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GENENTECH, INC.
460 Point San Bruno Boulevard, South San Francisco, CA 94080
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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:

1. The papers required for filing date under CFR §1.53(b):
   106 Pages of specification (including claims); 5 Sheets of drawings (_ formal / x informal)
2. x Declaration/Oath/Power of Attorney
3. __ Assignment of the invention to GENENTECH, INC.
4. 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.
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10. __ Information Disclosure Statement
11. x Return Receipt Postcard

By: Carolyn R. Adler
Name: Carolyn R. Adler
Registration No. 32,324

Dated June 14, 1991

FIGURE 1A: V_L DOMAIN

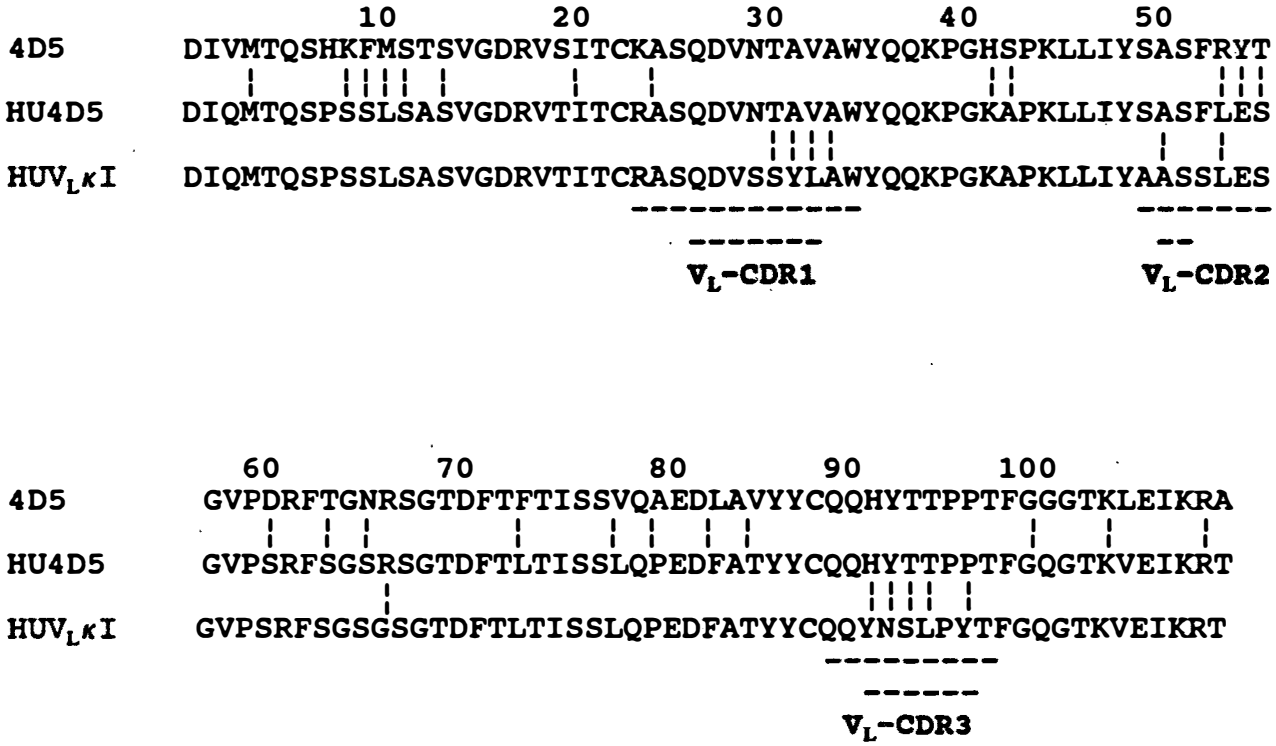


FIGURE 1B: V_H DOMAIN

```
                  10        20        30        40        50  A
4D5       EVQLQQSGPELVKPGASLKLSCTASGFNIKDTYIHWVKQRPEQGLEWIGRIYPTN
          || || | | | | |                | | || || |
HU4D5     EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTN
                                  ||| |||| | ||||
HUV_HIII  EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYAMSWVRQAPGKGLEWVAVISENG
                                  ----------  ------
                                  ---------   ----
                                  V_H-CDR1    V_H-CDR2

              60        70        80  ABC       90        100ABC
4D5       GYTRYDPKFQDKATITADTSSNTAYLQVSRLTSEDTAVYYCSRWGGDGFYAMDYW
          |||||||| | | ||| ||                          |
HU4D5     GYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDVW
          || |          | |   |                | || ||||||
HUV_HIII  SDTYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCARDRGGAVSYFDVW
          -----------                           -----------
                                                ---------
                                                V_H-CDR3

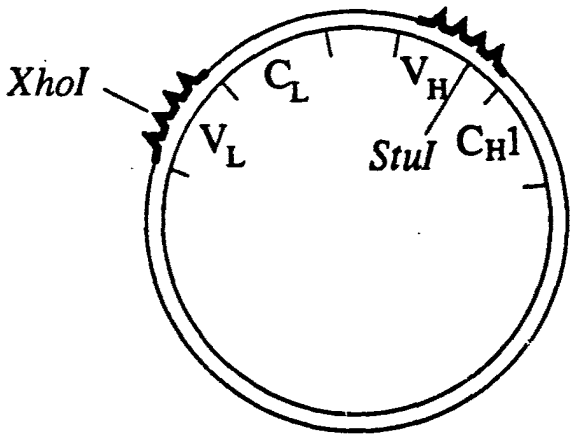
              110
4D5       GQGASVTVSS
             ||
HU4D5     GQGTLVTVSS

HUV_HIII  GQGTLVTVSS
```

FIGURE 2

Anneal huV_L or huV_H oligomers to pAK1 template

1. Ligate
2. Isolate assembled oligomers
3. Anneal to pAK1 template (*XhoI*$^-$, *StuI*$^+$)
4. Extend and ligate



1. Transform *E. coli*
2. Isolate phagemid pool
3. Enrich for huV_L and huV_H(*Xho I*$^+$, *StuI*$^-$)
4. Sequence verify

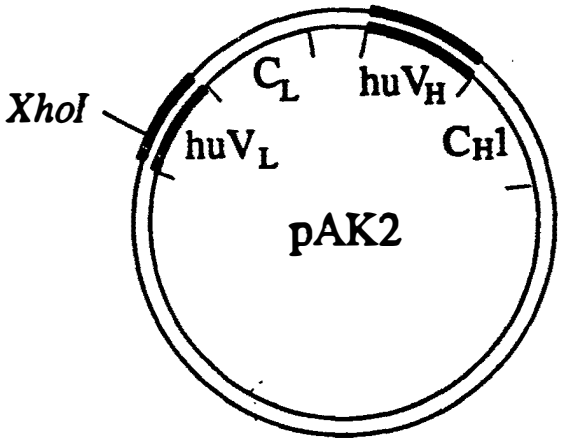
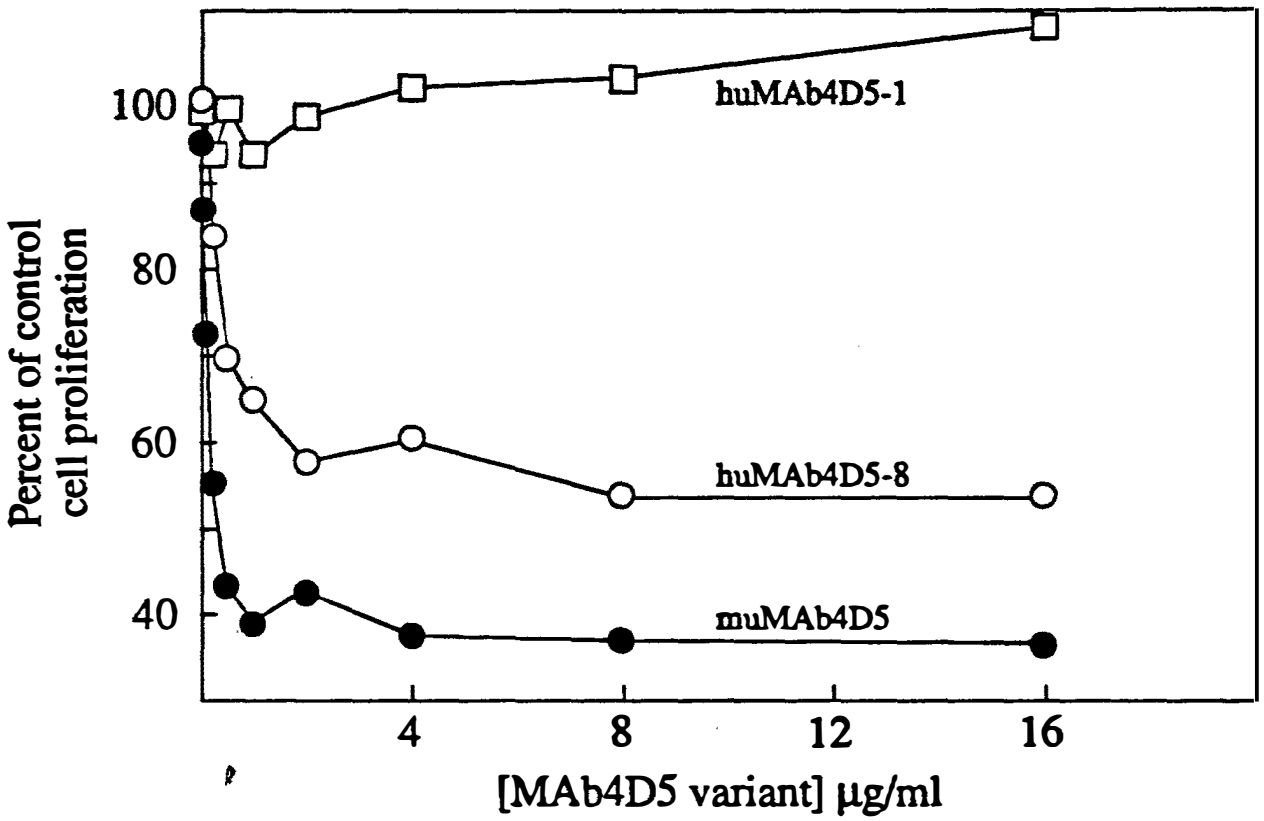


FIGURE 3


Percent of control cell proliferation
100
80
60
40
huMAb4D5-1
huMAb4D5-8
muMAb4D5
4
8
12
16
[MAb4D5 variant] µg/ml

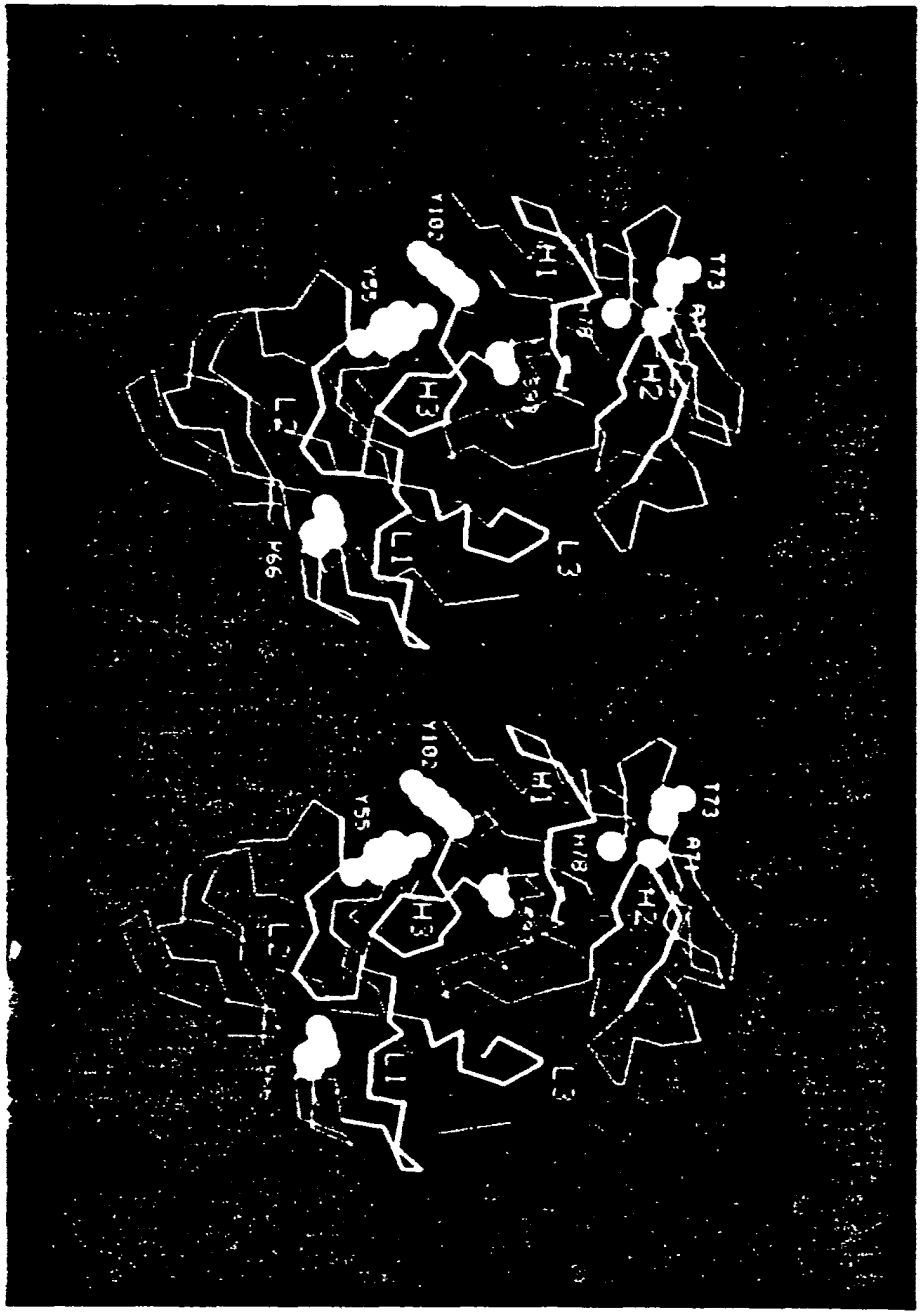

H1
H2
H3
L1
L2
L3
T23
Y102
Y55
H66
H1
H2
H3
L1
L2
L3
T23
Y102
Y55
H66

FIGURE 4

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IMMUNOGLOBULIN VARIANTS

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Field of the Invention

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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.

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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 25 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 30 chain. Particular amino acid residues are believed to form an interface between the light and heavy chain variable domains, see e.g. Chothia *et al.*, *J. Mol. Biol.* 186:651-663 (1985); Novotny and Haber, *Proc. Natl. Acad. Sci.*

USA 82:4592-4596 (1985).

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

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

The art has attempted to overcome this problem by constructing "chimeric" antibodies in which an animal antigen-binding variable domain is coupled to a human constant domain (Cabilly *et al.*, U.S. 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 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).

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

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

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

The three-dimensional structure of immunoglobulin chains has been studied, and crystal structures for intact immunoglobulins, for a variety of immunoglobulin fragments, and for antibody-antigen complexes have been published (see e.g., Saul *et al.*, *Journal of Biological Chemistry* 25:585-97 (1978); Sheriff *et al.*, *Proc. Natl. Acad. Sci. USA* 84:8075-79 (1987); Segal *et al.*, *Proc. Natl. Acad. Sci. USA* 71:4298-4302 (1974); Epp *et al.*, *Biochemistry* 14(22):4943-4952 (1975); Marquart *et al.*, *J. Mol. Biol.* 141:369-391 (1980); Furey *et al.*, *J. Mol. Biol.* 167:661-692 (1983); Snow and Amzel, *Protein: Structure, Function, and Genetics* 1:267-279, Alan R. Liss, Inc. pubs. (1986); Chothia and Lesk, *J. Mol. 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 ($p185^{HER2}$) that is related to and somewhat homologous to the human epidermal growth factor receptor

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

The murine monoclonal antibody known as muMAb4D5 (Fendly, B. M. *et al.*, *Cancer Res.* **50**:1550-1558 (1990)), directed against the extracellular domain (ECD) of $p185^{HER2}$, specifically inhibits the growth of tumor cell lines overexpressing $p185^{HER2}$ in monolayer culture or in soft agar (Hudziak, R. M. *et al.*, *Molec. Cell. Biol.* **9**:1165-1172 (1989); Lupu, R. *et al.*, *Science* **249**:1552-1555 (1990)). MuMAb4D5 also has the potential of enhancing tumor cell sensitivity to tumor necrosis factor, an important effector molecule in macrophage-mediated tumor cell cytotoxicity (Hudziak, *supra*, 1989; Shepard, H. M. and Lewis, G. D. *J. Clinical Immunology* **8**:333-395 (1988)). Thus muMAb4D5 has potential for clinical intervention in and imaging of carcinomas in which $p185^{HER2}$ is overexpressed. The muMAb4D5 and its uses are described in 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.

It is another object of this invention to provide humanized antibodies capable of binding $p185^{HER2}$.

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

Summary of the Invention

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

a. obtaining the amino acid sequences of at least a portion of an import antibody variable domain and of a consensus human variable domain;

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

c. substituting an import CDR amino acid sequence for the corresponding human CDR amino acid sequence;

d. aligning the amino acid sequences of a Framework Region (FR) of the import antibody and the corresponding FR of the consensus antibody;

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

f. determining if the non-homologous import amino acid residue is reasonably expected to have at least one of the following effects:

1. non-covalently binds antigen directly,

2. interacts with a CDR; or

3. participates in the V_L - V_H interface; and

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

Optionally, 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:

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP
KLLIYSASFLESGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHY
TTPPTFGQGTKVEIKRT

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

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLE
WVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDT
AVYYCSRWGGDGFYAMDVWGQGTLVTVSS

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:

SEQ. ID NO. 3 (light chain):
DIQMTQSPSSLSASVGDRVTITCRASQDVSSYLAWYQQKPGKAPK
LLIYAASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYN
SLPYTFGQGTKVEIKRT, and

SEQ. ID NO. 4 (heavy chain):
EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYAMSWVRQAPGKG
LEWVAVISENGGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAE
DTAVYYCSRWGGDGFYAMDVWGQGTLVTVSS

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.

The mismatches between sequences are shown by the vertical lines.

FIGURE 2 shows a scheme for humanization of muMAb4D5 V_L and V_H by gene conversion mutagenesis.

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).

FIGURE 4 shows a stereo view of α-carbon tracing for model of huMAb4D5-8 V_L and V_H. The CDR residues (Kabat, E. A. *et al., Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)) are shown in bold and side chains of V_H residues A71, T73, A78, S93, Y102 and V_L residues Y55 plus R66 (see Table 1) are shown.

Detailed Description of the Invention

Definitions

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

The murine monoclonal antibody known as muMAb4D5 (Fendly, B. M. *et al., Cancer Res.* 50:1550-1558 (1990)) is directed against the extracellular domain (ECD) of $p185^{HER2}$. The muMAb4D5 and its uses are described in 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.

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.

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.

The humanized antibody will be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including IgG1, IgG2, IgG3 and IgG4. Usually the constant domain is a complement fixing constant domain where it is desired that the humanized antibody exhibit cytotoxic activity, and the class is typically IgG_1. Where such cytotoxic activity is not desirable, the constant domain may be of the IgG_2 class. The humanized antibody may comprise sequences from more than one class or isotype, and selecting particular constant domains to optimize desired effector functions is within the ordinary skill in the art.

The FR and CDR regions of the humanized antibody need not correspond precisely to the parental sequences, e.g., the import CDR or the consensus FR may be mutagenized by substitution, insertion or deletion of 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 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, Cα, C, O, Cβ) by more than about 0.2 Angstroms. Backbone atoms of CDR sequences are displaced for example by residues that interrupt or modify organized structures such as beta sheets, helices or loops. Residues that can exert a profound affect on the conformation of neighboring sequences include proline and glycine, both of which are capable of introducing bends into the backbone. Other residues that can displace backbone atoms are those that are capable of participating in salt bridges and hydrogen bonds.

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

Immunoglobulin residues that affect the interface between heavy and light chain variable regions ("the V_L - V_H interface") are those that affect the proximity or orientation of the two chains with respect to one another.

Certain residues involved in interchain interactions are already known and include V_L residues 34, 36, 38, 44, 46, 87, 89, 91, 96, and 98 and V_H residues 35, 37, 39, 45, 47, 91, 93, 95, 100, and 103 (utilizing the nomenclature 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 interchain 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_L κ subgroup I and V_H group III. In such preferred embodiments, the V_L consensus domain has the amino acid sequence: DIQMTQSPSSLSASVGDRVTITCRASQDVSSYLAWYQQKPGKAPKLLIYAAS SLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSLPYTFGQGTKVEI KRT (SEQ. ID NO. 3);

the V_H consensus domain has the amino acid sequence: EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYAMSWVRQAPGKGLEWVAVI SENGGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGD GFYAMDVWGQGTLVTVSS (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^{HER2} antibodies are provided. These novel anti-p185^{HER2} antibodies are sometimes collectively referred to herein as huMAb4D5, and also sometimes as the light or heavy chain variable domains of huMAb4D5, and are defined herein to be any polypeptide sequence which possesses a biological property of a polypeptide comprising the following polypeptide sequence:

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

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLE WVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDT AVYYCSRWGGDGFYAMDVWGQGTLVTVSS (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 $p185^{HER2}$.

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.

In preferred embodiments, antigenically active huMAb4D5 is a polypeptide that binds with an affinity of at least about 10-9 l/mole to an antibody capable of binding huMAb4D5. Ordinarily the polypeptide binds with an affinity of at least about 10-8 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.

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

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% $NaDodSO_4$ 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 μg of plasmid or DNA fragment is used with about 1-2 units of enzyme in about 20 μl 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).

"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).

"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, 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*.

"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 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*.

"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 μg 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.

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.

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.

The distance from the template Cα to the analogous Cα in each of the superimposed structures is calculated for each residue position. Generally, if all (or nearly all) Cα-Cα distances for a given residue are $\leq$ 1Å, then that position is included in the consensus structure. In some cases the β-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, Cα, C, O and Cβ 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 Cα coordinates are fixed. The side chains of highly conserved residues, such as 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

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_H-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

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_L κ subgroup I and V_H group III, and have the sequences indicated in the definitions above.

While these steps may be taken in different order, typically a structure for the candidate humanized antibody is created by transferring the 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_L-CDR1 K24R, V_L-CDR2 R54L and V_L-CDR2 T56S.

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

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

a. obtaining the amino acid sequences of at least a portion of an import antibody variable domain and of a consensus human variable domain;

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

c. substituting an import CDR amino acid sequence for the corresponding human CDR amino acid sequence;

d. aligning the amino acid sequences of a Framework Region (FR) of the import antibody and the corresponding FR of the consensus antibody;

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

f. determining if the non-homologous import amino acid residue is reasonably expected to have at least one of the following effects:

1. non-covalently binds antigen directly,

2. interacts with a CDR; or

3. participates in the V_L - V_H interface; and

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

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

Additionally, in certain embodiments the corresponding consensus antibody residues identified in step (e) above are selected from the group consisting of 4L, 35L, 36L, 38L, 43L, 44L, 46L, 58L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 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 preferred embodiments, the method of this invention comprises the additional steps of searching either or both of the import, non-human and the consensus variable domain sequences for glycosylation sites, determining if the glycosylation is reasonably expected to be important for the desired antigen binding and biological activity of the antibody (i.e., determining if the glycosylation site binds to antigen or changes a side chain of an amino acid residue that binds to antigen, or if the glycosylation enhances or weakens antigen binding, or is important for maintaining antibody affinity). If the import sequence bears the glycosylation site, it is preferred to substitute that site for the corresponding residues in the consensus human sequence if the glycosylation site is reasonably expected to be important. If only the consensus sequence, and not the import, bears the glycosylation site, it is preferred to eliminate that glycosylation site or substitute therefor the corresponding amino acid residues from the import sequence.

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

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

a. (in the FR of the variable domain of the light chain) 4L, 35L, 36L, 38L, 43L, 44L, 58L, 46L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, or

b. (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

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, $SOCl_2$, or $R^1N = C = NR$, where R and R^1 are different alkyl groups.

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

Animals are typically immunized against the immunogenic conjugates or derivatives by combining 1 mg or 1 μg of conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with 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.

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

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

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

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

in WO 89/02922 published 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.

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

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

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

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

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

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

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

For alteration of the native DNA sequence (to generate amino acid sequence variants, for example), the oligonucleotide is hybridized to the single-stranded template under suitable hybridization conditions. A DNA polymerizing enzyme, usually the Klenow fragment of DNA polymerase 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.

In a specific example of PCR mutagenesis, template plasmid DNA (1 μg) is linearized by digestion with a restriction endonuclease that has a unique recognition site in the plasmid DNA outside of the region to be amplified. Of this material, 100 ng is added to a PCR mixture containing PCR buffer, which contains the four deoxynucleotide tri-phosphates and is included in the GeneAmp® kits (obtained from Perkin-Elmer Cetus, Norwalk, CT and Emeryville, CA), and 25 pmole of each oligonucleotide primer, to a final volume of 50 μl. The reaction mixture is overlayed with 35 μl mineral oil. The reaction is denatured for 5 minutes at 100°C, placed briefly on ice, and then 1 μl *Thermus aquaticus (Taq)* DNA polymerase (5 units/μl, purchased from Perkin-Elmer Cetus, Norwalk, 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.

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

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

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

Insertion of DNA into a Cloning Vehicle

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

(a) Signal Sequence Component

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

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

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

(b) Origin of Replication Component

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

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

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

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

(c) Selection Gene Component

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

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

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

For example, cells transformed with the DHFR selection gene are first identified by culturing all of the transformants in a culture medium that contains methotrexate (Mtx), a competitive antagonist of DHFR. An appropriate host cell when wild-type DHFR is employed is the Chinese hamster ovary (CHO) cell line deficient in DHFR activity, prepared and propagated as described by Urlaub and Chasin, Proc. Natl. Acad. Sci. USA, 77: 4216 [1980]. The transformed cells are then exposed to increased levels of methotrexate. This leads to the synthesis of multiple copies of the DHFR gene, and, concomitantly, multiple copies of other DNA comprising the expression vectors, such as the DNA encoding the target polypeptide. This amplification technique can be used with any otherwise suitable host, e.g., ATCC No. CCL61 CHO-K1, notwithstanding the presence of endogenous DHFR if, for example, a mutant DHFR gene that is highly resistant to Mtx is employed (EP 117,060). Alternatively, host cells (particularly wild-type hosts

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

A suitable selection gene for use in yeast is the *trp*1 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 *trp*1 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, *Leu*2-deficient yeast strains (ATCC 20,622 or 38,626) are complemented by known plasmids bearing the *Leu*2 gene.

(d) Promoter Component

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

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

Promoters suitable for use with prokaryotic hosts include the β-lactamase and lactose promoter systems (Chang *et al.*, Nature, 275: 615 [1978]; and Goeddel *et al.*, Nature, 281: 544 [1979]), alkaline phosphatase, a tryptophan (trp) promoter system (Goeddel, Nucleic Acids Res., 8: 4057 [1980] and EP 36,776) and hybrid promoters such as the 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 β-interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus, Canaani and Berg, Proc. Natl. Acad. Sci. USA, 79: 5166-5170 (1982) on expression of the human interferon β1 gene in cultured mouse and rabbit cells, and Gorman *et al.*, Proc. Natl. 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, α-fetoprotein and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, Nature, 297: 17-18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a

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

(f) Transcription Termination Component

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

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

For analysis to confirm correct sequences in plasmids constructed, the ligation mixtures are used to transform *E. coli* K12 strain 294 (ATCC 31,446) and successful transformants selected by ampicillin or tetracycline resistance where appropriate. Plasmids from the transformants are prepared, analyzed by restriction endonuclease digestion, and/or sequenced 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* χ1776 (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* [Louvencourt *et al.*, J. Bacteriol., 737 (1983)], *K. fragilis, K. bulgaricus, K. thermotolerans*, and *K. marxianus, yarrowia* [EP 402,226], *Pichia pastoris* [EP 183,070; Sreekrishna *et al.*, J. Basic Microbiol., 28: 265-278 (1988)], *Candida, Trichoderma reesia* [EP 244,234], *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, $CaPO_4$ 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*.

The mammalian host cells used to produce the target polypeptide of this invention may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ([MEM], Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ([DMEM], Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham and Wallace, Meth. Enz., 58: 44 (1979), Barnes and Sato, Anal. Biochem., 102: 255 (1980), U.S. 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 these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleosides (such as adenosine and thymidine), antibiotics (such as Gentamycin™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

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

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

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

Detecting Gene Amplification/Expression

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

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

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

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

Purification of The Target polypeptide

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

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

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

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

Covalent Modifications of Target Polypeptides

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

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

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

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

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

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

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

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

and glutamyl residues are converted to asparaginyl and glutaminyl residues by reaction with ammonium ions.

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

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

Other modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the α-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]).

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

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

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

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

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

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

Diagnostic and Related Uses of the Antibodies

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

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

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

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

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

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

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

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

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

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

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

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

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

In a further embodiment toxin-conjugates are made with Fab or $F(ab')_2$ fragments. Because of their relatively small size these fragments can better penetrate tissue to reach infected cells.

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

Antibody Dependent Cellular Cytotoxicity

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

Biological activity of antibodies is known to be determined, to a large extent, by the constant domains or Fc region of the antibody molecule (Uananue and Benacerraf, *Textbook of Immunology*, 2nd Edition, Williams & Wilkins, p. 218 (1984)). This includes their ability to activate complement and to mediate antibody-dependent cellular cytotoxicity (ADCC) as effected by leukocytes. Antibodies of different classes and subclasses differ in this respect, as do antibodies from the same subclass but different species; according to the present invention, antibodies of those classes having the desired biological activity are prepared. Preparation of these antibodies involves the selection of antibody constant domains are their incorporation in the humanized antibody by known technique. For example, mouse immunoglobulins of the IgG3 and IgG2a class are capable of activating serum complement upon binding to the target cells which express the cognate antigen, and therefore humanized antibodies which incorporate IgG3 and 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 $p185^{HER2}$ ECD and anti-proliferative activity against $p185^{HER2}$ overexpressing carcinoma cells.

MATERIALS and METHODS

Cloning of Variable Region Genes. The muMAb4D5 V_H and V_L genes were isolated by polymerase chain reaction (PCR) amplification of mRNA from the corresponding hybridoma (Fendly, B. M. *et al., Cancer Res.* 50:1550-1558 (1990)) as described by Orlandi et al. (Orlandi, R. *et al., Proc. Natl. Acad. Sci. USA* 86:3833-3837 (1989)). Amino terminal sequencing of muMAb4D5 V_L and V_H was used to design the sense strand PCR primers, whereas the anti-sense PCR primers were based upon consensus sequences of murine framework residues (Orlandi, R. *et al., Proc. Natl. Acad. Sci. USA* 86:3833-3837 (1989); Kabat, E. A. *et al., Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)) incorporating restriction sites for directional cloning shown by underlining and listed after the sequences: V_L sense, 5'-TCCGATATCCAGCTGACCCAGTCTCCA-3' (SEQ. ID NO. 7), *Eco*RV; V_L anti-sense, 5'-GTTTGATCTCCAGCTTGGTACCHSCDCCGAA-3' (SEQ. ID NO. 8), *Asp*718; V_H sense, 5'-AGGTSMARCTGCAGSAGTCWGG-3' (SEQ. ID NO. 9), *Pst*I and V_H anti-sense, 5'-TGAGGAGACGGTGACCGTGGTCCCTTGGCCCCAG-3' (SEQ. ID NO. 10), *Bst*EII; where H = A or C or T, S = C or G, D = A or G or T, M = A or C, R = A or G and W = A or T. The PCR products were cloned into pUC119

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

Molecular Modelling. Models for muMAb4D5 V_H and V_L domains were constructed separately from consensus coordinates based upon seven Fab structures from the Brookhaven protein data bank (entries 1FB4, 2RHE, 2MCP, 3FAB, 1FBJ, 2HFL and 1REI). The Fab fragment KOL (Marquart, M. *et al.*, *J. Mol. Biol.* **141**:369-391 (1980)) was first chosen as a template for V_L and V_H domains and additional structures were then superimposed upon this structure using their main chain atom coordinates (INSIGHT program, Biosym Technologies). The distance from the template Cα to the analogous Cα in each of the superimposed structures was calculated for each residue position. If all (or nearly all) Cα-Cα distances for a given residue were $\leq$ 1Å, then that position was included in the consensus structure. In most cases the β-sheet framework residues satisfied these criteria whereas the CDR loops did not. For each of these selected residues the average coordinates for individual N, Cα, C, O and Cβ atoms were calculated and then corrected for resultant deviations from non-standard bond geometry by 50 cycles of energy minimization using the DISCOVER program (Biosym Technologies) with the AMBER forcefield (Weiner, S. J. *et al.*, *J. Amer. Chem. Soc.* **106**:765-784 (1984)) and Cα coordinates fixed. The side chains of highly conserved residues, such as the disulfide-bridged cysteine residues, were then incorporated into the resultant consensus structure. Next the sequences of muMAb4D5 V_L and V_H were incorporated starting with the CDR residues and using the tabulations of CDR conformations from Chothia et al. (Chothia, C. *et al.*, *Nature* **342**:877-883 (1989)) as a guide. Side-chain conformations were chosen on the basis of Fab crystal structures, rotamer libraries (Ponder, J. W. & Richards, F. M., *J. Mol. Biol.* **193**:775-791 (1987)) and packing considerations. Since V_H-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_L κ subgroup I and V_H group III, and a molecular model generated for these sequences using the methods described above. A structure for huMAb4D5 was created by transferring the CDRs from the muMAb4D5 model into the consensus human structure. All huMAb4D5 variants contain human replacements of muMAb4D5 residues at three positions within CDRs as defined by sequence variability (Kabat, E. A. *et al.*, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)) but not as defined by structural variability (Chothia, C. & Lesk, A. M., *J. Mol. Biol.* 196:901-917 (1987)): V_L-CDR1 K24R, V_L-CDR2 R54L and V_L-CDR2 T56S. Differences between muMAb4D5 and the human consensus framework residues (Fig. 1) were individually modeled to investigate their possible influence on CDR conformation and/or binding to the $p185^{HER2}$ ECD.

Construction of Chimeric Genes. Genes encoding chMAb4D5 light and heavy chains were separately assembled in previously described phagemid vectors containing 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_L (Fig. 1A) and REI human κ_1 light chain C_L (Palm, W. & Hilschmann, N., *Z. Physiol. Chem.* **356**:167-191 (1975)) were precisely joined as were genes for muMAb4D5 V_H (Fig. 1B) and human γ1 constant region (Capon, D. J. *et al.*, *Nature* **337**:525-531 (1989)) by simple subcloning (Boyle, A., in *Current Protocols in Molecular Biology*, Chapter 3 (F. A. Ausubel *et al.*, eds., Greene Publishing & Wiley-Interscience, New York, 1990)) and site-directed mutagenesis (Carter, P., in *Mutagenesis: A Practical Approach*, Chapter 1 (IRL Press, Oxford, UK 1991)). The γ1 isotype was chosen as it has been found to be the preferred human isotype for

supporting ADCC and complement dependent cytotoxicity using matched sets of chimeric (Brüggemann, M. *et al., J. Exp. Med.* 166:1351-1361 (1987)) or humanized antibodies (Riechmann, L. *et al., Nature* 332:323-327 (1988)). The PCR-generated V_L and V_H fragments (Fig. 1) were subsequently mutagenized so that they faithfully represent the sequence of muMAb4D5 determined at the protein level: V_H Q1E, V_L V104L and T109A (variants are denoted by the amino acid residue and number followed by the replacement amino acid). The human γ1 constant regions are identical to those reported by Ellison et al. (Ellison, J. W. *et al., Nucleic Acids Res.* 13:4071-4079 (1982)) except for the mutations E359D and M361L (Eu numbering, as in Kabat, E. A. *et al., Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)) which we installed to convert the antibody from the naturally rare A allotype to the much more common non-A allotype (Tramontano, A. *et al., J. Mol. Biol.* 215:175-182 (1990)). This was an attempt to reduce the risk of anti-allotype antibodies interfering with therapy.

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

for 10 min at 14 °C. After electrophoresis on a 6% acrylamide sequencing gel the assembled oligonucleotides were located by autoradiography and recovered by electroelution. The assembled oligonucleotides (~0.3 pmol each) were simultaneously annealed to 0.15 pmol single-stranded deoxyuridine-containing pAK1 prepared according to Kunkel et al. (Kunkel, T. A. *et al., Methods Enzymol.* **154**:367-382 (1987)) in 10 μl 40 mM Tris-HCl (pH 7.5) and 16 mM $MgCl_2$ as above. Heteroduplex DNA was constructed by extending the primers with T7 DNA polymerase and transformed into *E. coli* BMH 71-18 *mut*L as previously described (Carter, P., in *Mutagenesis: A Practical Approach*, Chapter 1 (IRL Press, Oxford, UK 1991)). The resultant phagemid DNA pool was enriched first for huV_L by restriction purification using *Xho*I and then for huV_H by restriction selection using *Stu*I as described in Carter, P., in *Mutagenesis: A Practical Approach*, Chapter 1 (IRL Press, Oxford, UK 1991); and in Wells, J. A. *et al., Phil. Trans. R. Soc. Lond. A* **317**:415-423 (1986). Resultant clones containing both huV_L and huV_H genes were identified by nucleotide sequencing (Sanger, F. *et al., Proc. Natl. Acad. Sci. USA* 74:5463-5467 (1977)) and designated pAK2. Additional humanized variants were generated by site-directed mutagenesis (Carter, P., in *Mutagenesis: A Practical Approach*, Chapter 1 (IRL Press, Oxford, UK 1991)). The muMAb4D5 V_L and V_H gene segments in the transient expression vectors described above were then precisely replaced with their humanized versions.

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

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

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

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

RESULTS

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

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

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

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

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

antibody binds the $p185^{HER2}$ ECD 3-fold *more* tightly than does muMAb4D5 itself (Table 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 $p185^{HER2}$ ECD 80-fold *less* tightly than does the murine antibody and has no detectable anti-proliferative activity at the highest antibody concentration investigated (16 μg/ml).

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

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

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

From molecular modeling it appears that the tyrosyl side chain of muMAb4D5 V_L residue 55 may either stabilize the conformation of V_H-CDR3 or provide an interaction at the V_L-V_H interface. The latter function may be dependent upon the presence of V_H Y102. In the context of huMAb4D5-5 the mutations V_L E55Y (huMAb4D5-6) and V_H V102Y (huMAb4D5-7) individually increase the affinity for $p185^{HER2}$ ECD by 5-fold and 2-fold respectively, whereas together (huMAb4D5-8) they increase the affinity by 11-fold. This is consistent with either proposed role of V_L Y55 and V_H Y102.

Secondary Immune Function of huMAb4D5-8. MuMAb4D5 inhibits the growth of human breast tumor cells which overexpress $p185^{HER2}$ (Hudziak, R. M. *et al., Molec. Cell. Biol.* **9**:1165-1172 (1989)). The antibody, however, does not offer the possibility of direct tumor cytotoxic effects. This possibility does arise in huMAb4D5-8 as a result of its high affinity (K_d = 0.1 μM) and its human IgG_1 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 $p185^{HER2}$ and on SK-BR-3, which expresses a high level of $p185^{HER2}$. The results demonstrate that: (1) huMAb4D5 has a greatly enhanced ability to carry out ADCC as compared with its murine parent; and (2) that this activity may be selective for cell types which overexpress $p185^{HER2}$.

DISCUSSION

MuMAb4D5 is potentially useful for human therapy since it is cytostatic towards human breast and ovarian tumor lines overexpressing the *HER2*-encoded $p185^{HER2}$ receptor-like tyrosine kinase. Since both breast and ovarian carcinomas are chronic diseases it is anticipated that the optimal MAb4D5 variant molecule for therapy will have low immunogenicity and will be cytotoxic rather than solely cytostatic in effect. Humanization of muMAb4D5 should accomplish these goals. We have identified 5 different huMAb4D5 variants which bind tightly to $p185^{HER2}$ ECD ($K_d \leq$ 1 nM) and which have significant anti-proliferative activity (Table 1). Furthermore

huMAb4D5-8 but not muMAb4D5 mediates ADCC against human tumor cell lines overexpressing $p185^{HER2}$ 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)).

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

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

slightly less potent in blocking the proliferation of SK-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.

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

Table 1. $p185^{HER2}$ ECD binding affinity and anti-proliferative activities of MAb4D5 variants

	V_H Residue*					V_L Residue*			
MAb4D5 Variant	71 FR3	73 FR3	78 FR3	93 FR3	102 CDR3	55 CDR2	66 FR3	K_d† nM	Relative cell proliferation‡
huMAb4D5-1	R	D	L	A	V	E	G	103	82
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 $p185^{HER2}$ ECD were determined using the method of Friguet *et al.* (43) and the standard error of each estimate is ≤ ± 10%.

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

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

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

		WI-38*		SK-BR-3	
Effector:Target ratio†		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 $p185^{HER2}$ (0.6 pg per μg cell protein) and SK-BR-3 expresses a high level of $p185^{HER2}$ (64 pg $p185^{HER2}$ per μg cell protein), as determined by ELISA (Fendly *et al., J. Biol. Resp. Mod.* **9**:449-455 (1990)).

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

‡ Monoclonal antibody concentrations used were 0.1 μg/ml (A) and 0.1 μg/ml (B).

EXAMPLE 2. Schematic Method for Humanizing an Antibody Sequence

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

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

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

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

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

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

import residue.

7. Search the import sequence, the consensus sequence and the humanized sequence for glycosylation sites outside the CDRs, and determine if this glycosylation site is likely to have any affect on antigen binding and/or biological activity. If no effect is likely, use the human sequence at that site; if some affect is likely, eliminate the glycosylation site or use the import sequence at that site.

8. After completing the above analysis, determine the planned humanized sequence and prepare and test a sample. If the sample does not bind well to the target antigen, examine the particular residues listed below, regardless of the question of residue identity between the import and humanized residues.

   a. Examine particular peripheral (non-CDR) variable domain residues that may, due to their position, possibly interact directly with a macromolecular antigen, including the following residues (where the * indicates residues which have been found to interact with antigen based on crystal structures):

      i. Variable light domain: 36, 46, **49***, 63-70

      ii. Variable heavy domain: 2, **47***, 68, 70, 73-76.

   b. Examine particular variable domain residues which could interact with, or otherwise affect, the conformation of variable domain CDRs, including the following (not including CDR residues themselves, since it is assumed that, because the CDRs interact with one another, any residue in one CDR could potentially affect the conformation of another CDR residue) (L = LIGHT, H = HEAVY, residues appearing in **bold** are indicated to be structurally important according the Chothia *et al.*, Nature 342:877 (1989), and residues appearing in *italic* were altered during humanization by Queen *et al.* (PDL), Proc. Natl. Acad. Sci. USA 86:10029 (1989) and Proc. Natl. Acad. Sci. USA 88:2869 (1991).):

      i. Variable light domain:

a) CDR-1 (residues 24L-34L): **2L**, 4L, 66L-69L, **71L**

b) CDR-2 (residues 50L-56L): 35L, 46L, ***47L***, **48L**, ***49L***, 58L, 62L, **64L**-66L, 71L, 73L

c) CDR-3 (residues 89L-97L): 2L, 4L, 36L, 98L, 37H, 45H, 47H, 58H, 60H

ii. Variable heavy domain:

a) CDR-1 (residues 26H-35H): 2H, 4H, 24H, **36H**, 71H, 73H, 76H, **78H**, 92H, ***94H***

b) CDR-2 (residues 50H-55H): 49H, 69H, 69H, **71H**, 73H, 78H

c) CDR-3 (residues 95H-102H): examine all residues as possible interaction partners with this loop, because this loop varies in size and conformation much more than the other CDRs.

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

SEQUENCE LISTING

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(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: patin (Genentech)

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn
20 25 30

Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
35 40 45

Leu Leu Ile Tyr Ser Ala Ser Phe Leu Glu Ser Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile
65 70 75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
80 85 90

His Tyr Thr Thr Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu
95 100 105

Ile Lys Arg Thr
109

(2) INFORMATION FOR SEQ ID NO:2:

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

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys
20 25 30

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

Glu Trp Val Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr

50 55 60

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser
65 70 75

Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

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

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

(2) INFORMATION FOR SEQ ID NO:3:

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

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

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

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser
20 25 30

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

Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
65 70 75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
80 85 90

Tyr Asn Ser Leu Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu
95 100 105

Ile Lys Arg Thr
109

(2) INFORMATION FOR SEQ ID NO:4:

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

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

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
20 25 30

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

Glu Trp Val Ala Val Ile Ser Glu Asn Gly Gly Tyr Thr Arg Tyr
50 55 60

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser
65 70 75

Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

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

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

(2) INFORMATION FOR SEQ ID NO:5:

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

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

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

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

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

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

Arg Phe Thr Gly Asn Arg Ser Gly Thr Asp Phe Thr Phe Thr Ile
65 70 75

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

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

Ile Lys Arg Ala
109

(2) INFORMATION FOR SEQ ID NO:6:

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

(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 CCXXCDCCGA 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:

AGGTXXAXCT 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:

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

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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.

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
KLLIYSASFLESGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHY
TTPPTFGQGTKVEIKRT

13. A polypeptide comprising the sequence:
EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLE
WVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDT
AVYYCSRWGGDGFYAMDVWGQGTLVTVSS

14. A computer comprising the sequence data of the following amino acid sequence:
    a. DIQMTQSPSSLSASVGDRVTITCRASQDVSSYLAWYQQ
    KPGKAPKLLIYAASSLESGVPSRFSGSGSGTDFTLTISSLQ
    PEDFATYYCQQYNSLPYTFGQGTKVEIKRT, or
    b. EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYAMSWVR
    QAPGKGLEWVAVISENGGYTRYADSVKGRFTISADTSKN
    TAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDVWGQG
    TLVTVSS

15. A computer representation of the following amino acid sequence:
    a. DIQMTQSPSSLSASVGDRVTITCRASQDVSSYLAWYQQ
    KPGKAPKLLIYAASSLESGVPSRFSGSGSGTDFTLTISSLQ
    PEDFATYYCQQYNSLPYTFGQGTKVEIKRT, or
    b. EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYAMSWVR
    QAPGKGLEWVAVISENGGYTRYADSVKGRFTISADTSKN
    TAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDVWGQG

TLVTVSS

16. A method comprising storing a computer representation of the following amino acid sequence:

a. DIQMTQSPSSLSASVGDRVTITCRASQDVSSYLAWYQQ KPGKAPKLLIYAASSLESGVPSRFSGSGSGTDFTLTISSLQ PEDFATYYCQQYNSLPYTFGQGTKVEIKRT, or

b. EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYAMSWVR QAPGKGLEWVAVISENGGYTRYADSVKGRFTISADTSKN TAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDVWGQG TLVTVSS

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.

EXPRESS MAIL NO. B59937585
MAILED: 14 JUNE 1991

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) x 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.

301

Carolyn R. Adler - Reg. No. 32,324
Robert H. Benson - Reg. No. 30,446
Walter E. Buting - Reg. No. 23,092
Ginger R. Dreger - Reg. No. 33,055
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Janet E. Hasak - Reg. No. 28,616
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Dennis G. Kleid - Reg. No. 32,037
Nancy Olseki - Reg. No. 34,688
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Send correspondence to 601 Genentech, Inc.
602 Attn: Carolyn R. Adler
701 460 Point San Bruno Boulevard
702 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 4/8/00

Inventor's signature Date

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Full name of third joint inventor, if any

Third Inventor's signature Date

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BAR CODE LABEL

U.S. PATENT APPLICATION

SERIAL NUMBER	FILING DATE	CLASS	GROUP ART UNIT
07/715,272	06/14/91	530	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	SHEETS DRAWING	TOTAL CLAIMS	INDEPENDENT CLAIMS	FILING FEE RECEIVED	ATTORNEY DOCKET NO.
CA	5	16	8	$1,050.00	709

ADDRESS

GENENTECH, INC.
ATTN: CAROLYN R. ADLER
460 POINT SAN BRUNO BLVD.
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 00192 06/27/91 07715272 07-0630 030 101 930.00CH

PTO-1556
(5/87)

PATENT APPLICATION FEE DETERMINATION RECORD

Application or Docket Number: 715272

CLAIMS AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE	SMALL ENTITY FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY FEE
BASIC FEE				$ 315.00	OR		$ 630.00
TOTAL CLAIMS	16 minus 20 =	*	x $10=		OR	x $20 =	
INDEPENDENT CLAIMS	8 minus 3 =	* 5	x 30 =		OR	x 60 =	300
MULTIPLE DEPENDENT CLAIM PRESENT			+ 100 =		OR	+ 200 =	
			TOTAL		OR	TOTAL	930

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

CLAIMS AS AMENDED - PART II

AMENDMENT A

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE	SMALL ENTITY ADDITIONAL FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY ADDITIONAL FEE
Total	*	Minus	**	=	x $10=		OR	x $20 =	
Independent	*	Minus	***	=	x 30 =		OR	x 60=	
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM					+ 100 =		OR	+ 200 =	
					TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

AMENDMENT B

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE	SMALL ENTITY ADDITIONAL FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY ADDITIONAL FEE
Total	*	Minus	**	=	x $10 =		OR	x $20 =	
Independent	*	Minus	***	=	x 30 =		OR	x 60 =	
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM					+ 100 =		OR	+ 200 =	
					TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

AMENDMENT C

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE	SMALL ENTITY ADDITIONAL FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY ADDITIONAL FEE
Total	*	Minus	**	=	x $10 =		OR	x $20 =	
Independent	*	Minus	***	=	x 30 =		OR	x 60 =	
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM					+ 100 =		OR	+ 200 =	
					TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

* 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.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

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

Applicant

GENENTECH, INC. et al.

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.

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

1. [X] **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.
   - [X] the text has been established by this Authority to read as follows:

   METHOD FOR MAKING HUMANIZED ANTIBODIES.

5. With regard to the **abstract,**
   - [X] 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.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [ ] None of the figures.

Form PCT/ISA/210 (first sheet) (July 1992)

INTERNATIONAL S[redacted]CH REPORT

International application No.
PCT/[redacted]92/05126

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. [X] 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 searches 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.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 92/05126

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N15/13; C12P21/08; C07K13/00; C12N5/10
G06F15/00

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

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[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
Y	JOURNAL OF MOLECULAR BIOLOGY vol. 215, 1990, ACADEMIC PRESS pages 175 - 182 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' cited in the application See the whole document, especially paragraph 7	1-12,15
Y	WO,A,9 007 861 (PROTEIN DESIGN LABS, INC.) 26 July 1990 See pages 1-6; 9-25	1-12,15

-/--

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

2

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.

Form PCT/ISA/210 (second sheet) (January 1985) See notes on accompanying sheet

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 07/10/92
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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

EPO FORM P079

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	NATURE. vol. 342, December 1989, LONDON GB pages 877 - 883 Chothia, Cyrus; Lesk, Arthur M.; Tramontano, Anna; Levitt, Michael; Smith-Gill, Sandra J.; Air, Gillian; Sheriff, Steven; Padlan, 'Conformations of immunoglobulin hypervariable region' cited in the application See the whole document, especially 'Discussion'	1-12,15
P,X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 89, May 1992, WASHINGTON US pages 4285 - 4289 Carter, Paul et al. 'Humanization of an anti-p185HER2 antibody for human cancer therapy.' see the whole document	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

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Form Revision Date: December 8, 2006

NUMBER

FILING DATE	CLASS	SUBCLASS	GROUP ART UNIT	EXAMINER
06/14/91	530	387	~~145~~ 186	Feisee

APPLICANT: ...TER, SAN FRANCISCO, CA; LEONARD G. PRESTA, SAN FRANCISCO, CA.

CONTINUING DATA********
VERIFIED
None

FOREIGN/PCT APPLICATIONS********
VERIFIED
None

FOREIGN FILING LICENSE GRANTED 08/03/91

Foreign priority claimed / 35 USC 119 conditions met / Verified and Acknowledged (Examiner's Initials)	AS FILED	STATE OR COUNTRY	SHEETS DRWGS.	TOTAL CLAIMS	INDEP. CLAIMS	FILING FEE RECEIVED	ATTORNEY'S DOCKET NO.
☐ yes ☒ no / ☐ yes ☐ no	→	CA	5	16	8	$1,050.00	709

ADDRESS:
GENENTECH, INC.
ATTN: CAROLYN R. ADLER
460 POINT SAN BRUNO BLVD.
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		DRAWING		
Amount Due	Date Paid	Sheets	Figs. Drwg.	Print Fig.
			1	

Primary Examiner

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	9/29/92	VF
530	350	↓	↓
updated		4/29/93	VR
updated		10/15/93	VF
updated		1/10/94	VF

INTERFERENCE SEARCHED

Class	Sub.	Date	Exmr.

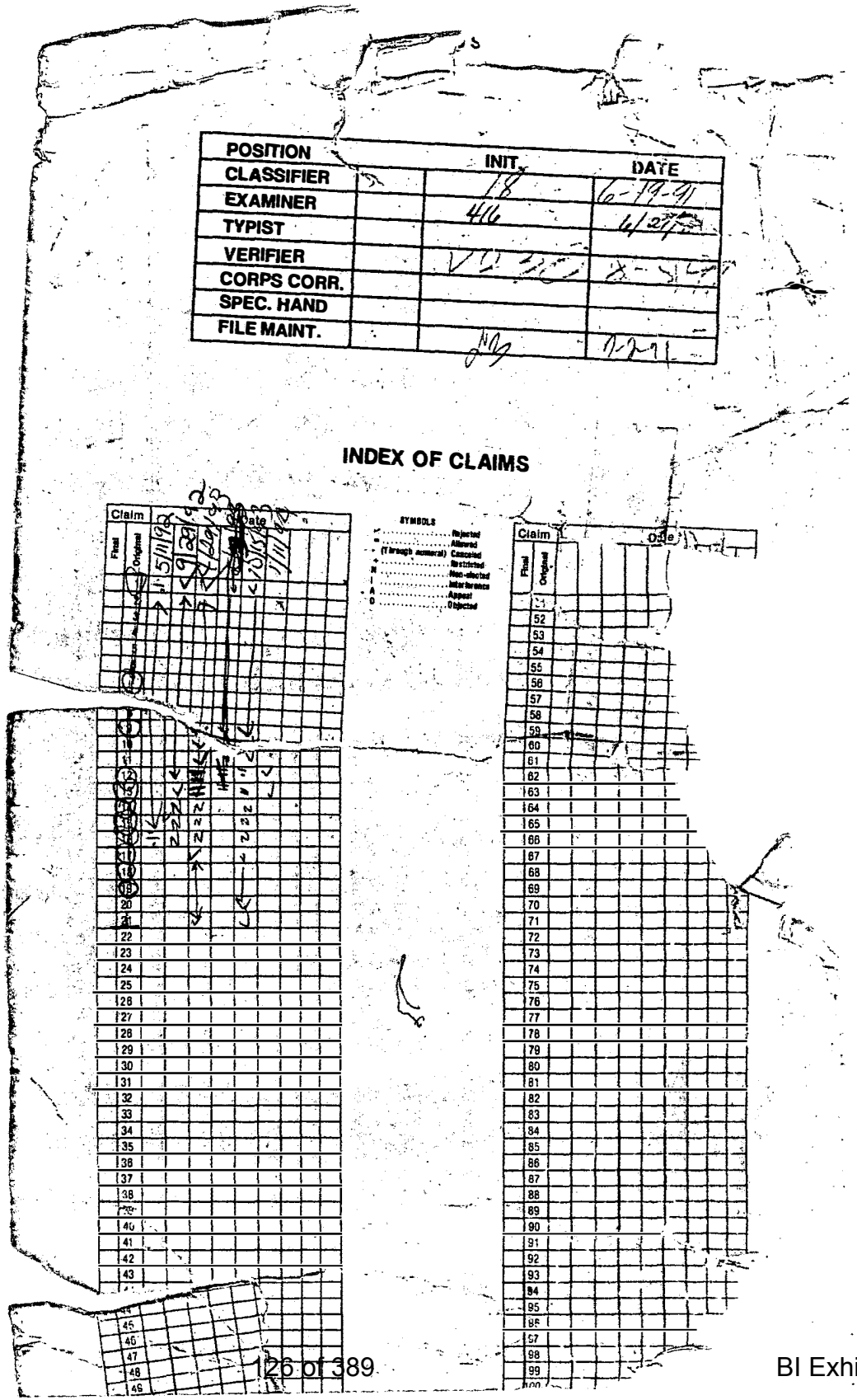
SEARCH NOTES

33. Request for access 6-21-02
34. Request for access
35. Request for access 10-18-02
36. Request for access 11-1-02
37. Request for access 12/18/02
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 2/4/03
42). Request for access 3/9/04
43). Request for access 2-04
44). Request for access
45). Request for access 6-04
46). Request for access 9/04
47). Request for access 8-16-04
48). Request for access
49. Request for access 11-04
50. Request for access 1-17-05
51. Request for access 1-21-06
52. Request for access 4-15-05
53. Request for access
59. Request for access 8/2/06

Dept.	4/29/93	VF
APS updated	10/15/93	VF
updated	1/10/94	VF

turn over please

BI Exhibit 1094



POSITION		INIT.	DATE
CLASSIFIER		JB	6-19-91
EXAMINER		416	6/27
TYPIST			
VERIFIER		VC	
CORPS CORR.			
SPEC. HAND			
FILE MAINT.		JMS	7-2-91

INDEX OF CLAIMS

#2

SEQUENCE LISTING

(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:

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

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn
20 25 30

Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
35 40 45

Leu Leu Ile Tyr Ser Ala Ser Phe Leu Glu Ser Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile
65 70 75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
80 85 90

His Tyr Thr Thr Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu
95 100 105

Ile Lys Arg Thr
109

(2) INFORMATION FOR SEQ ID NO:2:

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

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys
20 25 30

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

Glu Trp Val Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr
50 55 60

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser
65 70 75

Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

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

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

(2) INFORMATION FOR SEQ ID NO:3:

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

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

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

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser
20 25 30

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

Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
65 70 75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
80 85 90

Tyr Asn Ser Leu Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu
95 100 105

Ile Lys Arg Thr
109

(2) INFORMATION FOR SEQ ID NO:4:

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

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

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
20 25 30

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

Glu Trp Val Ala Val Ile Ser Glu Asn Gly Gly Tyr Thr Arg Tyr
                  50                  55                  60

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser
                  65                  70                  75

Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                  80                  85                  90

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

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


(2) INFORMATION FOR SEQ ID NO:5:

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

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

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

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

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

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

Arg Phe Thr Gly Asn Arg Ser Gly Thr Asp Phe Thr Phe Thr Ile
                  65                  70                  75

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

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

Ile Lys Arg Ala
             109

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

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

(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 CCXXCDCCGA A 31

X's are not valid according to the rule.

(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:

AGGTXXAXCT 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

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

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

LINE	ERROR	ORIGINAL TEXT
272	Wrong Nucleic Acid Designator	GTTTGATCTC CAGCTTGGTA CCXXCDCCGA A 31
269	Entered and Calc. Seq. Length differ	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
287	Wrong Nucleic Acid Designator	AGGTXXAXCT GCAGXAGTCX GG 22
284	Entered and Calc. Seq. Length differ	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

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

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

MANDATORY IDENTIFIER THAT WAS NOT FOUND

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

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

#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 filed under § 1.825(d). Specifically: _____

Other: _____

GIVEN ONE MONTH FROM THE DATE OF THIS LETTER WITHIN WHICH TO CORRECT 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(a). Any inquiry concerning the response to, and any questions about, this notice to the undersigned. A copy of this notice must be returned with your response.

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

APPLICATION NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTY DOCKET NO/TITLE
07/715,272	06/14/91	CARTER	P 709

#4

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

000

DATE MAILED: 07/03/91

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 RERQUIRED 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 surchage 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~~ 308-1202

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	P 709

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

000

DATE MAILED: 07/03/91

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 RERQUIRED FOR THIS ITEM.

8. ☐ A $50.00 processing fee is required for returned checks. (37 CFR 1.21(m)).

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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 surchage 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-

DS20157 07/12/91 715272 07 0630 020 105 120.00CR

FORM PTO-1533 (REV. 6-90) COPY TO BE RETURNED WITH RESPONSE

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
Reg. No. 32,324

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

Date: 9 July 1991

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:

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. 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 601 Genentech, Inc.
602 Attn: Carolyn R. Adler
701 460 Point San Bruno Boulevard
702 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 40100 Paul John Carter 18th June 1991

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 40208

Second Inventor's signature Date

Leonard G. Presta 6-19-91

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.
Attn: Carolyn K. Adler
460 Point San Bruno Blvd.
South San Francisco, CA 94080

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(e) 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 respons .

☑ C M Lawrence
For Manager, Application Processing Division

☐

SEQUENCE LISTING

(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: 07/715,272
(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:

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
 1               5                  10                  15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn
            20                  25                  30

Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
        35                  40                  45

Leu Leu Ile Tyr Ser Ala Ser Phe Leu Glu Ser Gly Val Pro Ser
    50                  55                  60
```

Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile
65 70 75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
80 85 90

His Tyr Thr Thr Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu
95 100 105

Ile Lys Arg Thr
109

(2) INFORMATION FOR SEQ ID NO:2:

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

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys
20 25 30

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

Glu Trp Val Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr
50 55 60

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser
65 70 75

Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

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

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

(2) INFORMATION FOR SEQ ID NO:3:

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

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

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

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser
20 25 30

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

Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
65 70 75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
80 85 90

Tyr Asn Ser Leu Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu
95 100 105

Ile Lys Arg Thr
109

(2) INFORMATION FOR SEQ ID NO:4:

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

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

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
20 25 30

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

Glu Trp Val Ala Val Ile Ser Glu Asn Gly Gly Tyr Thr Arg Tyr
50 55 60

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser
65 70 75

Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

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

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

(2) INFORMATION FOR SEQ ID NO:5:

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

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

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

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

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

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

Arg Phe Thr Gly Asn Arg Ser Gly Thr Asp Phe Thr Phe Thr Ile
65 70 75

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

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

Ile Lys Arg Ala
109

(2) INFORMATION FOR SEQ ID NO:6:

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

(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

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
Louise Strasbaugh
Signature of Depositing Party
July 12, 1991
Date of Signature

RESPONSE AND PRELIMINARY AMENDMENT

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

SEQUENCE LISTING

(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: 07/715,272
(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:

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

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn
20 25 30

Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
35 40 45

Leu Leu Ile Tyr Ser Ala Ser Phe Leu Glu Ser Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile
65 70 75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
80 85 90

His Tyr Thr Thr Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu
95 100 105

Ile Lys Arg Thr
109

(2) INFORMATION FOR SEQ ID NO:2:

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

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys
20 25 30

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

Glu Trp Val Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr
50 55 60

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser
65 70 75

Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

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

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

(2) INFORMATION FOR SEQ ID NO:3:

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

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

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

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser
20 25 30

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

Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
65 70 75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
80 85 90

Tyr Asn Ser Leu Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu
95 100 105

Ile Lys Arg Thr
109

(2) INFORMATION FOR SEQ ID NO:4:

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

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

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
20 25 30

```
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:
```

(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

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

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

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

LINE ORIGINAL TEXT

CORRECTED TEXT

18M Fersee

186

PATENT DOCKET 708

#18 5-18-92

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

RECEIVED
MAY 0 8 1992
GROUP 180

In re Application of

Paul J. Carter et al.

Serial No. 07/715272

Filed: June 14, 1991

For: Immunoglobulin Variants

Group Art Unit:

Examiner:

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

INFORMATION DISCLOSURE STATEMENT

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

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

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)

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

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

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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)

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

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

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

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

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

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

Respectfully submitted,
GENENTECH, INC.

Carolyn R. Adler
Reg. No. 32,324

Dated: April 30, 1992

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, I

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

ART UNIT	PAPER NUMBER
1806	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~~ restricted ☒ Responsive 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:

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 1-16 are pending in the application.

   Of the above, claims _____ are withdrawn from consideration.

2. ☐ Claims _____ have been cancelled.
3. ☐ Claims _____ are allowed.
4. ☐ Claims _____ are rejected.
5. ☐ Claims _____ are objected to.
6. ☒ Claims 1-16 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 _____, has been ☐ approved; ☐ disapproved (see explanation).
12. ☐ 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 _____.
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; 456 O.G. 213.
14. ☐ Other

EXAMINATION

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

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).

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.

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).

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.

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

PTO-FORM 948
(REV. 5-90)

U.S. DEPARTMENT OF COMMERCE
Patent and Trademark Office

ATTACHMENT TO PAPER NUMBER	9
APPLICATION NUMBER	715222

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

	Paper Size		
Margin	8 1/2 by 14 inches	8 1/2 by 13 inches	DIN size A4 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) Fig 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 4

8. ☐ Other.

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

_____
Reviewing Draftsman

8/6
Date

Elec #12 SRS 7/24/92

PATENT DOCKET 709

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 2 2 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.

10 July 1992

Carolyn R. Adler
Reg. No. 32,324

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

184

18C fee

PATENT DOCKET 709

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#11
Stn
7/24/92

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))

JUL 2 2 1992

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 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

Carolyn R. Adler

LA10099 07/17/92 07715272 07-0630 010 115 110.00CH

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE

ONLINE SEARCH REQUEST FORM

USER [redacted] Feisee SERIAL NUMBER 7/5272

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. [redacted]

[redacted]

See claims 1-13
especially!!

STAFF USE ONLY

9/15/92

Feise 715272

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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   synonyn for CDR
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 (COMPLE-
              MENTARY()DETERMINING OR HYPERVARIABLE)()REGION OR ANTIBODY()R-
              ELATED()BINDING()SITE? ? FROM 5,73,399   synonyms for CDR
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

 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 maurine 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 x 10(6) s-1 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 cytolysis 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

QH506.J67

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

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: FOP
Languages: ENGLISH

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 complementarily-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 complementarily-determing 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...

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

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; Avdalovic 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
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 x 10(9) M-1, 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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SYSTEM:OS  - DIALOG OneSearch
  File351: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

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Executing TD101
HILIGHT set on as '*'
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           2945  ANTIBODIES/TI
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>>>No valid files specified in FROM
>>>File 155 is not open
>>>No valid files specified in FROM
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>>>"S4" does not exist
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      S3       1  HUMANIZ?
      S4      26  HUMANIS?
      S5       0  S4 AND (HUMANIZ? OR HUMANIS?)
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>>>Set "S28" does not exist
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              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
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     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? ?
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                  (COMPLEMENTARY()DETERMING OR HYPERVARIABLE)()REGION OR
                  ANTIBODY()RELATED()BINDING()SITE? ?
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              45  25
     S26       8  7 AND 25
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 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 determn. 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 power 8 M-1, in which
    the complementarity determining regions (*CDR*) and human-type frame
    work 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 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′ and 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; KP ; 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 assciated 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; KP ; 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')2 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; KP; 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. Reynaud7s 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 Picornavididae, 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 comprises 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 derviced 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

26/7/7 (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	

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 numan-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.
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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 (COMPLE-
                MENTARY()DETERMINING OR HYPERVARIABLE)()REGION OR ANTIBODY()R-
                ELATED()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
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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')2 *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')2 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')2 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')2 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 Eiisme...
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

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 clonign 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. 109 M-1, 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

?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

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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
?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) culturirg 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
S10         0   (9 OR 7 OR 2 OR 4) NOT (7 OR 2 OR 4)
?
```

07/715,272 06/11/91

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Patent and Trademark Office

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

SERIAL NUMBER	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.

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

EXAMINER	
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 2/13/92 ☐ 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-1449. 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