Project No.

Book No.

TITLE Humanized 405

From Page No. 23

V_H

```
                1                                                  10
humiii   glu val gln leu val glu ser gly gly gly leu val gln pro gly gly
Hum4D5a
Hum4D5b
Hum4D5c

                        20                                                  30
humiii   ser leu arg leu ser cys ala ala ser | GLY PHE THR PHE SER ASP TYR
Hum4D5a                                      |                ASN ILE LYS     THR
Hum4D5b                                      |                ASN ILE LYS     THR
Hum4D5c                                      |                ASN ILE LYS     THR

                                            40
humiii   ALA MET | SER trp val arg gln ala pro gly lys gly leu glu trp val
Hum4D5a  TYR ILE | HIS
Hum4D5b  TYR ILE | HIS
Hum4D5c  TYR ILE | HIS

              50        52 | 52a  53                                  60
humiii   ala VAL ILE SER | GLU ASN GLY SER | ASP THR TYR TYR ALA ASP SER VAL
Hum4D5a      ARG     TYR | PRO THR ASN GLY | TYR     ARG
Hum4D5b      ARG     TYR | PRO THR ASN GLY | TYR     ARG
Hum4D5c      ARG     TYR | PRO THR ASN GLY | TYR     ARG

                                        70
humiii   LYS GLY arg phe thr ile ser arg asp asp ser lys asn thr leu tyr
Hum4D5a                               ala     thr                 ala
Hum4D5b                               ala     thr                 leu*
Hum4D5c                               ala     thr                 ala

          80      82 82a 82b 82c  83                                  90
humiii   leu gln met asn ser leu arg ala glu asp thr ala val tyr tyr cys
Hum4D5a
Hum4D5b
Hum4D5c

                                                100  a   b   c  101 |
humiii   ala arg ASP | ARG GLY GLY ALA VAL SER TYR GLY ASP | VAL trp gly gln
Hum4D5a  ser     TRP | GLY GLY ASP GLY PHE TYR ALA MET ASP |
Hum4D5b  ser     TRP | GLY GLY ASP GLY PHE TYR ALA MET ASP |
Hum4D5c  ser     TRP | GLY GLY ASP GLY PHE TYR ALA MET ASP | TYR*

humiii   gly thr leu val thr val ser ser
Hum4D5a
Hum4D5b
Hum4D5c
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To Page No. 25

Witnessed & Understood by me, | Date | Invented by Leonard Presta | Date

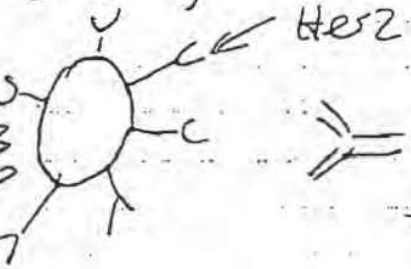
Recorded by Leonard Presta

From Page No.

AIM: To investigate the activity of Hu4D5 for inhibiting the binding of 4D5 to the cell line SKBR3 which over-expresses Her2

PROTOCOL

Assay performed by Jay Sarup + Kathy King



[125I] - 4D5 (0.1nM)

+ Varying amounts of Hu4D5 Fab (2/2 A shockate)

V_{assay} = 400µl

		$\bar{x}$	$\bar{x}$ corrected for non-specific binding (447cpm)	
Total counts	11665, 11567, 11603	11612	11365	
Test buffer	10862, 11473	11168	10921	
(200mM Tris-HCl, pH8.0; 80mM Glycine)				0.1nM
Hu4D5 (200ng)		2482	2235	[125I]-4D5
Hu4D5 (100ng)		4052	3805	

Test buffer	12746, 13147	12947	12700	0.1nM
Hu4D5 (200ng)	9044, 9583	9314	9067	[125I]-6.EQ
Hu4D5 (100ng)	10938, 9918	10428	10181	

another mab which binds her2

Interpretation

- Hu4D5 specifically inhib the interaction of 4D5 with the her2 expressing cell line SKBR3, but does not interfere with the interaction between this cell line and the antibody 6.EQ
- Orders of Magnitude estimate of Kd 10 - 100 fold) above from gel (approx) - estimate that sample contains ~1.6ng Hu4D5 Fab

To Page No.

Witnessed & Understood by me, | Date | Invented by Paul Carter | Date 1

EXHIBIT B

INTERLEUKIN-2–RECEPTOR BLOCKADE WITH DACLIZUMAB TO PREVENT ACUTE REJECTION IN RENAL TRANSPLANTATION

FLAVIO VINCENTI, M.D., ROBERT KIRKMAN, M.D., SUSAN LIGHT, M.D., GINNY BUMGARDNER, M.D., PH.D., MARK PESCOVITZ, M.D., PHILIP HALLORAN, M.D., PH.D., JOHN NEYLAN, M.D., ALAN WILKINSON, M.D., HENRIK EKBERG, M.D., PH.D., ROBERT GASTON, M.D., LARS BACKMAN, M.D., PH.D., AND JAMES BURDICK, M.D., FOR THE DACLIZUMAB TRIPLE THERAPY STUDY GROUP*

ABSTRACT

Background Monoclonal antibodies that block the high-affinity interleukin-2 receptor expressed on alloantigen-reactive T lymphocytes may cause selective immunosuppression. Daclizumab is a genetically engineered human IgG1 monoclonal antibody that binds specifically to the α chain of the interleukin-2 receptor and may thus reduce the risk of rejection after renal transplantation.

Methods We administered daclizumab (1.0 mg per kilogram of body weight) or placebo intravenously before transplantation and once every other week afterward, for a total of five doses, to 260 patients receiving first cadaveric kidney grafts and immunosuppressive therapy with cyclosporine, azathioprine, and prednisone. The patients were followed at regular intervals for 12 months. The primary end point was the incidence of biopsy-confirmed acute rejection within six months after transplantation.

Results Of the 126 patients given daclizumab, 28 (22 percent) had biopsy-confirmed episodes of acute rejection, as compared with 47 of the 134 patients (35 percent) who received placebo ($P=0.03$). Graft survival at 12 months was 95 percent in the daclizumab-treated patients, as compared with 90 percent in the patients given placebo ($P=0.08$). The patients given daclizumab did not have any adverse reactions to the drug, and at six months, there were no significant differences between the two groups with respect to infectious complications or cancers. The serum half-life of daclizumab was 20 days, and its administration resulted in prolonged saturation of interleukin-2α receptors on circulating lymphocytes.

Conclusions Daclizumab reduces the frequency of acute rejection in kidney-transplant recipients. (N Engl J Med 1998;338:161-5.)

ACUTE rejection is a strong risk factor for chronic rejection in recipients of renal grafts from cadaveric donors.[1] This fact has prompted the development of new immunosuppressive agents designed to reduce the incidence and severity of acute rejection.[2-6] All these agents, however, achieve reductions in the frequency and severity of acute rejection at the price of generalized immunosuppression, with its attendant risks of opportunistic infection and cancer.

One potential target for more specific immunosuppressive therapy with monoclonal antibodies is the interleukin-2 receptor.[7] The high-affinity interleukin-2 receptor is composed of three noncovalently bound chains: a 55-kd α chain (also referred to as CD25 or Tac), a 75-kd β chain, and a 64-kd γ chain.[7] This receptor is present on nearly all activated T cells but not on resting T cells. The interaction of interleukin-2 with this high-affinity receptor is required for the clonal expansion and continued viability of activated T cells. A variety of rodent monoclonal antibodies directed against the α chain of the receptor have been used in animals and humans to achieve selective immunosuppression by targeting only T-cell clones responding to the allograft.[8-13] Daclizumab, a molecularly engineered human IgG1 incorporating the antigen-binding regions of the parent murine monoclonal antibody, offers the potential for greater therapeutic use of interleukin-2–receptor blockade.[14-17] We compared the efficacy of daclizumab with placebo for the prevention of acute rejection in renal-transplant recipients.

METHODS

Study Design

We performed a randomized, double-blind, placebo-controlled trial at 11 transplantation centers in the United States, 3 in Canada, and 3 in Sweden. Adults receiving first renal allografts from cadaveric donors were eligible for the study. Patients were excluded if they were receiving multiple organ transplants or had a positive crossmatch for T-cell lymphocytes. The protocol was approved by the institutional review board or ethics committee at each participating center, and all patients gave written informed consent.

Immunosuppressive Treatment

All patients received cyclosporine, azathioprine, and prednisone. The first dose of cyclosporine was given during the period from 12 hours before to 24 hours after transplantation.

Daclizumab (Zenapax, Hoffmann-LaRoche) or placebo was

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*Other members of the Daclizumab Triple Therapy Study Group are listed in the Appendix.

administered intravenously over a period of 15 minutes. Each patient received five doses of either daclizumab (1 mg per kilogram of body weight, to a maximum of 100 mg per dose) or placebo (0.2 mg of polysorbate 80 per milliliter in 67 mM phosphate buffer). The first dose was administered within 24 hours before transplantation, with subsequent doses given two, four, six, and eight weeks after transplantation.

Primary and Secondary End Points

The primary end point of the study was the incidence of biopsy-confirmed acute rejection within the first six months after transplantation. All patients with an unexplained rise in the serum creatinine concentration or one or more symptoms of acute rejection (fever, pain over the graft, or a decrease in urinary volume) were required to undergo a renal biopsy within 24 hours after the initiation of antirejection therapy, which consisted initially of intravenous methylprednisolone (7 mg per kilogram per day) for three days. The histologic diagnosis of rejection was based on the presence of acute tubulitis or vasculitis and was made by the pathologist at each institution. Patients were considered to have presumptive rejection if they received a course of antirejection therapy in the absence of histologic confirmation of rejection. The diagnosis of any subsequent episodes of rejection in patients presenting with renal dysfunction was based on clinical criteria, such as the absence of evidence of nephrotoxicity or of urinary tract obstruction or infection, with a biopsy for confirmation performed at the investigator's discretion.

Secondary end points included patient survival and graft survival at one year, the time to the first episode of acute rejection, the number of acute rejection episodes per patient, the need for antilymphocyte therapy (OKT3 or polyclonal antithymocyte globulin) because of glucocorticoid-resistant rejection (defined as the absence of a response to intravenous methylprednisolone pulse therapy), graft function (as indicated by the serum creatinine concentration and glomerular filtration rate), and the cumulative dose of prednisone in the first six months after transplantation.

Pharmacokinetic Measurements

Blood samples were collected immediately before and after (for trough and peak concentrations, respectively) the first and fifth infusions of daclizumab or placebo and on days 70 and 84 after transplantation. A sandwich enzyme-linked immunosorbent assay was used to measure daclizumab in serum.[16]

In 20 consecutive patients at one U.S. center (University of California, San Francisco), lymphocyte analysis was performed to determine the saturation of the interleukin-2–receptor α chain, with the use of methods reported previously.[17]

Glomerular Filtration Rate

The glomerular filtration rate was measured in all patients with functioning grafts six months after transplantation. Measurements were based on iohexol, radioisotope, or inulin clearance.

Statistical Analysis

Differences in categorical variables between the two groups were determined with the use of the Mantel–Haenszel test (with stratification according to center). Differences in the time to the first biopsy-confirmed episode of rejection were determined with the use of the log-rank test (with stratification according to center). The log-rank test was also used to analyze the time to graft failure (or death with a functioning graft) because of the small number of events reported. Kaplan–Meier estimates of the probability of patient survival and graft survival and the cumulative probability of biopsy-confirmed rejection were plotted over time. Differences in the number of presumptive or biopsy-confirmed rejection episodes per patient in the first six months were analyzed with a normal regression model. The serum creatinine concentrations, glomerular filtration rates, and cumulative doses of prednisone administered during the first six months after transplantation in the two groups were compared with the use of the Wilcoxon rank-sum test. Logistic-regression analysis was used to determine the effects of various factors on the probability of biopsy-confirmed rejection. Proportional-hazards analysis was used to determine the effects of various factors on the time to biopsy-confirmed rejection. The results of lymphocyte and interleukin-2–receptor assays were compared with the use of Student's t-test. All statistical tests were two-sided.

All patients randomly assigned to a treatment group were included in the primary analyses of efficacy and safety, according to the intention-to-treat principle. Values are reported as means ±SD.

RESULTS

A total of 260 patients were enrolled in the study: 134 patients were assigned to the placebo group, and 126 to the daclizumab group. The two groups were similar with respect to age, sex, race, cause of end-stage renal disease, presence or absence of panel-reactive anti-HLA antibodies, number of HLA-DR mismatches between donor and recipient, and duration of cold ischemia for the graft (Table 1).

All patients received at least one dose of the study drug, and 107 of the patients in the placebo group (80 percent) and 107 of those in the daclizumab group (85 percent) received all five doses. Graft function was delayed in 39 patients in the placebo group (29 percent) and 27 patients in the daclizumab group (21 percent). The early use of prophylactic antilymphocyte therapy for delayed graft function led to the discontinuation of the study drug in nine patients in the placebo group (7 percent) and nine in the daclizumab group (7 percent).

Efficacy

Daclizumab prophylaxis resulted in a significant reduction in the incidence of biopsy-documented acute rejection during the first six months after transplantation (22 percent, vs. 35 percent in the placebo group; $P = 0.03$; odds ratio, 0.5; 95 percent confidence interval, 0.3 to 0.9) (Table 2). The proportion of patients with presumptive or biopsy-confirmed acute rejection and the number of rejection episodes per patient were also lower in the daclizumab group, and the time to the first rejection was longer. There was a trend toward a reduction in the number of patients with two or more rejection episodes and the number receiving antilymphocyte preparations for severe rejection in the daclizumab group. The beneficial effect of daclizumab was not influenced by delayed graft function, initial use of other antilymphocyte therapies, or exclusion of patients who did not receive all five infusions of the study drug (data not shown).

The patient-survival rates at one year were 98 percent in the daclizumab group and 96 percent in the placebo group (Table 3). The graft-survival rates in the daclizumab and placebo groups were 95 and 90 percent, respectively. None of the patients in the daclizumab group but three of those in the placebo group died of infections: one each of aspergillosis,

TABLE 1. BASE-LINE CHARACTERISTICS OF RENAL-ALLOGRAFT RECIPIENTS.*

CHARACTERISTIC	PLACEBO (N=134)	DACLIZUMAB (N=126)
Age — yr	47±13	47±13
Sex — no. of patients (%)		
Male	81 (60)	74 (59)
Female	53 (40)	52 (41)
Race or ethnic group — no. of patients (%)		
White	81 (60)	84 (67)
Black	27 (20)	24 (19)
Other	26 (19)	18 (14)
Cause of renal failure — no. of patients (%)		
Glomerulonephritis	40 (30)	33 (26)
Diabetes mellitus	29 (22)	32 (25)
Hereditary or polycystic kidney disease	20 (15)	24 (19)
Hypertension	19 (14)	18 (14)
Other	26 (19)	19 (15)
Panel-reactive serum antibodies — no. of patients (%)†		
0–10%	121 (90)	113 (89)
11–49%	10 (7)	12 (10)
50–100%	3 (2)	1 (1)
No. of HLA-DR mismatches — no. of patients (%)‡		
0	22 (16)	19 (15)
1	62 (46)	49 (39)
2	40 (30)	50 (40)
Graft cold-ischemia time — hr	21±9	22±8

*Plus–minus values are means ±SD. Percentages may not sum to 100 because of rounding.

†Panel-reactive antibodies are anti-HLA antibodies that have a cytotoxic effect on lymphocytes obtained from a panel of donors from the general population.

‡Data were missing for some patients.

TABLE 2. ACUTE REJECTION EPISODES IN THE FIRST SIX MONTHS AFTER RENAL TRANSPLANTATION IN THE PLACEBO AND DACLIZUMAB GROUPS.

REJECTION	PLACEBO (N=134)	DACLIZUMAB (N=126)	P VALUE
One or more biopsy-confirmed episodes — no. of patients (%)	47 (35)	28 (22)	0.03
One or more biopsy-confirmed or presumptive episodes — no. of patients (%)	52 (39)	32 (25)	0.04
Two or more biopsy-confirmed or presumptive episodes — no. of patients (%)	18 (13)	9 (7)	0.08
Mean no. of episodes/patient	0.6	0.3	0.01
Time to first episode — days*	30±27	75±59	0.008
Episode requiring antilymphocyte therapy — no. of patients (%)†	19 (14)	10 (8)	0.09

*Plus–minus values are means ±SD.

†Antilymphocyte therapy consisted of OKT3 or polyclonal antithymocyte globulin.

TABLE 3. CAUSES OF DEATH AND RENAL-GRAFT FAILURE AT ONE YEAR IN THE PLACEBO AND DACLIZUMAB GROUPS.

CAUSE	PLACEBO (N=134)	DACLIZUMAB (N=126)
	no. of patients (%)	
Death	5 (4)	3 (2)
Infection or lymphoma	3 (2)	1 (1)
Cardiovascular cause	1 (1)	0
Pulmonary embolism	1 (1)	0
Intracerebral bleeding	0	1 (1)
Suicide	0	1 (1)
Graft failure	13 (10)	6 (5)
Death	5 (4)	3 (2)
Rejection	3 (2)	1 (1)
Technical cause	4 (3)	2 (2)
Primary nonfunction	1 (1)	0

coccidioidomycosis, and pseudomonas sepsis. One patient in the daclizumab group died of lymphoma.

The mean serum creatinine concentrations six months after transplantation were the same in the two groups (1.7±0.7 mg per deciliter [150±60 μmol per liter]). The mean glomerular filtration rate was 55±23 ml per minute in the daclizumab group and 52±22 ml per minute in the placebo group. The average daily doses of prednisone and cyclosporine did not differ between the groups at any time during the study, nor was there a difference in the mean trough whole-blood cyclosporine concentrations at any time.

Adverse Events

The administration of daclizumab was not associated with any immediate side effects. There was no significant difference in reported adverse events between the two groups (Table 4). One patient in the placebo group and two patients in the daclizumab group had lymphoma during the first year after transplantation.

Pharmacokinetic Data

Pharmacokinetic data were available for 92 patients in the daclizumab group. The mean serum half-life of daclizumab was 20 days.

Circulating Peripheral-Blood Lymphocytes and Interleukin-2 α-Chain Receptor

There were no differences in absolute lymphocyte numbers between the placebo and daclizumab groups before transplantation or for six months afterward. Circulating CD3+ cell concentrations and T-cell subgroups were not measured, because they were not affected by daclizumab therapy in an earlier study.[17] There was a significant decrease in the percentage of circulating lymphocytes that stained with anti-

TABLE 4. ADVERSE EVENTS AT SIX MONTHS IN THE PLACEBO AND DACLIZUMAB GROUPS.

ADVERSE EVENTS	PLACEBO (N=134)	DACLIZUMAB (N=126)
	no. of patients (%)	
Serious event*	13 (10)	6 (5)
Fever	16 (12)	11 (9)
Sepsis and bacteremia	9 (7)	4 (3)
Pneumonia	4 (3)	3 (2)
Fungal infection	27 (20)	21 (17)
Fungemia	2 (1)	0
Local infection	25 (19)	21 (17)
Local infection†	70 (52)	59 (47)
Cellulitis and wound infection	4 (3)	7 (6)
Urinary tract infection	44 (33)	34 (27)
Other	38 (28)	36 (29)
Any viral infection†	32 (24)	29 (23)
Viremia	12 (9)	12 (10)
Local infection	21 (16)	20 (16)
Cytomegalovirus infection	14 (10)	15 (12)
Viremia	10 (7)	12 (10)
Tissue infection	4 (3)	3 (2)

*Serious adverse events were defined as complications other than death or rejection that prolonged or required hospitalization and were possibly or probably related to the study drug.

†Some patients had more than one type of infection.

CD25 antibody starting 10 hours after transplantation and lasting up to four months in the daclizumab group (data not shown). Similarly, there was a significant decrease in the percentage of circulating lymphocytes that stained with the fluorescein-conjugated antibody 7g7, which binds to an interleukin-2 α-chain–receptor epitope distinct from the epitope recognized by daclizumab and reflects total interleukin-2α–receptor expression (data not shown).

DISCUSSION

We found that the patients receiving daclizumab in addition to maintenance therapy with three immunosuppressive agents had a lower frequency of biopsy-confirmed acute rejection in the first six months after transplantation than the patients receiving placebo with the three immunosuppressive agents. In addition, the time to the first episode of acute rejection was significantly prolonged, and the mean number of episodes per patient significantly reduced in the daclizumab group. These results were obtained without a concomitant increase in infectious complications or cancers. The efficacy of daclizumab is probably related to its selective target, the α-chain component of the high-affinity interleukin-2 receptor, which is present almost exclusively on activated T cells. Use of the drug thus spares other immunocompetent cells.[7]

Only 10 percent of daclizumab is composed of murine sequences, which are from the antigen-binding regions of the parent antibody. These sequences are inserted into human immunoglobulin with the use of molecular biologic techniques.[14] Our study highlights the advantages of this type of antibody, including its prolonged serum half-life, approaching that of human IgG, and the absence of functional immunogenicity associated with its use.[15,16,19,20]

The exact mechanism or mechanisms of action of daclizumab are not known. A likely mechanism is that it binds to circulating lymphocytes with interleukin-2 α-chain receptors but does not activate the receptors, and the cells therefore have no free interleukin-2 α-chain receptors available for activation by interleukin-2. In addition, the decline in the percentage of circulating lymphocytes expressing CD25 (measured by staining with 7g7 antibody) without an accompanying decrease in the absolute number of lymphocytes suggests that the expression of interleukin-2 receptors is down-regulated or the shedding of the daclizumab-bound interleukin-2 α chain is increased.

In conclusion, when added to therapy with cyclosporine, azathioprine, and prednisone, daclizumab reduces the frequency of acute rejection and improves short-term graft survival in renal-transplant recipients.

Supported by a grant from Hoffmann–LaRoche.

We are indebted to Dr. Thomas A. Waldmann for his contribution to the development of daclizumab, and to Ms. Peggy Millar for her assistance in the preparation of the manuscript.

APPENDIX

In addition to the authors, the following investigators participated in the Daclizumab Triple Therapy Study Group: *Victoria General Hospital, Halifax, N.S., Canada* — B. Kibert; *Huddinge Hospital, Huddinge, Sweden* — G. Tyden; *University of Minnesota, Minneapolis* — A. Matas; *Beth Israel Deaconess Medical Center, Boston* — M. Shapiro; *Tampa General Hospital, Tampa, Fla.* — G. Chan; *Vancouver General Hospital, Vancouver, B.C., Canada* — P. Keown; *University of California, San Francisco* — M. Lantz; *University of Alberta, Edmonton, Alta., Canada* — K. Solez; and *Hoffmann–LaRoche, Nutley, N.J.* — A. Lin, I. Patel, K. Nieforth, A. Wolitzky, and J. Hakimi.

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Interview Summary

Application No.	Applicant(s)
08/146,206	Carter et al
Examiner	**Group Art Unit**
MINH TAM DAVIS	1642

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

(1) *MINH TAM DAVIS* (3) _____

(2) *Wendy Lee* (4) _____

Date of Interview *Dec 11, 2001*

Type: a)☒ Telephonic b)☐ Video Conference
c)☐ Personal [copy is given to 1)☐ applicant 2)☐ applicant's representative]

Exhibit shown or demonstration conducted: d)☐ Yes e)☒ No. If yes, brief description:

Claim(s) discussed: _____

Identification of prior art discussed:

Agreement with respect to the claims f)☐ was reached. g)☐ was not reached. h)☐ N/A.

Substance of Interview including description of the general nature of what was agreed to if an agreement was reached, or any other comments:

Pending claims 43-105, 113-131 are allowable.

(A fuller description, if necessary, and a copy of the amendments which the examiner agreed would render the claims allowable, if available, must be attached. Also, where no copy of the amendments that would render the claims allowable is available, a summary thereof must be attached.)

i)☒ It is not necessary for applicant to provide a separate record of the substance of the interview (if box is checked).

Unless the paragraph above has been checked, THE FORMAL WRITTEN REPLY TO THE LAST OFFICE ACTION MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP section 713.04). If a reply to the last Office action has already been filed, APPLICANT IS GIVEN ONE MONTH FROM THIS INTERVIEW DATE TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW. See Summary of Record of Interview requirements on reverse side or on attached

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

M-T DAVIS Re-Pwr

1600

RAW SEQUENCE LISTING DATE: 12/11/2001
PATENT APPLICATION: US/08/146,206C TIME: 13:58:59

Input Set : A:\p0709p1.txt
Output Set: N:\CRF3\12112001\H146206C.raw

#
67
ES
12-14-0

SEQUENCE LISTING

W--> 3 SEQUENCE LISTING
5 (1) GENERAL INFORMATION:
7 (i) APPLICANT: Carter, Paul J.
8 Presta, Leonard G.
10 (ii) TITLE OF INVENTION: Method for Making Humanized Antibodies
12 (iii) NUMBER OF SEQUENCES: 26
14 (iv) CORRESPONDENCE ADDRESS:
15 (A) ADDRESSEE: Genentech, Inc.
16 (B) STREET: 1 DNA Way
17 (C) CITY: South San Francisco
18 (D) STATE: California
19 (E) COUNTRY: USA
20 (F) ZIP: 94080
22 (v) COMPUTER READABLE FORM:
23 (A) MEDIUM TYPE: 3.5 inch, 1.44 Mb floppy disk
24 (B) COMPUTER: IBM PC compatible
25 (C) OPERATING SYSTEM: PC-DOS/MS-DOS
26 (D) SOFTWARE: WinPatin (Genentech)
28 (vi) CURRENT APPLICATION DATA:
C--> 29 **(A) APPLICATION NUMBER: US/08/146,206C**
C--> 30 **(B) FILING DATE: 17-Nov-1993**
31 (C) CLASSIFICATION:
33 (vii) PRIOR APPLICATION DATA:
34 (A) APPLICATION NUMBER: 07/715272
35 (B) FILING DATE: 14-JUN-1991
37 (viii) ATTORNEY/AGENT INFORMATION:
38 (A) NAME: Lee, Wendy M.
39 (B) REGISTRATION NUMBER: 40,378
40 (C) REFERENCE/DOCKET NUMBER: P0709P1
42 (ix) TELECOMMUNICATION INFORMATION:
43 (A) TELEPHONE: 650/225-1994
44 (B) TELEFAX: 650/952-9881
45 (2) INFORMATION FOR SEQ ID NO: 1:
47 (i) SEQUENCE CHARACTERISTICS:
48 (A) LENGTH: 109 amino acids
49 (B) TYPE: Amino Acid
50 (D) TOPOLOGY: Linear
52 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
54 Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
55  1               5                  10                  15
57 Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn
58              20                  25                  30
60 Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
61              35                  40                  45
63 Leu Leu Ile Tyr Ser Ala Ser Phe Leu Glu Ser Gly Val Pro Ser
64              50                  55                  60
```

ENTERED

RAW SEQUENCE LISTING DATE: 12/11/2001
PATENT APPLICATION: US/08/146,206C TIME: 13:58:59

Input Set : A:\p0709pl.txt
Output Set: N:\CRF3\12112001\H146206C.raw

```
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
```

RAW SEQUENCE LISTING DATE: 12/11/2001
PATENT APPLICATION: US/08/146,206C TIME: 13:58:59

Input Set : A:\p0709pl.txt
Output Set: N:\CRF3\12112001\H146206C.raw

```
                    95                  100                 105
 Ile Lys Arg Thr
             109
(2) INFORMATION FOR SEQ ID NO: 4:
     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 120 amino acids
          (B) TYPE: Amino Acid
          (D) TOPOLOGY: Linear
    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
   1               5                  10                  15
 Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
                   20                  25                  30
 Asp Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
                   35                  40                  45
 Glu Trp Val Ala Val Ile Ser Glu Asn Gly Ser Asp Thr Tyr Tyr
                   50                  55                  60
 Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser
                   65                  70                  75
 Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                   80                  85                  90
 Thr Ala Val Tyr Tyr Cys Ala Arg Asp Arg Gly Gly Ala Val Ser
                   95                 100                 105
 Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
                  110                 115                 120
(2) INFORMATION FOR SEQ ID NO: 5:
     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 109 amino acids
          (B) TYPE: Amino Acid
          (D) TOPOLOGY: Linear
    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:
 Asp Ile Val Met Thr Gln Ser His Lys Phe Met Ser Thr Ser Val
   1               5                  10                  15
 Gly Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asp Val Asn
                   20                  25                  30
 Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly His Ser Pro Lys
                   35                  40                  45
 Leu Leu Ile Tyr Ser Ala Ser Phe Arg Tyr Thr Gly Val Pro Asp
                   50                  55                  60
 Arg Phe Thr Gly Asn Arg Ser Gly Thr Asp Phe Thr Phe Thr Ile
                   65                  70                  75
 Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln
                   80                  85                  90
 His Tyr Thr Thr Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu
                   95                 100                 105
 Ile Lys Arg Ala
             109
(2) INFORMATION FOR SEQ ID NO: 6:
     (i) SEQUENCE CHARACTERISTICS:
```

RAW SEQUENCE LISTING DATE: 12/11/2001
PATENT APPLICATION: US/08/146,206C TIME: 13:58:59

Input Set : A:\p0709pl.txt
Output Set: N:\CRF3\12112001\H146206C.raw

(A) LENGTH: 120 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

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

```
Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly
 1               5                  10                  15
Ala Ser Leu Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys
            20                  25                  30
Asp Thr Tyr Ile His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu
        35                  40                  45
Glu Trp Ile Gly Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr
    50                  55                  60
Asp Pro Lys Phe Gln Asp Lys Ala Thr Ile Thr Ala Asp Thr Ser
65                  70                  75
Ser Asn Thr Ala Tyr Leu Gln Val Ser Arg Leu Thr Ser Glu Asp
                80                  85                  90
Thr Ala Val Tyr Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr
            95                  100                 105
Ala Met Asp Tyr Trp Gly Gln Gly Ala Ser Val Thr Val Ser Ser
        110                 115                 120
```

(2) INFORMATION FOR SEQ ID NO: 7:

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

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

TCCGATATCC AGCTGACCCA GTCTCCA 27

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

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

GTTTGATCTC CAGCTTGGTA CCHSCDCCGA A 31

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

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

AGGTSMARCT GCAGSAGTCW GG 22

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single

RAW SEQUENCE LISTING DATE: 12/11/2001
PATENT APPLICATION: US/08/146,206C TIME: 13:58:59

Input Set : A:\p0709pl.txt
Output Set: N:\CRF3\12112001\H146206C.raw

(D) TOPOLOGY: Linear

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

TGAGGAGACG GTGACCGTGG TCCCTTGGCC CCAG 34

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GTAGATAAAT CCTCTAACAC AGCCTATCTG CAAATG 36

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

GTAGATAAAT CCAAATCTAC AGCCTATCTG CAAATG 36

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

GTAGATAAAT CCTCTTCTAC AGCCTATCTG CAAATG 36

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 68 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

CTTATAAAGG TGTTTCCACC TATAACCAGA AATTCAAGGA TCGTTTCACG 50
ATATCCGTAG ATAAATCC 68

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

CTATACCTCC CGTCTGCATT CTGGAGTCCC 30

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 107 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

VERIFICATION SUMMARY DATE: 12/11/2001
PATENT APPLICATION: US/08/146,206C TIME: 13:59:00

Input Set : A:\p0709pl.txt
Output Set: N:\CRF3\12112001\H146206C.raw

L:3 M:244 W: Invalid beginning of sequence listing, Data=[SEQUENCE LISTING], Duplicate Sequence Listing Title!
L:29 M:220 C: Keyword misspelled or invalid format, [(A) APPLICATION NUMBER:]
L:30 M:220 C: Keyword misspelled or invalid format, [(B) FILING DATE:]

Genentech, Inc.

Anna S. Kan
Legal Department

(650) 225-2830
Fax (650) 952-9881
kan@gene.com

To: Examiner Minh-Tam Davis
From: Wendy Lee

This is the priority document for 08/146,206.

12/12/2001

Genentech Legal Department

1 DNA Way
South San Francisco, CA 94080
650-225-2830
Fax: 650-952-9881/9882

FAX TRANSMISSION COVER SHEET

Date: *December 12, 2001*

To: *Examiner Minh-Tam Davis*
Group Art 1642

Fax: *(703) 746-7145*

Re: *U.S. Ser. No 09/146,206*
filed August 1, 1995
Attorney Docket No.: P0709P1

Sender: *Anna Kan for Wendy Lee*

YOU SHOULD RECEIVE 12 PAGE(S), INCLUDING THIS COVER SHEET. IF YOU DO NOT RECEIVE ALL THE PAGES, PLEASE CALL 650-225-2830.

Dear Examiner Davis,

Pursuant to your request, attached are courtesy copies of the IDS Transmittals and PTO-1449 Forms filed on August 1, 1995 and February 1, 1999. We understand that you have the cited references but, if not, let us know and we will be happy to provide further copies.

Kindly send us initialed copies of the PTO-1449 Forms for the IDSs filed on the following dates. The reference nos. are noted below in parentheses.

09/02/97 (refs. 100-207)
08/24/98 (refs. 215-224)
02/01/99 (refs. 225-262)
03/09/99 (ref. 263)
08/30/01 (ref. 264-265)

Very truly yours,
Genentech, Inc.

Anna Kan

Anna Kan for Wendy Lee

WL/LS

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1642 Examiner: J. Reeves CERTIFICATE OF HAND DELIVERY I hereby certify that this correspondence is being hand delivered in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on February 1, 1999 K. H. Mitchell

Official
IDS
12/13/01

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

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

This Information Disclosure Statement:

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

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

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

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

should be charged or credited to this deposit account. A duplicate of this sheet is enclosed.

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

(f) (x) is filed after the mailing date of a final rejection, but a request to withdraw the finality thereof under 37 CFR § 1.129(a) was submitted on August 24, 1998. The U.S. Patent and Trademark Office is hereby authorized to charge Deposit Account No. 07-0630 to cover the cost of this Information Disclosure Statement in the event that any fees are due. A duplicate of this sheet is enclosed.

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

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

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

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

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

(x) each ( ) none ( ) only those listed below:

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

(x) not given

( ) given for each listed item

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

( ) In the form of an English language copy of a Search Report from a foreign patent

office, issued in a counterpart application, which refers to the relevant portions of the references.

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

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

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

Respectfully submitted,
GENENTECH, INC.

Date: January 29, 1999 By: _____
Wendy M. Lee
Reg. No. 40,378

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

In re Application of: Paul J. Carter et al.
Serial No.: 08/146,206
Filed On: 17 November 1993
Mailed On: 1 August 1995

Docket No.: 709P1
By: Wendy M. Lee
Reg. No.:

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

___ Amendment/Response
___ Extension of Time Request (dup)
___ Communication/Transmittal Letter (dup)
___ Notice of Appeal (dup)
___ Issue Fee Transmittal Form
_X_ Information Disclosure Statement (Supplemental)
_X_ Form 1449 with _3_ References
_X_ Certificate of Mailing
___ Certificate of Express Mailing
Express Mail Label No.:
_X_ Other: Limited Recognition

___ U.S. Patent Application
___ Rule 60 ___ Rule 62
___ Declaration/Power of Atty
___ Assignment
___ Recordation Form/Fee
___ Drawings: __ Sheets
___ Informal ___ Formal
___ Sequence Listing & Diskette
___ PCT Patent Application

In re Application of: Paul J. Carter et al.
Serial No.: 08/146,206
Filed On: 17 November 1993
Mailed On: 1 August 1995

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By: Wendy M. Lee
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___ Amendment/Response
___ Extension of Time Request (dup)
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___ Issue Fee Transmittal Form
_X_ Information Disclosure Statement (Supplemental)
_X_ Form 1449 with _3_ References
_X_ Certificate of Mailing
___ Certificate of Express Mailing
Express Mail Label No.:
_X_ Other: Limited Recognition

___ U.S. Patent Application
___ Rule 60 ___ Rule 62
___ Declaration/Power of Atty
___ Assignment
___ Recordation Form/Fee
___ Drawings: __ Sheets
___ Informal ___ Formal
___ Sequence Listing & Diskette
___ PCT Patent Application

MAIL ROOM 74 AUG 3 1995 PAT. & TRADEMARK OFF.

PATENT DOCKET 709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

Paul J. Carter et al.

Serial No. 08/146,206

Filed: 17 November 1993

For: METHOD FOR MAKING HUMANIZED ANTIBODIES

Group Art Unit: 1806

Examiner: D. Adams

CERTIFICATE OF MAILING

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

8/1/95

(Date of Deposit)

Duane Alexander Vick

Name of Depositing Party

Duane Alexander Vick

Signature of Depositing Party

8/1/95

Date of Signature

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

Official
TDes
12/13/01

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

Sir:

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

This Information Disclosure Statement:

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

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

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

(d) [X] is filed after the first Office Action and more than three months after the application's filing date or PCT national stage date of entry filing but, as far as is known to the undersigned, prior to the mailing date of either a final rejection or a notice of allowance, whichever occurs first, and is accompanied by either the fee ($210) set forth in 37 CFR §1.17(p) or a certification as specified in 37 CFR §1.97(e), as checked below. Should any fee be due, the U.S. Patent and Trademark Office is hereby authorized to charge Deposit Account No. 07-0630 in the amount of $210.00 to cover the cost of this

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

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

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

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

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

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

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

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

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

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

[X] not given

[] given for each listed item

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

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

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

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

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

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

Respectfully submitted,
GENENTECH, INC.

Date: August 1, 1995 By: _____
Wendy M. Lee

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

In re Application of: Paul J. Carter et al.
Serial No.: 08/146,206
Filed On: November 17, 1993
Hand Delivered On: ___ February 1999

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

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

_x_ Information Disclosure Statement
_x_ Form 1449 with 38 References
_x_ Communication with Exhibit A and two priority documents
_x_ Certificate of Hand Delivery

In re Application of: Paul J. Carter et al.
Serial No.: 08/146,206
Filed On: November 17, 1993
Hand Delivered On: ___ February 1999

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

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

_x_ Information Disclosure Statement
_x_ Form 1449 with 38 References
_x_ Communication with Exhibit A and two priority documents
_x_ Certificate of Hand Delivery

99 FEB -1 PM 12:21

TECH CENTER 1600/2900

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

LIST OF DISCLOSURES CITED BY APPLICANT

(Use several sheets if necessary)

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

U.S. PATENT DOCUMENTS

Examiner Initials		Document Number	Date	Name	Class	Subclass	Filing Date
	225	5,714,350	03.02.98	Co et al.			13.01.95
	226	5,821,337	13.10.98	Carter et al.			

FOREIGN PATENT DOCUMENTS

Examiner Initials		Document Number	Date	Country	Class	Subclass	Translation Yes	Translation No
	227	0 460 167 B1	11.12.91	EPO				
	228	0 519 596 A1	23.12.92	EPO				
	229	0 592 106 A1	13.04.94	EPO				
	230	120,694	03.10.84	EPO				
	231	125,023 A1	14.11.84	EPO				
	232	368,684	16.05.90	EPO				
	233	94/11509	26.05.94	PCT				
	234	WO 89/09622		PCT				
	235	WO 92/11383	09.07.92	PCT				
	236	2 188941	14.10.87	UNITED KINGDOM				

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

	237	"Biosym Technologies" in New Products, Chemical Design Automation 3" (December 1988)
	238	"Polygen Corporation" in New Products, Chemical Design Automation 3" (November 1988)
	239	Adair et al., "Humanization of the murine anti-human CD3 monoclonal antibody OKT3" Hum. Antibod. Hybridomas 5:41-47 (1994)
	240	Chothia et al., "Principles of protein-protein recognition" Nature 256:705-708 (1975)
	241	Chothia et al., "Transmission of conformational change in insulin" Nature 302:500-505 (1983)
	242	Corti et al., "Idiotope Determining Regions of a Mouse Monoclonal Antibody and Its Humanized Versions" J. Mol. Biol. 235:53-60 (1994)
	243	Couto et al., "Anti-BA46 Monoclonal Antibody Mc3 Humanization Using a Novel Positional Consensus and in Vivo and in Vitro Characterization" Cancer Research Supplement 55:1717-1722 (1995)
	244	Couto et al., "Humanization of KC4G3, an Anti-Human Carcinoma Antibody" Hybridoma 13:215-219 (1994)
	245	Ellis et al., "Engineered Anti-CD38 Monoclonal Antibodies for Immunotherapy of Multiple Myeloma" The Journal of Immunology pps. 925-937 (1995)
	246	Hieter et al., "Evolution of Human Immunoglobulin K J Region Genes" The Journal of Biological Chemistry 257:1516-1522 (1982)
	247	Lesk, Arthur M., "How Different Amino Acid Sequences Determine Similar Protein Structures: The Structure and Evolutionary Dynamics of the Globins" J. Mol. Biol. 136:225-270 (1980)

Examiner	Date Considered

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

USCOMM-DC 80-398.

FORM PTO-1449	U.S. Dept. of Commerce Patent and Trademark Office	Atty Docket No. P0709P1	Serial No. 08/146,206
LIST OF DISCLOSURES CITED BY APPLICANT (Use several sheets if necessary)		Applicant Carter et al.	
		Filing Date 17 Nov 1993	Group 1806

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

	248	Matsumura et al., "Hydrophobic stabilization in T4 lysozyme determined directly by multiple substitutions of Ile 3" Nature 334:406-410 (1988)
	249	Morrison, S. L., "Transfectomas Provide Novel Chimeric Antibodies" Science 229:1202-1207 (September 20, 1985)
	250	Nakatani et al., "Humanization of mouse anti-human IL-2 receptor antibody S-B10" Protein Engineering 7:435-443 (1994)
	251	Ohtomo et al., "Humanization of Mouse ONS-M21 Antibody with the Aid of Hybrid Variable Regions" Molecular Immunology 32:407-416 (1995)
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	256	Tramontano, "Structural Determinants of the Conformations of Medium-Sized Loops in Proteins" Proteins 6:382-394 (1989)
	257	Uchiyama et al., "A Monoclonal Antibody (ANTI-Tac) Reactive with Activated and Functionally Mature Human T Cells" Journal of Immunology 126:1393-1397 (1981)
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	260	Waldmann et al., "Interleukin 2 Receptor (Tac Antigen) Expression in HTLV-1-associated Adult T-Cell Leukemia" Cancer Research 45:4559s-4562s (1985)
	261	Waldmann, Thomas A., "The Structure, Function, and Expression of Interleukin-2 Receptors on Normal and Malignant Lymphocytes" Science 232:727-732 (1986)
	262	Wu et al., "An Analysis of the Sequences of the Variable Regions of Bence Jones Proteins and Myeloma Light Chains and Their Implications for Antibody Complementarity" Journal of Experimental Medicine 132:211-250 (1970)

Examiner	Date Considered

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USCOMM-DC 60-398.

UNITED STATES PATENT AND TRADEMARK OFFICE

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

NOTICE OF ALLOWANCE AND FEE(S) DUE

7590 12/18/2001

GENENTECH, INC.
1 DNA WAY
SOUTH SAN FRANCISCO, CA 940804990

EXAMINER
DAVIS, MINH TAM B

ART UNIT	CLASS-SUBCLASS
1642	530-387300

DATE MAILED: 12/18/2001

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
08/146,206	11/17/1993	PAUL J. CARTER	709P1	3992

TITLE OF INVENTION: METHOD FOR MAKING HUMANIZED ANTIBODIES

TOTAL CLAIMS	APPLN. TYPE	SMALL ENTITY	ISSUE FEE	PUBLICATION FEE	TOTAL FEE(S) DUE	DATE DUE
82	nonprovisional	NO	$1280	$0	$1280	03/18/2002

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT.

PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151.

HOW TO REPLY TO THIS NOTICE:

I. Review the SMALL ENTITY status shown above. If the SMALL ENTITY is shown as YES, verify your current SMALL ENTITY status:

A. If the status is changed, pay the PUBLICATION FEE (if required) and twice the amount of the ISSUE FEE shown above and notify the United States Patent and Trademark Office of the change in status, or

B. If the status is the same, pay the TOTAL FEE(S) DUE shown above.

If the SMALL ENTITY is shown as NO:

A. Pay TOTAL FEE(S) DUE shown above, or

B. If applicant claimed SMALL ENTITY status before, or is now claiming SMALL ENTITY status, check the box below and enclose the PUBLICATION FEE and 1/2 the ISSUE FEE shown above.

☐ Applicant claims SMALL ENTITY status. See 37 CFR 1.27.

II. PART B - FEE(S) TRANSMITTAL should be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). Even if the fee(s) have already been paid, Part B - Fee(s) Transmittal should be completed and returned. If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Box ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PTOL-85 (REV. 07-01) Approved for use through 01/31/2004.

PART B - FEE(S) TRANSMITTAL

Complete and mail this form, together with applicable fee(s), to:

Box ISSUE FEE
Assistant Commissioner for Patents
Washington, D.C. 20231

MAILING INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 4 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Legibly mark-up with any corrections or use Block 1)

7590 12/18/2001

GENENTECH, INC.
1 DNA WAY
SOUTH SAN FRANCISCO, CA 940804990

Note: The certificate of mailing below can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing.

Certificate of Mailing

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Box Issue Fee address above on the date indicated below.

(Depositor's name)

(Signature)

(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
08/146,206	11/17/1993	PAUL J. CARTER	709P1	3992

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TOTAL CLAIMS	APPLN. TYPE	SMALL ENTITY	ISSUE FEE	PUBLICATION FEE	TOTAL FEE(S) DUE	DATE DUE
82	nonprovisional	NO	$1280	$0	$1280	03/18/2002

EXAMINER	ART UNIT	CLASS-SUBCLASS
DAVIS, MINH TAM B	1642	530-387300

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363). Use of PTO form(s) and Customer Number are recommended, but not required.

☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47) attached.

2. For printing on the patent front page, list (1) the names of up to 3 registered patent attorneys or agents OR, alternatively, (2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 _____

2 _____

3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. Inclusion of assignee data is only appropriate when an assignment has been previously submitted to the USPTO or is being submitted under separate cover. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) ☐ individual ☐ corporation or other private group entity ☐ government

4a. The following fee(s) are enclosed:

☐ Issue Fee

☐ Publication Fee

☐ Advance Order - # of Copies _____

4b. Payment of Fee(s):

☐ A check in the amount of the fee(s) is enclosed.

☐ Payment by credit card. Form PTO-2038 is attached.

☐ The Commissioner is hereby authorized by charge the required fee(s), or credit any overpayment, to Deposit Account Number _____(enclose an extra copy of this form).

The COMMISSIONER OF PATENTS AND TRADEMARKS is requested to apply the Issue Fee and Publication Fee (if any) to the application identified above.

(Authorized Signature) (Date)

NOTE: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending on the needs of the individual case. Any comments on the amount of time required to complete this form should be sent to the Chief Information Officer, United States Patent and Trademark Office, Washington, D.C. 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND FEES AND THIS FORM TO: Box Issue Fee, Assistant Commissioner for Patents, Washington, D.C. 20231

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TRANSMIT THIS FORM WITH FEE(S)

PTOL-85 (REV. 07-01) Approved for use through 01/31/2004. OMB 0651-0033 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

UNITED STATES PATENT AND TRADEMARK OFFICE

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

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
08/146,206	11/17/1993	PAUL J. CARTER	709P1	3992

EXAMINER
DAVIS, MINH TAM B

ART UNIT	PAPER NUMBER
1642	

7590 12/18/2001

GENENTECH, INC.
1 DNA WAY
SOUTH SAN FRANCISCO, CA940804990

DATE MAILED: 12/18/2001

Determination of Patent Term Extension or Adjustment under 35 U.S.C. 154 (b)
(application filed prior to June 8, 1995)

This patent application was filed prior to June 8, 1995, thus no Patent Term Extension or Adjustment applies.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) system. (http://pair.uspto.gov)

PTOL-85 (REV. 07-01) Approved for use through 01/31/2004.

Notice of Allowability

Application No.	Applicant(s)
08/146,206	Carter et al
Examiner	**Art Unit**
MINH TAM DAVIS	1642

--The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance and Issue Fee Due or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *interview on 12/11/01*.

2. ☒ The allowed claim(s) is/are *43-105, 113-128, renumbered as 1-82*.

3. ☐ The drawings filed on _____ are acceptable as formal drawings.

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

   a) ☐ All b) ☐ Some* c) ☐ None of the:

   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____.
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   *Certified copies not received: _____.

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

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application. **THIS THREE-MONTH PERIOD IS NOT EXTENDABLE FOR SUBMITTING NEW FORMAL DRAWINGS, OR A SUBSTITUTE OATH OR DECLARATION.** ~~**This three-month period for complying with the REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL is extendable under 37 CFR 1.136(a).**~~

6. ☐ Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient. A SUBSTITUTE OATH OR DECLARATION IS REQUIRED.

7. ☒ Applicant MUST submit NEW FORMAL DRAWINGS

   (a) ☒ including changes required by the Notice of Draftsperson's Patent Drawing Review (PTO-948) attached

   1) ☐ hereto or 2) ☒ to Paper No. *12*.

   (b) ☐ including changes required by the proposed drawing correction filed _____, which has been approved by the examiner.

   (c) ☐ including changes required by the attached Examiner's Amendment/Comment or in the Office action of Paper No. _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings. The drawings should be filed as a separate paper with a transmittal letter addressed to the Official Draftsperson.

8. ☐ Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Any reply to this letter should include, in the upper right hand corner, the APPLICATION NUMBER (SERIES CODE/SERIAL NUMBER). If applicant has received a Notice of Allowance and Issue Fee Due, the ISSUE BATCH NUMBER and DATE of the NOTICE OF ALLOWANCE should also be included.

Attachment(s)

1 ☐ Notice of References Cited (PTO-892)
2 ☐ Notice of Informal Patent Application (PTO-152)
3 ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
4 ☐ Interview Summary (PTO-413), Paper No. _____.
5 ☒ Information Disclosure Statement(s) (PTO-1449), Paper No(s). *10 sheets*
6 ☐ Examiner's Amendment/Comment
7 ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
8 ☐ Examiner's Statement of Reasons for Allowance
9 ☐ Other

EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with Wendy Lee on 12/13/01.

The application has been amended as follows:

In the claims:

Claim 114. Delete "about", and replace it with --- up to ---

Delete "tightly", and replace it with --- in the binding affinity ---

Any inquiry concerning this communication or earlier communications from the examiner should be directed to MINH-TAM DAVIS whose telephone number is 703-305-2008. The examiner can normally be reached on 9:30AM-4:00PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, ANTHONY CAPUTA can be reached on 703-308-3995. The fax phone numbers for the organization where this application or proceeding is assigned are 703-308-4426 for regular communications and 703-308-4426 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 703-308-0916.

MINH TAM DAVIS
December 14, 2001

FORM PTO-1449

U.S. Dept. of Commerce
Patent and Trademark Office

Atty Docket No.	Serial No.
P0709P1	08/146,206
Applicant: Carter et al.	
Filing Date: 17 Nov 1993	Group: 1642

SEP 0 6 2001
PATENT & TRADEMARK OFFICE

LIST OF DISCLOSURES CITED BY APPLICANT

(Use several sheets if necessary)

U.S. PATENT DOCUMENTS

Examiner Initials		Document Number	Date	Name	Class	Subclass	Filing Date
MTD	264	5,834,598	10/11/98	Lowman et al.	530	399	

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

MTD	265	Rhodes, P., "Recombinant antibodies from CHO cells" ABSTR PAP AM CHEM SOC (Abstract No. 60 from the 199th American Chemical Society National Meeting held in Boston, MA April 22-27, 1990) 199(1-2):BIOT 60 (Apr 1990)

RECEIVED
SEP 0 6 2001
TECH CENTER 1600/2900

Examiner: M.T. DAVIS

Date Considered: 12/05/01

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

USCOMM-DC 80-398.

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

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U.S. PATENT DOCUMENTS

Examiner Initials		Document Number	Date	Name	Class	Subclass	Filing Date
JQR	263	5,859,205	12.01.99	Adair et al.	530	387.3	07.09.94

Examiner: Julie Reeves PhD Date Considered: 3/25/99

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

M. T. Davis 12/05/01

USCOMM-DC 80-398.

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

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TDS	225	5,714,350	03.02.98	Co et al.	435	69.6	13.01.95
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	236	2 188941	14.10.87	UNITED KINGDOM				

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TDS	237	"Biosym Technologies" in New Products, Chemical Design Automation 3" (December 1988)
	238	"Polygen Corporation" in New Products, Chemical Design Automation 3" (November 1988)
	239	Adair et al., "Humanization of the murine anti-human CD3 monoclonal antibody OKT3" Hum. Antibod. Hybridomas 5:41-47 (1994)
	240	Chothia et al., "Principles of protein-protein recognition" Nature 256:705-708 (1975)
	241	Chothia et al., "Transmission of conformational change in insulin" Nature 302:500-505 (1983)
	242	Corti et al., "Idiotope Determining Regions of a Mouse Monoclonal Antibody and Its Humanized Versions" J. Mol. Biol. 235:53-60 (1994)
	243	Couto et al., "Anti-BA46 Monoclonal Antibody Mc3 Humanization Using a Novel Positional Consensus and in Vivo and in Vitro Characterization" Cancer Research Supplement 55:1717-1722 (1995)
	244	Couto et al., "Humanization of KC4G3, an Anti-Human Carcinoma Antibody" Hybridoma 13:215-219 (1994)
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	246	Hieter et al., "Evolution of Human Immunoglobulin K J Region Genes" The Journal of Biological Chemistry 257:1516-1522 (1982)
	247	Lesk, Arthur M., "How Different Amino Acid Sequences Determine Similar Protein Structures: The Structure and Evolutionary Dynamics of the Globins" J. Mol. Biol. 136:225-270 (1980)

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

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USCOMM-DC 80-398

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

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MR	248	Matsumura et al., "Hydrophobic stabilization in T4 lysozyme determined directly by multiple substitutions of Ile 3" Nature 334:406-410 (1988)
	249	Morrison, S. L., "Transfectomas Provide Novel Chimeric Antibodies" Science 229:1202-1207 (September 20, 1985)
	250	Nakatani et al., "Humanization of mouse anti-human IL-2 receptor antibody B-B10" Protein Engineering 7:435-443 (1994)
	251	Ohtomo et al., "Humanization of Mouse ONS-M21 Antibody with the Aid of Hybrid Variable Regions" Molecular Immunology 32:407-416 (1995)
	252	~~Padlan et al., "Model-Building Studies of Antigen-Binding Sites: The Hapten-Binding Site of MOPC-315" C.S. Harbor Symp. Quant. Biol. 41:627-637 (1977)~~ Dupl.
	253	Rodrigues et al., "Engineering a humanized bispecific F(ab')$_2$ fragment for improved binding to T cells" Int. J. Cancer (Suppl.) 7:45-50 (1992)
	254	Sha et al., "A Heavy-Chain Grafted Antibody that Recognizes the Tumor-Associated TAG72 Antigen" Cancer Biotherapy 9:341-349 (1994)
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	256	Tramontano, "Structural Determinants of the Conformations of Medium-Sized Loops in Proteins" Proteins 6:382-394 (1989)
	257	Uchiyama et al., "A Monoclonal Antibody (ANTI-Tac) Reactive with Activated and Functionally Mature Human T Cells" Journal of Immunology 126:1393-1397 (1981)
	258	Vincenti et al., "Interleukin-2-Receptor Blockade with Daclizumab to Prevent Acute Rejection in Renal Transplantation" New Engl. J. Med. 338:161-165 (1998)
	259	Vitetta et al., "Redesigning Nature's Poisons to Create Anti-Tumor Reagents" Science 238:1098-1104 (1987)
	260	Waldmann et al., "Interleukin 2 Receptor (Tac Antigen) Expression in HTLV-1-associated Adult T-Cell Leukemia" Cancer Research 45:4559s-4562s (1985)
	261	Waldmann, Thomas A., "The Structure, Function, and Expression of Interleukin-2 Receptors on Normal and Malignant Lymphocytes" Science 232:727-732 (1986)
✓	262	Wu et al., "An Analysis of the Sequences of the Variable Regions of Bence Jones Proteins and Myeloma Light Chains and Their Implications for Antibody Complementarity" Journal of Experimental Medicine 132:211-250 (1970)

Examiner	Date Considered
M.T. Davis	12/04/01

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

USCOMM-DC 80-398.

copy. Attachment # 12 (11)

Sheet 1 of 1

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

LIST OF DISCLOSURES CITED BY APPLICANT

(Use several sheets if necessary)

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

FOREIGN PATENT DOCUMENTS

Examiner Initials		Document Number	Date	Country	Class	Subclass	Translation Yes	Translation No
TDB	79	WO 92/04381	19.03.92	PCT				
	80	WO 92/05274	02.04.92	PCT				
	81	WO 92/15683	17.09.92	PCT				

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

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

USCOMM-DC 80-398.

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

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

LIST OF DISCLOSURES CITED BY APPLICANT

(Use several sheets if necessary)

U.S. PATENT DOCUMENTS

Examiner Initials		Document Number	Date	Name	Class	Subclass	Filing Date
PN	81	5,225,539 7-6-93	~~06.07.93~~	Winter, G.	530/387.3	~~C07K~~	~~15/28~~ ~~25.10.91~~
PN	82	5,530,101 6-25-96	~~25.06.96~~	Queen et al.	530/387.3	~~A61K~~	39/395 ~~19.12.90~~

FOREIGN PATENT DOCUMENTS

Examiner Initials		Document Number	Date	Country	Class	Subclass	Translation Yes	Translation No
PN	83	85058/91 3-30-92	~~30.03.92~~	AUSTRALIA	C07K	15/12		
	84	328,404 A1 8-16-89	~~16.08.89~~	EPO	A61K	39/395		
	85	451,216 B1 1-24-96	~~24.01.96~~	EPO	C12P	21/08		
	86	WO 91/09966 7-11-91	~~11.07.91~~	PCT	C12P	21/08		
	87	WO 91/09968 7-11-91	~~11.07.91~~	PCT	C12P	21/08		
PN	88	WO 92/11018 9-7-92	~~07.09.92~~	PCT	A61K	35/14		

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

PN	89	Carter et al., "High level escherichia coli expression and production of a bivalent humanized antibody fragment" Bio/Technology 10:163-167 (1992)
	90	Foote et al., "Antibody Framework Residues Affecting the Conformation of the Hypervariable Loops" J. Mol. Biol. 224:487-499 (1992)
	91	Foote, J., "Humanized Antibodies" Nova acta Leopoldina 61(269):103-110 (1989)
	92	Kabat et al., "Sequences of Proteins of Immunological Interest", Bethesda, MD:National Institute of Health pps. 14-32 (1983)
	93	Kettleborough et al., "Humanization of a Mouse Monoclonal Antibody by CDR-grafting: the Importance of Framework Residues on Loop Conformation" Protein Engineering 4(7):773-783 (1991)
	94	Maeda et al., "Construction of Reshaped Human Antibodies with HIV-neutralizing Activity" Hum. Antibod. Hybridomas 2:124-134 (July 1991)
	95	Riechmann et al, "Expression of an Antibody Fv Fragment in Myeloma Cells" J. Mol. Biol. 203:825-828 (1988)
	96	Routledge et al., "A Humanized Monovalent CD3 Antibody which Can Activate Homologous Complement" European Journal of Immunology 21:2717-2725 (1991)
	97	Shearman et al., "Construction, Expression and Characterization of Humanized Antibodies Directed Against the Human α/β T Cell Receptor" J. Immunol. 147(12):4366-4373 (December 15, 1991)
PN	98	Tempest et al., "Reshaping a Human Monoclonal Antibody to Inhibit Human Respiratory Syncytial Virus Infection In Vivo" Bio/Technology 9:266-271 (March 1991)

Examiner: Patrick J. Nolan — Date Considered: 12-16-96

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

M.T. DAVIS 12/05/01

USCOMM-DC 80-398.

FORM PTO-1449

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Patent and Trademark Office

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OIPE JC43 AUG 26 1998 PATENT & TRADEMARK OFFICE

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

U.S. PATENT DOCUMENTS

Examiner Initials		Document Number	Date	Name	Class	Subclass	Filing Date
	*215	~~07/934,373~~		~~Carter et al.~~			~~21.08.92~~
	*216	~~08/437,642~~		~~Carter et al.~~			~~09.05.95~~
	*217	~~09/115,800~~		~~Carter et al.~~			~~15.07.98~~
MD	218	5,677,171	14.10.97	Hudziak et al.	435	7.23	05.08.94
	219	5,772,997	30.06.98	Hudziak et al.	424	130.1	23.05.95

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

MD	220	Brown, Jr. et al., "Anti-Tac-H, a humanized antibody to the interleukin 2 receptor, prolongs primate cardiac allograft survival" Proc. Natl. Acad. Sci. USA 88:2663-2667 (1991)
	221	Casale et al., "Use of an anti-IgE humanized monoclonal antibody in ragweed-induced allergic rhinitis" J. Allergy Clin. Immunol. 100:110-121 (1997)
	222	Fahy et al., "The Effect of an Anti-IgE Monoclonal Antibody on the Early- and Late-Phase Responses to Allergen Inhalation in Asthmatic Subjects" Am J. Respir. Crit. Care Med 155:1828-1834 (1997)
	223	Mathieson et al., "Monoclonal-Antibody Therapy in Systemic Vasculitis" New England J. of Medicine pps. 250-254 (July 1990)
✓	224	Presta et al., "Humanization of an anti-vascular endothelial growth factor monoclonal antibody for the therapy of solid tumors and other disorders" Cancer Research 57(20):4593-4599 (Oct 15, 1997)

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

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

USCOMM-DC 80-398.

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

LIST OF DISCLOSURES CITED BY APPLICANT

(Use several sheets if necessary)

Atty Docket No.	Serial No.
P0709P1	08/146,206
Applicant	
Carter et al.	
Filing Date	**Group**
17 Nov 1993	~~1806~~ 1816

FOREIGN PATENT DOCUMENTS

Examiner Initials		Document Number	Date	Country	Class	Subclass	Translation Yes	Translation No
PN	208	403,156 A1	19.12.90	EPO				
	209	438,310 A2	24.07.91	EPO				
	210	438,312 A2	24.07.91	EPO				
	211	440,351 A2	07.08.91	EPO				
	212	WO 91/07492	30.05.91	PCT				
PN	213	WO 92/16562*	01.10.92	PCT				
	214	WO 94/12214	09.06.94	PCT				

Examiner Patricia A-Nol Date Considered 12/22/97

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

M. T. DAVIS 12/05/01

USCOMM-DC 80-398.

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1642 #69M Examiner: Minh-Tam Davis **Date of Mailing of PTOL 85 entitled "Notice of Allowance and Issue Fee Due" December 18, 2001**

CERTIFICATE OF MAILING
I hereby certify that this correspondence is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated below and is addressed to: U.S. Patent and Trademark Office, Washington, D.C. 20231-9999

Express Mail Label No. EL 889 330 529 US

March 18, 2002

Wendy M. Lee

TRANSMITTAL OF NEW DRAWINGS TO CORRECT INFORMALITIES WITHIN THREE MONTH PERIOD OF RESPONSE SET IN NOTICE OF ALLOWABILITY (PTOL 37)

BOX ISSUE FEE
Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

1. To correct the informalities in the drawings as noted in the Draftsman's objections on PTO-948 applicant submits herewith new drawings for this application. Number of sheets of drawings submitted: 9.

2. The three month period of response set in the Notice of Allowability (PTOL 37) expires on March 18, 2002 and this submission is on or before this expiry date.

Respectfully submitted,
GENENTECH, INC.

Date: March 18, 2002 By: _____
Wendy M. Lee
Reg. No. 40,378
Telephone No. (650) 225-1994

FIG. 1A

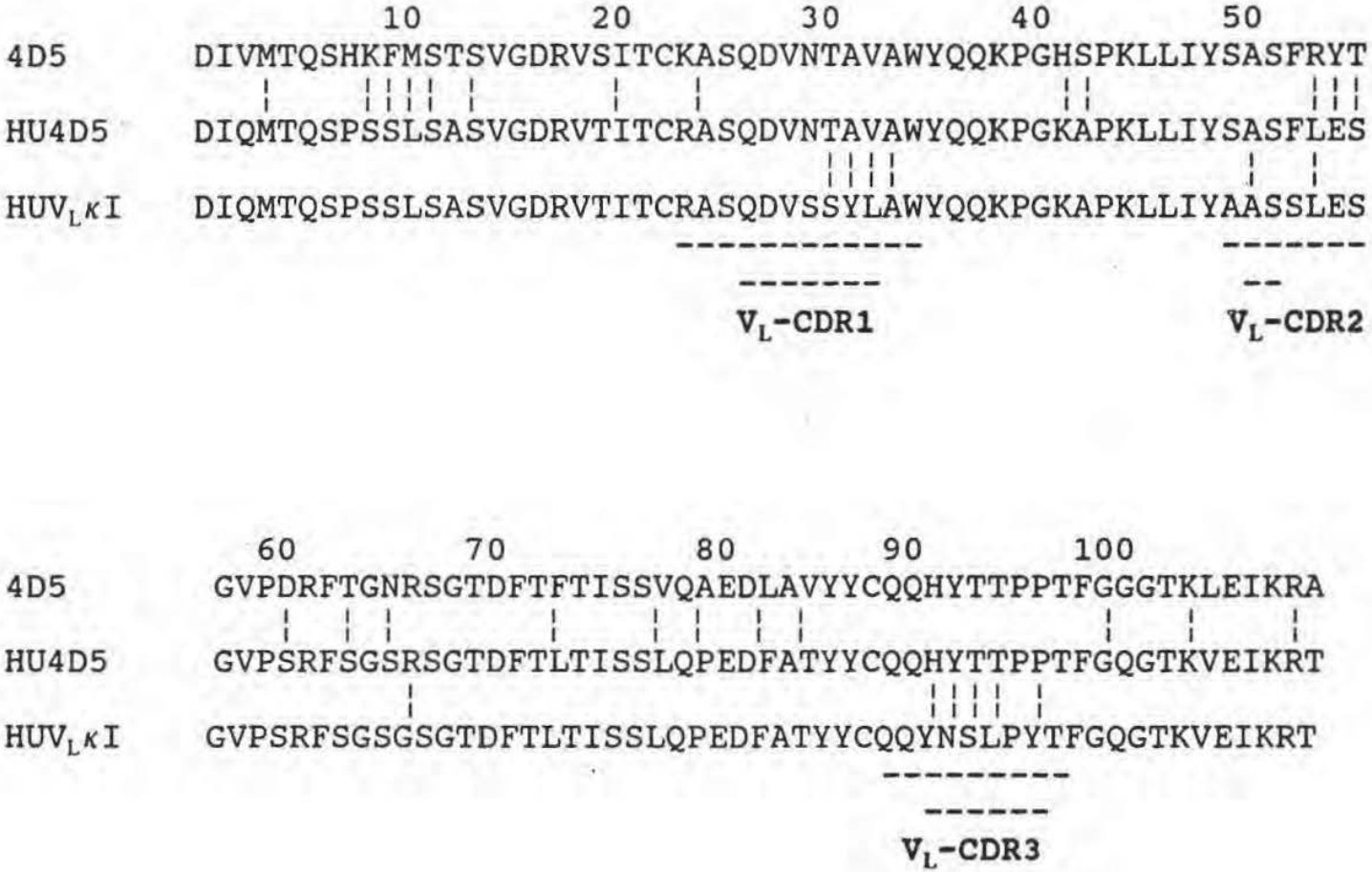
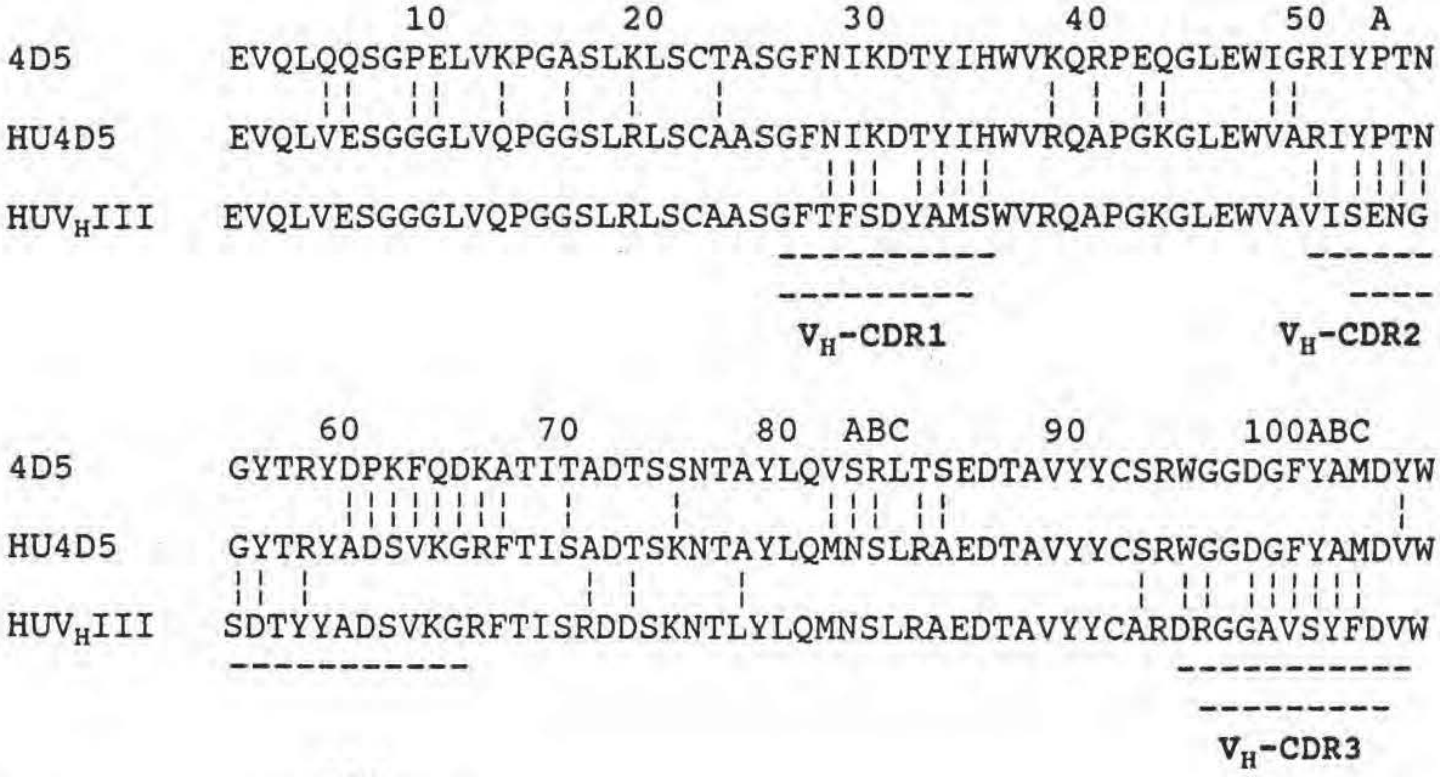

10 20 30 40 50
4D5 DIVMTQSHKFMSTSVGDRVSITCKASQDVNTAVAWYQQKPGHSPKLLIYSASFRYT
HU4D5 DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLES
HUV$_L$κI DIQMTQSPSSLSASVGDRVTITCRASQDVSSYLAWYQQKPGKAPKLLIYAASSLES
V$_L$-CDR1
V$_L$-CDR2
60 70 80 90 100
4D5 GVPDRFTGNRSGTDFTFTISSVQAEDLAVYYCQQHYTTPPTFGGGTKLEIKRA
HU4D5 GVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRT
HUV$_L$κI GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSLPYTFGQGTKVEIKRT
V$_L$-CDR3

FIG. 1B



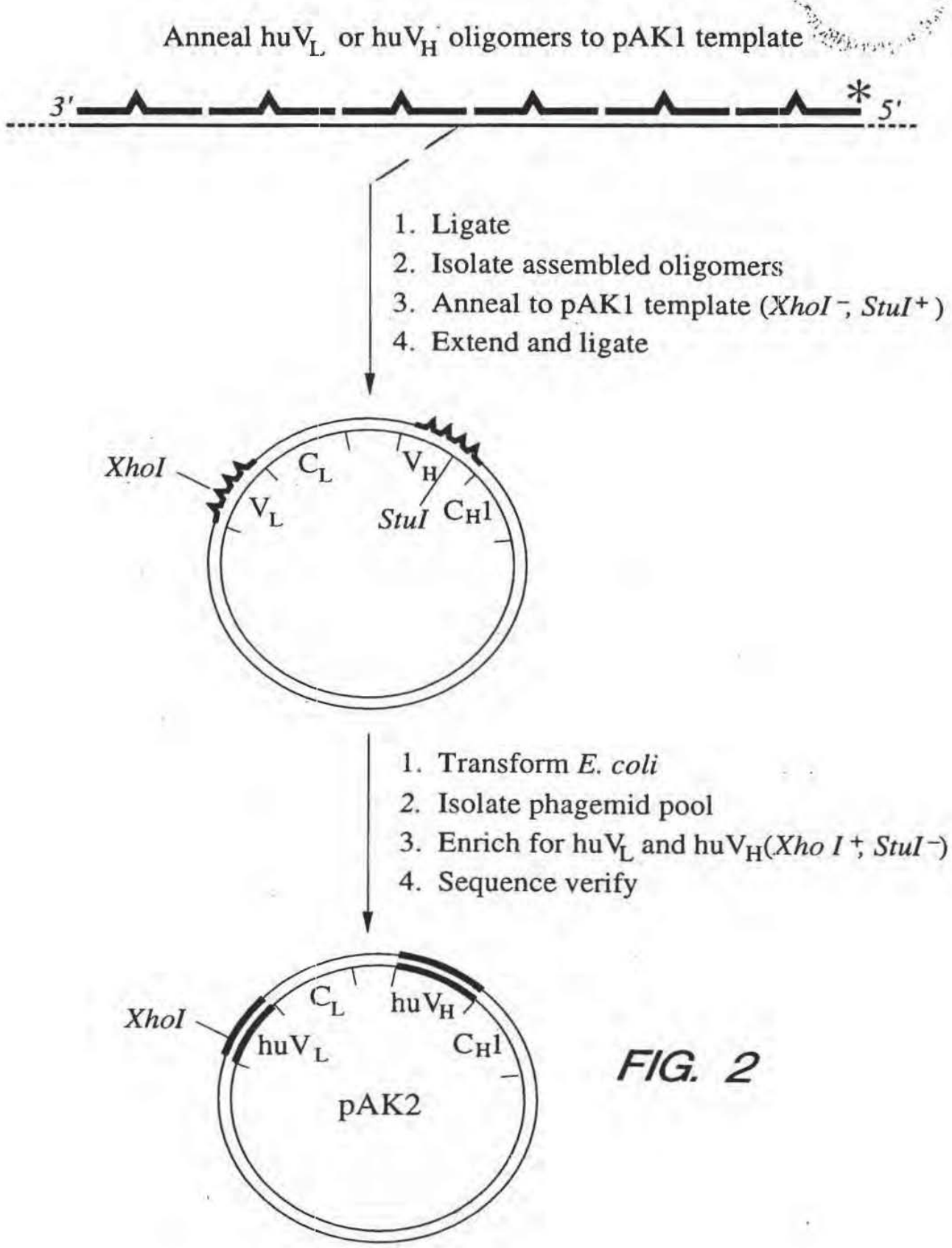


FIG. 2

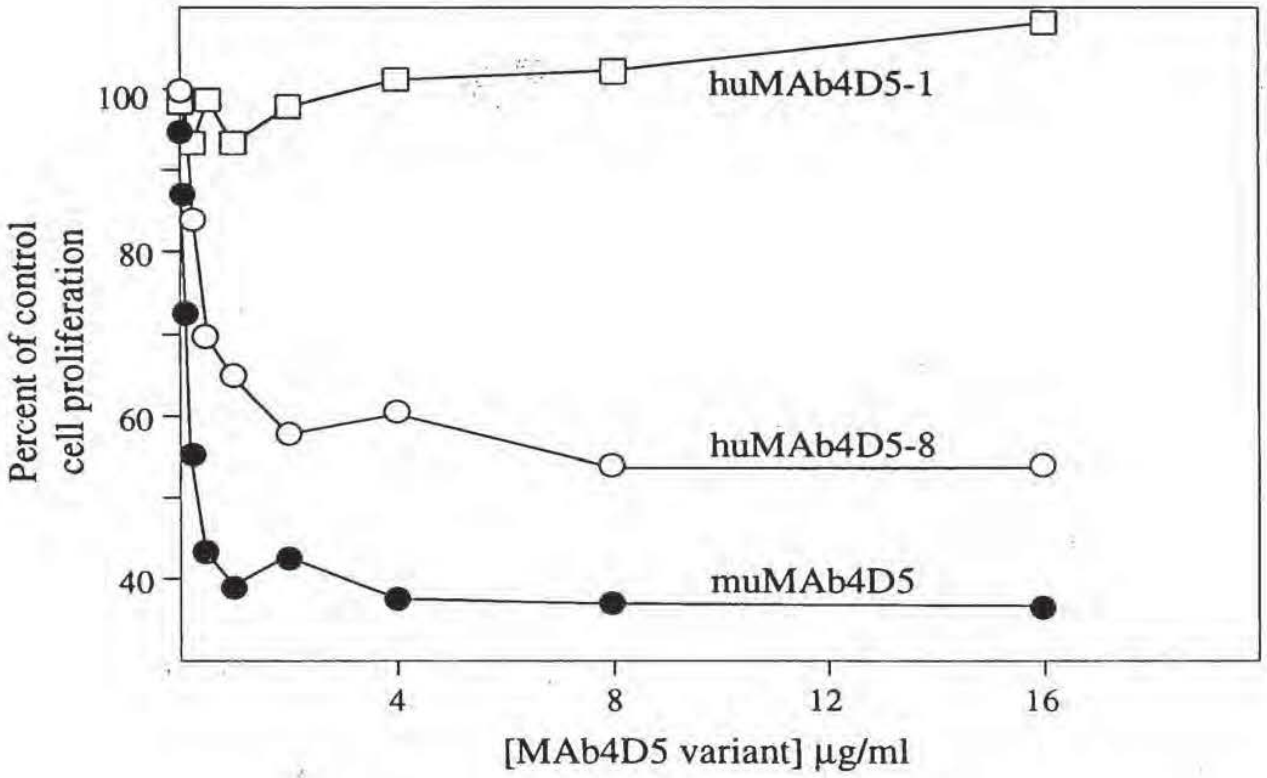

Percent of control cell proliferation
100
80
60
40
huMAb4D5-1
huMAb4D5-8
muMAb4D5
4
8
12
16
[MAb4D5 variant] µg/ml

FIG. 3

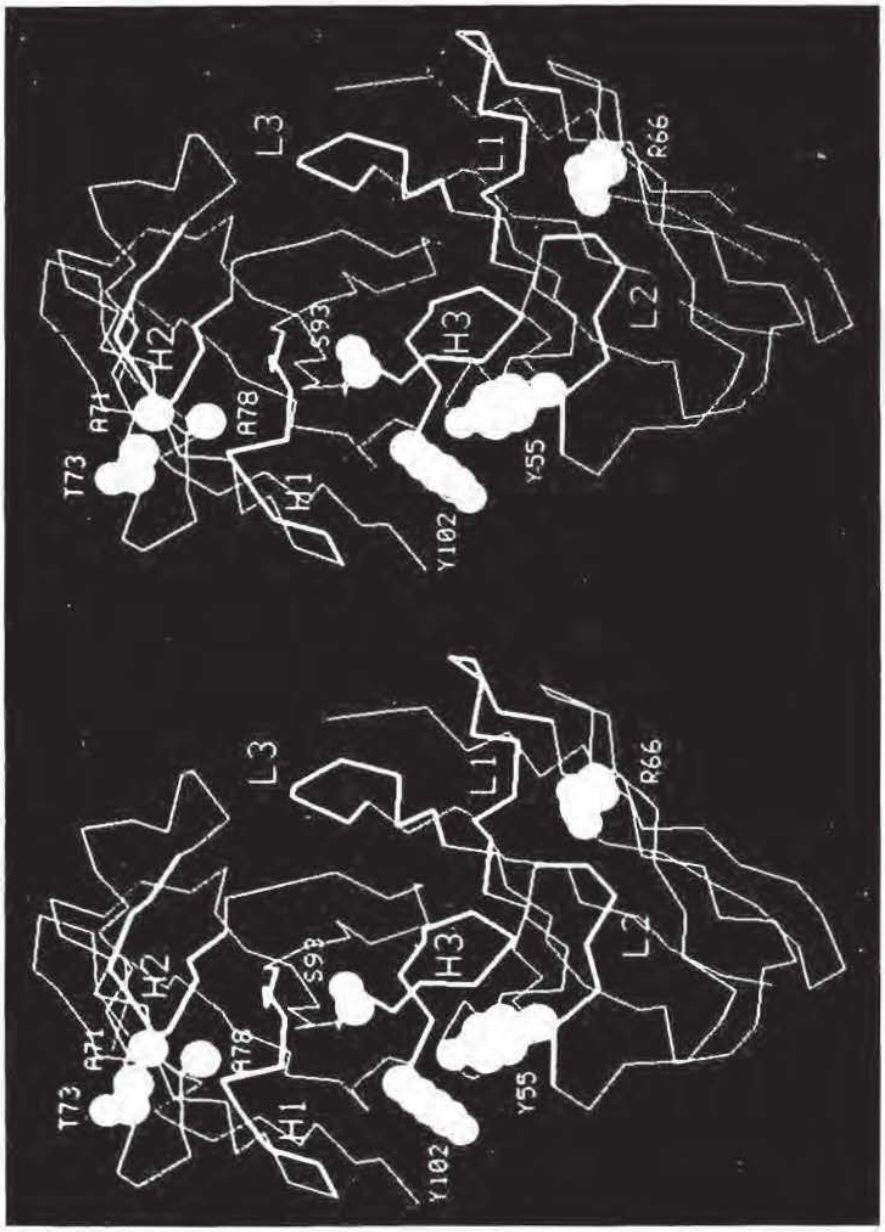


FIG. 4

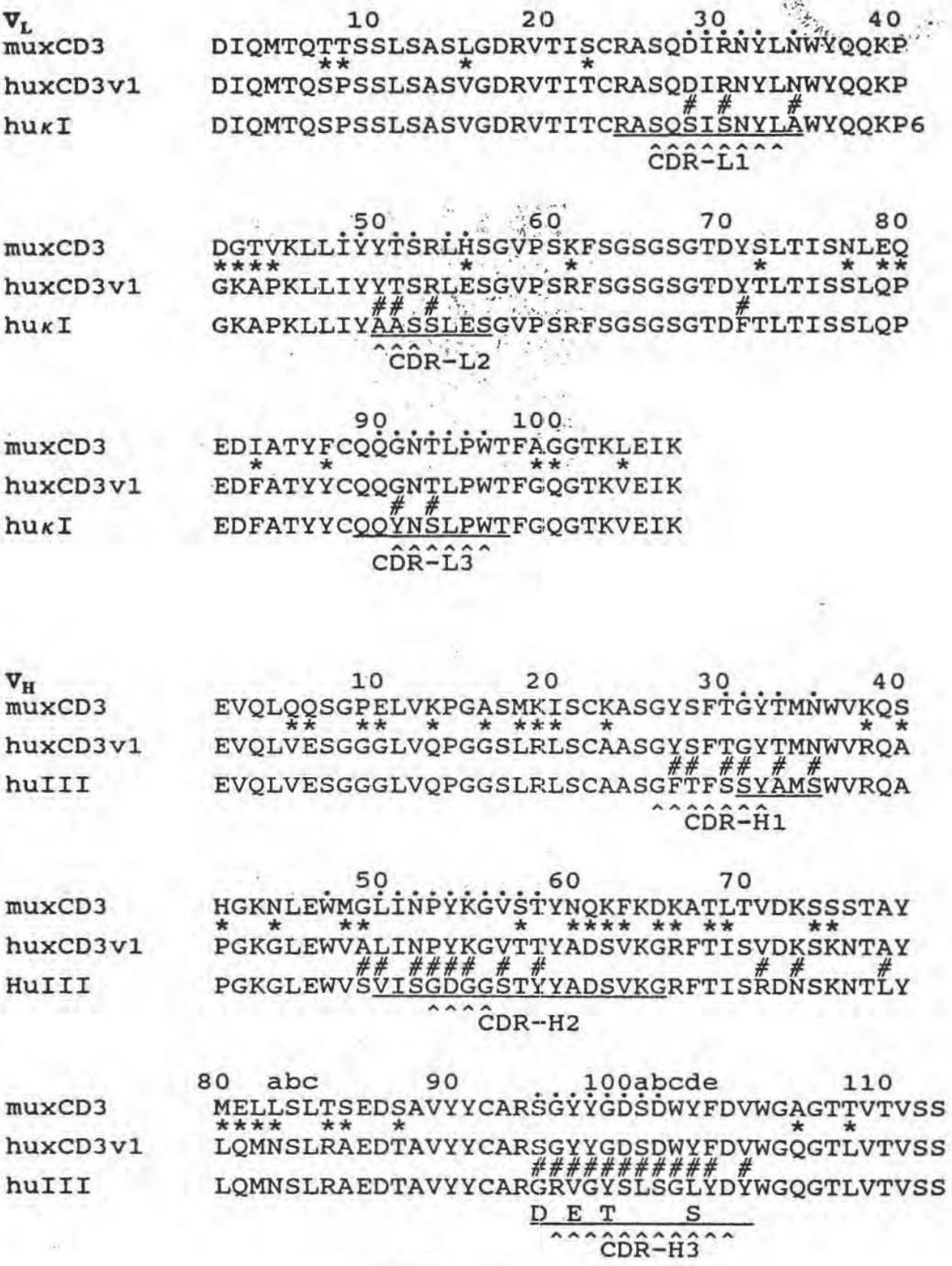


FIG. 5

FIG. 6A-1

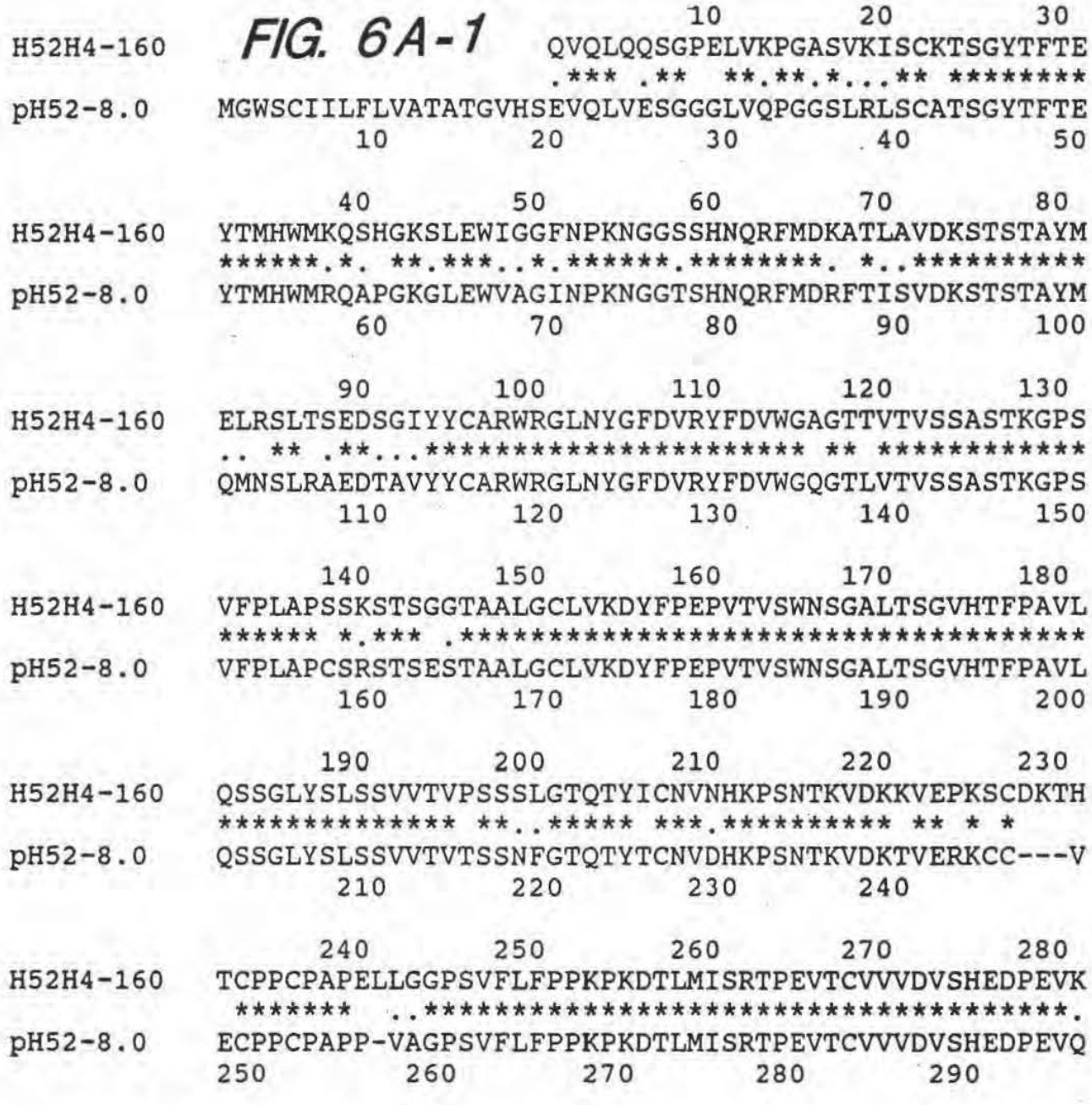


FIG. 6A-2

```
                290        300        310        320        330
H52H4-160       FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS
                *******.*************.***.********.***************
pH52-8.0        FNWYVDGMEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVS
                 300        310        320        330        340

                340        350        360        370        380
H52H4-160       NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP
                **.***********.************************************
pH52-8.0        NKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP
                 350        360        370        380        390

                390        400        410        420        430
H52H4-160       SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
                **********************.****************************
pH52-8.0        SDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFS
                 400        410        420        430        440

                440        450
H52H4-160       CSVMHEALHNHYTQKSLSLSPGK
                ***********************
pH52-8.0        CSVMHEALHNHYTQKSLSLSPGK
                 450        460
```

FIG. 6B

```
                                        10        20        30
H52L6-158                          DVQMTQTTSSLSASLGDRVTINCRASQDINN
                                   *.****. ******.****** *********
pH52-9.0    MGWSCIILFLVATATGVHSDIQMTQSPSSLSASVGDRVTITCRASQDINN
                     10        20        30        40        50

                    40        50        60        70        80
H52L6-158   YLNWYQQKPNGTVKLLIYYTSTLHSGVPSRFSGSGSGTDYSLTISNLDQE
            ********* . ****************************.****.*. *
pH52-9.0    YLNWYQQKPGKAPKLLIYYTSTLHSGVPSRFSGSGSGTDYTLTISSLQPE
                     60        70        80        90        100

                   90       100       110       120       130
H52L6-158   DIATYFCQQGNTLPPTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS
            *.***.*********** ******************************
pH52-9.0    DFATYYCQQGNTLPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS
                     110       120       130       140       150

                   140       150       160       170       180
H52L6-158   VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
            **************************************************
pH52-9.0    VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
                     160       170       180       190       200

                   190       200       210
H52L6-158    SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
             ********************************
pH52-9.0     SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
                     210       220       230
```

03-19-02

PART B - FEE(S) TRANSMITTAL

Complete and mail this form together with applicable fee(s), to: **Box ISSUE FEE**
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MAILING INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 4 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Legibly mark-up with any corrections or use Block 1)

7590 12/18/2001

GENENTECH, INC.
1 DNA WAY
SOUTH SAN FRANCISCO, CA 940804990

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I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Box Issue Fee address above on the date indicated below. [illegible]

Wendy Maria Lee (Typed or printed name)
(Signature)
3/18/02 (Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
08/146,206	11/17/1993	PAUL J. CARTER	7099.1	3992

TITLE OF INVENTION: METHOD FOR MAKING HUMANIZED ANTIBODIES

TOTAL CLAIMS	APPLN. TYPE	SMALL ENTITY	ISSUE FEE	PUBLICATION FEE	TOTAL FEE(S) DUE	DATE DUE
82	nonprovisional	NO	$1280	$0	$1280	03/18/2002

EXAMINER	ART UNIT	CLASS-SUBCLASS
DAVIS, MINH TAM B	1642	530-387300

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363). Use of PTO form(s) and Customer Number are recommended, but not required.

☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47) attached.

2. For printing on the patent front page, list (1) the names of up to 3 registered patent attorneys or agents OR, alternatively, (2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 Wendy M. Lee
2 _____
3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. Inclusion of assignee data is only appropriate when an assignment has been previously submitted to the USPTO or is being submitted under separate cover. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE: Genentech, Inc.

(B) RESIDENCE: (CITY and STATE OR COUNTRY): South San Francisco, California

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ individual ☒ corporation or other private group entity ☐ government

4a. The following fee(s) are enclosed:

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☐ Publication Fee
☒ Advance Order - # of Copies 4

4b. Payment of Fee(s):

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☐ Payment by credit card. Form PTO-2038 is attached.
☒ The Commissioner is hereby authorized by charge the required fee(s), or credit any overpayment, to Deposit Account Number 07-0030 (enclose an extra copy of this form).

The COMMISSIONER OF PATENTS AND TRADEMARKS is requested to apply the Issue Fee and Publication Fee (if any) to the application identified above.

(Authorized Signature) (Date) 3/18/02

Wendy M. Lee - Reg. No. 40,378

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9-25-02

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. U.S. Patent No.: 6,407,213 B1 Issued: June 18, 2002 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	CERTIFICATE OF MAILING I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on August 12, 2002 Wendy M. Lee

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 CFR 1.322

Assistant Commissioner of Patents
Washington, D.C. 20231

Certificate

AUG 27 2002

of Correction

Sir:

Enclosed is a Certificate of Correction for the above-referenced patent. Because the mistake occurred in the printing of the patent, it is not believed that any fee is required. However, if this is not the case, the Commissioner is hereby authorized to charge the required fee to Deposit Account No. 07-0630. Acceptance of this Certificate of Correction is respectfully requested.

Respectfully submitted,
GENENTECH, INC.

Date: August 12, 2002

By: Wendy M. Lee
Wendy M. Lee
Reg. No. 40,378
Telephone No. (650) 225-1994

09157
PATENT TRADEMARK OFFICE

AUG 27 2002

UNITED ST[illegible]ENT AND TRADEMA[illegible] OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : U.S. 6,407,213 B1

DATED : June 18, 2002

INVENTOR(S) : Carter et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In column 88, claim 65, line 63, please delete "63" and insert therefor --79--.

MAILING ADDRESS OF SENDER:

Wendy M. Lee

09157

PATENT TRADEMARK OFFICE

PATENT NO. U.S. 6,407,213

Form PTO1050(amended)

NOTICE RE: CERTIFICATES OF CORRECTION

DATE : 9-25-02 Paper No.: 11

TO : Supervisor, Art Unit 1642

SUBJECT : Certificate of Correction Request in Patent No.: 6,407,213

A response to the following question is requested with respect to the accompanying request for a certific of correction.

With respect to the change(s) requested, correcting Office and/or Applicant's errors, should the patent read as shown in the certificate of correction? No new matter should be introduced, nor should th scope or meaning of the claims be changed.

See red tags.

D. G. Olson

PLEASE COMPLETE THIS FORM AND
RETURN WITH FILE, WITHIN 7 DAYS,
TO CERTIFICATES OF CORRECTION BRANCH - PK 3-915/922
PALM LOCATION 7580 - TEL. NO. 305-8309

THANK YOU FOR YOUR ASSISTANCE!

Note your decision, regarding the changes requested in the Request for Certificate of Correction, placing a check mark (+) in the box that reflects your decision, which corresponds to the question checke above.

☒ YES ☐ NO ☐ Comments below

☐ Comments: Please enter the corrections
Ex: Micah -TAM DAVIS

ANTHONY C. CAPUTA
SUPERVISORY PATENT EXAMINER
TECHNOLOGY

Supervisor

8/16/02

Art Unit

PTOL-306 (REV. 2/02) U.S. DEPARTMENT OF COMMERCE Patent and Trademark O

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,407,213 B1
DATED : June 18, 2002
INVENTOR(S) : Carter et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 88,
Line 63, please delete "63" and insert therefor -- 79 --.

Signed and Sealed this

Third Day of December, 2002

JAMES E. ROGAN
Director of the United States Patent and Trademark Office

Dft

#45

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent of: Paul J. Carter *et al.* -- § 156

Patent No.: 6,407,213

Issued: June 18, 2002

Application No: 08/146,206

For: METHOD FOR MAKING HUMANIZED ANTIBODIES – Application for § 156 Patent Term Extension

Docket No: 22338-80060

Assignee: Genentech, Inc.

Unit: OPLA

CERTIFICATE OF MAILING - 37 C.F.R. § 1.10
EXPRESS MAIL LABEL NO. ER 736919973 US

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Signature	David Devenoe	Aug. 25, 2006
	Printed Name	Date

Mail Stop Patent Ext.
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

APPLICATION FOR EXTENSION OF PATENT TERM UNDER 35 U.S.C. § 156

Dear Sir:

Applicant, Genentech, Inc., hereby submits this application for extension of the term of United States Letters Patent 6,407,213 under 35 U.S.C. § 156 by providing the following information in accordance with the requirements specified in 37 C.F.R. § 1.740.

Applicant represents that it is the assignee of the entire interest in and to United States Letters Patent No. 6,407,213, granted to Paul J. Carter and Leonard G. Presta (Carter *et al.*) by virtue of an assignment of such patent to Genentech, Inc., recorded June 28, 1994, at Reel 7035, Frame 0272.

1. Identification of the Approved Product [§ 1.740(a)(1)]

The name of the approved product is LUCENTIS™. The name of the active ingredient of LUCENTIS™ is ranibizumab. Ranibizumab is a recombinant humanized monoclonal IgG_1 antibody antigen-binding fragment (Fab) based on a humanized framework with complementarity-determining regions (CDRs) derived from a murine monoclonal antibody that binds to human Vascular Endothelial Growth Factor (VEGF).

2. Federal Statute Governing Regulatory Approval of the Approved Product [§ 1.740(a)(2)]

The approved product was subject to regulatory review under, *inter alia*, the Public Health Service Act (42 U.S.C. § 201 *et seq.*) and the Federal Food, Drug and Cosmetic Act (21 U.S.C. § 355 *et seq.*).

3. Date of Approval for Commercial Marketing [§ 1.740(a)(3)]

LUCENTIS™ was approved for commercial marketing or use under § 351 of the Public Heath Service Act on **June 30, 2006**.

4. Identification of Active Ingredient and Certifications Related to Commercial Marketing of Approved Product [§ 1.740(a)(4)]

(a) The active ingredient of LUCENTIS™ is ranibizumab. Ranibizumab is a humanized monoclonal IgG_1 antibody antigen-binding fragment produced by an *E. coli* expression system. It contains human framework regions (FRs) and the complementarity-determining regions (CDRs) derived from a murine antibody that binds to VEGF.

(b) Applicant certifies that ranibizumab had not been approved for commercial marketing or use under the Federal Food, Drug and Cosmetic Act, the Public Health Service Act or the Virus-Serum-Toxin Act prior to the approval granted on June 30, 2006 to the present Applicant.

(c) Ranibizumab has been approved for the treatment of patients with neovascular (wet) age-related macular degeneration. *See* LUCENTIS™ product label, provided as Attachment A.

(d) LUCENTIS™ was approved for commercial marketing pursuant to § 351 of the Public Health Service Act (42 U.S.C. § 262) under Genentech's existing Department of Health and Human Services (DHHS) U.S. License No. 1048. *See* LUCENTIS™ approval letter, provided as Attachment B.

5. Statement Regarding Timeliness of Submission of Patent Term Extension Request [§ 1.740(a)(5)]

Applicant certifies that this application for patent term extension is being timely submitted within the sixty (60) day period permitted for submission specified in 35 U.S.C. § 156(d)(1) and 37 C.F.R. § 1.720(f). The last date on which this application may be submitted is August 28, 2006.

6. Complete Identification of the Patent for Which Extension Is Being Sought [§ 1.740(a)(6)]

The complete identification of the patent for which an extension is being sought is as follows:

(a) Names of the inventors: Paul J. Carter and Leonard G. Presta.

(b) Patent Number: 6,407,213 ("the '213 patent")

(c) Date of Issue: June 18, 2002

(d) Date of Expiration: June 18, 2019

7. Copy of the Patent for Which an Extension is Being Sought [§ 1.740(a)(7)]

A copy of U.S. Patent No. 6,407,213 is provided as Attachment C to the present application.

8. Copies of Disclaimers, Certificates of Correction, Receipt of Maintenance Fee Payment, or Reexamination Certificate [§ 1.740(a)(8)]

(a) U.S. Patent No. 6,407,213 is not subject to a terminal disclaimer.

(b) A Certificate of Correction was issued for U.S. Patent No. 6,407,213 on December 3, 2002. A copy of the Certificate of Correction is provided in Attachment D to the present application.

(c) The first maintenance fee for U.S. Patent No. 6,407,213 has been paid and there are no maintenance fees currently due, as provided in Attachment E.

(d) U.S. Patent No. 6,407,213 has not been the subject of a reexamination proceeding.

9. Statement Regarding Patent Claims Relative to Approved Product [§ 1.740(a)(9)]

The statements below are made solely to comply with the requirements of 37 C.F.R. § 1.740(a)(9). Applicant notes that, as the M.P.E.P. acknowledges, § 1.740(a)(9) does not require an applicant to show whether or how the listed claims would be infringed, and that this question cannot be answered without specific knowledge concerning acts performed by third parties. As such, these comments are not an assertion or an admission of Applicant as to the scope of the listed claims, or whether or how any of the listed claims would be infringed, literally or under the doctrine of equivalents, by the manufacture, use, sale, offer for sale or the importation of any product.

(a) At least claims 1-2, 4-5, 25, 29, 62-64, 66-67, 69, 71-73, 75-78, and 80-81 of U.S. Patent No. 6,407,213 claim the active pharmaceutical ingredient in the approved product or a method that may be used to make or use that ingredient.

(b) Pursuant to M.P.E.P. § 2753 and 37 C.F.R. § 1.740(a)(9), the following explanation is provided which shows how at least one of the above-listed claims of the '213 patent claim the approved product.

(1) Description of the approved product

The approved product is described in Section 11 of the approved label for LUCENTIS™ as follows, a copy of which is provided as Attachment A.

> LUCENTIS™ (ranibizumab injection) is a recombinant humanized IgG1 kappa isotype monoclonal antibody fragment designed for intraocular use. Ranibizumab binds to and inhibits the biologic activity of human vascular endothelial growth factor A (VEGF-A). Ranibizumab has a molecular weight of approximately 48 kilodaltons and is produced by an *E. coli* expression system in a nutrient medium containing the antibiotic tetracycline. Tetracycline is not detectable in the final product.
>
> LUCENTIS™ is a sterile, colorless to pale yellow solution in a single-use glass vial. LUCENTIS™ is supplied as a preservative-free, sterile solution in a single-use glass vial designed to deliver 0.05 mL of 10 mg/mL LUCENTIS™ aqueous solution with 10 mM histidine HCL, 10% α, α-trehalose dihydrate, 0.01% polysorbate 20, pH 5.5.

Ranibizumab is further characterized in a scientific reference, Chen *et al.* published in 1999 in the Journal of Molecular Biology (JMB) entitled "Selection and Analysis of an Optimized Anti-VEGF Antibody: Crystal Structure of an Affinity-matured Fab in Complex with Antigen."[1] The Chen *et al.* article discusses the lineage of the ranibizumab antibody fragment. In this respect, the article states that "[a] murine monoclonal antibody, A.4.6.1, was found to block VEGF-dependent cell proliferation *in vitro* and to antagonize tumor growth *in vivo*. [Citation omitted]. The murine mAb was previously humanized in Fab form to yield a variant known as Fab-12." [Citation omitted] *See* p. 866, left col., ¶1. The abstract explains that the authors affinity-matured Fab-12 and obtained Fab fragment Y0317, now known as ranibizumab. According to the article, ranibizumab was derived from the humanization and affinity-maturation of a non-human, murine monoclonal antibody that binds to VEGF. The Chen *et al.* article also describes the humanized structure of ranibizumab. *See, e.g.,* Figure 1.

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[1] 293:865-881 (1999) (Attachment F)

WO 98/45331 (Figures 1A, 1B, 10A, 10B, provided as Attachment G) also provides sequence data for the heavy and light chain variable domains of Y0317, together with the heavy and light chain variable domains of murine A.4.6.1, the heavy and light chain variable domains of humanized variant Fab-12, and the Kabat human consensus framework, humIII. WO 98/45331 confirms that, in addition to non-human CDRs derived from the sequence of the murine antibody, ranibizumab comprises framework substitutions in the variable domains at positions 4 and 46 in the light chain (V_L) and positions 49, 69, 71, 73, 76, 78, and 94 in the heavy chain (V_H).[2]

(2) Explanation Regarding Claim 29 of the '213 Patent Relative to Ranibizumab

As explained below, the active pharmaceutical ingredient of the approved product, ranibizumab, is a humanized Fab fragment that is covered by at least claim 29.

Claim 29 of the '213 patent reads as follows:

> 29. An antibody comprising the humanized variable domain of claim 1.

Claim 29 depends from claim 1, which reads as follows:

> 1. A humanized antibody variable domain comprising non-human Complementarity Determining Region (CDR) amino acid residues which bind an antigen incorporated into a human antibody variable domain, and further comprising a Framework Region (FR) amino acid substitution at a site selected from the group consisting of: 4L, 38L, 43L, 44L, 58L, 62L, 65L, 66L, 67L, 68L, 69L, 73L, 85L, 98L, 2H, 4H, 36H, 39H, 43H, 45H, 69H, 70H, 74H, and 92H, utilizing the numbering system set forth in Kabat.

The term "antibody," as defined in the '213 patent specification includes, in addition to full-length antibodies, antibody fragments such as Fab, Fab', $F(ab)_2$ and Fv so long as those fragments retain the desired biological activity, *i.e.*, binding to VEGF (*See, e.g.,* '213 at col. 8, lines 11-17; col. 24, lines 13-18). As recited in the '213 specification – "FAb fragments with specificity for the antigen are specifically encompassed within the term 'antibody' as it is defined, discussed, and claimed herein." '213 at col. 24, lines 13-18. Ranibizumab, being

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[2] Compare sequence data for the heavy and light chain variable domains of Y0317 (Figs. 10A-10B), A.4.6.1 (Figs. 1A-1B) and humIII (Figs. 1A-1B) as set forth in WO 98/45331, provided as Attachment G.

a Fab fragment that binds VEGF, falls within the scope of the term "antibody" as used in Claims 1 and 29.

The amino acid sequences of the V_L and V_H domains of ranibizumab include human framework substitutions at positions 4L, 46L, 49H, 69H, 71H, 73H, 76H, 78H and 94H.[3] Of these, substitutions at positions 4L and 69H are among those recited in the Markush group of claim 1. Figures 1A-1B of WO 98/45331, provided as Attachment G, show the heavy and light chain variable domains of sequences of the same import antibody ("A4.6.1") used to design ranibizumab on the lines above the variable domains of the Fab-12 sequence and the Kabat consensus sequences ("humIII").[4] The A4.6.1 antibody is a murine monoclonal antibody; its sequence is therefore "non-human." *See, e.g.,* Chen *et al.* Figures 10A-10B of WO 98/45331, provided as Attachment G (and Figure 1 of Chen *et al.*), show the variable domains of the Y0317 sequence. When the heavy and light chain variable domains of A4.6.1, Y0317 and humIII are aligned, the framework substitutions noted above are apparent utilizing the Kabat numbering system.

In each of the V_L and V_H domains of ranibizumab, "substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species" (*i.e.*, the murine antibody A4.6.1). *See, e.g.,* '213 at col. 2, lines 27-31. Ranibizumab is therefore "humanized" within the meaning of claims 1 and 29 of the '213 patent.

As also required in claim 1, ranibizumab includes non-human amino acid residues in its CDRs. The CDRs in ranibizumab are also functional to "bind an antigen" – here, the VEGF protein. *See* LUCENTIS™ label, provided as Attachment A.

Ranibizumab thus meets the limitations of dependent claim 29.

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3 *See* WO 98/45331 at Figures 1A-1B (humIII) and 10A-10B (Y0317).

4 The residues in a human Ig sequence that are substituted with residues from an "import antibody" are identified according to standard numbering conventions published by Kabat. *See* '213 at col. 10, line 45 through col. 11, line 26. The Kabat sequences represent consensus amino acid sequences for various human antibodies in each subclass. *See id.*

10. Relevant Dates Under 35 U.S.C. § 156 for Determination of Applicable Regulatory Review Period [§ 1.740(a)(10)]

(a) Patent Issue Date

U.S. Patent No. 6,407,213 was issued on June 18, 2002.

(b) IND Effective Date [35 U.S.C. § 156(g)(1)(B)(i); 37 C.F.R. § 1.740(a)(10)(i)(A)]

The date that an exemption under § 505(i) of the Federal Food, Drug and Cosmetic Act became effective (*i.e.*, the date that an investigational new drug application ("IND") became effective) for LUCENTIS ™ (referred to as "Humanized Monoclonal Antibody Fragment (rhuFab V2)(E. coli, Genentech) to Vascular Endothelial Growth Factor (VEGF), Intravitreal) was October 7, 1999. The IND was assigned number BB-IND # 8633. A copy of the letter from the FDA reflecting the effective date of the IND is provided in Attachment H. The application date for this IND was October 6, 1999.

(c) BLA Submission Date [35 U.S.C. § 156(g)(1)(B)(i); 37 C.F.R. § 1.740(a)(10)(i)(B)]

The BLA was submitted by Genentech to the FDA on December 29, 2005. The BLA was assigned number BL# 125156/0. A copy of the letter from the FDA acknowledging receipt of the BLA and reflecting the BLA submission date is provided in Attachment I.

(d) BLA Issue Date [35 U.S.C. § 156(g)(1)(B)(ii); 37 C.F.R. § 1.740(a)(10)(i)(C)]

The FDA approved biologic license application 125156/0 authorizing the marketing of LUCENTIS ™ on June 30, 2006. LUCENTIS ™ was approved under Department of Health and Human Services (DHHS) U.S. License No. 1048. A copy of the approval letter from the FDA is provided as Attachment B.

11. Summary of Significant Events During Regulatory Review Period [§ 1.740(a)(11)]

Pursuant to 37 C.F.R. § 1.740(a)(11), the following provides a brief description of the activities of Genentech, Inc. before the FDA in relation to the regulatory review of LUCENTIS™. The brief description lists the significant events that occurred during the regulatory review period for the approved product. In several instances, communications to or from the FDA are referenced. Pursuant to 37 C.F.R. § 1.740(a)(11), 21 C.F.R. § 60.20(a), and M.P.E.P. § 2753, copies of all such communications are not provided in this application, but can be obtained from records maintained by the FDA.

- On October 6, 1999, Genentech submitted to FDA (See Attachment H) an investigational new drug application for a recombinant humanized monoclonal antibody fragment (rhuFab V2, now known as Ranibizumab) against Vascular Endothelial Growth Factor (VEGF). The antibody was developed as a potential new therapeutic in treating patients with the exudative (wet or neovascular) form of age-related macular degeneration (AMD).

- On October 7, 1999 FDA made BB-IND #8633 effective via a communication mailed to Genentech on October 13, 1999 (*See* Attachment H). According to the FDA, initiation of trials could begin 30 days after October 7, 1999.

- The first human clinical trial (Phase I) was initiated on February 8, 2000 followed by Phase II human trials and Phase III human trials, some of which remain ongoing at the time of this application.

- On February 5, 2002, representatives of Genentech and the FDA (CBER and CDER) participated in a Type C meeting to discuss the proposed clinical development plan for ranibizumab in AMD.

- On October 31, 2002 representatives of Genentech and FDA (CBER and CDER) participated in an Type B End-of-Phase II meeting.

- Beginning in approximately March 2003, and continuing at the time of this application, Phase III studies have been conducted. The three Phase III trials forming the basis of the Biologics License Application (BLA), FVF2598g, FVF2587g, and FVF3192g are studies of two year duration with primary endpoints of one year. FVF2587g and FVF3192g, along with extension study FVF3426g and safety study FVF3689g, remain ongoing at the time of this application.

- On September 21, 2005 representatives of Genentech and CDER participated in a Type B Pre-BLA submission meeting to discuss information requirements for the BLA.

- Genentech submitted a BLA for ranibizumab for the treatment of patients with wet AMD on December 29, 2005 (*See* Attachment I).

- FDA acknowledged receipt of the BLA for ranibizumab via a communication mailed to Genentech dated January 27, 2006. The letter indicated that FDA had assigned the Submission Tracking Number (STN) of BL #125156/0 to the BLA (*See* Attachment I).

- By way of a communication mailed to Genentech on March 14, 2006 FDA made Genentech aware that the BLA for ranibizumab was filed on February 28, 2006 and that FDA had assigned a user fee goal date of June 30, 2006 (*See* Attachment J).

- On June 30, 2006 FDA approved BLA 125156/0, issuing marketing authorization for LUCENTIS™ (*See* Attachment B).

12. Statement Concerning Eligibility for and Duration of Extension Sought Under 35 U.S.C. § 156 [37 C.F.R. § 1.740(a)(12)]

(a) In the opinion of the Applicant, U.S. Patent No. 6,407,213 is eligible for an extension under § 156 because:

(i) one or more claims of the '213 patent claim the approved product or a method of making or using the approved product;

(ii) the term of the '213 patent has not been previously extended on the basis of § 156;

(iii) the '213 patent has not expired;

(iv) no other patent has been extended pursuant to § 156 on the basis of the regulatory review process associated with the approved product, LUCENTIS™;

(v) there is an eligible period of regulatory review by which the patent may be extended pursuant to § 156;

(vi) the applicant for marketing approval exercised due diligence within the meaning of § 156(d)(3) during the period of regulatory review;

(vii) the present application has been submitted within the 60-day period following the approval date of the approved product, pursuant to § 156(c); and

(viii) this application otherwise complies with all requirements of 35 U.S.C. § 156 and applicable rules and procedures.

(b) The period by which the term of the '213 patent is requested by Applicant to be extended is **378 days**.

(c) The requested period of extension of term for the '213 patent corresponds to the regulatory review period that is eligible for extension pursuant to § 156, based on the facts and circumstances of the regulatory review associated with the approved product LUCENTIS™ and the issuance of the '213 patent. The period was determined as follows.

(i) The relevant dates for calculating the regulatory review period, based on the events discussed in the section above, are the following.

Exemption under FDCA § 505(i) became effective	October 7, 1999
Patent was granted	June 18, 2002
Biologics License Application (BLA) under PHSA § 351 was filed	December 29, 2005
BLA was approved	June 30, 2006

(ii) The '213 patent was granted during the period specified in § 156(g)(1)(B)(i) (*i.e.*, the period from the date of the grant of the exemption under § 505(i) of the FDCA until the date of submission of the BLA). Pursuant to § 156(b) and (c)(2), the calculated regulatory review period therefore includes a component of time between when the patent was granted and when the BLA was submitted (1/2 of 1289 days or 644 days).

(iii) The patent was granted prior to the start of the period specified in § 156(g)(1)(B)(ii) (*i.e.*, the period from the date of submission of the BLA until the date of approval). The regulatory review period under § 156(b) therefore includes a component equal to the total number of days in that period that are after the BLA was submitted (184 days).

(iv) The period determined according to § 156(b), (c)(2), and (g)(1) for the approved product (*i.e.*, the number of days following the date of issue of the patent between the dates of submission and of approval of the BLA for LUCENTIS™) is 828 days.

(v) The '213 patent will expire on June 18, 2019.

(vi) The date of approval of the approved product is June 30, 2006.

(vii) The date that is fourteen years from the date of approval of the approved product is June 30, 2020.

(viii) The period measured from the date the patent expires (*i.e.*, June 18, 2019) until the end of the fourteen-year period specified in §156 (c)(3) (*i.e.*, June 30, 2020) is approximately 1 year and 13 days or 378 days.

(ix) The number of days in the regulatory review period determined pursuant to § 156(g)(1)(B)(ii) (i.e., 828 days) exceeds the number of days that the

patent may be extended pursuant to §156(c)(3). As such, the period by which the patent may be extended is limited by the fourteen-year rule of §156(c)(3) to **378 days**.

(x) The '213 patent issued after the effective date of Public Law No. 98-417. As such, the two- or three-year limit of 35 U.S.C. § 156(g)(6)(C) does not apply.

13. Statement Pursuant to 37 C.F.R. § 1.740(a)(13)

Pursuant to 37 C.F.R. § 1.740(a)(13), Applicant acknowledges its duty to disclose to the Director of the PTO and to the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension sought, particularly as that duty is defined in 37 C.F.R. § 1.765.

14. Applicable Fee [§ 1.740(a)(14)]

Our check in payment of the fee prescribed in 37 C.F.R. § 1.20(j) for a patent term extension application under 35 U.S.C. § 156 accompanies this application. Please deduct any additional required fees from, or credit any overpayments to our deposit account no. 18-1260.

15. Name and Address for Correspondence [§ 1.740(a)(14)]

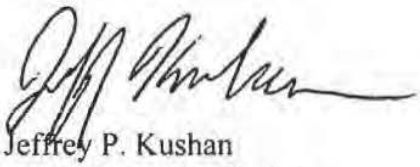
Please direct all inquiries, questions, and communications regarding this application for term extension to:

Jeffrey P. Kushan
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, D.C. 20005
Phone: 202-736-8914
Fax: 202-736-8111
email: jkushan@sidley.com

The correspondence address for U.S. Patent No. 6,407,213 is unchanged for all other purposes. A Power of Attorney granted to the undersigned by the patent assignee, a copy of which is included with this application as Attachment K, accompanies this communication.

Two additional copies of this application are enclosed, in compliance with 37 C.F.R. § 1.740(b). Applicant also provides herewith two further copies of the application for the convenience of the Office, pursuant to M.P.E.P. § 2763.

Sincerely,



Jeffrey P. Kushan
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Sidley Austin LLP
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Washington, D.C. 20005

Dated: August 25, 2006

INDEX OF ATTACHMENTS

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use LUCENTIS safely and effectively. See full prescribing information for LUCENTIS.

LUCENTIS™ (ranibizumab injection)

Initial U.S. Approval: 2006

----------INDICATIONS AND USAGE----------

LUCENTIS is indicated for the treatment of patients with neovascular (wet) age-related macular degeneration (1).

----------DOSAGE AND ADMINISTRATION----------

- FOR OPHTHALMIC INTRAVITREAL INJECTION ONLY (2.1)
- LUCENTIS 0.5 mg (0.05 mL) is recommended to be administered by intravitreal injection once a month (2.2).
- Although less effective, treatment may be reduced to one injection every three months after the first four injections if monthly injections are not feasible. Compared to continued monthly dosing, dosing every 3 months will lead to an approximate 5-letter (1-line) loss of visual acuity benefit, on average, over the following 9 months. Patients should be evaluated regularly (2.2).

----------DOSAGE FORMS AND STRENGTHS----------

- 10 mg/mL single-use vial (3)

----------CONTRAINDICATIONS----------

- Ocular or periocular infections (4.1)
- Hypersensitivity (4.2)

----------WARNINGS AND PRECAUTIONS----------

- Endophthalmitis and retinal detachments may occur following intravitreal injections. Patients should be monitored during the week following the injection (5.1).
- Increases in intraocular pressure have been noted within 60 minutes of intravitreal injection (5.2).

----------ADVERSE REACTIONS----------

The most common adverse reactions (reported ≥ 6% higher in LUCENTIS-treated subjects than control subjects) are conjunctival hemorrhage, eye pain, vitreous floaters, increased intraocular pressure, and intraocular inflammation (6.2).

To report SUSPECTED ADVERSE REACTIONS, contact Genentech at 1-888-835-2555 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

See Section 17 for PATIENT COUNSELING INFORMATION.

FULL PRESCRIBING INFORMATION: CONTENTS*

* Sections or subsections omitted from the Full Prescribing Information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

LUCENTIS is indicated for the treatment of patients with neovascular (wet) age-related macular degeneration.

2 DOSAGE AND ADMINISTRATION

2.1 General Dosing Information

FOR OPHTHALMIC INTRAVITREAL INJECTION ONLY.

2.2 Dosing

LUCENTIS 0.5 mg (0.05 mL) is recommended to be administered by intravitreal injection once a month.

Although less effective, treatment may be reduced to one injection every three months after the first four injections if monthly injections are not feasible. Compared to continued monthly dosing, dosing every 3 months will lead to an approximate 5-letter (1-line) loss of visual acuity benefit, on average, over the following 9 months. Patients should be evaluated regularly [*see Clinical Studies (14.2)*].

2.3 Preparation for Administration

Using aseptic technique, all (0.2 mL) of the LUCENTIS vial contents are withdrawn through a 5-micron 19-gauge filter needle attached to a 1-cc tuberculin syringe. The filter needle should be discarded after withdrawal of the vial contents and should not be used for intravitreal injection. The filter needle should be replaced with a sterile 30-gauge × 1/2-inch needle for the intravitreal injection. The contents should be expelled until the plunger tip is aligned with the line that marks 0.05 mL on the syringe.

2.4 Administration

The intravitreal injection procedure should be carried out under controlled aseptic conditions, which include the use of sterile gloves, a sterile drape, and a sterile eyelid speculum (or equivalent). Adequate anesthesia and a broad-spectrum microbicide should be given prior to the injection.

Following the intravitreal injection, patients should be monitored for elevation in intraocular pressure and for endophthalmitis. Monitoring may consist of a check for perfusion of the optic nerve head immediately after the injection, tonometry within 30 minutes following the injection, and biomicroscopy between two and seven days following the injection. Patients should be instructed to report any symptoms suggestive of endophthalmitis without delay.

Each vial should only be used for the treatment of a single eye. If the contralateral eye requires treatment, a new vial should be used and the sterile field, syringe, gloves, drapes, eyelid speculum, filter, and injection needles should be changed before LUCENTIS is administered to the other eye.

No special dosage modification is required for any of the populations that have been studied (e.g., gender, elderly).

2.5 Stability and Storage

LUCENTIS should be refrigerated at 2°-8°C (36°-46°F). DO NOT FREEZE. Do not use beyond the date stamped on the label. LUCENTIS vials should be protected from light. Store in the original carton until time of use.

3 DOSAGE FORMS AND STRENGTHS

Single-use glass vial designed to deliver 0.05 mL of 10 mg/mL.

4 CONTRAINDICATIONS

4.1 Ocular or Periocular Infections

LUCENTIS is contraindicated in patients with ocular or periocular infections.

4.2 Hypersensitivity

LUCENTIS is contraindicated in patients with known hypersensitivity to ranibizumab or any of the excipients in LUCENTIS.

5 WARNINGS AND PRECAUTIONS

5.1 Endophthalmitis and Retinal Detachments

Intravitreal injections, including those with LUCENTIS, have been associated with endophthalmitis and retinal detachments. Proper aseptic injection technique should always be used when administering LUCENTIS. In addition, patients should be monitored during the week following the injection to permit early treatment should an infection occur [*see Dosage and Administration (2.3, 2.4) and Patient Counseling Information (17)*].

5.2 Increases in Intraocular Pressure

Increases in intraocular pressure have been noted within 60 minutes of intravitreal injection with LUCENTIS. Therefore, intraocular pressure as well as the perfusion of the optic nerve head should be monitored and managed appropriately [*see Dosage and Administration (2.4)*].

5.3 Thromboembolic Events

Although there was a low rate (<4%) of arterial thromboembolic events observed in the LUCENTIS clinical trials, there is a theoretical risk of arterial thromboembolic events following intravitreal use of inhibitors of VEGF [*see Adverse Reactions (6.3)*].

6 ADVERSE REACTIONS

6.1 Injection Procedure

Serious adverse events related to the injection procedure have occurred in < 0.1% of intravitreal injections, including endophthalmitis [*see Warnings and Precautions (5.1)*], rhegmatogenous retinal detachments, and iatrogenic traumatic cataracts.

6.2 Clinical Trials Experience – Ocular Events

Other serious ocular adverse events observed among LUCENTIS-treated patients occurring in <2% of patients

included intraocular inflammation and increased intraocular pressure [*see Warnings and Precautions (5.1, 5.2)*].

The available safety data include exposure to LUCENTIS in 874 patients with neovascular age-related macular degeneration in three double-masked, controlled studies with dosage regimens of 0.3 mg (375 patients) or 0.5 mg (379 patients) administered monthly by intravitreal injection (Studies 1 and 2) [*see Clinical Studies (14.1)*] and dosage regimens of 0.3 mg (59 patients) or 0.5 mg (61 patients) administered once a month for 3 consecutive doses followed by a dose administered once every 3 months (Study 3) [*see Clinical Studies (14.2)*].

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in one clinical trial of a drug cannot be directly compared with rates in the clinical trials of the same or another drug and may not reflect the rates observed in practice.

Table 1 shows the most frequently reported ocular adverse events that were reported with LUCENTIS treatment. The ranges represent the maximum and minimum rates across all three studies for control, and across all three studies and both dose groups for LUCENTIS.

Table 1

Adverse Event	LUCENTIS	Control
Conjunctival hemorrhage	77%-43%	66%-29%
Eye pain	37%-17%	33%-11%
Vitreous floaters	32%-3%	10%-3%
Retinal hemorrhage	26%-15%	56%-37%
Intraocular pressure increased	24%-8%	7%-3%
Vitreous detachment	22%-7%	18%-13%
Intraocular inflammation	18%-5%	11%-3%
Eye irritation	19%-4%	20%-6%
Cataract	16%-5%	16%-6%
Foreign body sensation in eyes	19%-6%	14%-6%
Lacrimation increased	17%-3%	16%-0%
Eye pruritis	13%-0%	12%-3%
Visual disturbance	14%-0%	9%-2%
Blepharitis	13%-3%	9%-4%
Subretinal fibrosis	13%-0%	19%-10%
Ocular hyperemia	10%-5%	10%-1%
Maculopathy	10%-3%	11%-3%
Visual acuity blurred/decreased	17%-4%	24%-10%
Detachment of the retinal pigment epithelium	11%-1%	15%-3%
Dry eye	10%-3%	8%-5%
Ocular discomfort	8%-0%	5%-0%
Conjunctival hyperemia	9%-0%	7%-0%
Posterior capsule opacification	8%-0%	5%-0%
Retinal exudates	9%-1%	11%-3%

6.3 Clinical Trials Experience – Non-Ocular Events

Table 2 shows the most frequently reported non-ocular adverse events with LUCENTIS treatment. The ranges represent the maximum and minimum rates across all three studies for control, and across all three studies and both dose groups for LUCENTIS.

Table 2

Adverse Event	LUCENTIS	Control
Hypertension/elevated blood pressure	23%-5%	23%-8%
Nasopharyngitis	16%-5%	13%-5%
Arthralgia	11%-3%	9%-0%
Headache	15%-2%	10%-3%
Bronchitis	10%-3%	8%-2%
Cough	10%-3%	7%-2%
Anemia	8%-3%	8%-0%
Nausea	9%-2%	6%-4%
Sinusitis	8%-2%	6%-4%
Upper respiratory tract infection	15%-2%	10%-4%
Back pain	10%-1%	9%-0%
Urinary tract infection	9%-4%	8%-5%
Influenza	10%-2%	5%-1%
Arthritis	8%-0%	8%-2%
Dizziness	8%-2%	10%-2%
Constipation	7%-3%	8%-2%

The rate of arterial thromboembolic events in the three studies in the first year was 2.1% of patients (18 out of 874) in the combined group of patients treated with 0.3 mg or 0.5 mg LUCENTIS compared with 1.1% of patients (5 out of 441) in the control arms of the studies. In the second year of Study 1, the rate of arterial thromboembolic events was 3.0% of patients (14 out of 466) in the combined group of patients treated with 0.3 mg or 0.5 mg LUCENTIS compared with 3.2% of patients (7 out of 216) in the control arm [*see Warnings and Precautions (5.3)*].

6.4 Immunogenicity

The pre-treatment incidence of immunoreactivity to LUCENTIS was 0%-3% across treatment groups. After monthly dosing with LUCENTIS for 12 to 24 months, low titers of antibodies to LUCENTIS were detected in approximately 1%-6% of patients. The immunogenicity data reflect the percentage of patients whose test results were considered positive for antibodies to LUCENTIS in an electrochemiluminescence assay and are highly dependent on the sensitivity and specificity of the assay. The clinical significance of immunoreactivity to LUCENTIS is unclear at this time, although some patients with the highest levels of immunoreactivity were noted to have iritis or vitritis.

7 DRUG INTERACTIONS

Drug interaction studies have not been conducted with LUCENTIS.

LUCENTIS intravitreal injection has been used adjunctively with verteporfin photodynamic therapy (PDT). Twelve of 105 (11%) patients developed serious intraocular inflammation; in 10 of the 12 patients, this occurred when LUCENTIS was administered 7 days (± 2 days) after verteporfin PDT.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy
Pregnancy Category C. Animal reproduction studies have not been conducted with ranibizumab. It is also not known whether ranibizumab can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. LUCENTIS should be given to a pregnant woman only if clearly needed.

8.3 Nursing Mothers
It is not known whether ranibizumab is excreted in human milk. Because many drugs are excreted in human milk, and because the potential for absorption and harm to infant growth and development exists, caution should be exercised when LUCENTIS is administered to a nursing woman.

8.4 Pediatric Use
The safety and effectiveness of LUCENTIS in pediatric patients has not been established.

8.5 Geriatric Use
In the controlled clinical studies, approximately 94% (822/879) of the patients randomized to treatment with LUCENTIS were ≥ 65 years of age and approximately 68% (601/879) were ≥ 75 years of age. No notable difference in treatment effect was seen with increasing age in any of the studies. Age did not have a significant effect on systemic exposure in a population pharmacokinetic analysis after correcting for creatinine clearance.

8.6 Patients with Renal Impairment
No formal studies have been conducted to examine the pharmacokinetics of ranibizumab in patients with renal impairment. Sixty-eight percent of patients (136 of 200) in the population pharmacokinetic analysis had renal impairment (46.5% mild, 20% moderate, and 1.5% severe). Reduction in ranibizumab clearance is minimal in patients with renal impairment and is considered clinically insignificant. Dose adjustment is not expected to be needed for patients with renal impairment.

8.7 Patients with Hepatic Dysfunction
No formal studies have been conducted to examine the pharmacokinetics of ranibizumab in patients with hepatic impairment. Dose adjustment is not expected to be needed for patients with hepatic dysfunction.

10 OVERDOSAGE
Planned initial single doses of ranibizumab injection 1.0 mg were associated with clinically significant intraocular inflammation in 2 of 2 patients injected. With an escalating regimen of doses beginning with initial doses of ranibizumab injection 0.3 mg, doses as high as 2.0 mg were tolerated in 15 of 20 patients.

11 DESCRIPTION
LUCENTIS™ (ranibizumab injection) is a recombinant humanized IgG1 kappa isotype monoclonal antibody fragment designed for intraocular use. Ranibizumab binds to and inhibits the biologic activity of human vascular endothelial growth factor A (VEGF-A). Ranibizumab has a molecular weight of approximately 48 kilodaltons and is produced by an *E. coli* expression system in a nutrient medium containing the antibiotic tetracycline. Tetracycline is not detectable in the final product.

LUCENTIS is a sterile, colorless to pale yellow solution in a single-use glass vial. LUCENTIS is supplied as a preservative-free, sterile solution in a single-use glass vial designed to deliver 0.05 mL of 10 mg/mL LUCENTIS aqueous solution with 10 mM histidine HCl, 10% α, α-trehalose dihydrate, 0.01% polysorbate 20, pH 5.5.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action
Ranibizumab binds to the receptor binding site of active forms of VEGF-A, including the biologically active, cleaved form of this molecule, $VEGF_{110}$. VEGF-A has been shown to cause neovascularization and leakage in models of ocular angiogenesis and is thought to contribute to the progression of the neovascular form of age-related macular degeneration (AMD). The binding of ranibizumab to VEGF-A prevents the interaction of VEGF-A with its receptors (VEGFR1 and VEGFR2) on the surface of endothelial cells, reducing endothelial cell proliferation, vascular leakage, and new blood vessel formation.

12.2 Pharmacodynamics
Neovascular AMD is associated with foveal retinal thickening as assessed by optical coherence tomography (OCT) and leakage from CNV as assessed by fluorescein angiography.

In Study 3, foveal retinal thickness was assessed by OCT in 118/184 patients. OCT measurements were collected at baseline, Months 1, 2, 3, 5, 8, and 12. In patients treated with LUCENTIS, foveal retinal thickness decreased, on average, more than the sham group from baseline through Month 12. Retinal thickness decreased by Month 1 and decreased further at Month 3, on average. Foveal retinal thickness data did not provide information useful in influencing treatment decisions [*see Clinical Studies (14.2)*].

In patients treated with LUCENTIS, the area of vascular leakage, on average, decreased by Month 3 as assessed by fluorescein angiography. The area of vascular leakage for an individual patient was not correlated with visual acuity.

12.3 Pharmacokinetics
In animal studies, following intravitreal injection, ranibizumab was cleared from the vitreous with a half-life of approximately 3 days. After reaching a maximum at approximately 1 day,

the serum concentration of ranibizumab declined in parallel with the vitreous concentration. In these animal studies, systemic exposure of ranibizumab is more than 2000-fold lower than in the vitreous.

In patients with neovascular AMD, following monthly intravitreal administration, maximum ranibizumab serum concentrations were low (0.3 ng/mL to 2.36 ng/mL). These levels were below the concentration of ranibizumab (11 ng/mL to 27 ng/mL) thought to be necessary to inhibit the biological activity of VEGF-A by 50%, as measured in an in vitro cellular proliferation assay. The maximum observed serum concentration was dose proportional over the dose range of 0.05 to 1.0 mg/eye. Based on a population pharmacokinetic analysis, maximum serum concentrations of 1.5 ng/mL are predicted to be reached at approximately 1 day after monthly intravitreal administration of LUCENTIS 0.5 mg/eye. Based on the disappearance of ranibizumab from serum, the estimated average vitreous elimination half-life was approximately 9 days. Steady-state minimum concentration is predicted to be 0.22 ng/mL with a monthly dosing regimen. In humans, serum ranibizumab concentrations are predicted to be approximately 90,000-fold lower than vitreal concentrations.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

No carcinogenicity or mutagenicity data are available for ranibizumab injection in animals or humans.

No studies on the effects of ranibizumab on fertility have been conducted.

14 CLINICAL STUDIES

The safety and efficacy of LUCENTIS were assessed in three randomized, double-masked, sham- or active-controlled studies in patients with neovascular AMD. A total of 1323 patients (LUCENTIS 879, Control 444) were enrolled in the three studies.

14.1 Study 1 and Study 2

In Study 1, patients with minimally classic or occult (without classic) CNV lesions received monthly LUCENTIS 0.3 mg or 0.5 mg intravitreal injections or monthly sham injections. Data are available through Month 24. Patients treated with LUCENTIS in Study 1 received a mean of 22 total treatments out of a possible 24 from Day 0 to Month 24.

In Study 2, patients with predominantly classic CNV lesions received one of the following: 1) monthly LUCENTIS 0.3 mg intravitreal injections and sham PDT; 2) monthly LUCENTIS 0.5 mg intravitreal injections and sham PDT; or 3) sham intravitreal injections and active verteporfin PDT. Sham PDT (or active verteporfin PDT) was given with the initial LUCENTIS (or sham) intravitreal injection and every 3 months thereafter if fluorescein angiography showed persistence or recurrence of leakage. Data are available through Month 12. Patients treated with LUCENTIS in Study 2 received a mean of 12 total treatments out of a possible 13 from Day 0 through Month 12.

In both studies, the primary efficacy endpoint was the proportion of patients who maintained vision, defined as losing fewer than 15 letters of visual acuity at 12 months compared with baseline. Almost all LUCENTIS-treated patients (approximately 95%) maintained their visual acuity. 34%-40% of LUCENTIS-treated patients experienced a clinically significant improvement in vision, defined as gaining 15 or more letters at 12 months. The size of the lesion did not significantly affect the results. Detailed results are shown in the tables below.

Table 3
Outcomes at Month 12 and Month 24 in Study 1

Outcome Measure	Month	Sham n = 238	LUCENTIS 0.5 mg n = 240	Estimated Difference (95% CI)[a]
Loss of < 15 letters in visual acuity (%)[b]	Month 12	62%	95%	32% (26%, 39%)
	Month 24	53%	90%	37% (29%, 44%)
Gain of ≥ 15 letters in visual acuity (%)[b]	Month 12	5%	34%	29% (22%, 35%)
	Month 24	4%	33%	29% (23%, 35%)
Mean change in visual acuity (letters) (SD)[b]	Month 12	-10.5 (16.6)	+7.2 (14.4)	17.5 (14.8, 20.2)
	Month 24	-14.9 (18.7)	+6.6 (16.5)	21.1 (18.1, 24.2)

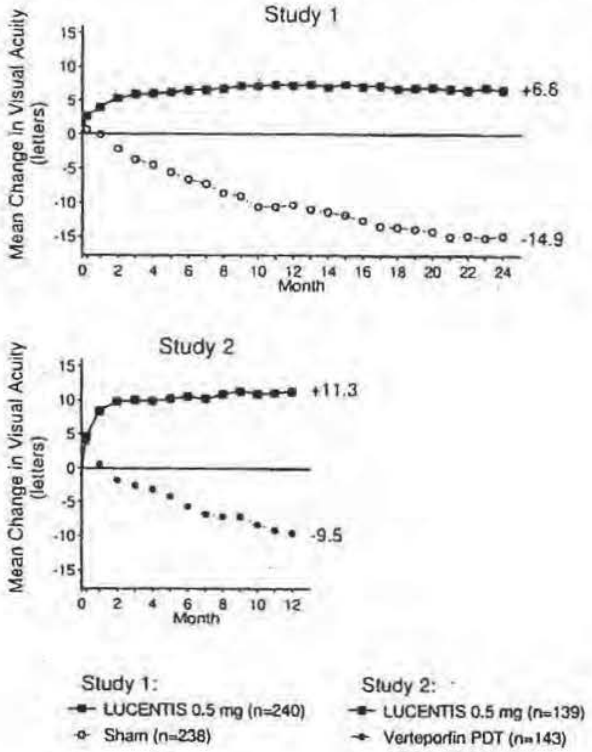
[a] Adjusted estimate based on the stratified model.
[b] $p<0.01$.

Table 4
Outcomes at Month 12 in Study 2

Outcome Measure	Verteporfin PDT n = 143	LUCENTIS 0.5 mg n = 140	Estimated Difference (95% CI)[a]
Loss of < 15 letters in visual acuity (%)[b]	64%	96%	33% (25%, 41%)
Gain of ≥ 15 letters in visual acuity (%)[b]	6%	40%	35% (26%, 44%)
Mean change in visual acuity (letters) (SD)[b]	-9.5 (16.4)	+11.3 (14.6)	21.1 (17.5, 24.6)

[a] Adjusted estimate based on the stratified model.
[b] $p<0.01$.

Figure 1
Mean Change in Visual Acuity from Baseline to Month 24 in Study 1 and to Month 12 in Study 2



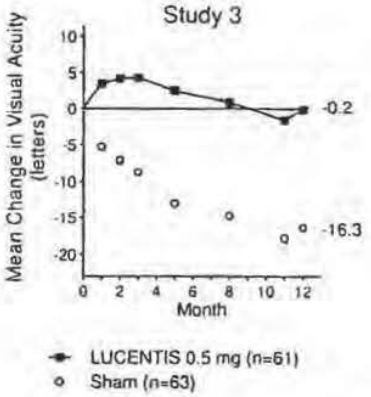
Patients in the group treated with LUCENTIS had minimal observable CNV lesion growth, on average. At Month 12, the mean change in the total area of the CNV lesion was 0.1-0.3 DA for LUCENTIS versus 2.3-2.6 DA for the control arms.

The use of LUCENTIS beyond 24 months has not been studied.

14.2 Study 3
Study 3 was a randomized, double-masked, sham-controlled, two-year study designed to assess the safety and efficacy of LUCENTIS in patients with neovascular AMD (with or without a classic CNV component). Data are available through Month 12. Patients received LUCENTIS 0.3 mg or 0.5 mg intravitreal injections or sham injections once a month for 3 consecutive doses, followed by a dose administered once every 3 months. A total of 184 patients were enrolled in this study (LUCENTIS 0.3 mg, 60; LUCENTIS 0.5 mg, 61; sham, 63); 171 (93%) completed 12 months of this study. Patients treated with LUCENTIS in Study 3 received a mean of 6 total treatments out of possible 6 from Day 0 through Month 12.

In Study 3, the primary efficacy endpoint was mean change in visual acuity at 12 months compared with baseline (see Figure 2). After an initial increase in visual acuity (following monthly dosing), on average, patients dosed once every three months with LUCENTIS lost visual acuity, returning to baseline at Month 12. In Study 3, almost all LUCENTIS-treated patients (90%) maintained their visual acuity at Month 12.

Figure 2
Mean Change in Visual Acuity from Baseline to Month 12 in Study 3



16 HOW SUPPLIED/STORAGE AND HANDLING
Each LUCENTIS carton, NDC 50242-080-01, contains one 2-cc glass vial of ranibizumab, one 5-micron, 19-gauge × 1-1/2-inch filter needle for withdrawal of the vial contents, one 30-gauge × 1/2-inch injection needle for the intravitreal injection, and one package insert [*see Dosage and*

Administration (2.4)]. VIALS ARE FOR SINGLE EYE USE ONLY.

17 PATIENT COUNSELING INFORMATION

In the days following LUCENTIS administration, patients are at risk of developing endophthalmitis. If the eye becomes red, sensitive to light, painful, or develops a change in vision, the patient should seek immediate care from an ophthalmologist [*see Warnings and Precautions (5.1)*].

LUCENTIS™ [ranibizumab injection]

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

8277700
LL1404
4833801
FDA Approval Date:
June 2006

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville, MD 20852

BLA 125156

Genentech, Inc.
Attention: Robert L. Garnick, Ph.D.
Senior Vice President, Regulatory Affairs, Quality & Compliance
1 DNA Way
South San Francisco, California 94080-4990

Dear Dr. Garnick:

We have approved your biologics' license application for Lucentis (ranibizumab injection) effective this date. You are hereby authorized to introduce or deliver for introduction into interstate commerce, ranibizumab injection under your existing Department of Health and Human Services U.S. License No. 1048. Lucentis (ranibizumab injection) is indicated for the treatment of patients with neovascular (wet) age-related macular degeneration.

Under this license, you are approved to manufacture ranibizumab drug substance at Genentech, Inc., South San Francisco, California; fill the final formulated product at [redacted] and label and package filled vials at Genentech, Inc., South San Francisco, California. You may label your product with the proprietary name Lucentis and market it in 10 mg/mL single use glass vials.

We acknowledge receipt of your submissions dated December 29, 2005, and January 31, February 10, 17, 21, and 24, March 17, 23, and 31, April 10, and 28, May 5, 10, 25 (2), 26 (2), and 31, and June 1, 5 (2), 6, 9, 13, 16, 23, 26, 27, 28 (3), and 29, 2006.

The final printed labeling (FPL) must be identical in content to the enclosed labeling text for the package insert, submitted June 28, 2006; the immediate vial container submitted March 31, 2006; and the carton labels submitted June 5, 2006. The statement "No U.S. standard of potency" should be added with the next printing of carton labels. Marketing this product with FPL that is not identical in content to the approved labeling text may render the product misbranded and an unapproved new drug.

The dating period for formulated drug product shall be 18 months from the date of manufacture when stored at 2°-8°C (36°-46°F). The date of manufacture shall be defined as the date of final sterile filtration of the formulated drug product. The dating period for ranibizumab drug substance shall be [redacted] when stored at -20 °C.

You currently are not required to submit samples of future lots of Lucentis to the Center for Drug Evaluation and Research (CDER) for release by the Director, CDER, under 21 CFR 610.2. We will continue to monitor compliance with 21 CFR 610.1 requiring completion of tests for conformity with standards applicable to each product prior to release of each lot.

You must submit information to your biologics license application for our review and written approval under 21 CFR 601.12 for any changes in the manufacturing, testing, packaging or labeling of Lucentis, or in the manufacturing facilities.

All applications for new active ingredients, new dosage forms, new indications, new routes of administration, and new dosing regimens are required to contain an assessment of the safety and effectiveness of the product in pediatric patients unless this requirement is waived or deferred. We are waiving the pediatric study requirement for this application.

The following are Postmarketing Studies that are subject to reporting requirements of 21 CFR 601.70:

1. Submit the final Clinical Study Report from Study FVF3689g by June 30, 2008.

2. Provide safety and efficacy data from a 2-year adequate and well-controlled clinical trial of a mutually acceptable design exploring multiple dosing frequencies of Lucentis.

   Date of submission of protocol: November 14, 2008.

   Date of start of study: September 21, 2009.

   Date of final clinical study report: April 1, 2013.

3. To detect and characterize immune responses to ranibizumab:

   a. Develop and validate a confirmatory assay capable of detecting both IgG and IgM isotype responses.

   b. Develop and validate an assay to detect neutralizing anti-ranibizumab antibodies.

   The assay methodology and validation reports: September 28, 2007.

4. To characterize further the immune response to ranibizumab, serum samples collected in studies FVF2587g, FVF2598g, FVF3192g will be assayed using the validated methods described above in Postmarketing Commitment #3. The data obtained will be analyzed to discover and evaluate any association between immunoreactivity and dosing frequency as well as any potential impact of immunoreactivity on efficacy or safety outcomes.

   The need for an additional clinical study will be determined based on the results from the analysis described above.

   Date of submission of protocol and statistical analysis plan: February 28, 2007.

   Date of submission of final study report: September 30, 2008.

The following are Postmarketing Studies that are not subject to reporting requirements of 21 CFR 601.70:

5. To revise release specifications, shelf-life specifications and in-process limits for ranibizumab drug substance and drug product after (b) (4) nmercial manufacturing runs to reflect increased manufacturing experience.

   These revisions to the Quality control system, the corresponding data from the (b) (4) commercial manufacturing runs and the analysis plan used to create the revisions will be submitted as a supplement on or before June 30, 2008.

6. To perform additional Lucentis stability studies at 40°C using Ion Exchange Chromatography (IEC) to demonstrate that the corrective actions taken at (b) (4) to address the atypical accelerated stability profile observed in the Lucentis 2005 qualification campaign have been sufficient.

   Specifically, a one time stability study consisting of (b) (4) centis Drug Product launch lots are placed at 40°C and tested by IEC at (b) (4) months. These (b) (4) Lucentis Drug Product lots are derived from the following:

   - (b) (4) of these Lucentis Drug Product lots are manufactured from distinct lots of (b) (4).
   - At least (b) (4) these (b) (4) lots are aliquoted and used to manufacture (b) (4) ucentis drug product lots.

   Data will be submitted as a supplement on or before March 31, 2007.

We request that you submit clinical protocols to your IND, with a cross-reference letter to this biologics license application. Submit nonclinical and chemistry, manufacturing, and controls protocols and all study final reports to this application. Please use the following designators to label prominently all submissions, including supplements, relating to these postmarketing study commitments as appropriate:

- **Postmarketing Study Protocol**
- **Postmarketing Study Final Report**
- **Postmarketing Study Correspondence**
- **Annual Report on Postmarketing Studies**

For each postmarketing study subject to the reporting requirements of 21 CFR 601.70, you must describe the status in an annual report on postmarketing studies for this product. The status report for each study should include:

- information to identify and describe the postmarketing commitment,
- the original schedule for the commitment,
- the status of the commitment (i.e. pending, ongoing, delayed, terminated, or submitted),

- an explanation of the status including, for clinical studies, the patient accrual rate (i.e. number enrolled to date and the total planned enrollment), and
- a revised schedule if the study schedule has changed and an explanation of the basis for the revision.

As described in 21 CFR 601.70(e), we may publicly disclose information regarding these postmarketing studies on our Web site (http://www.fda.gov/cder/pmc/default.htm). Please refer to the April 2001 Draft Guidance for Industry: Reports on the Status of Postmarketing Studies – Implementation of Section 130 of the Food and Drug Administration Modernization Act of 1997 (see http://www.fda.gov/cber/gdlns/post040401.htm) for further information.

You must submit adverse experience reports under the adverse experience reporting requirements for licensed biological products (21 CFR 600.80). You should submit postmarketing adverse experience reports to the Central Document Room, Center for Drug Evaluation and Research, Food and Drug Administration, 5901-B Ammendale Road, Beltsville, MD 20705-1266. Prominently identify all adverse experience reports as described in 21 CFR 600.80.

The MedWatch-to-Manufacturer Program provides manufacturers with copies of serious adverse event reports that are received directly by the FDA. New molecular entities and important new biologics qualify for inclusion for three years after approval. Your firm is eligible to receive copies of reports for this product. To participate in the program, please see the enrollment instructions and program description details at www.fda.gov/medwatch/report/mmp.htm.

You must submit distribution reports under the distribution reporting requirements for licensed biological products (21 CFR 600.81).

You must submit reports of biological product deviations under 21 CFR 600.14. You should promptly identify and investigate all manufacturing deviations, including those associated with processing, testing, packing, labeling, storage, holding and distribution. If the deviation involves a distributed product, may affect the safety, purity, or potency of the product, and meets the other criteria in the regulation, you must submit a report on Form FDA-3486 to the Division of Compliance Risk Management and Surveillance (HFD-330), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857. Biological product deviations sent by courier or overnight mail should be addressed to Food and Drug Administration, CDER, Office of Compliance, Division of Compliance Risk Management and Surveillance, HFD-330, Montrose Metro 2, 11919 Rockville Pike, Rockville, MD 20852.

Please submit all FPL at the time of use and include implementation information on FDA Form 356h. Please provide a PDF-format electronic copy as well as original paper copies (ten for circulars and five for other labels). In addition, you may wish to submit draft copies of the proposed introductory advertising and promotional labeling with a cover letter requesting advisory comments to the Food and Drug Administration, Center for Drug Evaluation and Research, Division of Drug Marketing, Advertising and Communication, 5901-B Ammendale Road, Beltsville, MD 20705-1266. Final printed advertising and promotional labeling should be submitted at the time of initial dissemination, accompanied by a FDA Form 2253.

All promotional claims must be consistent with and not contrary to approved labeling. You should not make a comparative promotional claim or claim of superiority over other products unless you have substantial evidence to support that claim.

Please refer to http://www.fda.gov/cder/biologics/default.htm for important information regarding therapeutic biological products, including the addresses for submissions.

If you have any questions, call Lori M. Gorski, Project Manager, at (301) 796-0722.

Sincerely,

Mark J. Goldberger, M.D., M.P.H.
Director
Office of Antimicrobial Products
Center for Drug Evaluation and Research

Enclosure

US006407213B1

(12) **United States Patent**
Carter et al.

(10) **Patent No.: US 6,407,213 B1**
(45) **Date of Patent: Jun. 18, 2002**

(54) **METHOD FOR MAKING HUMANIZED ANTIBODIES**

(75) Inventors: **Paul J. Carter**; **Leonard G. Presta**, both of San Francisco, CA (US)

(73) Assignee: **Genentech, Inc.**, South San Francisco, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **08/146,206**

(22) PCT Filed: **Jun. 15, 1992**

(86) PCT No.: **PCT/US92/05126**

§ 371 (c)(1),
(2), (4) Date: **Nov. 17, 1993**

Related U.S. Application Data

(63) Continuation-in-part of application No. 07/715,272, filed on Jun. 14, 1991, now abandoned.

(51) **Int. Cl.**[7] **C07K 16/00**
(52) **U.S. Cl.** **530/387.3**; 435/69.6; 435/69.7; 435/70.21; 435/91; 536/23.53; 424/133.1
(58) **Field of Search** 435/69.6, 69.7, 435/70.21, 91, 172.2, 240.1, 240.27, 252.3, 320.1, 328; 536/23.53; 424/133.1; 530/387.3

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(57) **ABSTRACT**

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

82 Claims, 9 Drawing Sheets

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FIG. 1A

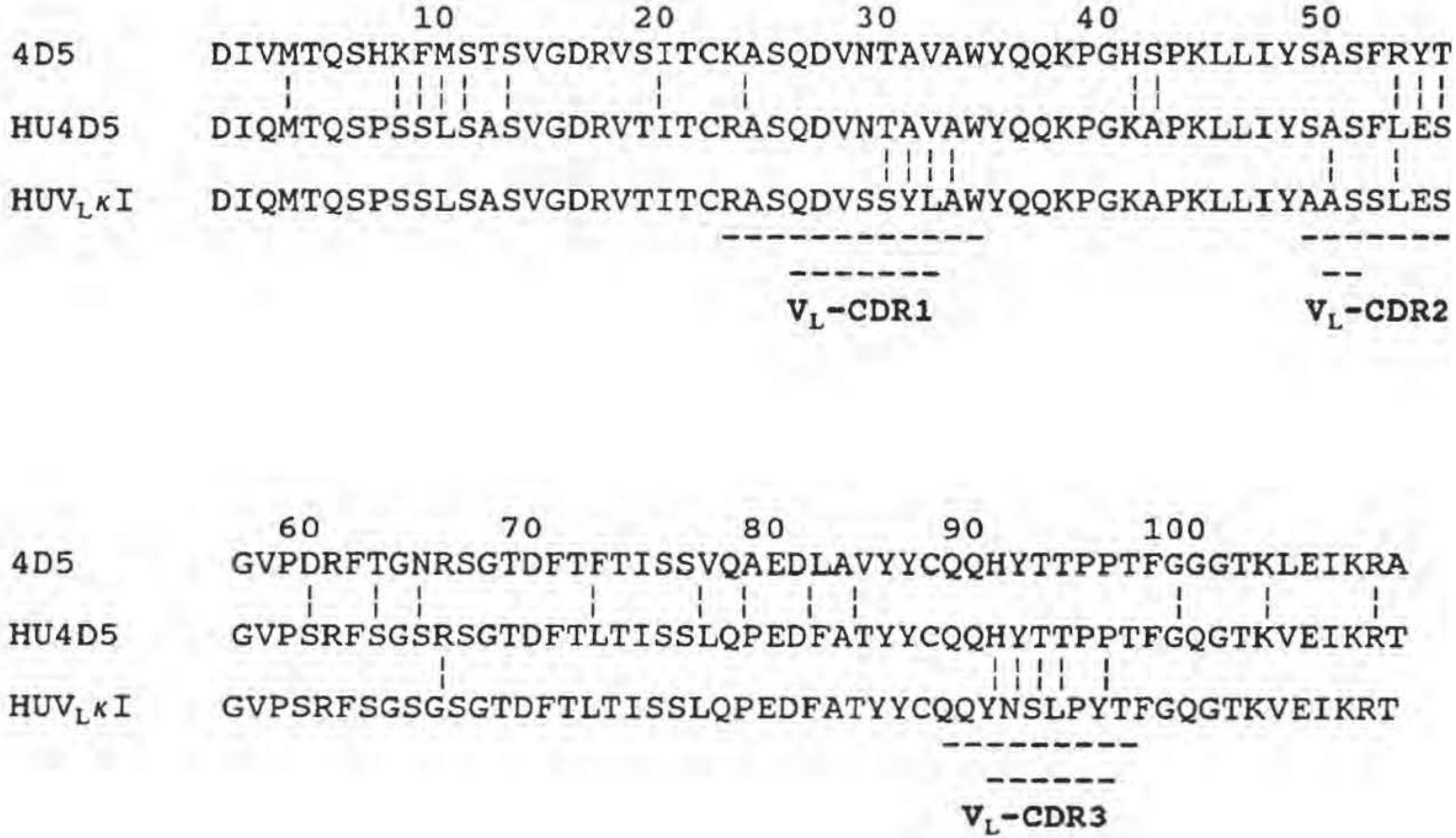
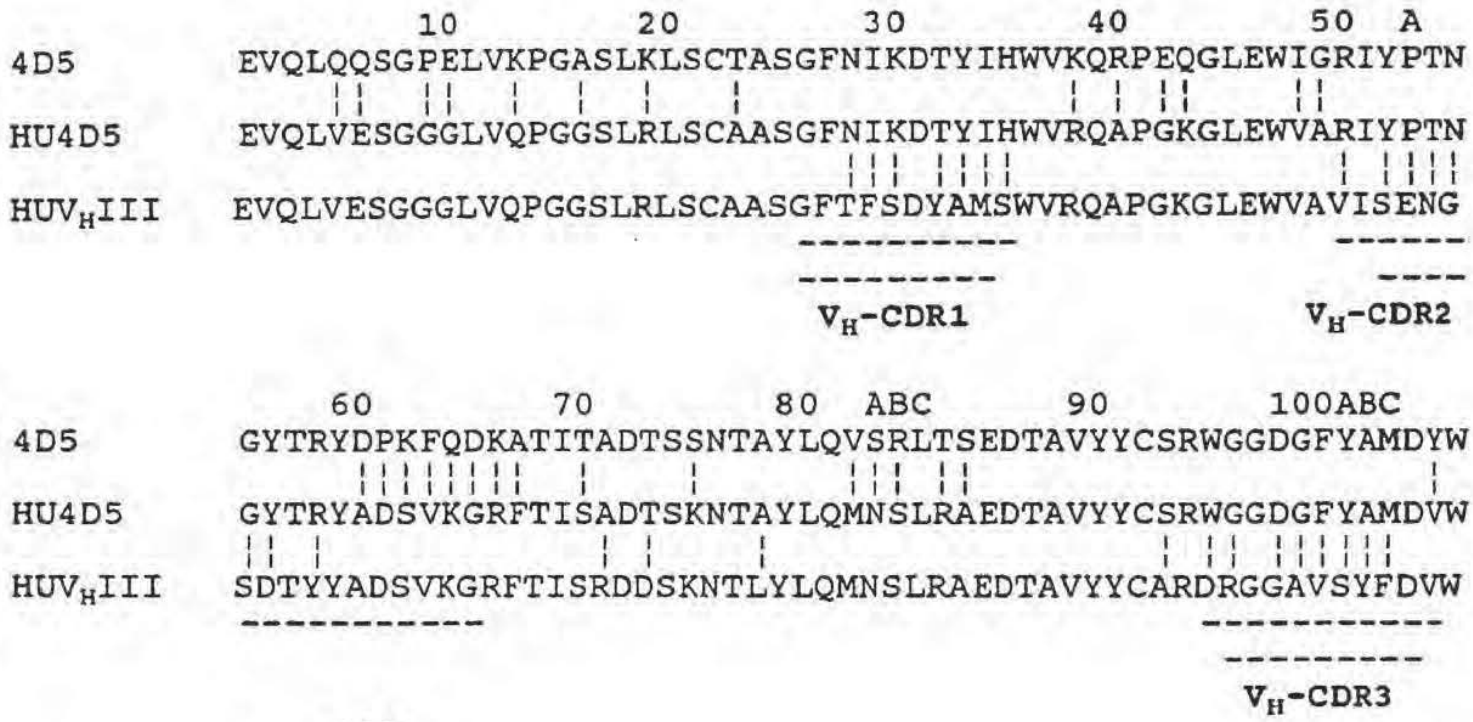

10 20 30 40 50
4D5 DIVMTQSHKFMSTSVGDRVSITCKASQDVNTAVAWYQQKPGHSPKLLIYSASFRYT
HU4D5 DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLES
HUVLκI DIQMTQSPSSLSASVGDRVTITCRASQDVSSYLAWYQQKPGKAPKLLIYAASSLES
VL-CDR1
VL-CDR2
60 70 80 90 100
4D5 GVPDRFTGNRSGTDFTFTISSVQAEDLAVYYCQQHYTTPPTFGGGTKLEIKRA
HU4D5 GVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRT
HUVLκI GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSLPYTFGQGTKVEIKRT
VL-CDR3

FIG. 1B



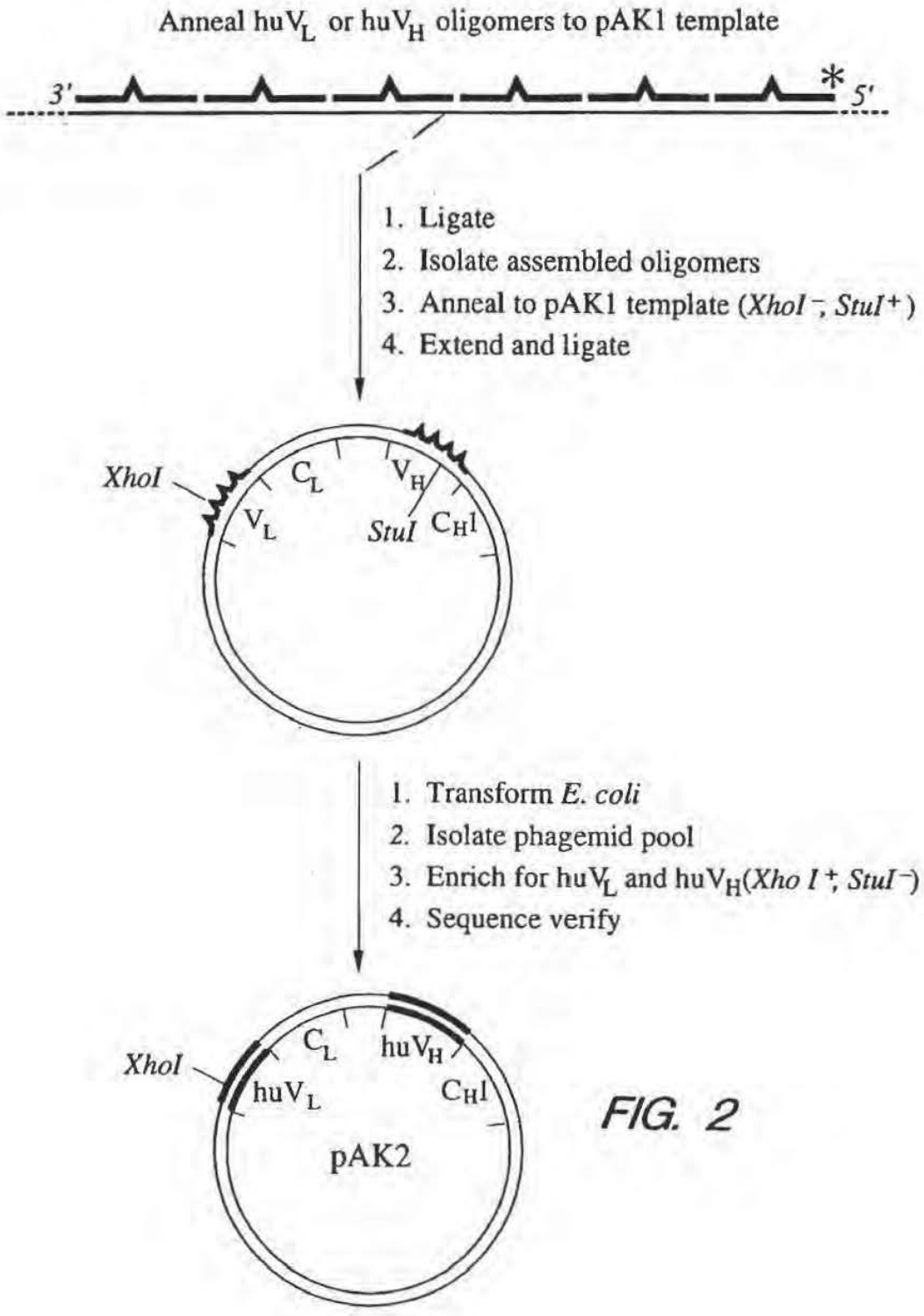

Anneal huV$_L$ or huV$_H$ oligomers to pAK1 template
3'
5'
*
1. Ligate
2. Isolate assembled oligomers
3. Anneal to pAK1 template (*XhoI*$^-$, *StuI*$^+$)
4. Extend and ligate
XhoI
C$_L$
V$_H$
V$_L$
StuI
C$_H$1
1. Transform *E. coli*
2. Isolate phagemid pool
3. Enrich for huV$_L$ and huV$_H$(*Xho I*$^+$, *StuI*$^-$)
4. Sequence verify
XhoI
C$_L$
huV$_H$
huV$_L$
C$_H$1
pAK2

FIG. 2

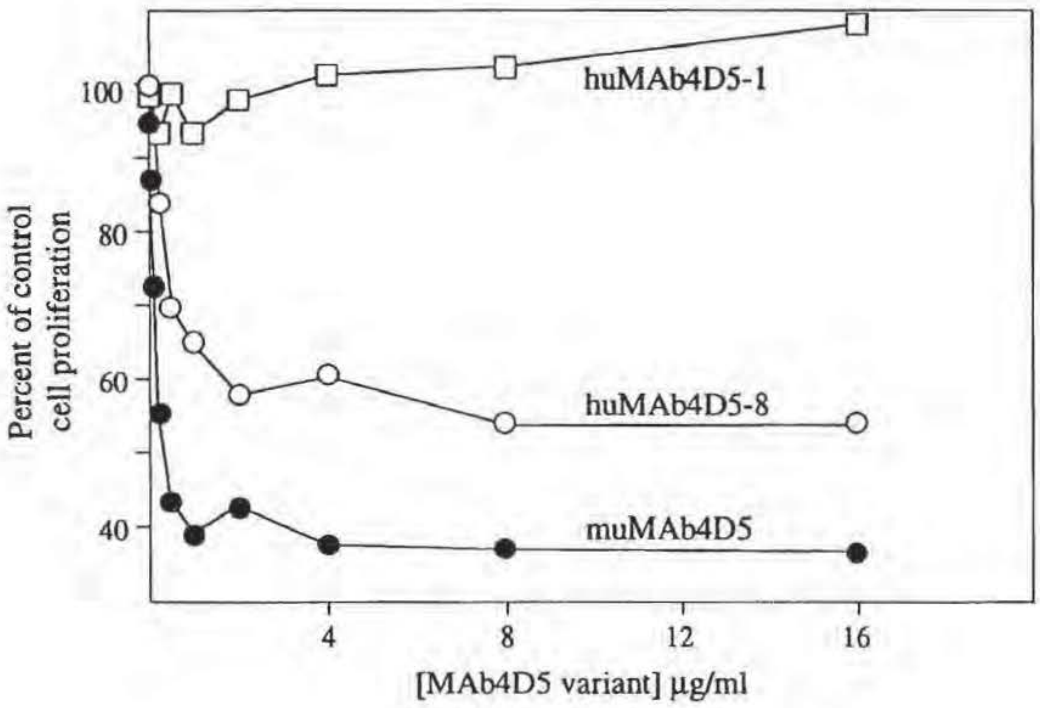

huMAb4D5-1
huMAb4D5-8
muMAb4D5
100
80
60
40
Percent of control cell proliferation
4
8
12
16
[MAb4D5 variant] µg/ml

FIG. 3

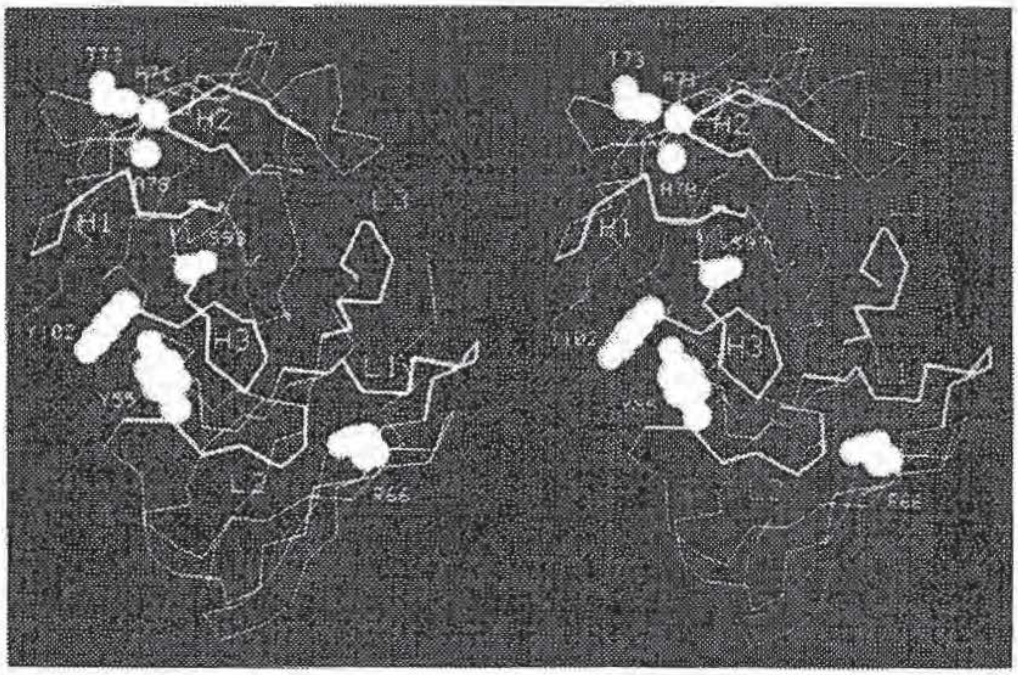


FIG. 4

```
VL                    10        20        30        40
muxCD3      DIQMTQTTSSLSASLGDRVTISCRASQDIRNYLNWYQQKP
                 **        *         *
huxCD3v1    DIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKP
                                      # # #
huκI        DIQMTQSPSSLSASVGDRVTITCRASQSISNYLAWYQQKP6
                                     ^^^^^^^^^
                                     CDR-L1

                    50        60        70        80
muxCD3      DGTVKLLIYYTSRLHSGVPSKFSGSGSGTDYSLTISNLEQ
            ****          *         *         *  * **
huxCD3v1    GKAPKLLIYYTSRLESGVPSRFSGSGSGTDYTLTISSLQP
                  ## #                    #
huκI        GKAPKLLIYAASSLESGVPSRFSGSGSGTDFTLTISSLQP
                   ^^^
                   CDR-L2

                    90        100
muxCD3      EDIATYFCQQGNTLPWTFAGGTKLEIK
              *   *           **   *
huxCD3v1    EDFATYYCQQGNTLPWTFGQGTKVEIK
                  # #
huκI        EDFATYYCQQYNSLPWTFGQGTKVEIK
                  ^^^^^^^
                  CDR-L3
```

```
VH                    10        20        30        40
muxCD3      EVQLQQSGPELVKPGASMKISCKASGYSFTGYTMNWVKQS
              **  **  *  * ***  *                 * *
huxCD3v1    EVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQA
                                    ## ## # #
huIII       EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQA
                                    ^^^^^^^
                                     CDR-H1

                    50        60        70
muxCD3      HGKNLEWMGLINPYKGVSTYNQKFKDKATLTVDKSSSTAY
            *  *   **         *  **** ** **    **
huxCD3v1    PGKGLEWVALINPYKGVTTYADSVKGRFTISVDKSKNTAY
                 ## #### # #              # #    #
HuIII       PGKGLEWVSVISGDGGSTYYADSVKGRFTISRDNSKNTLY
                    ^^^^
                    CDR-H2

          80  abc       90        100abcde        110
muxCD3      MELLSLTSEDSAVYYCARSGYYGDSDWYFDVWGAGTTVTVSS
            ****  **  *                   *  *
huxCD3v1    LQMNSLRAEDTAVYYCARSGYYGDSDWYFDVWGQGTLVTVSS
                              ########### #
huIII       LQMNSLRAEDTAVYYCARGRVGYSLSGLYDYWGQGTLVTVSS
                              D E T   S
                              ^^^^^^^^^^^
                              CDR-H3
```

FIG. 5

FIG. 6A-1

```
                                     10        20        30
H52H4-160                      QVQLQQSGPELVKPGASVKISCKTSGYTFTE
                               .*** .**  **.**.*...** ********
pH52-8.0    MGWSCIILFLVATATGVHSEVQLVESGGGLVQPGGSLRLSCATSGYTFTE
                     10        20        30        40        50

                 40        50        60        70        80
H52H4-160   YTMHWMKQSHGKSLEWIGGFNPKNGGSSHNQRFMDKATLAVDKSTSTAYM
            ******.*. **.***..*.******.********. *..**********
pH52-8.0    YTMHWMRQAPGKGLEWVAGINPKNGGTSHNQRFMDRFTISVDKSTSTAYM
                     60        70        80        90       100

                 90       100       110       120       130
H52H4-160   ELRSLTSEDSGIYYCARWRGLNYGFDVRYFDVWGAGTTVTVSSASTKGPS
            .. ** .**...********************** ** ************
pH52-8.0    QMNSLRAEDTAVYYCARWRGLNYGFDVRYFDVWGQGTLVTVSSASTKGPS
                    110       120       130       140       150

                140       150       160       170       180
H52H4-160   VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
            ****** *.*** .************************************
pH52-8.0    VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
                    160       170       180       190       200

                190       200       210       220       230
H52H4-160   QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH
            ************** **..***** ***.********** ** * *
pH52-8.0    QSSGLYSLSSVVTVTSSNFGTQTYTCNVDHKPSNTKVDKTVERKCC---V
                    210       220       230       240

                240       250       260       270       280
H52H4-160   TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK
             *******  ..*************************************.
pH52-8.0    ECPPCPAPP-VAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQ
            250        260       270       280       290
```

FIG. 6A-2

```
                290         300         310         320         330
H52H4-160       FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS
                *******.**************.***.********.***************
pH52-8.0        FNWYVDGMEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVS
                 300         310         320         330         340

                340         350         360         370         380
H52H4-160       NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP
                **.***********.**********************************
pH52-8.0        NKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP
                 350         360         370         380         390

                390         400         410         420         430
H52H4-160       SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
                **********************.****************************
pH52-8.0        SDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFS
                 400         410         420         430         440

                440         450
H52H4-160       CSVMHEALHNHYTQKSLSLSPGK
                ***********************
pH52-8.0        CSVMHEALHNHYTQKSLSLSPGK
                 450         460
```

FIG. 6B

```
                                        10        20        30
H52L6-158                          DVQMTQTTSSLSASLGDRVTINCRASQDINN
                                   *.****. ******.****** *********
pH52-9.0    MGWSCIILFLVATATGVHSDIQMTQSPSSLSASVGDRVTITCRASQDINN
                    10        20        30        40        50

                   40        50        60        70        80
H52L6-158   YLNWYQQKPNGTVKLLIYYTSTLHSGVPSRFSGSGSGTDYSLTISNLDQE
            ********* . ****************************.****.*. *
pH52-9.0    YLNWYQQKPGKAPKLLIYYTSTLHSGVPSRFSGSGSGTDYTLTISSLQPE
                    60        70        80        90        100

                  90        100       110       120       130
H52L6-158   DIATYFCQQGNTLPPTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS
            *.***.************ ********************************
pH52-9.0    DFATYYCQQGNTLPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS
                    110       120       130       140       150

                  140       150       160       170       180
H52L6-158   VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
            ************************************************
pH52-9.0    VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
                    160       170       180       190       200

                  190       200       210
H52L6-158    SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
             ********************************
pH52-9.0     SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
                    210       220       230
```

METHOD FOR MAKING HUMANIZED ANTIBODIES

CROSS REFERENCES

This application is a continuation-in-part of U.S. application Ser. No. 07/715,272 filed Jun. 14, 1991 (abandoned) which application is incorporated herein by reference and to which application priority is claimed under 35 USC §120.

FIELD OF THE INVENTION

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

BACKGROUND OF THE INVENTION

Naturally occurring antibodies (immunoglobulins) comprise two heavy chains linked together by disulfide bonds and two light chains, one light chain being linked to each of the heavy chains by disulfide bonds. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain (V_L) at one end and a constant domain at its other end, the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light and heavy chain variable domains, see e.g. Chothia et al., *J. Mol. Biol.* 186:651–663 (1985); Novotny and Haber, *Proc. Natl. Acad. Sci. USA* 82:4592–4596 (1985).

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

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

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

antigen binding portion of an antibody molecule linked to at least part of another protein (typically an immunoglobulin constant domain).

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

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

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

The therapeutic promise of this approach is supported by the clinical efficacy of a humanized antibody specific for the CAMPATH-1 antigen with two non-Hodgkin lymphoma patients, one of whom had previously developed an anti-globulin response to the parental rat antibody (Riechmann, L. et al., *Nature* 332:323–327 (1988); Hale, G. et al., *Lancet i:*1394–1399 (1988)). A murine antibody to the interleukin 2 receptor has also recently been humanized (Queen, C. et al., *Proc. Natl. Acad. Sci. USA* 86:10029–10033 (1989)) as a potential immunosuppressive reagent. Additional references related to humanization of antibodies include Co et al., *Proc. Natl. Acad. Sci. USA* 88:2869–2873 (1991); Gorman et al., *Proc. Natl. Acad. Sci. USA* 88:4181–4185 (1991); Daugherty et al., *Nucleic Acids Research* 19(9):2471–2476 (1991); Brown et al., *Proc. Natl. Acad. Sci. USA* 88:2663–2667 (1991); Junghans et al., *Cancer Research* 50:1495–1502 (1990).

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

For a given antibody a small number of FR residues are anticipated to be important for antigen binding. Firstly for example, certain antibodies have been shown to contain a few FR residues which directly contact antigen in crystal structures of antibody-antigen complexes (e.g., reviewed in Davies, D. R. et al., *Ann. Rev. Biochem.* 59:439–473 (1990)).

Secondly, a number of FR residues have been proposed by Chothia, Lesk and colleagues (Chothia, C. & Lesk, A. M., *J. Mol. Biol.* 196:901–917 (1987); Chothia, C. et al., *Nature* 342:877–883 (1989); Tramontano, A. et al., *J. Mol. Biol.* 215:175–182 (1990)) as critically affecting the conformation of particular CDRs and thus their contribution to antigen binding. See also Margolies et al., *Proc. Natl. Acad. Sci. USA* 72:2180–2184 (1975).

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

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

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

The proto-oncogene HER2 (human epidermal growth factor receptor 2) encodes a protein tyrosine kinase ($p185^{HER2}$) that is related to and somewhat homologous to the human epidermal growth factor receptor (see Coussens, L. et al., *Science* 230:1132–1139 (1985); Yamamoto, T. et al., *Nature* 319:230–234 (1986); King, C. R. et al., *Science* 229:974–976 (1985)). HER2 is also known in the field as c-erbB-2, and sometimes by the name of the rat homolog, neu. Amplification and/or overexpression of HER2 is associated with multiple human malignancies and appears to be integrally involved in progression of 25–30% of human breast and ovarian cancers (Slamon, D. J. et al., *Science* 235:177–182 (1987), Slamon, D. J. et al., *Science* 244:707–712 (1989)). Furthermore, the extent of amplification is inversely correlated with the observed median patient survival time (Slamon, supra, Science 1989).

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

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

It is a further object of this invention to provide methods for the efficient humanization is of antibodies, i.e. selecting non-human amino acid residues for importation into a human antibody background sequence in such a fashion as to retain or improve the affinity of the non-human donor antibody for a given antigen.

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

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

SUMMARY OF THE INVENTION

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

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

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

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

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

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

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

1. non-covalently binds antigen directly,

2. interacts with a CDR; or

3. participates in the V_L–V_H interface; and

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

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

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

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

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

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

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

b. (in the FR of the variable domain of the heavy chain) 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 67H, 68H, 69H, 70H, 73H, 74H, 75H, 78H, 91H, 92H, 93H, and 103H.

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

Optionally, this just-recited embodiment comprises the additional steps of following the method steps appearing at the beginning of this summary and determining whether a particular amino acid residue can reasonably be expected to have undesirable effects.

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

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

1. SEQ. ID NO. 1, which is the light chain variable domain of a humanized version of muMAb4D5:
DIQMTQSPSSLSASVGDRVTITCRASQD-
VNTAVAWYQQKPGKAPKLLIYSASFLES-
GVPSRFSGSRSGTDFTLTISSLQPEDFA-
TYYCQQHYTTPPTFGQGTKVEIKRT

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

In another aspect, this invention provides a consensus antibody variable domain amino acid sequence for use in the preparation of humanized antibodies, methods for obtaining, using, and storing a computer representation of such a consensus sequence, and computers comprising the sequence data of such a sequence. In one embodiment, the following consensus antibody variable domain amino acid sequences are provided:

SEQ. ID NO. 3 (light chain): DDIQMTQSPSSLSAS-
VGDRVTITCRASQDVSSYLAWYQQKPGKAPKLL
IYAASSLESGVPSRFSGSGSGTDFTLTISSLQP
EDFATYYCQQYNSLPYTFGQGTKVEIKRT, and

SEQ. ID NO. 4 (heavy chain): EVQLVESGGGLVQPG
GSLRLSCAASGFTFSDYAMSWVRQAPGKGL
EWVAVISENGGYTRYADSVKGRFTISADTSKNT
AYLQMNSLRAEDTAWYCSRWGGDGFYAMD
VWGQGTLVTVSS

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A shows the comparison of the V_L domain amino acid residues of muMAb4D5, huMAb4D5, and a consensus sequence (FIG. 1A, SEQ.ID NO. 5, SEQ. ID NO. 1 and SEQ. ID NO. 3, respectively). FIG. 1B shows the comparison between the V_H domain amino acid residues of the muMAb4D5, huMAb4D5, and a consensus sequence (FIG. 1B, SEQ. ID NO. 6, SEQ. ID NO. 2 and SEQ. ID NO. 4, respectively). Both FIGS. 1A and 1B use the generally accepted numbering scheme from Kabat, E. A., et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md. (1987)). In both FIG. 1A

and FIG. 1B, the CDR residues determined according to a standard sequence definition (as in Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)) are indicated by the first underlining beneath the sequences, and the CDR residues determined according to a structural definition (as in Chothia, C. & Lesk, A. M., *J. Mol. Biol.* 196:901–917 (1987)) are indicated by the second, lower underlines. The mismatches between genes are shown by the vertical lines.

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

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

FIG. 4 shows a stereo view of α-carbon tracing for a model of huMAb4D5-8 V_L and V_H. The CDR residues (Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)) are shown in bold and side chains of V_H residues A71, T73, A78, S93, Y102 and V_L residues Y55 plus R66 (see Table 3) are shown.

FIG. 5 shows an amino acid sequence comparison of V_L (top panel) and V_H (lower panel) domains of the murine anti-CD3 monoclonal Ab UCHT1 (muxCD3, Shalaby et al., *J. Exp. Med.* 175, 217–225 (1992) with a humanized variant of this antibody (huxCD3v1). Also shown are consensus sequences (most commonly occurring residue or pair of residues) of the most abundant human subgroups, namely V_L κ 1 and V_H III upon which the humanized sequences are based (Kabat, E. A. et al., *Sequences of Proteins of immunological Interest*, 5th edition, National Institutes of Health, Bethesda, Md., USA (1991)). The light chain sequences—muxCD3, huxCD3v1 and huKI—correspond to SEQ.ID. NOs 16, 17, and 18, respectively. The heavy chain sequences—muxCD3, huxCD3v1 and huxI—correspond to SEQ.ID.NOs 19, 26, and 21, respectively. Residues which differ between muxCD3 and huxCD3v1 are identified by an asterisk (*), whereas those which differ between humanized and consensus sequences are identified by a sharp sign (#). A bullet (•) denotes that a residue at this position has been found to contact antigen in one or more crystallographic structures of antibody/antigen complexes (Kabat et al., 1991; Mian, I. S. et al., *J. Mol. Biol* 217, 133–151 (1991)). The location of CDR residues according to a sequence definition (Kabat et al., 1991) and a structural definition (Chothia and Lesk, supra 1987) are shown by a line and carats (^) beneath the sequences, respectively.

FIG. 6A compares murine and humanized amino acid sequences for the heavy chain of an anti-CD18 antibody. H52H4-160 (SEQ. ID. NO. 22) is the murine sequence, and pH52–8.0 (SEQ. ID. NO. 23) is the humanized heavy chain sequence. pH52–8.0 residue 143S is the final amino acid in the variable heavy chain domain V_H, and residue 144A is the first amino acid in the constant heavy chain domain C_{H1}.

FIG. 6B compares murine and humanized amino acid sequences for the light chain of an anti-CD18 antibody. H52L6-158 (SEQ. ID. NO. 24) is the murine sequence, and pH52–9.0 (SEQ. ID. NO. 25) is the humanized light chain sequence. pH52–9.0 residue 128T is the final amino acid in the light chain variable domain V_L, and residue 129V is the first amino acid in the light chain constant domain C_L.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

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

The murine monoclonal antibody known as muMAb4D5 (Fendly, B. M. et al., *Cancer Res.* 50:1550–1558 (1990)) is directed against the extracellular domain (ECD) of $p185^{HER2}$. The muMAb4D5 and its uses are described in PCT application WO 89/06692 published Jul. 27, 1989. This murine antibody was deposited with the ATCC and designated ATCC CRL 10463. In this description and claims, the terms muMAb4D5, chMAb4D5 and huMAb4D5 represent murine, chimerized and humanized versions of the monoclonal antibody 4D5, respectively.

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

Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are referred to herein as "import" residues, which are typically taken from an "import" antibody domain, particularly a variable domain. An import residue, sequence, or antibody has a desired affinity and/or specificity, or other desirable antibody biological activity as discussed herein.

In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains (Fab, Fab', $F(ab')_2$, Fabc, Fv) in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. Ordinarily, the antibody will contain both the light chain as well as at least the variable domain of a heavy chain. The antibody also may include the CH1, hinge, CH2, CH3, and CH4 regions of the heavy chain.

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

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

In general, humanized antibodies prepared by the method of this invention are produced by a process of analysis of the parental sequences and various conceptual humanized products using three dimensional models of the parental and humanized sequences. Three dimensional immunoglobulin models are commonly available and are familiar to those

skilled in the art. Computer programs are available which illustrate and display probable three dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen.

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

A residue that noncovalently directly binds to antigen is one that, by three dimensional analysis, is reasonably expected to noncovalently directly bind to antigen. Typically, it is necessary to impute the position of antigen from the spatial location of neighboring CDRs and the dimensions and structure of the target antigen. In general, only those humanized antibody residues that are capable of forming salt bridges, hydrogen bonds, or hydrophobic interactions are likely to be involved in non-covalent antigen binding, however residues which have atoms which are separated from antigen spatially by 3.2 Angstroms or less may also non-covalently interact with antigen. Such residues typically are the relatively larger amino acids having the side chains with the greatest bulk, such as tyrosine, arginine, and lysine. Antigen-binding FR residues also typically will have side chains that are oriented into an envelope surrounding the solvent oriented face of a CDR which extends about 7 Angstroms into the solvent from the CDR domain and about 7 Angstroms on either side of the CDR domain, again as visualized by three dimensional modeling.

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

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

Immunoglobulin residues that affect the interface between heavy and light chain variable regions ("the V_L–V_H interface") are those that affect the proximity or orientation of the two chains with respect to one another. Certain residues involved in interchain interactions are already known and include V_L residues 34, 36, 38, 44, 46, 87, 89, 91, 96, and 98 and V_H residues 35, 37, 39, 45, 47, 91, 93, 95, 100, and 103 (utilizing the nomenclature setforth in Kabat et al., *Sequences of Proteins of immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)). Additional residues are newly identified by the inventors herein, and include 43L, 85L, 43H and 60H. While these residues are indicated for IgG only, they are applicable across species. In the practice of this invention, import antibody residues that are reasonably expected to be involved in interchain interactions are selected for substitution into the consensus sequence. It is believed that heretofore no humanized antibody has been prepared with an intrachain-affecting residue selected from an import antibody sequence.

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

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

Throughout this description, reference is made to the numbering scheme from Kabat, E. A., et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md. (1987) and (1991). In these compendiums, Kabat lists many amino acid sequences for antibodies for each subclass, and lists the most commonly occurring amino acid for each residue position in that subclass. Kabat uses a method for assigning a residue number to each amino acid in a listed sequence, and this method for assigning residue numbers has become standard in the field. The Kabat numbering scheme is followed in this description.

For purposes of this invention, to assign residue numbers to a candidate antibody amino acid sequence which is not included in the Kabat compendium, one follows the following steps. Generally, the candidate sequence is aligned with any immunoglobulin sequence or any consensus sequence in Kabat. Alignment may be done by hand, or by computer using commonly accepted computer programs; an example of such a program is the Align 2 program discussed in this description. Alignment may be facilitated by using some amino acid residues which are common to most Fab

sequences. For example, the light and heavy chains each typically have two cysteines which have the same residue numbers; in V_L domain the two cysteines are typically at residue numbers 23 and 88, and in the V_H domain the two cysteine residues are typically numbered 22 and 92. Framework residues generally, but not always, have approximately the same number of residues, however the CDRs will vary in size. For example, in the case of a CDR from a candidate sequence which is longer than the CDR in the sequence in Kabat to which it is aligned, typically suffixes are added to the residue number to indicate the insertion of additional residues (see, e.g. residues 100abcde in FIG. 5). For candidate sequences which, for example, align with a Kabat sequence for residues 34 and 36 but have no residue between them to align with residue 35, the number 35 is simply not assigned to a residue.

Thus, in humanization of an import variable sequence, where one cuts out an entire human or consensus CDR and replaces it with an import CDR sequence, (a) the exact number of residues may be swapped, leaving the numbering the same, (b) fewer import amino acid residues may be introduced than are cut, in which case there will be a gap in the residue numbers, or (c) a larger number of amino acid residues may be introduced then were cut, in which case the numbering will involve the use of suffixes such as 100abcde.

The terms "consensus sequence" and "consensus antibody" as used herein refers to an amino acid sequence which comprises the most frequently occurring amino acid residues at each location in all immunoglobulins of any particular subclass or subunit structure. The consensus sequence may be based on immunoglobulins of a particular species or of many species. A "consensus" sequence, structure, or antibody is understood to encompass a consensus human sequence as described in certain embodiments of this invention, and to refer to an amino acid sequence which comprises the most frequently occurring amino acid residues at each location in all human immunoglobulins of any particular subclass or subunit structure. This invention provides consensus human structures and consensus structures which consider other species in addition to human.

The subunit structures of the five immunoglobulin classes in humans are as follows:

Class	Heavy Chain	Subclasses	Light Chain	Molecular Formula
IgG	γ	$\gamma 1, \gamma 2, \gamma 3, \gamma 4$	κ or λ	$(\gamma_2\kappa_2), (\gamma_2\lambda_2)$
IgA	α	$\alpha 1, \alpha 2$	κ or λ	$(\alpha_2\kappa_2)_n^*, (\alpha_2\lambda_2)_n^*$
IgM	μ	none	κ or λ	$(\mu_2\kappa_2)_5, (\mu_2\lambda_2)_5$
IgD	δ	none	κ or λ	$(\delta_2\kappa_2), (\delta_2\lambda_2)$
IgE	ϵ	none	κ or λ	$(\epsilon_2\kappa_2), (\epsilon_2\lambda_2)$

(*n may equal 1, 2, or 3)

In preferred embodiments of an IgGγ1 human consensus sequence, the consensus variable domain sequences are derived from the most abundant subclasses in the sequence compilation of Kabat et al., *Sequences of Proteins of Immunological Interest,* National Institutes of Health, Bethesda Md. (1987), namely V_L κ subgroup I and V_H group III. In such preferred embodiments, the V_L consensus domain has the amino acid sequence:

DIQMTQSPSSLSASVGDRVTITCRASQD-VSSYLAWYQQKPGKAPKLLIYAASSLES-GVPSRFSGSGSGTDFTLTISSLQPEDFA-TYYCQQYNSLPYTFGQGTKVEIKRT (SEQ. ID NO. 3);

the V_H consensus domain has the amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYAMSW VRQAPGKGLEWVAVISENGGYTRYADSVKGRFT ISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGD GFYAMDVWGQGTLVTVSS (SEQ. ID NO. 4).

These sequences include consensus CDRs as well as consensus FR residues (see for example in FIG. 1).

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

Identity or homology with respect to a specified amino acid sequence of this invention is defined herein as the percentage of amino acid residues in a candidate sequence that are identical with the specified residues, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent homology, and not considering any conservative substitutions as part of the sequence identity. None of N-terminal, C-terminal or internal extensions, deletions, or insertions into the specified sequence shall be construed as affecting homology. All sequence alignments called for in this invention are such maximal homology alignments. While such alignments may be done by hand using conventional methods, a suitable computer program is the "Align 2" program for which protection is being sought from the U.S. Register of Copyrights (Align 2, by Genentech, Inc., application filed Dec. 9, 1991).

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

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

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

DIQMTQSPSSLSASVGDRVTITCRASODVNTAVAWY QQKPGKAPKLLIYSASFLESGVPSRFSGSRSGT DFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTK VEIKRT (SEQ. ID NO. 1, which is the light chain variable domain of huMAb4D5); or

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHW VRQAPGKGLEWVARIYPTNGYTRYADSVKGRFT ISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGD GFYAMDVWGQGTLVTVSS (SEQ. ID NO. 2, which is the heavy chain variable domain of huMAb4D5).

"Biological property", as relates for example to anti-p185^{HER2}, for the purposes herein means an in vivo effector or antigen-binding function or activity that is directly or indirectly performed by huMAb4D5 (whether in its native or denatured conformation). Effector functions include p185^{HER2} binding, any hormonal or hormonal antagonist activity, any mitogenic or agonist or antagonist activity, any

cytotoxic activity. An antigenic function means possession of an epitope or antigenic site that is capable of cross-reacting with antibodies raised against the polypeptide sequence of huMAb4D5.

Biologically active huMAb4D5 is defined herein as a polypeptide that shares an effector function of huMAb4D5. A principal known effector function of huMAb4D5 is its ability to bind to $p185^{HER2}$.

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

An "isolated" polypeptide means polypeptide which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, for example, a polypeptide product comprising huMAb4D5 will be purified from a cell culture or other synthetic environment (1) to greater than 95% by weight of protein as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a gas- or liquid-phase sequenator (such as a commercially available Applied Biosystems sequenator Model 470, 477, or 473), or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated huMAb4D5 includes huMAb4D5 in situ within recombinant cells since at least one component of the huMAb4D5 natural environment will not be present. Ordinarily, however, isolated huMAb4D5 will be prepared by at least one purification step.

In accordance with this invention, huMAb4D5 nucleic acid is RNA or DNA containing greater than ten bases that encodes a biologically or antigenically active huMAb4D5, is complementary to nucleic acid sequence encoding such huMAb4D5, or hybridizes to nucleic acid sequence encoding such huMAb4D5 and remains stably bound to it under stringent conditions, and comprises nucleic acid from a muMAb4D5 CDR and a human FR region.

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

Stringent conditions are those that (1) employ low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate/0/1% $NaDodSO_4$ at 50° C.; (2) employ during hybridization a denaturing agent such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serumalbumin/0/1% Ficoll/0/1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42° C.; or (3) employ 50% formamide, 5×SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5×Denhardt's solution, sonicated salmon sperm DNA (50 g/ml), 0.1% SDS, and 10% dextran sulfate at 42 C., with washes at 42 C. in 0.2×SSC and 0.1% SDS.

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

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

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

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

"Oligonucleotides" are short-length, single- or double-stranded polydeoxynucleotides that are chemically synthesized by known methods (such as phosphotriester, phosphite, or phosphoramidite chemistry, using solid phase techniques such as described in EP 266,032 published May

4, 1988, or via deoxynucleoside H-phosphonate intermediates as described by Froehler et al., *Nucl. Acids Res.*, 14: 5399–5407 [1986]). They are then purified on polyacrylamide gels.

The technique of "polymerase chain reaction," or "PCR," as used herein generally refers to a procedure wherein minute amounts of a specific piece of nucleic acid, RNA and/or DNA, are amplified as described in U.S. Pat. No. 4,683,195 issued Jul. 28, 1987. Generally, sequence information from the ends of the region of interest or beyond needs to be available, such that oligonucleotide primers can be designed; these primers will be identical or similar in sequence to opposite strands of the template to be amplified. The 5' terminal nucleotides of the two primers may coincide with the ends of the amplified material. PCR can be used to amplify specific RNA sequences, specific DNA sequences from total genomic DNA, and cDNA transcribed from total cellular RNA, bacteriophage or plasmid sequences, etc. See generally Mullis et al., *Cold Spring Harbor Symp. Quant. Biol.*, 51: 263 (1987); Erlich, ed., *PCR Technology*, (Stockton Press, N.Y., 1989). As used herein, PCR is considered to be one, but not the only, example of a nucleic acid polymerase reaction method for amplifying a nucleic acid test sample, comprising the use of a known nucleic acid (DNA or RNA) as a primer and utilizes a nucleic acid polymerase to amplify or generate a specific piece of nucleic acid or to amplify or generate a specific piece of nucleic acid which is complementary to a particular nucleic acid.

Suitable Methods for Practicing the Invention

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

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

Molecular Modeling

An integral step in our approach to antibody humanization is construction of computer graphics models of the import and humanized antibodies. These models are used to determine if the six complementarity-determining regions (CDRs) can be successfully transplanted from the import framework to a human one and to determine which framework residues from the import antibody, if any, need to be incorporated into the humanized antibody in order to maintain CDR conformation. In addition, analysis of the sequences of the import and humanized antibodies and reference to the models can help to discern which framework residues are unusual and thereby might be involved in antigen binding or maintenance of proper antibody structure.

All of the humanized antibody models of this invention are based on a single three-dimensional computer graphics structure hereafter referred to as the consensus structure. This consensus structure is a key distinction from the approach of previous workers in the field, who typically begin by selecting a human antibody structure which has an amino acid sequence which is similar to the sequence of their import antibody.

The consensus structure of one embodiment of this invention was built in five steps as described below.

Step 1

Seven Fab X-ray crystal structures from the Brookhaven Protein Data Bank were used (entries 2FB4, 2RHE, 3FAB, and 1 REI which are human structures, and 2MCP, 1 FBJ, and 2HFL which are murine structures). For each structure, protein mainchain geometry and hydrogen bonding patterns were used to assign each residue to one of three secondary structure types: alpha-helix, beta-strand or other (i.e. non-helix and non-strand). The immunoglobulin residues used in superpositioning and those included in the consensus structure are shown in Table 1.

TABLE I

	Immunoglobulin Residues Used in Superpositioning and Those Included in the Consensus Structure							
Ig[a]	2FB4	2RHE	2MCP	3FAB	1FBJ	2HFL	1REI	Consensus[b]
				$V_L\kappa$ domain				
								2–11
	18–24	18–24	19–25	18–24	19–25	19–25	19–25	16–27
	32–37	34–39	39–44	32–37	32–37	32–37	33–38	33–39
								41–49
	60–66	62–68	67–72	53–66	60–65	60–65	61–66	59–77
	69–74	71–76	76–81	69–74	69–74	69–74	70–75	
	84–88	86–90	91–95	84–88	84–88	84–88	85–89	82–91
								101–105
RMS[c]		0.40	0.60	0.53	0.54	0.48	0.50	
				V_H domain				
								3–8
	18–25		18–25	18–25	18–25	18–25		17–23
	34–39		34–39	34–39	34–39	34–39		33–41
	46–52		46–52	46–52	46–52	46–52		45–51
	57–61		59–63	56–60	57–61	57–61		57–61
	68–71		70–73	67–70	68–71	68–71		66–71
	78–84		80–86	77–83	78–84	78–84		75–82
	92–99		94–101	91–98	92–99	92–99		88–94
								102–108

TABLE I-continued

Immunoglobulin Residues Used in Superpositioning and Those Included in the Consensus Structure								
Ig[a]	2FB4	2RHE	2MCP	3FAB	1FBJ	2HFL	1REI	Consensus[b]
RMS[c]			0.43	0.85	0.62	0.91		
RMS[d]	0.91		0.73	0.77	0.92			

[a]Four-letter code for Protein Data Bank file.
[b]Residue numbers for the crystal structures are taken from the Protein Data Bank files. Residue numbers for the consensus structure are according to Kabat et al.
[c]Root-mean-square deviation in Å for (N, Cα, C) atoms superimposed on 2FB4.
[d]Root-mean-square deviation in Å for (N, Cα, C) atoms superimposed on 2HFL.

Step 2

Having identified the alpha-helices and beta-strands in each of the seven structures, the structures were superimposed on one another using the INSIGHT computer program (Biosym Technologies, San Diego, Calif.) as follows: The 2FB4 structure was arbitrarily chosen as the template (or reference) structure. The 2FB4 was held fixed in space and the other six structures rotated and translated in space so that their common secondary structural elements (i.e. alpha-helices and beta-strands) were oriented such that these common elements were as close in position to one another as possible. (This superpositioning was performed using accepted mathematical formulae rather than actually physically moving the structures by hand.)

Step 3

With the seven structures thus superimposed, for each residue in the template (2FB4) Fab one calculates the distance from the template alpha-carbon atom (Cα) to the analogous Cα atom in each of the other six superimposed structures. This results in a table of Cα-Cα distances for each residue position in the sequence. Such a table is necessary in order to determine which residue positions will be included in the consensus model. Generally, is if all Cα-Cα distances for a given residue position were ≦1.0 Å, that position was included in the consensus structure. If for a given position only one Fab crystal structure was >1.0 Å, the position was included but the outlying crystal structure was not included in the next step (for this position only). In general, the seven β-strands were included in the consensus structure while some of the loops connecting the β-strands, e.g. complementarity-determining regions (CDRs), were not included in view of Cα divergence.

Step 4

For each residue which was included in the consensus structure after step 3, the average of the coordinates for individual mainchain N, Cα, C, O and Cβ atoms were calculated. Due to the averaging procedure, as well as variation in bond length, bond angle and dihedral angle among the crystal structures, this "average" structure contained some bond lengths and angles which deviated from standard geometry. For purposes of this invention, "standard geometry" is understood to include geometries commonly accepted as typical, such as the compilation of bond lengths and angles from small molecule structures in Weiner, S. J. et. al., *J. Amer. Chem. Soc.*, 106: 765–784 (1984).

Step 5

In order to correct these deviations, the final step was to subject the "average" structure to 50 cycles of energy minimization (DISCOVER program, Biosym Technologies) using the AMBER (Weiner, S. J. et. al., *J. Amer. Chem. Soc.*, 106: 765–784 (1984)) parameter set with only the Ca coordinates fixed (i.e. all other atoms are allowed to move) (energy minimization is described below). This allowed any deviant bond lengths and angles to assume a standard (chemically acceptable) geometry. See Table II.

TABLE II

Average Bond Lengths and Angles for "Average" (Before) and Energy-Minimized Consensus (After 50 Cycles) Structures

	V_LK before (Å)	V_LK after (Å)	V_H before (Å)	V_H after (Å)	Standard Geometry (Å)
N—Cα	1.459(0.012)	1.451(0.004)	1.451(0.023)	1.452(0.004)	1.449
Cα-C	1.515(0.012)	1.523(0.005)	1.507(0.033)	1.542(0.005)	1.522
O=C	1.208(0.062)	1.229(0.003)	1.160(0.177)	1.231(0.003)	1.229
C—N	1.288(0.049)	1.337(0.002)	1.282(0.065)	1.335(0.004)	1.335
Cα-Cβ	1.508(0.026)	1.530(0.002)	1.499(0.039)	1.530(0.002)	1.526
	(°)	(°)	(°)	(°)	(°)
C—N—Cα	123.5(4.2)	123.8(1.1)	125.3(4.6)	124.0(1.1)	121.9
N—Cα-C	110.0(4.0)	109.5(1.9)	110.3(2.8)	109.5(1.6)	110.1
Cα-C—N	116.6(4.0)	116.6(1.2)	117.6(5.2)	116.6(0.8)	116.6
O=C—N	123.1(4.1)	123.4(0.6)	122.2(4.9)	123.3(0.4)	122.9
N—Cα-Cβ	110.3(2.1)	109.8(0.7)	110.6(2.5)	109.8(0.6)	109.5
Cβ-Cα-C	111.4(2.4)	111.1(0.7)	111.2(2.2)	111.1(0.6)	111.1

Values in parentheses are standard deviations. Note that while some bond length and angle averages did not change appreciably after energy-minimization, the corresponding standard deviations are reduced due to deviant geometries assuming standard values after energy-minimization. Standard geometry values are from the AMBER forcefield as implemented in DISCOVER (Biosym Technologies).

The consensus structure might conceivably be dependent upon which crystal structure was chosen as the template on which the others were superimposed. As a test, the entire procedure was repeated using the crystal structure with the worst superposition versus 2FB4, i.e. the 2HFL Fab structure, as the new template (reference). The two consensus structures compare favorably (root-mean-squared deviation of 0.11 Å for all N, Cα and C atoms).

Note that the consensus structure only includes mainchain (N, Cα, C, O, Cβ atoms) coordinates for only those residues which are part of a conformation common to all seven X-ray crystal structures. For the Fab structures, these include the common β-strands (which comprise two β-sheets) and a few non-CDR loops which connect these β-strands. The consensus structure does not include CDRs or sidechains, both of which vary in their conformation among the seven structures. Also, note that the consensus structure includes only the V_L and V_H domains.

This consensus structure is used as the archetype. It is not particular to any species, and has only the basic shape without side chains. Starting with this consensus structure

the model of any import, human, or humanized Fab can be constructed as follows. Using the amino acid sequence of the particular antibody V_L and V_H domains of interest, a computer graphics program (such as INSIGHT, Biosym Technologies) is used to add sidechains and CDRs to the consensus structure. When a sidechain is added, its conformation is chosen on the basis of known Fab crystal structures (see the Background section for publications of such crystal structures) and rotamer libraries (Ponder, J. W. & Richards, F. M., *J. Mol. Biol.* 193: 775–791 (1987)). The model also is constructed so that the atoms of the sidechain are positioned so as to not collide with other atoms in the Fab.

CDRs are added to the model (now having the backbone plus side chains) as follows. The size (i.e. number of amino acids) of each import CDR is compared to canonical CDR structures tabulated by Chothia et al., *Nature,* 342:877–883 (1989)) and which were derived from Fab crystals. Each CDR sequence is also reviewed for the presence or absence of certain specific amino acid residues which are identified by Chothia as structurally important: e.g. light chain residues 29 (CDR1) and 95 (CDR3), and heavy chain residues 26, 27, 29 (CDR1) and 55 (CDR2). For light chain CDR2, and heavy chain CDR3, only the size of the CDR is compared to the Chothia canonical structure. If the size and sequence (i.e. inclusion of the specific, structurally important residues as denoted by Chothia et al.) of the import CDR agrees in size and has the same structurally important residues as those of a canonical CDR, then the mainchain conformation of the import CDR in the model is taken to be the same as that of the canonical CDR. This means that the import sequence is assigned the structural configuration of the canonical CDR, which is then incorporated in the evolving model.

However, if no matching canonical CDR can be assigned for the import CDR, then one of two options can be exercised. First, using a program such as INSIGHT (Biosym Technologies), the Brookhaven Protein Data Bank can be searched for loops with a similar size to that of the import CDR and these loops can be evaluated as possible conformations for the import CDR in the model. Minimally, such loops must exhibit a conformation in which no loop atom overlaps with other protein atoms. Second, one can use available programs which calculate possible loop conformations, assuming a given loop size, using methods such as described by Bruccoleri et al., *Nature* 335: 564–568 (1988).

When all CDRs and sidechains have been added to the consensus structure to give the final model (import, human or humanized), the model is preferably subjected to energy minimization using programs which are available commercially (e.g. DISCOVER, Biosym Technologies). This technique uses complex mathematical formulae to refine the model by performing such tasks as checking that all atoms are within appropriate distances from one another and checking that bond lengths and angles are within chemically acceptable limits.

Models of a humanized, import or human antibody sequence are used in the practice of this invention to understand the impact of selected amino acid residues of the activity of the sequence being modeled. For example, such a model can show residues which may be important in antigen binding, or for maintaining the conformation of the antibody, as discussed in more detail below. Modeling can also be used to explore the potential impact of changing any amino acid residue in the antibody sequence.

Methods for Obtaining a Humanized Antibody Sequence

In the practice of this invention, the first step in humanizing an import antibody is deriving a consensus amino acid sequence into which to incorporate the import sequences. Next a model is generated for these sequences using the methods described above. In certain embodiments of this invention, the consensus human sequences are derived from the most abundant subclasses in the sequence compilation of Kabat et al. (Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)), namely V_L κ subgroup I and V_H group III, and have the sequences indicated in the definitions above.

While these steps may be taken in different order, typically a structure for the candidate humanized antibody is created by transferring the at least one CDR from the non-human, import sequence into the consensus human structure, after the entire corresponding human CDR has been removed. The humanized antibody may contain human replacements of the non-human import residues at positions within CDRs as defined by sequence variability (Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)) or as defined by structural variability (Chothia, C. & Lesk, A. M., *J. Mol. Biol.* 196:901–917 (1987)). For example, huMAb4D5 contains human replacements of the muMAb4D5 residues at three positions within CDRs as defined by sequence variability (Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)) but not as defined by structural variability (Chothia, C. & Lesk, A. M., *J. Mol Bol.* 196:901–917 (1987)): V_L-CDR1 K24R, V_L-CDR2 R54L and V_L-CDR2 T56S.

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

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

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

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

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

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

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

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

1. non-covalently binds antigen directly,

2. interacts with a CDR; or

3. participates in the V_L–V_H interface; and

g. for any non-homologous import antibody amino acid residue which is reasonably expected to have at least

one of these effects, substituting that residue for the corresponding amino acid residue in the consensus antibody FR sequence.

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

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

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

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

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

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

b. fin the FR of the variable domain of the heavy chain) 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 67H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H.

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

If after making a humanized antibody according to the steps above and testing its activity one is not satisfied with the humanized antibody, one preferably reexamines the potential effects of the amino acids at the specific locations recited above. Additionally, it is desirable to reinvestigate any buried residues which are reasonably expected to affect the V_L–V_H interface but may not directly affect CDR conformation. It is also desirable to reevaluate the humanized antibody utilizing the steps of the methods claimed herein.

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

Antibodies

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

In accordance with certain aspects of this invention, antibodies to be humanized (import antibodies) are isolated from continuous hybrid cell lines formed by the fusion of antigen-primed immune lymphocytes with myeloma cells. in certain embodiments, the antibodies of this invention are obtained by routine screening. Polyclonal antibodies to an antigen generally are raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the antigen and an adjuvant. It may be useful to conjugate the antigen or a fragment containing the target amino acid sequence to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic is anhydride, $SOCl_2$, or $R^1N{=}C{=}NR$, where R and R^1 are different alkyl groups.

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

Animals are typically immunized against the immunogenic conjugates or derivatives by combining 1 mg or 1 μg of conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with ⅓ to ⅒ the original amount of conjugate in Freund's complete adjuvant (or other suitable adjuvant) by subcutaneous injection at multiple sites. 7 to 14 days later animals are bled and the serum is assayed for antigen titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different protein and/or through a different cross-linking agent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are used to enhance the immune response.

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

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

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

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

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

Amino Acid Sequence Variants

Amino acid sequence variants of the antibodies and polypeptides of this invention (referred to in herein as the target polypeptide) are prepared by introducing appropriate nucleotide changes into the DNA encoding the target polypeptide, or by in vitro synthesis of the desired target polypeptide. Such variants include, for example, humanized variants of non-human antibodies, as well as deletions from, or insertions or substitutions of, residues within particular amino acid sequences. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the target polypeptide, such as changing the number or position of glycosylation sites, altering any membrane anchoring characteristics, and/or altering the intra-cellular location of the target polypeptide by inserting, deleting, or otherwise affecting any leader sequence of the native target polypeptide.

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

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

expressed target polypeptide variants are screened for the optimal combination of desired activity.

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

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

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

Other insertional variants of the target polypeptide include the fusion to the N- or C-terminus of the target polypeptide of immunogenic polypeptides, e.g., bacterial polypeptides such as beta-lactamase or an enzyme encoded by the *E. coli* trp locus, or yeast protein, and C-terminal fusions with proteins having a long half-life such as immunoglobulin constant regions (or other immunoglobulin regions), albumin, or ferritin, as described in WO 89/02922 published Apr. 6, 1989.

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

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

Substantial modifications in function or immunological identity of the target polypeptide are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (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, gin, his, lys, arg;

(5) residues that influence chain orientation: gly, pro; and

(6) aromatic: trp, tyr, phe.

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

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

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

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

Generally, oligonucleotides of at least 25 nucleotides in length are used. An optimal oligonucleotide will have 12 to 15 nucleotides that are completely complementary to the template on either side of the nucleotide(s) coding for the mutation. This ensures that the oligonucleotide will hybridize properly to the single-stranded DNA template molecule. The oligonucleotides are readily synthesized using techniques known in the art such as that described by Crea et al. (*Proc. Natl. Acad. Sci. USA*, 75: 5765 [1978]).

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

For alteration of the native DNA sequence (to generate amino acid sequence variants, for example), the oligonucleotide is hybridized to the single-stranded template under suitable hybridization conditions. A DNA polymerizing enzyme, usually the Klenow fragment of DNA polymerase 1, is then added to synthesize the complementary strand of the template using the oligonucleotide as a primer for

synthesis. A heteroduplex molecule is thus formed such that one strand of DNA encodes the mutated form of the target polypeptide, and the other strand (the original template) encodes the native, unaltered sequence of the target polypeptide. This heteroduplex molecule is then transformed into a suitable host cell, usually a prokaryote such as *E. coli* JM101. After the cells are grown, they are plated onto agarose plates and screened using the oligonucleotide primer radiolabeled with 32-phosphate to identify the bacterial colonies that contain the mutated DNA. The mutated region is then removed and placed in an appropriate vector for protein production, generally an expression vector of the type typically employed for transformation of an appropriate host.

The method described immediately above may be modified such that a homoduplex molecule is created wherein both strands of the plasmid contain the mutation(s). The modifications are as follows: The single-stranded oligonucleotide is annealed to the single-stranded template as described above. A mixture of three deoxyribonucleotides, deoxyriboadenosine (dATP), deoxyriboguanosine (dGTP), and deoxyribothymidine (dTTP), is combined with a modified thio-deoxyribocytosine called dCTP-(aS) (which can be obtained from Amersham Corporation). This mixture is added to the template-oligonucleotide complex. Upon addition of DNA polymerase to this mixture, a strand of DNA identical to the template except for the mutated bases is generated. In addition, this new strand of DNA will contain dCTP-(aS) instead of dCTP, which serves to protect it from restriction endonuclease digestion.

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

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

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

The alternative method involves two or more rounds of mutagenesis to produce the desired mutant. The first round is as described for the single mutants: wild-type DNA is used for the template, an oligonucleotide encoding the first desired amino acid substitution(s) is annealed to this template, and the heteroduplex DNA molecule is then generated. The second round of mutagenesis utilizes the mutated DNA produced in the first round of mutagenesis as the template. Thus, this template already contains one or more mutations. The oligonucleotide encoding the additional desired amino acid substitution(s) is then annealed to this template, and the resulting strand of DNA now encodes mutations from both the first and second rounds of mutagenesis. This resultant DNA can be used as a template in a third round of mutagenesis, and so on.

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

If the ratio of template to product material is extremely low, the vast majority of product DNA fragments incorporate the desired mutation(s). This product material is used to replace the corresponding region in the plasmid that served as PCR template using standard DNA technology. Mutations at separate positions can be introduced simultaneously by either using a mutant second primer, or performing a second PCR with different mutant primers and ligating the two resulting PCR fragments simultaneously to the vector fragment in a three (or more)-part ligation.

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

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

Another method for preparing variants, cassette mutagenesis, is based on the technique described by Wells et al. (*Gene,* 34: 315 [1985]). The starting material is the plasmid (or other vector) comprising the target polypeptide DNA to be mutated. The codon(s) in the target polypeptide DNA to be mutated are identified. There must be a unique restriction endonuclease site on each side of the identified mutation site(s). If no such restriction sites exist, they may be generated using the above-described oligonucleotide-mediated mutagenesis method to introduce them at appropriate locations in the target polypeptide DNA. After the restriction sites have been introduced into the plasmid, the plasmid is cut at these sites to linearize it. A double-stranded oligonucleotide encoding the sequence of the DNA between the restriction sites but containing the desired mutation(s) is synthesized using standard procedures. The two strands are synthesized separately and then hybridized together using standard techniques. This double-stranded oligonucleotide is referred to as the cassette. This cassette is designed to have 3' and 5' ends that are compatible with the ends of the linearized plasmid, such that it can be directly ligated to the plasmid. This plasmid now contains the mutated target polypeptide DNA sequence.

Insertion of DNA into a Cloning Vehicle

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

(a) Signal Sequence Component

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

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

(b) Origin of Replication Component

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

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

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

(c) Selection Gene Component

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

One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene express a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin (Southern et al., *J. Molec. Appl. Genet.,* 1: 327 [1982]), mycophenolic acid (Mulligan et al., *Science:* 1422 [1980]) or hygromycin (Sugden et al., *Mol. Cell. Biol.,* 5: 410–413 [1985]). The three examples given above employ bacterial genes under eukaryotic control to convey resistance to the appropriate drug G418 or neomycin (geneticin), xgpt (mycophenolic acid), or hygromycin, respectively.

Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the target polypeptide nucleic acid, such as dihydrofolate reductase (DHFR) or thymidine kinase. The mammalian cell transformants are placed under selection pressure which only the transformants are uniquely adapted to survive by virtue of having taken up the marker. Selection pressure is imposed by culturing the transformants

under conditions in which the concentration of selection agent in the medium is successively changed, thereby leading to amplification of both the selection gene and the DNA that encodes the target polypeptide. Amplification is the process by which genes in greater demand for the production of a protein critical for growth are reiterated in tandem within the chromosomes of successive generations of recombinant cells. Increased quantities of the target polypeptide are synthesized from the amplified DNA.

For example, cells transformed with the DHFR selection gene are first identified by culturing all of the transformants in a culture medium that contains methotrexate (Mtx), a competitive antagonist of DHFR. An appropriate host cell when wild-type DHFR is employed is the Chinese hamster ovary (CHO) cell line deficient in DHFR activity, prepared and propagated as described by Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA,* 77: 4216 [1980]. The transformed cells are then exposed to increased levels of methotrexate. This leads to the synthesis of multiple copies of the DHFR gene, and, concomitantly, multiple copies of other DNA comprising the expression vectors, such as the DNA encoding the target polypeptide. This amplification technique can be used with any otherwise suitable host, e.g., ATCC No. CCL61 CHO-K1, notwithstanding the presence of endogenous DHFR if, for example, a mutant DHFR gene that is highly resistant to Mtx is employed (EP 117,060). Alternatively, host cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding the target polypeptide, wild-type DHFR protein, and another selectable marker such as aminoglycoside 3' phosphotransferase (APH) can be selected by cell growth in medium containing a selection agent for the selectable marker such as an aminoglycosidic antibiotic, e.g., kanamycin, neomycin, or G418. See U.S. Pat. No. 4,965,199.

A suitable selection gene for use in yeast is the trp1 gene present in the yeast plasmid YRp7 (Stinchcomb et al., *Nature,* 22: 39 [1979]; Kingsman et al., *Gene,* 7: 141 [1979]; or Tschemper et al., *Gene,* 10: 157 [1980]). The trp1 gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1 (Jones, *Genetics,* 5: 12 [1977]). The presence of the trp1 lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan. Similarly, Leu2-deficient yeast strains (ATCC 20,622 or 38,626) are complemented by known plasmids bearing the Leu2 gene.

(d) Promoter Component

Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to the target polypeptide nucleic acid. Promoters are untranslated sequences located upstream (5') to the start codon of a structural gene (generally within about 100 to 1000 bp) that control the transcription and translation of a particular nucleic acid sequence, such as that encoding the target polypeptide, to which they are operably linked. Such promoters typically fall into two classes, inducible and constitutive. Inducible promoters are promoters that initiate increased levels of transcription from DNA under their control in response to some change in culture conditions, e.g. the presence or absence of a nutrient or a change in temperature. At this time a large number of promoters recognized by a variety of potential host cells are well known. These promoters are operably linked to DNA encoding the target polypeptide by removing the promoter from the source DNA by restriction enzyme digestion and inserting the isolated promoter sequence into the vector. Both the native target polypeptide promoter sequence and many heterologous promoters may be used to direct amplification and/or expression of the target polypeptide DNA. However, heterologous promoters are preferred, as they generally permit greater transcription and higher yields of expressed target polypeptide as compared to the native target polypeptide promoter.

Promoters suitable for use with prokaryotic hosts include the β-lactamase and lactose promoter systems (Chang et al., *Nature,* 275: 615 [1978]; and Goeddel et al., *Nature,* 281: 544 [1979]), alkaline phosphatase, a tryptophan (trp) promoter system (Goeddel, *Nucleic Acids Res.,* 8: 4057 [1980] and EP 36,776) and hybrid promoters such as the 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.,* 2: 149 [1968]; and Holland, *Biochemistry,* 17: 4900 [1978]), such asenolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in Hitzeman et al., EP 73,657A. Yeast enhancers also ate advantageously used with yeast promoters.

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

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

The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that