I am indebted to Drs. Rose Mage Margulies made helpful text. Dr. Lawrence A. use  $V_H$  and  $V_L$  families as  $V_H$  and  $V_L$  sequences into

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of  
Paul J. Carter et al.  
Serial No. 07/715,272  
Filed: 14 June 1991  
For: Immunoglobulin Variants

Group Art Unit: 1806  
Examiner: L. Feisee

460 Point San Bruno Boulevard  
South San Francisco, CA 94080  
(415) 225-2614

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

Honorable Commissioner of Patents  
and Trademarks  
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Sir:

Applicant petitions the Commissioner of Patents and Trademarks to extend the time for response to the Office action dated 05 October 1992 for one month(s) from 5 January 1993 to 5 February 1993. The extended time for response does not exceed the statutory period.

Please charge Deposit Account Number 07-0630 in the amount of \$110. 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.

*Carolyn R. Adler*  
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Reg. No. 32,324

Date: 29 January 1993

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#15  
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Attorney Docket No. 709  
 Examiner: L. Feisee  
 Group Art Unit 1806

In re Application of: Paul J. Carter et al.

Serial No.: 07/715,272

Filed: 14 June 1991

For: Immunoglobulin Variants

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ATTN: CAROLYN R. ADLER  
460 POINT SAN BRUNO BLVD.  
SOUTH SAN FRANCISCO, CA 94080

ART UNIT PAPER NUMBER

1805

17

DATE MAILED: 05/19/93

This is a communication from the examiner in charge of your application.  
COMMISSIONER OF PATENTS AND TRADEMARKS

This application has been examined  Responsive to communication filed on 2/3/93  This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s) 0 days from the date of this letter.  
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

**Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:**

- 1.  Notice of References Cited by Examiner, PTO-892.
- 2.  Notice re Patent Drawing, PTO-948.
- 3.  Notice of Art Cited by Applicant, PTO-1449.
- 4.  Notice of Informal Patent Application, Form PTO-152.
- 5.  Information on How to Effect Drawing Changes, PTO-1474.
- 6.  \_\_\_\_\_

**Part II SUMMARY OF ACTION**

1.  Claims 1-23 are pending in the application.

Of the above, claims 14-16 are withdrawn from consideration.

2.  Claims \_\_\_\_\_ have been cancelled.

3.  Claims 12 and 13 are allowed.

4.  Claims 1-13, 17-21 are rejected.

5.  Claims \_\_\_\_\_ are objected to.

6.  Claims \_\_\_\_\_ are subject to restriction or election requirement.

7.  This application has been filed with Informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.

8.  Formal drawings are required in response to this Office action.

9.  The corrected or substitute drawings have been received on \_\_\_\_\_ Under 37 C.F.R. 1.84 these drawings are  acceptable.  not acceptable (see explanation or Notice re Patent Drawing, PTO-948).

10.  The proposed additional or substitute sheet(s) of drawings, filed on \_\_\_\_\_ has (have) been  approved by the examiner.  disapproved by the examiner (see explanation).

11.  The proposed drawing correction, filed on \_\_\_\_\_, has been  approved.  disapproved (see explanation).

12.  Acknowledgment is made of the claim for priority under U.S.C. 119. The certified copy has  been received  not been received  been filed in parent application, serial no. \_\_\_\_\_; filed on \_\_\_\_\_

13.  Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.

14.  Other

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Some of the rejections under 35 USC 112 second paragraph have been obviated in view of the amendments to the claims. However, the following rejection still remain. The language "consensus human variable domain" is still unclear despite the description in the specification. It is unclear whether the consensus human variable domain is a culmination of different variable domains or a single universal variable domain which is homologous to other human variable domains.

With regards to the language "import amino acid", it is suggested the import amino acid be described in the following manner: "an import antibody comprising the amino acid sequence of a non-human antibody which binds to ...". The language "reasonably expected" is unclear since it is not known what criteria determines what is "reasonable".

Claim 1 remains rejected and new claims 19-21 are rejected under 35 USC 112 first paragraph as lacking enablement for the language "at least a portion" for the same reasons as set forth in pages 3 and 4 of paper #13.

Applicant states that this language has been deleted from claim 1, but, this is not the case. This language has been moved to the beginning of the claim and the claim contains the same objectionable language, therefore, the rejection set forth

*Withdraw*  
*Withdraw*  
*Withdraw*

previously still applies.

The rejection of claims 1-4, 6-8 under 35 USC 101 is withdrawn in view of the amendment to the claims.

The rejection of claims 9-13 as lacking utility is withdrawn in view of the argument set forth in the letter of 2/3/93.

The objection to the specification and the rejection of claims 1-11 under 35 USC 112 first paragraph is maintained and newly added claims 17-21 are rejected for the reasons of record.

The language "at least a portion" still remains in claim 1 and newly added claims 19-21. Therefore, the rejection set forth previously on pages 3-4 of paper #13 still applies. With regards to substituting an import CDR in place of the human CDR, the rejection still applies, since there is no clear guidance in the specification to enable one of ordinary skill in the art to make the human "consensus variable region" which is to contain the claimed substitution. It is true that once the amino acid sequences are known, it is routine to determine the CDRs according to Kabat, and substitute the rodent CDRs in place of the human CDRs. However, the only guidance presented in the specification with regards to the substitutions is the amino acid sequences of SEQ ID NO: 3 and 4, which are specific variable regions. The specification vaguely alludes to variable domain sequences which are derived from the most abundant subclasses but shows no way of making such variable domains. The fact remains

that applicant has not clearly taught how to determine which amino acids are the ones to be substituted since there is only a single example of the appropriate variable region which is to support the substitutions.

The rejection of claim 2 with regards to determining which residues are surface or buried residues is withdrawn in view of the argument presented explaining that computer modeling is well known in the art to determine the position of various amino acid residues.

The rejection of claims 1 and 3 with regards to the language "reasonably" and newly~~added~~ claim 19 is maintained, since there is no set standard for determining what is reasonable interaction, or interfacing or what amount of glycosylation reasonably affects binding. *Withdraw*

The rejection of claims 6,7 and 9 based on the specific amino acids sequences which are only relevant to IgG is maintained. Applicant argues that he is not required to exemplify every embodiment, however, if the claim requires the presence of a certain sequence which does not exist in a particular isotype, than clearly there is a lack of enablement for making that particular embodiment of the claim.

The rejections of claims 1,2,5-10 under 35 USC 102(a) and 102(b) is maintained and newly added claims 17-21 are rejected under 35 USC 102(a) and 35 USC 102(b) as being anticipated by

Serial No.

-5-

Art Unit

Queen et. al. or Co et. al. for the same reasons as set forth in the previous Office action.

Applicant argues that the distinction between the prior art and the instant invention is that the framework amino acids are chosen from a consensus human variable region. However, as previously mentioned there is no clear indication of what is meant by consensus variable regions and as it is stated by applicant on page 14 of the response the chosen amino acids in the references may indeed be the same as what applicant calls consensus variable domain sequences.

The rejection of claims 3 and 4 under 35 USC 103 is maintained for the same reasons as set forth in the previous Office action. Applicant again argues that the use of "consensus region variable domains" is different from the prior art methods, however, as previously mentioned, the consensus amino acids may be the same as the most homologous murine antibodies of the references. The lack of clarity of the language "consensus amino acid region" is what allows this particular interpretation of the claims.

Claims 17,18, 20 and 21 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject, matter which applicant regards as the invention. New claims 17,18,20 and 21 are indefinite in that there are no discrete method steps.



Serial No.

-6-

Art Unit


**THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 C.F.R. § 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 C.F.R. § 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.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Lila Feisee whose telephone number is (703) 308-2731.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Feisee/em  
May 18, 1993

  
DAVID L. LACEY  
SUPERVISORY PATENT EXAMINER  
GROUP 180  
5/19/93

FORM PTO-1449

U.S. Dept. of Commerce  
Patent and Trademark Office

Atty Docket No.  
709

Serial No.  
07/715,272

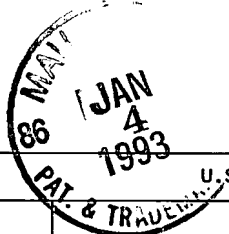
LIST OF DISCLOSURES CITED BY APPLICANT

(Use several sheets if necessary)

Applicant  
Paul J. Carter et al. #14

Filing Date  
June 14, 1991

Group  
1806



U.S. PATENT DOCUMENTS

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

FOREIGN PATENT DOCUMENTS

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

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

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

Examiner

*[Signature]*

Date Considered

4/29/93

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

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25 SEP 22 1993  
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GENENTECH, INC.  
460 Point San Bruno Boulevard  
South San Francisco, CA 94080  
(415) 225-2614

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25 SEP 23 1993  
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Attorney Docket No. 709  
Examiner: L. FEISEE  
Group Art Unit 1806

In re Application of: Paul J. Carter et al.

Serial No.: 07/715272

Filed: June 14, 1991

For: Immunoglobulin Variants

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

Sir:

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

The fee has been calculated as shown below.

|   | (Col. 1)                         |       | (Col. 2)                        |   | (Col. 3)      |             |            |
|---|----------------------------------|-------|---------------------------------|---|---------------|-------------|------------|
|   | Claims Remaining After Amendment |       | Highest No. Previously Paid For |   | Present Extra | Rate        | Addit. Fee |
| Total   | * 17                             | Minus | ** 21                           | = | 0             | x 22=       | \$ 0       |
| Indep.  | * 6                              | Minus | *** 10                          | = | 0             | x 74=       | \$ 0       |
| _____ First Presentation of Multiple Dep. Claim |                                  |       |                                 |   |               | + 230=      | \$         |
|   |                                  |       |                                 |   |               | TOTAL . . . | \$ 0       |

\*If the entry in Col. 1 is less than the entry in Col. 2, write "0" in Col. 3.  
 \*\*If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, write "20" in this space.  
 \*\*\*If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, write "3" in this space.  
 The "Highest Number Previously Paid For" (Total or Independent is the highest number found from the equivalent box in col. 1 of a prior amendment or the number of claims originally filed.)

- No additional fee is required.
  - Please charge any additional fees, including any fees necessary for extensions of time, or credit overpayment to Deposit Account No. 07-0630. **A duplicate copy of this sheet is enclosed.**
- Any additional filing fees required under 37 CFR 1.16.  
 Any patent application processing fees under 37 CFR 1.17.

Dated: September 20, 1993

*Janet E. Hasak*  
(Attorney of Record)

Janet E. Hasak  
Registration No. 28,616

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the U.S. Postal Service on the date below as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Dated: 20 Sept 1993

*Louise Strasbaugh*  
Louise Strasbaugh

81806

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PATENT DOCKET 709

BOX AF

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE



In 1993 application of  
arter et al.

Serial No. 07/715272

Filed: June 14, 1991

For: Immunoglobulin Variants

) Group Art Unit: 1806

) Examiner: L. FEISEE

) 460 Point San Bruno Boulevard  
) South San Francisco, CA 94080  
) (415) 225-1896

#1806  
SUGG  
9-20-93

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

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

Sir:

Applicant petitions the Commissioner of Patents and Trademarks to extend the time for response to the Office action dated 19 May 1993 for one month(s) from 19 August 1993 to 19 September 1993. The extended time for response does not exceed the statutory period.

Please charge Deposit Account Number 07-0630 in the amount of \$110 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.

*Janet E. Hasak*

Janet E. Hasak  
Reg. No. 28,616

Date: September 20, 1993

CS14005 09/24/93 07715272

07-0630 140 115 110.00CH

CERTIFICATE OF MAILING (37 CFR 1.8a)

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

*Louise Strasbaugh*  
Louise Strasbaugh

Date: September 20, 1993

Amendment under 37 CFR 1.116  
Expedited Procedure  
Examining Group 1806

PATENT DOCKET 709



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

|                              |   |                               |
|------------------------------|---|-------------------------------|
| Application of               | ) | Group Art Unit: 1806          |
| Paul J. Carter et al.        | ) | Examiner: L. FEISEE           |
| Serial No. 07/715272         | ) |                               |
| Filed: June 14, 1991         | ) |                               |
| For: Immunoglobulin Variants | ) | 460 Point San Bruno Boulevard |
|                              | ) | South San Francisco, CA 94080 |
|                              | ) | (415) 225-1896                |

19/23 (ne)  
SUGG  
9-20-93

AMENDMENT AFTER FINAL REJECTION PURSUANT TO 37 CFR § 1.116

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

Sir:

This is responsive to the Office Action mailed May 19, 1993, which is a final rejection of claims 1 to 11 and 17 to 21. Claims 12 & 13 have been allowed. A request for a one-month extension of time to respond is submitted herewith, bringing the due date for this response to September 20, 1993 as September 19 is a Sunday. This response is timely filed.

IN THE SPECIFICATION:

On page 19, line 3, please delete "effect or" and insert --effector--.

On page 87, please amend Table 1 as follows:

line 6, please amend the heading of the second to last column to read

--K<sub>d</sub><sup>+</sup>--;

lines 6 & 7, please amend the heading of the last column to read --Relative

w014.u

Do not enter

cell proliferation<sup>±</sup>--;

line 8, please delete "proliferation<sup>±</sup>";

line 10, please amend the second to last column to read --25-- and the last column to read --102--.

**IN THE CLAIMS:**

Please cancel claims 14-16 and 18 from the application, without prejudice. Please amend claims 1, 3, 7, 17, 19, 20 and 21 as follows:

- 1: (Twice amended) A method for making [at least a portion of] a humanized antibody variable domain comprising amino acid sequences of an import antibody comprising a non-human antibody which is desired to be humanized [(import antibody)] and a human antibody, comprising the steps of:
- a. obtaining the amino acid sequences of an import variable domain and of a consensus human variable domain of a human immunoglobulin subgroup; *New*
  - b. identifying Complementarity Determining Region (CDR) amino acid sequences in the import and the human amino variable domain sequences;
  - c. substituting an import CDR amino acid sequence for the corresponding human CDR amino acid sequence;
  - d. aligning the amino acid sequences of a Framework Region (FR) of the import antibody and the corresponding FR of the consensus antibody;
  - e. identifying import antibody FR residues in the aligned FR sequences that are non-homologous to the corresponding consensus antibody residues;
  - f. determining if the non-homologous import amino acid residue is [reasonably] expected to have at least one of the following effects:
    1. non-covalently binds antigen directly,
    2. interacts with a CDR; or
    3. participates in the V<sub>L</sub> - V<sub>H</sub> interface by affecting the proximity or orientation of the V<sub>L</sub> and V<sub>H</sub> regions with respect to one another;

- g. for any non-homologous import antibody amino acid residue which is expected to have at least one of these effects, substituting that residue for the corresponding amino acid residue in the consensus antibody FR sequence; and
- h. preparing a humanized antibody variable domain having amino acid sequences determined in steps a-g.

In claim 3, line 4, please delete "reasonably".

- 7. (Twice amended) A method for making a humanized antibody comprising providing an import antibody comprising a non-human antibody variable domain amino acid sequence which is desired to be humanized [(import antibody)] having a CDR and a FR, obtaining the amino acid sequence of [at least a portion of] a consensus human antibody variable domain of a human immunoglobulin subgroup, having a CDR and a FR, substituting the non-human CDR for the human CDR in the consensus human antibody variable domain, and then substituting an amino acid residue for the consensus amino acid residue at at least one of the following sites:  
4L, 35L, 36L, 38L, 43L, 44L, 46L, 58L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H.
- 17. (Amended) A method of making a humanized antibody variable domain comprising the step of substituting Complementary Determining Region (CDR) amino acid residues of a variable domain of a non-human antibody for the corresponding CDR amino acid residues of [using] a consensus human antibody variable domain amino acid sequence of a human immunoglobulin subgroup [in the preparation of a humanized antibody].

19. (Amended) A method for making an improved antibody, comprising amino acid sequences from an import antibody comprising 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 antibody variable domain and of a consensus human antibody variable domain of a human immunoglobulin subgroup;
  - b. identifying Complementarity Determining Region (CDR) amino acid sequences in the import and the human [amino] variable domain sequences;
  - c. substituting an import CDR amino acid sequence for the corresponding human CDR amino acid sequence;
  - d. aligning the amino acid sequences of a Framework Region (FR) of the import antibody and the corresponding FR of the consensus antibody;
  - e. identifying import antibody FR residues in the aligned FR sequences that are non-homologous to the corresponding consensus antibody residues;
  - f. determining if the non-homologous import amino acid residue is [reasonably] expected to have at least one of the following effects:
    1. non-covalently binds antigen directly,
    2. interacts with a CDR; or
    3. participates in the  $V_L - V_H$  interface by affecting the proximity or orientation of the  $V_L$  and  $V_H$  regions with respect to one another;
  - g. for any non-homologous import antibody amino acid residue which is [reasonably] expected to have at least one of these effects, substituting that residue for the corresponding amino acid residue in the consensus antibody FR sequence[; and] ; and
  - h. preparing an improved, humanized antibody having amino acid sequences determined in steps a-g; and
  - i. evaluating the antigen binding or immunogenicity of the improved,



humanized antibody with respect to the parental antibody.

20. A method of making a humanized antibody comprising the step of making the antibody identified [, following the identification of an antibody] by the method of any one of claims [1,] 7[,] or 17 [-19, the manufacture of the antibody].
21. A method of making a humanized antibody comprising the step of expressing nucleic acid encoding the antibody identified [, following the identification of an antibody] by the method of any one of claims 1, 7, [or] 17, [-] or 19 [, the expression of nucleic acid encoding the antibody].

#### REMARKS

The claims pending in this application are claims 1 to 13, 17 and 19 to 21. Applicants have canceled claims 14 to 16 and 18, without prejudice to file divisional applications directed thereto.

The proposed amendments to the claims are purely in response to the rejections of the Final Action. No new matter has been introduced by the claim amendments. These amendments should be considered under Rule 116 because they do not introduce issues not already fully joined in this case and because they are believed to place the claims in better condition for appeal. Further, they are offered in a good faith effort to place this case in condition for allowance.

#### I. Amendments

The specification has been amended to correct obvious typographical errors. With respect to the amendment to Table 1 on page 87, a copy of Carter *et al.*, *Proc. Natl. Acad. Sci.*, 89, (1992) is attached, which is a publication of the experimental data disclosed in the above application, and was published after the filing date thereof. It is clear that the

last two column headings of Table 1 were inadvertently superimposed and the amendment to the specification serves merely to correct these errors. It would have been obvious from the information provided on page 87 of the specification, that the last two headings were intended to be "Kd nM", and "Relative cell proliferation", respectively, as the key under Table 1 discloses what the headings indicated by <sup>+</sup> and <sup>+</sup> are. Also, it is clear that the figures in the last two columns of the first line of data in Table 1 were intended to be 25 and 102 respectively, and were inadvertently superimposed. Applicants respectfully request that the specification be amended to correct the obvious typographical errors discussed above.

Claims 1, 7, 17 and 19 have been amended to refer to the consensus human variable domain "of a human immunoglobulin subgroup", with support for the amendment found on at least page 16, lines 29-32 and page 17, line 4. Claim 17, 19, and 20 have been amended to recite a preamble and a positive step, which steps are clear from at least the original set of claims filed.

## II. Rejections under 35 U.S.C. § 112, second paragraph

Most of the rejections under 35 U.S.C. § 112, second paragraph, which were raised in the earlier Office Action dated October 5, 1992 have been withdrawn. Applicants thank the Examiner for withdrawing these rejections.

The Examiner has, however, maintained some of the rejections under 35 U.S.C. § 112, second paragraph, which relate to claims 1, 3-5 and 7. The separate sets of rejections are addressed separately below.

A. The Examiner has maintained the rejection of claim 1 with respect to the phrase "consensus human variable domain" because it is allegedly not clear whether the consensus domain is a culmination of different variable domains or a single universal variable domain which is homologous to other human variable domains.

In the interests of expediting examination, claims 1, 7, 17 and 19 have been amended to recite that the consensus human variable domain is "of a human

immunoglobulin subgroup". Information concerning the amino acid sequences of the *variable* domains of antibodies belonging to various human immunoglobulin subgroups was compiled by Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, Fourth Edition, U.S. Dept. of Health & Human Services, pubs., (1987), a copy of which is attached to the enclosed Kelley Declaration as Exhibit "B". 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 160-167 of Kabat *et al.*). The "occurrences of most common amino acids" at each position of the variable domain are provided in the second to last column for each immunoglobulin subgroup in Kabat *et al.* The consensus human variable domain claimed in the above application is an amino acid sequence comprising the most commonly occurring amino acid residues at each position of the variable domain for a particular human immunoglobulin subgroup as defined by Kabat *et al.* It would have been readily apparent, to the ordinarily skilled biochemist, what constitutes a consensus human variable domain of a human immunoglobulin subgroup upon reading the above application.

Applicants respectfully request the withdrawal of the rejection of claim 1 as indefinite in light of the above submissions.

B. The Examiner has suggested that the "import amino acid" be described as "an import antibody comprising the amino acid sequence of a non-human antibody which binds to ...". Applicants understand that the Examiner considers that inclusion of the wording "import antibody" in parentheses is unclear and that the rejection relates to claims 1, 3, 4, 5 and 7. In order to overcome the rejection, claims 1, 7 and 19 have been amended to recite "an import antibody comprising a non-human antibody...". The non-human, import antibody may be the muMAb4D5 disclosed in Example 1 of the application, for example. Claims 3-5 depend on claim 1 and because there is clear antecedence basis for the phrases "import antibody variable domain amino acid

sequence", "import sequence" and "import antibody" in claim 1, the rejection of these claims is also rendered moot.

C. The Examiner has maintained the rejection of claim 1 under 35 U.S.C. §112, second paragraph, with respect to the wording "reasonably expected" on the grounds that it is not known what criteria determines what is "reasonable". In order to obviate the rejection, Applicants have deleted the word "reasonably" from claims 1, 3 and 19. Applicants respectfully submit that the amendment to the claims renders the rejection moot.

Applicants respectfully request that the maintained rejections of claims 1, 3-5 and 7 under 35 U.S.C. § 112, second paragraph, be withdrawn in light of the amendments to the claims and the submissions under paragraphs A to C above.

### III. Objection and Rejections under 35 U.S.C. § 112, first paragraph

The Examiner has maintained the objection to the specification and the rejection of claims 1 to 11 under 35 U.S.C. § 112, first paragraph as lacking enablement. New claims 17 to 21 have also been rejected under 35 U.S.C. § 112, first paragraph as lacking enablement. The various sets of rejections are addressed separately below.

A. The Examiner has maintained the rejection of claim 1 and has rejected claims 19 to 21 for including the language "at least a portion". In the interests of expediting examination, claims 1, 7 and 19 have been amended by deleting the wording "at least a portion of" therefrom. Applicants submit that the amendment of the claims renders the rejection of claims 1 and 19-20 under 35 U.S.C. § 112, first paragraph, moot and respectfully request the withdrawal thereof.

B. The Examiner has maintained the rejection that step c) of claim 1 (i.e. the step of substituting an import CDR amino acid sequence for the corresponding human CDR amino acid sequence) is not enabled by the specification. The Examiner asserts that there is no clear guidance in the specification to enable one of ordinary skill in the art to make the human "consensus variable domain". The Examiner further asserts that the

only guidance presented in the specification with regards to the substitutions is the amino acid sequences of SEQ ID NO: 3 and 4. Applicants understand that the basis for the Examiner's rejection is that the information provided in the specification would not have enabled the ordinarily skilled biochemist to carry out the methods claimed in order to produce a humanized antibody.

Applicants respectfully traverse this rejection on the grounds that the specification is enabling for the method claimed. In support of the above position, a Declaration pursuant to 37 C.F.R. § 1.132 by Robert Kelley is attached. See specifically his opinion in paragraph 3 and the bases for this opinion set forth in paragraphs 4 to 7.

This Declaration was not earlier submitted because it was believed, in good faith, that the rejection would be overcome without the need for a Declaration. Applicants respectfully request the entry of this Declaration in the above application pursuant to Rule 116, because it does not introduce issues not already fully joined in this case. The Declaration is offered in a good faith effort to place this case in condition for allowance.

As discussed under section II (A) above and in paragraph 4 of the Kelley Declaration, the consensus human variable domain constitutes an amino acid sequence comprising the most commonly occurring amino acids at each position in the variable domain of a particular human immunoglobulin subgroup as defined by Kabat *et al.* The immunoglobulin subgroups referred to in Kabat *et al.* were grouped according to the amino acid sequence homology between human immunoglobulin *variable* domains, and the most commonly occurring amino acids at each position in the variable domain for each subgroup were identified (i.e. the "consensus human variable domain"). The skilled biochemist could have used the consensus human variable domains of the light chain and heavy chain subgroups having the greatest number of sequences therein (i.e. light chains kappa subgroup I and heavy chains subgroup III) as disclosed in Kabat *et al.* (see page 17, first paragraph of the specification) to humanize the non-human antibody of interest. Alternatively, the skilled biochemist could have chosen the consensus human variable domain of another human immunoglobulin subgroup as defined in Kabat *et al.*

i.e. the consensus human variable domain for human kappa light chains subgroups II to IV, human lambda light chains subgroups I to VI, or human heavy chains subgroups I or II (see pages 41-76 and 160-167 of Kabat *et al.*). Therefore, the skilled biochemist could have elected to use a consensus human variable domain other than those defined as SEQ ID NO: 3 & 4 on page 17 of the above application, as the consensus human variable domains for other subgroups were compiled in Kabat *et al.* Page ix of Kabat *et al.* identifies the residues forming the CDR regions of heavy and light chain variable domains tabulated from human and mouse variable domains. Kabat *et al.* have adopted standardized numbering for each of the residue locations. Accordingly, the skilled biochemist could have identified the CDR regions of the consensus human variable domain and the import variable domain using the teachings of Kabat *et al.* Alternatively, the structural definition of Chothia *et al.*, *J. Mol. Biol.*, **196**: 901-917 (1987) (see page 16, third paragraph of the specification) could have been adopted to identify the CDR regions of the consensus and import variable domains. See paragraph 4 of the Kelley Declaration. The above submissions show that steps a & b of claim 1 were enabled by the specification as filed.

Also, step c of claim 1 could have been carried out by the ordinarily skilled biochemist using the information provided in the specification and techniques such as manual tabulation of amino acid sequences or a computer program which was known in the art prior to June 14, 1991. See paragraph 5 of the Kelley Declaration.

Steps d to g of claim 1 would similarly have been straightforward to perform. These steps of claim 1 relate to the identification of Framework Region (FR) residues in the consensus human variable domain which are non-homologous to the corresponding import FR residues and replacement of such non-homologous human residues with corresponding import residues, if the residues are expected to have any one of the effects specified in step f. The locations of FR residues in human and mouse variable domains are indicated in Kabat *et al.* (see page ix) and the structural definition of the FR's was available (see Chothia *et al.*) Hence, it would have been straightforward for the skilled

immunologist to identify the FR residues in the consensus human variable domain and the import sequence. Using computer programs available before June 14, 1991, the skilled biochemist would have been able to study the 3-dimensional structure of the antibody in order to establish whether a particular non-homologous import amino acid residue is likely to have one of the effects discussed in section f of claim 1. Information is provided on pages 14 to 16 of the specification which would have enabled the skilled biochemist to determine whether any non-homologous residue(s) would be expected to have the effects claimed. The techniques claimed in steps d to g of claim 1 could have been carried out routinely by a person versed in the relevant art, prior to June 14, 1991. See paragraph 6 of the Declaration.

As discussed in paragraph 7 of the Declaration, once the primary amino acid sequence of the antibody had been characterized, it would have been routine to make the protein using recombinant techniques or a peptide synthesizer, which techniques were well known in the art prior to the filing date of the above application.

Applicants conclude that, contrary to the Examiner's assertions, the ordinarily skilled biochemist would have been able to carry out the method claimed in the above application, using the information provided in the specification and techniques which were well known in the relevant art, prior to June 14, 1991.

Accordingly, Applicants request that the rejection of claim 1 under 35 U.S.C. § 112, first paragraph, be reconsidered and withdrawn in light of the above submissions and the Declaration.

C. The Examiner has maintained the rejection of claims 1 and 3, and has rejected claim 19 under 35 U.S.C. § 112, first paragraph, with respect to the wording "reasonably" therein. In order to obviate the rejection, the wording "reasonably" has been deleted from claims 1, 3 and 19.

Accordingly, Applicants request that the rejection of claims 1, 3 and 19 under 35 U.S.C. § 112, first paragraph, be withdrawn.

D. The Examiner has maintained the rejection of claims 6, 7 and 9 as lacking

enablement under 35 U.S.C. § 112, first paragraph, the Examiner's position being that the amino acids are relevant to IgG only and not to other isotypes. Applicants respectfully traverse this rejection on the basis that the immunoglobulin *sites* claimed would have been relevant with respect to antibodies, other than IgG antibodies. Applicants refer the Examiner to paragraphs 8 & 9 of the Kelley Declaration which support this position. The Examiner appears to suggest that the rejected claims cover *sequences* which would not be found in immunoglobulin isotypes, other than IgG isotypes. However, as pointed out in paragraph 9 of the Kelley Declaration, the claims refer to *positions* or *sites* of the variable domain, not specific amino acid residues. These sites relate to the position of a residue in the 3-D structure of the variable domain. Kabat *et al.* have used universal numbering for the amino acid residue locations of the variable domains for each of the immunoglobulin subgroups mentioned therein. The FR residue sites indicated may be occupied by an amino acid residue which is non-homologous to the corresponding consensus human variable domain residue, and which residue is likely to have at least one of the effects discussed in step f of claim 1. The residue at the particular site can be any amino acid residue, depending on the antibody in which it is located. These residue locations or sites are applicable *across species* (see page 16, line 8). Accordingly, it is likely that an amino acid residue located at one of the sites indicated in claims 6, 7 and 9 will have one of the effects of claim 1 (step f) regardless of the antibody in which it is located. It is apparent that the particular sites claimed are applicable to immunoglobulins other than IgG.

Accordingly, Applicants submit that the rejection of claims 6, 7 & 9 under 35 U.S.C. § 112, first paragraph, should be reconsidered and withdrawn in light of the above submissions and Declaration.

In light of the submissions presented in paragraphs A to D above, Applicants respectfully request that the objection to the specification and the rejection of claims 1-11 and 17-21 under 35 U.S.C. §112, first paragraph, be withdrawn.

Applicants thank the Examiner for withdrawing the rejections which were raised



under 35 U.S.C. § 101 in the earlier Office Action dated October 5, 1992 .

#### IV. Rejection of claims 1, 2 and 5-10 under 35 U.S.C. 102 (a) and 102(b)

The rejection of claims 1, 2 and 5-10 under 35 U.S.C. § 102(a) and 102(b) has been maintained and newly added claims 17-21 have been rejected under 35 U.S.C. § 102(a) and 102(b) as being anticipated by Queen *et al.*, *Proc. Natl. Acad. Sci.*, 86:10029-10033 (1989) and Co *et al.*, *Proc. Natl. Acad. Sci.*, 88:2869-2873 (1991). The basis for the rejection is that there is allegedly no clear indication as to what is meant by the consensus human variable domain claimed in the above application.

To constitute anticipation, all material elements of a claim must be found in one prior art source. *In re Marshall*, 198 USPQ 344 (CCPA 1978), *In re Kalm*, 154 USPQ 10 (CCPA 1967). Applicants will show that Queen *et al.* and Co *et al.* do not contain all material elements of claims 1, 2, 5-10 and 17-21.

The nature of the "consensus human variable domain of a human immunoglobulin subgroup" as defined in the claims as amended has been discussed above under Section II(A) of this response and in paragraph 4 of the Kelley Declaration, those discussions being incorporated herein. Applicants submit that the meaning of the phrase consensus human variable domain of a human immunoglobulin subgroup would have been clearly understood by those skilled in the art upon reading the specification. The prior art relied upon in the Office Action fails to disclose a method of making a humanized antibody using a consensus human variable domain to "humanize" a non-human antibody. The Declaration by Kelley supports this position. In particular, Applicants direct the Office's attention to paragraphs 11-13 of the attached Declaration. It is apparent from the information given in Table 1 of Exhibit C and in the Figures of Exhibits D and E of the Kelley Declaration (see paragraphs 12 & 13 thereof), that the variable domains of the human immunoglobulin sequences used by Queen *et al.* and Co *et al.* are not a consensus human variable domain of any human immunoglobulin subgroup as set forth in the claims of the above application.

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Since, as shown above, Queen *et al.* and Co *et al.* do not teach all the material elements of the instant claims as required under *Marshall* and *Kalm, supra*, Applicants respectfully submit that the rejection of claims 1, 2, 5-10 and 17-21 under 35 U.S.C. § 102(a) and (b) can not be upheld and therefore request that the rejections be withdrawn.

#### V. Rejection of claims 3 and 4 under 35 U.S.C. § 103

The rejection of claims 3 and 4 as unpatentable under 35 U.S.C. § 103 over Queen *et al.* or Co *et al., supra*, in view of Wallick *et al., J. Exp. Med., 168* (1988) has been maintained. The basis for the rejection relates to the alleged lack of clarity of the language "consensus human variable domain" in the claims of the above application. The consensus human variable domain as defined in the above application would have been readily understood by the ordinarily skilled biochemist (see paragraph 4 of the Kelley Declaration). Claim 1 of the above application relates to a method of using a consensus human variable domain to "humanize" a non-human antibody (e.g. muMAb4D5). As established in section IV above, use of a consensus human variable domain from a human immunoglobulin subgroup is not disclosed in Queen *et al.* or Co *et al.*

The publication by Wallick *et al.* does not compensate for the deficiencies in the primary references. Wallick *et al.* refer to the importance of glycosylation for maintaining antigen binding affinity of monoclonal antibodies. Wallick *et al.* fail to disclose or suggest a method of humanizing a non-human antibody, much less a method of humanizing a non-human antibody using a consensus human variable domain of a immunoglobulin subgroup. The skilled biochemist would have had no motivation to use a consensus human variable domain based on the prior art referred to in the Office Action, because the prior art techniques had all relied upon using a human variable domain sequence which has the closest sequence homology to the non-human variable sequence (to be humanized) in order to reduce the likelihood of introducing distortions into the CDR's (see column 2 on page 10031 of Queen *et al.*) and "to retain high binding affinity in the humanized antibody" (see column 1 on page 2871 of Co *et al.*). The method claimed in

the above application does not rely on a high degree of homology between the variable domain of the non-human sequence and the consensus variable domain which is used to humanize the non-human sequence.

Also, as supported by paragraph 15 of the Kelley Declaration, the invention claimed in the above application resulted in an unexpected result which could not have been reasonably predicted from the prior art. It was surprising that a consensus variable domain of a selected immunoglobulin subgroup could be used to humanize a non-human antibody, regardless of the degree of homology between the human and non-human amino acid sequences. It was also surprising that the humanized antibody so formed retained, and in some instances, had increased antigen binding affinity compared to the non-human antibody from which it was derived. The above application shows that the huMAb4D5-8 variant actually binds the p185<sup>HER2</sup> ECD 3-fold more tightly than muMAb4D5 (see page 82 lines 31 & 32 to page 83, line 1 of the specification) which could not have been predicted by the ordinarily skilled biochemist. See paragraph 15 of the Kelley Declaration. The evidence of unexpected results in Applicants' application is sufficient to support a conclusion of nonobviousness. *Ralston Purina Co. Far-Mar-Co., Inc.*, 222 USPQ 863 (DC KS, 1984).

It is apparent that the invention claimed in claim 1 was novel and nonobvious over the citations because the combination of the prior art failed to disclose, or suggest, the invention claimed in claim 1 and, moreover, the method resulted in a new and unexpected result which could not have been reasonably predicted from the art.

Claims 3 & 4 depend on claim 1 which, as established above, is novel and nonobvious over the citations. Claim 3 refers to the step of finding any glycosylation site which is likely to affect the antigen binding or affinity in the import antibody and substituting the glycosylation site *into* the *consensus* amino acid sequence. Claim 4 refers to the step of *replacing* glycosylation sites of the consensus domain with the corresponding import amino acid residues if such glycosylation sites are not present in the import sequence. These claims would not have been obvious over the prior art of

record because the prior art failed to disclose the use of a human consensus variable domain to humanize the non-human antibody. Accordingly, the skilled biochemist would have had no motivation to replace or insert glycosylation sites into a consensus amino acid sequence, as claimed in claims 3 and 4 of the application. See paragraph 15 of the Kelley Declaration.

The law is clear that obviousness cannot be established by combining the teachings of the references to produce the claimed invention, absent some teaching, suggestion, or incentive supporting the combination. *ACS Hospital Systems, Inc. v. Montefiore Hospital*, 221 USPQ 929, 933 (Fed. Cir. 1984). The above discussion shows that the cited references, alone or in combination, lack the requisite teaching of the use of a consensus human variable domain to humanize a non-human antibody. In this case, the combined art would not have reasonably enabled or motivated the skilled practitioner to use a human consensus variable domain in this manner, which provides a method of making improved humanized antibodies. Accordingly, it is clear that the invention claimed in claims 3 & 4 is novel and nonobvious over the prior art of record.

Applicants submit that the rejection of claims 3 and 4 under 35 U.S.C. § 103 should be reconsidered and withdrawn in light of the above submissions and the Declaration.

VI. Rejection of claims 17,18, 20 and 21 under 35 U.S.C. §112, second paragraph.

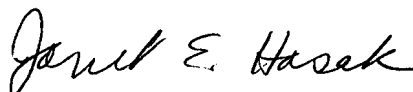
Claims 17, 18, 20 and 21 have been rejected under 35 U.S.C. § 112, second paragraph, as being indefinite in that there are allegedly no discrete method steps. In order to obviate the rejection, claims 17, 20 and 21 have been amended to each recite a definite method step and claim 18 has been deleted.

Applicants respectfully request the withdrawal of the rejection of claims 17, 20, and 21 under 35 U.S.C. § 112, second paragraph, in light of the amendments to the claims.

As all objections and rejections have been addressed and overcome, Applicants

believe that the claims are now in condition for allowance. Notice to that effect is respectfully requested. If the Examiner has any questions concerning the response, she should feel free to call the undersigned attorney at the number indicated above.

Respectfully submitted,  
GENENTECH, INC.



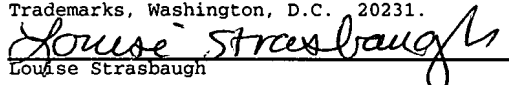
Janet E. Hasak  
Reg. No. 28,616

Date: September 20, 1993

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CERTIFICATE OF MAILING (37 CFR 1.8a)

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

  
Louise Strasbaugh

Date: September 20, 1993



PATENT DOCKET 709

THE UNITED STATES PATENT AND TRADEMARK OFFICE

|                              |   |                               |
|------------------------------|---|-------------------------------|
| In re Application of         | ) | Group Art Unit: 1806          |
| Paul J. Carter et al.        | ) | Examiner: L. FEISEE           |
| Serial No. 07/715272         | ) |                               |
| Filed: June 14, 1991         | ) |                               |
| For: Immunoglobulin Variants | ) | 460 Point San Bruno Boulevard |
|                              | ) | South San Francisco, CA 94080 |
|                              | ) |                               |
|                              | ) |                               |

#20098  
SEP 18  
9-20-93

**DECLARATION OF ROBERT F. KELLEY PURSUANT TO 37 CFR §1.132**

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

Sir:

I, ROBERT F. KELLEY, do hereby declare as follows:

1. I received my Ph.D. in Biochemistry in 1984 from the University of Iowa. Following my Ph.D, I was a NIH postdoctoral fellow in the Department of Molecular Biophysics & Biochemistry at Yale University from July 1984 to December 1985. In 1986, I joined the Biocatalysis Department at Genentech, Inc. as an Associate Scientist. In September 1988, I was promoted to Scientist and I am employed in that capacity at present. (The Biocatalysis Department has been renamed "Protein Engineering"). I am the author or co-author of 22 publications relating to the 3-D structures and folding of various proteins. A copy of my curriculum vitae is attached as Exhibit "A".

2. I understand that the Patent Office has rejected the above application on the basis that the application as filed does not provide sufficient disclosure to enable a skilled biochemist to carry out the method of claim 1 because the Examiner believes no clear guidance exists in the specification to allow a skilled biochemist to make the "consensus human variable domain" and substitute an import (i.e. non-human) Complementary Determining Region (CDR) amino acid sequence for the corresponding human CDR amino acid sequence, as set forth in claim 1. I further understand that the Office considers that

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the only guidance in the specification with regards to the substitutions is the amino acid sequences of SEQ ID NO: 3 and 4.

3. I have read the above application, the Office Action date May 19, 1992 (Paper # 17) rejecting the claims of the application, and the proposed amendment of the claims in response to the rejection. In my opinion, the skilled biochemist could have readily carried out the method of claim 1 in order to make a humanized antibody, using the general knowledge available in the field on and before June 14, 1991, and the information given in the above application. The bases for my opinion are given in paragraphs 4 to 7 below.

4. Claim 1 relates to a method of making a humanized antibody. Steps a and b of claim 1, as amended, discuss identification of the CDR amino acid sequences of a non-human import antibody (to be humanized) and a consensus human variable domain of a human immunoglobulin subgroup. The consensus human variable domain constitutes an amino acid sequence comprising the most commonly occurring amino acids at each position in the variable domain of a particular human immunoglobulin subgroup as defined by Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, Fourth Edition, U.S. Dept. of Health & Human Services, pubs., (1987), a copy of which is attached as Exhibit "B". The immunoglobulin subgroups referred to in Kabat *et al.* were grouped according to the amino acid sequence homology between human immunoglobulin *variable* domains, and the most commonly occurring amino acids at each position in the variable domain for each subgroup were identified (i.e. the "consensus human variable domain"). The skilled biochemist could have used the consensus human variable domains of the light chain and heavy chain subgroups having the greatest number of sequences (i.e. light chains kappa subgroup I and heavy chains subgroup III) as disclosed in Kabat *et al.* (see page 17, first paragraph of the specification) to humanize the non-human antibody of interest. Alternatively, the skilled biochemist could have chosen the consensus human variable domain of another human immunoglobulin subgroup as defined in Kabat *et al.* (i.e. the consensus human variable domain for human kappa light chains subgroups II to IV, human lambda light chains subgroups I to VI, or human

heavy chains subgroups I or II [see pages 41-76 and 160-167 of Kabat *et al.*]). Therefore, the skilled biochemist could have elected to use a consensus human variable domain other than those defined as SEQ ID NO: 3 & 4 on page 17 of the above application, as the consensus human variable domains for other subgroups were compiled in Kabat *et al.* Page ix of Kabat *et al.* identifies the residues forming the CDR regions of heavy and light chain variable domains tabulated from human and mouse variable domains. Kabat *et al.* have adopted standardized numbering for each of the residue locations. Accordingly, the skilled biochemist could have identified the CDR regions of the consensus human variable domain and the import variable domain using the teachings of Kabat *et al.* Alternatively, the structural definition of Chothia *et al.*, *J. Mol. Biol.*, **196**: 901-917 (1987) (see page 16, third paragraph of the specification) could have been adopted to identify the CDR regions of the consensus and import variable domains. Hence, it would have been straightforward for the skilled biochemist to carry out steps a and b of claim 1 using the information provided in the specification.

5. Step c of claim 1 discloses the step of replacing the corresponding human CDR sequence with the import CDR amino acid sequence. This step could have been carried out routinely by the skilled biochemist by manual tabulation or using a computer program such as the ALIGN program, (Dayhoff *et al.*, *Meth. Enzymol.*, **91**:524-545 [1983]) which was available prior to June 14, 1991. Steps a to c of claim 1 would have resulted in the characterization of a primary amino acid sequence encoding a humanized variable domain with import (non-human) CDR regions.

6. Steps d to g of claim 1 relate to the identification of Framework Region (FR) residues in the consensus human variable domain which are non-homologous to the corresponding import FR residues and replacement of such non-homologous human residues with corresponding import residues, if the residues are expected to have any one of the effects specified in step f. The locations of FR residues in human and mouse variable domains are indicated in Kabat *et al.* (see page ix) and the structural definition of the FR's was available (see Chothia *et al.*) Hence, it would have been straightforward for the skilled immunologist to identify the FR residues in the consensus human variable domain and the



import sequence. Using computer programs (such as the INSIGHT program [Biosym Technologies], available before June 14, 1991), the skilled biochemist would have been able to study the 3-dimensional structure of an antibody in order to establish whether a particular non-homologous import amino acid residue is likely to have one of the effects discussed in section f of claim 1. Information is provided on pages 14 to 16 of the specification which would have enabled the skilled biochemist to determine whether any non-homologous residue(s) would be expected to have the effects claimed. The techniques claimed in steps d to g of claim 1 could have been carried out routinely by a person versed in the relevant art, prior to June 14, 1991.

7. Steps a to g of claim 1 would have lead to the characterization of an amino acid sequence of a humanized antibody having non-human CDR amino acid residues and, optionally, having one or more non-human FR residues. In order to prepare the humanized antibody as claimed in claim 1, step h, the skilled biochemist could have synthesized the antibody using a peptide synthesizer which was commercially available before June 14, 1991. Alternatively, the antibody could have been made in recombinant cell culture (see page 26, last paragraph of the specification). Preparation of the antibody would have been straightforward to perform by the person skilled in the art, once the amino acid sequence of the humanized antibody had been characterized.

8. I understand that the Patent Office has rejected the above application on the basis that the sites in the variable domain referred to in claims 6, 7, and 9 are relevant to IgG antibodies only. It is my opinion that the sites referred to in claims 6, 7, and 9 would be relevant to other immunoglobulins. The basis for my opinion is given in paragraph 9 below.

9. The sites referred to in claims 6, 7, and 9 are the residue locations, or sites, of the FR residues in the heavy or light chain forming the variable domain of immunoglobulins. The residue sites referred to in claims 6, 7 & 9 relate to the position of a residue in the 3-D structure of the variable domain. Kabat *et al.* have used universal numbering for the amino acid residue locations of the variable domains for each of the immunoglobulin subgroups mentioned in the reference. The FR residue sites

indicated may be occupied by an amino acid residue which is non-homologous to the corresponding consensus human variable domain residue, and which is likely to have at least one of the effects discussed in step f of claim 1. These residue locations or sites are applicable *across species* (see page 16, line 8 of the specification). Accordingly, it is likely that an amino acid residue located at one of the sites indicated in claims 6, 7 and 9 will have one of the effects of claim 1 (step f), regardless of the antibody in which it is located, because it will be in the same position in the 3-D structure of the antibody variable domain as the residue sites referred to in the rejected claims. Accordingly, the examples of residue locations to be substituted in the variable domains would be applicable to antibodies, other than IgG antibodies.

10. I understand that the Patent Office has rejected the above application on the grounds that the invention as claimed is disclosed in Queen *et al.*, *Proc. Natl. Acad. Sci.*, **86**:10029-10033 (1989) or Co *et al.*, *Proc. Natl. Acad. Sci.*, **88**:2869-2873 (1991) and that the Office has suggested that the human variable domains disclosed in these references may have the same amino acid sequences as one of the consensus human variable domains disclosed in Kabat *et al.*

11. The above statements regarding the state of knowledge as of June 14, 1991, do not establish that the invention claimed in this application was known, or would have been obvious, to the skilled biochemist at the time the invention was made. To the contrary, after having read the citations relied upon by the Patent Office, it is my judgement that these documents would not have disclosed, nor suggested, the methods claimed. The basis for my opinion is given below.

12. The invention of the above application can be distinguished on the basis that a *consensus human variable domain* is used to "humanize" a non-human antibody of interest. The Queen *et al.* and Co *et al.* publications fail to disclose a consensus human variable domain. Instead, these publications refer to the use of a human variable domain having the closest sequence homology to the variable domain of the non-human antibody to be humanized. Queen *et al.* used the Eu human variable domain sequence (see Fig 2 thereof) and Co *et al.* used the variable domains of the Pom or Eu human

antibodies (see Fig 1 thereof). The sequences used in Queen *et al.* and Co *et al.* do not constitute a consensus human variable domain of a human immunoglobulin subgroup. The sequence identity between the amino acid sequences of the FR residues of the variable domains of the Pom or Eu heavy or light chains compared to the FR residues of the consensus human variable domains of each of the human immunoglobulin subgroups as defined by Kabat *et al.* is illustrated in Table 1 (see Exhibit "C", attached hereto). The CDR residues were not used in the comparison because of the large number of differences between these residues for variable domains of different antibodies. The Pom and Eu variable domain sequences were taken from Kabat *et al.* The consensus human variable domains of the  $V_L$  lambda subgroups IV and V were not compared, as these subgroups have too few members. While the variable domain of Eu is classified in subgroups  $V_L$  kappa I and  $V_H$  I, and the variable domain of Pom is classified in subgroups  $V_L$  kappa III and  $V_H$  III, it is apparent that the Eu and Pom variable domain amino acid sequences are not consensus human variable domains of any immunoglobulin subgroup. This is further demonstrated in the following paragraph.

13. Exhibits "D" and "C" attached hereto, show the differences in the amino acid sequences of the Pom and Eu heavy and light chain variable domains compared to the consensus human variable domain of the subgroup in which they are classified. Exhibit D illustrates an alignment of the amino acid sequences of the light chain variable domains of Eu, Pom and the consensus variable domain of the  $V_L$  kappa subgroup I (in which the light chain variable domain of Eu is classified). Exhibit E illustrates an alignment of the amino acid sequences of the heavy chain variable domains of Eu, Pom and the consensus variable domain of the  $V_H$  subgroup III (in which the heavy chain variable domain of Pom is classified). Even though Eu is classified in  $V_L$  kappa I, it has seven framework residues which are different from the framework residues of the kappa I consensus sequence. Furthermore, while Pom is classified in the  $V_H$  III subgroup, eight of its framework residues differ from the corresponding framework residues of the  $V_H$  III consensus sequence. There are, of course, many differences between the CDR residues of the consensus sequences and the corresponding CDR residues of Pom and Eu.

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It is clear from the information in Exhibits C, D, & E that the Queen *et al.* and Co *et al.* publications fail to disclose a method wherein a non-human import antibody is humanized using a consensus human variable domain of an immunoglobulin subgroup.

14. I understand the Patent Office has rejected the above application on the basis that the invention claimed in claims 3 & 4 would have been obvious in light of Queen *et al.*, or Co *et al.*, when read in conjunction with Wallick *et al.*, *J. Exp. Med.*, **168** (1988). After reading these references, it is my opinion that the invention claimed in claims 3 and 4 is novel and would not have been obvious in light of the citations. The basis for my opinion is given in the following paragraph.

15. Claim 1 of the above application relates to a method of using a consensus human variable domain to "humanize" a non-human antibody (e.g. muMAb4D5). Use of a consensus human variable domain from a human immunoglobulin subgroup to humanize a non-human antibody is not disclosed in Queen *et al.*, Co *et al.* or Wallick *et al.* Wallick *et al.* does not relate to a method of humanizing a non-human antibody, much less a method of humanizing a non-human antibody using a consensus human variable domain of a human immunoglobulin subgroup. The skilled biochemist would have had no motivation at the filing date of this application to use a consensus human variable domain to humanize a non-human antibody, because the techniques in the prior literature had all relied upon using a human variable domain sequence which has the closest sequence homology to the non-human variable sequence (to be humanized) in order to reduce the likelihood of introducing distortions into the CDR's (see column 2 on page 10031 of Queen *et al.*) or to "retain high binding affinity in the humanized antibodies" (see column 1 on page 2871 of Co *et al.*). The method claimed in the above application does not rely on a high degree of homology between the variable domain of the non-human sequence and the consensus variable domain which is used to humanize the non-human sequence. It was surprising that a consensus variable domain of a selected immunoglobulin subgroup could be used to humanize a non-human antibody, regardless of the degree of homology between the human and non-human amino acid sequences. It was also surprising that the humanized antibody so formed retained,

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and in some instances, had increased antigen binding affinity compared to the non-human antibody from which it was derived. The above application shows that the huMAb4D5-8 variant actually binds the p185<sup>HER2</sup> ECD 3-fold more tightly than muMAb4D5 (see page 82 lines 31 & 32 to page 83, line 1 of the specification), which could not have been predicted by the ordinarily skilled biochemist at the time the specification was filed. Claim 3 refers to the step of finding any glycosylation site which is likely to affect the antigen binding or affinity in the import antibody and substituting the glycosylation site *into* the *consensus* amino acid sequence. Claim 4 refers to the step of *replacing* glycosylation sites of the consensus domain with the corresponding import amino acid residues if such glycosylation sites are not present in the import sequence. In my opinion, these claims would not have been obvious over the prior literature because the reference failed to disclose the use of a human consensus variable domain to humanize the non-human antibody. Accordingly, the skilled biochemist would have had no motivation to replace or insert glycosylation sites into a consensus amino acid sequence, as claimed in claims 3 and 4 of the application.

16. 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: 9/20/93

Signed: Robert F. Kelley  
ROBERT F. KELLEY

CERTIFICATE OF MAILING

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### Scientific publications

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TABLE 1  
SEQUENCE IDENTITY - (%)

| CONSENSUS VARIABLE DOMAIN<br>SUBGROUP | EU | POM |
|---------------------------------------|----|-----|
| V <sub>L</sub> kappa I                | 92 | 76  |
| V <sub>L</sub> kappa II               | 61 | 71  |
| V <sub>L</sub> kappa III              | 72 | 85  |
| V <sub>L</sub> kappa IV               | 73 | 78  |
| V <sub>L</sub> lambda I               | 61 | 59  |
| V <sub>L</sub> lambda II              | 57 | 54  |
| V <sub>L</sub> lambda III             | 59 | 56  |
| V <sub>L</sub> lambda VI              | 52 | 49  |
| V <sub>H</sub> I                      | 83 | 64  |
| V <sub>H</sub> II                     | 53 | 62  |
| V <sub>H</sub> III                    | 61 | 91  |



Variable Light Domain

|         |                     |              |                         |         |
|---------|---------------------|--------------|-------------------------|---------|
|         | 10                  | 20           | 30                      | 40      |
| EU      | DIQMTQSPSTLSASV     | GDRVTITCRASQ | SINTWLAWYQOKPGKAPKLLMY  |         |
|         | *                   |              | @ @ @                   | *       |
| Kappa-I | DIQMTQSPSSLSASV     | GDRVTITCRASQ | --ISNYLAWYQOKPGKAPKLLIY |         |
|         | * *                 | ** * * * * * | @ @ @ @                 | * * * * |
| POM     | EIVMTQSPVTLSPGERATL | SCRASQ       | SISNSYLAWYQOKPSGSPRLLIY |         |

-----  
CDR-L1

|         |   |     |    |    |    |         |
|---------|---|-----|----|----|----|---------|
|         | 50  | 60  | 70 | 80 | 90 | 100     |
| EU      | KASSLESGVPSRFIGSGSGTEFTLTISSLOPDDFATYYCOQYNSDSKMFQ  |     |    |    |    |         |
|         | @   | *   | *  | *  |    | @ @ @ @ |
| Kappa-I | AASSLESGVPSRFIGSGSGTDFTLTISSLOPEDFATYYCOQYNSLPWTFQ  |     |    |    |    |         |
|         | @ @ @ @   | * * | *  | *  | *  | @ @ @   |
| POM     | GASTRATGIPARFSGSGSGTEFTLTISSLOSEDFAVYYCOQYNNWPPTFGQ |     |    |    |    |         |

-----  
CDR-L2

-----  
CDR-L3

|         |           |
|---------|-----------|
| EU      | GTKVEVKGT |
|         | * *       |
| Kappa-I | GTKVEIKRT |
|         | *         |
| POM     | GTRVEIKR  |

KEY: \* = differences in FR residues  
@ = differences in CDR residues

Variable Heavy Domain

|           |                       |                |          |           |
|-----------|-----------------------|----------------|----------|-----------|
|           | 10                    | 20             | 30       | 40        |
| EU        | QVQLVQSGAEVKKPGSSVKV  | SCKASGGTFSRS   | AIWV     | QAPGGGLEW |
| human-III | EVQLVESGGGLVQPGGSLRLS | CAASGFTFSSYAMS | WVRQAPGK | GLEWVS    |
| POM       | EVOLLESGGGLVQPGGSLRLS | CAASGFTFSSSAMS | WVRQAPGK | GLEWVA    |

-----  
CDR-H1

|           |                       |                   |                |    |    |     |    |
|-----------|-----------------------|-------------------|----------------|----|----|-----|----|
|           | 50                    | a                 | 60             | 70 | 80 | abc | 90 |
| EU        | GIVPMFGPPNYAOKFOGRVTI | TADESTNTAYMELSSLR | SEDTAFYFCAG    |    |    |     |    |
| human-III | VISGDGGSTIYADSVKGRFTI | SRDNSKNTLYLQMN    | SLRAEDTAVYYCAR |    |    |     |    |
| POM       | WKYENGNDKHYADSVNGRFTI | SRDNSKNTLYLLMNS   | LQAE           |    |    |     |    |

-----  
CDR-H2

|           |                       |     |
|-----------|-----------------------|-----|
|           |                       | 110 |
| EU        | GYGIYSPE-----EYNGGLVT | VSS |
| human-III | GRGGSDY-----WGQGLVT   | VSS |
| POM       | DAGPYVSPITFFAHYGQGLVT |     |

-----  
CDR-H3

KEY: \* = differences in FR residues

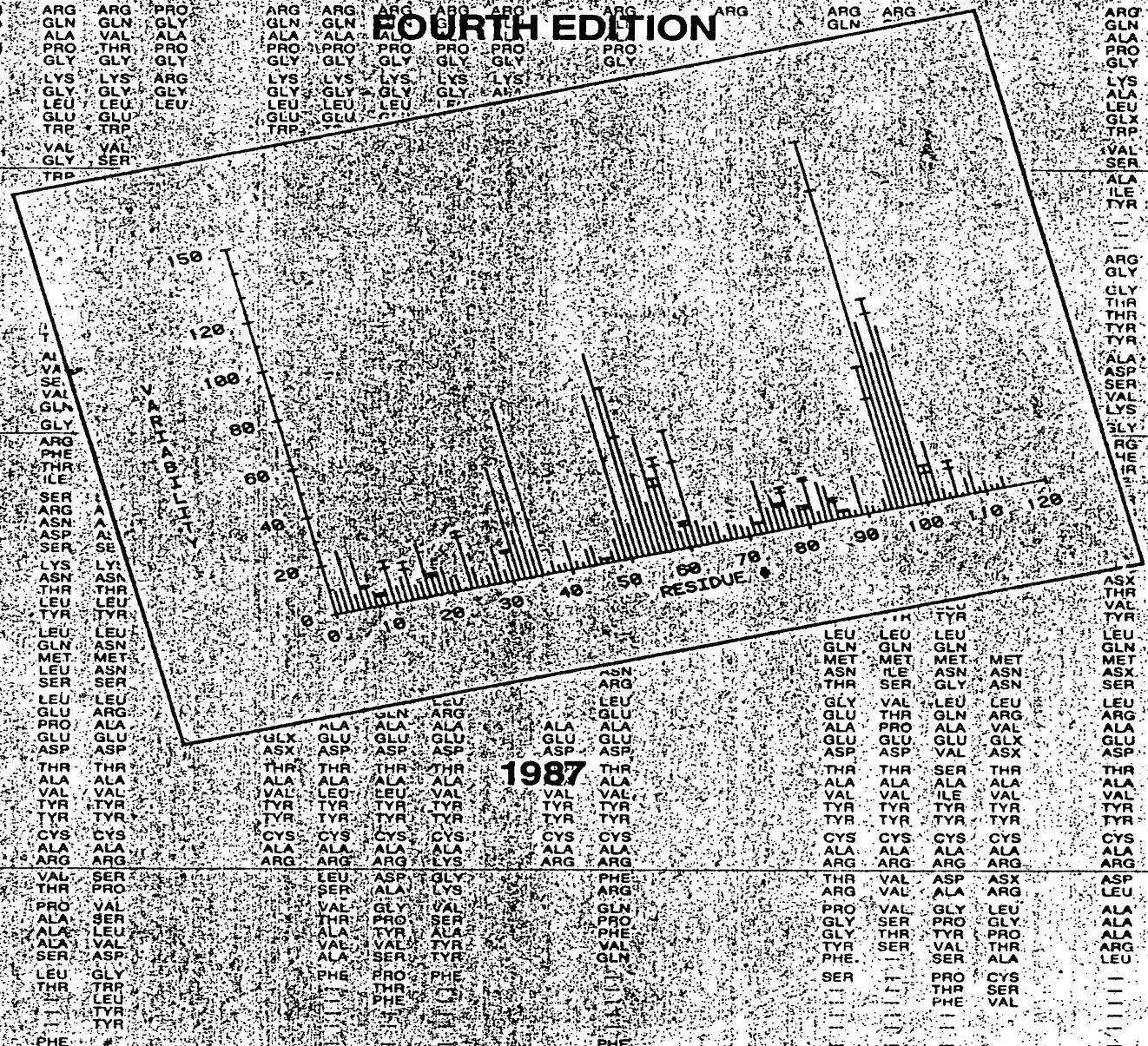
@ = differences in CDR residues

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# SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST

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# SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST

## FOURTH EDITION

Tabulation and Analysis of  
Amino Acid and Nucleic Acid Sequences of  
Precursors, V-Regions, C-Regions, J-Chain,  
T-Cell Receptor for Antigen, T-Cell Surface Antigens,  
 $\beta_2$ -Microglobulins, Major Histocompatibility Antigens,  
Thy-1, Complement, C-Reactive Protein, Thymopoietin,  
Post-gamma Globulin, and  $\alpha_2$ -Macroglobulin

1987

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| CODONS OF HUMAN T-LYMPHOCYTE RECEPTOR FOR ANTIGEN ALPHA CHAIN J-MINIGENES             | 677 |
| CODONS OF HUMAN T-LYMPHOCYTE RECEPTOR FOR ANTIGEN BETA CHAIN J-MINIGENES              | 680 |
| CODONS OF HUMAN T-LYMPHOCYTE RECEPTOR FOR ANTIGEN GAMMA CHAIN J-MINIGENES             | 683 |
| CODONS OF MOUSE T-LYMPHOCYTE RECEPTOR FOR ANTIGEN ALPHA CHAIN J-MINIGENES             | 684 |
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considered uncertain by the authors have not been included in the table. In some instances the symbol # is used to indicate that several amino acid residues were found in one position, and these residues are listed in the notes. The four columns at the end of each table give:

1. the number of residues sequenced at that position,
2. the number of different amino acids found at that position,
3. the number of times the most common amino acid occurred and that amino acid in parentheses, and
4. the variability.

Variability is calculated (11) as:

$$\text{Variability} = \frac{\text{Number of different amino acids occurring at a given position}}{\text{Frequency of the most common amino acid at that position}}$$

An invariant position would have a variability of one; if 20 amino acids occurred with equal frequency, the variability would be 20 divided by 0.05 equals 400. If, for example, four different amino acids Ser, Asp, Pro, and Thr occurred at a given position, and of 100 sequences available at that position, Ser occurred 80 times, the variability would be  $4/0.8 = 5$ . When any of the amino acid residues sequenced were not identified completely and are listed as Glx (or Asx), two values, separated by a colon, are given in the last three columns. The first value in each of these columns is calculated assuming that only one of the two possibilities, e.g., Glu or Gln (or Asp or Asn) occurred, while the second considers that both were present and maximizes variability. In the variability plots, the horizontal bars indicate the two values.

When two or more amino acids are most common and occur with equal frequency, they are tabulated as a note, and the symbol + is used in the next to last column. If no sequence data have been reported for any position, there are no entries in the last four columns. Variability is not calculated for insertions or if only a single sequence is known. When the translated sequence of a clone corresponds to a previously listed sequence of a plasmacytoma from which it was prepared, only one sequence is listed so that the variability computations are not affected, and a note is included.

If a given sequence is associated with any antibody activity, this is indicated by an asterisk alongside the protein heading, and the antibody specificities are given in a separate list with binding constants if available. The notes list the a-allotypes for the rabbit heavy chain V-region and the b-allotypes for the constant domain of the rabbit kappa light chain. A key reference to the sequence is given; generally the most recent reference since it is usually the most nearly complete, but often several references are included, especially when revisions of a sequence have been made. Notes are now of two types; general notes about a table indicated by the symbol #, and specific notes indicated by the sequence number.

## Signal Sequences

The signal (precursor) amino acid sequences of immunoglobulin chains are listed in three tables: one for kappa light chains, one for lambda light chains, and one for heavy chains. They were obtained either by direct sequencing of signal proteins (12-14) or by translating nucleotide sequences from DNA clones. Signal segments range from 17-29 amino acid residues in length and are thus numbered from -29 to -1. Genomic DNA clones contain introns of varying length that interrupt the coding sequence of the precursor within the codon for position -4, and in rare cases for position -6. Thus, the L-gene encodes the leader peptide to position -4 and the 5' end of the V-gene codes for positions -4 to -1.

The signal amino acid sequences of the T-cell receptors for antigens,  $\beta_2$ -microglobulins, major histocompatibility complex proteins, and complement components are listed in separate tables.

By conformational energy calculations, the core  $V_{\kappa}$  hydrophobic Leu-Leu-Leu-Trp-Val-Leu-Leu-Leu (MOPC321, MOPC63) exists in an alpha helical conformation, terminated by chain reversal conformations in the four C-terminal residues Trp-Val-Pro-Gly; the four amino terminal residues are compatible with the alpha helix (15).

## Variable Region Sequences

The variable regions (16) of immunoglobulins have been shown to contain hypervariable segments in their light (11, 17-23) and heavy (22, 24-27) chains, of which certain residues have been affinity labeled (28-41). Three hypervariable segments of light chain were delineated from a statistical examination

of sequences of human  $V_{\kappa}$ , human  $V_{\lambda}$ , and mouse  $V_{\kappa}$  light chains aligned for maximum homology (11,22). These and the three corresponding segments of the heavy chains (22,26,27) were hypothesized (11,22) to be the complementarity-determining regions or segments (CDR) containing the residues which make contact with various antigenic determinants, and this has been verified by X-ray diffraction studies at high resolution (42-67). The rest of the V-region constitutes the framework (11,22,66-68). It is convenient to identify the framework segments (FR1, FR2, FR3, and FR4) and the complementarity-determining segments (CDR1, CDR2, and CDR3) with the three CDRs separating the four FRs. The residue numbers for these segments are as follows:

| Segment | Light Chain  | Heavy Chain  |
|---------|--|--|
| FR1     | 1-23 (with an occasional residue at 0, and a deletion at 10 in $V_{\lambda}$ chains) | 1-30 (with an occasional residue at 0)                                 |
| CDR1    | 24-34 (with possible insertions numbered as 27A,B,C,D,E,F)                           | 31-35 (with possible insertions numbered as 35A,B)                     |
| FR2     | 35-49  | 36-49  |
| CDR2    | 50-56  | 50-65 (with possible insertions numbered as 52A,B,C) <sup>a</sup>      |
| FR3     | 57-88  | 66-94 (with possible insertions numbered as 82A,B,C)                   |
| CDR3    | 89-97 (with possible insertions numbered as 95A,B,C,D,E,F)                           | 95-102 (with possible insertions numbered as 100A,B,C,D,E,F,G,H,I,J,K) |
| FR4     | 98-107 (with a possible insertion numbered as 106A)                                  | 103-113  |

<sup>a</sup> In the rabbit, Mage *et al.* (69) consider position 65 in  $V_H$  to be in FR3, since it is allotype related.

In the tables of V-regions, the FR and CDR are separated by horizontal lines for convenience in reading. One mouse kappa light chain, MPC11, has an extra segment of 12 amino acid residues between position 1 and the signal sequence (70). Several chains have internal deletions.

In the tables, the V-genes for the light chains code to amino acid position 95, and the J-minigenes from position 97 to 107 for lambda and 108 for kappa light chains. Position 96 is usually the site of V-J joining by recombination and may be coded partly by the V-gene and partly by the J-minigene. Because the site of V-J recombination could occur at different positions within a codon, different amino acid residues may result at this position. We have changed the location of the inserted residues from 97A-F (2) to 95A-F, since it makes for better alignment by confining chains of different lengths to the V-gene region. In  $V_{\kappa}$  chains, J1 and J2 were used 5 to 10 times more frequently than J4 and J5 (71).

The V-genes for the heavy chains code up to amino acid position 94 and are followed by the D- and J-minigenes. Because of the extensive variation in the lengths of D-minigenes, the exact boundary between D and J is not always located at the same amino acid position. In addition, the lengths of the J encoded amino acid sequences vary by a few amino acid residues. Moreover, the process of D-J joining appears to involve insertions of extra nucleotides between V and D and between D and J, termed the N region (72-76) and correlates with the appearance of terminal deoxytransferase in B cells (75). The original numbering system for the heavy chains has therefore been retained. Wysocki *et al.* (76) have provided some evidence suggesting a non-random origin for the  $V_H$ - $D_H$  junction, perhaps a minigene, rather than random addition of the N nucleotides.

It has become evident that a critical understanding of the architecture of antibody combining sites and the genetics of the generation of diversity and of antibody complementarity will depend to a great extent on the evaluation of a large number of sequences of the variable regions and especially of the complementarity-determining segments of light and heavy chains of immunoglobulins of different species. Ability to locate residues in the site making contact with antigenic determinants (77) and to predict (67,78-82) the structures of antibody combining sites will depend heavily upon such sequences.

Figures 1 and 2 are stereoviews of the  $\alpha$ -carbon skeletons of the four Fv regions for which high resolution X-ray structures have been determined, NEWM (44), KOL (62), MCPC603 (47, 48, 63), and J539 (64). The residues in the CDRs are shown as solid circles. In Fig. 1 the combining site is at the













## HUMAN KAPPA LIGHT CHAINS SUBGROUP I (cont'd)

## VARIABILITY

|     |      |           |
|-----|------|-----------|
|     | 0    |           |
|     | 1    | 3.2 : 4.4 |
|     | 2    | 4.2       |
|     | 3    | 8.9 : 9.3 |
|     | 4    | 4.6       |
|     | 5    | 3.1       |
|     | 6    | 1. : 2.1  |
|     | 7    | 3.2       |
|     | 8    | 3.1       |
|     | 9    | 4.2       |
| 1FR | 10   | 6.4       |
|     | 11   | 5.7       |
|     | 12   | 4.2       |
|     | 13   | 4.4       |
|     | 14   | 7.9       |
|     | 15   | 3.1       |
|     | 16   | 2.        |
|     | 17   | 3.2 : 4.7 |
|     | 18   | 6.6       |
|     | 19   | 3.1       |
|     | 20   | 4.2       |
|     | 21   | 4.2       |
|     | 22   | 8.2       |
|     | 23   | 1.        |
|     | 24   | 8.7       |
|     | 25   | 4.2       |
|     | 26   | 4.3       |
|     | 27   | 4.4 : 5.4 |
|     | 27A  |           |
|     | 27B  |           |
|     | 27C  |           |
|     | 27D  |           |
|     | 27E  |           |
|     | 27F  |           |
| 2FR | 28   | 23. : 26. |
|     | 29   | 5.8       |
|     | 30   | 19. : 21. |
|     | 31   | 28.       |
|     | 32   | 14.       |
|     | 33   | 4.3       |
|     | 34   | 18. : 22. |
|     | 35   | 1.        |
|     | 36   | 2.1       |
|     | 37   | 4.3 : 4.9 |
|     | 38   | 4.2 : 4.6 |
|     | 39   | 4.4       |
|     | 40   | 4.2       |
|     | 41   | 3.3       |
|     | 42   | 6.8       |
|     | 43   | 2.2       |
|     | 44   | 1.        |
|     | 45   | 6.9       |
|     | 46   | 9.8       |
|     | 47   | 2.        |
|     | 48   | 2.        |
|     | 49   | 4.3       |
|     | 50   | 21. : 24. |
|     | 51   | 5.8       |
|     | 52   | 4.3       |
|     | 53   | 12. : 14. |
|     | 54   | 2         |
|     | 55   | 15.       |
|     | 56   | 11.       |
|     | 57   | 1.        |
|     | 58   | 2.        |
|     | 59   | 4.3       |
|     | 60   | 1.        |
|     | 61   | 3.1       |
|     | 62   | 3.1       |
|     | 63   | 8.4       |
|     | 64   | 1.        |
|     | 65   | 4.4       |
|     | 66   | 3.1       |
|     | 67   | 3.2       |
|     | 68   | 3.2       |
|     | 69   | 3.2       |
|     | 70   | 8.2 : 11. |
|     | 71   | 4.4       |
|     | 72   | 4.3       |
|     | 73   | 3.9       |
|     | 74   | 4.3       |
|     | 75   | 3.2       |
|     | 76   | 2.1       |
|     | 77   | 7.4       |
|     | 78   | 2.3       |
|     | 79   | 2.1 : 3.4 |
|     | 80   | 3.6       |
|     | 81   | 4.1 : 7.7 |
|     | 82   | 1. : 2.2  |
|     | 83   | 5.7       |
|     | 84   | 2.1       |
|     | 85   | 4.3       |
|     | 86   | 2.        |
|     | 87   | 2.1       |
|     | 88   | 1.        |
|     | 89   | 3.2 : 4.6 |
|     | 90   | 3.3 : 5.1 |
|     | 91   | 19. : 21. |
|     | 92   | 25. : 28. |
|     | 93   | 21.       |
|     | 94   | 38.       |
|     | 95   | 6.4       |
|     | 95A  |           |
|     | 95B  |           |
|     | 95C  |           |
|     | 95D  |           |
|     | 95E  |           |
|     | 95F  |           |
|     | 96   | 4.3       |
|     | 97   | 4.6       |
|     | 98   | 3.2       |
|     | 99   | 1.        |
|     | 100  | 6.9 : 9.1 |
|     | 101  | 1.        |
|     | 102  | 2.1       |
|     | 103  | 5.4       |
|     | 104  | 10.6      |
|     | 105  | 4.5 : 6.3 |
|     | 106  | 14.       |
|     | 106A |           |
|     | 107  | 2.1       |
|     | 108  | 2.2       |
|     | 109  | 1.        |

## ANTIBODY SPECIFICITIES: HUMAN KAPPA LIGHT CHAINS SUBGROUP I

- 8) WEA: ANTI-3,4-PYRUVYLATED GALACTOSE MONOCLONAL  
 25) LOW: COLD AGGLUTININ WITH ANTI-BLOOD GROUP I ACTIVITY  
 39) LAY: ANTI-HUMAN GAMMA G1 AND G3 GLOBULINS; PO IDIOTYPE  
 53) HEI: COLD AGGLUTININ WITH ANTI-GD (MEMBRANE-GLYCOLIPID-DEPENDENT) ACTIVITY  
 66) DAV: ANTI-HUMAN GAMMA G GLOBULIN  
 67) FIN: ANTI-HUMAN GAMMA G GLOBULIN  
 92) WAG: ANTI-DINITROPHENYL  
 104) MAR: ANTI-LIPOPROTEIN LIPASE

## ALLOTYPE: HUMAN KAPPA LIGHT CHAINS SUBGROUP I

- 79) KUE: INV(2)

## CLASS: HUMAN KAPPA LIGHT CHAINS SUBGROUP I

- 8) WEA: IGM-KAPPA  
 33) F-GUI: IGG3-KAPPA  
 55) S-GUI: IGG3-KAPPA  
 74) PW: IGG1-KAPPA  
 82) RI: IGG1-KAPPA

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 37) JOH: CAPRA,J.D. & KUNKEL,H.G. (1970) PROC.NAT.ACAD.SCI.USA,67,87-92. (CHECKED BY AUTHOR)  
 38) KER: MILSTEIN,C. (1966) BIOCHEM.J.,101,352-368. (CHECKED BY AUTHOR WHO PROVIDED ADDITIONAL RESIDUES TO THOSE PUBLISHED)  
 39) LAY: KAPLAN,A.P. & METZGER,H. (1969) BIOCHEMISTRY,8,3944-3951. (CHECKED BY AUTHOR); KLAPPER,D.G. & CAPRA,J.D. (1976) ANN.IMMUNOL.(INST.PASTEUR),127C,261-271. (CHECKED BY AUTHOR 08/01/79)  
 40) BRA: WANG,A.C.,WELLS,J.V.,FUDENBERG,H.H. & GERGELY,J. (1974) IMMUNOCHEM.,11,341-345. (CHECKED BY AUTHOR)  
 41) WES: KRATZIN,H.,YANG,C.Y.,KRUSCHE,J.U. & HILSCHMANN,N. (1980) Z.PHYSIOL.CHEM.,361,1591-1598.  
 42) Vb'CL: PECH,M.,JAENICHEN,H.-R.,POHLENZ,H.-D.,NEUMAIER,P.S.,KLOBECK,H.-G. & ZACHAU,H.G. (1984) J.MOL.BIOL.,176,189-204. (CHECKED BY AUTHOR 12/14/84)  
 43) Vb''CL: PECH,M.,JAENICHEN,H.-R.,POHLENZ,H.-D.,NEUMAIER,P.S.,KLOBECK,H.-G. & ZACHAU,H.G. (1984) J.MOL.BIOL.,176,189-204. (CHECKED BY AUTHOR 12/14/84)  
 44) HK102'CL: BENTLEY,D.L. & RABBITTS,T.H. (1980) NATURE,288,730-733. (CHECKED BY AUTHOR 11/30/82)  
 45) EU: GOTTLIEB,P.D.,CUNNINGHAM,B.A.,RUTISHAUSER,U. & EDELMAN,G.M. (1970) BIOCHEMISTRY,9,3155-3161. (CHECKED BY AUTHOR)  
 46) DEN: YANG,C.Y.,PAULY,E.,KRATZIN,H. & HILSCHMANN,N. (1981) Z.PHYSIOL.CHEM.,362,1131-1146.  
 47) PAU: DAYHOFF,M.O. (1972) ATLAS OF PROTEIN SEQUENCE & STRUCTURE,5,D-245. SUBMITTED BY SMITHIES,O.,GIBSON,D.M. AND FANNING,E.M. (CHECKED BY AUTHOR)  
 48) HBJ4: SMITH,G.P.,HOOD,L. & FITCH,W.M. (1971) ANN.REV.BIOCHEM.,40,969-1012.  
 49) FRA: MEINKE,G.C.,SIGRIST,P.H. & SPIEGELBERG,H.L. (1974) IMMUNOCHEM.,11,457-460. (CHECKED BY AUTHOR WHO PROVIDED ADDITIONAL RESIDUES TO THOSE PUBLISHED); MEINKE,G.C. & SPIEGELBERG,H.L. (1976) IMMUNOCHEM.,13,915-919. (CHECKED BY AUTHOR 10/17/77)  
 50) GR: FAIR,D.S.,SLEDGE,C.,KRUEGER,R.G.,MANN,K.G. & HOOD,L.E. (1975) BIOCHEMISTRY,14,5561-5568.  
 51) PAUL: SMITH,G.P.,HOOD,L. & FITCH,W.M. (1971) ANN.REV.BIOCHEM.,40,969-1012.  
 52) MON: NIAL,H.D. & EDMAN,P. (1967) NATURE,216,262-263. (CHECKED BY AUTHOR 07/25/79)  
 53) HEI: RIESEN,W.F.,MAJANIEMI,I.,HUSER,H.,BRAUN,D.G. & ROELCKE,D. (1978) SCAND.J.IMMUNOL.,8,145-148. (CHECKED BY AUTHOR 10/10/79)  
 54) POT: CAPRA,J.D. & KUNKEL,H.G. (1970) PROC.NAT.ACAD.SCI.USA,67,87-92. (CHECKED BY AUTHOR WHO CORRECTED RESIDUE 9 AS GIVEN IN TABLE)  
 55) S-GUI: WANG,A.C.,FUDENBERG,H.H. & CREYSEL,R. (1982) ACTA HAEMAT.,68,187-195. (CHECKED BY AUTHOR 05/26/83)  
 56) AMYLOID BAN: DWULET,F.E.,O'CONNOR,T.P. & BENSON,M.D. (1986) MOL.IMMUNOL.,23,73-78.  
 57) BJ19: ALESCIO-ZONTA,L. & BAGLIONI,C. (1970) EUR.J.BIOCHEM.,15,450-463. (CHECKED BY AUTHOR)  
 58) BEL: MILSTEIN,C. (1969) PROC. 5TH FEBS SYMP. 15,43-56. (CHECKED BY AUTHOR WHO PROVIDED ADDITIONAL RESIDUES TO THOSE PUBLISHED AND CORRECTED RESIDUES 1,3,6,27,79 AND 82 AS GIVEN IN TABLE)  
 59) JBL: SEON,B.K. (1982) MOL.IMMUNOL.,19,83-86. (CHECKED BY AUTHOR 05/23/83)  
 60) PAP: NIAL,H.D. & EDMAN,P. (1967) NATURE,216,262-263. (CHECKED BY AUTHOR 07/25/79)  
 61) CAR: MILSTEIN,C.P. & DEVERSON,E.V. (1974) EUR.J.BIOCHEM.,49,377-391. (CHECKED BY AUTHOR)  
 62) MEV: EULITZ,M. & LINKE,R.P. (1982) Z.PHYSIOL.CHEM.,363,1347-1358. (CHECKED BY AUTHOR 10/10/83)



**NOTES: HUMAN KAPPA LIGHT CHAINS SUBGROUP I (cont'd)****GENERAL NOTES:**

# SEE SIGNAL PEPTIDE TABLE IF # OCCURS AT POSITION 0.

**SPECIFIC NOTES:**

- 5) **HK101'CL**: THE SEQUENCE IS OBTAINED BY TRANSLATING THE NUCLEOTIDE SEQUENCE OF A CLONE OF HUMAN FOETAL LIVER DNA.  
 7) **AG**: THE AMINO ACID RESIDUES AT POSITIONS 39 AND 41 WERE REPORTED BY THE AUTHORS AS GLY AND LYS RESPECTIVELY; HOWEVER, THE PROOF WAS NOT ABSOLUTE. THUS, THEY ARE OMITTED.  
 9) **HK137'CL**: THE AMINO ACID SEQUENCE IS OBTAINED BY TRANSLATING THE NUCLEOTIDE SEQUENCE OF A CLONE OF HUMAN FETAL DNA.  
 10) **HK134'CL**: THE AMINO ACID SEQUENCE IS OBTAINED BY TRANSLATING THE NUCLEOTIDE SEQUENCE OF A CLONE OF HUMAN FETAL DNA.  
 17) **BJ26**: ACID RESIDUES AT POSITIONS 39 AND 41 OF BJ26 WERE REPORTED BY THE AUTHORS AS GLY AND LYS RESPECTIVELY. SINCE THIS PROTEIN WAS SEQUENCED BEFORE THE SEQUENCES OF MANY OTHER PROTEINS WERE KNOWN AT THESE TWO POSITIONS, WE HAVE OMITTED THEM.  
 33) **F-GUI**: THE SEQUENCES OF F-GUI AND S-GUI WERE FROM THE SAME PATIENT.  
 44) **HK102'CL**: THE SEQUENCE IS OBTAINED BY TRANSLATING THE NUCLEOTIDE SEQUENCE OF A CLONE OF HUMAN FOETAL LIVER DNA.  
 55) **S-GUI**: THE SEQUENCES OF F-GUI AND S-GUI WERE FROM THE SAME PATIENT.  
 56) **AMYLOID BAN**: AMINO ACID RESIDUES FOUND AT POSITIONS 104 AND 105 ARE VAL,LEU AND GLN,GLU RESPECTIVELY.  
 57) **BJ19**: THE AMINO ACID RESIDUES AT POSITIONS 39 AND 41 WERE REPORTED BY THE AUTHORS AS GLY AND LYS RESPECTIVELY. SINCE THIS PROTEIN WAS SEQUENCED BEFORE THE SEQUENCES OF MANY OTHER PROTEINS WERE KNOWN AT THESE TWO POSITIONS, WE HAVE OMITTED THEM.  
 59) **JBL**: THE AMINO ACID RESIDUE FOUND AT POSITION 34 WAS ALA OR SER.  
 64) **AMYLOID ES305**: THE AMINO ACID RESIDUES AT POSITIONS 21 AND 29 WERE ILE OR LEU.  
 74) **PW**: THE SEQUENCE WAS FROM A PATIENT WITH TRANSITIONAL CELL CARCINOMA OF THE URINARY BLADDER.  
 82) **RI**: THE SEQUENCE WAS FROM A PATIENT WITH TRANSITIONAL CELL CARCINOMA OF THE URINARY BLADDER.  
 109) **AMYLOID MS**: THE AMINO ACID RESIDUE AT POSITION 2 MS WAS ILE OR LEU.  
 111) **GM131'CL**: FROM AN EPSTEIN-BARR VIRUS-TRANSFORMED HUMAN LYMPHOID CELL LINE

+ THE FOLLOWING WERE EQUALLY AND MOST FREQUENTLY OCCURRING:

| AT POSITION | RESIDUES      |
|-------------|---------------|
| 27C         | (LEU,VAL)     |
| 27D         | (TRP,GLU)     |
| 50          | (ALA,ASP)     |
| 92          | (TYR,ASP,ASN) |
| 95A         | (SER,GLY)     |
| 95B         | (TRP,GLY)     |





## ANTIBODY SPECIFICITIES: HUMAN KAPPA LIGHT CHAINS SUBGROUP II

- 5) **ROB**: COLD AGGLUTININ WITH ANTI-PR10 ACTIVITY  
 10) **WILS**: COLD AGGLUTININ WITH ANTI-BLOOD GROUP I ACTIVITY  
 14) **FR**: ANTI-PHOSPHOCHOLINE (BINDING CONSTANT=6.4X10EXP4)  
 24) **GLI**: ANTI-IGG  
 27) **TH**: COLD AGGLUTININ WITH ANTI-PR2 ACTIVITY (RBC MEMBRANE ANTIGEN ON HUMAN, RAT AND GUINEA PIG ERYTHROCYTES INACTIVATED BY PROTEOLYTIC ENZYMES AND NEURAMINIDASE)

## REFERENCE: HUMAN KAPPA LIGHT CHAINS SUBGROUP II

- 1) **TEW**: PUTNAM,F.W.,WHITLEY,E.J.,JR., PAUL,C. & DAVIDSON,J.N. (1973) BIOCHEMISTRY,12,3763-3780. (CHECKED BY AUTHOR 06/15/83)  
 2) **MIL**: DREYER,W.J.,GRAY,W.R. & HOOD,L. (1967) COLD SPRING HARBOR SYMP. QUANTITATIVE BIOL.,32,353-367.  
 3) **NIM**: EULLITZ,M. & KLEY,H.-P. (1977) IMMUNOCHEM.,14,289-297. (CHECKED BY AUTHOR 10/18/77)  
 4) **CUM**: HILSCHMANN,N. & CRAIG,L.C. (1965) PROC.NAT.ACAD.SCI.USA,53,1403-1409; HILSCHMANN,N. (1967) Z.PHYSIOL.CHEM.,348,1718-1722; HILSCHMANN,N. (1969) NATURE.,56,195-205. (CHECKED BY AUTHOR)  
 5) **GM 607**: KLOBECK,H.G.,SOLOMON,A. & ZACHAU,H.G. (1984) NATURE,309,73-76.  
 6) **BAT**: DAYHOFF,M.O. (1972) ATLAS OF PROTEIN SEQUENCE & STRUCTURE,5,D-246. SUBMITTED BY SMITHIES,O.,GIBSON,D.M. AND FANNING,E.M. (CHECKED BY AUTHOR)  
 7) **BATES**: SMITH,G.P.,HOOD,L. & FITCH,W.M. (1971) ANN.REV.BIOCHEM.,40,969-1012.  
 8) **ROB**: GERGELY,J.,WANG,A.C. & FUDENBERG,H.H. (1973) VOX SANG.,24,432-440. (CHECKED BY AUTHOR)  
 9) **SLO**: WANG,A.C.,TUNG,E.,WANG,I.,FUDENBERG,H.H.,PICK,A.I. & FROELICHMAN,R. (1980) CANCER IMMUNOL.IMMUNOTHER.,9,81-86. (CHECKED BY AUTHOR 03/18/81)  
 10) **WILS**: CAPRA,J.D.,KEHOE,J.M.,WILLIAMS,R.C.,JR.,FEIZI,T. & KUNKEL,H.G. (1972) PROC.NAT.ACAD.SCI.USA,69,40-43. (CHECKED BY AUTHOR)  
 11) **GLI**: FRANGIONE,B.,FRANKLIN,E.C. & PRELLI,F. (1976) SCAND.J.IMMUNOL.,5,623-627. (CHECKED BY AUTHOR 10/17/77)  
 12) **AMYLOID TEW**: TERRY,W.D.,PAGE,D.L.,KIMURA,S.,ISOBE,T.,OSSELMAN,E.F. & GLENNER,G.G. (1973) J.CLIN.INVEST.,52,1276-1281. (CHECKED BY AUTHOR 03/02/84)  
 13) **RAI**: MILSTEIN,C.P. & MILSTEIN,C. (1971) BIOCHEM.J.,121,211-215. (CHECKED BY AUTHOR WHO PROVIDED ADDITIONAL RESIDUES TO THOSE PUBLISHED)  
 14) **FR**: RIESEN,W.,RUDIKOFF,S.,ORIOLE,R. & POTTER,M. (1975) BIOCHEMISTRY,14,1052-1057; RIESEN,W.F.,BRAUN,D.G. & JATON,J.C. (1976) PROC.NAT.ACAD.SCI.USA,73,2096-2100; RIESEN,W.F. & JATON,J.C. (1976) BIOCHEMISTRY,15,3829-3833. (CHECKED BY AUTHOR 12/05/77)  
 15) **YOS**: WANG,A.C.,TUNG,E.,WANG,I.,FUDENBERG,H.H.,PICK,A.I. & FROELICHMAN,R. (1980) CANCER IMMUNOL.IMMUNOTHER.,9,81-86. (CHECKED BY AUTHOR 03/18/81)  
 16) **RPM1-6410**: HIETER,P.A.,MAX,E.E.,SEIDMAN,J.G.,MAIZEL,J.V.,JR. & LEDER,P. (1980) CELL,22,197-207; KLOBECK,H.G.,MEINDL,A.,COMBRIATO,G.,SOLOMON,A. & ZACHAU,H.G. (1985) NUC.ACIDS RES.,13,6499-6513.  
 17) **MAN**: MILSTEIN,C. (1969) PROC. 5TH FEBS SYMP.,15,43-56. (CHECKED BY AUTHOR WHO PROVIDED ADDITIONAL RESIDUES TO THOSE PUBLISHED)  
 18) **KIR**: SLETTEN,K.,HANNESTAD,K. & HARBOE,M. (1974) SCAND.J.IMMUNOL.,3,215-218. (CHECKED BY AUTHOR 12/05/77)  
 19) **HYL**: SLETTEN,K.,HANNESTAD,K. & HARBOE,M. (1974) SCAND.J.IMMUNOL.,3,215-218. (CHECKED BY AUTHOR 12/05/77)  
 20) **MAG**: SLETTEN,K.,HANNESTAD,K. & HARBOE,M. (1974) SCAND.J.IMMUNOL.,3,215-218. (CHECKED BY AUTHOR 12/05/77)  
 21) **TVE**: SLETTEN,K.,HANNESTAD,K. & HARBOE,M. (1974) SCAND.J.IMMUNOL.,3,215-218. (CHECKED BY AUTHOR 12/05/77)  
 22) **EID**: SLETTEN,K.,HANNESTAD,K. & HARBOE,M. (1974) SCAND.J.IMMUNOL.,3,215-218. (CHECKED BY AUTHOR 12/05/77)  
 23) **GAL(II)**: MILSTEIN,C.,JARVIS,J.M. & MILSTEIN,C.P. (1969) J.MOL.BIOL.,46,599-602. (CHECKED BY AUTHOR)  
 24) **GI**: ABRAHAM,G.N.,BROWN,P.,JOHNSTON,S.L.,NELLIS,L.,MARKS,S. & WELCH,E.H. (1978) IMMUNOLOGY,35,447-453. (CHECKED BY AUTHOR 07/23/79)  
 25) **MEH**: SLETTEN,K.,HANNESTAD,K. & HARBOE,M. (1974) SCAND.J.IMMUNOL.,3,215-218. (CHECKED BY AUTHOR 12/05/77)  
 26) **SC**: SEON,B.K.,YAGI,Y. & PRESSMAN,D. (1973) J.IMMUNOL.,110,345-349. (CHECKED BY AUTHOR)  
 27) **TH**: GERGELY,J.,WANG,A.C. & FUDENBERG,H.H. (1973) VOX SANG.,24,432-440. (CHECKED BY AUTHOR)  
 28) **SYV**: SLETTEN,K.,HANNESTAD,K. & HARBOE,M. (1974) SCAND.J.IMMUNOL.,3,215-218. (CHECKED BY AUTHOR 12/05/77)  
 29) **LUT**: SLETTEN,K.,HANNESTAD,K. & HARBOE,M. (1974) SCAND.J.IMMUNOL.,3,215-218. (CHECKED BY AUTHOR 12/05/77)  
 30) **ROB2**: MOULIN,A. & FOUGEREAU,M. (1973) NATURE NEW BIOLOGY,246,176-178.  
 31) **RAI2**: MOULIN,A. & FOUGEREAU,M. (1973) NATURE NEW BIOLOGY,246,176-178.

## NOTES: HUMAN KAPPA LIGHT CHAINS SUBGROUP II

## IDENTICAL SETS OF FRAMEWORK SEGMENTS:

- FR1: SET 1: TEW[1],MIL[2],NIM[3],CUM[4],GM 607 'CL[5],BAT[6],BATES[7],ROB[8],SLO[9],WILS[10],GLI[11],AMYLOID TEW[12],RAI[13]. (13 IDENTICAL)  
 FR2: SET 1: MIL[2],NIM[3],GM 607 'CL[5]. (3 IDENTICAL HUMAN V-KAPPA-II; ALSO 2 MOUSE V-KAPPA-II: VKAPPA 24B'CL[63],2S1[367].)  
 SET 2: MIL[2],FR[14]. (2 IDENTICAL)  
 FR3: SET 1: TEW[1],GM 607 'CL[5],RPM1-6410'CL[16]. (3 IDENTICAL)  
 FR4: SET 1: GM 607 'CL[5],RPM1-6410'CL[16]. (2 IDENTICAL HUMAN V-KAPPA-II; ALSO 3 HUMAN V-KAPPA-I: AU[2],GAL[II][36],CL'11[10]; 7 HUMAN V-KAPPA-III: WOL[2],PAY[7],PIE[11],GLO[15],CUR[20],REE[57],VKAPPA3'CL[82]; AND 1 HUMAN V-KAPPA-IV: PB17IV'CL[3].)  
 SET 2: NIM[3],FR[14]. (2 IDENTICAL HUMAN V-KAPPA-II; ALSO 3 HUMAN V-KAPPA-I: AGI[7],DEN[46],BI[63]; 6 HUMAN V-KAPPA-III: NEUI[5],GOT[6],GAR[10],FLO[12],FR4[21],IARC/BL4'CL[28]; AND 1 HUMAN V-KAPPA-IV: LEN[4].)  
 SET 3: TEW[1]. (IDENTICAL TO 2 HUMAN V-KAPPA-I: WALKER'CL[12],OU[100][34].)

## IDENTICAL SETS OF COMPLEMENTARITY DETERMINING REGIONS:

- CDR1:  
 CDR2: SET 1: MIL[2],NIM[3],GM 607 'CL[5]. (3 IDENTICAL)  
 CDR3:

## IDENTICAL SETS OF J-MINIGENES:

- SET 1: RPM1-6410'CL[16]. (IDENTICAL TO 1 HUMAN V-KAPPA-I: AU[2]; 2 HUMAN V-KAPPA-III: PIE[11],VKAPPA3'CL[82]; AND 1 HUMAN V-KAPPA-IV: PB17IV'CL[3].)  
 SET 2: TEW[1]. (IDENTICAL TO 1 HUMAN V-KAPPA-I: WALKER'CL[12].)  
 SET 3: FR[14]. (IDENTICAL TO 2 HUMAN V-KAPPA-I: DEN[46],BI[63]; AND 3 HUMAN V-KAPPA-III: GAR[10],FLO[12],IARC/BL4'CL[28].)

## SPECIFIC NOTES:

- 12) **AMYLOID TEW**: IT HAS THE SAME SEQUENCE AS THAT OF TEW SO FAR AS THE SEQUENCED POSITIONS ARE CONCERNED.  
 14) **FR**: AN IDIOTYPIC ANTIBODY TO FR NOT INHIBITABLE BY PHOSPHORYLCHOLINE REACTED BETTER WITH THE FR HEAVY CHAIN THAN WITH THE LIGHT CHAIN. THE CROSS-REACTION WITH MOPC167 WAS 10,000 TIMES WEAKER. (RIESEN,W.F. (1979) EUR.J.IMMUNOL.,9,421-425.)  
 16) **RPM1-6410**: THE AMINO ACID SEQUENCE IS OBTAINED BY TRANSLATING THE NUCLEOTIDE SEQUENCE OF A CLONE OF HUMAN ADULT DNA.

+ THE FOLLOWING WERE EQUALLY AND MOST FREQUENTLY OCCURRING:

| AT POSITION | RESIDUES              |
|-------------|-----------------------|
| 27F         | (GLY,ASN) ; (GLY,ASP) |
| 28          | (ASP,ASN)             |
| 31          | (THR,ASP)             |
| 34          | (ASP,ASN)             |
| 45          | (GLU,GLN)             |
| 79          | (GLU,GLN)             |
| 94          | (THR,SER)             |
| 104         | (LEU,VAL)             |











**ANTIBODY SPECIFICITIES: HUMAN KAPPA LIGHT CHAINS SUBGROUP III**

- 2) **WOL:** ANTI-HUMAN GAMMA G GLOBULIN; WA IDIOTYPE
- 3) **SIE:** ANTI-HUMAN GAMMA G GLOBULIN; WA IDIOTYPE
- 5) **NEU:** CRYOGLOBULIN WITH ANTI-IGG ACTIVITY; B IDIOTYPE (KUNKEL,H.G.,WINCHESTER,R.J.,JOSLIN,F.G. & CAPRA,J.D. (1974) J.EXP.MED.,139,128)
- 6) **GOT:** CRYOGLOBULIN WITH ANTI-IGG ACTIVITY; B IDIOTYPE
- 7) **PAY:** CRYOGLOBULIN WITH ANTI-IGG ACTIVITY; B IDIOTYPE
- 8) **SON:** CRYOGLOBULIN WITH ANTI-LOW-DENSITY LIPOPROTEIN ACTIVITY; B IDIOTYPE
- 9) **WEI:** CRYOGLOBULIN WITH ANTI-LOW-DENSITY LIPOPROTEIN ACTIVITY; B IDIOTYPE
- 10) **GAR:** CRYOGLOBULIN WITH ANTI-IGG ACTIVITY; B IDIOTYPE
- 11) **PIE:** AUTOANTIBODY WHICH BINDS SPECIFICALLY TO INTERMEDIATE FILAMENTS
- 12) **FLO:** CRYOGLOBULIN WITH ANTI-IGG ACTIVITY; B IDIOTYPE
- 13) **LOP:** CRYOGLOBULIN WITH ANTI-IGG ACTIVITY; B IDIOTYPE
- 14) **SCA:** CRYOGLOBULIN WITH ANTI-LOW-DENSITY LIPOPROTEIN ACTIVITY; B IDIOTYPE
- 15) **GLO:** ANTI-HUMAN GAMMA G GLOBULIN; WA IDIOTYPE; CRYOGLOBULIN WITH ANTI-IGG ACTIVITY; B IDIOTYPE
- 18) **MA:** COLD AGGLUTININ WITH ANTI-BLOOD GROUP I ACTIVITY (GROUP 1)
- 19) **NIC:** COLD AGGLUTININ WITH ANTI-BLOOD GROUP SMALL I ACTIVITY
- 20) **CUR:** CRYOGLOBULIN WITH ANTI-IGG ACTIVITY; B IDIOTYPE
- 22) **DRE:** COLD AGGLUTININ WITH ANTI-BLOOD GROUP I ACTIVITY
- 23) **PER:** COLD AGGLUTININ WITH ANTI-BLOOD GROUP I ACTIVITY
- 25) **STE:** COLD AGGLUTININ WITH ANTI-BLOOD GROUP I ACTIVITY
- 26) **GJ:** COLD AGGLUTININ WITH ANTI-BLOOD GROUP I ACTIVITY (ATYPICAL)
- 27) **TAK:** COLD AGGLUTININ WITH ANTI-BLOOD GROUP I ACTIVITY
- 35) **AJ:** COLD AGGLUTININ WITH ANTI-BLOOD GROUP I ACTIVITY
- 42) **CLA:** CRYOGLOBULIN WITH ANTI-IGG ACTIVITY; B IDIOTYPE
- 43) **SHE:** CRYOGLOBULIN WITH ANTI-IGG ACTIVITY; B IDIOTYPE
- 48) **POM:** ANTI-HUMAN GAMMA G1 GLOBULIN; PO IDIOTYPE
- 54) **GOEII:** ANTI-MEASLES VIRUS (WOODFOLK STRAIN); ANTI-SUBACUTE SCLEROSING PANENCEPHALITIS VIRUS (LEC STRAIN)
- 62) **TEH:** ANTI-HUMAN GAMMA G GLOBULIN
- 63) **CRA(III):** ANTI-HUMAN GAMMA G GLOBULIN
- 64) **PLA:** ANTI-HUMAN GAMMA G GLOBULIN
- 65) **PIN:** ANTI-HUMAN GAMMA G GLOBULIN
- 70) **BOR:** COLD AGGLUTININ WITH ANTI-BLOOD GROUP I ACTIVITY
- 71) **DRI:** ANTI-HUMAN GAMMA G GLOBULIN
- 72) **WAL:** ANTI-HUMAN GAMMA G GLOBULIN
- 73) **GOL:** ANTI-HUMAN GAMMA G GLOBULIN
- 74) **GAG:** COLD AGGLUTININ WITH ANTI-BLOOD GROUP I ACTIVITY

**CLASS: HUMAN KAPPA LIGHT CHAINS SUBGROUP III**

- 5) **NEU:** IGM-KAPPA
- 6) **GOT:** IGM-KAPPA
- 7) **PAY:** IGM-KAPPA
- 8) **SON:** IGM-KAPPA
- 9) **WEI:** IGM-KAPPA
- 10) **GAR:** IGM-KAPPA
- 11) **PIE:** IGM-KAPPA
- 12) **FLO:** IGM-KAPPA
- 13) **LOP:** IGM-KAPPA
- 14) **SCA:** IGM-KAPPA
- 15) **GLO:** IGM-KAPPA
- 20) **CUR:** IGM-KAPPA
- 42) **CLA:** IGM-KAPPA
- 43) **SHE:** IGM-KAPPA

**REFERENCE: HUMAN KAPPA LIGHT CHAINS SUBGROUP III**

- 1) **TI:** SUTER,L.,BARNIKOL,H.U.,WATANABE,S. & HILSCHMANN,N. (1969) Z.PHYSIOL.CHEM.,350,275-278; (1972) Z.PHYSIOL.CHEM.,353,189-208. (CHECKED BY AUTHOR)
- 2) **WOL:** ANDREWS,D.W. & CAPRA,J.D. (1981) PROC.NAT.ACAD.SCI.USA,78,3799-3803. (CHECKED BY AUTHOR 08/25/81); ANDREWS,D.W. & CAPRA,J.D. (1981) BIOCHEMISTRY,20,5816-5822.
- 3) **SIE:** CAPRA,J.D. (1975) ADV.IMMUNOLOGY,20,1-40. (CHECKED BY AUTHOR); ANDREWS,D.W. & CAPRA,J.D. (1981) PROC.NAT.ACAD.SCI.USA,78,3799-3803. (CHECKED BY AUTHOR 08/25/81 WHO SUGGESTED THAT THE SEQUENCE DETERMINED IN 1975 WAS INCORRECT AND SHOULD BE DELETED); ANDREWS,D.W. & CAPRA,J.D. (1981) BIOCHEMISTRY,20,5816-5822.
- 4) **NG9CL:** BENTLEY,D.L. (1984) NATURE,307,77-80.
- 5) **NEU:** LEDFORD,D.K.,GONI,F.,PIZZOLATO,M.,FRANKLIN,E.C.,SOLOMON,A. & FRANGIONE,B. (1983) J.IMMUNOL.,131,1322-1325. (CHECKED BY AUTHOR 03/23/84); GONI,F.,CHEN,P.P.,PONS-ESTEL,B.,CARSON,D.A. & FRANGIONE,B. (1985) J.IMMUNOL.,135,4073-4079.
- 6) **GOT:** LEDFORD,D.K.,GONI,F.,PIZZOLATO,M.,FRANKLIN,E.C.,SOLOMON,A. & FRANGIONE,B. (1983) J.IMMUNOL.,131,1322-1325. (CHECKED BY AUTHOR 03/23/84); PONS-ESTEL,B.,GONI,F.,SOLOMON,A. & FRANGIONE,B. (1984) J.EXP.MED.,160,893-904; GONI,F.,CHEN,P.P.,PONS-ESTEL,B.,CARSON,D.A. & FRANGIONE,B. (1985) J.IMMUNOL.,135,4073-4079.
- 7) **PAY:** LEDFORD,D.K.,GONI,F.,PIZZOLATO,M.,FRANKLIN,E.C.,SOLOMON,A. & FRANGIONE,B. (1983) J.IMMUNOL.,131,1322-1325. (CHECKED BY AUTHOR 03/23/84); GONI,F.,CHEN,P.P.,PONS-ESTEL,B.,CARSON,D.A. & FRANGIONE,B. (1985) J.IMMUNOL.,135,4073-4079.
- 8) **SON:** LEDFORD,D.K.,GONI,F.,PIZZOLATO,M.,FRANKLIN,E.C.,SOLOMON,A. & FRANGIONE,B. (1983) J.IMMUNOL.,131,1322-1325. (CHECKED BY AUTHOR 03/23/84); PONS-ESTEL,B.,GONI,F.,SOLOMON,A. & FRANGIONE,B. (1984) J.EXP.MED.,160,893-904.
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- 10) **GAR:** LEDFORD,D.K.,GONI,F.,PIZZOLATO,M.,FRANKLIN,E.C.,SOLOMON,A. & FRANGIONE,B. (1983) J.IMMUNOL.,131,1322-1325. (CHECKED BY AUTHOR 03/23/84); PONS-ESTEL,B.,GONI,F.,SOLOMON,A. & FRANGIONE,B. (1984) J.EXP.MED.,160,893-904; GONI,F.,CHEN,P.P.,PONS-ESTEL,B.,CARSON,D.A. & FRANGIONE,B. (1985) J.IMMUNOL.,135,4073-4079.
- 11) **PIE:** PONS-ESTEL,B.,GONI,F.,SOLOMON,A. & FRANGIONE,B. (1984) J.EXP.MED.,160,893-904. (CHECKED BY AUTHOR 05/16/86)
- 12) **FLO:** LEDFORD,D.K.,GONI,F.,PIZZOLATO,M.,FRANKLIN,E.C.,SOLOMON,A. & FRANGIONE,B. (1983) J.IMMUNOL.,131,1322-1325. (CHECKED BY AUTHOR 03/23/84); GONI,F.,CHEN,P.P.,PONS-ESTEL,B.,CARSON,D.A. & FRANGIONE,B. (1985) J.IMMUNOL.,135,4073-4079.
- 13) **LOP:** LEDFORD,D.K.,GONI,F.,PIZZOLATO,M.,FRANKLIN,E.C.,SOLOMON,A. & FRANGIONE,B. (1983) J.IMMUNOL.,131,1322-1325. (CHECKED BY AUTHOR 03/23/84)
- 14) **SCA:** LEDFORD,D.K.,GONI,F.,PIZZOLATO,M.,FRANKLIN,E.C.,SOLOMON,A. & FRANGIONE,B. (1983) J.IMMUNOL.,131,1322-1325. (CHECKED BY AUTHOR 03/23/84)
- 15) **GLO:** CAPRA,J.D. (1975) ADV.IMMUNOLOGY,20,1-40. (CHECKED BY AUTHOR); LEDFORD,D.K.,GONI,F.,PIZZOLATO,M.,FRANKLIN,E.C.,SOLOMON,A. & FRANGIONE,B. (1983) J.IMMUNOL.,131,1322-1325. (CHECKED BY AUTHOR 03/23/84); GONI,F.,CHEN,P.P.,PONS-ESTEL,B.,CARSON,D.A. & FRANGIONE,B. (1985) J.IMMUNOL.,135,4073-4079.
- 16) **SAL:** CAPRA,J.D.,KEHOE,J.M.,WINCHESTER,R.J. & KUNKEL,H.G. (1971) ANN.N.Y.ACAD.SCI.,190,371-381. (CHECKED BY AUTHOR)
- 17) **WIL:** CAPRA,J.D.,KEHOE,J.M.,WINCHESTER,R.J. & KUNKEL,H.G. (1971) ANN.N.Y.ACAD.SCI.,190,371-381. (CHECKED BY AUTHOR)
- 18) **MA:** CAPRA,J.D.,KEHOE,J.M.,WILLIAMS,R.C.,JR.,FEIZI,T. & KUNKEL,H.G. (1972) PROC.NAT.ACAD.SCI.USA,69,40-43. (CHECKED BY AUTHOR)
- 19) **NIC:** CAPRA,J.D.,KEHOE,J.M.,WILLIAMS,R.C.,JR.,FEIZI,T. & KUNKEL,H.G. (1972) PROC.NAT.ACAD.SCI.USA,69,40-43. (CHECKED BY AUTHOR)
- 20) **CUR:** LEDFORD,D.K.,GONI,F.,PIZZOLATO,M.,FRANKLIN,E.C.,SOLOMON,A. & FRANGIONE,B. (1983) J.IMMUNOL.,131,1322-1325. (CHECKED BY AUTHOR 03/23/84); GONI,F.,CHEN,P.P.,PONS-ESTEL,B.,CARSON,D.A. & FRANGIONE,B. (1985) J.IMMUNOL.,135,4073-4079.
- 21) **FR4:** MILSTEIN,C. (1969) FEBS LETTERS,2,301-304. (CHECKED BY AUTHOR WHO PROVIDED ADDITIONAL RESIDUES TO THOSE PUBLISHED)
- 22) **DRE:** GERGELY,J.,WANG,A.C. & FUDENBERG,H.H. (1973) VOX SANG.,24,432-440. (CHECKED BY AUTHOR)
- 23) **PER:** GERGELY,J.,WANG,A.C. & FUDENBERG,H.H. (1973) VOX SANG.,24,432-440. (CHECKED BY AUTHOR)
- 24) **CAM:** HOPPER,J.E.,NOYES,C.,HSU,R.,HEINRIKSON,R. & GALLAGHER,W. (1979) J.IMMUNOL.,122,2007-2010. (CHECKED BY AUTHOR 01/26/83)
- 25) **STE:** EDMAN,P. & COOPER,A.G. (1968) FEBS LETTERS,2,33-35. (CHECKED BY AUTHOR)
- 26) **GJ:** CAPRA,J.D.,KEHOE,J.M.,WILLIAMS,R.C.,JR.,FEIZI,T. & KUNKEL,H.G. (1972) PROC.NAT.ACAD.SCI.USA,69,40-43. (CHECKED BY AUTHOR)
- 27) **TAK:** GERGELY,J.,WANG,A.C. & FUDENBERG,H.H. (1973) VOX SANG.,24,432-440. (CHECKED BY AUTHOR)
- 28) **IARC/BL41'CL:** KLOBECK,H.G.,MEINDL,A.,COMBRIATO,G.,SOLOMON,A. & ZACHAU,H.G. (1985) NUC.ACIDS RES.,13,6499-6513.
- 29) **RAD:** MILSTEIN,C. (1969) FEBS LETTERS,2,301-304. (CHECKED BY AUTHOR)
- 30) **DIL:** DAYHOFF,M.O. (1972) ATLAS OF PROTEIN SEQUENCE & STRUCTURE,5,D-250. SUBMITTED BY SMITHIES,O.,GIBSON,D.M. AND FANNING,E.M. (CHECKED BY AUTHOR 07/24/79)

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- 31) **CAS**: NIALL,H.D. & EDMAN,P. (1967) NATURE,216,262-263. (CHECKED BY AUTHOR 07/25/79)
- 32) **MCE**: MIDDAGH,C.R.,KEHOE,J.M.,PRYSTOWSKY,M.B.,GERBER-JENSON,B.,JENSON,J.C. & LITMAN,G.W. (1978) IMMUNOCHEM.,15,171-187. (CHECKED BY AUTHOR 10/22/80)
- 33) **KEA**: WANG,A.C. & FUDENBERG,H.H. (1975) IMMUNOL.COMMUN.,4,483-497. (CHECKED BY AUTHOR 09/23/77); WANG,A.C.,TUNG,E.,WANG,I.,FUDENBERG,H.H.,PICK,A.I. & FROELICHMAN,R. (1980) CANCER IMMUNOL.IMMUNOTHER.,9,81-86. (CHECKED BY AUTHOR 03/18/81)
- 34) **SMI**: NIALL,H.D. & EDMAN,P. (1967) NATURE,216,262-263. (CHECKED BY AUTHOR 07/25/79)
- 35) **AJ**: CAPRA,J.D.,KEHOE,J.M.,WILLIAMS,R.C.,JR.,FEIZI,T. & KUNKEL,H.G. (1972) PROC.NAT.ACAD.SCI.USA,69,40-43. (CHECKED BY AUTHOR)
- 36) **BJ'GG**: HOPPER,J.E.,NOYES,C.,HEINRIKSON,R. & KESSEL,J.W. (1976) J.IMMUNOL.,116,743-746. (CHECKED BY AUTHOR 01/26/83)
- 37) **NIG**: NIALL,H.D. & EDMAN,P. (1967) NATURE,216,262-263. (CHECKED BY AUTHOR 07/25/79)
- 38) **IKE**: CAPRA,J.D. (1975) ADV.IMMUNOLOGY,20,1-40. (CHECKED BY AUTHOR)
- 39) **TIL**: PINK,J.R.L.,WANG,A.C. & FUDENBERG,H.H. (1971) ANN.REV.MED.,22,145-170. (CHECKED BY AUTHOR)
- 40) **AMYLOID KSA**: SLETTEN,K.,WESTERMARK,P.,PITKANEN,P.,THYRESSON,N. & OLSTAD,O.K. (1983) SCAND.J.IMMUNOL.,18,557-560. (CHECKED BY AUTHOR 04/26/84)
- 41) **WANG,A.C.,WELLS,J.V.,FUDENBERG,H.H. & GERGELY,J.** (1974) IMMUNOCHEM.,11,341-345. (CHECKED BY AUTHOR)
- 42) **CLA**: LEDFORD,D.K.,GONI,F.,PIZZOLATO,M.,FRANKLIN,E.C.,SOLOMON,A. & FRANGIONE,B. (1983) J.IMMUNOL.,131,1322-1325. (CHECKED BY AUTHOR 03/23/84)
- 43) **SHE**: LEDFORD,D.K.,GONI,F.,PIZZOLATO,M.,FRANKLIN,E.C.,SOLOMON,A. & FRANGIONE,B. (1983) J.IMMUNOL.,131,1322-1325. (CHECKED BY AUTHOR 03/23/84)
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- 45) **WIN**: NIALL,H.D. & EDMAN,P. (1967) NATURE,216,262-263. (CHECKED BY AUTHOR 07/25/79)
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- 48) **POM**: KLAPPER,D.G. & CAPRA,J.D. (1976) ANN.IMMUNOL.(INST.PASTEUR),127C,261-271. (CHECKED BY AUTHOR 08/01/79)
- 49) **VAND**: SEON,B.K.,GAILANI,S.,HENDERSON,E.S. & PRESSMAN,D. (1977) IMMUNOCHEM.,14,567-572. (CHECKED BY AUTHOR 08/28/78)
- 50) **AMYLOID SO124**: SLETTEN,K.,WESTERMARK,P.,PITKANEN,P.,THYRESSON,N. & OLSTAD,O.K. (1983) SCAND.J.IMMUNOL.,18,557-560. (CHECKED BY AUTHOR 04/26/84)
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- 52) **SHM**: WANG,A.C.,TUNG,E.,WANG,I.,FUDENBERG,H.H.,PICK,A.I. & FROELICHMAN,R. (1980) CANCER IMMUNOL.IMMUNOTHER.,9,81-86. (CHECKED BY AUTHOR 03/18/81)
- 53) **GRA**: NIALL,H.D. & EDMAN,P. (1967) NATURE,216,262-263. (CHECKED BY AUTHOR 07/25/79)
- 54) **GOEII**: STROSBERG,A.D.,KARCHER,D. & LOWENTHAL,A. (1975) J.IMMUNOL.,115,157-160. (CHECKED BY AUTHOR)
- 55) **LOW**: WANG,A.C.,TUNG,E.,WANG,I.,FUDENBERG,H.H.,PICK,A.I. & FROELICHMAN,R. (1980) CANCER IMMUNOL.IMMUNOTHER.,9,81-86. (CHECKED BY AUTHOR 03/18/81)
- 56) **VER**: CHERSIA, & NATALI,P.G. (1978) IMMUNOCHEMISTRY,15,585-589. (CHECKED BY AUTHOR 09/13/79)
- 57) **REE**: PRELLI,F.,TUMMOLO,D.,SOLOMON,A. & FRANGIONE,B. (1986) J.IMMUNOL., IN PRESS.
- 58) **WE**: DWORSKY,E.,SLETTEN,K.,HARBOE,M. & WETTELAND,P. (1980) SCAND.J.IMMUNOL.,12,281-287. (CHECKED BY AUTHOR 02/28/1984)
- 59) **HOW**: KAPLAN,A.P. & METZGER,H. (1969) BIOCHEMISTRY,8,3944-3951. (CHECKED BY AUTHOR)
- 60) **HS4**: HOOD,L.,GRAY,W.R.,SANDERS,B.G. & DREYER,W.J. (1967) COLD SPRING HARBOR SYMP. QUANTITATIVE BIOL.,32,133-145.
- 61) **HBJS**: HOOD,L.,GRAY,W.R.,SANDERS,B.G. & DREYER,W.J. (1967) COLD SPRING HARBOR SYMP. QUANTITATIVE BIOL.,32,133-145.
- 62) **TEH**: JOHNSTON,S.L.,ABRAHAM,G.N. & WELCH,E.H. (1975) BIOCHEM.BIOPHYS.RES.COMMUN.,66,842-847. (CHECKED BY AUTHOR 10/17/77)
- 63) **CRA(III)**: JOHNSTON,S.L.,ABRAHAM,G.N. & WELCH,E.H. (1975) BIOCHEM.BIOPHYS.RES.COMMUN.,66,842-847. (CHECKED BY AUTHOR 10/17/77)
- 64) **PLA**: JOHNSTON,S.L.,ABRAHAM,G.N. & WELCH,E.H. (1975) BIOCHEM.BIOPHYS.RES.COMMUN.,66,842-847. (CHECKED BY AUTHOR 10/17/77)
- 65) **PIN**: JOHNSTON,S.L.,ABRAHAM,G.N. & WELCH,E.H. (1975) BIOCHEM.BIOPHYS.RES.COMMUN.,66,842-847. (CHECKED BY AUTHOR 10/17/77)
- 66) **MCE**: CAPRA,J.D.,KEHOE,J.M.,WILLIAMS,R.C.,JR.,FEIZI,T. & KUNKEL,H.G. (1972) PROC.NAT.ACAD.SCI.USA,69,40-43. (CHECKED BY AUTHOR)
- 67) **HAC**: HOOD,L. & TALMAGE,D.W. (1970) SCIENCE,168,325-334.
- 68) **K- EV15'CL**: STAVNEZER,J.,KEKISH,O.,BATTER,D.,GRENIER,J.,BALAZS,I.,HENDERSON,E. & ZEGERS,B.J.M. (1985) NUC.ACIDS RES.,13,3495-3514.
- 69) **BER**: WANG,A.C.,WELLS,J.V.,FUDENBERG,H.H. & GERGELY,J. (1974) IMMUNOCHEM.,11,341-345. (CHECKED BY AUTHOR)
- 70) **BOR**: GERGELY,J.,WANG,A.C. & FUDENBERG,H.H. (1973) VOX SANG.,24,432-440. (CHECKED BY AUTHOR)
- 71) **DRI**: CAPRA,J.D. (1975) ADV.IMMUNOLOGY,20,1-40. (CHECKED BY AUTHOR)
- 72) **WAL**: CAPRA,J.D. (1975) ADV.IMMUNOLOGY,20,1-40. (CHECKED BY AUTHOR)
- 73) **GOL**: CAPRA,J.D. (1975) ADV.IMMUNOLOGY,20,1-40. (CHECKED BY AUTHOR)
- 74) **GAG**: CAPRA,J.D.,KEHOE,J.M.,WILLIAMS,R.C.,JR.,FEIZI,T. & KUNKEL,H.G. (1972) PROC.NAT.ACAD.SCI.USA,69,40-43. (CHECKED BY AUTHOR)
- 75) **DOB**: HOOD,L. & TALMAGE,D.W. (1970) SCIENCE,168,325-334.
- 76) **HS8**: HOOD,L.,GRAY,W.R.,SANDERS,B.G. & DREYER,W.J. (1967) COLD SPRING HARBOR SYMP. QUANTITATIVE BIOL.,32,133-145.
- 77) **HBJ12**: HOOD,L.,GRAY,W.R.,SANDERS,B.G. & DREYER,W.J. (1967) COLD SPRING HARBOR SYMP. QUANTITATIVE BIOL.,32,133-145.
- 78) **BUR(K)**: MOULIN,A. & FOUGEREAU,M. (1973) NATURE NEW BIOLOGY,246,176-178. (CHECKED BY AUTHOR)
- 79) **LEG**: MOULIN,A. & FOUGEREAU,M. (1973) NATURE NEW BIOLOGY,246,176-178. (CHECKED BY AUTHOR)
- 80) **B6**: MILSTEIN,C. (1969) FEBS LETTERS,2,301-304. (CHECKED BY AUTHOR)
- 81) **AMYLOID WR**: WESTERMARK,P.,SLETTEN,K.,PITKANEN,P.,NATVIG,J.B. & LINDHOLM,C.E. (1982) MOL.IMMUNOL.,19,447-450. (CHECKED BY AUTHOR 06/01/83)
- 82) **VKAPPA3'CL**: BENTLEY,D.L. & RABBITTS,T.H. (1981) CELL,24,613-623. (CHECKED BY AUTHOR 12/07/81)

## NOTES: HUMAN KAPPA LIGHT CHAINS SUBGROUP III

## IDENTICAL SETS OF FRAMEWORK SEGMENTS:

- FR1**: SET 1: TI[1],WOL[2],SIE[3],NG9'CL[4],NEU[5],GOT[6],PAY[7],SON[8],WEI[9],GAR[10],PIE[11],FLO[12],LOP[13],SCA[14],GLO[15],SAL[16],WIL[17],MAI[18],NIC[19],CUR[20],FR4[21],DRE[22],PER[23],CAM[24]. (24 IDENTICAL)
- SET 2: GJ[28],TAK[27]. (2 IDENTICAL)
- SET 3: RAD[29],DIL[30],CAS[31]. (3 IDENTICAL)
- SET 4: KEA[33],SMI[34]. (2 IDENTICAL HUMAN V-KAPPA-III; ALSO 1 MOUSE V-KAPPA-V: Vg'CL[122].)
- SET 5: DRE[22],PER[23],BRO'GG[36]. (3 IDENTICAL)
- SET 6: CLA[42],SHE[43]. (2 IDENTICAL)
- FR2**: SET 1: TI[1],WOL[2],SIE[3],NG9'CL[4],NEU[5],GOT[6],SON[8],GAR[10],PIE[11],FLO[12],GLO[15],CUR[20]. (12 IDENTICAL HUMAN V-KAPPA-III; ALSO 1 MOUSE V-KAPPA-IV: Vh'CL[12]; AND 1 MOUSE V-KAPPA-V: Vg'CL[122].)
- FR3**: SET 1: TI[1],WOL[2]. (2 IDENTICAL)
- SET 2: GOT[6],PAY[7],GAR[10],PIE[11],FLO[12],GLO[15],CUR[20]. (7 IDENTICAL)
- FR4**: SET 1: WOL[2],PAY[7],PIE[11],GLO[15],CUR[20],REE[57],VKAPPA3'CL[82]. (7 IDENTICAL HUMAN V-KAPPA-III; ALSO 3 HUMAN V-KAPPA-I: AU[2],RPM1-6410'CL[16]; AND 1 HUMAN V-KAPPA-IV: PB17IV'CL[3].)
- SET 2: POM[48]. (IDENTICAL TO 1 HUMAN V-KAPPA-I: HAU[4].)
- SET 3: NEU[5],GOT[6],GAR[10],FLO[12],FR4[21],IARC/BL41'CL[28]. (6 IDENTICAL HUMAN V-KAPPA-III; ALSO 3 HUMAN V-KAPPA-I: AGI[7],DEN[46],BI[63]; 2 HUMAN V-KAPPA-II: NIM[3],FR[14]; AND 1 HUMAN V-KAPPA-IV: LEN[4].)
- SET 4: SON[8]. (IDENTICAL TO 1 HUMAN V-KAPPA-IV: VJ'CL[1].)

## IDENTICAL SETS OF COMPLEMENTARITY DETERMINING REGIONS:

- CDR1**: SET 1: SIE[3],IKE[38]. (2 IDENTICAL)
- SET 2: NG9'CL[4],PAY[7],SON[8],WEI[9],GAR[10],PIE[11],FLO[12],GLO[15],CUR[20],DRE[22],CAM[24]. (11 IDENTICAL)
- SET 3: TIL[39]. (IDENTICAL TO 1 MOUSE V-KAPPA-V: Vg'CL[122].)
- CDR2**: SET 1: WOL[2],SIE[3],NEU[5],GOT[6],PAY[7],SON[8],GAR[10],PIE[11],FLO[12],GLO[15],CUR[20]. (11 IDENTICAL)
- SET 2: POM[48]. (IDENTICAL TO 1 MOUSE V-KAPPA-IV: Vh'CL[12].)
- CDR3**: SET 1: POM[48]. (IDENTICAL TO 1 HUMAN V-KAPPA-I: LAY[39].)
- SET 2: GOT[6],CUR[20]. (2 IDENTICAL)
- SET 3: PAY[7],GLO[15]. (2 IDENTICAL)
- SET 4: GAR[10],FLO[12]. (2 IDENTICAL)

## IDENTICAL SETS OF J-MINIGENES:

- SET 1: PIE[11],VKAPPA3'CL[82]. (2 IDENTICAL HUMAN V-KAPPA-III; ALSO 1 HUMAN V-KAPPA-I: AU[2]; 1 HUMAN V-KAPPA-II: RPM1-6410'CL[16]; AND 1 HUMAN V-KAPPA-IV: PB17IV'CL[3].)
- SET 2: GOT[6]. (IDENTICAL TO 1 HUMAN V-KAPPA-I: AGI[7].)
- SET 3: GAR[10],FLO[12],IARC/BL41'CL[28]. (3 IDENTICAL HUMAN V-KAPPA-III; ALSO 2 HUMAN V-KAPPA-I: DEN[46],BI[63]; AND 1 HUMAN V-KAPPA-II: FR[14].)
- SET 4: WOL[2],CUR[20]. (2 IDENTICAL)
- SET 5: PAY[7],GLO[15]. (2 IDENTICAL)

## SPECIFIC NOTES:

- 4) **NG9'CL**: THE AMINO ACID SEQUENCE IS TRANSLATED FROM THE NUCLEOTIDE SEQUENCE OF A CLONE OF HUMAN CDNA.
- 32) **MCE**: IT IS A CRYOIMMUNOGLOBULIN. THE AUTHORS ORIGINALLY DESIGNATED IT AS MCE. BUT IN ORDER TO DIFFERENTIATE IT FROM ANOTHER MCE SEQUENCED BY CAPRA ET AL., IT IS DENOTED AS MCE'.
- 42) **CLA**: THE AMINO ACID RESIDUES FOUND AT POSITION 9 WERE GLY AND ALA.
- 43) **SHE**: THE AMINO ACID RESIDUES FOUND AT POSITION 9 WERE GLY AND ALA.

**NOTES: HUMAN KAPPA LIGHT CHAINS SUBGROUP III (cont'd)**

44) JH: THE NAME WAS GIVEN TO US BY THE AUTHORS. IT IS NOT INCLUDED IN THE PAPER.

58) WE: AT POSITIONS 20,29 AND 33 OF AMINO ACID SEQUENCE WERE FOUND BOTH LEU AND ILE. IN THE SAME SEQUENCE TWO RESIDUES WERE FOUND IN POSITIONS 1,3,4,9,10,15,17,19,20,21,22 AND 29. THE SECOND RESIDUES WERE GLU,VAL,LEU,GLY,THR,PRO,GLU,ALA,THR,LEU,SER AND VAL, RESPECTIVELY. A DETERMINATION WAS NOT MADE IN THE ARTICLE AS TO WHETHER THE SEQUENCE BELONGED TO SUBGROUP I OR TO SUBGROUP III.

81) AMYLOID WR: AMINO ACID RESIDUES FOUND AT POSITION 54 ARE LEU AND ALA.

82) VKAPPA3'CL: THE AMINO ACID SEQUENCE IS OBTAINED BY TRANSLATING THE NUCLEOTIDE SEQUENCE OF A CLONE OF CDNA FROM A MOUSE-HUMAN HYBRID CELL LINE.



## HUMAN KAPPA LIGHT CHAINS SUBGROUP IV (cont'd)

|      | OCCURRENCES<br>OF MOST COMMON<br>AMINO ACID | VARIABILITY |
|------|---|-------------|
| 0    |   |             |
| 1    | 15(ASP)                                     | 1.          |
| 2    | 14(LEU)                                     | 2.1         |
| 3    | 15(VAL)                                     | 1.          |
| 4    | 13(MET)                                     | 2.3         |
| 5    | 14(THR)                                     | 2.1         |
| 6    | 15(GLN)                                     | 1.          |
| 7    | 14(SER)                                     | 1.          |
| 8    | 15(PRO)                                     | 1.          |
| 9    | 10(ASP) : 7(ASP)                            | 7.5 : 11.   |
| 10   | 11(SER)                                     | 2.4         |
| 11   | 14(LEU)                                     | 1.          |
| 12   | 14(ALA)                                     | 1.          |
| 13   | 14(VAL)                                     | 1.          |
| 14   | 11(SER)                                     | 1.          |
| 15   | 8(LEU)                                      | 2.8         |
| 16   | 11(GLY)                                     | 1.          |
| 17   | 7(GLU) : 5(GLU)                             | 3.1 : 6.6   |
| 18   | 8(ARG)                                      | 4.1         |
| 19   | 12(ALA)                                     | 1.          |
| 20   | 12(THR)                                     | 1.          |
| 21   | 9(ILE)                                      | 4.          |
| 22   | 7(ASN) : 4(+)                               | 5.1 : 9.    |
| 23   | 10(CYS)                                     | 1.          |
| 24   | 5(LYS)                                      | 6.          |
| 25   | 5(SER)                                      | 5.4         |
| 26   | 6(SER)                                      | 2.3         |
| 27   | 7(GLN) : 6(GLN)                             | 1. : 2.3    |
| 27A  | 5(SER)                                      |             |
| 27B  | 6(VAL)                                      |             |
| 27C  | 6(LEU)                                      |             |
| 27D  | 5(TYR)                                      |             |
| 27E  | 4(SER)                                      |             |
| 27F  | 4(SER)                                      |             |
| 28   | 3(ASN)                                      | 3.3         |
| 29   | 3(ASN)                                      | 2.7         |
| 30   | 5(LYS)                                      | 1.          |
| 31   | 4(ASN)                                      | 1.          |
| 32   | 4(TYR)                                      | 1.          |
| 33   | 4(LEU)                                      | 1.          |
| 34   | 4(ALA)                                      | 1.          |
| 35   | 4(TRP)                                      | 1.          |
| 36   | 4(TYR)                                      | 1.          |
| 37   | 4(GLN)                                      | 1.          |
| 38   | 4(GLN)                                      | 1.          |
| 39   | 4(LYS)                                      | 1.          |
| 40   | 5(PRO)                                      | 1.          |
| 41   | 5(GLY)                                      | 1.          |
| 42   | 5(GLN)                                      | 1.          |
| 43   | 4(PRO)                                      | 2.5         |
| 44   | 5(PRO)                                      | 1.          |
| 45   | 5(LYS)                                      | 1.          |
| 46   | 5(LEU)                                      | 1.          |
| 47   | 5(LEU)                                      | 1.          |
| 48   | 5(ILE)                                      | 1.          |
| 49   | 5(TYR)                                      | 1.          |
| 50   | 5(TRP)                                      | 1.          |
| 51   | 4(ALA)                                      | 2.5         |
| 52   | 4(SER)                                      | 2.5         |
| 53   | 4(THR)                                      | 1.          |
| 54   | 4(ARG)                                      | 1.          |
| 55   | 4(GLU)                                      | 1.          |
| 56   | 4(SER)                                      | 1.          |
| 57   | 4(GLY)                                      | 1.          |
| 58   | 4(VAL)                                      | 1.          |
| 59   | 4(PRO)                                      | 1.          |
| 60   | 4(ASP)                                      | 1.          |
| 61   | 4(ARG)                                      | 1.          |
| 62   | 4(PHE)                                      | 1.          |
| 63   | 5(SER)                                      | 1.          |
| 64   | 5(GLY)                                      | 1.          |
| 65   | 5(SER)                                      | 1.          |
| 66   | 5(GLY)                                      | 1.          |
| 67   | 5(SER)                                      | 1.          |
| 68   | 4(GLY)                                      | 2.5         |
| 69   | 4(THR)                                      | 1.          |
| 70   | 4(ASP)                                      | 1.          |
| 71   | 4(PHE)                                      | 1.          |
| 72   | 4(THR)                                      | 1.          |
| 73   | 4(LEU)                                      | 1.          |
| 74   | 4(THR)                                      | 1.          |
| 75   | 4(ILE)                                      | 1.          |
| 76   | 4(SER)                                      | 1.          |
| 77   | 4(SER)                                      | 1.          |
| 78   | 4(LEU)                                      | 1.          |
| 79   | 4(GLN)                                      | 1.          |
| 80   | 4(ALA)                                      | 1.          |
| 81   | 4(GLU)                                      | 1.          |
| 82   | 4(ASP)                                      | 1.          |
| 83   | 4(VAL)                                      | 1.          |
| 84   | 4(ALA)                                      | 1.          |
| 85   | 4(VAL)                                      | 1.          |
| 86   | 4(TYR)                                      | 1.          |
| 87   | 4(TYR)                                      | 1.          |
| 88   | 4(CYS)                                      | 1.          |
| 89   | 4(GLN)                                      | 1.          |
| 90   | 4(GLN)                                      | 1.          |
| 91   | 4(TYR)                                      | 1.          |
| 92   | 3(TYR)                                      | 2.7         |
| 93   | 2(SER)                                      | 6.          |
| 94   | 2(THR)                                      | 6.          |
| 95   | 4(PRO)                                      | 1.          |
| 95A  |   |             |
| 95B  |   |             |
| 95C  |   |             |
| 95D  |   |             |
| 95E  |   |             |
| 95F  |   |             |
| 96   | 1(+)  | 4.          |
| 97   | 2(THR)                                      | 3.          |
| 98   | 3(PHE)                                      | 1.          |
| 99   | 3(GLY)                                      | 1.          |
| 100  | 2(GLN)                                      | 3.          |
| 101  | 3(GLY)                                      | 1.          |
| 102  | 3(THR)                                      | 1.          |
| 103  | 3(LYS)                                      | 1.          |
| 104  | 2(+)  | 4.          |
| 105  | 4(GLU)                                      | 1.          |
| 106  | 4(ILE)                                      | 1.          |
| 106A |   |             |
| 107  | 3(LYS)                                      | 2.7         |
| 108  | 3(ARG)                                      | 1.          |
| 109  | 1(THR)                                      | 1.          |



**ANTIBODY SPECIFICITIES: HUMAN KAPPA LIGHT CHAINS SUBGROUP IV**

- 3) **PB17IV'CL**: ANTI-STREPTOCOCCUS GROUP A CARBOHYDRATE WITH SPECIFICITY FOR N-ACETYL GLUCOSAMINE  
 5) **R.K.:** COLD AGGLUTININ WITH ANTI-PR1H ACTIVITY (RBC MEMBRANE ANTIGEN ON HUMAN ERYTHROCYTES INACTIVATED BY PROTEOLYTIC ENZYMES AND NEURAMINIDASE)  
 6) **L.TH.:** COLD AGGLUTININ WITH ANTI-PR2 ACTIVITY (RBC MEMBRANE ANTIGEN ON HUMAN, RAT AND GUINEA PIG ERYTHROCYTES INACTIVATED BY PROTEOLYTIC ENZYMES AND NEURAMINIDASE)  
 7) **TUR:** COLD AGGLUTININ WITH ANTI-PR ACTIVITY

**REFERENCE: HUMAN KAPPA LIGHT CHAINS SUBGROUP IV**

- 1) **VJ'CL:** KLOBECK,H.G.,BORNKAMMM,G.W.,COMBRIATO,G.,MOCIKAT,R.,POHLENZ,H.D. & ZACHAU,H.G. (1985) NUC.ACIDS RES.,13,6515-6529. (CHECKED BY AUTHOR 02/25/86)  
 2) **VKAPPA IV GERMLINE'CL:** KLOBECK,H.G.,BORNKAMMM,G.W.,COMBRIATO,G.,MOCIKAT,R.,POHLENZ,H.D. & ZACHAU,H.G. (1985) NUC.ACIDS RES.,13, 6515-6529.  
 3) **PB17IV'CL:** MARSH,P.,MILLS,F. & GOULD,H. (1985) NUC.ACIDS RES.,13,6531-6544. (CHECKED BY AUTHOR 03/19/86 WHO CORRECTED A MISPRINT IN THE ORIGINAL PAPER FOR RESIDUE 50)  
 4) **LEN:** SCHNEIDER,M. & HILSCHMANN,N. (1974) Z.PHYSIOL.CHEM.,355,1164-1168. (CHECKED BY AUTHOR)  
 5) **R.K.:** WANG,A.C.,FUDENBERG,H.H.,WELLS,J.V. & ROELCKE,D. (1973) NATURE NEW BIOLOGY,243,126-128. (CHECKED BY AUTHOR)  
 6) **L.TH.:** WANG,A.C.,FUDENBERG,H.H.,WELLS,J.V. & ROELCKE,D. (1973) NATURE NEW BIOLOGY,243,126-128. (CHECKED BY AUTHOR)  
 7) **TUR:** CAPRA,J.D.,KEHOE,J.M.,WILLIAMS,R.C.,JR.,FEIZI,T. & KUNKEL,H.G. (1972) PROC.NAT.ACAD.SCI.USA,69,40-43. (CHECKED BY AUTHOR)  
 8) **AH:** PICK,A.I.,WANG,A.C.,FROHLICHMAN,R. & FUDENBERG,H.H. (1982) ACTA HAEMAT.,68,207-214. (CHECKED BY AUTHOR 05/26/83)  
 9) **DA:** WANG,A.C.,ZHANG,H.S.,BONWALD,L.,TUNG,E.,BOUVET,J.P. & LIACOPOULOS,P. (1985) MIAMI WINTER SYMP.,17,335-336. (CHECKED BY AUTHOR 02/25/86 WHO CORRECTED RESIDUES AS SHOWN)  
 10) **DA-H:** BOUVET,J.P.,LIACOPOULOS,P.,PILLOT,J.,BANDA,R.,TUNG,E. & WANG,A.C. (1980) J.IMMUNOL.,125,213-220. (CHECKED BY AUTHOR 08/04/80); BOUVET,J.P.,LIACOPOULOS,P.,PILLOT,J.,BANDA,R.,TUNG,E. & WANG,A.C. (1982) J.IMMUNOL.,129,1519-1524.  
 11) **DA-N:** BOUVET,J.P.,LIACOPOULOS,P.,PILLOT,J.,BANDA,R.,TUNG,E. & WANG,A.C. (1980) J.IMMUNOL.,125,213-220. (CHECKED BY AUTHOR 08/04/80); BOUVET,J.P.,LIACOPOULOS,P.,PILLOT,J.,BANDA,R.,TUNG,E. & WANG,A.C. (1982) J.IMMUNOL.,129,1519-1524.  
 12) **JAH:** SLETTEN,K.,HANNESTAD,K. & HARBOE,M. (1974) SCAND.J.IMMUNOL.,3,219-222. (CHECKED BY AUTHOR 12/05/77)  
 13) **SCH:** SLETTEN,K.,HANNESTAD,K. & HARBOE,M. (1974) SCAND.J.IMMUNOL.,3,219-222. (CHECKED BY AUTHOR 12/05/77)  
 14) **JUV:** SLETTEN,K.,HANNESTAD,K. & HARBOE,M. (1974) SCAND.J.IMMUNOL.,3,219-222. (CHECKED BY AUTHOR 12/05/77)  
 15) **AMYLOID GAB:** PRAS,M.,FRANGIONE,B. & FRANKLIN,E.C. (1980) IN AMYLOID AND AMYLOIDOSIS,G.G.GLENNER,P.P.E COSTA & F.DE FREITAS EDS., EXCERPTA MEDICA AMSTERDAM OXFORD-PRINCETON,249-252. (CHECKED BY AUTHOR 11/18/81)

**NOTES: HUMAN KAPPA LIGHT CHAINS SUBGROUP IV****IDENTICAL SETS OF FRAMEWORK SEGMENTS:**

- FR1:** SET 1: VJ'CL[1],VKAPPA IV GERMLINE'CL[2],PB17IV'CL[3],R.K.[5]. (4 IDENTICAL)  
 SET 2: LEN[4],R.K.[5]. (2 IDENTICAL)  
 SET 3: DA[9],DA-H[10]. (2 IDENTICAL)  
**FR2:** SET 1: VJ'CL[1],VKAPPA IV GERMLINE'CL[2],PB17IV'CL[3],LEN[4]. (4 IDENTICAL HUMAN V-KAPPA-IV; ALSO 2 HUMAN V-KAPPA-I; V19B'CL[88], V18B'CL[89]; 1 MOUSE V-KAPPA-I; MCPC603[47]; 30 MOUSE V-KAPPA-III: MPC11'CL[6],TEPC11[17],PC3741(NZB)[8],TEPC124[9], MOPC321[12],PC7043(NZB)[13],PC7183(NZB)[14],PC6308(NZB)[15],PC6684(NZB)[17],PC7940(NZB)[18],PC7175(NZB)[19], PC2485(NZB)[20],PC4039(NZB)[21],PC7210(NZB)[23],H36-15[26],2242[29],V-21E1,5KB'CL[30],V-21C9,5KB'CL[31], PC7461(NZB)[33],PC2960(NZB)[34],37,C(A,SY)[35],10,A(TH)[39],H36-5[48],40,C(A,TH)[52],MOPC63[54],ABPC2[55], PC9245(NZB)[56],PC4050(NZB)[57],V-21B16KB'CL[58],11949[62]; 1 MOUSE V-KAPPA-VI: BFPC61A'CL[64]; AND 15 RABBIT V-KAPPA: K9-335-[19],3369[20],BS-5[38],BS-1[39],K49-501[45],3547[47],K4820[57],K30-267[61],311[65],4422[66],17D9'CL[68], 4192[71],4363[85],120[103],K-25[112].)  
**FR3:** SET 1: VJ'CL[1],VKAPPA IV GERMLINE'CL[2],PB17IV'CL[3],LEN[4]. (4 IDENTICAL)  
**FR4:** SET 1: PB17IV'CL[3]. (IDENTICAL TO 3 HUMAN V-KAPPA-I; AU[2],GAL[1][36],CL\*[110]; 2 HUMAN V-KAPPA-II; GM 607 'CL[5], RPM1-6410'CL[16]; AND 7 HUMAN V-KAPPA-III: WOL[2],PAY[7],PIE[11],GLO[15],CUR[20],REE[57],VKAPPA3'CL[82].)  
 SET 2: LEN[4]. (IDENTICAL TO 3 HUMAN V-KAPPA-I; AG[7],DEN[46],B[63]; 2 HUMAN V-KAPPA-II; NIM[3],FR[14]; AND 6 HUMAN V-KAPPA-III: NEU[5],GOT[6],GAR[10],FLO[12],FR4[21],IARC[BL41'CL[28].)  
 SET 3: VJ'CL[1]. (IDENTICAL TO 1 HUMAN V-KAPPA-III: SON[8].)

**IDENTICAL SETS OF COMPLEMENTARITY DETERMINING REGIONS:**

- CDR1:** SET 1: VJ'CL[1],VKAPPA IV GERMLINE'CL[2]. (2 IDENTICAL)  
**CDR2:** SET 1: VJ'CL[1],VKAPPA IV GERMLINE'CL[2],PB17IV'CL[3],LEN[4]. (4 IDENTICAL HUMAN V-KAPPA-IV; ALSO 1 MOUSE V-KAPPA-VI: KPN16 'CL[70].)  
**CDR3:**

**IDENTICAL SETS OF J-MINGENES:**

- SET 1: FB17IV'CL[3]. (IDENTICAL TO 1 HUMAN V-KAPPA-I; AU[2]; 1 HUMAN V-KAPPA-II: RPM1-6410'CL[16]; AND 2 HUMAN V-KAPPA-III: PIE[11],VKAPPA3'CL[82].)

+ THE FOLLOWING WERE EQUALLY AND MOST FREQUENTLY OCCURRING:

| AT POSITION | RESIDUES      |
|-------------|---------------|
| 22          | (SER,ASP,ASN) |
| 96          | (TRP,TYR)     |
| 104         | (LEU,VAL)     |



## HUMAN LAMBDA LIGHT CHAINS SUBGROUP 1 (cont'd)

|       | 24<br>FUL<br># | # OF<br>SEQUENCES | # OF<br>AMINO<br>ACIDS | OCCURRENCES<br>OF MOST COMMON<br>AMINO ACID | VARIABILITY |
|-------|----------------|-------------------|------------------------|---|-------------|
|       | 0              |                   |                        |   |             |
|       | 1              | 20                | 2                      | 19(PCA)                                     | 2.1         |
|       | 2              | 20                | 1                      | 20(SER)                                     | 1.          |
|       | 3              | 21                | 1                      | 20(VAL)                                     | 2.1         |
|       | 4              | 21                | 1                      | 21(LEU)                                     | 1.          |
|       | 5              | 22                |                        | 22(THR)                                     | 1.          |
|       | 6              | 21                | 1 : 2                  | 21(GLN) : 20(GLN)                           | 1. : 2.1    |
|       | 7              | 21                | 1                      | 21(PRO)                                     | 1.          |
|       | 8              | 21                | 1                      | 21(PRO)                                     | 1.          |
|       | 9              | 21                | 1                      | 21(SER)                                     | 1.          |
| FR 1  | 10             |                   |                        |   |             |
|       | 11             | 21                | 3                      | 11(ALA)                                     | 5.7         |
|       | 12             | 22                | 1                      | 22(SER)                                     | 1.          |
|       | 13             | 22                | 2                      | 16(GLY)                                     | 2.8         |
|       | 14             | 22                | 3                      | 11(THR)                                     | 6.          |
|       | 15             | 21                | 2                      | 20(PRO)                                     | 2.1         |
|       | 16             | 21                | 1                      | 21(GLY)                                     | 1.          |
|       | 17             | 21                | 2                      | 20(GLN) : 19(GLN)                           | 2.1 : 2.2   |
|       | 18             | 21                | 2                      | 14(ARG)                                     | 2.1         |
|       | 19             | 20                | 2                      | 19(VAL)                                     | 2.1         |
|       | 20             | 20                | 4                      | 16(THR)                                     | 5.          |
|       | 21             | 19                | 2                      | 18(ILE)                                     | 2.1         |
|       | 22             | 19                | 2                      | 18(SER)                                     | 2.1         |
|       | 23             | CYS<br>SER        | 1<br>3                 | 19(CYS)<br>15(SER)                          | 1.<br>3.8   |
|       | 24             | GLY               | 18                     | 18(GLY)                                     | 1.          |
|       | 25             | ASN               | 17                     | 13(SER)                                     | 3.9         |
|       | 26             | SER               | 16                     | 12(SER)                                     | 6.7         |
|       | 27             | ---               |                        |   |             |
|       | 27A            | ---               |                        |   |             |
|       | 27B            | ---               |                        |   |             |
| CDR 1 | 27C            | SER               | 15                     | 12(SER)                                     |             |
|       | 27D            |                   | 3                      | 12(ASN)                                     |             |
|       | 27E            |                   | 3                      | 2(ILE)                                      | 7.5         |
|       | 27F            |                   | 4                      | 10(ILE)                                     |             |
|       | 28             |                   | 5                      |   | 3.5         |
|       | 29             |                   | 3                      | 12(GLY)                                     | 25          |
|       | 30             |                   | 7                      | 4(SER)                                      | 5.1         |
|       | 31             |                   | 4                      | 11(ASN)                                     | 17.         |
|       | 32             |                   | 6                      | 5(TYR)                                      | 1.          |
|       | 33             |                   | 1                      | 14(VAL)                                     | 25.         |
|       | 34             |                   | 7                      | 4(+)  |             |
|       | 35             |                   | 1                      | 14(TRP)                                     | 1.          |
|       | 36             |                   | 2                      | 13(TYR)                                     | 2.2         |
|       | 37             |                   | 3                      | 12(GLN)                                     | 3.5         |
|       | 38             |                   | 3                      | 9(GLN)                                      | 4.7         |
|       | 39             |                   | 4                      | 9(LEU)                                      | 6.2         |
|       | 40             |                   | 1                      | 14(PRO)                                     | 1.          |
|       | 41             |                   | 1                      | 14(GLY)                                     | 1.          |
|       | 42             |                   | 3                      | 12(THR)                                     | 3.5         |
|       | 43             |                   | 2                      | 13(ALA)                                     | 2.2         |
|       | 44             |                   | 1                      | 14(PRO)                                     | 1.          |
|       | 45             |                   | 2                      | 13(LYS)                                     | 2.2         |
|       | 46             |                   | 1                      | 14(LEU)                                     | 1.          |
|       | 47             |                   | 2                      | 13(LEU)                                     | 2.2         |
|       | 48             |                   | 2                      | 13(ILE)                                     | 2.2         |
|       | 49             |                   | 2                      | 12(TYR)                                     | 2.3         |
| CDR 2 | 50             |                   | 8                      | 4(SER)                                      | 28.         |
|       | 51             |                   | 3                      | 8(ASN)                                      | 5.3         |
|       | 52             |                   | 3                      | 8(ASN)                                      | 5.3         |
|       | 53             |                   | 5                      | 6(GLN)                                      | 12.         |
|       | 54             |                   | 3                      | 12(ARG)                                     | 3.5         |
|       | 55             |                   | 3                      | 10(PRO)                                     | 3.6         |
|       | 56             |                   | 1                      | 12(SER)                                     | 1.          |
|       | 57             |                   | 1                      | 12(GLY)                                     | 1.          |
|       | 58             |                   | 2                      | 9(VAL)                                      | 2.7         |
|       | 59             |                   | 2                      | 10(PRO)                                     | 2.4         |
|       | 60             |                   | 2                      | 11(ASP)                                     | 2.2         |
|       | 61             |                   | 1                      | 13(ARG)                                     | 1.          |
|       | 62             |                   | 2                      | 12(PHE)                                     | 2.3         |
|       | 63             |                   | 1                      | 14(SER)                                     | 1.          |
|       | 64             |                   | 3                      | 9(GLY)                                      | 4.7         |
|       | 65             |                   | 1                      | 14(SER)                                     | 1.          |
|       | 66             |                   | 1                      | 14(LYS)                                     | 1.          |
|       | 67             |                   | 1                      | 14(SER)                                     | 1.          |
|       | 68             |                   | 1                      | 14(GLY)                                     | 1.          |
|       | 69             |                   | 3                      | 12(THR)                                     | 3.5         |
|       | 70             |                   | 1                      | 14(SER)                                     | 1.          |
|       | 71             |                   | 1                      | 14(ALA)                                     | 1.          |
|       | 72             |                   | 2                      | 9(SER)                                      | 3.1         |
|       | 73             |                   | 1                      | 14(LEU)                                     | 1.          |
|       | 74             |                   | 2                      | 11(ALA)                                     | 2.5         |
|       | 75             |                   | 1                      | 14(ILE)                                     | 1.          |
|       | 76             |                   | 2                      | 9(SER)                                      | 3.1         |
|       | 77             |                   | 1                      | 14(GLY)                                     | 1.          |
|       | 78             |                   | 1                      | 14(LEU)                                     | 1.          |
|       | 79             |                   | 4                      | 9(GLN)                                      | 6.2         |
|       | 80             |                   | 4                      | 8(SER)                                      | 7.          |
|       | 81             |                   | 2                      | 10(GLU)                                     | 2.8         |
|       | 82             |                   | 2                      | 13(ASP)                                     | 2.2         |
|       | 83             |                   | 1                      | 14(GLU)                                     | 1.          |
|       | 84             |                   | 3                      | 11(ALA)                                     | 3.8         |
|       | 85             |                   | 3                      | 12(ASP)                                     | 3.5         |
|       | 86             |                   | 1                      | 14(TYR)                                     | 1.          |
|       | 87             |                   | 3                      | 11(TYR)                                     | 3.8         |
|       | 88             |                   | 1                      | 14(CYS)                                     | 1.          |
|       | 89             |                   | 3                      | 10(ALA)                                     | 4.2         |
|       | 90             |                   | 3                      | 7(THR)                                      | 6.          |
|       | 91             |                   | 2                      | 12(TRP)                                     | 2.3         |
|       | 92             |                   | 2                      | 12(ASP)                                     | 2.3         |
|       | 93             |                   | 5                      | 8(ASP)                                      | 8.8         |
|       | 94             |                   | 2                      | 12(SER)                                     | 2.3         |
|       | 95             |                   | 2                      | 13(LEU)                                     | 2.2         |
|       | 95A            |                   | 1                      | 8(ASP)                                      |             |
|       | 95B            |                   | 4                      | 6(GLY)                                      |             |
|       | 95C            |                   |                        |   |             |
|       | 95D            |                   |                        |   |             |
|       | 95E            |                   |                        |   |             |
|       | 95F            |                   |                        |   |             |
|       | 96             | 14                | 7                      | 6(PRO)                                      | 16.         |
|       | 97             | 14                | 3                      | 12(VAL)                                     | 3.5         |
|       | 98             | 14                | 1                      | 14(PHE)                                     | 1.          |
|       | 99             | 14                | 1                      | 14(GLY)                                     | 1.          |
|       | 100            | 14                | 2                      | 13(GLY)                                     | 2.2         |
|       | 101            | 14                | 1                      | 14(GLY)                                     | 1.          |
|       | 102            | 14                | 1                      | 14(THR)                                     | 1.          |
| FR 4  | 103            | 14                | 5                      | 10(LYS)                                     | 7.          |
|       | 104            | 14                | 2                      | 7(+)  | 4.          |
|       | 105            | 14                | 1                      | 14(THR)                                     | 1.          |
|       | 106            | 14                | 1                      | 14(VAL)                                     | 1.          |
|       | 106A           | 14                | 3                      | 12(LEU)                                     | 1.          |
|       | 107            | 14                | 3                      | 11(GLY)                                     | 3.8         |
|       | 108            | 12                | 1                      | 12(GLN)                                     | 1.          |
|       | 109            | 12                | 1                      | 12(PRO)                                     | 1.          |

## ANTIBODY SPECIFICITIES: HUMAN LAMBDA LIGHT CHAINS SUBGROUP I

- 1) **NEWM:** ANTI-3-(3'-HYDROXY-3',7',11',15'-TETRAMETHYL HEXADECYL) 2-METHYL 1,4 NAPHTHOQUINONE(VIT.K10H)  
 16) **KOH:** ANTI-HUMAN GAMMA G GLOBULIN

## REFERENCE: HUMAN LAMBDA LIGHT CHAINS SUBGROUP I

- 1) **NEWM:** CHEN,B.L. & POLJAK,R.J. (1974) BIOCHEMISTRY,13,1295-1302. (CHECKED BY AUTHOR 01/24/78)  
 2) **HA:** SHINODA,T.,TITANI,K. & PUTNAM,F.W. (1970) J.BIOL.CHEM.,245,4475-4487. (CHECKED BY AUTHOR 06/15/83)  
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## NOTES: HUMAN LAMBDA LIGHT CHAINS SUBGROUP I

## IDENTICAL SETS OF FRAMEWORK SEGMENTS:

- FR1: SET 1: WAH[7],NIG-77[8],VOR[9],RHE[10],LOC[11],OKA[12]. (6 IDENTICAL)  
 FR2: SET 1: NEWM[1],AMYLOID EPS[13]. (2 IDENTICAL)  
 SET 2: HA[2],NIG-64[4]. (2 IDENTICAL)  
 SET 3: NIG-77[8],LOCI[11]. (2 IDENTICAL)  
 FR3: SET 1: NIG-64[4],BL2 'CL[6]. (2 IDENTICAL)  
 FR4: SET 1: NEWM[1]. (IDENTICAL TO 1 HUMAN V-LAMBDA-II: WHI[3]; AND 1 HUMAN V-LAMBDA-V: BOI[1])  
 SET 2: NEW[5],VOR[9],COX[15]. (3 IDENTICAL HUMAN V-LAMBDA-I; ALSO 1 HUMAN V-LAMBDA-VI: AMYLOID-AR[1]; AND 6 MOUSE V-LAMBDA: MOPC315[25],TEPC952[26],MA8-13[27],5-7[29],MOPC315-26'CL[30],MOPC315-37'CL[32])  
 SET 3: BL2 'CL[6],RHE[10],OKA[12],NIG-51[19]. (4 IDENTICAL HUMAN V-LAMBDA-I; ALSO 5 HUMAN V-LAMBDA-II: MES[2],ES492[8],TRO[14],VIL[17],WIN[21]; 4 HUMAN V-LAMBDA-III: HIL[1],CAPI[4],BAU[12],DEL[14]; 1 HUMAN V-LAMBDA-IV: SHI[1]; 3 HUMAN V-LAMBDA-VI: SUT[2],THO[4],LBV[15]; AND 24 MOUSE V-LAMBDA: MOPC104E[1],J558[2],XS104[3],HOPC[14],J698[5],H2061[6],W3159[7],Y5431[8],Y5485[9],Y5830[10],Y5669[11],MOPC511(L)[12],S178[13],Y5444[14],Y5606[15],S176[16],H2020[17],RPC20[18],IG 303LAMBDA'CL[19],S43'CL[21],S2H5'CL[38],S2E9'CL[39],S1F12'CL[40],IG 25LAMBDA'CL[41].)  
 SET 4: LOC[11]. (IDENTICAL TO 1 HUMAN V-LAMBDA-V: MCG[3].)

## IDENTICAL SETS OF COMPLEMENTARITY DETERMINING REGIONS:

- CDR1:  
 CDR2: SET 1: NIG-64[4],BL2 'CL[6]. (2 IDENTICAL)  
 CDR3: SET 1: VOR[9],NIG-51[19]. (2 IDENTICAL)

## IDENTICAL SETS OF J-MINIGENES:

- SET 1: NEW[5]. (IDENTICAL TO 1 HUMAN V-LAMBDA-VI: AMYLOID-AR[1].)  
 SET 2: BL2 'CL[6]. (IDENTICAL TO 2 HUMAN V-LAMBDA-VI: SUT[2],THO[4]. AND 24 MOUSE V-LAMBDA: MOPC104E[1],J558[2],XS104[3],HOPC[14],J698[5],H2061[6],W3159[7],Y5431[8],Y5485[9],Y5830[10],Y5669[11],MOPC511(L)[12],S178[13],Y5444[14],Y5606[15],S176[16],H2020[17],RPC20[18],IG 303LAMBDA'CL[19],S43'CL[21],S2H5'CL[38],S2E9'CL[39],S1F12'CL[40],IG 25LAMBDA'CL[41].)  
 SET 3: VOR[9],COX[15]. (2 IDENTICAL)  
 SET 4: OKA[12],NIG-51[19]. (2 IDENTICAL)

## SPECIFIC NOTES:

- 24) **FUL:** SOX AND HOOD HAVE REPORTED FOUR HUMAN V KAPPA AND ONE V LAMBDA CHAINS WITH ASN-SER/THR TO CONTAIN CARBOHYDRATE.

## + THE FOLLOWING WERE EQUALLY AND MOST FREQUENTLY OCCURRING:

| AT POSITION | RESIDUES  |
|-------------|-----------|
| 34          | (SER,ASN) |
| 104         | (LEU,VAL) |



## HUMAN LAMBDA LIGHT CHAINS SUBGROUP II (cont'd)

|      | 25<br>WAL | 26<br>4A<br>CL | # OF<br>SEQUENCES | # OF<br>AMINO<br>ACIDS | OCCURRENCES<br>OF MOST COMMON<br>AMINO ACID | VARIABILITY |
|------|-----------|----------------|-------------------|------------------------|---|-------------|
| 0    | ---       | ---            |                   |                        |   |             |
| 1    | PCA       | gln            | 26                | 3                      | 24(PCA)                                     | 3.3         |
| 2    | SER       | thr            | 26                | 2                      | 25(SER)                                     | 2.1         |
| 3    | val       | val            | 26                | 2                      | 23(ALA)                                     | 3.4         |
| 4    | LEU       | val            | 26                | 2                      | 25(LEU)                                     | 2.1         |
| 5    | THR       | THR            | 26                | 3                      | 23(THR)                                     | 3.4         |
| 6    | GLN       | GLN            | 26                | 1                      | 26(GLN) : 25(GLN)                           | 1. : 2.1    |
| 7    | PRO       | glu            | 26                | 3                      | 24(PRO)                                     | 3.3         |
| 8    | pro       | pro            | 26                | 3                      | 18(ALA)                                     | 4.3         |
| 9    | SER       | SER            | 26                | 2                      | 25(SER)                                     | 2.1         |
| 10   | ---       | ---            |                   |                        |   |             |
| 11   | ala       | leu            | 26                | 3                      | 23(VAL)                                     | 3.4         |
| 12   | SER       | thr            | 26                | 2                      | 25(SER)                                     | 2.1         |
| 13   | GLY       | val            | 26                | 4                      | 23(GLY)                                     | 4.5         |
| 14   | thr       | SER            | 26                | 2                      | 25(SER)                                     | 2.1         |
| 15   | PRO       | PRO            | 26                | 2                      | 25(PRO)                                     | 2.1         |
| 16   | GLY       | GLY            | 26                | 1                      | 26(GLY)                                     | 1.          |
| 17   | GLN       | gly            | 26                | 4                      | 23(GLN)                                     | 4.5         |
| 18   | arg       | thr            | 26                | 3                      | 23(SER)                                     | 3.4         |
| 19   |           | val            | 25                | 3                      | 18(ILE)                                     | 4.2         |
| 20   | THR       |                | 25                | 1                      | 25(THR)                                     | 1.          |
| 21   | leu       |                | 19                | 3                      | 17(ILE)                                     | 3.4         |
| 22   | thr       |                | 18                | 2                      | 17(SER)                                     | 2.1         |
| 23   | CYS       |                | 18                | 1                      | 18(CYS)                                     | 1.          |
| 24   | ALA       |                | 15                | 4                      | 9(THR)                                      | 6.7         |
| 25   | SER       |                | 15                | 2                      | 14(GLY)                                     | 2.1         |
| 26   | SER       |                | 15                | 5                      | 10(THR)                                     | 7.5         |
| 27   | THR       |                | 15                | 5                      | 7(SER)                                      | 11.         |
| 27A  | ---       |                |                   |                        |   |             |
| 27B  | ---       |                |                   |                        |   |             |
| 27C  | ---       |                |                   |                        |   |             |
| 27D  | GLY       |                | 15                | 4                      | 12(SER)                                     | 12.         |
| 27E  | ALA       |                | 15                | 4                      | 11(ASP)                                     | 9.3         |
| 27F  | VAL       |                | 15                | 2                      | 14(VAL)                                     | 14. : 14.   |
| 28   | THR       |                | 15                | 5                      | 10(GLY)                                     | 7.5         |
| 29   | SER       |                | 14                | 5                      | 6(GLY)                                      | 12.         |
| 30   | GLY       |                | 14                | 6                      | 9(TYR)                                      | 9.3         |
| 31   | TYR       |                | 14                | 7                      | 8(ASN) : 7(ASN)                             | 12. : 14.   |
| 32   | TYR       |                | 14                | 5                      | 5(TYR)                                      | 14.         |
| 33   | PRO       |                | 13                | 3                      | 11(VAL)                                     | 3.5         |
| 34   | ASN       |                | 13                | 2                      | 12(SER)                                     | 2.2         |
| 35   | TRP       |                | 14                | 1                      | 14(TRP)                                     | 1.          |
| 36   | PHE       |                | 14                | 2                      | 10(TYR)                                     | 2.8         |
| 37   | GLN       |                | 14                | 1 : 2                  | 14(GLN) : 13(GLN)                           | 1. : 2.2    |
| 38   | GLN       |                | 14                | 2 : 3                  | 13(GLN) : 12(GLN)                           | 2.2 : 3.5   |
| 39   | LYS       |                | 14                | 5                      | 10(HIS)                                     | 7.          |
| 40   | PRO       |                | 14                | 1                      | 14(PRO)                                     | 1.          |
| 41   | GLY       |                | 14                | 2                      | 13(GLY)                                     | 2.2         |
| 42   | GLN       |                | 14                | 4                      | 11(LYS)                                     | 5.1         |
| 43   | ALA       |                | 14                | 2                      | 13(ALA)                                     | 2.2         |
| 44   | PRO       |                | 14                | 1                      | 14(PRO)                                     | 1.          |
| 45   | ARG       |                | 14                | 2                      | 13(LYS)                                     | 2.2         |
| 46   | ALA       |                | 14                | 3                      | 12(LEU)                                     | 3.5         |
| 47   | LEU       |                | 14                | 3                      | 5( - )                                      | 8.4         |
| 48   | ILE       |                | 14                | 1                      | 14(ILE)                                     | 1.          |
| 49   | TYR       |                | 14                | 3                      | 9(TYR)                                      | 4.7         |
| 50   | SER       |                | 14                | 5                      | 7(ASP)                                      | 10.         |
| 51   | THR       |                | 14                | 4                      | 11(VAL)                                     | 5.1         |
| 52   | SER       |                | 14                | 5                      | 5(SER)                                      | 14.         |
| 53   | ASN       |                | 14                | 6                      | 4( - )                                      | 21          |
| 54   | LYS       |                | 14                | 2                      | 13(ARG)                                     | 2.2         |
| 55   | HIS       |                | 14                | 2                      | 13(PRO)                                     | 2.2         |
| 56   | SER       |                | 14                | 1                      | 14(SER)                                     | 1.          |
| 57   | TRP       |                | 14                | 2                      | 13(GLY)                                     | 2.2         |
| 58   | THR       |                | 14                | 3                      | 10(VAL)                                     | 4.2         |
| 59   | PRO       |                | 14                | 2                      | 7( - )                                      | 4.          |
| 60   | ALA       |                | 14                | 7                      | 5(ASP)                                      | 20.         |
| 61   | ARG       |                | 14                | 1                      | 14(ARG)                                     | 1.          |
| 62   | PHE       |                | 15                | 2                      | 14(PHE)                                     | 2.1         |
| 63   | SER       |                | 15                | 1                      | 15(SER)                                     | 1.          |
| 64   | GLY       |                | 15                | 1                      | 15(GLY)                                     | 1.          |
| 65   | SER       |                | 15                | 1                      | 15(SER)                                     | 1.          |
| 66   | LEU       |                | 15                | 1                      | 13(LYS)                                     | 3.5         |
| 67   | LEU       |                | 14                | 2                      | 13(SER)                                     | 2.2         |
| 68   | GLY       |                | 14                | 2                      | 12(GLY)                                     | 2.3         |
| 69   | GLY       |                | 14                | 4                      | 10(ASN) : 9(ASN)                            | 5.6 : 6.2   |
| 70   | LYS       |                | 14                | 3                      | 12(THR)                                     | 3.5         |
| 71   | ALA       |                | 14                | 1                      | 14(ALA)                                     | 1.          |
| 72   | ALA       |                | 14                | 2                      | 13(SER)                                     | 2.2         |
| 73   | LEU       |                | 14                | 1                      | 14(LEU)                                     | 1.          |
| 74   | THR       |                | 14                | 1                      | 14(THR)                                     | 1.          |
| 75   | LEU       |                | 14                | 2                      | 13(ILE)                                     | 2.2         |
| 76   | SER       |                | 14                | 1                      | 14(SER)                                     | 1.          |
| 77   | GLY       |                | 14                | 1                      | 14(GLY)                                     | 1.          |
| 78   | VAL       |                | 14                | 2                      | 13(LEU)                                     | 2.2         |
| 79   | GLN       |                | 14                | 3                      | 12(GLN)                                     | 3.5         |
| 80   | PRO       |                | 14                | 3                      | 10(ALA)                                     | 4.2         |
| 81   | GLU       |                | 14                | 3                      | 11(GLU)                                     | 3.8         |
| 82   | ASP       |                | 14                | 2                      | 13(ASP)                                     | 2.2         |
| 83   | GLU       |                | 14                | 1                      | 14(GLU)                                     | 1.          |
| 84   | ALA       |                | 14                | 1                      | 14(ALA)                                     | 1.          |
| 85   | GLU       |                | 14                | 3 : 4                  | 11(ASP) : 10(ASP)                           | 3.8 : 5.6   |
| 86   | TYR       |                | 14                | 1                      | 14(TYR)                                     | 1.          |
| 87   | TYR       |                | 14                | 2                      | 12(TYR)                                     | 2.3         |
| 88   | CYS       |                | 14                | 1                      | 14(CYS)                                     | 1.          |
| 89   | LEU       |                | 14                | 4                      | 8(SER)                                      | 7.          |
| 90   | LEU       |                | 14                | 2                      | 13(SER)                                     | 2.2         |
| 91   | TYR       |                | 14                | 2                      | 12(TYR)                                     | 2.3         |
| 92   | TYR       |                | 14                | 7                      | 5(ALA)                                      | 20.         |
| 93   | GLY       |                | 14                | 4                      | 7(GLY)                                      | 8.          |
| 94   | GLY       |                | 14                | 5 : 6                  | 5(SER)                                      | 14. : 17.   |
| 95   | VAL       |                | 13                | 7                      | 3( - )                                      | 30.         |
| 95A  | ---       |                | 11                | 3                      | 5( - )                                      |             |
| 95B  | ---       |                | 2                 | 2                      | 1( - )                                      |             |
| 95C  | ---       |                |                   |                        |   |             |
| 95D  | ---       |                |                   |                        |   |             |
| 95E  | ---       |                |                   |                        |   |             |
| 95F  | ---       |                |                   |                        |   |             |
| 96   | ---       |                | 13                | 8                      | 5(VAL)                                      | 21.         |
| 97   | VAL       |                | 16                | 3                      | 10(VAL)                                     | 4.8         |
| 98   | PHE       |                | 16                | 1                      | 16(PHE)                                     | 1.          |
| 99   | GLY       |                | 18                | 1                      | 18(GLY)                                     | 1.          |
| 100  | SER       |                | 18                | 4                      | 10(GLY)                                     | 7.2         |
| 101  | GLY       |                | 18                | 1                      | 18(GLY)                                     | 1.          |
| 102  | THR       |                | 18                | 1                      | 18(THR)                                     | 1.          |
| 103  | LYS       |                | 18                | 5                      | 13(LYS)                                     | 6.9         |
| 104  | VAL       |                | 15                | 2                      | 9(LEU)                                      | 3.3         |
| 105  | THR       |                | 15                | 3                      | 13(THR)                                     | 3.5         |
| 106  | ---       |                | 13                | 1                      | 13(WAL)                                     | 1.          |
| 106A | ---       |                | 13                | 1                      | 13(LEU)                                     | 1.          |
| 107  | ---       |                | 13                | 3                      | 8(GLY)                                      | 4.9         |
| 108  | ---       |                | 10                | 1 : 2                  | 10(GLN) : 9(GLN)                            | 1. : 2.2    |
| 109  | ---       |                | 10                | 1                      | 10(PRO)                                     | 1.          |

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## NOTES: HUMAN LAMBDA LIGHT CHAINS SUBGROUP II

## IDENTICAL SETS OF FRAMEWORK SEGMENTS:

- FR1: SET 1: NIG-84[1],MES[2],WH[3],NEI[4],KAR[5],RIM[6],SLA[7]. (7 IDENTICAL)  
 SET 2: TRO[14],BOH[15]. (2 IDENTICAL)
- FR2: SET 1: WH[3],BOH[15],NIG-58[16],BUR[22]. (4 IDENTICAL)
- FR3:
- FR4: SET 1: WH[3]. (IDENTICAL TO 1 HUMAN V-LAMBDA-I: NEWM[1]; AND 1 HUMAN V-LAMBDA-V: BO[1].)  
 SET 2: MES[2],ES492[8],TRO[14],VIL[17],WIN[21]. (5 IDENTICAL HUMAN V-LAMBDA-II; ALSO 4 HUMAN V-LAMBDA-I: BL2 [CL[6],RHE[10],OKAI[12],NIG-51[19]; 4 HUMAN V-LAMBDA-III: HIL[1],CAP[4],BAU[12],DEL[14]; 1 HUMAN V-LAMBDA-IV: SH[1]; 3 HUMAN V-LAMBDA-VI: SUT[2],THO[4],LBV[CL[5]; AND 24 MOUSE V-LAMBDA: MOPC104E[1],J558[2],XS104[3],HOPC114[4],J698[5],H2061[6],W3159[7],Y5431[8],Y5485[9],Y5830[10],Y5669[11],MOPC511[L][12],S1781[13],Y5444[14],Y5606[15],S1761[16],H2020[17],RPC201[18],IG 303[LAMBDA'CL[19],S43[CL[21],S2H5[CL[38],S2E9[CL[39],S1F12[CL[40],IG 25[LAMBDA'CL[41].)  
 SET 3: NIG-84[1]. (IDENTICAL TO 1 HUMAN V-LAMBDA-III: GAR[7].)

## IDENTICAL SETS OF COMPLEMENTARITY DETERMINING REGIONS:

- CDR1: SET 1: MES[2],VIL[17]. (2 IDENTICAL HUMAN V-LAMBDA-II; ALSO 1 HUMAN V-LAMBDA-V: MCG[3].)
- CDR2: SET 1: NIG-84[1],TOG[10]. (2 IDENTICAL)
- CDR3:

## IDENTICAL SETS OF J-MINIGENES:

- SET 1: MES[2],TRO[14]. (2 IDENTICAL HUMAN V-LAMBDA-II; ALSO 1 HUMAN V-LAMBDA-III: BAU[12].)
- SET 2: ES492[8],VIL[17]. (2 IDENTICAL HUMAN V-LAMBDA-II; ALSO 1 HUMAN V-LAMBDA-III: DEL[14].)

## SPECIFIC NOTES:

- 1) **SM:** IT HAS O-LINKED CARBOHYDRATE ATTACHED TO SER AT POSITION 22 AND N-LINKED CARBOHYDRATE ATTACHED TO ASX AT POSITION 25.

## + THE FOLLOWING WERE EQUALLY AND MOST FREQUENTLY OCCURRING:

| AT POSITION | RESIDUES  |
|-------------|-----------|
| 47          | (ILE,MET) |
| 53          | (LYS,ASN) |
| 59          | (PRO,SER) |
| 95          | (SER,ASN) |
| 95A         | (THR,SER) |
| 95B         | (LEU,ARG) |







ANTIBODY SPECIFICITIES: HUMAN LAMBDA LIGHT CHAINS SUBGROUP III

7) GAR: ANTI-RIBOFLAVIN

REFERENCE: HUMAN LAMBDA LIGHT CHAINS SUBGROUP III

- 1) HIL: LOPEZ DE CASTRO, J.A., CHIU, Y.Y.H. & POLJAK, R.J. (1978) BIOCHEMISTRY, 17, 1718-1723. (CHECKED BY AUTHOR 07/16/79)
- 2) YO: TISCHENDORF, F.W., TISCHENDORF, M.M. & WITTMANN-LIEBOLD, B. (1976) Z.NATURFORSCH. 31C, 758-760.
- 3) PS: KOCHWA, S., TERRY, W.D., CAPRA, J.D. & YANG, N.Y. (1971) ANN.N.Y.ACAD.SCI. 190, 49-70. (CHECKED BY AUTHOR)
- 4) CAP: ZHU, D., KIM, H.S. & DEUTSCH, H.F. (1983) MOL.IMMUNOL., 20, 1107-1116.
- 5) LOY A: WOLFENSTEIN-TODEL, C., FRANKLIN, E.C. & RUDDERS, R.A. (1974) J.IMMUNOL., 112, 871-876. (CHECKED BY AUTHOR)
- 6) LOY G: WOLFENSTEIN-TODEL, C., FRANKLIN, E.C. & RUDDERS, R.A. (1974) J.IMMUNOL., 112, 871-876. (CHECKED BY AUTHOR)
- 7) GAR: KIEFER, C.R., MCGUIRE, B.S., JR., OSSERMAN, E.F. & GARVER, F.A. (1983) J.IMMUNOL., 131, 1871-1875. (CHECKED BY AUTHOR 02/20/84)
- 8) CH: OKADA, Y., NOZU, Y., TITANI, K., WATANABE, S., HARA, H. & KITAGAWA, M. (1972) IMMUNOCHEM., 9, 207-210.
- 9) X(PET): MILSTEIN, C., CLEGG, J.B. & JARVIS, J.M. (1968) BIOCHEM.J., 110, 631-652. (CHECKED BY AUTHOR)
- 10) KERN: PONSTINGL, H., HESS, M. & HILSCHMANN, N. (1968) Z.PHYSIOL.CHEM., 349, 867-871; (1971) Z.PHYSIOL.CHEM., 352, 247-266. (CHECKED BY AUTHOR)
- 11) TA: TONNELLE, C. (1973) BIOCHIM.BIOPHYS.RES.COMM., 55, 1112-1116. (CHECKED BY AUTHOR 11/16/80)
- 12) BAU: BACZKO, K., BRAUN, D.G., HESS, M. & HILSCHMANN, N. (1970) Z.PHYSIOL.CHEM., 351, 763-767; BACZKO, K., BRAUN, D.G. & HILSCHMANN, N. (1974) Z.PHYSIOL.CHEM., 355, 131-154. (CHECKED BY AUTHOR)
- 13) AMYLOID 758: NATVIG, J.B., WESTERMARK, P., SLETTEN, K., HUSBY, G. & MICHAELSON, T. (1981) SCAND.J.IMMUNOL., 14, 89-94. (CHECKED BY AUTHOR 06/01/83)
- 14) DEL: EULITZ, M. (1974) EUR.J.BIOCHEM., 50, 49-69. (CHECKED BY AUTHOR 10/18/77)
- 15) LYN: MEINKE, G.C., SIGRIST, P.H. & SPIEGELBERG, H.L. (1974) IMMUNOCHEM., 11, 457-460. (CHECKED BY AUTHOR WHO PROVIDED ADDITIONAL RESIDUES TO THOSE PUBLISHED); MEINKE, G.C. & SPIEGELBERG, H.L. (1976) IMMUNOCHEM., 13, 915-919. (CHECKED BY AUTHOR 10/17/77)
- 16) NIG-68: KAMETANI, F., YOSHIMURA, K., TONOIKE, H., HOSHI, A., SHINODA, T. & ISOBE, T. (1985) BIOCHEM.BIOPHYS.RES.COMMUN., 126, 848-852.
- 17) AMYLOID 808: WESTERMARK, P., NATVIG, J.B., ANDERS, R.F., SLETTEN, K. & HUSBY, G. (1976) SCAND.J.IMMUNOL., 5, 31-36. (CHECKED BY AUTHOR 06/01/83)
- 18) MOT: KOJIMA, M., ODANI, S. & IKENAKA, T. (1980) MOL.IMMUNOL., 17, 1407-1414.
- 19) WIG: FETT, J.W. & DEUTSCH, H.F. (1976) IMMUNOCHEM., 13, 149-155. (CHECKED BY AUTHOR)
- 20) WHI: WANG, A.C., WELLS, J.V., FUDENBERG, H.H. & GERGELY, J. (1974) IMMUNOCHEM., 11, 341-345. (CHECKED BY AUTHOR)
- 21) DU: BUCHWALD, B.M. (1971) CAN.J.BIOCHEM., 49, 900-902. (CHECKED BY AUTHOR)
- 22) LON: JOHNSTON, S.L., ABRAHAM, G.N. & WELCH, E.H. (1975) BIOCHEM.BIOPHYS.RES.COMMUN., 66, 842-847. (CHECKED BY AUTHOR 10/17/77)
- 23) SG: TISCHENDORF, F.W., TISCHENDORF, M.M. & WITTMANN-LIEBOLD, B. (1976) Z.NATURFORSCH. 31C, 758-760.
- 24) GIM: HESS, M., HILSCHMANN, N., RIVET, L., RIVET, C. & ROPARTZ, C. (1971) NATURE NEW BIOLOGY, 234, 58-61. (CHECKED BY AUTHOR)
- 25) 111: LANGER, B., STEINMETZ-KAYNE, M. & HILSCHMANN, N. (1968) Z.PHYSIOL.CHEM., 349, 945-951. (CHECKED BY AUTHOR)
- 26) 119: HESS, M., HILSCHMANN, N., RIVET, L., RIVET, C. & ROPARTZ, C. (1971) NATURE NEW BIOLOGY, 234, 58-61. (CHECKED BY AUTHOR)
- 27) VIN: PINK, J.R.L. & MILSTEIN, C. (1969) PROC. 5TH FEBS SYMP., 15, 177-182. (CHECKED BY AUTHOR)
- 28) MIL: LANGER, B., STEINMETZ-KAYNE, M. & HILSCHMANN, N. (1968) Z.PHYSIOL.CHEM., 349, 945-951. (CHECKED BY AUTHOR)

NOTES: HUMAN LAMBDA LIGHT CHAINS SUBGROUP III

IDENTICAL SETS OF FRAMEWORK SEGMENTS:

FR1: SET 1: HIL[1], YO[2], PS[3], CAP[4]. (4 IDENTICAL)  
SET 2: LOY A[5], LOY G[6]. (2 IDENTICAL)

FR2:

FR3:

FR4: SET 1: HIL[1], CAP[4], BAU[12], DEL[14]. (4 IDENTICAL HUMAN V-LAMBDA-III; ALSO 4 HUMAN V-LAMBDA-I; BL2 CL[6], RHE[10], OKA[12], NIG-51[19]; 5 HUMAN V-LAMBDA-II; MES[2], ES492[8], TRO[14], VIL[17], WIN[21]; 1 HUMAN V-LAMBDA-IV; SH[1]; 3 HUMAN V-LAMBDA-VI; SUT[2], THO[4], LBV[CL]5; AND 24 MOUSE V-LAMBDA: MOPC104E[1], J558[2], XS104[3], HOPC[14], W3159[7], Y5431[8], Y5485[9], Y5830[10], Y5669[11], MOPC511(L)[12], S178[13], Y5444[14], Y5608[15], S176[16], H2020[17], RPC20[18], IG 303LAMBDA'CL[19], S43 CL[21], S2H5'CL[38], S2E9 CL[39], S1F12 CL[40], IG 25LAMBDA'CL[41].)  
SET 2: GAR[7]. (IDENTICAL TO 1 HUMAN V-LAMBDA-II; NIG-84[1].)  
SET 3: KERN[10]. (IDENTICAL TO 1 HUMAN V-LAMBDA-VI; NIG-48[10].)

IDENTICAL SETS OF J-MINIGENES:

SET 1: BAU[12]. (IDENTICAL TO 2 HUMAN V-LAMBDA-II; MES[2], TRO[14].)  
SET 2: DEL[14]. (IDENTICAL TO 2 HUMAN V-LAMBDA-II; ES492[8], VIL[17].)

SPECIFIC NOTES:

18) MOT: THERE ARE TWO RESIDUES IN FRONT OF POSITION 1; THEY ARE VAL AND THR.

+ THE FOLLOWING WERE EQUALLY AND MOST FREQUENTLY OCCURRING:

| AT POSITION | RESIDUES             |
|-------------|----------------------|
| 30          | (ASP, ASN, GLN)      |
| 81          | (MET, GLU)           |
| 94          | (ILE, ARG, SER, GLY) |
| 95A         | (TYR, ALA, GLY, ASP) |
| 95B         | (HIS, GLU)           |



**REFERENCE: HUMAN LAMBDA LIGHT CHAINS SUBGROUP IV**

- 1) **SH:** TITANI,K.,WIKLER,M.,SHINODA,T. & PUTNAM,F.W. (1970) J.BIOL.CHEM.,245,2171-2176. (CHECKED BY AUTHOR 06/15/83)
- 2) **NEV:** WANG,A.C.,WELLS,J.V.,FUDENBERG,H.H. & GERGELY,J. (1974) IMMUNOCHEM.,11,341-345. (CHECKED BY AUTHOR)
- 3) **USH:** TISCHENDORF,F.W.,TISCHENDORF,M.M. & WITTMANN-LIEBOLD,B. (1976) Z.NATURFORSCH.31C,758-760.
- 4) **PFA:** TISCHENDORF,F.W.,TISCHENDORF,M.M. & WITTMANN-LIEBOLD,B. (1976) Z.NATURFORSCH.31C,758-760.
- 5) **FRA:** WANG,A.C. & FUDENBERG,H.H. (1974) J.IMMUNOGENETICS,1,303-313. (CHECKED BY AUTHOR)

**NOTES: HUMAN LAMBDA LIGHT CHAINS SUBGROUP IV**

## IDENTICAL SETS OF FRAMEWORK SEGMENTS:

FR1: SET 1: SH1|1,NEV|2|. (2 IDENTICAL)

FR2:

FR3:

FR4: SET 1: SH|1|. (IDENTICAL TO 4 HUMAN V-LAMBDA-I: BL2 'CL|6|,RHE|10|,OKA|12|,NIG-51|19|; 5 HUMAN V-LAMBDA-II: MES|2|,ES492|8|,TRO|14|,VIL|17|,WIN|21|; 4 HUMAN V-LAMBDA-III: HIL|1|,CPI|4|,BAU|12|,DEL|14|; 3 HUMAN V-LAMBDA-VI: SUT|2|,THO|4|,LBV|CL|5|; AND 24 MOUSE V-LAMBDA: MOPC104E|1|,J558|2|,XS104|3|,HOPC|1|4|,J698|5|,H206|1|6|,W3159|7|,Y5431|8|,Y5485|9|,Y5830|10|,Y5669|11|,MOPC511|L|12|,S178|13|,Y5444|14|,Y5608|15|,S176|16|,H2020|17|,RPC20|18|,IG 303LAMBDA'CL|19|,S43'CL|21|,S2H5'CL|38|,S2E9'CL|39|,S1F12'CL|40|,IG 25LAMBDA'CL|41|.)

+ THE FOLLOWING WERE EQUALLY AND MOST FREQUENTLY OCCURRING:

| AT POSITION | RESIDUES          |
|-------------|-------------------|
| 19          | (VAL,ALA)         |
| 27          | (LYS,SER)         |
| 30          | (ALA,GLY,ASP,GLN) |
| 32          | (TYR,ASP,ASN)     |
| 34          | (ILE,ALA,SER)     |



**ANTIBODY SPECIFICITIES: HUMAN LAMBDA LIGHT CHAINS SUBGROUP V**

- 3) **MCG:** ANTI-EPSILON-DNP-LYS, EPSILON-DNP-AMINOCAPROATE, DNP-LEU, TRIACETIN, SODIUM MERTHIOLATE, METHADONE, 1,10-PHENANTHROLINE, CAFFEINE, THEOPHYLLINE, DI-DNP-LYS, DNP-TRP, DNP-PHE, DI-DNP-TYR, COLCHICINE, P-NITROANILINE, P-NITROPHENYLPHOSPHORYL CHOLINE, 5-ACETYLRACIL, MENADIONE, MEPERIDINE, TRIBUTYRIN, OMEGA-BROMOHEPTANOATE, O-CHLOROMERCURIPHENOL, P-CHLOROMERCURIPHENOL, PHENYLMERCURIC COMPOUNDS, METHYL-MERCURIC CHLORIDE.

**REFERENCE: HUMAN LAMBDA LIGHT CHAINS SUBGROUP V**

- 1) **BO:** WIKLER.M. & PUTNAM.F.W. (1970) J.BIOL.CHEM..245,4488-4507. (CHECKED BY AUTHOR 06/15/83)  
 2) **HBJ2:** HOOD.L.,GRAY,W.R.,SANDERS.B.G. & DREYER,W.J. (1967) COLD SPRING HARBOR SYMP. QUANTITATIVE BIOL..32,133-145.  
 3) **MCG:** FETT.J.W. & DEUTSCH.H.F. (1974) BIOCHEMISTRY,13,4102-4114. (CHECKED BY AUTHOR)

**NOTES: HUMAN LAMBDA LIGHT CHAINS SUBGROUP V****IDENTICAL SETS OF FRAMEWORK SEGMENTS:**

- FR1: SET 1: BO[1],HBJ2[2]. (2 IDENTICAL)  
 FR2: -  
 FR3: -  
 FR4: SET 1: BO[1]. (IDENTICAL TO 1 HUMAN V-LAMBDA-I: NEWM[1]; AND 1 HUMAN V-LAMBDA-II: WH[3].)  
 SET 2: MCG[3]. (IDENTICAL TO 1 HUMAN V-LAMBDA-I: LOC[11].)

**IDENTICAL SETS OF COMPLEMENTARITY DETERMINING REGIONS:**

- CDR1: SET 1: MCG[3]. (IDENTICAL TO 2 HUMAN V-LAMBDA-II: MES[2],VIL[17].)  
 CDR2: -  
 CDR3: -

+ THE FOLLOWING WERE EQUALLY AND MOST FREQUENTLY OCCURRING:

| AT POSITION | RESIDUES  |
|-------------|-----------|
| 29          | (GLY,ASP) |
| 30          | (TYR,ASN) |
| 31          | (LYS,ASN) |
| 40          | (PRO,ALA) |
| 42          | (LYS,ARG) |
| 46          | (LEU,VAL) |
| 47          | (ILE,VAL) |
| 49          | (TYR,PHE) |
| 52          | (SER,ASN) |
| 53          | (LYS,GLY) |
| 68          | (GLY,ASP) |
| 79          | (ARG,GLN) |
| 92          | (VAL,GLU) |
| 93          | (GLY,ASP) |
| 94          | (SER,ASN) |
| 95          | (ASP,ASN) |
| 100         | (THR,GLY) |
| 104         | (LEU,VAL) |
| 107         | (ARG,GLY) |









**ANTIBODY SPECIFICITIES: HUMAN HEAVY CHAINS SUBGROUP I**

- 2) **SIE:** ANTI-HUMAN GAMMA G GLOBULIN; WA IDIOTYPE  
 3) **WOL:** ANTI-HUMAN GAMMA G GLOBULIN; WA IDIOTYPE  
 10) **STE:** COLD AGGLUTININ WITH ANTI-BLOOD GROUP I ACTIVITY  
 16) **MAR:** ANTI-LIPOPROTEIN LIPASE  
 25) **KOH:** ANTI-HUMAN GAMMA G GLOBULIN

**CLASS: HUMAN HEAVY CHAINS SUBGROUP I**

- 1) **EU:** IGG1-KAPPA  
 2) **SIE:** IGM-KAPPA  
 4) **WOL:** IGM-KAPPA  
 5) **CA:** IGG1-  
 6) **ND'CL:** IGE-  
 7) **MOT:** IGG-  
 8) **BRO'IGG:** IGG-KAPPA  
 10) **STE:** IGG1-  
 11) **BEN(I):** IGG3-  
 12) **ZUC:** IGG3-  
 13) **DI:** IGM-  
 14) **BOT:** IGM-  
 15) **OMM'CL:** IGG3-  
 16) **MAR:** IGM-  
 19) **WAR:** IGG1-  
 20) **VIL:** IGG3-LAMBDA  
 21) **DUN:** IGG4-  
 22) **ADA:** IGA-  
 23) **NOR:** IGA-  
 24) **SAW:** IGG2-  
 25) **KOH:** IGM-LAMBDA  
 26) **RIC:** IGG3-  
 27) **WIS:** IGG3-  
 28) **VAU:** IGG1-  
 29) **LEB:** IGG1-  
 30) **SAC:** IGG1-KAPPA  
 34) **HUS:** IGG3-

**REFERENCE: HUMAN HEAVY CHAINS SUBGROUP I**

- 1) **EU:** CUNNINGHAM,B.A.,RUTISHAUSER,U.,GALL,W.E.,GOTTLIEB,P.D.,WAXDAL,M.J. & EDELMAN,G.M. (1970) BIOCHEMISTRY,9,3161-3170. (CHECKED BY AUTHOR)  
 2) **SIE:** ANDREWS,D.W. & CAPRA,J.D. (1981) PROC.NAT.ACAD.SCI.USA,78,3799-3803; ANDREWS,D.W. & CAPRA,J.D. (1981) BIOCHEMISTRY,20,5816-5822. (CHECKED BY AUTHOR 11/15/82); ANDREWS,D.W. & CAPRA,J.D. (1981) BIOCHEMISTRY,20,5822-5830.  
 3) **HG3'CL:** RECHAVI,G.,RAM,D.,GLAZER,L.,ZAKUT,R. & GIVOLD,D. (1983) PROC.NAT.ACAD.SCI.USA,80,855-859. (CHECKED BY AUTHOR 01/04/83)  
 4) **WOL:** ANDREWS,D.W. & CAPRA,J.D. (1981) PROC.NAT.ACAD.SCI.USA,78,3799-3803; ANDREWS,D.W. & CAPRA,J.D. (1981) BIOCHEMISTRY,20,5816-5822. (CHECKED BY AUTHOR 11/15/82); ANDREWS,D.W. & CAPRA,J.D. (1981) BIOCHEMISTRY,20,5822-5830.  
 5) **CA:** PITCHER,S.E. & KONIGSBERG,W. (1970) J.BIOL.CHEM.,245,1267-1274. (CHECKED BY AUTHOR)  
 6) **ND'CL:** BENNICH,H. & VON BAHR-LINDSTROM,H. (1974) PROGRESS IN IMMUNOLOGY,1,49-58; BENNICH,H.H.,JOHANSSON,S.G.O. & VON BAHR-LINDSTROM,H. (1978) IN IMMEDIATE HYPERSENSITIVITY: MODERN CONCEPTS AND DEVELOPMENTS. BACH,M.K., ED.,PP.1-36. MARCEL DEKKER,NEW YORK; KENTEN,J.H.,MOLGAARD,H.V.,HOUGHTON,M.,DERBYSHIRE,R.B.,VINEY,J.,BELL,L.O. & GOULD,H.J. (1982) PROC.NAT.ACAD.SCI.USA,79,6661-6665.  
 7) **MOT:** KOJIMA,M.,ODANI,S. & ONO,T. (1982) MOL.IMMUNOL.,19,1095-1103; KOJIMA,M.,KOIDE,T.,ODANI,S. & ONO,T. (1986) MOL.IMMUNOL.,23,169-174. (CHECKED BY AUTHOR 08/08/86)  
 8) **BRO'IGG:** HOPPER,J.E.,NOYES,C.,HEINRIKSON,R. & KESSEL,J.W. (1976) J.IMMUNOL.,116,743-746; HOPPER,J.E. & BRAHN,E. (1977) J.IMMUNOL.,119,847-849. (CHECKED BY AUTHOR 08/25/78) WHO POINTED OUT THAT BRO' IS SAME AS BRIGG AND SUGGESTED THAT IT SHOULD BE RENAMED AS BROIGG)  
 9) **THO:** HOPPER,J.E. & BRAHN,E. (1977) J.IMMUNOL.,119,847-849. (CHECKED BY AUTHOR 08/25/78)  
 10) **STE:** FISHER,C.E.,PALM,W.H. & PRESS,E.M. (1969) FEBS LETTERS,5,20-22. (CHECKED BY AUTHOR)  
 11) **BEN(I):** KAPLAN,A.P.,HOOD,L.,TERRY,W.D. & METZGER,H. (1971) IMMUNOCHEMISTRY,8,801-811. (CHECKED BY AUTHOR)  
 12) **ZUC:** FRANGIONE,B. & MILSTEIN,C. (1969) NATURE,224,597-599. (CHECKED BY AUTHOR)  
 13) **DI:** KOHLER,H.,SHIMIZU,A.,PAUL,C.,MOORE,V. & PUTNAM,F.W. (1970) NATURE,227,1318-1320; FLORENT,G.,LEHMAN,D. & PUTNAM,F.W. (1974) BIOCHEMISTRY,13,2482-2498. (CHECKED BY AUTHOR 06/15/83)  
 14) **BOT:** BARNIKOL-WATANABE,S.,MIHAESCO,E.,MIHAESCO,C.,BARNIKOL,H.U. & HILSCHMANN,N. (1984) Z.PHYSIOL.CHEM.,365,105-118.  
 15) **OMM'CL:** ALEXANDER,A.,STEINMETZ,M.,BARRITAU,D.,FRANGIONE,B.,FRANKLIN,E.C.,HOOD,L. & BUXBAUM,J.N. (1982) PROC.NAT.ACAD.SCI.USA,79,3260-3264. (CHECKED BY AUTHOR 06/17/83)  
 16) **MAR:** KAPLAN,A.P.,HOOD,L.,TERRY,W.D. & METZGER,H. (1971) IMMUNOCHEMISTRY,8,801-811. (CHECKED BY AUTHOR)  
 17) **FI:** MONTGOMERY,P.C.,BELLO,A.C. & ROCKEY,J.H. (1970) BIOCHIM.BIOPHYS.ACTA,200,258-266. (CHECKED BY AUTHOR)  
 18) **VU:** MONTGOMERY,P.C.,BELLO,A.C. & ROCKEY,J.H. (1970) BIOCHIM.BIOPHYS.ACTA,200,258-266. (CHECKED BY AUTHOR)  
 19) **WAR:** KAPLAN,A.P.,HOOD,L.,TERRY,W.D. & METZGER,H. (1971) IMMUNOCHEMISTRY,8,801-811. (CHECKED BY AUTHOR)  
 20) **VIL:** KAPLAN,A.P.,HOOD,L.,TERRY,W.D. & METZGER,H. (1971) IMMUNOCHEMISTRY,8,801-811. (CHECKED BY AUTHOR)  
 21) **DUN:** KAPLAN,A.P.,HOOD,L.,TERRY,W.D. & METZGER,H. (1971) IMMUNOCHEMISTRY,8,801-811. (CHECKED BY AUTHOR)  
 22) **ADA:** KAPLAN,A.P.,HOOD,L.,TERRY,W.D. & METZGER,H. (1971) IMMUNOCHEMISTRY,8,801-811. (CHECKED BY AUTHOR)  
 23) **NOR:** KAPLAN,A.P.,HOOD,L.,TERRY,W.D. & METZGER,H. (1971) IMMUNOCHEMISTRY,8,801-811. (CHECKED BY AUTHOR)  
 24) **SAW:** KAPLAN,A.P.,HOOD,L.,TERRY,W.D. & METZGER,H. (1971) IMMUNOCHEMISTRY,8,801-811. (CHECKED BY AUTHOR)  
 25) **KOH:** KAPLAN,A.P.,HOOD,L.,TERRY,W.D. & METZGER,H. (1971) IMMUNOCHEMISTRY,8,801-811. (CHECKED BY AUTHOR)  
 26) **RIC:** KAPLAN,A.P.,HOOD,L.,TERRY,W.D. & METZGER,H. (1971) IMMUNOCHEMISTRY,8,801-811. (CHECKED BY AUTHOR)  
 27) **WIS:** FRANKLIN,E.C.,PRELLI,F. & FRANGIONE,B. (1979) PROC.NAT.ACAD.SCI.USA,76,452-456. (CHECKED BY AUTHOR 07/18/79)  
 28) **VAU:** FRANKLIN,E.C.,KYLE,R.,SELIGMANN,M. & FRANGIONE,B. (1979) MOL.IMMUNOL.,16,919-921. (CHECKED BY AUTHOR 12/10/82)  
 29) **LEB:** FRANKLIN,E.C.,KYLE,R.,SELIGMANN,M. & FRANGIONE,B. (1979) MOL.IMMUNOL.,16,919-921. (CHECKED BY AUTHOR 12/10/82)  
 30) **SAC:** PARR,D.M. (1981) MOL.IMMUNOL.,18,257-259. (CHECKED BY AUTHOR 03/02/82)  
 31) **DEE:** FRANGIONE,B. & MILSTEIN,C. (1967) NATURE,216,939-941. (CHECKED BY AUTHOR)  
 32) **LEA:** FRANGIONE,B. & FRANKLIN,E.C. (1977) PROG.IMMUNOL.,3,278-288. (CHECKED BY AUTHOR 07/18/79)  
 33) **HAR:** FRANGIONE,B. & FRANKLIN,E.C. (1977) PROG.IMMUNOL.,3,278-288. (CHECKED BY AUTHOR 07/18/79)  
 34) **HUS:** WANG,A.C. & FUDENBERG,H.H. (1975) ARCH.BIOCHEM.BIOPHYS.,168,657-664. (CHECKED BY AUTHOR 09/23/77)

**NOTES: HUMAN HEAVY CHAINS SUBGROUP I****IDENTICAL SETS OF FRAMEWORK SEGMENTS:**

- FR1: SET 1: VAU[28],LEB[29]. (2 IDENTICAL)  
 FR2: SET 1: EU[1],HG3'CL[3]. (2 IDENTICAL)  
 SET 2: WOL[4]. (IDENTICAL TO 2 HUMAN V-H-III: TI[4],TE[10].)  
 FR3: SET 1: ND'CL[6]. (IDENTICAL TO 1 HUMAN V-H-III: U266'CL[106].)  
 FR4: SET 1: WOL[4]. (IDENTICAL TO 2 HUMAN V-H-III: MCE[4],NZU[15]; 4 HUMAN V-H-III: TI[4],DOB[31],WEA[33],NIE[34]; AND 1 MOUSE V-H-III: MOPC47A[48].)  
 SET 2: ND'CL[6]. (IDENTICAL TO 1 HUMAN V-H-III: HIG1'CL[10]; 1 HUMAN V-H-III: U266'CL[106]; AND 1 MOUSE V-H-III: HDX12[15].)

**IDENTICAL SETS OF COMPLEMENTARITY DETERMINING REGIONS:**

- CDR1:  
 CDR2:  
 CDR3: SET 1: HG3'CL[3]. (IDENTICAL TO 1 HUMAN V-H-III: LAMBDA-VH26'CL[2]; 1 MOUSE V-H-III: PJ14'CL[22]; AND 5 MOUSE V-H-III: 186-2'CL[3]. 186-1'CL[5],102'CL[15],23'CL[18],3'CL[26].)  
 SET 2: ND'CL[6]. (IDENTICAL TO 1 HUMAN V-H-III: U266'CL[106].)

**IDENTICAL SETS OF J-MINIGENES:**

- SET 1: ND'CL[6]. (IDENTICAL TO 1 HUMAN V-H-III: HIG1'CL[10]; AND 1 HUMAN V-H-III: U266'CL[106].)

**NOTES: HUMAN HEAVY CHAINS SUBGROUP I (cont'd)****SPECIFIC NOTES:**

- 3) **HG3'CL:** THE AMINO ACID SEQUENCE IS OBTAINED BY TRANSLATING THE NUCLEOTIDE SEQUENCE OF A CLONE OF HUMAN FETAL LIVER GENOMIC DNA.
- 6) **ND'CL:** THE AMINO ACID SEQUENCE IS OBTAINED BY TRANSLATING THE NUCLEOTIDE SEQUENCE OF A CLONE OF MOUSE CDNA. IT CORRESPONDS TO THE AMINO ACID SEQUENCE DETERMINED EARLIER EXCEPT THAT THE AMINO ACID SEQUENCE DETERMINATION GAVE PCA AT POSITION 1, VAL AT 2, VAL AT 34, GLY AT 35, ILE AT 48 AND HIS AT 49.
- 7) **MOT:** PAPAINE CLEAVES BETWEEN ARG 56 AND THR 57, AND BETWEEN ARG 62 AND SER 63.
- 12) **ZUC:** IT WAS FROM A CASE OF HEAVY CHAIN DISEASE.
- 14) **BOT:** IT WAS FROM A CASE OF IGM HEAVY CHAIN DISEASE.
- 15) **OMM'CL:** THE AMINO ACID SEQUENCE IS OBTAINED BY TRANSLATING THE NUCLEOTIDE SEQUENCE OF A CLONE OF HUMAN CELL LINE CDNA. IT WAS FROM A CASE OF HEAVY CHAIN DISEASE.
- 27) **WIS:** IT WAS FROM A CASE OF HEAVY CHAIN DISEASE. ITS RESIDUES AT POSITIONS 108 AND 109 ARE ASN AND CYS RESPECTIVELY, WHICH DO NOT CORRESPOND TO THE USUAL RESIDUES FOUND AT THESE POSITIONS IN HUMAN HEAVY CHAIN SUBGROUP I.
- 28) **VAU:** IT WAS FROM A CASE OF HEAVY CHAIN DISEASE.
- 29) **LEB:** IT WAS FROM A CASE OF HEAVY CHAIN DISEASE.
- 30) **SAC:** IT WAS FROM A CASE OF HEAVY CHAIN DISEASE.

**+ THE FOLLOWING WERE EQUALLY AND MOST FREQUENTLY OCCURRING:**

| AT POSITION | RESIDUES                      |
|-------------|-------------------------------|
| 16          | (ALA,SER)                     |
| 19          | (LYS,ARG)                     |
| 33          | (TYR,ALA)                     |
| 43          | (LYS,ARG,GLN)                 |
| 50          | (TRP,ILE,VAL,SER,GLY,GLU,GLN) |
| 54          | (PHE,SER)                     |
| 56          | (PRO,GLY)                     |
| 62          | (LYS,ARG)                     |
| 69          | (VAL,MET)                     |
| 71          | (LEU,ARG)                     |
| 73          | (PRO,THR)                     |
| 75          | (PHE,THR)                     |
| 95          | (GLY,GLU)                     |
| 100D        | (TYR,PRO,SER,ASN)             |
| 100E        | (PHE,GLY)                     |
| 100F        | (THR,ASP)                     |
| 100G        | (TYR,SER)                     |
| 100H        | (LEU,SER)                     |
| 100K        | (TYR,PHE,LEU)                 |
| 101         | (PRO,ASP)                     |



HUMAN HEAVY CHAINS SUBGROUP II (cont'd)

VARIABILITY

|         |      |           |
|---------|------|-----------|
|         | 0    |           |
|         | 1    | 4.5       |
|         | 2    | 5.3       |
|         | 3    | 8.        |
|         | 4    | 2.2       |
|         | 5    | 11. : 14. |
|         | 6    | 2.2 : 2.4 |
|         | 7    | 3.7       |
|         | 8    | 1.        |
|         | 9    | 2.2       |
|         | 10   | 7.5       |
|         | 11   | 1.        |
|         | 12   | 1.        |
|         | 13   | 2.5       |
|         | 14   | 2.2       |
| F R 1   | 15   | 6.        |
|         | 16   | 6.        |
|         | 17   | 2.2       |
|         | 18   | 1.        |
|         | 19   | 3.3       |
|         | 20   | 1.        |
|         | 21   | 1.        |
|         | 22   | 1.        |
|         | 23   | 3.6       |
|         | 24   | 6.        |
|         | 25   | 2.2       |
|         | 26   | 1.        |
|         | 27   | 14.       |
|         | 28   | 3.7       |
|         | 29   | 8.        |
|         | 30   | 5.7       |
| C D R 1 | 31   | 13.       |
|         | 32   | 35.       |
|         | 33   | 8.        |
|         | 34   | 10.       |
|         | 35   | 40.       |
|         | 35A  |           |
|         | 35B  |           |
|         | 36   | 1.        |
|         | 37   | 2.2       |
| F R 2   | 38   | 1.        |
|         | 39   | 2.2       |
|         | 40   | 3.8       |
|         | 41   | 1.        |
|         | 42   | 1.        |
|         | 43   | 5.        |
|         | 44   | 4.        |
|         | 45   | 1.        |
|         | 46   | 1.        |
|         | 47   | 1.        |
|         | 48   | 3.3       |
|         | 49   | 3.3       |
|         | 50   | 23.       |
|         | 51   | 6.7       |
|         | 52   | 20.       |
|         | 52A  |           |
|         | 52B  |           |
|         | 52C  |           |
| C D R 2 | 53   | 11.       |
|         | 54   | 10. : 13. |
|         | 55   | 3.3       |
|         | 56   | 8.        |
|         | 57   | 15.       |
|         | 58   | 6.7       |
|         | 59   | 5.7       |
|         | 60   | 17. : 20. |
|         | 61   | 6.        |
|         | 62   | 2.2       |
| 63      | 1.   |           |
| 64      | 7.5  |           |
|         | 65   | 6.        |
|         | 66   | 1.        |
|         | 67   | 3.3       |
|         | 68   | 3.8       |
|         | 69   | 6.7       |
|         | 70   | 4.3       |
|         | 71   | 8.        |
|         | 72   | 2.2       |
|         | 73   | 2.2       |
|         | 74   | 1.        |
|         | 75   | 2.9       |
|         | 76   | 1.        |
|         | 77   | 2.2       |
|         | 78   | 3.3       |
|         | 79   | 3.3       |
| F R 3   | 80   | 1.        |
|         | 81   | 20.       |
|         | 82   | 10.       |
|         | 82A  |           |
|         | 82B  |           |
|         | 82C  |           |
|         | 83   | 11.       |
|         | 84   | 3.1       |
|         | 85   | 6.6       |
|         | 86   | 1.        |
| 87      | 2.4  |           |
| 88      | 2.4  |           |
| 89      | 4.7  |           |
| 90      | 1.   |           |
| 91      | 2.4  |           |
| 92      | 1.   |           |
| 93      | 2.2  |           |
| 94      | 2.8  |           |
|         | 95   | 26.       |
|         | 96   | 50.       |
|         | 97   | 19.       |
|         | 98   | 26.       |
|         | 99   | 35.       |
|         | 100  | 40.       |
|         | 100A |           |
| C D R 4 | 100B |           |
|         | 100C |           |
|         | 100D |           |
|         | 100E |           |
|         | 100F |           |
|         | 100G |           |
|         | 100H |           |
|         | 100I |           |
|         | 100J |           |
|         | 100K |           |
|         | 101  | 3.6       |
|         | 102  | 6.3       |
|         | 103  | 2.2       |
|         | 104  | 2.2       |
|         | 105  | 6.        |
| F R 4   | 106  | 2.2       |
|         | 107  | 5.3       |
|         | 108  | 12.       |
|         | 109  | 2.2       |
|         | 110  | 5.3       |
|         | 111  | 2.2       |
|         | 112  | 2.2       |
| 113     | 4.   |           |

## ANTIBODY SPECIFICITIES: HUMAN HEAVY CHAINS SUBGROUP II

8) **NEWM**: ANTI-3-(3'-HYDROXY-3',7',11',15',TETRAMETHYL HEXADECYL) 2-METHYL 1,4 NAPHTHOQUINONE(VIT.K10H)

## CLASS: HUMAN HEAVY CHAINS SUBGROUP II

- 1) **COR**: IGG1-
- 2) **DAW**: IGG1-LAMBDA
- 3) **OU**: IGM-KAPPA
- 4) **MCE**: IGM-KAPPA
- 6) **HE**: IGG1-
- 8) **NEWM**: IGG1-LAMBDA
- 9) **WAH**: IGD-LAMBDA
- 12) **SA**: IGG2-LAMBDA
- 15) **NZU**: IGM-
- 16) **ERI**: IGD-

## REFERENCE: HUMAN HEAVY CHAINS SUBGROUP II

- 1) **COR**: PRESS,E.M. & HOGG,N.M. (1970) BIOCHEM.J.,117,641-660. (CHECKED BY AUTHOR)
- 2) **DAW**: PRESS,E.M. & HOGG,N.M. (1970) BIOCHEM.J.,117,641-660. (CHECKED BY AUTHOR)
- 3) **OU**: PUTNAM,F.W.,SHIMIZU,A.,PAUL,C.,SHINODA,T. & KOHLER,H. (1971) ANN.N.Y.ACAD.SCI.,190,83-103. (CHECKED BY AUTHOR 06/15/83)
- 4) **MCE**: GERBER-JENSON,B.,KAZIN,A.,KEHOE,J.M.,SCHEFFEL,C.,ERICKSON,B.W. & LITMAN,G.W. (1981) J.IMMUNOL.,126,1212-1216. (CHECKED BY AUTHOR 12/15/80)
- 5) **CE-1 'CL**: TAKAHASHI,N.,NOMATA,T. & HONJO,T. (1984) PROC.NAT.ACAD.SCI.USA,81,5194-5198.
- 6) **HE**: CUNNINGHAM,B.A.,GOTTLIEB,P.D.,PFLUMM,M.N. & EDELMAN,G.M. (1971) PROGRESS IN IMMUNOLOGY (B.AMOS,ED.),ACADEMIC PRESS,N.Y.,PP.3-24. (CHECKED BY AUTHOR)
- 7) **SUP-T1 VH-JA'CL**: DENNY,C.T.,YOSHIKAI,Y.,MAK,T.W.,SMITH,S.D.,HOLLIS,G.F. & KIRSCH,I.R. (1986) NATURE,320,549-551.
- 8) **NEWM**: POLJAK,R.J.,AMZEL,L.M.,CHEN,B.L.,PHIZACKERLEY,R.P. & SAULF,F. (1974) PROC.NAT.ACAD.SCI.USA,71,3440-3444. (CHECKED BY AUTHOR WHO CORRECTED RESIDUES 6,9,15,16,24,26,27,29 THROUGH 35B,59,60 AS GIVEN IN TABLE OF THE FIRST EDITION OF THIS BOOK, AND HAS MORE RECENTLY REVISED RESIDUES 5,24,28,29,30,31,33,34,35,35A,35B,59,60 AND 101); POLJAK,R.J.,AMZEL,L.M.,CHEN,B.L.,CHIU,Y.Y.,PHIZACKERLEY,R.P.,SAULF,F. & YSERN,X. (1976) COLD SPRING HARBOR SYMP. QUANTITATIVE BIOL.,41,639-645; POLJAK,R.J.,NAKASHIMA,Y.,CHEN,B.L. & KONIGSBERG,W. (1977) BIOCHEMISTRY,16,3412-3420. THE SEQUENCE LISTED IN THE LAST REFERENCE IS GIVEN IN THE TABLE. (CHECKED BY AUTHOR, W.K., 09/30/78)
- 9) **WAH**: PUTNAM,F.W.,TAKAHASHI,N.,TETAERT,D.,DEBUIRE,B. & LIN,L.C. (1981) PROC.NAT.ACAD.SCI.USA,78,6168-6172. (CHECKED BY AUTHOR 11/30/81); TAKAHASHI,N.,TETAERT,D.,DEBUIRE,B.,LIN,L.C. & PUTNAM,F.W. (1982) PROC.NAT.ACAD.SCI.USA,79,2850-2854.
- 10) **HIG1'CL**: KUDO,A.,ISHIHARA,T.,NISHIMURA,Y. & WATANABE,T. (1985) GENE,33,181-189. (CHECKED BY AUTHOR 10/01/85)
- 11) **CAR**: FRANGIONE,B. (1968) PH.D. THESIS, UNIVERSITY OF CAMBRIDGE. (CHECKED BY AUTHOR)
- 12) **SA**: MILSTEIN,C. & FRANGIONE,B. (1971) BIOCHEM.J.,121,217-225. (CHECKED BY AUTHOR)
- 13) **IO**: MONTGOMERY,P.C.,BELLO,A.C. & ROCKEY,J.H. (1970) BIOCHIM.BIOPHYS.ACTA,200,258-266. (CHECKED BY AUTHOR)
- 14) **SPA**: FRANGIONE,B. & FRANKLIN,E.C. (1979) J.IMMUNOL.,122,1177-1179. (CHECKED BY AUTHOR 07/18/79)
- 15) **NZU**: ERICKSON,B.W.,GERBER-JENSON,B.,WANG,A.C. & LITMAN,G.W. (1981) MOL.IMMUNOL.,19,357-365. (CHECKED BY AUTHOR 11/30/81)
- 16) **ERI**: MILSTEIN,C.P. & DEVERSON,E.V. (1980) IMMUNOLOGY,40,657-664. (CHECKED BY AUTHOR 11/30/82)

## NOTES: HUMAN HEAVY CHAINS SUBGROUP II

## IDENTICAL SETS OF FRAMEWORK SEGMENTS:

- FR1:  
FR2: SET 1: SUP-T1 VH-JA'CL[7],WAH[9]. (2 IDENTICAL)  
FR3:  
FR4: SET 1: MCE[4],NZU[15]. (2 IDENTICAL HUMAN V-H-II; ALSO 1 HUMAN V-H-I: WOL[4]; 4 HUMAN V-H-III: TIL[4],DOBI[31],WEA[33],NIE[34]; AND 1 MOUSE V-H-III: MOPC47A[48].)  
SET 2: HIG1'CL[10]. (IDENTICAL TO 1 HUMAN V-H-I: ND'CL[6]; 1 HUMAN V-H-III: U266'CL[106]; AND 1 MOUSE V-H-IIA: HDX12[15].)

## IDENTICAL SETS OF J-MINIGENES:

- SET 1: HIG1'CL[10]. (IDENTICAL TO 1 HUMAN V-H-I: ND'CL[6]; AND 1 HUMAN V-H-III: U266'CL[106].)

## SPECIFIC NOTES:

- 4) **MCE**: IT IS A CRYOIMMUNOGLOBULIN AND IS DESIGNATED BY THE AUTHORS AS MCE. IN ORDER TO DIFFERENTIATE IT FROM ANOTHER MCE SEQUENCED BY CAPRA ET AL., IT IS DENOTED AS MCE.
- 5) **CE-1 'CL**: CELL LINE CESS
- 7) **SUP-T1 VH-JA'CL**: IT IS FROM A PATIENT SUFFERING FROM CHILDHOOD T-CELL LYMPHOMA WITH inv(14)(q11.2;q32.2). THE INVERSION ON CHROMOSOME 14 BRINGS THE VH GENE AND JA MINIGENE TOGETHER, GIVING RISE TO A HYBRID MOLECULE CONTAINING PART OF THE IMMUNOGLOBULIN GENE AND PART OF THE T-LYMPHOCYTE RECEPTOR FOR ANTIGEN GENE.
- 14) **SPA**: IT WAS FROM A CASE OF HEAVY CHAIN DISEASE.
- 15) **NZU**: IT IS A CRYOIMMUNOGLOBULIN.

## + THE FOLLOWING WERE EQUALLY AND MOST FREQUENTLY OCCURRING:

| AT POSITION | RESIDUES             |
|-------------|----------------------|
| 5           | (ARG, GLN)           |
| 10          | (ALA, GLY)           |
| 32          | (THR, SER, ASP)      |
| 35          | (CYS, SER)           |
| 44          | (ALA, GLY)           |
| 52A         | (TYR, HIS)           |
| 60          | (SER, ASN)           |
| 81          | (LYS, THR)           |
| 82          | (LEU, MET)           |
| 82A         | (THR, SER)           |
| 82B         | (SER, ASN)           |
| 82C         | (VAL, MET)           |
| 85          | (VAL, ALA)           |
| 96          | (PRO, LEU)           |
| 99          | (PRO, ARG, GLY)      |
| 100         | (TYR, PHE)           |
| 100A        | (ALA, THR)           |
| 100D        | (TYR, LEU)           |
| 100F        | (TYR, GLY)           |
| 100H        | (TYR, SER, ASP, ASN) |
| 100I        | (SER, GLY, ASP)      |





PATENT DOCKET NO. 709

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#23  
8/20/93  
11-12-93

In re Application of )  
Paul J. Carter et al. )  
Serial No. 07/715,272 )  
Filed: 14 June 1991 )  
For: Immunoglobulin Variants )

Group Art Unit: 1806  
Examiner: L. Feisee

RECEIVED  
NOV 05 1993  
GROUP 1800

460 Point San Bruno Boulevard  
South San Francisco, CA 94080  
(415) 225-1896

NOTICE OF APPEAL

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

Sir:

Applicant hereby appeals to the Board of Appeals and Interferences from the decision dated May 19, 1993, of the Primary Examiner finally rejecting claims 1-11 and 17-21.

The Commissioner is hereby authorized to charge Deposit Account No. 07-0630 in the amount of \$270 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.

*Janet E. Hasak*

Janet E. Hasak  
Reg. No. 28,616

Dated: October 15, 1993

CERTIFICATE OF MAILING (37 CFR 1.8a)

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

*Louise Strasbaugh*  
Louise Strasbaugh

Date: October 15, 1993

RP14167 11/04/93 07715272 07-0630 140 119 270.00CH  
w020.u





GP1806

PATENT DOCKET 709

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

18D1/10/25/93

In re Application of )  
 Paul J. Carter et al. )  
 Serial No. 07/715,272 )  
 Filed: 14 June 1991 )  
 For: Immunoglobulin Variants )

Group Art Unit: 1806

Examiner: L. Feisee

#22098  
 RECEIVED  
 NOV 05 1993  
 GROUP 1800

460 Point San Bruno Boulevard  
 South San Francisco, CA 94080  
 (415) 225-1896

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

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

Sir:

Applicant petitions the Commissioner of Patents and Trademarks to extend the time for response to the Office action dated May 19, 1993 for an additional month, from September 19, 1993 to October 19, 1993. The extended time for response does not exceed the statutory period.

Please charge Deposit Account Number 07-0630 in the amount of \$250 to cover the cost of the second month extension fee less the first month extension fee paid in relation to the request for a one month extension of time filed on September 20, 1993. Any deficiency or overpayment should be charged or credited to this deposit account. A duplicate of this sheet is enclosed.

Respectfully submitted,  
 GENENTECH, INC.

Janet E. Hasak  
 Reg. No. 28,616

Date: October 15, 1993

RP14166 11/04/93 07715272 07-0630 140 116 250.00CH

CERTIFICATE OF MAILING (37 CFR 1.8a)

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

Louise Strasbaugh

Date: October 15, 1993



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

| SERIAL NUMBER | FILING DATE | FIRST NAMED APPLICANT | ATTORNEY DOCKET NO. |
|---------------|-------------|-----------------------|---------------------|
| 07715,272     | 06/14/91    | CARTER                | 19                  |

GENENTECH, INC.  
 ATTN: CAROLYN R. ADLER  
 460 POINT SAN BRUNO BLVD.  
 SOUTH SAN FRANCISCO, CA 94080

18M2/1021

| EXAMINER |              |
|----------|--------------|
| ART UNIT | PAPER NUMBER |
| 1802     | 21           |

DATE MAILED:

10/21/93

Below is a communication from the EXAMINER in charge of this application  
 COMMISSIONER OF PATENTS AND TRADEMARKS

ADVISORY ACTION

THE PERIOD FOR RESPONSE:

- is extended to run 4 months or continues to run \_\_\_\_\_ from the date of the final rejection
- expires three months from the date of the final rejection or as of the mailing date of this Advisory Action, whichever is later. In no event however, will the statutory period for the response expire later than six months from the date of the final rejection.

Any extension of time must be obtained by filing a petition under 37 CFR 1.136(a), the proposed response and the appropriate fee. The date on which the response, the petition, and the fee have been filed is the date of the response and also the date for the purposes of determining the period of extension and the corresponding amount of the fee. Any extension fee pursuant to 37 CFR 1.17 will be calculated from the date of the originally set shortened statutory period for response or as set forth in b) above.

- Appellant's Brief is due in accordance with 37 CFR 1.192(a).
- Applicant's response to the final rejection, filed 9/20/93 has been considered with the following effect, but it is not deemed to place the application in condition for allowance:

- 1.  The proposed amendments to the claim and/or specification will not be entered and the final rejection stands because:
  - a.  There is no convincing showing under 37 CFR 1.116(b) why the proposed amendment is necessary and was not earlier presented.
  - b.  They raise new issues that would require further consideration and/or search. (See Note).
  - c.  They raise the issue of new matter. (See Note).
  - d.  They are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal.
  - e.  They present additional claims without cancelling a corresponding number of finally rejected claims.

NOTE: The language "of a human immunoglobulin subgroup" has not been defined with respect to the variable domain of a consensus antibody.

- 2.  Newly proposed or amended claims \_\_\_\_\_ would be allowed if submitted in a separately filed amendment cancelling the non-allowable claims.

- 3.  Upon the filing an appeal, the proposed amendment  will be entered  will not be entered and the status of the claims will be as follows:
 

Claims allowed: None 12 and 13

Claims objected to: None

Claims rejected: 1-11, 17-21

DAVID L. LACEY  
 SUPERVISORY PATENT EXAMINER  
 GROUP 180  
 10/11/93

However;

- Applicant's response has overcome the following rejection(s): The rejection under 35 USC 112 first paragraph regarding "at least a portion" and "reasonably".
- 4.  The affidavit, exhibit or request for reconsideration has been considered but does not overcome the rejection because the introduction of the language "human subgroup" is deemed in proper but not in the application. Also, it is not clear
- 5.  The affidavit or exhibit will not be considered because applicant has not shown good and sufficient reasons why it was not earlier presented.

- The proposed drawing correction  has  has not been approved by the examiner.

Other

that the "consensus antibody" in the application wouldn't be identical to 846 or 839 of Queen et al. The difference is not added in the application. Exhibit 1094

AF

PATENT DOCKET 709



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

Paul J. Carter et-al.

Serial No. 07/715272

Filed: June 14, 1991

For: Immunoglobulin Variants GROUP 1800

Group Art Unit: 1806

Examiner: L. FEISEE

RECEIVED

DEC 29 1993

1806

Handwritten notes and signatures in the top right corner, including a large signature and the number 1594.

460 Point San Bruno Boulevard  
South San Francisco, CA 94080  
(415) 225-1896

AMENDMENT PURSUANT TO 37 CFR § 1.116(a)

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

Sir:

Pursuant to 37 CFR § 1.116(a), please cancel claims 1-11, 17, and 19-21 of the above application. Claim 18 was canceled in the Amendment filed September 20, 1993. Applicants trust that the above-mentioned application with allowed claims 12 and 13 will be in condition for allowance following the entry of this amendment and look forward to receiving the Notice to this effect.

OK to enter 11/11/94

Respectfully submitted,  
GENENTECH, INC.

*Janet E. Hasak*

Dated: December 13, 1993

Janet E. Hasak  
Reg. No. 28,616

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service in first class envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on the date shown below.

Dated: 13 DEC 1993

*Louise Strasbaugh*  
Louise Strasbaugh



UNITED STATES DEPARTMENT OF COMMERCE  
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D. C. 20231

|               |             |                       |                     |
|---------------|-------------|-----------------------|---------------------|
| SERIAL NUMBER | FILING DATE | FIRST NAMED APPLICANT | ATTORNEY DOCKET NO. |
|---------------|-------------|-----------------------|---------------------|

EXAMINER

|          |              |
|----------|--------------|
| ART UNIT | PAPER NUMBER |
|----------|--------------|

25

DATE MAILED:

EXAMINER INTERVIEW SUMMARY RECORD

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

(1) Wardy Lee (3) \_\_\_\_\_

(2) Nita Feiser (4) \_\_\_\_\_

Date of interview 1/11/94

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

Exhibit shown or demonstration conducted:  Yes  No. If yes, brief description: \_\_\_\_\_

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

Claims discussed: \_\_\_\_\_

Identification of prior art discussed: \_\_\_\_\_

Description of the general nature of what was agreed to if an agreement was reached, or any other comments:

Finality would be withdrawn.  
Action both coming.

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendments which would render the claims allowable is available, a summary thereof must be attached.)

Unless the paragraphs below have been checked to indicate to the contrary, A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW (e.g., items 1-7 on the reverse side of this form). If a response to the last Office action has already been filed, then applicant is given one month from this interview date to provide a statement of the substance of the interview.

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

Since the examiner's interview summary above (including any attachments) reflects a complete response to each of the objections, rejections and requirements that may be present in the last Office action, and since the claims are now allowable, this completed form is considered to fulfill the response requirements of the last Office action.

Examiner's Signature



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

| SERIAL NUMBER | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. |
|---------------|-------------|----------------------|---------------------|
|---------------|-------------|----------------------|---------------------|

07/715,272 06/14/91 CARTER

P 709  
 EXAMINER  
 FEISEE, L

18M2/0203

GENENTECH, INC.  
 ATTN: CAROLYN R. ADLER  
 460 POINT SAN BRUNO BLVD.  
 SOUTH SAN FRANCISCO, CA 94080

ART UNIT PAPER NUMBER

1806

26

DATE MAILED: 02/03/94

This is a communication from the examiner in charge of your application.  
 COMMISSIONER OF PATENTS AND TRADEMARKS

This application has been examined  Responsive to communication filed on 12/17/93  This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s), 0 days from the date of this letter.  
 Failure to respond within the period for response will cause the application to become abandoned, 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

- 1.  Notice of References Cited by Examiner, PTO-892.
- 2.  Notice of Draftsman's Patent Drawing Review, PTO-948.
- 3.  Notice of Art Cited by Applicant, PTO-1449.
- 4.  Notice of Informal Patent Application, PTO-152.
- 5.  Information on How to Effect Drawing Changes, PTO-1474.
- 6.

Part II SUMMARY OF ACTION

- 1.  Claims 12 and 13 are pending in the application.  
 Of the above, claims \_\_\_\_\_ are withdrawn from consideration.
- 2.  Claims 1-11, 14-21 have been cancelled.
- 3.  Claims \_\_\_\_\_ are allowed.
- 4.  Claims 12 and 13 are rejected.
- 5.  Claims \_\_\_\_\_ are objected to.
- 6.  Claims \_\_\_\_\_ are subject to restriction or election requirement.
- 7.  This application has been filed with Informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.
- 8.  Formal drawings are required in response to this Office action.
- 9.  The corrected or substitute drawings have been received on \_\_\_\_\_ Under 37 C.F.R. 1.84 these drawings are  acceptable;  not acceptable (see explanation or Notice of Draftsman's Patent Drawing Review, PTO-948).
- 10.  The proposed additional or substitute sheet(s) of drawings, filed on \_\_\_\_\_ has (have) been  approved by the examiner;  disapproved by the examiner (see explanation).
- 11.  The proposed drawing correction, filed \_\_\_\_\_, has been  approved;  disapproved (see explanation).
- 12.  Acknowledgement is made of the claim for priority under 35 U.S.C. 119. The certified copy has  been received  not been received  been filed in parent application, serial no. \_\_\_\_\_; filed on \_\_\_\_\_.
- 13.  Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.
- 14.  Other

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The finality of the previous Office action is withdrawn in view of the following new grounds of rejection.

Claims 12 and 13 are pending in this application, and these claims are humanized light and heavy chain variable regions of a  
5 previously referenced antibody 4D5.

Claims 12 and 13 are rejected under 35 U.S.C. § 103 as being unpatentable over Hudziak et. al. or Fendly et. al. in view of Queen et. al.

Hudziak et. al. and Fendly et. al. both teach the production  
10 and characterization of the 4D5 antibody (see Hudziak et. al. 1166-1167 and Fendly et. al. pages 1553-1554). Hudziak et. al. suggests the possible therapeutic role of the 4D5 antibody in human neoplasias which overexpress p185-HER2 (pages 1171, last paragraph) while Fendly et. al. disclose the possible use of anti-p185 HER2  
15 antibodies for in vivo radioimaging for detection of relevant primary tumors. They do not describe the production of these antibodies in the humanized form.

Queen et. al. teach the production of antibodies against 1L-2 receptor in the humanized form, using computer modeling in order to  
20 determine the modification of certain framework regions in conjunction with CDR grafting. The antibodies produced are than to be used for in vivo administration to human patients, either for diagnosis or therapy. It is known in the art that murine and even chimeric antibodies have characteristics which may severely limit  
25 their use in human therapy. As foreign proteins, murine and chimeric antibodies may elicit immune reactions that reduce or destroy their therapeutic efficacy and/or evoke allergic or hypersensitivity reactions in patients. The probable need for readministration of such therapeutic modalities in neoplastic

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disorders increases these risks. The result would be tissue injury by virtue of antigen-antibody deposition.

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to make  
5 humanized antibodies having the sequences of the 4D5 antibody. The methods of Queen et. al. were clear and self explanatory, and resulted in a high affinity antibody. One of ordinary skill in the art would have been motivated to humanize the 4D5 antibody in light of its potential therapeutic and diagnostic applicability.

10 Although the claims are drawn to specific amino acid sequences, it is maintained that the differences in amino acid sequence which would have been obtained using the method of Queen et. al. would not have been patentably distinct from the claimed amino acid sequences. Absent sufficient factual evidence to the  
15 contrary the claims are obvious over the cited prior art.

35 U.S.C. § 101 reads as follows:

20 **Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter or any new and useful improvement thereof, may obtain a patent therefore, subject to the conditions and requirements of this title.**

Claims 12 and 13 are rejected under 35 U.S.C. § 101 because the claimed invention lacks patentable utility. These claims are  
25 drawn to a light chain variable region polypeptide and a heavy chain variable region polypeptide which in and of themselves have no patentable utility. The specification does not disclose any

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practical utility for the individual polypeptides and does not present evidence that these polypeptides are capable of binding in any particular manner when not in association with each other.

Claims 12 and 13 are directed to an invention not patentably  
5 distinct from claims 1, 3-9, and 40 of commonly assigned  
07/977,453.

Specifically, the claims of the instant invention are drawn to the humanized version of the 4D5 antibody which is disclosed in copending application.

10 Commonly assigned 07/977,453, discussed above, would form the basis for a rejection of the noted claims under 35 U.S.C. § 103 if the commonly assigned case qualifies as prior art under 35 U.S.C. §  
15 102(f) or (g) and the conflicting inventions were not commonly owned at the time the invention in this application was made. In order for the examiner to resolve this issue, the assignee is  
20 required under 37 C.F.R. 1.78(c) and 35 U.S.C. § 132 to either show that the conflicting inventions were commonly owned at the time the invention in this application was made or to name the prior inventor of the conflicting subject matter. Failure to comply with  
this requirement will result in a holding of abandonment of the application. A showing that the inventions were commonly owned at the time the invention in this application was made will preclude a rejection under 35 U.S.C. § 103 based upon the commonly assigned case as a reference under 35 U.S.C. § 102(f) or (g).



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Claims 12 and 13 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1,3-9, and 40 of copending application Serial No. 07/977,453 in view of Queen et. al.. The  
5 instant claims are drawn to the heavy chain and light chain variable regions of the 4D5 antibody. Copending application 07/977,453 claims an antibody with the same characteristics as 4D5, and also states within the claims that 4D5 antibody was useful for diagnosis and therapy of tumors expressing the p185 HER2 antigen on  
10 their surface. The induction of HAMA responses upon repeated administration of rodent antibodies has led to the desirability of producing antibodies which are even more "near human" than chimeric antibodies. Queen et. al. describes the production of antibodies which contain essentially the CDR of rodents and are grafted into  
15 human framework regions. These antibodies are also mutated in certain framework residues in order to produce functional and high affinity molecules. The procedure in Queen et. al. clearly teaches the particular framework residues that need to be changed in order to yield high affinity antibodies, and they teach how to determine  
20 the appropriate residues using computer modeling programs. This protocol is adaptable to any number of antibodies. Therefore, not only was the production of non-immunogenic 4D5 antibodies desirable, but the procedure for producing the antibodies was also well known and practiced. It would have been prima facie obvious

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Art Unit 1806

to one of ordinary skill in the art at the time the invention was made to use the claims of the copending application in combination with the reference of Queen et. al. in order to obtain high affinity functional humanized antibodies.

5           The obviousness-type double patenting rejection is a judicially established doctrine based upon public policy and is primarily intended to prevent prolongation of the patent term by prohibiting claims in a second patent not patentably distinct from claims in a first patent. In re Vogel, 164 U.S.P.Q. 619 (CCPA  
10 1970). A timely filed terminal disclaimer in compliance with 37 C.F.R. 1.321(b) would overcome an actual or provisional rejection on this ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 C.F.R. 1.78(d).

15           Claims 12 and 13 are provisionally rejected under 35 U.S.C. § 103 as being obvious over copending application Serial No. 07/977,453 in view of Queen et. al.

          The instant claims are drawn to the heavy chain and light chain variable regions of the 4D5 antibody. Copending application  
20 07/977,453 discloses an antibody with the same characteristics as 4D5, and also discloses that 4D5 antibody is useful for diagnosis and therapy of tumors expressing the p185 HER2 antigen on their surface. The induction of HAMA responses upon repeated administration of rodent antibodies has led to the desirability of

Serial No. 715272

Art Unit 1806

producing antibodies which are even more "near human" than chimeric antibodies. Queen et. al. describes the production of antibodies which contain essentially the CDR of rodents and are grafted into human framework regions. These antibodies are also mutated in  
5 certain framework residues in order to produce functional and high affinity molecules. The procedure in Queen et. al. clearly teaches the particular framework residues that need to be changed in order to yield high affinity antibodies, and they teach how to determine the appropriate residues using computer modeling programs. This  
10 protocol is adaptable to any number of antibodies. Therefore, not only was the production of non-immunogenic 4D5 antibodies desirable, but the procedure for producing the antibodies was also well known and practiced. It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was  
15 made to use the claims of the copending application in combination with the reference of Queen et. al. in order to obtain high affinity functional humanized antibodies.

Copending application Serial No. 07/977,453 has a common assignee with the instant application. Based upon the earlier  
20 effective U.S. filing date of the copending application, it would constitute prior art under 35 U.S.C. § 102(e) if patented. This provisional rejection under 35 U.S.C. § 103 is based upon a presumption of future patenting of the conflicting application.

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This provisional rejection might be overcome either by a showing under 37 C.F.R. 1.132 that any unclaimed invention disclosed in the copending application was derived from the inventor of this application and is thus not the invention "by  
5 another", or by a showing of a date of invention prior to the effective U.S. filing date of the copending application.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Lila Feisee whose telephone number is (703) 308-2731.

10 Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Feisee/lf  
January 11, 1994

15

  
PAULA K. HUTZELL  
PRIMARY EXAMINER  
GROUP 1800

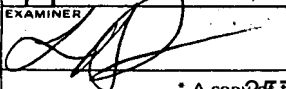
TO SEPARATE. D TOP AND BOTTOM EDGES, SNAP-APART AND DISCARD CARBON

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|-----------------------------|--|------------------------------|------------------------|---|
| FORM PTO-892<br>(REV. 2-92) | U.S. DEPARTMENT OF COMMERCE<br>PATENT AND TRADEMARK OFFICE | SERIAL NO.<br>07/715272      | GROUP/ART UNIT<br>1806 | ATTACHMENT<br>TO<br>PAPER<br>NUMBER<br>26 |
| NOTICE OF REFERENCES CITED  |  | APPLICANT(S)<br>Carter et al |                        |   |

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

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

| OTHER REFERENCES (Including Author, Title, Date, Pertinent Pages, Etc.) |   |
|---|---|
| R   | Queen et al. PNAS 86: 10029-10033 (1989)                        |
| S   | Fendley et al. Cancer Research 5: 1550-1558 (1985)              |
| T   | Hudziak et al. Molecular and Cellular Biology 1989 p. 1165-1172 |
| U   |   |

|   |                  |
|---|------------------|
| EXAMINER<br> | DATE<br>11/11/94 |
|---|------------------|

with this office action.  
on 707.05 (a)  
Celltrion, Inc., Exhibit 1094



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

|               |             |                       |                     |
|---------------|-------------|-----------------------|---------------------|
| SERIAL NUMBER | FILING DATE | FIRST NAMED APPLICANT | ATTORNEY DOCKET NO. |
|---------------|-------------|-----------------------|---------------------|

07/715,272    06/14/91    CARTER    EXAMINER P    709

18M2/0906    FEISEE, L  
 ART UNIT    PAPER NUMBER

GENENTECH, INC.  
 ATTN: CAROLYN R. ADLER  
 460 POINT SAN BRUNO BLVD.  
 SOUTH SAN FRANCISCO, CA 94080

DATE MAILED: 1806

**NOTIFICATION OF DEFECTIVE NOTICE OF APPEAL OR DEFECTIVE BRIEF**

09/06/94

1.  The Notice of Appeal filed \_\_\_\_\_ is:
- A.  Not acceptable for reason(s) that:
- (1)  The Appeal fee required by 35 U.S.C. 41 (a)(6) and 37 CFR 1.17(e) was not submitted with the Notice of Appeal.
  - (2)  The submitted fee of \$ \_\_\_\_\_ is insufficient. The appeal fee required by 37 CFR 1.17(e) is \$ \_\_\_\_\_.
  - (3)  The Notice of Appeal was not timely filed.
  - (4)  The Appeal fee received on \_\_\_\_\_ was not timely filed.
  - (5)  The Appeal is not in compliance with 37 CFR 1.191 in that the claims have not been finally or twice rejected.
  - (6)  A Notice of Allowability was mailed by the Office on \_\_\_\_\_.
- B.  Defective and should be corrected as indicated. Applicant is given a TIME LIMIT of ONE MONTH from the date of this letter OR the TIME REMAINING IN THE RESPONSE PERIOD OF THE LAST OFFICE ACTION, whichever is longer, to complete the appeal. NO EXTENSION OF THIS ONE MONTH PERIOD MAY BE GRANTED UNDER 37 CFR 1.136(a) or (b) BUT THE PERIOD FOR RESPONSE SET IN THE LAST ACTION MAY POSSIBLY BE EXTENDED. If the appeal is not timely completed, the application will be abandoned.
- (1)  The Notice of Appeal is not signed.
  - (2)  Identification of the appealed claim or claims is required under 37 CFR 1.191 (b).

2.  The Brief filed \_\_\_\_\_ is NOT acceptable for the reason(s) indicated below.
- The Appeal in this application will be dismissed unless the applicant makes the Brief acceptable. Extensions of time may be obtained under 37 CFR 1.136(a).
- A.  The Brief and/or Brief fee is untimely. See 37 CFR 1.192.
  - B.  The requisite fee which must accompany the Brief has been omitted. See 37 CFR 1.17(f).
  - C.  The submitted Brief fee of \_\_\_\_\_ is not the proper amount. The Brief fee required by 37 CFR 1.17(f) is \_\_\_\_\_.

3.  The Appeal in this application is DISMISSED because
- A.  The fee for filing the Brief as required under 37 CFR 1.17(f) was not submitted or timely submitted and the period for obtaining an extension of time to file the brief under 37 CFR 1.136 has expired.
  - B.  The Brief was not filed, or was not timely filed and the period for obtaining an extension of time to file the brief under 37 CFR 1.136 has expired.

4.  As the result of the dismissal in "3" above, this application:
- A.  is abandoned since there are no allowed claims.
  - B.  is being returned to the examiner for disposition since it contains allowed claims. Prosecution on the merits is CLOSED.

*David L. Lacey*  
 DAVID L. LACEY  
 SUPERVISORY PATENT EXAMINER  
 GROUP 180  
 9/1/94

REQUEST FOR ACCESS OF ABANDONED APPLICATION UNDER 37 CFR 1.14(a)

PROCES HI-BI

OCT 19 1999

F 11

In re Application of

Application Number

Filed

07/715,272

6-14-91

Group Art Unit

Examiner

Paper No. #28

Assistant Commissioner for Patents  
Washington, DC 20231

I hereby request access under 37 CFR 1.14(a)(3)(iv) to the application file record of the above-identified ABANDONED application, which is: (CHECK ONE)

(A) referred to in United States Patent Number 5821337, column 1

(B) referred to in an application that is open to public inspection as set forth in 37 CFR 1.11, i.e., Application No. \_\_\_\_\_ filed \_\_\_\_\_ on page \_\_\_\_\_ of paper number \_\_\_\_\_

(C) an application that claims the benefit of the filing date of an application that is open to public inspection, i.e., Application No. \_\_\_\_\_ filed \_\_\_\_\_ or

(D) an application in which the applicant has filed an authorization to lay open the complete application to the public.

Please direct any correspondence concerning this request to the following address:

2001 Jefferson Davis Hwy Apt. No. 22202  
Suite # 806

Brian Willingham  
Signature

10 / 19 / 99  
Date

Brian Willingham  
Typed or printed name

FOR PTO USE ONLY  
Approved by: [Signature]  
(Initials)  
Unit: Bill Tomich

RECEIVED  
NOV 30 1999  
File Information Unit

In re Application of

Application Number

Filed

07/715272

6-14-91

Group Art Unit

Examiner

Paper No. #29

Assistant Commissioner for Patents  
Washington, DC 20231

I hereby request access under 37 CFR 1.14(a)(3)(iv) to the application file record of the above-identified ABANDONED application, which is: (CHECK ONE)

- (A) referred to in United States Patent Number 5821337, column \_\_\_\_\_
- (B) referred to in an application that is open to public inspection as set forth in 37 CFR 1.11, i.e., Application No. \_\_\_\_\_, filed \_\_\_\_\_, on page \_\_\_\_\_ of paper number \_\_\_\_\_
- (C) an application that claims the benefit of the filing date of an application that is open to public inspection, i.e., Application No. \_\_\_\_\_, filed \_\_\_\_\_, or
- (D) an application in which the applicant has filed an authorization to lay open the complete application to the public.

Please direct any correspondence concerning this request to the following address:

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

*Michael P. ...*  
Signature

11-30-99  
Date

\_\_\_\_\_  
Typed or printed name

FOR PTO USE ONLY  
Approved by: *JW*  
(Initials)  
Unit: *FIC*

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comment on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patents and Trademarks Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.



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## REQUEST FOR ACCESS OF ABANDONED APPLICATION UNDER 37 CFR 1.14(a)

|  |   |
|--|---|
| In re Application of   |   |
| Application Number<br><span style="font-size: 1.5em; font-family: cursive;">07/715272</span> | Filed<br><span style="font-size: 1.5em; font-family: cursive;">6/14/91</span> |
| Group Art Unit   | Examiner  |

Paper No. 30

Assistant Commissioner for Patents  
 Washington, DC 20231

I hereby request access under 37 CFR 1.14(a)(3)(iv) to the application file record of the above-identified ABANDONED application, which is: (CHECK ONE):

- (A) referred to in United States Patent Number 6054297, column \_\_\_\_\_
- (B) referred to in an application that is open to public inspection as set forth in 37 CFR 1.11, i.e., Application No. \_\_\_\_\_, filed \_\_\_\_\_, on page \_\_\_\_\_ of paper number \_\_\_\_\_.
- (C) an application that claims the benefit of the filing date of an application that is open to public inspection, i.e., Application No. \_\_\_\_\_, filed \_\_\_\_\_, or
- (D) an application in which the applicant has filed an authorization to lay open the complete application to the public.

Please direct any correspondence concerning this request to the following address:

\_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_

Wayne Croteau  
 Signature

9-10-01  
 Date

WAYNE CROTEAU  
 Typed or printed name

RECEIVED  
 TECH CENTER  
 1600/2900  
 01 SEP 12 AM 11:15

**FOR PTO USE ONLY**

Approved by: \_\_\_\_\_  
 (Initials)

Unit: \_\_\_\_\_

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

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### REQUEST FOR ACCESS OF ABANDONED APPLICATION UNDER 37 CFR 1.14(a)

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| In re Application of |          |
| Application Number   | Filed    |
| 07/715272            | 6-14-91  |
| Group Art Unit       | Examiner |
|                      |          |

Assistant Commissioner for Patents  
Washington, DC 20231

Paper No. #31

I hereby request access under 37 CFR 1.14(a)(3)(iv) to the application file record of the above-identified ABANDONED application, which is: (CHECK ONE)

(A) referred to in United States Patent Number 6054997 column \_\_\_\_\_

(B) referred to in an application that is open to public inspection as set forth in 37 CFR 1.11, i.e., Application No. \_\_\_\_\_, filed \_\_\_\_\_ on page \_\_\_\_\_ of paper number \_\_\_\_\_

(C) an application that claims the benefit of the filing date of an application that is open to public inspection, i.e., Application No. \_\_\_\_\_, filed \_\_\_\_\_ or

(D) an application in which the applicant has filed an authorization to lay open the complete application to the public.

Please direct any correspondence concerning this request to the following address:

\_\_\_\_\_

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### REQUEST FOR ACCESS TO AN APPLICATION UNDER 37 CFR 1.14(e)

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| In re Application of<br><i>Carter</i>  |                         |
| Application Number<br><i>07/715272</i> | Filed<br><i>6/14/91</i> |
| Art Unit                               | Examiner                |

Paper No. *#32*

Assistant Commissioner for Patents  
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1.  I hereby request access under 37 CFR 1.14(e)(2) to the application file record of the above-identified ABANDONED Application, which is not within the file jacket of a pending Continued Prosecution Application (CPA) (37 CFR 1.53(d)) and is: (CHECK ONE)

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2.  I hereby request access under 37 CFR 1.14(e)(1) to an application in which the applicant has filed an authorization to lay open the complete application to the public.

*Devita Myles*  
\_\_\_\_\_  
Signature

*3/11/02*  
\_\_\_\_\_  
Date

*Devita Myles*  
\_\_\_\_\_  
Typed or printed name

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# REQUEST FOR ACCESS TO AN APPLICATION UNDER 37 CFR 1.14(e)

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| In re Application of                    |                               |
| Application Number<br><b>07/715,272</b> | Filed<br><b>Jun. 14, 1991</b> |
| Art Unit                                | Examiner                      |

Paper No. **#33**

Assistant Commissioner for Patents  
Washington, DC 20231

1.  I hereby request access under 37 CFR 1.14(e)(2) to the application file record of the above-identified ABANDONED Application, which is not within the file jacket of a pending Continued Prosecution Application (CPA) (37 CFR 1.53(d)) and is: (CHECK ONE)

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United States Patent Application Publication No. \_\_\_\_\_, page \_\_\_\_\_, line \_\_\_\_\_,  
United States Patent Number **6,407,213**, column **63**, line \_\_\_\_\_, or  
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2.  I hereby request access under 37 CFR 1.14(e)(1) to an application in which the applicant has filed an authorization to lay open the complete application to the public.

Chris Riley  
Signature

6/24/02  
Date

Chris Riley  
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| Application Number<br><b>07/715,272</b> | Filed<br><b>6-14-91</b> |
| Art Unit _____                          | Examiner _____          |

Paper No. **#34**

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United States Patent Number **6407213**, column \_\_\_\_\_, line \_\_\_\_\_, or

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(B) referred to in an application that is open to public inspection as set forth in 37 CFR 1.11(b) or

1.14(e)(2)(i), i.e., Application No. \_\_\_\_\_, paper No. \_\_\_\_\_, page \_\_\_\_\_, line \_\_\_\_\_.

2.  I hereby request access under 37 CFR 1.14(e)(1) to an application in which the applicant has filed an authorization to lay open the complete application to the public.

Brian Willingham  
Signature

8/21/02  
Date

Brian Willingham  
Typed or printed name

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# REQUEST FOR ACCESS TO AN APPLICATION UNDER 37 CFR 1.14(e)

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| In re Application of                    |                           |
| Application Number<br><b>07/715,272</b> | Filed<br><b>6-14-91</b>   |
| Art Unit<br><b>186</b>                  | Examiner<br><b>Keisee</b> |

Paper No. 35

Assistant Commissioner for Patents  
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1.  I hereby request access under 37 CFR 1.14(e)(2) to the application file record of the above-identified ABANDONED Application, which is not within the file jacket of a pending Continued Prosecution Application (CPA) (37 CFR 1.53(d)) and is: (CHECK ONE)

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(B) referred to in an application that is open to public inspection as set forth in 37 CFR 1.11(b) or 1.14(e)(2)(i), i.e., Application No. \_\_\_\_\_, paper No. \_\_\_\_\_, page \_\_\_\_\_, line \_\_\_\_\_.

2.  I hereby request access under 37 CFR 1.14(e)(1) to an application in which the applicant has filed an authorization to lay open the complete application to the public.

Keisha Jenkins  
Signature

10/18/02  
Date

Keisha Jenkins  
Typed or printed name

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| In re Application of<br><i>0407,213</i> |                         |
| Application Number<br><i>07/715,272</i> | Filed<br><i>0-14-91</i> |
| Art Unit                                | Examiner                |

Paper No. *#36*

Assistant Commissioner for Patents  
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2.  I hereby request access under 37 CFR 1.14(e)(1) to an application in which the applicant has filed an authorization to lay open the complete application to the public.

*Lucey Smith*  
 \_\_\_\_\_  
 Signature

*11-1-02*  
 \_\_\_\_\_  
 Date

*Lucey Smith*  
 \_\_\_\_\_  
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| In re Application of                   |                          |
| Application Number<br><u>07-715272</u> | Filed<br><u>06-14-91</u> |
| Art Unit                               | Examiner                 |

Paper No. # 37

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an International Application which was filed on or after November 29, 2000 and which  
designates the United States, WIPO Pub. No. \_\_\_\_\_, page \_\_\_\_\_, line \_\_\_\_\_.

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Shoaib Ahayour  
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Shoaib Ahayour  
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US006407213B1

(12) **United States Patent**  
**Carter et al.**

(10) **Patent No.: US 6,407,213 B1**  
(45) **Date of Patent: Jun. 18, 2002**

- (54) **METHOD FOR MAKING HUMANIZED ANTIBODIES**
- (75) **Inventors: Paul J. Carter; Leonard G. Presta, both of San Francisco, CA (US)**
- (73) **Assignee: Genentech, Inc., South San Francisco, CA (US)**
- (\* ) **Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.**
- (21) **Appl. No.: 08/146,206**
- (22) **PCT Filed: Jun. 15, 1992**
- (86) **PCT No.: PCT/US92/05126**  
§ 371 (c)(1),  
(2), (4) **Date: Nov. 17, 1993**

**Related U.S. Application Data**

- (63) **Continuation-in-part of application No. 07/715,272, filed on Jun. 14, 1991, now abandoned.**
- (51) **Int. Cl.<sup>7</sup> ..... C07K 16/00**
- (52) **U.S. Cl. .... 530/387.3; 435/69.6; 435/69.7; 435/70.21; 435/91; 536/23.53; 424/133.1**
- (58) **Field of Search ..... 435/69.6, 69.7, 435/70.21, 91, 172.2, 240.1, 240.27, 252.3, 320.1, 328; 536/23.53; 424/133.1; 530/387.3**

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(List continued on next page.)

*Primary Examiner*—Anthony C. Caputa  
*Assistant Examiner*—Minh-Tam Davis  
(74) *Attorney, Agent, or Firm*—Wendy M. Lee

(57) **ABSTRACT**

Variant immunoglobulins, particularly humanized antibody polypeptides are provided, along with methods for their preparation and use. Consensus immunoglobulin sequences and structural models are also provided.

**82 Claims, 9 Drawing Sheets**

**REQUEST FOR ACCESS TO AN APPLICATION UNDER 37 CFR 1.14(e)**

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|--|-----------------------------|
| In re Application of<br><i>Carer</i>   |                             |
| Application Number<br><i>07/715272</i> | Filed<br><i>June 14, 91</i> |
| Art Unit                               | Examiner                    |

Paper No. 38

Assistant Commissioner for Patents  
 Washington, DC 20231

1.  I hereby request access under 37 CFR 1.14(e)(2) to the application file record of the above-identified ABANDONED Application, which is not within the file jacket of a pending Continued Prosecution Application (CPA) (37 CFR 1.53(d)) and is: (CHECK ONE)

(A) referred to in:  
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(B) referred to in an application that is open to public inspection as set forth in 37 CFR 1.11(b) or 1.14(e)(2)(i), i.e., Application No. \_\_\_\_\_, paper No. \_\_\_\_\_, page \_\_\_\_\_, line \_\_\_\_\_.

2.  I hereby request access under 37 CFR 1.14(e)(1) to an application in which the applicant has filed an authorization to lay open the complete application to the public.

*Viola Ponder*  
 Signature

5/20/03  
 Date

Viola Ponder  
 Typed or printed name

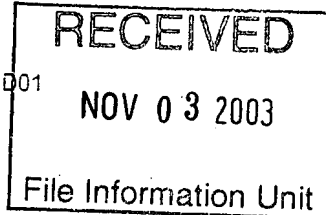
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In re Application of \_\_\_\_\_

Application Number

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07/715272 6-14-91

Paper No. #39

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United States Patent Number 6639055 column \_\_\_\_\_, line \_\_\_\_\_ or

WIPO Pub. No. \_\_\_\_\_, page \_\_\_\_\_, line \_\_\_\_\_

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David Brown  
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7/4182200

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In re Application of

*Caeter*

Application Number

*07/15 272*

Date

*06/14/91*

Paper No.

*#40*

I hereby request access under 37 CFR 1.114(a)(1)(iv) to the application file record of the above-identified ABANDONED application, which is identified in, or to which a benefit is claimed, in the following document (as shown in the attachment):

United States Patent Application Publication No. \_\_\_\_\_, page \_\_\_\_\_, line \_\_\_\_\_

United States Patent Number *605427*, column \_\_\_\_\_, line \_\_\_\_\_, or

WIPO Pub. No. \_\_\_\_\_, page \_\_\_\_\_, line \_\_\_\_\_

**Related Information about Access to Pending Applications (37 CFR 1.114):**

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  - any document in the file of the pending application.
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*Urseltha Bunchester*  
 Signature

*11/19/03*  
 Date

*Urseltha Bunchester*  
 Typed or printed name

Registration Number, if applicable

*7/480-1150*  
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| In re Application of _____             |                         |
| Application Number<br><b>07/715272</b> | Filed<br><b>6-14-91</b> |

Paper No. **#41**

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United States Patent Application Publication No. \_\_\_\_\_, page \_\_\_\_\_, line \_\_\_\_\_.

United States Patent Number **6407213**, column \_\_\_\_\_, line \_\_\_\_\_ or \_\_\_\_\_

WIPO Pub. No. \_\_\_\_\_, page \_\_\_\_\_, line \_\_\_\_\_.

#### Related Information about Access to Pending Applications (37 CFR 1.14):

Direct access to pending applications is not available to the public but copies may be available and may be purchased from the Office of Public Records upon payment of the appropriate fee (37 CFR 1.19(b)), as follows: For **published** applications that are still pending, a member of the public may obtain a copy of:

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- (2) If the application is **incorporated by reference or otherwise identified** in a U.S. patent, a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:
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*Wayne Croteman*  
Signature

**12-4-03**  
Date

**WAYNE CROTEMAN**  
Typed or printed name

Registration Number, if applicable \_\_\_\_\_

Telephone Number \_\_\_\_\_

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| In re Application of _____              |                         |
| Application Number<br><b>07/715,272</b> | Filed<br><b>6/14/91</b> |

Paper No. **742**

I hereby request access under 37 CFR 1.14(a)(1)(iv) to the application file record of the above-identified ABANDONED application, which is identified in, or to which a benefit is claimed, in the following document (as shown in the attachment):

United States Patent Application Publication No. \_\_\_\_\_, page, \_\_\_\_\_ line \_\_\_\_\_,

United States Patent Number **6,054,297**, column \_\_\_\_\_, line, \_\_\_\_\_ or

WIPO Pub. No. \_\_\_\_\_, page \_\_\_\_\_, line \_\_\_\_\_.

#### Related Information about Access to Pending Applications (37 CFR 1.14):

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- For unpublished applications that are still pending:
- (1) If the benefit of the pending application is claimed under 35 U.S.C. 119(e), 120, 121, or 365 in another application that has: (a) issued as a U.S. patent, or (b) published as a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:
    - the file contents;
    - the pending application as originally filed; or
    - any document in the file of the pending application.
  - (2) If the application is incorporated by reference or otherwise identified in a U.S. patent, a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:
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Gary Wan  
Signature

3/9/04  
Date

GARY WASSERMAN  
Typed or printed name

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In re Application of

*CARTER et al*

Application Number:

*07/715272*

Filed:

*6/14/91*

Page No. *#43*

I hereby request access under 37 CFR 1.14(a)(1)(iv) to the application file record of the above-identified ABANDONED application, which is identified in, or to which a benefit is claimed, in the following document (as shown in the attachment):

United States Patent Application Publication No. \_\_\_\_\_, page \_\_\_\_\_, line \_\_\_\_\_.

United States Patent Number *6639055*, column \_\_\_\_\_, line \_\_\_\_\_, or

WIPO Pub. No. \_\_\_\_\_, page \_\_\_\_\_, line \_\_\_\_\_.

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*[Signature]*

Signature

*Rob Johnson*

Typed or printed name

*4/2/04*

Date

Registration Number, if applicable

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Telephone Number

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In re Application of

*CARTER*

Application Number:

*07/715272*

Filed

*6/14/91*

PEPSP No.

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United States Patent Application Publication No. \_\_\_\_\_, page \_\_\_\_\_, line \_\_\_\_\_

United States Patent Number *6407213*, column \_\_\_\_\_, line \_\_\_\_\_ or

WIPO Pub. No. \_\_\_\_\_, page \_\_\_\_\_, line \_\_\_\_\_

**Related Information about Access to Pending Applications (37 CFR 1.14):**

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For unpublished applications that are still pending:

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*[Signature]*  
 \_\_\_\_\_  
 Signature

*5/4/03*  
 \_\_\_\_\_  
 Date

*Rick Terzag*  
 \_\_\_\_\_  
 Typed or printed name

Registration Number, if applicable  
*703-418-2848*  
 Telephone Number

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In re Application of

*Carter*

Application Number

*07/715272*

Filed

*6/14/91*

Paper No. *#45*

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United States Patent Application Publication No. \_\_\_\_\_, page, \_\_\_\_\_ line \_\_\_\_\_.

United States Patent Number *6407213*, column \_\_\_\_\_, line, \_\_\_\_\_ or

WIPO Pub. No. \_\_\_\_\_, page \_\_\_\_\_, line \_\_\_\_\_.

#### Related Information about Access to Pending Applications (37 CFR 1.14):

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For unpublished applications that are still pending:

- (1) If the benefit of the pending application is claimed under 35 U.S.C. 119(e), 120, 121, or 365 in another application that has: (a) issued as a U.S. patent, or (b) published as a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:
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*Anda Myles*

Signature

*Anda Myles*

Typed or printed name

*6/7/04*

Date

Registration Number, if applicable  
*(703) 4154630*

Telephone Number

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JUN 07 2004  
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In re Application of \_\_\_\_\_

Application Number

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07/715,272

06-14-91

Paper No. #46

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United States Patent Application Publication No. \_\_\_\_\_, page, \_\_\_\_\_ line \_\_\_\_\_,

United States Patent Number US 6,407,213 B1, column \_\_\_\_\_, line, \_\_\_\_\_ or

WIPO Pub. No. \_\_\_\_\_, page \_\_\_\_\_, line \_\_\_\_\_.

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Rachel Hail

Signature

06-09-04

Date

Rachel Hail

Typed or printed name

Registration Number, if applicable

703-486-1150

Telephone Number

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|----------|--------------------------------|
| Approved | <u>[Signature]</u><br>Initials |
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| In re Application of<br><i>Carter</i>  |                         |
| Application Number<br><i>07/715222</i> | Filed<br><i>6/14/91</i> |
| Paper No. <i># 47</i>                  |                         |

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United States Patent Application Publication No. \_\_\_\_\_, page, \_\_\_\_\_ line \_\_\_\_\_.

United States Patent Number *640723*, column \_\_\_\_\_, line, \_\_\_\_\_ or \_\_\_\_\_

WIPO Pub. No. \_\_\_\_\_, page \_\_\_\_\_, line \_\_\_\_\_.

**Related Information about Access to Pending Applications (37 CFR 1.14):**  
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*Diobala Ponder*  
Signature

*8/16/04*  
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*Diobala Ponder*  
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In re Application of \_\_\_\_\_

Application Number

07715272

Filed

Jun. 15, 91

Paper No. 48

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United States Patent Application Publication No. 6800730, page, \_\_\_\_\_ line \_\_\_\_\_.

United States Patent Number \_\_\_\_\_, column \_\_\_\_\_, line, \_\_\_\_\_ or

WIPO Pub. No. \_\_\_\_\_, page \_\_\_\_\_, line \_\_\_\_\_.

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For published applications that are still pending, a member of the public may obtain a copy of:

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For unpublished applications that are still pending:

- (1) If the benefit of the pending application is claimed under 35 U.S.C. 119(e), 120, 121, or 365 in another application that has: (a) issued as a U.S. patent, or (b) published as a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:
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  - the pending application as originally filed; or
  - any document in the file of the pending application.
- (2) If the application is incorporated by reference or otherwise identified in a U.S. patent, a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:
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Kevin Rodriguez

Signature

Kevin Rodriguez

Typed or printed name

Registration Number, if applicable

(703) 418-2777

Telephone Number

11-104

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| In re Application of<br><b>Cartier</b> |                         |
| Application Number<br><b>07/215272</b> | Filed<br><b>7/14/91</b> |
| Paper No. <b>#49</b>                   |                         |

I hereby request access under 37 CFR 1.14(a)(1)(iv) to the application file record of the above-identified ABANDONED application, which is identified in, or to which a benefit is claimed, in the following document (as shown in the attachment):

United States Patent Application Publication No. \_\_\_\_\_, page \_\_\_\_\_, line \_\_\_\_\_

United States Patent Number **6407213**, column \_\_\_\_\_, line \_\_\_\_\_

WIPO Pub. No. \_\_\_\_\_, page \_\_\_\_\_, line \_\_\_\_\_

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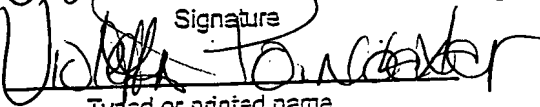
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**11/29/04**  
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In re Application of \_\_\_\_\_

Application Number

07/715272

Filed

June 14, 1991

Paper No. 450

I hereby request access under 37 CFR 1.14(a)(1)(iv) to the application file record of the above-identified ABANDONED application, which is not within the file jacket of a pending Continued Prosecution Application (CPA) (37 CFR 1.53(d)) and which is identified in, or to which a benefit is claimed, in the following document (as shown in the attachment):

United States Patent Application Publication No. \_\_\_\_\_, page, \_\_\_\_\_ line \_\_\_\_\_,

United States Patent Number 6054297, column \_\_\_\_\_, line, \_\_\_\_\_ or

WIPO Pub. No. \_\_\_\_\_, page \_\_\_\_\_, line \_\_\_\_\_.

**Related Information About Access to Applications Maintained in the Image File Wrapper System (IFW) and Access to Pending Applications in General**

A member of the public, acting without a power to inspect, cannot order applications maintained in the IFW system through the FIU. If the member of the public is entitled to a copy of the application file, then the file is made available through the Public Patent Application Information Retrieval system (Public PAIR) on the USPTO internet web site (www.uspto.gov). Terminals that allow access to Public PAIR are available in the Public Search Room. The member of the public may also be entitled to obtain a copy of all or part of the application file upon payment of the appropriate fee. Such copies must be purchased through the Office of Public Records upon payment of the appropriate fee (37 CFR 1.19(b)).

For published applications that are still pending, a member of the public may obtain a copy of: the file contents; the pending application as originally filed; or any document in the file of the pending application.

For unpublished applications that are still pending:

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- (2) If the application is incorporated by reference or otherwise identified in a U.S. patent, a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of the pending application as originally filed.

Darlene Jones

Signature

Darlene Jones

Typed or printed name

Jan. 18. 05

Date

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JAN 18 2005

Approved by: \_\_\_\_\_

(initials)

Unit: \_\_\_\_\_

Registration Number, if applicable

7/418-0330

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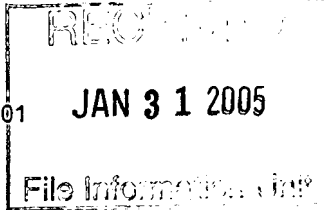
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|   |                         |
|---|-------------------------|
| In re Application of<br><i>Carter et al</i> |                         |
| Application Number<br><i>07/715,272</i>     | Filed<br><i>6/14/91</i> |

Paper No. *#51*

I hereby request access under 37 CFR 1.14(a)(1)(iv) to the application file record of the above-identified ABANDONED application, which is identified in, or to which a benefit is claimed, in the following document (as shown in the attachment):

United States Patent Application Publication No. \_\_\_\_\_, page, \_\_\_\_\_ line \_\_\_\_\_.

United States Patent Number *6,800,738*, column *1*, line, \_\_\_\_\_ or

WIPO Pub. No. \_\_\_\_\_, page \_\_\_\_\_, line \_\_\_\_\_.

#### Related Information about Access to Pending Applications (37 CFR 1.14):

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For unpublished applications that are still pending:

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  - the pending application as originally filed.

*Rayline K. Petitt*

Signature

Rayline K. Petitt

Typed or printed name

n/a

Registration Number, if applicable

703-415-3060

Telephone Number

*Jan. 31, 2005*

Date

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| In re Application of                   |                         |
| Application Number<br><b>07/715272</b> | Filed<br><b>6-14-91</b> |

Paper No. **#52**

I hereby request access under 37 CFR 1.14(a)(1)(iv) to the application file record of the above-identified ABANDONED application, which is identified in, or to which a benefit is claimed, in the following document (as shown in the attachment):

United States Patent Application Publication No. \_\_\_\_\_, page, \_\_\_\_\_ line \_\_\_\_\_.

United States Patent Number **5821337**, column \_\_\_\_\_, line, \_\_\_\_\_ or

WIPO Pub. No. \_\_\_\_\_, page \_\_\_\_\_, line \_\_\_\_\_.

**Related Information about Access to Pending Applications (37 CFR 1.14):**  
 Direct access to pending applications is not available to the public but copies may be available and may be purchased from the Office of Public Records upon payment of the appropriate fee (37 CFR 1.19(b)), as follows:  
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 (1) If the benefit of the pending application is claimed under 35 U.S.C. 119(e), 120, 121, or 365 in another application that has: (a) issued as a U.S. patent, or (b) published as a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:  
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Wayne Proteau  
 Signature  
WAYNE PROTEAU  
 Typed or printed name  
 \_\_\_\_\_  
 Registration Number, if applicable  
415-1077  
 Telephone Number

4-15-05  
 Date

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SEP 12 2005

File Information Unit

Application Number

07/715272

File

6/4/91

Paper No. 533

I hereby request access under 37 CFR 1.114(e)(1)(iv) to the application file record of the above-identified ABANDONED application, which is identified in, or to which a benefit is claimed, in the following document(s) as shown in the attachment:

United States Patent Application Publication No. \_\_\_\_\_, page \_\_\_\_\_, line \_\_\_\_\_

United States Patent Number 6407213, column \_\_\_\_\_, line \_\_\_\_\_, or

WIPO Pub. No. \_\_\_\_\_, page \_\_\_\_\_, line \_\_\_\_\_

Related Information about Access to Pending Applications (37 CFR 1.114):

Direct access to pending applications is not available to the public but copies may be available and may be purchased from the Office of Public Records upon payment of the appropriate fee (37 CFR 1.15(b)), as follows: For published applications that are still pending, a member of the public may obtain a copy of:

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any document in the file of the pending application.

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- (1) If the benefit of the pending application is claimed under 35 U.S.C. 119(a), 120, 121, or 365 in another application that has: (a) issued as a U.S. patent, or (b) published as a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:
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  - the pending application as originally filed; or
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  - the pending application as originally filed.

*Prickly Garcia*  
Signature

PRICKLY GARCIA  
Typed or printed name

9/12/05  
Date

Registration Number, if applicable

703 413 0600

Telephone Number

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(initials)

Unit: File Information Unit

The collection of information is required by 37 CFR 1.114. The information is required to obtain or retain a benefit by the publication in the file of the USPTO to process an application. Confidentiality is governed by 35 U.S.C. 110 and 37 CFR 1.114. This collection is estimated to take 10 minutes to complete, including copying, preparing, and submitting the completed application form to the USPTO. This will vary depending upon the individual case. Any comments on the amount of time you require to complete this form should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22304-1450. DO NOT SEND FEE OR COMPLETED FORMS TO THIS ADDRESS. BRING TO: File Information Unit, Crystal Plaza Three, Room 1001, 2021 South Clark Place, Arlington, VA.

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In re Application of

*CATER*

Application Number

*07/715272*

Filed

*1/15/02*

Paper No.

*459*

I hereby request access under 37 CFR 1.14(a)(1)(iv) to the application file record of the above-identified ABANDONED application, which is identified in, or to which a benefit is claimed, in the following document (as shown in the attachment):

United States Patent Application Publication No. *0407213* page \_\_\_\_\_ line \_\_\_\_\_

United States Patent Number \_\_\_\_\_, column \_\_\_\_\_, line \_\_\_\_\_ or

WIPO Pub. No. \_\_\_\_\_, page \_\_\_\_\_, line \_\_\_\_\_

#### Related Information about Access to Pending Applications (37 CFR 1.14):

Direct access to pending applications is not available to the public but copies may be available and may be purchased from the Office of Public Records upon payment of the appropriate fee (37 CFR 1.19(b)), as follows:

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- the file contents;
- the pending application as originally filed; or
- any document in the file of the pending application.

For unpublished applications that are still pending:

- (1) If the benefit of the pending application is claimed under 35 U.S.C. 119(e), 120, 121, or 365 in another application that has: (a) issued as a U.S. patent, or (b) published as a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:

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- (2) If the application is incorporated by reference or otherwise identified in a U.S. patent, a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:

- the pending application as originally filed.

*Eric White*  
Signature

*3/27/06*  
Date

*ERIC WHITE*  
Typed or printed name

Registration Number, if applicable

Telephone Number

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In re Application of \_\_\_\_\_

Application Number \_\_\_\_\_

Filed \_\_\_\_\_

07/715272

Jun 14, 1991

SEP 01 2006

Paper No. #53

I hereby request access under 37 CFR 1.14(a)(1)(iv) to the application file record of the above-identified ABANDONED application, which is not within the file jacket of a pending Continued Prosecution Application (CPA) (37 CFR 1.53(d)) and which is identified in, or to which a benefit is claimed, in the following document (as shown in the attachment):

United States Patent Application Publication No. \_\_\_\_\_, page, \_\_\_\_\_ line \_\_\_\_\_,

United States Patent Number 5821337, column \_\_\_\_\_, line, \_\_\_\_\_ or

WIPO Pub. No. \_\_\_\_\_, page \_\_\_\_\_, line \_\_\_\_\_.

#### Related Information About Access to Applications Maintained in the Image File Wrapper System (IFW) and Access to Pending Applications in General

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For unpublished applications that are still pending:

- (1) If the benefit of the pending application is claimed under 35 U.S.C. 119(e), 120, 121, or 365 in another application that has: (a) issued as a U.S. patent, or (b) published as a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of: the file contents; the pending application as originally filed; or any document in the file of the pending application.
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*S. Funes*

Signature

*Sal Funes*

Typed or printed name

Registration Number, if applicable

*703-413-3667*

Telephone Number

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6-14-91

Paper No. 84

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United States Patent Application Publication No. \_\_\_\_\_, page, \_\_\_\_\_ line \_\_\_\_\_,

United States Patent Number 6407213 column \_\_\_\_\_, line, \_\_\_\_\_ or

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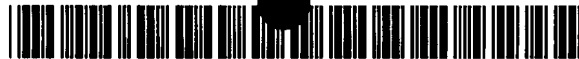
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US006407213B1

(12) **United States Patent**  
Carter et al.(10) **Patent No.:** US 6,407,213 B1  
(45) **Date of Patent:** Jun. 18, 2002(54) **METHOD FOR MAKING HUMANIZED ANTIBODIES**(75) **Inventors:** Paul J. Carter; Leonard G. Presta,  
both of San Francisco, CA (US)(73) **Assignee:** Genentech, Inc., South San Francisco,  
CA (US)(\*) **Notice:** Subject to any disclaimer, the term of this  
patent is extended or adjusted under 35  
U.S.C. 154(b) by 0 days.(21) **Appl. No.:** 08/146,206(22) **PCT Filed:** Jun. 15, 1992(86) **PCT No.:** PCT/US92/05126§ 371 (c)(1),  
(2), (4) **Date:** Nov. 17, 1993**Related U.S. Application Data**(63) **Continuation-in-part of application No. 07/715,272, filed on**  
Jun. 14, 1991, now abandoned.(51) **Int. Cl.<sup>7</sup>** ..... C07K 16/00(52) **U.S. Cl.** ..... 530/387.3; 435/69.6; 435/69.7;  
435/70.21; 435/91; 536/23.53; 424/133.1(58) **Field of Search** ..... 435/69.6, 69.7,  
435/70.21, 91, 172.2, 240.1, 240.27, 252.3,  
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(57) **ABSTRACT**

Variant immunoglobulins, particularly humanized antibody polypeptides are provided, along with methods for their preparation and use. Consensus immunoglobulin sequences and structural models are also provided.

**82 Claims, 9 Drawing Sheets**