TABLE 1 CDR-GRAFTED GENE CONSTRUCTS

CODE	MOUSE SEQUENCE CONTENT	METHOD OF CONSTRUCTION	KOZAK SEQUENCE -	KOZAK SEQUENCE +
LIGHT CHAIN	ALL HUMAN FRAMEWORK REI			
121	26-32, 50-56, 91-96 inclusive	SDM and gene assembly	+	n.d.
121A	26-32, 50-56, 91-96 inclusive +1, 3, 46, 47	Partial gene assembly	n.d.	+
121B	26-32, 50-56, 91-96 inclusive + 46, 47	Partial gene assembly	n.d.	+
221	24-24, 50-56, 91-96 inclusive	Partial gene assembly	+	+
221A	24-34, 50-56, 91-96 inclusive +1, 3, 46, 47	Partial gene assembly	+	+
221B	24-34, 50-56, 91-96 inclusive +1, 3	Partial gene assembly	+	+
221C	24-34, 50-56, 91-96 inclusive	Partial gene assembly	+	+
HEAVY CHAIN	ALL HUMAN FRAMEWORK KOL			
121	26-32, 50-56, 95-100B inclusive	Gene assembly	n.d.	+
131	26-32, 50-58, 95-100B inclusive	Gene assembly	n.d.	+
141	26-32, 50-65, 95-100B inclusive	Partial gene assembly	+	n.d.
321	26-35, 50-56, 95-100B inclusive	Partial gene assembly	+	n.d.
331	26-35, 50-58, 95-100B inclusive	Partial gene assembly	+	
		Gene assembly		+
341	26-35, 50-65, 95-100B inclusive	SDM	+	
		Partial gene assembly		+
341A	26-35, 50-65, 95-100B inclusive +6, 23, 24, 48, 49, 71, 73, 76, 78, 88, 91 (+63 - human) (SEQ ID NO: 8-28)	Gene assembly	n.d.	+
341B	26-35, 50-65, 95-100B inclusive + 48, 49, 71, 73, 76, 78, 88, 91 (+63 + human)	Gene assembly	n.d.	+

KEY

n.d.	not done
SDM	Site directed mutagenesis
Gene assembly	Variable region assembled entirely from oligonucleotides
Partial gene assembly	Variable region assembled by combination of restriction fragments either from other genes originally created by SDM and gene assembly or by oligonucleotide assembly of part of the variable region and reconstruction with restriction fragments from other genes originally created by SDM and gene assembly

14. EXPRESSION OF CDR-GRAFTED GENES

14.1. PRODUCTION OF ANTIBODY CONSISTING OF GRAFTED LIGHT (gL) CHAINS WITH MOUSE HEAVY (mH) OR CHIMERIC HEAVY (cH) CHAINS

All gL chains, in association with mH or cH produced reasonable amounts of antibody. Insertion of the Kozak consensus sequence at a position 5' to the ATG (kgL constructs) however, led to a 2-5 fold improvement in net expression. Over an extended series of experiments expression levels were raised from approximately 200ng/ml to approximately 500 ng/ml for kgL/cH or kgL/mH combinations.

When direct binding to antigen on HUT 78 cells was measured, a construct designed to include mouse sequence based on loop length (gL121) did not lead to active antibody in association with mH or cH. A construct designed to include mouse sequence based on Kabat CDRs (gL221 (SEQ ID NO:28)) demonstrated some weak binding in association with mH or cH. However, when framework residues 1, 3, 46, 47 were changed from the human to the murine OKT3 equivalents based on the arguments outlined in Section 12.1 antigen binding was demonstrated when both of the new constructs, which were termed 121A and 221A were co-expressed with cH. When the effects of these residues were examined in more detail, it appears that residues 1 and 3 are not major contributing residues as the product of the gL221B gene (SEQ ID NO:27) shows little detectable binding activity in association with cH. The light chain product of gL221C (SEQ ID NO:28), in which mouse sequences are present at 46 and 47, shows good binding activity in association with cH.

14.2 PRODUCTION OF ANTIBODY CONSISTING OF GRAFTED HEAVY (gH) CHAINS WITH MOUSE LIGHT (mL) OR CHIMERIC LIGHT (cL) CHAINS

Expression of the gH genes proved to be more difficult to achieve than for gL. First, inclusion of the Kozak sequence appeared to have no marked effect on expression of gH genes. Expression appears to be slightly improved but not to the same degree as seen for the grafted light chain.

Also, it proved difficult to demonstrate production of expected quantities of material when the loop choice (amino acid 26-32) for CDR1 is used, e.g. gH121, 131, 141 and no conclusions can be drawn about these constructs. (SEQ ID NO:11)

Moreover, co-expression of the gH341 gene with cL or mL has been variable and has tended to produce lower amounts of antibody than the cH/cL or mH/mL combinations. The alterations to gH341 to (SEQ ID NO:11) produce gH341A (SEQ ID NO:12) and gH341B (SEQ ID NO:21) lead to improved levels of expression.

This may be due either to a general increase in the fraction of mouse sequence in the variable region, or to the alteration at position 63 where the residue is returned to the human amino acid Valine (Val) from Phenylalanine (Phe) to avoid possible internal packing problems with the rest of the human framework. This arrangement also occurs in gH331 and gH321.

When gH321 or gH331 were expressed in association with cL, antibody was produced but antibody binding activity was not detected. (SEQ ID NO:11)

When the more conservative gH341 gene was used antigen binding could be detected in association with cL or mL, but the activity was only marginally above the background level.

When further mouse residues were substituted based on the arguments in 12.1, antigen binding could be clearly demonstrated for the antibody produced when kgH341A and kgH341B were expressed in association with cL.

14.3 PRODUCTION OF FULLY CDR-GRAFTED ANTIBODY

The kgL221A gene was co-expressed with kgH341, kgH341A or kgH341B. For the combination kgH221A/kgH341 very little material was produced in a normal COS cell expression.

For the combinations kgL221A/kgH341A or kgH221A/kgH341B amounts of antibody similar to gL/cH was produced.

In several experiments no antigen binding activity could be detected with kgH221A/gH341 or kgH221A/kgH341 combinations, although expression levels were very low.

Antigen binding was detected when kgL221A/kgH341A or kgH221A/kgH341B combinations were expressed. In the case of the antibody produced from the kgL221A/kgH341A combination the antigen binding was very similar to that of the chimeric antibody.

An analysis of the above results is given below.

15. DISCUSSION OF CDR-GRAFTING RESULTS

In the design of the fully humanised antibody the aim was to transfer the minimum number of mouse amino acids that would confer antigen binding onto a human antibody framework.

15.1. LIGHT CHAIN

15.1.1. EXTENT OF THE CDRs

For the light chain the regions defining the loops known from structural studies of other antibodies to contain the antigen contacting residues, and

those hypervariable sequences defined by Kabat et al (refs. 4 and 5) as Complementarity Determining Regions (CDRs) are equivalent for CDR2. For CDR1 the hypervariable region extends from residues 24-34 inclusive while the structural loop extends from 26-32 inclusive. In the case of OKT3 (SEQ ID NO:5) there is only one amino acid difference between the two options, at amino acid 24, where the mouse sequence is a serine and the human framework RE1 has glutamine. For CDR3 the loop extends from residues 91-96 inclusive while the Kabat hypervariability extends from residues 89-97 inclusive. For OKT3 amino acids 89, 90 and 97 (SEQ ID NO. 8 AND 9) are the same between OKT3 and RE1 (Fig. 3). When constructs based on the loop choice for CDR1 (gL121) and the Kabat choice (gL221) were made and co-expressed with mH or cH no evidence for antigen binding activity could be found for gL121, but trace activity could be detected for the gL221, suggesting that a single extra mouse residue in the grafted variable region could have some detectable effect. Both gene constructs were reasonably well expressed in the transient expression system.

15.1.2. FRAMEWORK RESIDUES

The remaining framework residues were then further examined, in particular amino acids known from X-ray analysis of other antibodies to be close to the CDRs and also those amino acids which in OKT3 showed differences from the consensus framework for the mouse subgroup (subgroup VI) to which OKT3 shows most homology. Four positions 1, 3, 46 and 47 were identified and their possible contribution was examined by substituting the mouse amino acid for the human amino acid at each position. Therefore gL221A (gL221 + D1Q, Q3V, L46R, L47W,

see Figure 3 and Table 1) was made, cloned in EE6hCMVneo and co-expressed with cH (pJA144). The resultant antibody was well expressed and showed good binding activity. When the related genes gL221B (SEQ ID NO:28) (gL221 + D1Q, Q3V) and gL221C (gL221 + L46R, L47W) were made and similarly tested, while both genes produced antibody when co-expressed with cH, only the gL221C/cH combination showed good antigen binding. When the gL121A (gL121 + D1Q, Q3V, L46R, L47W) gene was made and co-expressed with cH, antibody was produced which also bound to antigen.

15.2. HEAVY CHAIN

15.2.1. EXTENT OF THE CDRs

For the heavy chain the loop and hypervariability analyses agree only in CDR3. For CDR1 the loop region extends from residues 26-32 inclusive whereas the Kabat CDR extends from residues 31-35 inclusive. For CDR2 the loop region is from 50-58 inclusive while the hypervariable region covers amino acids 50-65 inclusive. Therefore humanised heavy chains were constructed using the framework from antibody KOL and with various combinations of these CDR choices, including a shorter choice for CDR2 of 50-56 inclusive as there was some uncertainty as to the definition of the end point for the CDR2 loop around residues 56 to 58. The genes were co-expressed with mL or cL initially. In the case of the gH genes with loop choices for CDR1 e.g. gH121, gH131, gH141 very little antibody was produced in the culture supernatants. As no free light chain was detected it was presumed that the antibody was being made and assembled inside the cell but that the heavy chain was aberrant in some way, possibly incorrectly folded, and therefore the antibody was

being degraded internally. In some experiments trace amounts of antibody could be detected in ^{35}S labelling studies.
As no net antibody was produced, analysis of these constructs was not pursued further.
When, however, a combination of the loop choice and the Kabat choice for CDR1 was tested (mouse amino acids 26-35 inclusive) and in which residues 31 (Ser to Arg), 33 (Ala to Thr), and 35 (Tyr to His) were changed from the human residues to the mouse residue and compared to the first series, antibody was produced for gH321, kgH331 and kgH341 when co-expressed with cL. Expression was generally low and could not be markedly improved by the insertion of the Kozak consensus sequence 5' to the ATG of the signal sequence of the gene, as distinct from the case of the gL genes where such insertion led to a 2-5 fold increase in net antibody production. However, only in the case of gH341/mL or kgH341/cL could marginal antigen binding activity be demonstrated. When the kgH341 gene was co-expressed with kgL221A (SEQ ID NO: 26), the net yield of antibody was too low to give a signal above the background level in the antigen binding assay.

15.2.2. FRAMEWORK RESIDUES

As in the case of the light chain the heavy chain frameworks were re-examined. Possibly because of the lower initial homology between the mouse and human heavy variable domains compared to the light chains, more amino acid positions proved to be of interest. Two genes kgH341A and kgH341B were constructed, with 11 or 8 human residues respectively substituted by mouse residues compared to gH341, and with the CDR2 residue 63 returned to the human amino acid potentially to

improve domain packing. Both showed antigen binding when combined with cL or kgL221A, the kgH341A gene with all 11 changes appearing to be the superior choice.

15.3 INTERIM CONCLUSIONS

It has been demonstrated, therefore, for OKT3 that to transfer antigen binding ability to the humanised antibody, mouse residues outside the CDR regions defined by the Kabat hypervariability or structural loop choices are required for both the light and heavy chains. Fewer extra residues are needed for the light chain, possibly due to the higher initial homology between the mouse and human kappa variable regions.

Of the changes seven (1 and 3 from the light chain and 6, 23, 71, 73 and 76 from the heavy chain) are predicted from a knowledge of other antibody structures to be either partly exposed or on the antibody surface. It has been shown here that residues 1 and 3 in the light chain are not absolutely required to be the mouse sequence; and for the heavy chain the gH341B heavy chain in combination with the 221A light chain generated only weak binding activity. Therefore the presence of the 6, 23 and 24 changes are important to maintain a binding affinity similar to that of the murine antibody. It was important, therefore, to further study the individual contribution of othe other 8 mouse residues of the kgH341A gene compared to kgH341.

16. FURTHER CDR-GRAFTING EXPERIMENTS

Additional CDR-grafted heavy chain genes were prepared substantially as described above. With reference to Table 2 the further heavy chain genes were based upon the gh341 (plasmid pJA178) and

gH341A (plasmid pJA185) with either mouse OKT3 or human KOL residues at 6, 23, 24, 48, 49, 63, 71, 73, 76, 78, 88 and 91, as indicated. The CDR-grafted light chain genes used in these further experiments were gL221, gL221A, gL221B and gL221C as described above.

TABLE 2

OKT3 HEAVY CHAIN CDR GRAFTS

1. gH341 and derivatives

RES NUM	6	23	24	48	49	63	71	73	76	78	88	91	
OKT3vh	Q	K	A	I	G	F	T	K	S	A	A	Y	
gH341	E	S	S	V	A	F	R	N	N	L	G	F	JA178
gH341A	Q	K	A	I	G	V	T	K	S	A	A	Y	JA185
gH341E	Q	K	A	I	G	V	T	K	S	A	G	G	JA198
gH341*	Q	K	A	I	G	V	T	K	N	A	G	F	JA207
gH341*	Q	K	A	I	G	V	R	N	N	A	G	F	JA209
gH341D	Q	K	A	I	G	V	T	K	N	L	G	F	JA197
gH341*	Q	K	A	I	G	V	R	N	N	L	G	F	JA199
gH341C	Q	K	A	V	A	F	R	N	N	L	G	F	JA184
gH341*	Q	S	A	I	G	V	T	K	S	A	A	Y	JA203
gH341*	E	S	A	I	G	V	T	K	S	A	A	Y	JA205
gH341B	E	S	S	I	G	V	T	K	S	A	A	Y	JA183
gH341*	Q	S	A	I	G	V	T	K	S	A	G	F	JA204
gH341*	E	S	A	I	G	V	T	K	S	A	G	F	JA206
gH341*	Q	S	A	I	G	V	T	K	N	A	G	F	JA208
KOL	E	S	S	V	A		R	N	N	L	G	F	

A 5 (SEQ ID NO: 30 7, 10 and 11-24)

OKT3 LIGHT CHAIN CDR GRAFTS

2. gL221 and derivatives

RES NUM	1	3	46	47	
OKT3v1	Q	V	R	W	
GL221	D	Q	L	L	DA221
gL221A	Q	V	R	W	DA221A
gL221B	Q	V	L	L	DA221B
GL221C	D	Q	R	W	DA221C
RE1	D	Q 29	L	L	

A 5 (SEQ ID NO: 8, 8, 9 and 25-28)

MURINE RESIDUES ARE UNDERLINED

The CDR-grafted heavy and light chain genes were co-expressed in COS cells either with one another in various combinations but also with the corresponding murine and chimeric heavy and light chain genes substantially as described above. The resultant antibody products were then assayed in binding and blocking assays with HPB-ALL cells as described above.

The results of the assays for various grafted heavy chains co-expressed with the gL221C light chain (SEQ ID NO:28) are given in Figures 7 and 8 (for the JA184, JA185, JA197 and JA198 constructs - see Table 2), in Figure 9 (for the JA183, JA184, JA185 and JA197 constructs) in Figure 10 a and b (for the chimeric, JA185, JA199, JA204, JA205, JA207, JA208 and JA209 constructs) and in Figure 11 a and b (for the JA183, JA184, JA185, JA198, JA203, JA205 and JA206 constructs).

The basic grafted product without any human to murine changes in the variable frameworks, i.e. gL221 (SEQ ID NO:25) co-expressed with gh341 (JA178) (SEQ ID NO:11), and also the "fully grafted" product, having most human to murine changes in the grafted heavy chain framework, i.e. gL221C (SEQ ID NO:28) co-expressed with gh341A (JA185) (SEQ ID NO:12), were assayed for relative binding affinity in a competition assay against murine OKT3 reference standard, using HPB-ALL cells. The assay used was as described above in section 3.3. The results obtained are given in Figure 12 for the basic grafted product and in Figure 13 for the fully grafted product. These results indicate that the basic grafted product has neglibible binding ability as compared with the OKT3 murine reference standard; whereas the "fully grafted" product has a binding ability very similar to that of the OKT3 murine reference standard.

The binding and blocking assay results indicate the following:

The JA198 and JA207 constructs appear to have the best binding characteristics and similar binding abilities, both substantially the same as the chimeric and fully grafted gH341A products. This indicates that positions 88 and 91 and position 76 are not highly critical for maintaining the OKT3 binding ability; whereas at least some of positions 6, 23, 24, 48, 49, 71, 73 and 78 are more important.

This is borne out by the finding that the JA209 and JA199, although of similar binding ability to one another, are of lower binding ability than the JA198 and JA207 constructs. This indicates the importance of having mouse residues at positions 71, 73 and 78, which are either completely or partially human in the JA199 and JA209 constructs respectively.

Moreover, on comparing the results obtained for the JA205 and JA183 constructs it is seen that there is a decrease in binding going from the JA205 to the JA183 constructs. This indicates the importance of retaining a mouse residue at position 23, the only position changed between JA205 and JA183.

These and other results lead us to the conclusion that of the 11 mouse framework residues used in the gH341A (JA185) construct, it is important to retain mouse residues at all of positions 6, 23, 24, 48 and 49, and possibly for maximum binding affinity at 71, 73 and 78.

Similar Experiments were carried out to CDR-graft a number of the rodent antibodies including antibodies having specificity for CD4 (OKT4), ICAM-1 (R6-5), TAG72 (B72.3), and TNFα(61E71, 101.4, hTNF1, hTNF2 and hTNF3).

EXAMPLE 2

CDR-GRAFTING OF A MURINE ANTI-CD4 T CELL RECEPTOR ANTIBODY, OKT4A

Anti OKT4A CDR-grafted heavy and light chain genes were prepared, expressed and tested substantially as described above in Example 1 for CDR-grafted OKT3. The CDR grafting of OKT4A is described in detail in Ortho patent application PCT/GB 90..... of even date herewith entitled "Humanised Antibodies". The disclosure of this Ortho patent application PCT/GB 90 ..... is incorporated herein by reference. A number of CDR-grafted OKT4 antibodies have been prepared. Presently the CDR-grafted OKT4A of choice is the combination of the grafted light chain LCDR2 and the grafted heavy chain HCDR10.

THE LIGHT CHAIN

The human acceptor framework used for the grafted light chains was RE1. (SEQ ID NO: 8 and 9) The preferred LCDR2 light chain has human to mouse changes at positions 33, 34, 38, 49 and 89 in addition to the structural loop CDRs. Of these changed positions, positions 33, 34 and 89 fall within the preferred extended CDRs of the present invention (positions 33 and 34 in CDR1 and position 89 in CDR3). The human to murine changes at positions 38 and 49 corresponds to positions at which the amino acid residues are preferably donor murine amino acid residues in accordance with the present invention.

A comparison of the amino acid sequences of the donor murine light chain variable domain and the RE1 human acceptor light chain variable further reveals that the murine and human residues are identical at all of positions 46, 48 and 71 and at all of positions 2, 4, 6, 35, 36, 44, 47, 62, 64-69, 85, 87, 98, 99 and 101 and 102. However the amino acid residue at position 58 in LCDR2 is

the human RE1 framework residue not the mouse OKT4 residue as would be preferred in accordance with the present invention.

THE HEAVY CHAIN

The human acceptor framework used for the grafted heavy chains was KOL (SEQ ID NO:10)

The preferred CDR graft HCDR10 heavy chain has human to mouse changes at positions 24, 35, 57, 58, 60, 88 and 91 in addition to the structural loop CDRs.

Of these positions, positions 35 (CDR1) and positions 57, 58 and 60 (CDR2) fall within the preferred extended CDRs of the present invention. Also the human to mouse change at position 24 corresponds to a position at which the amino acid residue is a donor murine residue in accordance with the present invention. Moreover, the human to mouse changes at positions 88 and 91 correspond to positions at which the amino acid residues are optionally donor murine residues.

Moreover, a comparison of the murine OKT4A and human KOL heavy chain variable amino acid sequences reveals that the murine and human residues are identical at all of positions 23, 49, 71, 73 and 78 and at all of positions 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

Thus the OKT4A CDR-grafted heavy chain HCDR10 corresponds to a particularly preferred embodiment according to the present invention.

EXAMPLE 3

CDR-GRAFTING OF AN ANTI-MUCIN SPECIFIC MURINE ANTIBODY, B72.3

The cloning of the genes coding for the anti-mucin specific murine monoclonal antibody B72.3 and the preparation of B72.3 mouse-human chimeric antibodies has been described previously (ref. 13 and WO 89/01783). CDR-grafted versions of B72.3 were prepared as follows.

(a) B72.3 Light Chain

CDR-grafting of this light chain was accomplished by direct transfer of the murine CDRs into the framework of the human light chain RE1. The regions transferred were:

CDR Number	Residues
1	24-34
2	50-56
3	90-96

The activity of the resulting grafted light chain was assessed by co-expression in COS cells, of genes for the combinations:

B72.3 cH/B72.3 cL

and B72.3 cH/B72.3 gL

Supernatants were assayed for antibody concentration and for the ability to bind to microtitre plates coated with mucin. The results obtained indicated that, in combination with the B72.3 cH chain, B72.3 cL and B72.3 gL had similar binding properties.

Comparison of the murine B72.3 and REI light chain amino acid sequences (SEQ ID NO: 8 and 9) reveals that the residues are identical at positions 46, 58 and 71 but are different at position 48.

Thus changing the human residue to the donor mouse residue at position 48 may further improve the binding characteristics of the CDR-grafted light chain, (B72.3 gL) in accordance with the present invention.

(b) B72.3 heavy chain

i. Choice of framework

At the outset it was necessary to make a choice of human framework. Simply put, the question was as follows: Was it necessary to use the framework regions from an antibody whose crystal structure was known or could the choice be made on some other criteria?

For B72.3 heavy chain, it was reasoned that, while knowledge of structure was important, transfer of the CDRs from mouse to human frameworks might be facilitated if the overall homology between the donor and receptor frameworks was maximised.

Comparison of the B72.3 heavy chain sequence with those in Kabat (ref. 4) for human heavy chains showed clearly that B72.3 had poor homology for KOL (SEQ ID NO:10) and NEWM (for which crystal structures are available) but was very homologous to the heavy chain for EU.

On this basis, EU was chosen for the CDR-grafting and the following residues transferred as CDRs.

CDR Number	Residues
1	27-36
2	50-63
3	93-102

Also it was noticed that the FR4 region of EU was unlike that of any other human (or mouse) antibody. Consequently, in the grafted heavy chain genes this was also changed to produce a "consensus" human sequence. (Preliminary experiments showed that grafted heavy chain genes containing the EU FR4 sequence expressed very poorly in transient expression systems.)

ii. Results with grafted heavy chain genes

Expression of grafted heavy chain genes containing all human framework regions with either gL or cL genes produced a grafted antibody with little ability to bind to mucin. The grafted antibody had about 1% the activity of the chimeric antibody.

In these experiments, however, it was noted that the activity of the grafted antibody could be increased to ~ 10% of B72.3 by exposure to pHs of 2-3.5.

This observation provided a clue as to how the activity of the grafted antibody could be improved without acid treatment. It was postulated that acid exposure brought about the protonation of an acidic residue (pKa of aspartic acid = 3.86 and of glutamine acid = 4.25) which in turn caused a change in structure of the CDR loops, or allowed better access of antigen.

From comparison of the sequences of B72.3 (ref. 13) and EU (refs. 4 and 5), it was clear that, in going from the mouse to human frameworks, only two positions had been changed in such a way that acidic residues had been introduced. These

positions are at residues 73 and 81, where K to E and Q to E changes had been made, respectively.

Which of these positions might be important was determined by examining the crystal structure of the KOL antibody. In KOL heavy chain (SEQ ID NO:10), position 81 is far removed from either of the CDR loops.

Position 73, however, is close to both CDRs 1 and 3 of the heavy chain and, in this position it was possible to envisage that a K to E change in this region could have a detrimental effect on antigen binding.

iii. Framework changes in B72.3 gH gene

On the basis of the above analysis, E73 was mutated to a lysine (K). It was found that this change had a dramatic effect on the ability of the grafted Ab to bind to mucin. Further the ability of the grafted B72.3 produced by the mutated gH/gL combination to bind to mucin was similar to that of the B72.3 chimeric antibody.

iv. Other framework changes

In the course of the above experiments, other changes were made in the heavy chain framework regions. Within the accuracy of the assays used, none of the changes, either alone or together, appeared beneficial.

v. Other

All assays used measured the ability of the grafted Ab to bind to mucin and, as a whole, indicated that the single framework change at position 73 is sufficient to generate an antibody with similar binding properties to B72.3.

Comparison of the B72.3 murine and EU heavy chain sequences reveals that the mouse and human residues are identical at positions 23, 24, 71 and 78.
Thus the mutated CDR-grafted B72.3 heavy chain corresponds to a preferred embodiment of the present invention.

EXAMPLE 4

CDR-GRAFTING OF A MURINE ANTI-ICAM-1 MONOCLONAL ANTIBODY

A murine antibody, R6-5-D6 (EP 0314863) having specificity for Intercellular Adhesion Molecule 1 (ICAM-1) was CDR-grafted substantially as described above in previous examples. This work is described in greater detail in co-pending application, British Patent Application No. 9009549.8, the disclosure of which is incorporated herein by reference.

The human EU framework was used as the acceptor framework for both heavy and light chains. The CDR-grafted antibody currently of choice is provided by co-expression of grafted light chain gL221A (SEQ ID NO: 26) and grafted heavy chain gH341D (SEQ ID NO:16) which has a binding affinity for ICAM 1 of about 75% of that of the corresponding mouse-human chimeric antibody.

LIGHT CHAIN

gL221A has murine CDRs at positions 24-34 (CDR1), 50-56 (CDR2) and 89-97 (CDR3). In addition several framework residues are also the murine amino acid. These residues were chosen after consideration of the possible contribution of these residues to domain packing and stability of the conformation of the antigen binding region. The residues which have been retained as mouse are at positions 2, 3, 48 (?), 60, 84, 85 and 87. Comparison of the murine anti-ICAM 1 and human EU light chain amino acid sequences reveals that the murine and human residues are identical at positions 46, 58 and 71.

HEAVY CHAIN

gH341D has murine CDRs at positions 26-35 (CDR1), 50-56 (CDR2) and94-100B (CDR3). In addition murine residues were used in gH341D at positions 24, 48, 69, 71, 73, 80, 88 and 91. Comparison of the murine anti-ICAM 1 and human EU heavy chain amino acid sequences are identical at positions 23, 49 and 78.

EXAMPLE 5

CDR-Grafting of murine anti-TNFα antibodies

A number of murine anti-TNFα monoclonal antibodies were CDR-grafted substantially as described above in previous examples. These antibodies include the murine monoclonal antibodies designated 61 E71, hTNF1, hTNF3 and 101.4 A brief summary of the CDR-grafting of each of these antibodies is given below.

61E71

A similar analysis as described above (Example 1, Section 12.1.) was done for 61E71 and for the heavy chain 10 residues were identified at 23, 24, 48, 49, 68, 69, 71, 73, 75 and 88 as residues to potentially retain as murine. The human frameworks chosen for CDR-grafting of this antibody, and the hTNF3 and 101.4 antibodies were REI for the light chain and KOL for the heavy chain.
Three genes were built, the first of which contained 23, 24, 48, 49, 71 and 73 [gH341(6)] as murine residues. The second gene also had 75 and 88 as murine residues [gH341(8)] while the third gene additionally had 68, 69, 75 and 88 as murine residues [gH341(10)]. Each was co-expressed with gL221, the minimum grafted light chain (CDRs only). The gL221/gH341(6) and gL221/gH341(8) antibodies both bound as well to TNF as murine 61E71. The gL221/gH341(10) antibody did not express and this combination was not taken further.
Subsequently the gL221/gH341(6) antibody was assessed in an L929 cell competition assay in which the antibody competes against the TNF receptor on L929 cells for binding to TNF in solution. In this assay the gL221/gH341(6) antibody was approximately 10% as active as murine 61E71.

hTNF1

hTNF1 is a monoclonal antibody which recognises an epitope on human TNF- . The EU human framework was used for CDR-grafting of both the heavy and light variable domains.

Heavy Chain

In the CDR-grafted heavy chain (ghTNF1) mouse CDRs were used at positions 26-35 (CDR1), 50-65 (CDR2) and 95-102 (CDR3). Mouse residues were also used in the frameworks at positions 48, 67, 69, 71, 73, 76, 89, 91, 94 and 108. Comparison of the TNF1 mouse and EU human heavy chain residues reveals that these are identical at positions 23, 24, 29 and 78.

Light Chain

In the CDR-grafted light chain (gLhTNF1) mouse CDRs wre used at positions 24-34 (CDR1), 50-56 (CDR2) and 89-97 (CDR3). In addition mouse residues were used in the frameworks at positions 3, 42, 48, 49, 83, 106 and 108. Comparison of the hTNF1 mouse and EU human light chain residues reveals that these are identical at positions 46, 58 and 71.

The grafted hTNF1 heavy chain was co-expressed with the chimeric light chain and the binding ability of the product compared with that of the chimeric light chain/chimeric heavy chain product in a TNF binding assay. The grafted heavy chain product appeared to have binding ability for TNF slightly better than the fully chimeric product.

Similarly, a grafted heavy chain/grafted light chain product was co-expressed and compared with the fully chimeric product and found to have closely similar binding properties to the latter product.

hTNF3

hTNF3 recognises an epitope on human TNF-α. The sequence of hTNF3 shows only 21 differences compared to 61E71 in the light and heavy chain variable regions, 10 in the light chain (2 in the CDRs at positions 50, 96 and 8 in the framework at 1, 19, 40, 45, 46, 76, 103 and 106) and 11 in the heavy chain (3 in the CDR regions at positions 52, 60 and 95 and 8 in the framework at 1, 10, 38, 40, 67, 73, 87 and 105). The light and heavy chains of the 61E71 and hTNF3 chimeric antibodies can be exchanged without loss of activity in the direct binding assay. However 61E71 is an order of magnitude less able to compete with the TNF receptor on L929 cells for TNF-a compared to hTNF3. Based on the 61E71 CDR grafting data gL221 and gH341(+23, 24, 48, 49 71 and 73 as mouse) genes have been built for hTNF3 and tested and the resultant grafted antibody binds well to TNF-a, but competes very poorly in the L929 assay. It is possible that in this case also the framework residues identified for OKT3 programme may improve the competitive binding ability of this antibody.

101.4

101.4 is a further murine monoclonal antibody able to recognise human TNF-a. The heavy chain of this antibody shows good homology to KOL and so the CDR-grafting has been based on REI for the light chain and KOL for the heavy chain. Several grafted heavy chain genes have been constructed with conservative choices for the CDR's (gH341) (SEQ ID NO:11) and which have one or a small number of non-CDR residues at positions 73, 78 or 77-79 inclusive, as the mouse amino acids. These have been co-expressed with cL or gL221. In all cases binding to TNF equivalent to the chimeric antibody is seen and when co-expressed with cL the resultant antibodies are able to compete well in the L929 assay. However, with gL221 the resultant antibodies

are at least an order of magnitude less able to compete for TNF against the TNF receptor on L929 cells.

Mouse residues at other positions in the heavy chain, for example, at 23 and 24 together or at 76 have been demonstrated to provide no improvement to the competitive ability of the grafted antibody in the L929 assay.

A number of other antibodies including antibodies having specificity for interleukins e.g. IL1 and cancer markers such as carcinoembryonic antigen (CEA) e.g. the monoclonal antibody A5B7 (ref. 21), have been successfully CDR-grafted according to the present invention.
It will be appreciated that the foregoing examples are given by way of illustration only and are not intended to limit the scope of the claimed invention. Changes and modifications may be made to the methods described whilst still falling within the spirit and scope of the invention.

References

1. Kohler & Milstein, Nature, 265, 295-497, 1975.

2. Chatenoud et al, (1986), J. Immunol. 137, 830-838.

3. Jeffers et al, (1986), Transplantation, 41, 572-578.

4. Begent et al, Br. J. Cancer 62: 487 (1990).

5. Verhoeyen et al, Science, 239, 1534-1536, 1988.

6. Riechmann et al, Nature, 332, 323-324, 1988.

7. Kabat, E.A., Wu, T.T., Reid-Miller, M., Perry, H.M., Gottesman, K.S., 1987, in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA.

8. Wu, T.T., and Kabat, E.A., 1970, J. Exp. Med. 132 211-250.

9. Queen et al, (1989), Proc. Natl. Acad. Sci. USA, 86, 10029-10033 and WO 90/07861

10. Maniatis et al, Molecular Cloning, Cold Spring Harbor, New York, 1989.

11. Primrose and Old, Principles of Gene Manipulation, Blackwell, Oxford, 1980.

12. Sanger, F., Nicklen, S., Coulson, A.R., 1977, Proc. Natl. Acad. Sci. USA, 74 5463

13. Kramer, W., Drutsa, V., Jansen, H.-W., Kramer, B., Plugfelder, M., Fritz, H.-J., 1984, Nucl. Acids Res. 12, 9441

14. Whittle, N., Adair, J., Lloyd, J.C., Jenkins, E., Devine, J., Schlom, J., Raubitshek, A., Colcher, D., Bodmer, M., 1987, Protein Engineering 1, 499.

15. Sikder, S.S., Akolkar, P.N., Kaledas, P.M., Morrison, S.L., Kabat, E.A., 1985, J. Immunol. 135, 4215.

16. Wallick, S.C., Kabat, E.A., Morrison, S.L., 1988, J. Exp. Med. 168, 1099

17. Bebbington, C.R., Published International Patent Application WO 89/01036.

18. Granthan and Perrin 1986, Immunology Today 7, 160.

19. Kozak, M., 1987, J. Mol. Biol. 196, 947.

20. Jones, T.P., Dear, P.H., Foote, J., Neuberger, M.S., Winter, G., 1986, Nature, 321, 522

21. Harwood et al, Br. J. Cancer, 54, 75-82 (1986).

CLAIMS

1. A CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

2. A CDR-grafted heavy chain according to Claim 1 comprising donor residues at positions 23, 24, 49, 71, 73 and 78, or at positions 23, 24 and 49.

3. A CDR-grafted heavy chain according to Claim 2 comprising donor residues at positions 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

4. A CDR-grafted heavy chain according to Claim 2 or 3, comprising donor residues at one, some or all of positions:
1 and 3,
69 (if 48 is different between donor and acceptor),
38 and 46 (if 48 is the donor residue),
67,
82 and 18 (if 67 is the donor residue),
91, and
any one or more of 9, 11, 41, 87, 108, 110 and 112.

5. A CDR-grafted heavy chain according to any of the preceding comprising donor CDRs at positions 26-35, 50-65 and 95-100.

6. A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 1 and/or 3 and 46 and/or 47.

7. A CDR-grafted light chain according to Claim 6 comprising donor residues at positions 46 and 47.

8. A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, 58 and 71.

9. A CDR-grafted light chain according to Claim 8 comprising donor residues at positions 46, 48, 58 and 71.

10. A CDR-grafted light chain according to Claim 8 or 9, comprising donor residues at positions 2, 4, 6, 35, 36, 38, 44, 47, 49, 62, 64-69, 85, 87, 98, 99, 101 and 102.

11. A CDR-grafted light chain according to Claim 9 or 10, comprising donor residues at one, some or all of positions:
1 and 3,
63,
60 (if 60 and 54 are able to form a potential saltbridge),
70 (if 70 and 24 are able to form a potential saltbridge),
73 and 21 (if 47 is different between donor and acceptor),
37 and 45 (if 47 if different between donor and acceptor), and
any one or more of 10, 12, 40, 83, 103 and 105.

12. A CDR-grafted light chain according to any one of Claims 6-11, comprising donor CDRs at positions 24-34, 50-56 and 89-97.

13. A CDR-grafted antibody molecule comprising at least one CDR-grafted heavy chain according to any one of Claims 1-5 and at least one CDR-grafted light chain according to any one of Claims 6-12.

14. A CDR-grafted antibody molecule according to Claim 13, which is a site-specific antibody molecule.

15. A CDR-grafted antibody molecule according to Claim 13 which has specificity for an interleukin, hormone or other biologically active compound or a receptor therefor.

16. A CDR-grafted antibody heavy or light chain or molecule according to any one of the preceding claims comprising human acceptor residues and non-human donor residues.

17. A DNA sequence which codes for a CDR-grafted heavy chain according to Claim 1 or a CDR-grafted light chain according to Claim 6 or Claim 8.

18. A cloning or expression vector containing a DNA sequence according to Claim 17.

19. A host cell transformed with a DNA sequence according to Claim 17.

20. A process for the production of a CDR-grafted antibody sequence according to Claim 17 in a transformed host cell.

21. A process for producing a CDR-grafted antibody product comprising:

Amt B1

(a) producing in an expression vector an operon having a DNA sequence which encodes an antibody heavy chain according to Claim 1;

and/or

(b) producing in an expression vector an operon having a DNA sequence which encodes a complementary antibody light chain according to Claim 6 or Claim 8;

(c) transfecting a host cell with the or each vector; and

(d) culturing the transfected cell line to produce the CDR-grafted antibody product.

22. A therapeutic or diagnostic composition comprising a CDR-grafted antibody heavy chain according to Claim 1, or a CDR-grafted light chain according to Claim 6 or Claim 8, or a CDR-grafted antibody molecule according to Claim 13 in combination with a pharmaceutically acceptable carrier, diluent or excipient.

23. A method of therapy or diagnosis comprising administering an effective amount of a CDR-grafted heavy chain according to Claim 1, or a CDR-grafted light chain according to Claim 6 or Claim 8, or a CDR-grafted antibody molecule according to Claim 13 to a human or animal subject.

ABSTRACT

CDR-grafted antibody heavy and light chains comprise acceptor framework and donor antigen binding regions, the heavy chains comprising donor residues at at least one of positions (6, 23) and/or (24, 48) and/or (49, 71) and/or (73, 75) and/or (76) and/or (78) and (88) and/or (91). The CDR-grafted light chains comprise donor residues at at least one of positions (1) and/or (3) and (46) and/or (47) or at at least one of positions (46, 48, 58) and (71). The CDR-grafted antibodies are preferably humanised antibodies, having non human, e.g. rodent, donor and human acceptor frameworks, and may be used for *in vivo* therapy and diagnosis. A generally applicable protocol is disclosed for obtaining CDR-grafted antibodies.

```
   1  GAATTCCCAA AGACAAAatg gattttcaag tgcagatttt cagcttcctg
  51  ctaatcagtg cctcagtcat aatatccaga ggacaaattg ttctcaccca
 101  gtctccagca atcatgtctg catctccagg ggagaaggtc accatgacct
 151  gcagtgccag ctcaagtgta agttacatga actggtacca gcagaagtca
 201  ggcacctccc ccaaaagatg gatttatgac acatccaaac tggcttctgg
 251  agtccctgct cacttcaggg gcagtgggtc tgggacctct tactctctca
 301  caatcagcgg catggaggct gaagatgctg ccacttatta ctgccagcag
 351  tggagtagta acccattcac gttcggctcg gggacaaagt tggaaataaa
 401  ccgggctgat actgcaccaa ctgtatccat cttcccacca tccagtgagc
 451  agttaacatc tggaggtgcc tcagtcgtgt gcttcttgaa caacttctac
 501  cccaaagaca tcaatgtcaa gtggaagatt gatggcagtg aacgacaaaa
 551  tggcgtcctg aacagttgga ctgatcagga cagcaaagac agcacctaca
 601  gcatgagcag caccctcacg ttgaccaagg acgagtatga acgacataac
 651  agctatacct gtgaggccac tcacaagaca tcaacttcac ccattgtcaa
 701  gagcttcaac aggaatgagt gtTAGAGACA AAGGTCCTGA GACGCCACCA
 751  CCAGCTCCCA GCTCCATCCT ATCTTCCCTT CTAAGGTCTT GGAGGCTTCC
 801  CCACAAGCGC tTACCACTGT TGCGGTGCTC tAAACCTCCT CCCACCTCCT
 851  TCTCCTCCTC CTCCCTTTCC TTGGCTTTTA TCATGCTAAT ATTTGCAGAA
 901  AATATTCAAT AAAGTGAGTC TTTGCCTTGA AAAAAAAAAA AAA
```

Fig. 1(a)

```
   1  MDFOVQIFSF LLISASVIIS RGQIVLTQSP AIMSASPGEK VTMTCSASSS
  51  VSYMNWYQQK SGTSPKRWIY DTSKLASGVP AHFRGSGSGT SYSLTISGME
 101  AEDAATYYCQ QWSSNPFTFG SGTKLEINRA DTAPTVSIFP PSSEQLTSGG
 151  ASVVCFLNNF YPKDINVKWK IDGSERQNGV LNSWTDQDSK DSTYSMSSTL
 201  TLTKDEYERH NSYTCEATHK TSTSPIVKSF NRNEC*
```

Fig. 1(b)

SUBSTITUTE SHEET

```
   1 GAATTCCCCT CTCCACAGAC ACTGAAAACT CTGACTCAAC ATGGAAAGGC
  51 ACTGGATCTT TCTACTCCTG TTGTCAGTAA CTGCAGGTGT CCACTCCCAG
 101 GTCCAGCTGC AGCAGTCTGG GGCTGAACTG GCAAGACCTG GGGCCTCAGT
 151 GAAGATGTCC TGCAAGGCTT CTGGCTACAC CTTTACTAGG TACACGATGC
 201 ACTGGGTAAA ACAGAGGCCT GGACAGGGTC TGGAATGGAT TGGATACATT
 251 AATCCTAGCC GTGGTTATAC TAATTACAAT CAGAAGTTCA AGGACAAGGC
 301 CACATTGACT ACAGACAAAT CCTCCAGCAC AGCCTACATG CAACTGAGCA
 351 GCCTGACATC TGAGGACTCT GCAGTCTATT ACTGTGCAAG ATATTATGAT
 401 GATCATTACT GCCTTGACTA CTGGGGCCAA GGCACCACTC TCACAGTCTC
 451 CTCAGCCAAA ACAACAGCCC CATCGGTCTA TCCACTGGCC CCTGTGTGTG
 501 GAGATACAAC TGGCTCCTCG GTGACTCTAG GATGCCTGGT CAAGGGTTAT
 551 TTCCCTGAGC CAGTGACCTT GACCTGGAAC TCTGGATCCC TGTCCAGTGG
 601 TGTGCACACC TTCCCAGCTG TCCTGCAGTC TGACCTCTAC ACCCTCAGCA
 651 GCTCAGTGAC TGTAACCTCG AGCACCTGGC CCAGCCAGTC CATCACCTGC
 701 AATGTGGCCC ACCCGGCAAG CAGCACCAAG GTGGACAAGA AAATTGAGCC
 751 CAGAGGGCCC ACAATCAAGC CCTGTCCTCC ATGCAAATGC CCAGCACCTA
 801 ACCTCTTGGG TGGACCATCC GTCTTCATCT TCCCTCCAAA GATCAAGGAT
 851 GTACTCATGA TCTCCCTGAG CCCCATAGTC ACATGTGTGG TGGTGGATGT
 901 GAGCGAGGAT GACCCAGATG TCCAGATCAG CTGGTTTGTG AACAACGTGG
 951 AAGTACACAC AGCTCAGACA CAAACCCATA GAGAGGATTA CAACAGTACT
1001 CTCCGGGTGG TCAGTGCCCT CCCCATCCAG CACCAGGACT GGATGAGTGG
1051 CAAGGAGTTC AAATGCAAGG TCAACAACAA AGACCTCCCA GCGCCCATCG
1101 AGAGAACCAT CTCAAAACCC AAAGGGTCAG TAAGAGCTCC ACAGGTATAT
1151 GTCTTGCCTC CACCAGAAGA AGAGATGACT AAGAAACAGG TCACTCTGAC
1201 CTGCATGGTC ACAGACTTCA TGCCTGAAGA CATTTACGTG GAGTGGACCA
1251 ACAACGGGAA AACAGAGCTA AACTACAAGA ACACTGAACC AGTCCTGGAC
1301 TCTGATGGTT CTTACTTCAT GTACAGCAAG CTGAGAGTGG AAAAGAAGAA
1351 CTGGGTGGAA AGAAATAGCT ACTCCTGTTC AGTGGTCCAC GAGGGTCTGC
1401 ACAATCACCA CACGACTAAG AGCTTCTCCC GGACTCCGGG TAAATGAGCT
1451 CAGCACCCAC AAAACTCTCA GGTCCAAAGA GACACCCACA CTCATCTCCA
1501 TGCTTCCCTT GTATAAATAA AGCACCCAGC AATGCCTGGG ACCATGTAAA
1551  AAAAAAAAAA AAAGGAATTC
```

Fig. 2(a)

SUBSTITUTE SHEET

OKT 3 HEAVY CHAIN PROTEIN SEQUENCE DEDUCED FROM DNA SEQUENCE

```
  1  MERHWIFLLL LSVTAGVHSQ VQLQQSGAEL ARPGASVKMS CKASGYTFTR
 51  YTMHWVKQRP GQGLEWIGYI NPSRGYTNYN QKFKDKATLT TDKSSSTAYM
101  QLSSLTSEDS AVYYCARYYD DHYCLDYWGQ GTTLTVSSAK TTAPSVYPLA
151  PVCGDTTGSS VTLGCLVKGY FPEPVTLTWN SGSLSSGVHT FPAVLQSDLY
201  TLSSSVTVTS STWPSQSITC NVAHPASSTK VDKKIEPRGP TIKPCPPCKC
251  PAPNLLGGPS VFIFPPKIKD VLMISLSPIV TCVVVDVSED DPDVQISWFV
301  NNVEVHTAQT QTHREDYNST LRVVSALPIQ HQDWMSGKEF KCKVNNKDLP
351  APIERTISKP KGSVRAPQVY VLPPPEEEMT KKQVTLTCMV TDFMPEDIYV
401  EWTNNGKTEL NYKNTEPVLD SDGSYFMYSK LRVEKKNWVE RNSYSCSVVH
451  EGLHNHHTTK SFSRTPGK*
```

Fig. 2(b)

```
                1                       23                  42
                NN      N                N        N     N
RES TYPE        SBspSPESssBSbSsSssPSPSPsPSsse*s*p*Pi^ISsSe
Okt3v1          QIVLTQSPAIMSASPGEKVTMTCSASS.SVSYMNWYQQKSGT
REI             DIQMTQSPSSLSASVGDRVTITCQASQDIIKYLNWYQQTPGK
                ? ?
                  CDR1   (LOOP)          *******
                  CDR1   (KABAT)        ***********

                          56                              85
                N  NN
RES TYPE        *IsiPpIeesesssSBEsePsPSBSSEsPspsPsseesSPePb
Okt3v1          SPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAAT
REI             APKLLIYEASNLQAGVPSRFSGSGSGTDYTFTISSLQPEDIAT
                ?  ??                          ?  ?
                     *******  CDR2 (LOOP/KABAT)

                               102   108
RES TYPE        PiPIPies**iPIIsPPSPSPSS
Okt3v1          YYCQQWSSNPFTFGSGTKLEINR
REIv1           YYCQQYQSLPYTFGQGTKLQITR
                              ?    ?
                   ******        CDR3 (LOOP)
                  *********      CRD3 (KABAT)
```

Fig. 3

SUBSTITUTE SHEET

```
          NN N                  23 26    32 35 N39  43
RES TYPE  SESPs^SBssS^sSSsSpSpSPsPSEbSBssBePiPIpiesss
Okt3h     QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQ
KOL       QVQLVESGGGVVQPGRSLRLSCSSSGFIFSSYAMYWVRQAPGK
               ?              . .??
                                      ******    CDR1 (LOOP)
                                         ***** CDR1 (KABAT)

                    52a      60  65     N N  N    82abc     89
RES TYPE IIeIppp^sssssssss^ps^pSSsbSpseSsSseSp^pSpsSBssS^ePb
Okt3vh   GLEWIGYINPSRGYTNTNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAV
KOL      GLEWVAIIWDDGSDQHYADSVKGRFTISRDNSKNTLFLQMDSLRPEDTGV
            ??                      ? ?  ?  ?            ?
               ************          CDR2  (LOOP)
               ********************  CDR2  (KABAT)

           92 N                  107    113
RES TYPE  PiPIEisssssiiisssbibi*EIPIP*spSBSS
Okt3vh    YYCARYYDDHY.......CLDYWGQGTTLTVSS
KOL       YFCARDGGHGFCSSASCFGPDYWGQGTPVTVSS
               ****************** CRD3 (KABAT/LOOP)
```

Fig. 4

OKT 3 HEAVY CHAIN CDR GRAFTS

1. gh341 and derivatives

```
          1                          26        35  39  43
Okt3vh    QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQ
gH341     QVQLVESGGGVVQPGRSLRLSCSSSGYTFTRYTMHWVRQAPGK   JA178
gH341A    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA185

gH341E    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA198
gH341*    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA207
gH341*    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA209
gH341D    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA197
gH341*    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA199
gH341C    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA184

gH341*    QVQLVQSGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA203
gH341*    QVQLVESGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA205
gH341B    QVQLVESGGGVVQPGRSLRLSCSSSGYTFTRYTMHWVRQAPGK   JA183
gH341*    QVQLVQSGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA204
gH341*    QVQLVESGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA206
gH341*    QVQLVQSGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA208
KOL       QVQLVESGGGVVQPGRSLRLSCSSSGFIFSSYAMYWVRQAPGK
```

Fig. 5(i)

```
          44    50              65                  83
Okt3vh    GLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLT
gH341     GLEWVAYINPSRGYTNYNQKFKDRFTISRDNSKNTLFLQMDSLR JA178
gH341A    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR JA185

gH341E    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR JA198
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKNTAFLQMDSLR JA207
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTAFLQMDSLR JA209
gH341D    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKNTLFLQMDSLR JA197
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTLFLQMDSLR JA199
gH341C    GLEWVAYINPSRGYTNYNQKFKDRFTISRDNSKNTLFLQMDSLR JA184

gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR JA207
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR JA205
gH341B    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR JA183
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR JA204
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR JA206
gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKNTAFLQMDSLR JA208
KOL       GLEWVAIIWDDGSDQHYADSVKGRFTISRDNSKNTLFLQMDSLR
```

Fig. 5(ii)

SUBSTITUTE SHEET

	84	95	102	113	
Okt3vh	SEDSAVYYCARYYDDHY			CLDYWGQGTTLTVSS	
gH341	PEDTGVYFCARYYDDHY			CLDYWGQGTTLTVSS	JA178
gH341A	PEDTAVYYCARYYDDHY			CLDYWGQGTTLTVSS	JA185
gH341E	PEDTGVYFCARYYDDHY			CLDYWGQGTTLTVSS	JA198
gH341*	PEDTGVYFCARYYDDHY			CLDYWGQGTTLTVSS	JA207
gH341D	PEDTGVYFCARYYDDHY			CLDYWGQGTTLTVSS	JA197
gH341*	PEDTGVYFCARYYDDHY			CLDYWGQGTTLTVSS	JA209
gH341*	PEDTGVYFCARYYDDHY			CLDYWGQGTTLTVSS	JA199
gH341C	PEDTGVYFCARYYDDHY			CLDYWGQGTTLTVSS	JA184
gH341*	PEDTAVYYCARYYDDHY			CLDYWGQGTTLTVSS	JA203
gH341*	PEDTAVYYCARYYDDHY			CLDYWGQGTTLTVSS	JA205
gH341B	PEDTAVYYCARYYDDHY			CLDYWGQGTTLTVSS	JA183
gH341*	PEDTGVYFCARYYDDHY			CLDYWGQGTTLTVSS	JA204
gH341*	PEDTGVYFCARYYDDHY			CLDYWGQGTTLTVSS	JA206
gH341*	PEDTGVYFCARYYDDHY			CLDYWGQGTTLTVSS	JA208
KOL	PEDTGVYFCARDGGHGFCSSASCFGPDYWGQGTPVTVSS				

Fig. 5(iii)

OKT3 LIGHT CHAIN CDR GRAFTING

1. gL221 and derivatives

```
          1                       24        34      42
Okt3v1    QIVLTQSPAIMSASPGEKVTMTCSASS.SVSYMNWYQQKSGT
gL221     DIQMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
gL221A    QIVMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
gL221B    QIVMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
gL221C    DIQMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
REI       DIQMTQSPSSLSASVGDRVTITCQASQDIIKYLNWYQQTPGK

          43      50    56                          85
Okt3v1    SPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAAT
gL221     APKLLIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
gL221A    APKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
gL221B    APKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
gL221C    APKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
REI       APKLLIYEASNLQAGVPSRFSGSGSGTDYTFTISSLQPEDIAT

          86   91   96          108
Okt3v1    YYCQQWSSNPFTFGSGTKLEINR
gL221     YYCQQWSSNPFTFGQGTKLQITR
gL221A    YYCQQWSSNPFTFGQGTKLQITR
gL221B    YYCQQWSSNPFTFGQGTKLQITR
gL221C    YYCQQWSSNPFTFGQGTKLQITR
REI       YYCQQYQSLPYTFGQGTKLQITR
```

CDR'S ARE UNDERLINED

FRAMEWORK RESIDUES INCLUDED IN THE GENE ARE DOUBLE UNDERLINED

Fig. 6

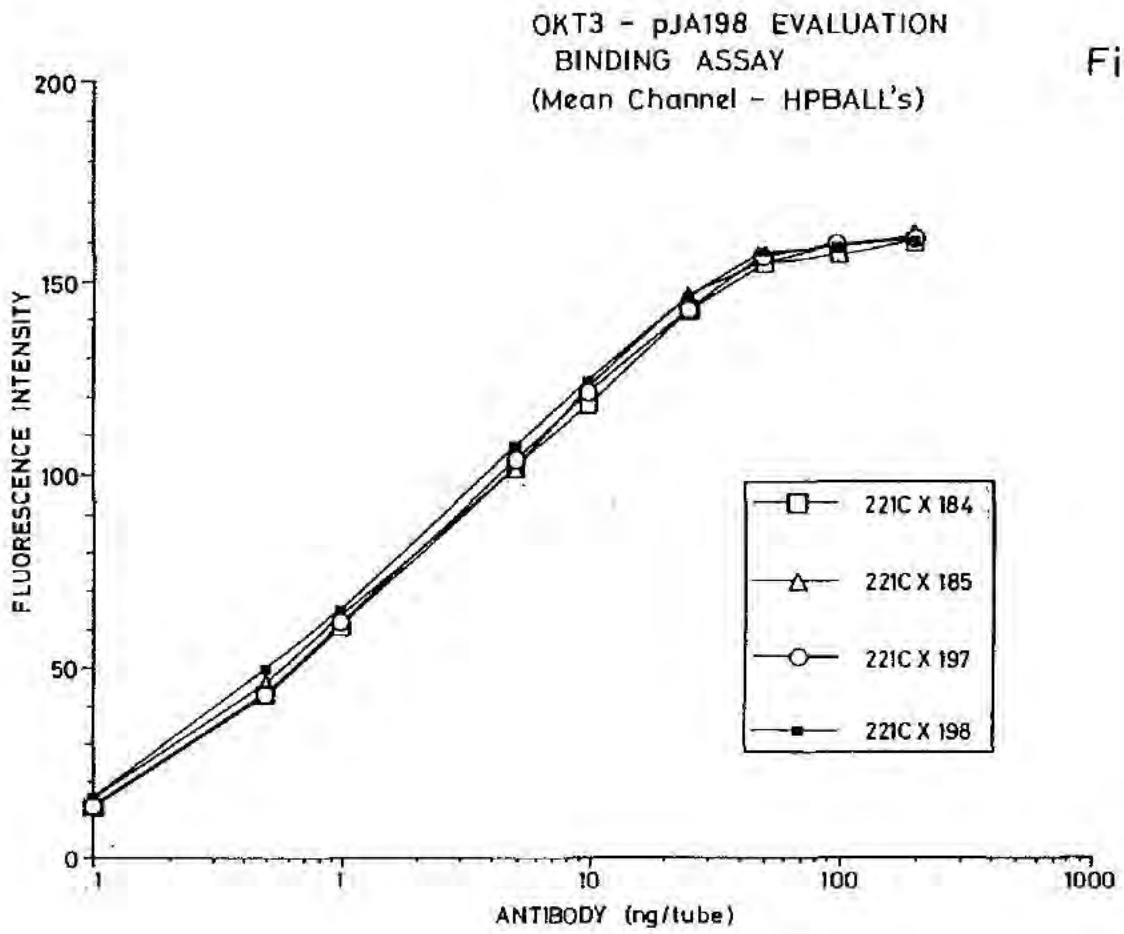
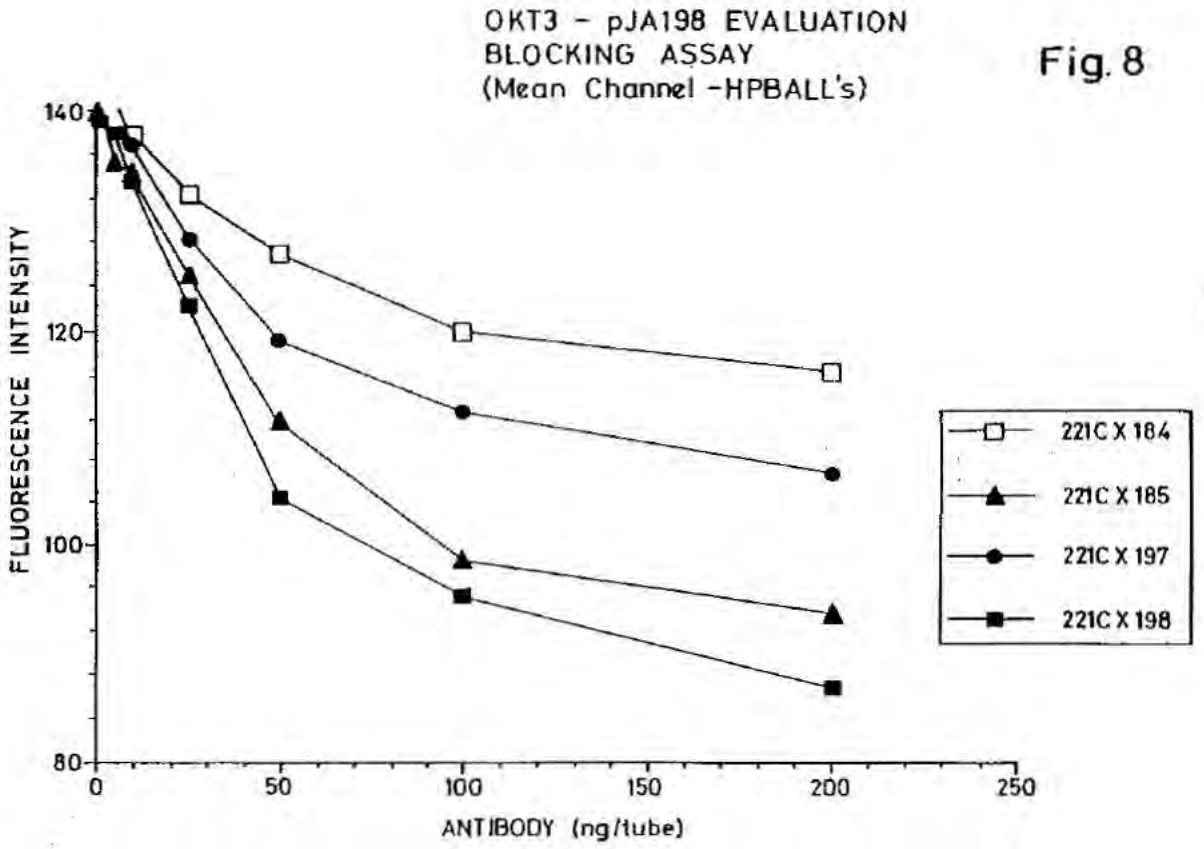


Fig. 7



SUBSTITUTE SHEET

10/15

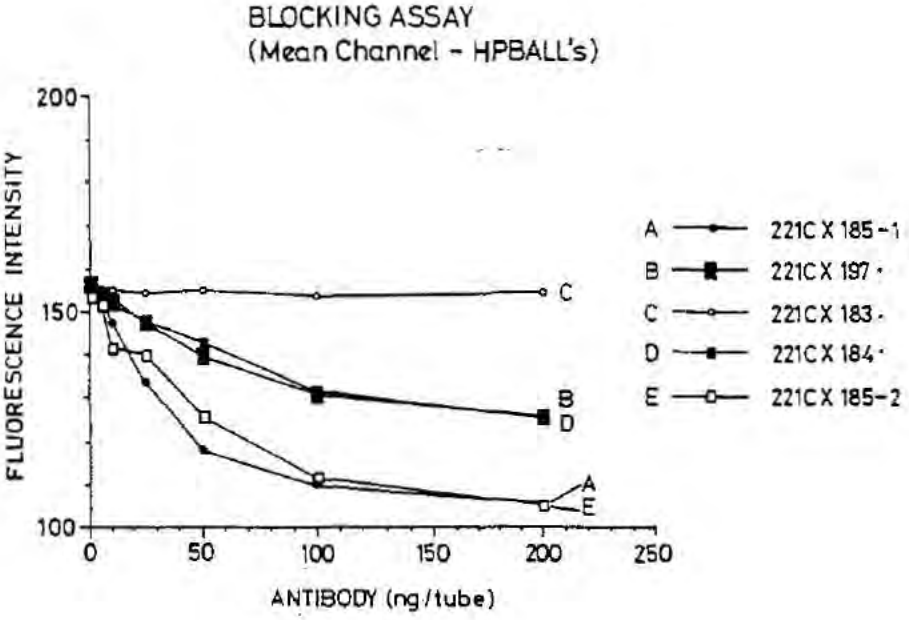
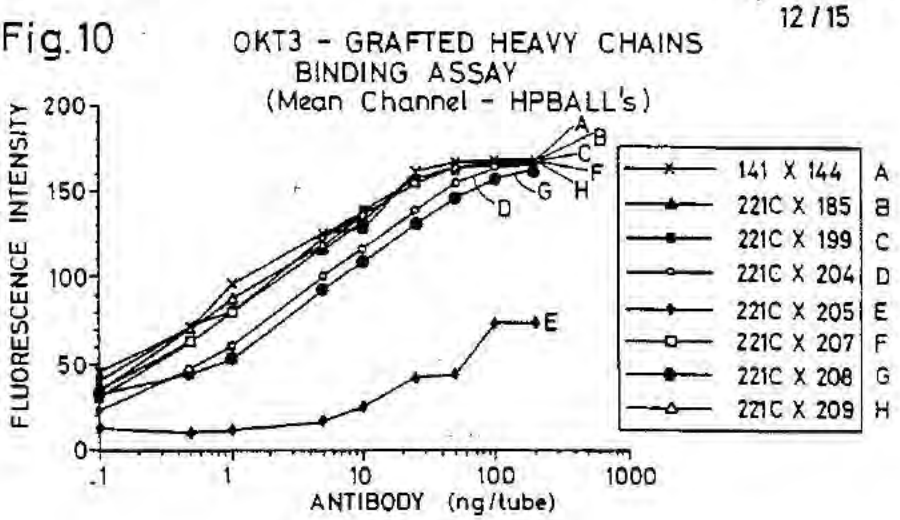
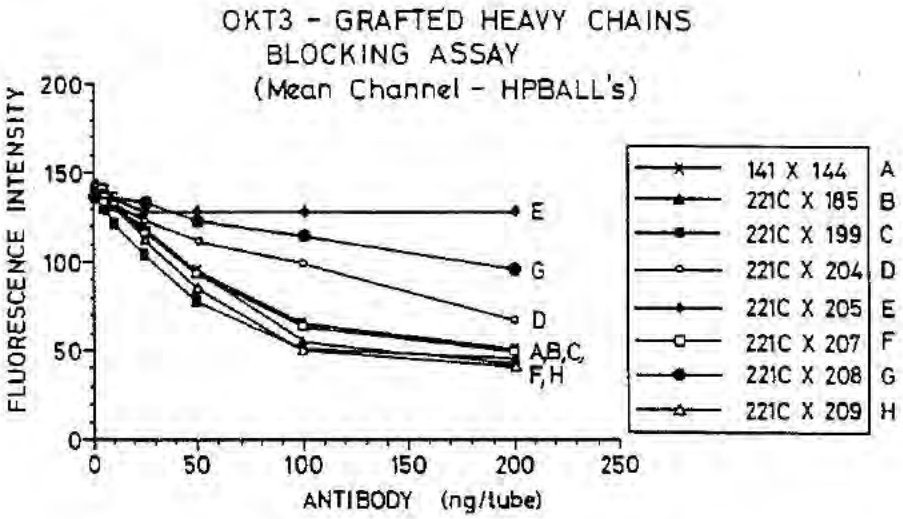
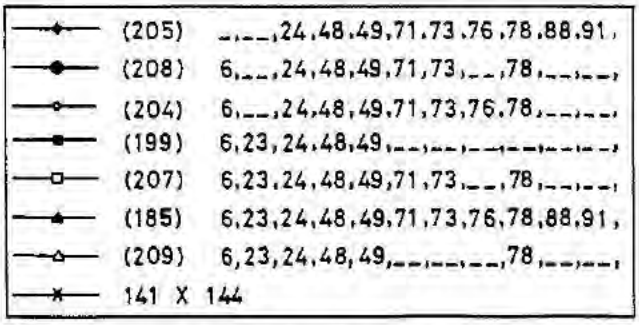

BLOCKING ASSAY
(Mean Channel - HPBALL's)
FLUORESCENCE INTENSITY
200
150
100
0
50
100
150
200
250
ANTIBODY (ng/tube)
A
B
C
D
E
A 221C X 185-1
B 221C X 197
C 221C X 183
D 221C X 184
E 221C X 185-2

Fig. 9

Fig.10

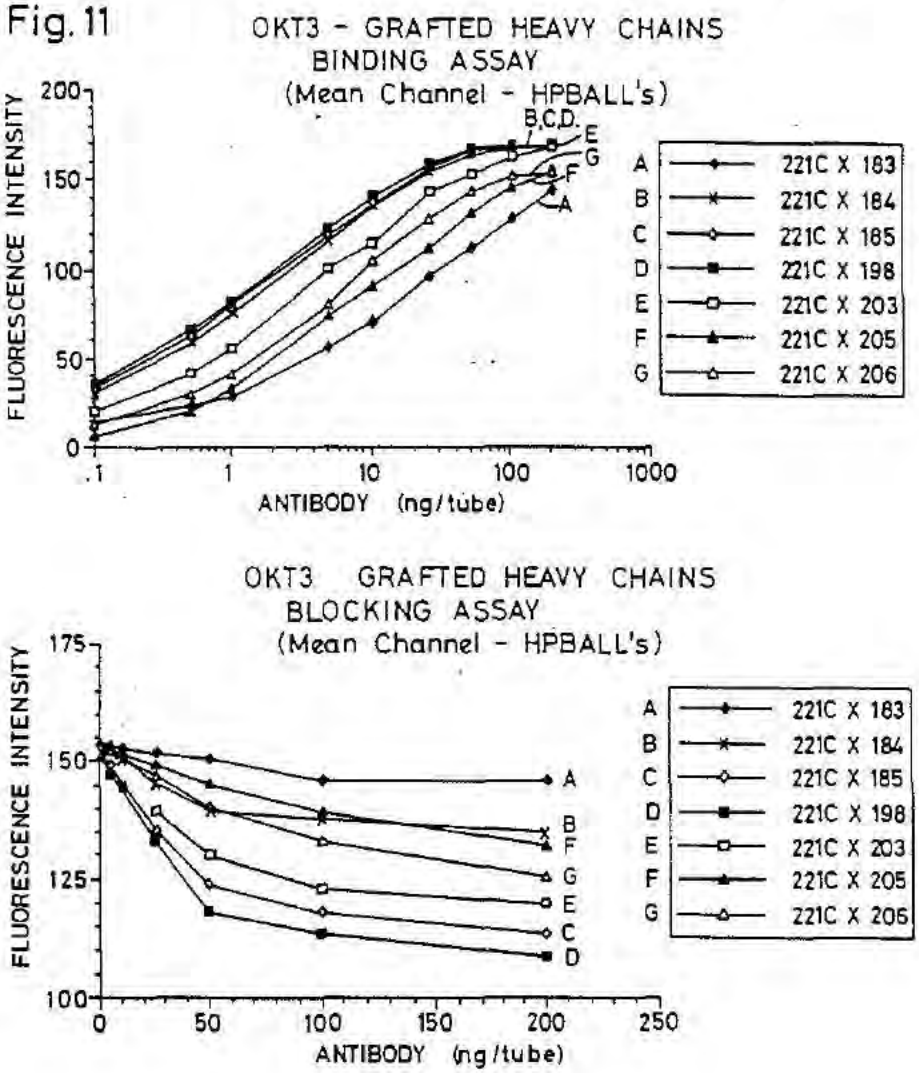






SUBSTITUTE SHEET

Fig. 11



SUBSTITUTE SHEET

PCT/GB90/02017

Fig. 12

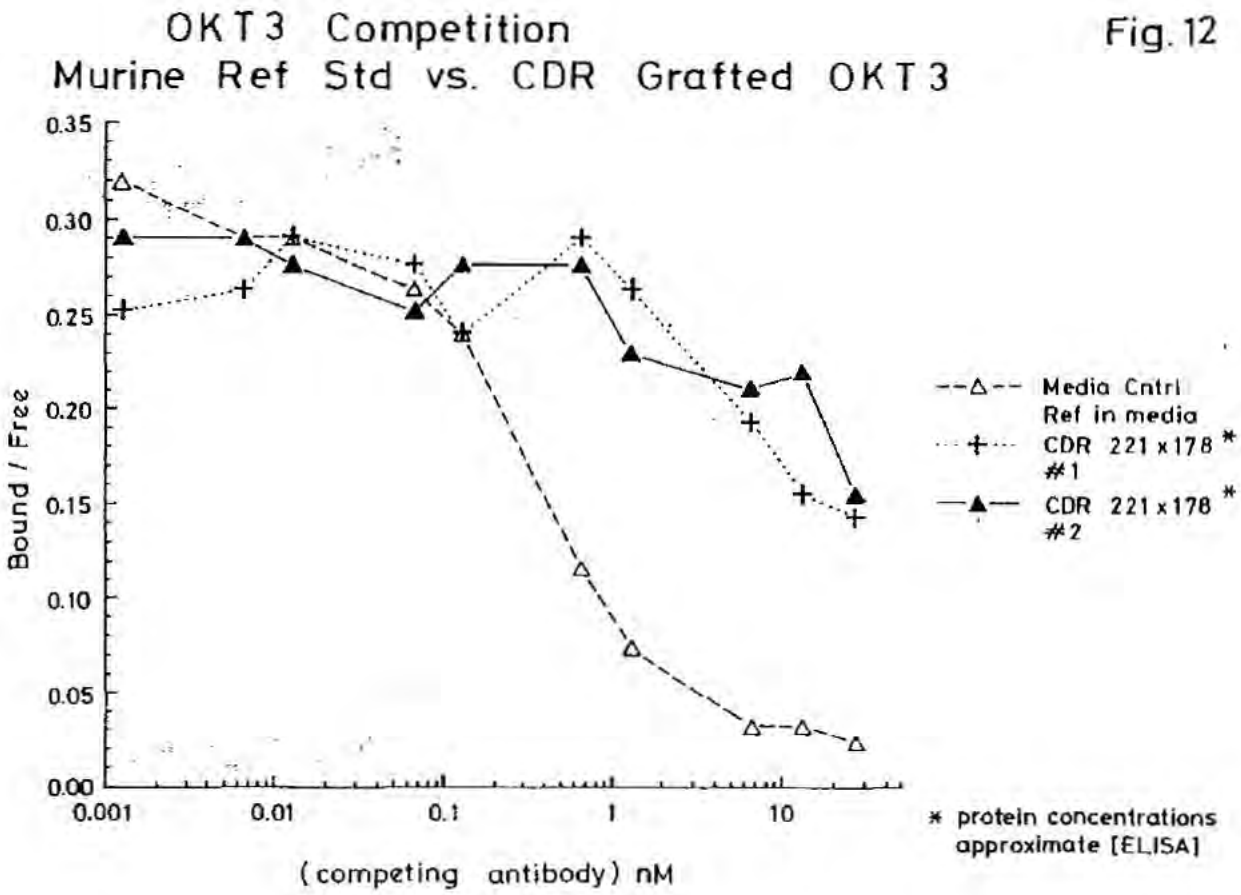
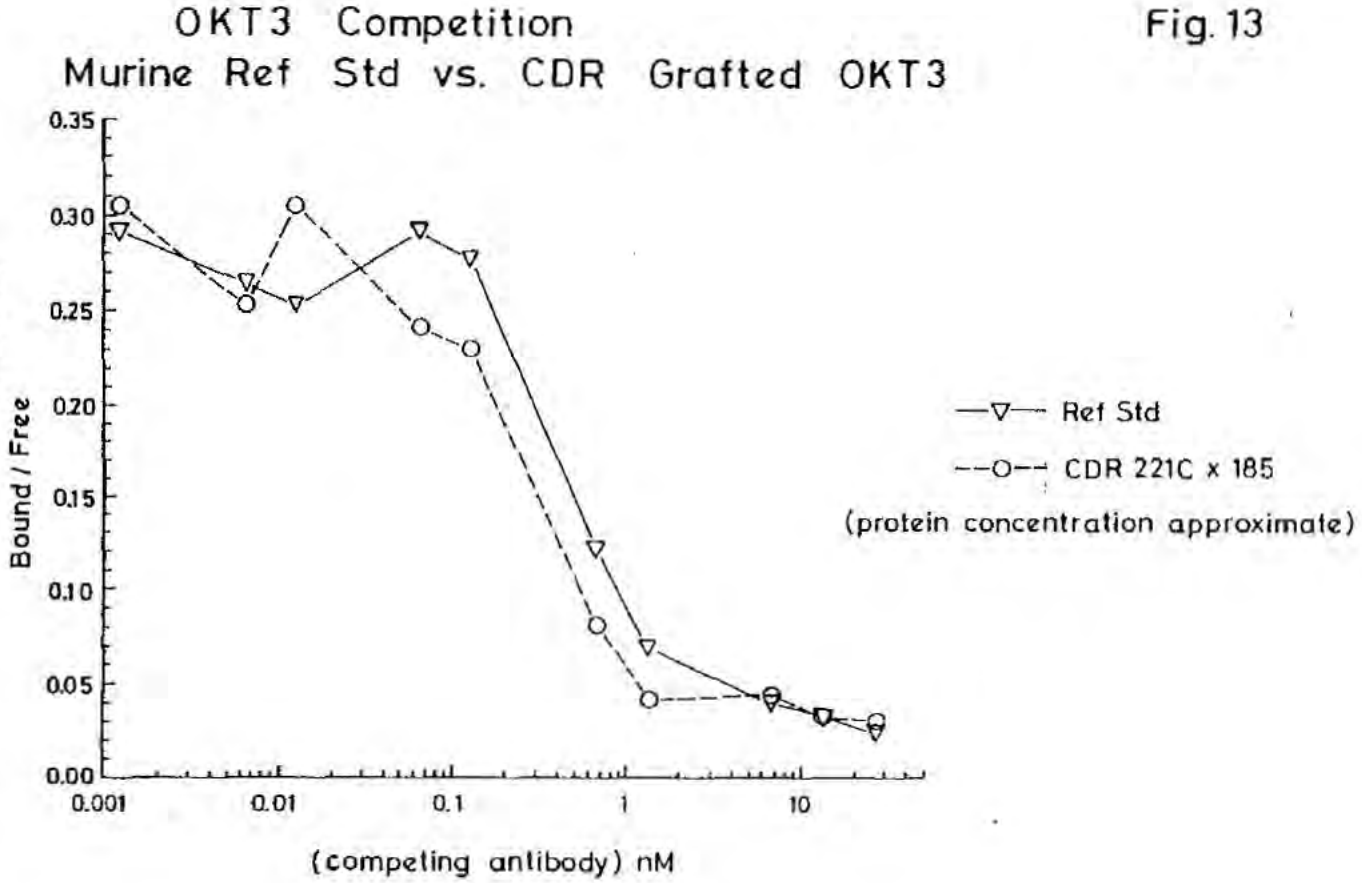


Fig. 13



#4

FORM PTO-1390 (REV. 5-93) — U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)

ATTORNEY'S DOCKET NUMBER: CARP-0009

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB90/02017	21 December 1990	21 December 1989

TITLE OF INVENTION: HUMANISED ANTIBODIES

APPLICANT(S) FOR DO/EO/US: ADAIR, John, Robert, ATHWAL, Diljeet, Singh, and EMTAGE, John, Spencer

Applicant herewith submits to the United States Designated/ Elected Office (DO/EO/US) the following items under 35 U.S.C. 371:

1. ☐ This express request to immediately begin national examination procedures (35 U.S.C. 371(f)).
2. ☑ The U.S. National Fee (35 U.S.C. 371(c)(1)) and other fees as follows:

CLAIMS	(1) FOR	(2) NUMBER FILED	(3) NUMBER EXTRA	(4) RATE	(5) CALCULATIONS
	TOTAL CLAIMS	-20-		X$ 20.00	$
	INDEPENDENT CLAIMS	-3-		X$ 60.00	
	MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+$ 200.00	
	BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(4)): ☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) ..... $ 330 ☐ No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445 (a)(2)) ..... $ 370 ☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO..... $ 500 ☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2) to (4)..... $ 50				
	Surcharge of $120 for furnishing the National fee or oath or declaration later than ☑20 ☐30 mos. from the earliest claimed priority date (37 CFR 1.492(e)).				$120.00
			TOTAL OF ABOVE CALCULATIONS		-$120.00
	Reduction by 1/2 for filing by small entity, if applicable. Affidavit musts be filed also. (Note 37 CFR 1.9, 1.27, 1.28.)				$60.00
			SUBTOTAL		+$60.00
	Processing fee of $30 for furnishing the English Translation later than ☐20 ☐30 mos. from the earliest claimed priority date (37 CFR 1.492(f)).				
			TOTAL NATIONAL FEE		$ 60.00
	Fee for recording the enclosed assignment (37 CFR 1.21(h)).				+
			TOTAL FEES ENCLOSED		$ 60.00

a. ☑ A check in the amount of $ 60.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 23-3050. A duplicate copy of this sheet is enclosed.

10/16/91 060 01 743329 25 60

"EXPRESS MAIL" Mailing Label No. RB567260177

Date of Deposit September 17, 1991

I hereby certify that this paper or fee 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 above and is addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231

Diane M. Kushner

(TYPED)

Diane M. Kushner

(SIGNATURE)

ATTORNEY'S DOCKET NUMBER
CARP-0009

3. A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
   c. ☐ has been transmitted by the International Bureau.
4. ☐ A translation of the International Application into English (35 U.S.C. 371 (c)(2)).
5. Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
6. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
7. ☒ An oath or declaration of the inventor (35 U.S.C. 371(c)(4)).
8. ☐ A translation of the Annexes to the International Preliminary Examination Report under PCT Article 36(35 U.S.C. 371(c)(5)).

Other document(s) or information included:

9. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
10. ☐ An assignment document for recording.
    Please mail the recorded assignment document to:
    a. ☐ the person whose signature, name & address appears at the bottom of this page.
    b. ☐ the following:

11. The above checked items are being transmitted
    a. ☐ before the 18th month publication.
    b. ☐ after publication and the Article 20 communication but before 20 months from the priority date.
    c. ☒ after 20 months but before 22 months (surcharge and/or processing fee included).
    d. ☐ after 22 months (surcharge and/or processing fee included).
       Note: Petition to revive (37 CFR 1.137(a) or (b)) is necessary if 35 U.S.C. 371 requirements submitted after 22 months *and no proper demand for International Preliminary Examination was made by 19 months from the earliest claimed priority date.*
    e. ☐ by 30 months and a proper demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
    f. ☐ after 30 months but before 32 months and a proper demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date (surcharge and/or processing fee included).
    g. ☐ after 32 months (surcharge and/or processing fee included).
       Note: Petition to revive (37 CFR 1.137(a) or (b)) is necessary if 35 U.S.C. 371 requirements submitted after 32 months and a proper demand for International Preliminary Examination was made by 19 months from the earliest claimed priority date.
12. At the time of transmittal, the time limit for amending claims under Article 19
    a. ☐ has expired and no amendments were made.
    b. ☐ has not yet expired.
13. ☐ Certain requirements under 35 U.S.C. 371 were previously submitted by the applicant on _____ (date), namely:
14. Submission of Verified Statement Claiming Small Entity Status and Request for Refund

Francis A. Paintin
NAME

Woodcock Washburn Kurtz Mackiewicz & Norris
ADDRESS

One Liberty Place - 46th Floor

Philadelphia, PA 19103

SIGNATURE

19,386
REGISTRATION NUMBER

Form PTO-1390 (Rev. 8-87)

USCOMM-DC-87-3805

#74

DOCKET NO.: CARP-0009 **PATENT**

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name; and

I verily believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: HUMANISED ANTIBODIES the specification of which:

___ is attached hereto.

✓ International was filed on 21 December 1990 as Application Serial No. PCT/GB90/00201 and was amended on _____. (if applicable)

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

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with 37 CFR § 1.56(a).

I hereby claim foreign priority benefits under 35 U.S.C. § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of any application on which priority is claimed:

Country	Number	Date Filed	Priority Claimed
U.K.	8928874.0	21.12.89	yes

PTO-19

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose material information as defined in 37 CFR § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	**Filing Date**	**Status (patented, pending)**

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

Francis A. Paintin

Registration Nos. 19,386

of the firm of **WOODCOCK WASHBURN KURTZ MACKIEWICZ & NORRIS**, One Liberty Place - 46th Floor, Philadelphia, Pennsylvania 19103, and

Address all telephone calls and correspondence to:

Francis A. Paintin

WOODCOCK WASHBURN KURTZ MACKIEWICZ & NORRIS
One Liberty Place - 46th Floor
Philadelphia, PA 19103
Telephone No. **215-568-3100.**

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the

United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-00	**Full Name** JOHN ROBERT ADAIR	**Inventor's Signature** John Robert Adair	**Date** 13/8/91
1	**Residence** 23 George Road, Stokenchurch, High Wycombe, Buckinghamshire HP14 3RN, U.K. GBX	**Citizenship** U.K.	
	Post Office Address 23 George Road, Stokenchurch, High Wycombe, Buckinghamshire HP14 3RN, U.K.		
2-00	**Full Name** DILJEET SINGH ATHWAL	**Inventor's Signature**	**Date** 13/8/91
2	**Residence** Flat 35, Knollys House, Tavistock Square, London WC1, U.K. GBX	**Citizenship** U.K.	
	Post Office Address Flat 35, Knollys House, Tavistock Square, London WC1, U.K.		
3-00	**Full Name** JOHN SPENCER EMTAGE	**Inventor's Signature** John Spencer Emtage	**Date** 13/8/91
3	**Residence** 49 Temple Mill Island, Temple Marlow, Buckinghamshire, SL7 1SQ, U.K. GBX	**Citizenship** U.K.	
	Post Office Address 49 Temple Mill Island, Temple, Marlow, Buckinghamshire SL7 1SQ, U.K.		
4	**Full Name**	**Inventor's Signature**	**Date**
	Residence	**Citizenship**	
	Post Office Address		
5	**Full Name**	**Inventor's Signature**	**Date**
	Residence	**Citizenship**	
	Post Office Address		

#15

DOCKET NO.: CARP-0009 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

John R. Adair et al.

Serial No.: 07/743,329 Group No.: 1807

Filed: August 16, 1991 Examiner: L. Bennett

For: HUMANISED ANTIBODIES

Certificate of Facsimile Transmission

I hereby certify that this paper is being facsimile transmitted to the Patent and Trademark Office on the date shown below.

On January 19, 1993

Dawn L. McMahon

Commissioner of Patents & Trademarks
Washington, DC 20231

Sir:

RESPONSE TO OFFICE ACTION

This is in response to the Office Action of November 18, 1992, the time for a response to which is set to expire on January 18, 1993. January 18, 1993 is a Federal Holiday in the District of Columbia (observance of Martin Luther King, Jr.'s birthday). This response is timely filed under 37 CFR §1.7 since it is filed on January 19, 1993, the next succeeding day that is not a Saturday, Sunday or Federal Holiday within the District of Columbia. Please amend the above-identified patent application as follows.

In the claims:

21. (amended) A process for producing [a CDR-grafted antibody product] an antigen-binding molecule comprising:

Carter Exhibit 2007
Carter v. Adair
Interference No. 105,744

(a) producing in an expression vector an operon having a DNA sequence which encodes a heavy chain according to [Claim 1] Claim 24 or Claim 25;

[and/or]

(b) producing in an expression vector an operon having a DNA sequence which encodes a complementary light chain according to Claim [6] 42 or Claim [8] 43;

(c) transfecting a host cell with the [or each vector] expression vectors of steps (a) and (b) to form a transfected cell containing said expression vectors;

and

(d) culturing [the] said transfected cell [line] to produce [the CDR-grafted antibody product] said antigen binding molecule.

Please cancel claims 1-20, 22 and 23 without prejudice and substitute therefor the following new claims.

24. An antigen-binding molecule having affinity for a predetermined antigen, comprising a heavy chain and a complementary light chain, said heavy chain having a variable domain comprising framework regions from an acceptor antibody heavy chain and antigen binding regions from the heavy chain of a donor antibody which has affinity for said predetermined antigen, wherein amino acid residues 23, 24, 31 to 35, 49 to 58 and 95 to

102, according to the Kabat numbering system, of said heavy chain are donor antibody residues.

25. The antigen binding molecule of claim 24 wherein residues 71, 73 and 78, according to the Kabat numbering system, in the heavy chain are additionally donor antibody residues.

26. The antigen-binding molecule of claim 24, wherein residues 26 to 30, according to the Kabat numbering system, in the heavy chain are additionally donor antibody residues.

27. The antigen-binding molecule of claim 25, wherein residues 26 to 30, according to the Kabat numbering system, in the heavy chain are additionally donor antibody residues.

B2 cont.

28. The antigen-binding molecule of claim 24, wherein residues 59 to 65, according to the Kabat numbering system, in the heavy chain are additionally donor antibody residues.

29. The antigen-binding molecule of claim 25, wherein residues 59 to 65, according to the Kabat numbering system, in the heavy chain are additionally donor antibody residues.

30. The antigen-binding molecule of claim 24, wherein residues 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107, according to the Kabat numbering system, in the heavy chain are additionally donor antibody residues.

31. The antigen-binding molecule of claim 25, wherein residues 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106

and 107, according to the Kabat numbering system, in the heavy chain are additionally donor antibody residues.

32. The antigen-binding molecule of claim 30, wherein, according to the Kabat numbering system, if residue 48 in the donor antibody heavy chain is different from residue 48 in the acceptor antibody heavy chain, then residue 69 in the heavy chain is additionally a donor antibody residue.

33. The antigen-binding molecule of claim 31, wherein, according to the Kabat numbering system, if residue 48 in the donor antibody heavy chain is different from residue 48 in the acceptor antibody heavy chain, then residue 69 in the heavy chain is additionally a donor antibody residue.

B2 cont.

34. The antigen-binding molecule of claim 30, wherein residues 38 and 46, according to the Kabat numbering system, in the heavy chain are additionally donor antibody residues.

35. The antigen-binding molecule of claim 31, wherein residues 38 and 46, according to the Kabat numbering system, in the heavy chain are additionally donor antibody residues.

36. The antigen-binding molecule of claim 32, wherein residues 20 and 80, according to the Kabat numbering system, in the heavy chain are additionally donor antibody residues.

37. The antigen-binding molecule of claim 33, wherein residues 20 and 80, according to the Kabat numbering system, in the heavy chain are donor antibody residues.

38. The antigen-binding molecule of claim 30, wherein residues 18, 67 and 82, according to the Kabat numbering system, in the heavy chain are donor antibody residues.

39. The antigen-binding molecule of claim 31, wherein residues 18, 67 and 82, according to the Kabat numbering system, in the heavy chain are donor antibody residues.

40. The antigen-binding molecule of claim 30, wherein residues 1, 3, 9, 11, 41, 72, 76, 87, 88, 91, 108, 110 and 112, according to the Kabat numbering system, in the heavy chain are donor antibody residues.

B2 cont.

41. The antigen-binding molecule of claim 31, wherein residues 1, 3, 9, 11, 41, 72, 76, 87, 88, 91, 108, 110 and 112, according to the Kabat numbering system, in the heavy chain are donor antibody residues.

42. An antigen-binding molecule having affinity for a predetermined antigen, comprising a light chain and a complementary heavy chain, the light chain having a variable domain comprising framework regions from an acceptor antibody light chain and antigen binding regions from the light chain of a donor antibody which has affinity for said predetermined antigen, wherein amino acid residues 24 to 34, 46, 48, 50 to 56, 58, 71 and 89 to 97, according to the Kabat numbering system, are donor antibody residues.

43. The antigen-binding molecule of claim 42, wherein residues 2, 4, 6, 35, 38, 44, 47, 49, 64 to 69, 85, 87, 98, 99, 101 and 102, according to the Kabat numbering system, in the heavy chain are donor antibody residues.

44. The antigen-binding molecule of claim 42, wherein, according to the Kabat numbering system, if residue 60 in the light chain can form a salt bridge with residue 54 in the light chain, then residue 60 in the light chain is a donor antibody residue.

B2 cont. 45. The antigen-binding molecule of claim 42, wherein, according to the Kabat numbering system, if residue 70 in the light chain can form a salt bridge with residue 24 in the light chain, then residue 70 in the light chain is a donor antibody residue.

46. The antigen-binding molecule of claim 43, wherein, according to the Kabat numbering system, residues 21 and 73 in the light chain are additionally donor antibody residues.

47. The antigen-binding molecule of claim 43, wherein, according to the Kabat numbering system, residues 37 and 45 in the light chain are additionally donor antibody residues.

48. The antigen-binding molecule of claim 43, wherein residues 1, 3, 10, 12, 40, 63, 80, 103 and 105, according to the Kabat numbering system, in the light chain are additionally donor antibody residues.

49. An antigen-binding molecule having affinity for a predetermined antigen, comprising a heavy chain and a complementary light chain:

the heavy chain having a variable domain comprising framework regions from an acceptor antibody heavy chain and antigen binding regions from the heavy chain of a donor antibody which has affinity for said predetermined antigen, wherein amino acid residues 23, 24, 31 to 35, 49 to 58 and 95 to 102, accordingly to the Kabat numbering system, are donor antibody residues; and

B2 Cont.

the complementary light chain having a variable domain comprising framework regions from an acceptor antibody light chain and antigen binding regions from said donor antibody light chain wherein amino acid residues 24 to 34, 46, 48, 50 to 56, 58, 71 and 89 to 97 according to the Kabat numbering system are donor antibody residues.

50. The antigen-binding molecule of claim 49 wherein residues 71, 73, 78 and 95 to 102, according to the Kabat numbering system, of said heavy chain are additionally donor antibody residues.

51. The antigen-binding molecule of claim 49 or claim 50, which is a site specific antibody molecule.

52. The antigen-binding molecule of claim 49 or claim 50, which has specificity for an antigen selected from the group

consisting of interleukins, hormones, biologically active compounds and receptors therefor.

53. A DNA molecule which encodes an antigen-binding molecule heavy chain having a variable domain comprising framework regions from the heavy chain of a donor antibody which has affinity for said predetermined antigen wherein amino acid residues 23, 24, 31 to 35, 49 to 58 and 95 to 102 of said heavy chain, according to the Kabat numbering system, are donor antibody residues.

54. The DNA molecule of claim 53 wherein residues 71, 73 and 78, according to the Kabat numbering system, of said heavy chain are additionally donor antibody residues.

53 cont.

55. A DNA molecule which encodes an antigen-binding molecule light chain having a variable domain comprising framework regions from an acceptor antibody light chain and antigen binding regions from the light chain of an antibody having affinity for a predetermined antigen wherein amino acid residues 24 to 34, 46, 48, 50 to 56, 58, 71 and 89 to 97, according to the Kabat numbering system, of said light are donor antibody residues.

56. A cloning vector comprising a DNA molecule according to claims 53, 54 or 55.

57. An expression vector comprising a DNA molecule according to claims 53, 54 or 55.

58. A host cell transformed with a DNA molecule according to claims 53, 54 or 55.

59. A therapeutic composition comprising a therapeutically effective amount of an antigen-binding molecule according to claims 24, 25 or 42 in combination with a pharmaceutically acceptable carrier.

60. A therapeutic composition comprising a therapeutically effective amount of an antigen-binding molecule according to claim 49 or claim 50 in combination with a pharmaceutically acceptable carrier.

B2 Cont.

61. A diagnostic composition comprising a diagnostically effective amount of an antigen-binding molecule according to claims 24, 25 or 42 in combination with a diagnostically acceptable carrier.

62. A diagnostic composition comprising a diagnostically effective amount of an antigen-binding molecule according to claim 49 or claim 50 in combination with a diagnostically acceptable carrier.

63. A method of therapy comprising administering to a human or animal subject an antigen-binding molecule according to claims 24, 25 or 42.

64. A method of diagnosis comprising administering to a human or animal subject a composition according to claims 24, 25 or 42.

65. A method for producing an antigen-binding molecule having affinity for a predetermined antigen comprising the steps of:

(1) providing a heavy chain for an antigen-binding molecule, said heavy chain having acceptor framework regions and donor antigen binding regions wherein amino acid residues 23, 24, 31 to 35, 49 to 58 and 95 to 102 according to the Kabat numbering system are donor antibody residues;

(2) associating the heavy chain of step (1) with a complementary light chain to form an antigen-binding molecule;

(3) determining the affinity of the antigen-binding molecule formed in step (2) for said predetermined antigen;

(4) if the affinity determined in step (3) is not equivalent to that of the donor antibody, providing a heavy chain as described in step (1) in which amino acid residues 71, 73 and 78, according to the Kabat numbering system, are additionally donor antibody residues;

(5) associating the heavy chain of step (4) with a complementary light chain to form an antigen-binding molecule;

(6) determining the affinity of the antigen-binding molecule formed in step (5) for said predetekmined antigen;

(7) if the affinity determined in step (6) is not equivalent to that of the donor antibody, providing a heavy chain as described in step (4) in which amino acid residues 26 to 30,

according to the Kabat numbering system, are additionally donor antibody residues;

(8) associating the heavy chain of step (7) with a complementary light chain to form an antigen-binding molecule;

(9) determining the affinity of the antigen-binding molecule formed in step (8) for said predetermined antigen;

(10) if the affinity determined in step (9) is not equivalent to that of the donor antibody, providing a heavy chain as described in step (7) in which amino acid residues 59 to 65, according to the Kabat numbering system, are additionally donor antibody residues;

B2 Cont.

(11) associating the heavy chain of step (10) with a complementary light chain to form an antigen-binding molecule;

(12) determining the affinity of the antigen-binding molecule formed in step (11) for said predetermined antigen;

(13) if the affinity determined in step (12) is not equivalent to that of the donor antibody, providing a heavy chain as described in step (10) in which amino acid residues 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107, according to the Kabat numbering system, are additionally donor antibody residues; and

(14) associating the heavy chain produced in step (13) with a complementary light chain to form an antigen-binding molecule.

66. A method for producing an antigen-binding molecule having affinity for a predetermined antigen comprising the steps of:

(1) providing a light chain for an antigen-binding molecule, said light chain having acceptor framework regions and donor antigen binding regions wherein amino acid residues 24 to 34, 46, 48, 50 to 56, 58, 71 and 89 to 97 according to the Kabat numbering system are donor antibody residues;

(2) associating the light chain of step (1) with a complementary heavy chain to form an antigen-binding molecule;

(3) determining the affinity of the antigen-binding molecule formed in step (2) for said predetermined antigen;

B2 Cont.

(4) if the affinity determined in step (3) is not equivalent to that of the donor antibody, providing a light chain as described in step (1) in which amino acid residues 2, 4, 6, 35, 38, 44, 47, 49, 64 to 69, 85, 87, 98, 99, 101 and 102, according to the Kabat numbering system, are additionally donor antibody residues;

(5) associating the light chain of step (4) with a complementary heavy chain to form an antigen-binding molecule;

(6) determining the affinity of the antigen-binding molecule formed in step (5) for said predetermined antigen;

(7) if the affinity determined in step (6) is not equivalent to that of the donor antibody, providing a light chain

as described in step (4) in which amino acid residues 21, 37, 45, 60, 70 and 73, accordingly to the Kabat numbering system, are additionally donor antibody residues;

(8) associating the light chain of step (7) with a complementary heavy chain to form an antigen-binding molecule;

(9) determining the affinity of the antigen-binding molecule formed in step (8) for said predetermined antigen;

B2 cont'd

(10) if the affinity determined in step (9) is not equivalent to that of the donor antibody, providing a light chain as described in step (7) in which amino acid residues 1, 3, 10, 12, 40, 63, 80, 103 and 105, according to Kabat numbering system, are additionally donor antibody residues; and

(11) associating the light chain of step (10) with a complementary heavy chain to form an antigen-binding molecule.

REMARKS

The Office Action of November 18, 1992 has been carefully considered and this response prepared. Claims 1-20, 22 and 23 have been cancelled without prejudice and replaced with new claims 24-66. Claim 21 has been amended to more clearly point out aspects of the claimed method. Support for the newly submitted claims can be found throughout the specification, particularly at pages 17 and 18, page 19, line 23, page 41, Table 1 (each relating to the heavy chain), pages 17-19 (relating to

the light chain), and pages 17-23 (relating to methods for production of antigen-binding molecules).

At paragraph 15 of the Office Action, the Examiner objected to the disclosure, stating that various words in the specification are misspelled.

It is respectfully submitted that the spellings referred to by the Examiner are not incorrect. The use of an "s" instead of a "z" in such words as "recognise" is acceptable English spelling. In fact, the use of "s" is the original English spelling and the use of "z" has only more recently been recognized as being acceptable. In this respect, the Examiner is referred to the enclosed page from Chambers 20th Century Dictionary (1973 Edition) which illustrates this point. Therefore, no amendments have been made. Withdrawal of this objection to the specification is respectfully requested.

At paragraph 16 of the Office Action, the Examiner objected to claims 5, 11-16, 22 and 23 under 37 CFR §1.75(c) as being in improper form because a multiple dependent claims cannot depend from any other multiple dependent claim.

Claims 5, 11-16, 22 and 23 have been cancelled without prejudice and substituted with new claims having proper dependent form. Withdrawal of this objection to the specification is respectfully requested.

At paragraph 17 of the Office Action, the Examiner objected to claims 1-23 stating that the term "CDR-grafted" is improper because abbreviations and acronyms are not appropriate in claim language.

The term CDR-grafted has been deleted from the claims. Withdrawal of this objection to the claims is respectfully requested.

At paragraph 18 of the Office Action, the Examiner rejected claims 1-12 under 35 USC §101. This rejection is divided into four parts which will be answered separately.

In Part A of this section 101 rejection, claims 1-12 were rejected as being inoperative and thus lacking utility. It was stated that the claims are drawn to single heavy or light chains and that there is no evidence in the specification indicating that heavy and light chains alone have activity.

Applicants respectfully traverse this rejection.

It is respectfully submitted that there is no reason to show that the isolated antibody heavy and light chains of the present invention have utility in binding to antigen. As the Examiner has correctly pointed out, the assembled antibodies of the invention have been shown to be able to bind to antigen. What has also been shown is that the isolated heavy and light chains have utility in that they can be used to form the antigen-binding antibody. Thus, the isolated chains have patentable

utility as intermediates for use in CDR-grafted antibodies. There is no requirement that the claimed invention be the ultimate product.

Nonetheless, in order to reduce the number of issues to be dealt with, claims 1-12 have been cancelled without prejudice and new claims have been submitted that are drawn to antibody-binding molecules having heavy and light chains. However, the Applicants reserve the right to file a divisional application relating to the isolated chains.

In Part B of this section 101 rejection, claim 17 was rejected as being drawn to non-statutory subject matter. The Examiner stated that claim 17 is drawn to a DNA sequence coding for a CDR-grafted heavy of light chain, but that "DNA sequences" are not patentable because they are algorithms. The Examiner suggested amending the claims to recite "DNA molecule" instead of "DNA sequence".

Applicants respectfully traverse this rejection. An algorithm is a procedure for solving a given type of mathematical problem. DNA sequences are chemical compounds. Thus the prohibition of section 101 against granting patents on mathematical algorithms does not apply to DNA sequences.

Applicants respectfully submit that the term DNA sequence is proper claim language. This language appears in the claims of issued patents as well as the rules issued by the.

Patent and Trademark Office for applications containing nucleic acid and/or amino acid sequences (see 37 CFR §§1.821 - 1.825). In order to advance prosecution of this application, however, the term "DNA molecule" has been substituted for "DNA sequence" in the claims.

In part C of this section 101 rejection, the Examiner rejected claims 22-23 as inoperative and therefor lacking utility. The Examiner stated that the specification fails to establish the utility of the claim method and pharmaceutical composition using a CDR-grafted antibody in humans or any other animal. The Examiner further stated that the specification does not present any *in vivo* or *in vitro* data to support the claims, and that pharmaceutical therapy with reshaped monoclonal antibodies is unpredictable in the absence of *in vivo* clinical data.

Applicants respectfully traverse this rejection. The Examiner asserts that because the application does not contain any *in vivo* data, it is not possible for a person of ordinary skill in the art to use the humanized antibodies shown in the application in human therapy and diagnosis *in vivo*. It is submitted that at the priority date of the application a person of ordinary skill, having read the application, would have had every reason to expect that a humanized antibody, for instance

one directed against the CD3 antigen, would be useful diagnostically or therapeutically *in vivo*.

For any antibody to be useful *in vivo* in therapy or diagnosis, there are two main factors to consider. The first is the affinity of the antibody for the antigen. The second is the bioavailability of the antibody after administration. As regards the affinity, clearly the antibody would be of no use if its affinity were so low that it did not bind to the antigen, however much of the antibody was available to be bound. Similarly, if the antibody can never reach the antigen, for instance because it is degraded too fast, it will be of no use, however strongly it binds to the antigen.

In the present case, it is clearly shown that the humanized antibodies according to the invention have equivalent binding affinity to that of the prototype murine antibody such as OKT3, OKT4, 61E71 or B72.3. It is therefore plain to anyone with reasonable knowledge in this field that, if the humanized antibody can reach its site of action, it will be able to bind effectively to the appropriate antigen. The *in vitro* data given in the application clearly show that the humanized antibody would be expected to be equally effective as the prototype antibody once it reaches the relevant site.

As to bioavailability, it is plain to anyone with reasonable knowledge of the art that the humanized antibodies of

the present invention are predominantly human in appearance to the human immune system. The only part which may appear foreign is the antigen binding site. However, in this respect, the humanized antibody is no different from a natural human antibody. The antigen binding site in a natural human antibody is also, eventually, regarded as being foreign and the body mounts an immune response against it. This is called an anti-idiotype response and may be involved in the regulation of the immune system.

In view of the close, overall similarity between the humanized antibodies of the present invention and a natural human antibody, anyone with reasonable knowledge in the field would expect that the humanized antibody would have about the same bioavailability as a normal human antibody. The knowledgeable person would therefore expect that the humanized antibody would have very good bioavailability.

The skilled person would certainly expect that the humanized antibody would have better bioavailability than the prototype murine antibody. The prototype antibody is entirely of mouse origin and would therefore be regarded as being foreign by a human immune system. The human immune system would therefore

set up a response against the murine antibody which would lead to its more rapid clearance from the body.

The knowledgeable person would therefore have expected the humanized antibody of the present invention to have good bioavailability and would have seen from the *in vitro* data in the application that it has acceptable affinity. There would therefore have been every reason to expect that the humanized antibody would have good therapeutic and diagnostic uses *in vivo*.

The skilled person would certainly have known that OKT3, even though it is a totally murine antibody, is a highly effective therapeutic agent, especially in the treatment of acute organ transplant rejection episodes. This is very well documented in the prior art. On the basis of the data given in the present application, it would be expected that the humanized antibody could be used in the same way as is OKT3 to treat the same conditions.

The skilled person would also have known that OKT4, 61E71 and B72.3, while not approved for general use, have been used therapeutically and diagnostically in certain limited circumstances, demonstrating their utility. It would again be expected that this same utility could be demonstrated by the humanized versions of these murine antibodies.

The knowledgeable person would not need any data correlating the *in vitro* activity with *in vivo* use to come to

this conclusion. There is nothing in the art which would lead him in any way to expect that the *in vitro* data did not give a reasonable basis for a confident prediction of *in vivo* utility. Certainly, the Applicants are not aware of any studies which show that *in vitro* results with antibodies cannot be extrapolated to *in vivo* results.

The Examiner raises various possible reasons why the humanized antibodies might not work. However, the Examiner has provided no documentary evidence to support these suggestions. In the absence of such documentary evidence, it is respectfully submitted that these reasons represent no more than speculation. In any event, the speculations are unfounded. As to (1), there is no reason why a humanized antibody should be any more subject to proteolytic degradation than the murine prototype antibodies or normal human antibodies. Both murine and normal human antibodies are subjected to such degradation in the human body, in order to clear unwanted antibodies from the system. However, since the rate of clearance is generally of the order of a week or two, such degradation will not prevent their efficacious use. As to (2), it has been shown that murine antibodies reach the required target site. Why should a humanized antibody not reach the target site also? As to (3), the data in the specification show that acceptable affinity can be obtained. The specification also shows how the choice of the heavy chain constant domains

leads to desired effector functions. Thus, there is no basis for the Examiner's assertions. As to (4), all antibodies are eventually recognized as foreign and cleared from circulation. There is no reason to expect a rejection reaction, as discussed above, since the antibodies are predominantly human in appearance.

It is therefore submitted that there is no force in any of the arguments put forward by the Examiner. Accordingly, one skilled in the art is adequately taught by the specification how to practice the claimed invention. Withdrawal of this 35 USC §101 rejection is respectfully requested.

At paragraph 19 of the Office Action, the Examiner objected to the specification and rejected claims 1-12 under 37 USC §112, first paragraph as failing to adequately teach how to make and use the claimed invention. The Examiner stated that isolated heavy and light chains appear to be inoperable for binding antigen and as a consequence undue experimentation would be required of the skilled artisan in order to practice the invention.

It is respectfully submitted that the points made in response to the section 101 rejection above also apply here. The isolated chains have patent utility as intermediates in the preparation of the antibodies which have been demonstrated as having end use utility. The point is now moot, however, in view

of the cancellation of claims 1 through 12 and the submission of new claims drawn to antigen-binding molecule having both heavy and light chains. Withdrawal of this 35 USC §112, first paragraph rejection is respectfully requested.

At paragraph 20 of the Office Action, the Examiner objected to the specification and rejected claims 22 and 23 under 35 USC §112, first paragraph as failing to adequately teach how to make and use the claimed invention. The Examiner stated that the claimed invention appears to be inoperable without supporting *in vivo* data for the reasons discussed in the rejection made under 35 USC §101, and therefore undue experimentation would be required of the skilled artisan in order to practice the claimed invention.

Applicants respectfully traverse this rejection.

A person of ordinary skill in the art could, without the need for undue experimentation, practice the invention disclosed in the present application. It should be borne in mind that the use of murine monoclonal antibody OKT3 for therapy and murine monoclonal antibodies OKT3, OKT4, 61E71 and B72.3 for diagnosis has been reported in the prior art. Thus, therapeutic treatment regimes, amounts used per treatment, intervals between treatments, routes of administration, etc. for OKT3 and diagnostic regimes for all four antibodies are well known in the art. Since the *in vitro* results show that the humanized

antibodies of the present invention have the same affinity as the prototype antibodies and since it can reasonably be predicted that the humanized antibodies will have as good, if not better, bioavailability as the prototype antibodies, the knowledgeable person will confidently adopt the same sort of treatment regimes as are adopted for the prototype antibodies.

It is therefore submitted that the invention claimed in the present application can readily be put in to effect by the skilled person. Withdrawal of this 35 USC §112, first paragraph rejection is respectfully requested.

At paragraph 21 of the Office Action, the Examiner rejected claims 13-16 under 35 USC §112, first paragraph stating that the disclosure is enabling only for claims limited to specific CDR-grafted antibodies disclosed in the specification as having effective binding affinities for their specific antibody, i.e. which are similar to the non-humanized donor antibodies.

Applicants respectfully traverse this rejection. The Examiner contends that the present specification is only enabling in respect of the exemplified humanized antibodies because, in the Examiner's view, it would require undue experimentation to adapt the teaching in the present application to other antibodies. This view appears to be based on an analysis of the prior art rather than an analysis of the present invention.

It is indeed true that in the prior art, there is very little guidance as to how to go about producing a recombinant antibody which has the same antigen binding affinity as the prototype antibody. The most guidance is provided by Queen et al. (PNAS-USA, 86, 10029-10033, 1989). However, the amount of guidance provided is small. Although it is not explicitly stated in Queen et al., it is clear that it is necessary before beginning the process described therein to determine the amino acid sequence of the antibody chain to be recombinant. The skilled person reading Queen et al. is told firstly to select a human chain which is as closely comparable to the murine chain as possible. It is then necessary to carry out computer modelling of the chain to determine which residues outside the Kabat CDRs may be important for antigen binding or retaining the appropriate shape in the antigen binding region. This computer modelling is by no means trivial as it requires the modeler to make a number of critical choices of parameters will lead to a different model and may well lead to a different determination of non-CDR residues.

By carrying out this procedure, Queen et al. identified a number of residues outside the CDR which are altered in order to improve the affinity of the recombinant antibody. However, Queen et al. provides no guidance as to which residues are critical for improving affinity in the particular case referred

to by Queen et al. (the anti-TAC antibody). Moreover, there is no indication that it might be possible to change the same residues in a different antibody in the expectation of achieving good affinity.

It is also to be noted that Queen et al. does not use the Kabat numbering for the amino acid residues. The sequences are merely numbered in a linear fashion. It is thus not possible readily to determine which residues according to the Kabat numbering system were altered. This makes the teaching in Queen et al. even more specific to the particular antibody shown by Queen et al.

Thus, the teaching of Queen et al. is that, in order to reshape any antibody, it is necessary to treat each antibody individually and to carry out for each antibody the steps of sequence determination, acceptor sequence selection and computer modelling. Clearly, this requires undue experimentation to apply the teaching of Queen et al. to other antibodies.

In contrast, the teaching in the present application can be applied without any undue experimentation to any antibody. All that is required is experimentation following a protocol which is clearly set out in the description, in particular at page 16, line 30 to page 19, line 9. In order to follow this protocol, as a first step, it is necessary to determine the amino acid sequence of the donor chain. The sequence of the acceptor

chain will already be known, for instance from a sequence data base.

There is then no need to carry out computer modelling to determine which donor residues to substitute into the acceptor sequence. The protocol in the present application provides the teaching directly. It instructs the skilled person to compare the two sequences and change certain specified residues in the acceptor sequence to donor residues.

Moreover, the present application provides a hierarchical structure of residues which can be considered. Thus, if changing the residues identified at the top of the structure does not provide adequate affinity, then a lower level of residues are considered, and so on until acceptable affinity is obtained.

The manipulation of an acceptor sequence using recombinant DNA technology is a matter of routine. For instance, a known sequence can be altered using site directed mutagenesis. Alternatively, a complete sequence can be synthesized from isolated nucleotides. The chain having the altered amino acid sequence can be tested for affinity using assays of the type described in the application. Thus, producing recombinant chains and testing them for affinity merely involves routine experimentation following a protocol which is clearly defined in the application.

It is submitted that this identifies where the present invention makes a significant departure from the prior art. The prior art indicates that each antibody has to be treated individually. In contrast, the present invention teaches that, by following the protocol set forth in the present application, it is possible to reshape any antibody.

The Examiner pointed to the fact that the present application refers to only three antibodies (OKT3, OKT4 and anti-ICAM) and contends that this is no basis for predicting that the protocol is generally application. It is respectfully submitted that the Examiner is incorrect. In the first place, the application refers to nine different antibodies (OKT3, OKT4, RG-5, B72.3, 61R71, 101.4, hTNF1, hTNF2 and hTNF3) (see page 52, last paragraph) which have been humanized successfully using the protocol set out in the application. It therefore cannot be seen how it can be contended that the concept underlying the present claims has not been fully developed. Since it has proved possible to humanize all nine antibodies, it is submitted that a reasonably skilled person would readily predict that the concept is applicable to other antibodies.

The Examiner refers to the absence from the application of binding affinity values and alleges that the description of binding affinity is only qualitative. It is submitted that this is not the case. Figures 7 to 13 clearly show data. On page 60,

second paragraph, the binding affinity of the humanized anti-ICAM antibody is given a quantitative value. On page 62, quantitative statements regarding binding activity are also given. Thus, the application does contain quantitative data showing that the concept is applicable to produce a useful antigen-binding molecule.

It is therefore submitted that the description is fully enabling without the need for undue experimentation. Withdrawal of this 35 USC §112 rejection is therefore respectfully requested.

At paragraph 22 of the Office Action, the Examiner rejected claims 1-23 under 35 USC §112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. This rejection is in several parts which will answered separately.

In Part A of the rejection, the Examiner stated that claims 1-5 are indefinite due to the language of the claims relating to donor positions and because the position numbers are arbitrary.

Claims 1-5 have been cancelled without prejudice. The newly submitted claims do not contain the language found objectionable by the Examiner. The residue numbers in the newly submitted claims refers to the numbering system devised by Kabat.

This is a standard numbering system used to enable comparisons of antibody chain amino acid sequences to be made.

The Kabat numbering system was developed by studying the amino acid sequences of a large number of heavy and light chains. It was discovered that, within the variable domains, there were some residues which are highly conserved, some areas in which sequence variation is low and some areas in which the sequence variation is high. On the basis of the highly conserved residues and the low variability areas, it proved to be possible to assign numbers to residues in all heavy and light chains.

In some cases, it was found that there had been deletions in the sequence. In other cases, there were additional residues. Generally, these additions or deletions occurred in the highly variable areas. In order to allow for this, in some sequences the numbering has a jump in it to account for a deletion and in other sequences there are a number of residues with the same number, followed by a, b, c, etc., to account for insertions. Nonetheless, it is still readily possible to align antibody chain sequences on the basis of the Kabat numbering. This is a system which is widely used and commonly recognized in the art. In this respect, reference can be made to Riechmann et al., page 325, first sentence after "Strategy".

It is therefore believed that the language now used in the claims is in accordance with the requirements of section 112.

In Part B of the rejection, the Examiner stated that claims 6-12 are also indefinite due to the language of the claims relating to donor positions and because the position numbers are arbitrary.

Claims 6-12 have been cancelled without prejudice. The newly submitted claims do not contain the language found objectionable by the Examiner. The position numbers of the donor residues refer to the numbering system devised by Kabat, as discussed in the response to Part A of this rejection.

In Part C of the rejection, the Examiner stated that claims 4 and 11 are indefinite and confusing because they are in an improper Markush listing.

Claims 4 and 11 have been cancelled without prejudice. This rejection is now moot.

In Part D of the rejection, the Examiner stated that claim 21 is indefinite. Claim 21 has been amended to correct an inadvertent clerical in step (c), so that step (c) now recites "transfecting a host cell with the expression vectors of steps (a) and (b) to form a transfected cell containing said expression vectors". Step (c) indicates that the expression vectors are transfected into one host cell. The amendment to step (c) also provides an antecedent basis for "the transfected cell line" in step (d).

The use of the term "complementary" in claim 21 does not render the claim indefinite. It is submitted that this is clear to the person skilled in the art. It is well known that it is possible for almost any heavy chain to associate to some extent with almost any light chain. However, randomly associated heavy/light chain dimers will generally not have any antigen binding activity. Antigen binding activity will only be obtainable if the heavy and light chains are of such sequence and configuration as to provide antigen binding regions which fit together to form a site which binds to a desired antigen. It is well known to the art that a heavy and a light chain which, when associated, form a binding site for a desired antigen are said to be complementary. Thus, in the present claims, "complementary" is used in the way it is normally understood by those skilled in the art and does not introduce any lack of clarity in the claims. Withdrawal of this entire 35 USC §112, second paragraph rejection is respectfully requested.

At paragraph 23 of the Office Action the Examiner rejected the claims under 35 USC §102(b). This rejection is divided into two parts with will be answered separately.

In Part A of this rejection, claims 1, 5, 6-8, and 12-22 were rejected as anticipated by Riechmann et al. The Examiner stated that claim 1 and claim 6 were interpreted to mean that the framework has donor residues in at least one of any of positions

6, 23, 24, 48, 49, 71, 73, 75, 76, 78, 88, or 91 in the heavy chain and (1, 3, 46, or 47) or 46, 48, 58, or 71) in the light chain, and thus, the teachings of Riechmann et al. anticipate the invention as claimed.

The Examiner contends that the original claims lacked novelty over Riechmann et al. Claims 1, 5, 6-8, 12 and 22 have been cancelled without prejudice and submitted as new claims that more distinctly point out certain aspects of the present invention.

In present claims 24 and 25, it is specified that residues 23 and 24 in the heavy chain should be donor residues. However, as can be seen from Fig. 1, panel (a) in Riechmann et al., in the recombinant antibody shown there, residues 23 and 24 are acceptor residues.

In the present claim 42, it is specified that residue 58 in the light chain should be a donor residue. However, as can be seen from Fig. 1, panel (b) in Riechmann et al., in the recombinant antibody shown there, residue 58 is an acceptor residue. Applicants' claimed antigen-binding molecules are thus not anticipated by Riechmann et al.

In Part B of the rejection, the Examiner rejected claims 1-6 and 12-22 as anticipated by Queen et al.

Claims 1-6, 12-20 and 22 have been cancelled without prejudice and submitted as new claims that more distinctly point out certain aspects of the present invention.

In present claims 24 and 25, it is specified that residues 48, 66, 67, 68, 93, 103 to 108 and 110 should all be acceptor residues. However, in Queen et al., as can be seen from Fig. 2B, in these positions Queen et al. uses donor, rather than acceptor, residues. It should again be borne in mind that Queen et al. does not use the Kabat numbering and it is therefore necessary to look carefully at the disclosure in Queen et al. before it is possible to come to any final conclusion.

In present claim 38, it is specified that residue 71 should be a donor residue. However, as can be seen from Fig. 2A of Queen et al., in that position Queen et al. uses an acceptor, rather than a donor residue.

Applicants' claimed antigen-binding molecules are thus not anticipated by Queen et al. Withdrawal of this entire 35 USC §102(b) rejection is respectfully requested.

At paragraph 24 of the Office Action, the Examiner rejected claims 1-21 under 35 USC §103 as being obvious over Riechmann et al. in view of Queen et al. The Examiner states

> It would have been *prima facie* obvious to one of ordinary skill in the art in the art at the time the invention was made to use the guidelines taught by Riechmann et al. and Queen et al. to reshape any given antibody to

> "humanize" that antibody by making changes in the framework regions of the human acceptor to the donor residue when those residues are close to the CDR's and when those amino acids affect the conformation of the CDR's.

Applicants respectfully traverse this rejection.

The Examiner contended that all the previous claims lacked an inventive step over either Riechmann et al. or Queen et al. It is submitted, for the following reasons, that the Examiner's contention is unfounded.

With respect to Riechmann et al., it is submitted that this document does not go much beyond the original idea of Winter et al. (see WO-A) 89/07452 referred to on page 4 of the present application) of transferring only the CDRs to a human framework. Thus, Riechmann et al. shows transferring the hypervariable regions identified according to Kabat (i.e. the Kabat CDRs) to a human framework (see Riechmann et al., page 325, after "Strategy"). This basic concept was to a certain extent modified for the heavy chain on the basis of the difference between Kabat CDR1 (as defined by sequence) and CDR1 as defined by structural studies.

On the basis of specific sequence differences in structural CDR1 between the rat (donor) and human (acceptor) sequences, Riechmann et al. decided to change residues 27 and 30 in the acceptor sequence to the equivalent residues in the donor sequence. It is made clear that these sequence changes were made

because the rat (donor) sequence was unusual. It is also to be noted that Riechmann et al. made no other changes outside the CDRs in the heavy chain.

As regards the light chain, all that Riechmann et al. did was to transfer the rat CDRs (as determined by Kabat) to the human acceptor framework. No residue changes outside the CDRs were made.

It can thus be seen that, at best, Riechmann et al. teaches the skilled person to transfer the six CDRs and to look at the possibility of transferring a composite CDR1 comprising a combination of the Kabat and structural CDR1, but only if there are any unusual residues in the area of the structural CDR1 not covered by the Kabat CDR1. Even this teaching, however, is specific to the particular antibody considered by Riechmann et al. There is nothing in Riechmann et al. to suggest that this teaching is generally applicable to other antibodies.

In any event, Riechmann et al. does not in any way suggest that altering residues remote from the CDRs might be effective in improving affinity. Certainly, Riechmann et al. in no way suggest that there might be a hierarchy of residues which should be considered if improvements in affinity are to be sought. Still less does Riechmann et al. provide any teaching which would allow the skilled person to identify the hierarchy of residues as set forth in the present application.

It is therefore submitted that the subject matter of present claims is in no way suggested by Riechmann et al. Thus, the present invention is not at all obvious over Riechmann et al.

As regards Queen et al., as the Examiner has noted, the teaching there is quite different from that in the present application. Queen et al. teaches that the amino acid sequence of the antibody chain in question (donor) should be determined and then compared to that of known acceptor chains. An acceptor chain should then be chosen which is as homologous as possible to the donor chain.

The next step is to carry out a computer modelling exercise to determine which residues might be involved in antigen binding or in ensuring that the antigen binding site adopts the correct conformation. It is again to be pointed out that computer modelling can lead to different results, depending on the parameter choices made. Thus, following Queen et al. may not always lead to the same results. That this is the case can be seen from page 10031, paragraph 3, lines 13 to 15, which shows that an earlier model differed from a later model.

It is also to be pointed out that the criteria for making the choices are indeterminate. All that Queen et al. indicates is that the residues need to be "close enough" to the CDR's (see page 10031, paragraph 3). There is no indication as to how close is "close enough". It is therefore difficult for

the skilled person to follow the teaching of Queen et al. without having to make critical decisions on which no guidance is given.

Once these exercises have been carried out, the acceptor sequence is altered so that not only the Kabat CDR's but also all the other residues outside the CDRs identified by the modelling procedure are changed to donor residues.

The fact that the donor sequence is compared to a number of possible acceptor sequences and that a computer model of the donor antibody must be made shows that the whole procedure in Queen et al. is specific to one antibody at a time. Underlying these facts is the assumption that each antibody is different from other antibodies and that it is not possible to predict from work carried on one antibody how to deal with another antibody.

It is no doubt true that implication of Queen et al. is that carrying out the procedure disclosed therein will enable one to produce a recombinant antibody having at least some of the affinity of the prototype antibody. (In the case shown in Queen et al. the affinity of the recombinant antibody was only one third that of the prototype antibody.) However, there is no suggestion in Queen et al. that changing the same residues as were changed when reshaping the anti-TAC antibody could be expected to provide acceptable binding affinity in another recombinant antibody.

Even if (which is denied) a skilled person did try to change residues in accordance with the numbers determined by Queen et al., this still does not lead to the present invention. As has been discussed above, Queen et al. changes residues which, according to the present invention, need not be changed or leaves as acceptor residues ones which should be changed. Thus, following the Queen et al. procedure cannot lead to the production of an antibody as now claimed.

The Queen et al. procedure can be contrasted with the protocol set forth in the present application. In the case of the present invention, there is no need to compare the donor sequence with a number of possible acceptor sequences. Any acceptor sequence can be used. Moreover, there is no need to carry out any computer modelling. All that is required is for the skilled person to go through the hierarchy of residue changes set forth in the application, beginning at page 16, and to make the minimum number of changes required to obtain acceptable activity.

It is submitted that there is nothing in Queen et al. which in any way suggests that this simple hierarchical protocol can be adopted in the expectation of being able to reshape any desired antibody. Queen et al. teaches that each antibody requires its own reshaping procedure. The present invention

teaches that one reshaping procedure can be applied to any antibody.

In order to establish a *prima facie* case of obviousness, it must be shown that the prior art would have suggested to those or ordinary skill in the art that they should make the claimed composition or device, or carry out the claimed process; and that the prior art would also have revealed that in so making or carrying out, those of ordinary skill would have a reasonable expectation of success. Both the suggestion and the reasonable expectation of success must be founded in the prior art, not in the Applicants' disclosure.

As discussed above, at best, Riechmann et al. teaches the skilled person to transfer the six CDRs and to look at the possibility of transferring a composite CDR1 comprising of the Kabat and structural CDR1, but only if there are any unusual residues in the area of the structural CDR1 not covered by the Kabat CDR1. Riechmann et al. does not in any way suggest that altering residues remote from the CDR's might be effective in improving affinity of the CDR-grafted antibody. Additionally, Riechmann et al. in no way suggests that there might be a hierarchy of residues which should be considered if improvements in affinity are to be sought. Still less does Riechmann et al. provide any teaching which would allow the skilled person to

identify the hierarchy of residues as set forth in the present application.

Regarding Queen et al., the fact that the donor sequence is compared to a number of possible acceptor sequences and that a computer model of the donor antibody must be made shows that the whole procedure in Queen et al. is specific to one antibody at a time. Underlying these facts is the assumption that it is not possible to predict from work carried out on one antibody how to deal with another antibody. There is no suggestion in Queen et al. that changing the same residues as were changed when reshaping the anti-TAC antibody could be expected to provide acceptable binding affinity in another recombinant antibody. Queen et al. thus teaches that each antibody requires its own reshaping procedure thereby teaching away from the present invention.

In summary, there is neither a suggestion in the prior art to make the claimed CDR-grafted antibodies, nor does the prior art show a reasonable expectation of success of making the claimed CDR-grafted antibodies. Applicants' claims are thus not *prima facie* obvious over Riechmann et al. and Queen et al. Withdrawal of this 35 USC §103 rejection is respectfully requested.

DOCKET NO.: CARP-0009 **PATENT**

In view of the above, the present application is believed to be in a condition ready for allowance. Reconsideration of the application is respectfully requested and an early Notice of Allowance is earnestly solicited.

Respectfully submitted,

Date: January 19, 1993

Signature
Liza D. Hohenschutz
Registration No. 33,712

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS
One Liberty Place - 46th Floor
Philadelphia, PA 19103
(215) 568-3100

19/C
B. White
4-21-93

Docket No: CARP-0009 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: John R. ADAIR et al.
Serial No.: 07/743 329
Group Art Unit: 1807
Filed: 17th September 1991
Examiner: L. Bennett
For: Humanised Antibodies

Honorable Commissioner of Patents and Trademarks
Washington DC 20231.

Dear Sir,

AMENDMENT

1. This amendment is filed following the interview with Examiners Bennett and Chambers on 27th January 1993.

2. Amendments

Please cancel all the present claims and replace them with the new claims as follows.

67. An antibody molecule having affinity for a predetermined antigen and comprising a "composite" heavy chain and a complementary light chain, said composite heavy chain having a variable domain comprising human acceptor antibody heavy chain framework residues and donor antibody heavy chain antigen-binding residues, said donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain, amino acid residues 5, 8, 10, 12 to 17, 19, 21, 22, 40, 42 to 44, 66, 68, 70, 74, 77, 79, 81, 83 to 85, 90, 92, 105, 109, 111 and 113 at least are accepter residues and amino acid residues (23), (24),

31 to 35, 49 to 58, 71, 73, 78 and 95 to 102 at least are donor residues.

68. The antibody molecule of claim 67, wherein amino acid residues 26 to 30 and 59 to 65 in said composite heavy chain are additionally donor residues.

69. The antibody molecule of claim 67, wherein at least one of amino acid residues 1, 3, and 76 in said composite heavy chain are additionally donor residues.

70. The antibody molecule of claim 67, wherein at least one of amino acid residues 36, 94, 104, 106 and 107 in said composite heavy chain are additionally donor residues.

71. The antibody molecule of claim 70, wherein at least one of amino acid residues 2, 4, 6, 38, 48, 67 and 69 in said composite heavy chain are additionally donor residues.

72. The antibody molecule of claim 67, wherein amino acid residues 7, 9, 11, 18, 20, 25, 37, 39, 41, 45, 47, 48, 72, 75, 80, 82, 86 to 89, 91, 93, 103, 108, 110 and 112 in said composite heavy chain are additionally acceptor residues.

73. A tumour-specific antibody molecule having affinity for a predetermined tumour antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain comprising human acceptor antibody heavy chain framework residues and donor antibody heavy chain antigen-binding residues, said donor antibody having affinity for said predetermined tumour antigen, wherein, according to the Kabat numbering system, in said composite heavy chain, amino acid residues 5, 8, 10, 12 to 17, 19, 21, 22, 40, 42 to 44, 66, 68, 70, 74, 77, 79, 81, 83 to 85, 90, 92, 105, 109, 111 and 113 at least are acceptor residues and amino acid residues 23, 24, 31 to 35, 49 to 58 and 95 to 102 at least are donor residues.

74. The antibody molecule of claim 73, wherein amino acid residues 71, 73 and 78 in said composite heavy chain are additionally donor residues.

75. The antibody molecule of claim 73, wherein amino acid residues 26 to 30 and 59 to 65 in said composite heavy chain are additionally donor residues.

76. The antibody molecule of claim 73, wherein at least one of amino acid residues 1, 3, and 76 in said composite heavy chain are additionally donor residues.

77. The antibody molecule of claim 73, wherein at least one of amino acid residues 36, 94, 104, 106 and 107 in said composite heavy chain are additionally donor residues.

78. The antibody molecule of claim 77, wherein at least one of amino acid residues 2, 4, 6, 38, 48, 67 and 69 in said composite heavy chain are additionally donor residues.

79. The antibody molecule of claim 73, wherein amino acid residues 7, 9, 11, 18, 20, 25, 37, 39, 41, 45, 47, 48, 72, 75, 80, 82, 86 to 89, 91, 93, 103, 108, 110 and 112 in said composite heavy chain are additionally acceptor residues.

80. An interleukin-specific antibody molecule having affinity for a predetermined interleukin and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain comprising human acceptor antibody heavy chain framework residues and donor antibody heavy chain antigen-binding residues, said donor antibody having affinity for said predetermined interleukin wherein, according to the Kabat numbering system, in said composite heavy chain, amino acid residues 5, 8, 10, 12 to 17, 19, 21, 22, 40, 42 to 44, 66, 68, 70, 74, 77, 79, 81, 83 to 85, 90, 92, 105, 109, 111 and 113 at least are acceptor residues and amino acid residues 23, 24, 31 to 35, 49 to 58 and 95 to 102 at least are donor residues.

81. The antibody molecule of claim 80, wherein amino acid residues 71, 73 and 78 in said composite heavy chain are additionally donor residues.

82. The antibody molecule of claim 80, wherein amino acid residues 26 to 30 and 59 to 65 in said composite heavy chain are additionally donor residues.

83. The antibody molecule of claim 80, wherein at least one of amino acid residues 1, 3 and 76 in said composite heavy chain are additionally donor residues.

84. The antibody molecule of claim 80, wherein at least one of amino acid residues 36, 94, 104, 106 and 107 in said composite heavy chain are additionally donor residues.

85. The antibody molecule of claim 84, wherein at least one of amino acid residues 2, 4, 6, 38, 48, 67 and 69 in said composite heavy chain are additionally donor residues.

86. The antibody molecule of claim 80, wherein amino acid residues 7, 9, 11, 18, 20, 25, 37, 39, 41, 45, 47, 48, 72, 75, 80, 82, 86 to 89, 91, 93, 103, 108, 110 and 112 in said composite heavy chain are additionally acceptor residues.

87. An anti-CD3 antibody molecule having affinity for the CD3 antigen comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain comprising human acceptor antibody heavy chain framework residues and donor antibody heavy chain antigen-binding residues from a donor antibody heavy chain, said donor antibody having affinity for said CD3 antigen, wherein, according to the Kabat numbering system, in said composite heavy chain, amino acid residues 5, 8, 10, 12 to 17, 19, 21, 22, 40, 42 to 44, 66, 68, 70, 74, 77, 79, 81, 83 to 85, 90, 92, 105, 109, 111 and 113 at least are acceptor residues and amino acid residues 23, 24, 31 to 35, 49 to 58 and 95 to 102 at least are donor residues.

88. The antibody molecule of claim 87, wherein amino acid residues 71, 73 and 78 in said composite heavy chain are additionally donor residues.

89. The antibody molecule of claim 87, wherein amino acid residues 26 to 30 and 59 to 65 in said composite heavy chain are additionally donor residues.

90. The antibody molecule of claim 87, wherein at least one of amino acid residues 1, 3, and 76 in said composite heavy chain are additionally donor residues.

91. The antibody molecule of claim 87, wherein at least one of amino acid residues 36, 94, 104, 106 and 107 in said composite heavy chain are additionally donor residues.

92. The antibody molecule of claim 91, wherein at least one of amino acid residues 2, 4, 6, 38, 48, 67 and 69 in said composite heavy chain are additionally donor residues.

93. The antibody molecule of claim 87, wherein amino acid residues 7, 9, 11, 18, 20, 25, 37, 39, 41, 45, 47, 48, 72, 75, 80, 82, 86 to 89, 91, 93, 103, 108, 110 and 112 in said composite heavy chain are additionally acceptor residues.

94. An anti-CD4 antibody molecule having affinity for the CD4 antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain comprising human acceptor antibody heavy chain framework residues and donor antibody heavy chain antigen-binding residues, said donor antibody having affinity for said CD4 antigen, wherein, according to the Kabat numbering system, in said composite heavy chain, amino acid residues 5, 8, 10, 12 to 17, 19, 21, 22, 40, 42 to 44, 66, 68, 70, 74, 77, 79, 81, 83 to 85, 90, 92, 105, 109, 111 and 113 at least are acceptor residues and amino acid residues 23, 24, 31 to 35, 49 to 58 and 95 to 102 at least are donor residues.

95. The antibody molecule of claim 94, wherein amino acid residues 71, 73 and 78 in said composite heavy chain are additionally donor residues.

96. The antibody molecule of claim 94, wherein amino acid residues 26 to 30 and 59 to 65 in said composite heavy chain are additionally donor residues.

97. The antibody molecule of claim 94, wherein at least one of amino acid residues 1, 3, and 76 in said composite heavy chain are additionally donor residues.

98. The antibody molecule of claim 94, wherein at least one of amino acid residues 36, 94, 104, 106 and 107 in said composite heavy chain are additionally donor residues.

99. The antibody molecule of claim 98, wherein at least one of amino acid residues 2, 4, 6, 38, 48, 67 and 69 in said composite heavy chain are additionally donor residues.

100. The antibody molecule of claim 94, wherein amino acid residues 7, 9, 11, 18, 20, 25, 37, 39, 41, 45, 47, 48, 72, 75, 80, 82, 86 to 89, 91, 93, 103, 108, 110 and 112 in said composite heavy chain are additionally acceptor residues.

101. An anti-adhesion molecule antibody molecule having affinity for an adhesion molecule and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain comprising human acceptor antibody heavy chain framework residues and donor antibody heavy chain antigen-binding residues from a donor antibody heavy chain, said donor antibody having affinity for said adhesion molecule wherein, according to the Kabat numbering system, in said composite heavy chain, amino acid residues 5, 8, 10, 12 to 17, 19, 21, 22, 40, 42 to 44, 66, 68, 70, 74, 77, 79, 81, 83 to 85, 90, 92, 105, 109, 111 and 113 at least are acceptor residues and amino acid residues 23, 24, 31 to 35, 49 to 58 and 95 to 102 at least are donor residues.

102. The antibody molecule of claim 101, wherein amino acid residues 71, 73 and 78 in said composite heavy chain are additionally donor residues.

103. The antibody molecule of claim 101, wherein amino acid residues 26 to 30 and 59 to 65 in said composite heavy chain are additionally donor residues.

104. The antibody molecule of claim 101, wherein at least one of amino acid residues 1, 3, and 76 in said composite heavy chain are additionally donor residues.

105. The antibody molecule of claim 101, wherein at least one of amino acid residues 36, 94, 104, 106 and 107 in said composite heavy chain are additionally donor residues.

106. The antibody molecule of claim 105, wherein at least one of amino acid residues 2, 4, 6, 38, 48, 67 and 69 in said composite heavy chain are additionally donor residues.

107. The antibody molecule of claim 101, wherein amino acid residues 7, 9, 11, 18, 20, 25, 37, 39, 41, 45, 47, 48, 72, 75, 80, 82, 86 to 89, 91, 93, 103, 108, 110 and 112 in said composite heavy chain are additionally acceptor residues.

108. The antibody molecule of claim 67, wherein said complementary light chain is a composite light chain having a variable domain comprising human acceptor antibody light chain framework residues and donor antibody light chain antigen-binding residues, said donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite light chain, amino acid residues 5, 7 to 9, 11, 13 to 18, 20, 22, 23, 39, 41 to 43, 57, 59, 61, 72, 74 to 79, 81, 82, 84, 86, 88, 100, 104, 106 and 107 at least are acceptor residues and amino acid residues 24 to 34, 46, 48, 50 to 56, 58, 71 and 89 to 97 at least are donor residues.

109. The antibody molecule of claim 73, wherein said complementary light chain is a composite light chain having a variable domain comprising human acceptor antibody light chain framework residues and donor antibody light chain antigen-binding residues, said donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite light chain, amino acid residues 5, 7 to 9, 11, 13 to 18, 20, 22, 23, 39, 41 to 43, 57, 59, 61, 72, 74 to 79, 81, 82, 84, 86, 88, 100, 104, 106 and 107 at least are acceptor residues and amino acid residues 24 to 34, 46, 48, 50 to 56, 58, 71 and 89 to 97 at least are donor residues.

110. The antibody molecule of claim 80, wherein said complementary light chain is a composite light chain having a variable domain comprising human acceptor antibody light chain framework residues and donor antibody light chain antigen-binding residues, said donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite light chain, amino acid residues 5, 7 to 9, 11, 13 to 18, 20, 22, 23, 39, 41 to 43, 57, 59, 61, 72, 74 to 79, 81, 82, 84, 86, 88, 100, 104, 106 and 107 at least are acceptor residues and amino acid residues 24 to 34, 46, 48, 50 to 56, 58, 71 and 89 to 97 at least are donor residues.

111. The antibody molecule of claim 87, wherein said complementary light chain is a composite light chain having a variable domain comprising human acceptor antibody light chain framework residues and donor antibody light chain antigen-binding residues, said donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite light chain, amino acid residues 5, 7 to 9, 11, 13 to 18, 20, 22, 23, 39, 41 to 43, 57, 59, 61, 72, 74 to 79, 81, 82, 84, 86, 88, 100, 104, 106 and 107 at least are acceptor residues and amino acid residues 24 to 34, 46, 48, 50 to 56, 58, 71 and 89 to 97 at least are donor residues.

112. The antibody molecule of claim 94, wherein said complementary light chain is a composite light chain having a variable domain comprising human acceptor antibody light chain framework residues and donor antibody light chain antigen-binding residues, said donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite light chain, amino acid residues 5, 7 to 9, 11, 13 to 18, 20, 22, 23, 39, 41 to 43, 57, 59, 61, 72, 74 to 79, 81, 82, 84, 86, 88, 100, 104, 106 and 107 at least are acceptor residues and amino acid residues 24 to 34, 46, 48, 50 to 56, 58, 71 and 89 to 97 at least are donor residues.

113. The antibody molecule of claim 101, wherein said complementary light chain is a composite light chain having a variable domain comprising human acceptor antibody light chain framework residues and donor antibody light chain antigen-binding residues, said donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite light chain, amino acid residues 5, 7 to 9, 11, 13 to 18, 20, 22, 23, 39, 41 to 43, 57, 59, 61, 72, 74 to 79, 81, 82, 84, 86, 88, 100, 104, 106 and 107 at least are acceptor residues and amino acid residues 24 to 34, 46, 48, 50 to 56, 58, 71 and 89 to 97 at least are donor residues.

114. The antibody molecule of claim 108, wherein amino acid residues 1, 3 and 47 in said composite light chain are additionally donor residues.

115. The antibody molecule of claim 108, wherein amino acid residues 36, 44, 47, 85 and 87 in said composite light chain are additionally donor residues.

116. The antibody molecule of claim 108, wherein at least one of amino acid residues 2, 4, 6, 49, 62, 64 to 69, 98, 99, 101 and 102 in said composite lightly chain are additionally donor residues.

117. The antibody molecule of claim 108, wherein at least one of amino acid residues 1, 3, 10, 12, 21, 40, 60, 63, 70, 73, 80, 103 and 105 in said composite light chain are additionally donor residues.

118. A method for producing a recombinant antigen binding molecule having affinity for a predetermined antigen comprising the steps of:

[1] determining the amino acid sequence of the variable domain of the heavy chain of a donor antibody which has affinity for said predetermined antigen;

[2] determining the amino acid sequence of the variable domain of the heavy chain of a non-specific acceptor antibody;

[3] providing a composite heavy chain for an antibody molecule, said composite heavy chain having acceptor framework residues and donor antigen binding residues wherein, according to the Kabat numbering system, amino acid residues 5, 8, 10, 12 to 17, 19, 21, 22, 40, 42 to 44, 66, 68, 70, 74, 77, 79, 81, 83 to 85, 90, 92, 105, 109, 111 and 113 at least are acceptor residues and amino acid residues 23, 24, 31 to 35, 49 to 58 and 95 to 102 at least are donor residues;

[4] associating the heavy chain produced in step [3] with a complementary light chain to form an antibody molecule;

[5] determining the affinity of the antibody molecule formed in step [4] for said predetermined antigen;

[6] if the affinity determined in step [5] is not equivalent to that of the donor antibody, providing a heavy chain as described in [3] above but in which amino acid residues 71, 73 and 78 are additionally donor residues;

[7] associating the heavy chain produced in step [6] with a complementary light chain to form an antibody molecule;

[8] determining the affinity of the antibody molecule formed in step [7] for said predetermined antigen;

[9] if the affinity determined in step [8] is not equivalent to that of the donor antibody, providing a heavy chain as described in [6] above but in which amino acid residues 26 to 30 are additionally donor residues;

[10] associating the heavy chain produced in step [9] with a complementary light chain to form an antibody molecule;

[11] determining the affinity of the antibody molecule formed in step [10] for said predetermined antigen;

[12] if the affinity determined in step [11] is not equivalent to that of the donor antibody, providing a heavy chain as described in [9] above but in which at least one of amino acid residues 1, 3, and 76 are additionally donor residues;

[13] associating the heavy chain produced in step [12] with a complementary light chain to form an antibody molecule;

[14] determining the affinity of the antibody molecule formed in step [13] for said predetermined antigen;

[15] if the affinity determined in step [14] is not equivalent to that of the donor antibody, providing a heavy chain as described in [12] above but in which at least one of amino acid residues 36, 94, 104, 106, 107 are additionally donor residues;

[16] associating the heavy chain produced in step [15] with a complementary light chain to form an antibody molecule.

[17] determining the affinity of the antibody molecule formed in step [16] for said predetermined antigen;

[18] if the affinity determined in step [17] is not equivalent to that of the donor antibody, providing a heavy chain as described in [15] above but in which at least one of amino acid residues 2, 4, 6, 38, 48, 67 and 69 are additionally donor residues; and

[19] associating the heavy chain produced in step [18] with a complementary light chain to form an antibody molecule.

119. The method of claim 118, further comprising the steps of:

[1] determining the amino acid sequence of the variable domain of the light chain of said donor antibody which has affinity for said predetermined antigen;

[2] determining the amino acid sequence of the variable domain of the light chain of a non-specific acceptor antibody;

[3] providing a composite light chain for an antibody molecule, said composite light chain having acceptor framework residues and donor antigen binding residues wherein, according to the Kabat numbering system, amino acid residues 5, 7 to 9, 11, 13 to 18,

20, 22, 23, 39, 41 to 43, 57, 59, 61, 72, 74 to 79 to 79, 81, 82, 84, 86, 88, 100, 104 and 106 to 109 at least are acceptor residues and amino acid residues 24 to 34, 46, 48, 50 to 56, 58, 71 and 89 to 97 at least are donor residues;

[4] associating the light chain produced in step [3] with a complementary heavy chain to form an antibody molecule;

[5] determining the affinity of the antibody molecule formed in step [4] for said predetermined antigen;

[6] if the affinity determined in step [5] is not equivalent to that of the donor antibody, providing a light chain as described in [3] above but in which amino acid residues 1, 2, 3 and 47 are additionally donor residues;

[7] associating the light chain produced in step [6] with a complementary heavy chain to form an antigen-binding molecule;

[8] determining the affinity of the antigen-binding molecule formed in step [7] for said predetermined antigen;

[9] if the affinity determined in step [8] is not equivalent to that of the donor antibody, providing a light chain as described in [6] above but in which amino acid residues 36, 44, 47, 85 and 87 are additionally donor residues;

[10] associating the light chain produced in step [9] with a complementary heavy chain to form an antibody molecule;

[11] determining the affinity of the antibody molecule formed in step [10] for said predetermined antigen;

[12] if the affinity determined in step [11] is not equivalent to that of the donor antibody, providing a light chain as described in [9] above but in which at least one of amino acid residues 2, 4, 6, 49, 62, 64 to 69, 98, 99, 101 are additionally donor residues; and

[13] associating the light chain produced in step [9] with a complementary heavy chain to form an antibody molecule.

---

3. Remarks

The undersigned would like to thank Examiners Lisa Bennett and Scott Chambers for the very cordial and helpful interview on January 27, 1993 relating to this application. The amendments to the claims reflect the discussions of the interview as recorded in the Examiner

Interview Summary Record.

Having considered the Examiner's concerns that the language of the claims might be indefinite, because it was not clear whether the specified residues were the only or the minimum number of residues to be donor residues, the Applicants have amended the claims. In all the claims it is made clear that there is a minimum number of residues which have to be donor residues and a minimum number which have to be acceptor residues. Those residues which are not specified in the claims may be either donor or acceptor.

The claims have been amended to delete "antigen-binding molecule" and recite instead "antibody molecule". In order to reduce issues, the claims have also been amended recite that the acceptor antibody heavy or light chain is a human acceptor antibody heavy or light chain. These amendments to the claims address the Examiner's concern relating to utility and enablement of the claims. In view of the great conservation of structure between antibodies of different species, however, Applicants believe the acceptor and donor antibodies can come from any species.

In order to reduce the issues further, the claims have been limited to antibodies in which the heavy chain has been "superhumanised". Claims referring to the light chain have been made dependent on the claims referring to the heavy chain.

Regarding claim 67, it can be seen from the description (see page 6 line 29 to page 7, line 28; page 17, lines 9 to 11, Sections 2.1 to 2.3 bridging pages 17 and 18; Section 1 bridging pages 19 and 20; Sections 2.1.1, 2.2.1, 2.3.1 and 2.4.1 in the passage bridging pages 20 to 23; and Section 15.2.1 on pages 46 and 47) that a number of residues are mentioned which can be considered for changing from acceptor to donor residues. It follows that if a residue has not been considered for changing, it must remain as in the acceptor chain. In order to make this clear in the claim, it has been specified in claim 67 that all the unmentioned residues must be acceptor residues.

As regards the recitation of the donor residues in claim 67, this comprises a combination of the minimum residues need to define the CDR loops or the Kabat CDRs. For CDR1, this comprises residues 31 to 35 (see page 46, line 18) and for CDR2 this comprises residues 50 to 58 (see page 46, line 20). For CDR3, the Kabat definition of residues 95 to 102 was used (see page 17, line 11).

In addition claim 67 recites as donor residues those identified on page 20, line 25 and page 21, line 9 as being key residues outside the CDRs.

In claim 67, it has been specified that residues 71, 73 and 78 are all donor residues in order to ensure that claim 67 is novel over the anti-TAC antibody disclosed by Queen. This anti-TAC antibody has an acceptor residue at residue 73. However, as can be seen from page 7, lines 1 to 5, the Applicant considers that in general, residues 71, 73 and 78 can be either all donor or all acceptor.

Claim 68 increases the sizes of CDR1 and CDR2 to the prudent definition given on page 17, line 9 and page 20, lines 6 to 9.

Claim 69 is based on the passage at page 20, lines 26 and 27. Claim 70 is based on the passage at page 21, lines 10 to 12. Claim 71 is based on the passage on page 21, lines 13 to 16.

Claim 72 is derived by taking all the donor residues mentioned in claims 67 to 71 and specifying that all apart from these residues are acceptor residues.

Claim 73 is based on page 15, line 27 and pages 55 to 59 of the description. Claim 80 is based on page 15, line 33 of the description. Claim 87 is based on page 15, line 31 and pages 25 to 52 of the description. Claim 94 is based on page 15, line 31 and pages 53 and 54 of the description. Claim 101 is based on page 15, line 32 and pages 60 and 61 of the description.

None of the prior art CDR-grafted antibodies has specificity for a tumour-specific antigen, an interleukin, the CD3 or CD4 antigen, or an adhesion molecule. It is therefore submitted that claims 73, 80, 87, 94 and 101 are all novel.

It is stated on page 7, lines 1 to 5 that residues 71, 73 and 78 should all be either acceptor or donor. Claims 73, 80, 87, 94 and 101 cover the first alternative and claims 74, 81, 88, 95 and 102 cover the second alternative.

Claims 75 to 90, 82 to 86, 89 to 93, 96 to 100 and 103 to 107 are equivalent to claims 68 to 72.

Claims 108 to 113 are based on previously filed claim 42 and is derived in the same way that present claim 67 was derived. The residues which can be donor residues are listed on page 9, line 7 to page 10, line 15; page 17, lines 12 to 14; Section 3 bridging pages 18 and 19; Section 2.1.2 on page 20; Section 2.2.2 on page 21; Section 2.3.2 on page 22 and Section 2.4.2 on page 23.

Claims 114 to 117 are based on the passage from page 9, line 7 to page 10, line 11.

Claims 118 and 119 are based on previous claims 65 and 66 but with amendments to make them consistent with claims 67 onwards.

It is therefore believed that all the claims are fully supported by the description.

Although all the previous claims have been cancelled, the applicants reserve the right to file divisional applications relating to the deleted subject matter.

At the interview, the Examiner expressed concerns that the claims may lack novelty and that the method disclosed in the application might not be universally applicable. In an effort to resolve these questions, the applicants send herewith three Tables relating to a

number of antibodies which have been "superhumanised" by the method of the invention. The first Table relates to the heavy chain and the second Table relates to the light chain. On the third Table is set out the degree of affinity recovered as a percentage of the affinity of the original monoclonal antibody. There is also provided on the Tables a comparison with antibodies which have been produced by prior art proposals.

On each of the Tables, the top line shows the residue numbering according to the Kabat scheme. It can be seen that this includes, for the heavy chain, residues 52a-52c, 82a-82c and 100a to 100i.

Underneath the residue numbering is set out schematically the residue assignment for each of the antibodies referred to. This is schematic in that it does not give the actual residue but instead indicates whether it is a donor (D), acceptor (A) or common (c) residue. "Common" means that the same residue was present in both the donor and the acceptor sequences. Some of the common residues are also highly or completely conserved, but this has not been marked on the Tables.

In the heavy chain Table, the first five residues of 39D10 are marked by a question mark. This is because, due to the cloning method used, the first five residues in the donor chain were not determined. Thus, no comparison can be made.

At the end of each line is given the name of the acceptor sequence used to produce the chain.

It should be noted that for the antibodies 61E71 and hTNF3, the projects were terminated before the method had been fully applied. The applicants are confident that, had they had the funds to complete the work on these antibodies, good recovery of affinity would have been obtained.

In the Tables, the B1.8, D1.3, CAMPATH and anti-TAC entries represent prior art antibodies. There is no entry in the light chain Table for

B1.8 because the B1.8 light chain was never CDR-grafted.

B1.8 recognises an artificial antigen, the nitrophenyl hapten. Its heavy chain has acceptor residues at positions 23, 24, 73 and 78.

D1.3 recognises lysosyme. Its heavy chain has acceptor residues at positions 71, 73 and 78. Its light chain has acceptor residues at positions 48 and 71.

CAMPATH recognises the CD52 differentiation antigen found on various leukocytes. Its heavy chain has acceptor residues at positions 23, 24, 71, 73 and 78. Its light chain has an acceptor residue at position 58.

Anti-TAC is the antibody described by Queen. It recognises an epitope on a surface receptor for IL-2. (It does not recognise IL-2 itself). Its heavy chain has an acceptor residue at position 73. Its light chain has an acceptor residue at position 71.

Looking at the heavy chain Table, it can be seen that in all the successfully "superhumanised" antibodies produced by the applicants, in the heavy chain residues 23, 24, 31 to 35, 49 to 58 and 95 to 102 are all donor residues. Also in each case residues 26 to 30 are donor residues. Moreover, in most cases, residues 59 to 65 are also donor residues.

In one chain, OKT3 209, residues 71 and 73 were left as acceptor. For this particular antibody, it was shown that as long as residue 78 is donor, it does not make much difference whether residues 71 and 73 are donor or acceptor. This seems to be a peculiarity of this antibody, but which highlights the importance of residue 78.

In the heavy chains for 61E71 and hTNF3, residue 78 remained as acceptor. In both cases, the donor residue is alanine and the acceptor residue is leucine. Since leucine is more bulky than alanine, this may explain why the affinity of these chains was lower than desirable.

In hTNF1 a number of residues (12, 66 and 83) are donor residues whereas claim 67 specifies them as being acceptor residues. This antibody was prepared as the first in a series and the aim was to ensure that it worked. Therefore, any residues which were in the slightest unusual were changed to donor residues, even though it was suspected that some of the changes were unnecessary. As can be seen from, for instance 101/4, a later antibody in the series, if residues 12, 66 and 83 remain as acceptor, reasonable affinity is recovered. It is submitted that this is evidence that hTNF1 was overengineered.

In P67.6, residue 44 is a donor residue whereas claim 67 specifies that it should be an acceptor residue. In the donor antibody in this case, residue 44 was a very unusual amino acid for that position and it was therefore felt that, in that particular case, the unusual amino acid should be adopted.

At residue 77, which is specified as an acceptor residue, 101/4 and 39D10 have the donor residue. The change here in both cases is of a surface residue and is from leucine to valine. These are both hydrophobic residues and it is unlikely that this change will significantly affect the recovery of activity.

In 101/4 residue 79 is donor whereas claim 67 specifies it as acceptor. The donor residue is tyrosine and the acceptor is phenylalanine. This is a very conservative change and it is highly unlikely that this change will significantly affect the recovery of affinity.

For residue 105, which should be acceptor, B1RR0001, CTM01 and P67.6 all have donor residues. B1RR0001 gave very low recovery of affinity and it is believed that retaining the acceptor sequence at residue 105 would have improve the recovery of affinity. For the other two antibodies, the change is asparagine for glutamine, a very conservative change. It is highly unlikely that this change will have had any significant effect on the recovery of affinity.

Heavy chain residue 78 is specified as donor. In 61E71 and hTNF3, it is acceptor. However, these antibodies have low recovery of affinity. The projects on these antibodies were terminated at an early stage. If residue 78 had been changed to donor, it is believed that better recovery of affinity would have been obtained.

It is submitted that the data given in the three Tables amply demonstrate that successful "superhumanisation" can be achieved by following the method of the invention.

The Examiners expressed some concern that the language of the claims could read on naturally-occurring antibody heavy or light chains. The claims recite a "donor" antibody and an "acceptor" antibody as sources of residues in the heavy or light chains. Such an antibody, as claimed, cannot exist in nature. Therefore, the claims cannot read on a naturally-occurring antibody.

It is submitted that the present claims define inventive subject matter, for the reasons set forth in the Amendment mailed 19th January 1993.

In view of the above amendments and discussion, the present application is believed to be in a condition ready for allowance. Reconsideration of the application is respectfully requested and an early Notice of Allowance is respectfully requested.

Respectfully submitted,

Date: April 7, 1993

Signature
Francis A. Paintin
Registration No. 19,386

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS
One Liberty Place - 46th Floor
Philadelphia, PA 19103
(215) 568-3100

```
HEAVY CHAIN                                                                                                              1              1 1
RESNUM              1         2         3         4         5            6         7         8            9         0              1 1
          1234567890123456789012345678901234567890123456789012abc345678901234567890123456789012abc34567890123456789 0abcde1234567890123   ACCEPTOR
B72.3     ccccAccAccAAcccAcccAccccccDccDDDDDDcAAcAcAccccAcDDDDDDDDDDDDDDDDDDDAAcAcccDcAAccccAcAccAcccAcAcccDDDDDDD.......DDDcDAAcccc   EU
OKT3 209  ccccADccAAAAAccAcAAAccDDccDDcDDDDDDccAcAccAccccDDDDDDDDDDDDDDDDDDDADDAAcAAAcAcAAcDAAcAAccAAccAAccAcccDDDDDDDD...DDcccccAAcccc   KOL
OKT3 207  ccccADccAAAAAccAcAAAccDDccDDcDDDDDDccAcAccAccccDDDDDDDDDDDDDDDDDDDADDAAcAADcDcAAcDAAcAAccAAccAAccAcccDDDDDDDD...DDcccccAAcccc   KOL
OKT4A     AcAccccccAAcAccAccAccccDcccDccDDDDDccccAcAcAccccccDDDDDDDDDDDDDDDDDDDccccccccAccccAcccAcccAcccDAcDcccDDDDDDDDD..DDcccccAAcccc   KOL
BIRR0001  ccccAcccAcAAAccAcccAcccDccDccDDDDDDccAAAAAAAcccDcDDDDDDDDDDDDDDDDDDDDDAAcDcDcDcAccccDccAAcAcccAcDcDccDDDDDDDDD...DDDDDcDDAcccc   EU
A5B7      DcAcAcccccccccccccccccDcccccDDDDDDccccAcccAcccDDDDDDDDDDDDDDDDDDDDDccccccDDcADAccccccDcAccDccAcccDcDDDDDDDD...DDcccccAAcccc   LAY
CTM01     cDccAcccAcAAccçAcccAccccccDccDDDDDDcDAcAcccccccDcDDDDDDDDDDDDDDDDDDDDADcDcDcDcAAccccAccccAcccccAccccDDDDDDDDDD..DDDDDcDAccccA   EU
P67.6     DcccAcccAccAcccAcccAccccccDcDDDDDDDccAcADcADcccDcDDDDDDDDDDDDDDDDDDDDDADcDcDcDDAAccccccAccAcccAcAcDcDDDDDDD......DDDDDcDcccccc   EU
A33       DcAcAcccccccAcccccAccccccccDccDDDDDccccAcAcAcccccDDDDDDDDDDDDDDDDDDDDccccccDDAAcccccccADcAAcDAcAccccDDDDDDD.....DDcccccccccA   LAY
61E71     cAcccAccAAAAAccAAAAAccDDccDDcDDDDDDccAcAcccccAcDDDDDDDDDDDDDDDDDDDDDcAAAcDADcAAcAAccAAAcAAccAAAAccccDDDDDDD....DDcccccAAcccc   KOL
hTNF3     AAcccAccAAAAAccAAAAAccDDccDDcDDDDDDccAcccccccAcDDDDDDDDDDDDDDDDDDDDDDccAAcDADcAAcAAccAAAcAAcccAAccccDDDDDDD....DDcccccAAcccc   KOL
101/4     AcADAcccccDccccAcAAcccDDcccDccDDDDDccccAcAcccccccDDDDDDDDDDDDDDDDDDDDDcccccccDccADDDcccAAcAAccccAcDcDDD..........DDcccccDccccA   KOL
hTNF1     AcAcAcccAcADcccAcccAAcccccDccDDDDDDccccAAcAAcDcDcDDDDDDDDDDDDDDDDDDDDDDcDcDcDcADcccccAccDcccccDcDccDDDDDDDDDDD.DDDDAcDDccccc   EU
39D10     ?????AAcccAcccAAAcAcAcDDccDDDDDDDDDcDccAcccccccADDDDDDDDDDDDDDDDDDDDDcAAccccDccDDDADAccccAAccccAcAcccDDDDDD.....DDcccccAccccc   HIL
L243      cAccccccAcAccccAAccAcccccccDccDDDDDDccAccccAccAcDcDDDDDDDDDDDDDDDDDDDDcDADAcDccDDcccAAAAAcAAccccAcAccDDDDDDDDDDD.DDcccccAAcccc   SGI consensus
HLT29     cDcccccccccccccAccAcccAcccccDDDDDDDcAcccccccccccDDDDDDDDDDDDDDDDDDDDDDccccccccccDccccccccccAccccAcAcccDDDDDDDDDDDDDccccAAAcccc   SGIII consensus

B1.8      cccccAAcAAccAcAAAAAcAcAAccAccADDDDDccAcAccccccccccDDDDDDDDDDDDDDDDDDDDAAcAAccAAAAAAAAAcccAcAAcAcccccccDDDDDDD....DDccccAAAcccc   NEW
D1.3      ccccAccccccccAcccAccAccccccAAAADDDDDcccccccAccccAcDDDDDDDDDDDDDDDDDDDDcAAAAAcAccAcAAcAAAcAAAAcccAcccccDDDDDD.....DDccccAAAcccc   NEW
CAMPATH   AcAcAcccAcccAcAAAAAcAcAAccDccDDDDDDcAcccAcAAAccAcDDDDDDDDDDDDDDDDDDDDcAcAAAcAAAcAAAcAAAAAAcAcccAcccccDDDDDDD....DDccccAAAcccc   NEW
Anti-TAC  ccccAccccccAAcccAcccAccccccDccDDDDDDccAcAcccccccDcDDDDDDDDDDDDDDDDDDDDDDcAcccAcAAccccAccccAAccAcccccccDDDDDD......DDDccccAAcccc   EU(PDL)
          1234567890123456789012345678901234567890123456789012abc345678901234567890123456789012abc34567890123456789 0abcde1234567890123
```

c CONSERVED BETWEEN DONOR AND ACCEPTOR

D DONOR RESIDUE IN HUMANIZED FORM

A ACCEPTOR RESIDUE IN HUMANIZED FORM

SGI consensus means the acceptor sequence was the consensus sequence of the human antibodies in subgroup I of the Kabat et al 1987 compendium
SGIII consensus means the acceptor sequence was the consensus sequence of the human antibodies in subgroup III of the Kabat et al 1987 compendium
EU(PDL)) is a modified form of the EU sequence in which certain residues have been altered to make the sequence more homologous to the consensus sequence for the subgroup.

```
LIGHT CHAIN                                                                                                        1   1
                         1          2          3                4          5          6          7          8          9          0   0
           1234567890123456789012345678900abcdef12345678901234567890123456789012345678901234567890123456789012345678901234567   ACCEPTOR

B72.3      ccccccccAcccAcccAAcccccDDDDDDD......DDDDccccAAccAcAccAcDDDDDDDcccccccccccccAcAAAcAcccAccAAAcccDDDDDDDDDccAccAcAcA   REI
OKT3       AcAAccccAAAcccAcAAccAccDDDDDD.......DDDDccccAAcAAccDDccDDDDDDDcccAAcAccccccAcAAcccAAAAccAccccDDDDDDDDDccAccccAcA   REI
OKT4a      cccccccccccccAcAAcccAcDDDDDDD......DDDDcccDAcccAcAcccDDDDDDDDcAccccccccccAccAcAccAcAccccccccDDDDDDDDDccAccccAcA   REI
BIRR0001   cDDcccccAAcAAcAccAAAcAcDDDDDDDDDDDD.DDDDccAccAcAcccccDcDDDDDDDcccDccAccccccDcccAccAAAAAcADDcDcDDDDDDDDDccAcccAcAc   EU
A5B7       DDDDAcccAAccAcAcAAccAccDDDDDD.......DDDDcccccccAAccDAccDDDDDDDcccAccccccccccADAAcccAAAAccAccccDDDDDDDDDccAcccAcAc   LAY
CTM01      ccAcccAAAAAAAAAcAAAAcAcDDDDDDDDDDDD.DDDDcDAcAccAAcAccAcDDDDDDDcccAccDccccccAcccAAAAAAAAcAAAcccDDDDDDDDDccAcccAcAc   EU
P67.6      ccADccccAAcAAcAcAcAccAcDDDDDDDDDDD..DDDDcDccAccAAccccDcDDDDDDDcccAccDcccccccccAcAcAAAAAccAcAcAcDDDDDDDDDccAcccAcAc   EU
A33        ccAccccAAAAcAccccccAcccDDDDDDD......DDDDcccccccAAccDcccDDDDDDDcccAccAccccccccccAcccAAcAccDcAcDcDDDDDDDDDccAcccAccc   LAY
61E71      AcAcccAcAAcAAcAcccccccccDDDDDDD......DDDDccccAAcAAccAcccDDDDDDDcccAccAcccAcccAccccAAAcAccAcAcAcDDDDDDDDDccAccccAcA   REI
hTNF3      AcAcccAcAAcAAcAcccAccccDDDDDDD......DDDDccccAccAAcAccccDDDDDDDcccAccAcccAcccAccccAAAcAccAcAcAcDDDDDDDDDccAccAcAcA   REI
101/4      DcDDccccAAAcccAcAAccAccDDDDDDD......DDDDccccDccAAcAccccDDDDDDDcccAccccccccccAcAccccAAAAccAccccDDDDDDDDDccAcccADcD   REI
hTNF1      ccDcAccccAcAAcccAAccAAcDDDDDDDDDDDDDDDDDcccccccDAccccDDDDDDDDDcccAccAccccccAccccccccAAAAcAcAcccDDDDDDDDDccAcAcAcDA   REI
39D10      cccccccAcccccAcAAAAcAcDDDDDDD......DDDDccccccccAAcAccccDDDDDDDcccccccccccAcADAAAcccAccccAAAcAcDDDDDDDDDccAcccAcAc   LAY
L243       ccccccccAcccAcccAAcccccDDDDDDD......DDDDccAccAccAcDccADDDDDDDDccccccccccccDDAcAcAcccAcccAAcccDDDDDDDDDccAccAAccc   SGI consensus
HLT29      ccccccccccAccAcccccccccDDDDDDD......DDDDcDccAcccAcDcDccDDDDDDDcccccccccccDccAcccAccAAccAcAcccDDDDDDDDDccAcccAccc   SGI consensus

D1.3       ccccccccAcccccccAAcccccDDDDDDD......DDDDcccccAccAcAccAcDDDDDDDcccccccccccccAAAAAcAccccccAAAcccDDDDDDDDDccAcccAccc   REI(LMB)
CAMPATH-1  ccAccccccAcccccccccccAAcDDDDDDD......DDDDcccccAcAAccccccDDDDDDDcAcccccccccccccAccccccccccAcccAcDDDDDDDDDccAcccAcAc   REI(LMB)
anti-TAC   AcAAccccAAAcccAcAAcccccDDDDDD.......DDDDcAccccAAcccAccDDDDDDDcccccccccccccAAAccccAAAAAcAccccDDDDDDDDDccAcccAcAc   EU(PDL)
           1234567890123456789012345678900abcdef12345678901234567890123456789012345678901234567890123456789012345678901234567
```

c CONSERVED BETWEEN DONOR AND ACCEPTOR

D DONOR RESIDUE IN HUMANIZED FORM

A ACCEPTOR RESIDUE IN HUMANIZED FORM

SGI consensus means the acceptor sequence was the consensus sequence of the human antibodies in subgroup I of the Kabat et al 1987 compendium
REI(LMB) is a modified form of the REI sequence in which certain residues have been altered to make the sequence more homologous to the consensus sequence for the subgroup
EU(PDL)) is a modified form of the EU sequence in which certain residues have been altered to make the sequence more homologous to the consensus sequence for the subgroup.

TABLE HUMANIZED ANTIBODIES

ANTIBODY	SPECIFICITY	POTENCY[1] RELATIVE TO RODENT ANTIBODY %
ANTI-TUMOUR		
B72.3	Tumour associated glycoprotein 72 (TAG72)	30
P67.6	CD33	85
CTM01	Polymorphic epithelial mucin (PEM)	>100
A5B7	Carcinoembryonic antigen (CEA)	50
A33	Epithelial antigen	75
ANTI-CYTOKINE		
HTNF1	Tumour necrosis factor (TNF) α	100[2]
101/4	TNFα	30[2]
61E71	TNFα	100; < 1[2]
HTNF3	TNFα	100; < 1[2]
HLT29	Lymphotoxin (LT)	> 70[2]
39D10	Interleukin-5	> 80
ANTI-LYMPHOCYTE		
OKT3	CD3	100
OKT4	CD4	68
L243	MHC CLASS II	60
ANTI-ADHESION MOLECULE		
BIRR-001	ICAM-1	25

1. POTENCY IS MEASURED AS RELATIVE ABILITY TO COMPETE WITH THE RODENT ANTIBODY FOR BINDING TO ANTIGEN

2. CYTOKINE NEUTRALIZATION ASSAY. ANTIBODY BINDS TO CYTOKINE AND THE COMPLEX IS TESTED FOR ABILITY TO AFFECT THE GROWTH OF L929 CELLS.

TOTAL P.02

35

CARPMAELS & RANSFORD

CHARTERED PATENT ATTORNEYS EUROPEAN PATENT ATTORNEYS TRADE MARK AGENTS

35

TELEPHONE 071 242 8692
TELEX 267209
FACSIMILE 071 405 4186
071 831 8501

43 BLOOMSBURY SQUARE
LONDON · WC1A 2RA
AND AT MUNICH

IAN B. P. de M. DEVAUX
S. DAVID VOTIER — N. KEITH HOWICK
JOHN W. M. CARPMAEL — ADRIAN J. FISHER
ALAN J. JONES — CHRIS P. MERCER
STEPHEN J. COLGAN — HUW G. HALLYBONE

B. V. INGRAM — ANNE WONG
M. J. DONNAH — R. E. JACKSON
P. M. JOHNSTON — P. N. HOWARD
R. W. BISHOP — C. S. TUNSTALL
R. N. FIELD — M. HOLMES

J. A. MURPHY (MANAGER)

CONSULTANTS
DEREK G. R. GRUNDY — JOHN A. COOPER

European Patent Office,
Munich.

→ 21747

9th September 1993

YOUR REF

OUR REF P07856EP/CPM/EK

Dear Sirs,

Re: European Patent Application No. 91901433.2 2116
Celltech Limited

1. Accelerated Prosecution

1.1. It is hereby requested that the above-referenced application be subjected to accelerated prosecution. The Examination Fee was paid on 23rd May 1991. It is requested that the first communication on Substantive Examination be issued as soon as possible.

1.2. The present application relates to "humanised" antibody molecules and to methods for producing them. In order to "humanise" an antibody, a donor antibody, generally a mouse monoclonal antibody, having a desired specificity is produced. The sequence of the variable domain in each of the heavy and light chains of the donor antibody is then determined. An acceptor antibody, usually a human antibody, is taken and the sequences of its variable domains are changed so that the complementarity determining regions (CDRs) and a number of the framework residues in the original acceptor antibody correspond to the equivalent residues in the donor antibody. In this way, the antibody thus produced, which is a hybrid of donor and acceptor, has donor antigen-binding properties but mainly acceptor non-antigen-binding functions.

1.3. The technique of humanising antibodies is growing in importance in the medical field. It is anticipated that the market for humanized antibodies for use in medical treatments will grow considerably over the next few years.

1.4. The Applicant believes that the present application covers a pioneering invention in this field as it allows any combination of donor and acceptor antibody to be used to produce the hybrid antibody. Moreover, it allows the production of a hybrid antibody having about the same (or in some cases better) affinity for the target antigen as the donor antibody. It is believed that these possibilities have not previously been available.

15. 09. 93

1.5. The Applicant is aware that there are a number of companies which are offering antibody humanization services. It is likely that the antibodies they produce will infringe valid claims to be granted on the present application. As evidence of the existence of such companies, I enclose copies of three extracts from SCRIP (of: 9/8/91, page 14; 19/2/92, page 15; and 12/2/93, page 11). These relate to one company which, the Applicant believes, is intending to commercialize at least one of its humanized antibodies in the near future.

1.6. The Applicant would like to be in a position to take action against the company mentioned in SCRIP, and any other similar company, as soon as possible in order to prevent its legitimate rights from being infringed. For the above reasons, it is submitted that the request for Accelerated Prosecution is justified.

2. Amendments

2.1. In order to assist in accelerating the prosecution of this application, it is requested that the present claims be deleted and that new claims 1 to 20 (on new pages 67 to 74) (enclosed in triplicate) be used as the basis for substantive examination.

2.2. At present, no amendments to the description are offered. However, it is appreciated that it will be necessary to amend the description. It is suggested that it would be more efficient to agree on an allowable set of claims. Thereafter, the description can be amended to bring it into conformity with the agreed claims.

2.3. On review of the Application, it was felt that the language of the claims might be imprecise, because it was not clear whether the specified residues were the only or the minimum number of residues to be donor residues. The Applicants have therefore amended the claims to make it clear that there is a minimum number of residues which have to be donor residues and a minimum number which have to be acceptor residues. Those residues which are not specified in the claims may be either donor or acceptor.

2.4. In order to limit the issues raised in this case, the main independent claims have been limited to antibodies comprising both a heavy chain and a complementary light chain. Claims to the separate chains have been cancelled. Also, claims relating to changes in the light chain have been made dependent on the main independent claims.

2.5. Further, claims to DNA sequences, vectors, transformed host cells and methods of culturing such cells have been cancelled. Claim 23 has also been cancelled, in view of Article 52(4) EPC.

2.6. Since claims have been cancelled in order to limit the issues in the present application, the Applicant reserves the right to file divisional applications relating to the deleted subject matter.

2.7. Regarding new claim 1, it can be seen from the description (see page 6, line 29 to page 7, line 28; page 17, lines 9 to 11,

Sections 2.1 to 2.3 bridging pages 17 and 18; Section 1 bridging pages 19 and 20; Sections 2.1.1, 2.2.1, 2.3.1 and 2.4.1 in the passage bridging pages 20 to 23; and Section 15.2.1 on pages 46 and 47) that a number of residues are mentioned which can be considered for changing from acceptor to donor residues. It follows that if a residue has not been considered for changing, it must remain as in the acceptor chain. In order to make this clear, it has been specified in new claim 1 that all the unmentioned residues must be acceptor residues.

2.8. As regards the recitation of the donor residues in new claim 1, this comprises a combination of the minimum residues need to define the CDR loops or the Kabat CDRs. For CDR1, this comprises residues 31 to 35 (see page 46, line 18) and for CDR2 this comprises residues 50 to 58 (see page 46, line 20). For CDR3, the Kabat definition of residues 95 to 102 was used (see page 17, line 11).

2.9. In addition, new claim 1 recites as donor residues those identified on page 20, line 25 and page 21, line 9 as being key residues outside the CDRs.

2.10. In new claim 1, it has been specified that residues 71, 73 and 78 are all donor residues in order to ensure that new claim 1 is novel over the anti-TAC antibody disclosed in PNAS-USA, 86, 10029-10033, 1989 (Queen) (cited in the International Search Report). This anti-TAC antibody has an acceptor residue at residue 73. However, as can be seen from page 7, lines 1 to 5, the Applicant considers that in general, residues 71, 73 and 78 can be either all donor or all acceptor.

2.11. Claim 2 is based on page 15, line 27 and pages 55 to 59 of the description. Claim 3 is based on page 15, line 33 of the description. Claim 4 is based on page 15, line 31 and pages 25 to 52 of the description. Claim 5 is based on page 15, line 31 and pages 53 and 54 of the description. Claim 6 is based on page 15, line 32 and pages 60 and 61 of the description.

2.12. It is stated on page 7, lines 1 to 5 that residues 71, 73 and 78 should all be either acceptor or donor. Claims 2 to 6 cover the first alternative and claim 7 covers the second alternative.

2.13. Claim 8 increases the sizes of CDR1 and CDR2 to the prudent definition given on page 17, line 9 and page 20, lines 6 to 9.

2.14. Claim 9 is based on the passage at page 20, lines 26 and 27. Claim 10 is based on the passage at page 21, lines 10 to 12. Claim 11 is based on the passage on page 21, lines 13 to 16.

2.15. Claim 12 is derived by taking all the donor residues mentioned in claims 1 and 7 to 11 and specifying that all apart from these residues are acceptor residues.

2.16. Claim 13 is derived in the same way that present claim 1 was derived. The residues which can be donor residues are listed on page 9, line 7 to page 10, line 15; page 17, lines 12 to 14; Section 3 bridging pages 18 and 19; Section 2.1.2 on page 20; Section 2.2.2 on

page 21; Section 2.3.2 on page 22 and Section 2.4.2 on page 23.

2.17. Claims 14 to 17 are based on the passage from page 9, line 7 to page 10, line 11.

2.18. Claim 18 is equivalent to original claim 22.

2.19. Claims 19 and 20 put into claim format the Protocol set forth on pages 16 to 23 of the description in a way which is consistent with new claims 1 to 17.

2.20. It is therefore believed that all the claims are fully supported by the description.

3. The Prior art

3.1. In the International Search Report, the first cited document is EP-A-0 403 156 (Genzyme). The present application claims a priority date of 21st December 1989. The Genzyme application was published on 19th December 1990 and therefore cannot be used as a citation under Article 54(2) EPC. It can be used as a citation under Article 54(3) EPC, but only as long as the relevant disclosures in the citation are entitled to a priority date earlier than that of the present application.

3.2. The Genzyme application claims priority from US Patent Applications Nos. 362 549 and 529 979. The '549 application was filed before the priority date of the present application but the '979 application was not. Therefore, only subject matter which is supported by the '549 application can be cited against the present application. We enclose a copy of the '549 application.

3.3. Reference is made to Examples 8 to 12 of the Genzyme application. Examples 8 to 11, at least as far as page 7, line 55 of the Genzyme application, are equivalent to Examples 8 to 11 in the '549 application. However, these Examples relate solely to the production of chimeric antibodies, that is an antibody in which the whole variable domain is derived from a donor antibody. There is no disclosure of any antibody in which the variable domains comprise a mixture of donor and acceptor residues. Thus, these Examples do not deprive the present claims of novelty.

3.4. Example 12 and Tables 6A, 6B, 7A and 7B of the Genzyme application do not appear in the '549 application and thus cannot be cited against the present claims.

3.5. It is therefore believed that the present claims are novel over the parts of the Genzyme application which are entitled to the date of the '549 application.

3.6. Regarding the Queen article referred to above, this discloses a CDR-grafted antibody which has an acceptor residue at position 73 in the heavy chain whereas new claim 1 requires a donor residue at this position. Thus, it is submitted that new claim 1 is novel over Queen.

3.7. The antibody disclosed in the Queen article is directed against the p55 chain of the human interleukin 2 receptor. It does not have specificity for a tumour-specific antigen, an interleukin, the CD3 or CD4 antigen, or an adhesion molecule. It is therefore not specific for any of the types of antigen specified in claims 2 to 6. It is therefore submitted that all these claims are novel over Queen.

3.8. Since claims 7 to 18 are dependent on claims 1 to 6, it is submitted that all these claims are novel over Queen.

3.9. The Queen article discloses a method for "humanising" antibodies which involves molecular modelling and selection of residues to be changed to donor residues on the basis of comparisons of molecular models. This is an entirely different process from the one set forth in claims 19 and 20. Thus, these claims are novel over Queen.

3.10. In fact, there is nothing in the Queen paper which even suggests that it might be possible to determine a hierarchy of residues to be changed to donor which will work for any antibody with any framework. The disclosure in Queen suggests that each donor antibody needs to be treated as an individual case in comparison with the acceptor antibody. There is no suggestion that results obtained with one pair of donor and acceptor antibodies could be applied successfully to a different pair of antibodies. In contrast, the present invention shows that it is possible to apply general rules to any pair of antibodies in the expectation of success.

3.11. It is therefore submitted that the present claims are both novel and inventive over Queen.

3.12. EP-A-0 239 400 (Winter) is also cited in the International Search Report. The basic disclosure in the Winter application is limited to grafting only the CDRs from one antibody to another. All the Examples in the Winter application relate to this. There is a passage in the Winter application which indicates that it may be necessary to alter some framework residues. However, there is no guidance at all as to which residues should be changed or as to how such residues should be selected. There is certainly no suggestion that there is a hierarchy of residues which should be considered. Thus, it is submitted that the present claims are both novel and inventive over the Winter application.

3.13. It is believed that none of the other documents cited in the International Search Report is more relevant to the present claims than the three documents referred to above. Therefore, no discussion of any of these documents is provided. However, the Applicant would be pleased to address any concern the Examiner may have in this respect.

4. Procedure

4.1. It is suggested that, once the Examiner has had an opportunity to study the file and familiarize himself with the case, it would be useful to hold an interview to address any concerns that the Examiner may have. If, after consideration of the file, the Examiner agrees

M 13.09.93 40

with this suggestion, it is requested that the Examiner sends out a Communication identifying any areas of concern and any documents which need to be discussed so that the Applicant can address those points fully.

4.2. If the Examiner feels that prosecution could be expedited in any other way, he is requested to telephone the undersigned to discuss the matter.

4.3. As a precautionary measure, the Applicant hereby requests Oral Proceedings, in the event that the Examining Division should feel minded to refuse the application.

Your faithfully,

MERCER, CHRISTOPHER PAUL

priority doc' '549 in rechter Tasche

17.09.93 EH

CLAIMS

1. An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain comprising acceptor antibody heavy chain framework residues and donor antibody heavy chain antigen-binding residues, said donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain, amino acid residues 5, 8, 10, 12 to 17, 19, 21, 22, 40, 42 to 44, 66, 68, 70, 74, 77, 79, 81, 83 to 85, 90, 92, 105, 109, 111 and 113 at least are acceptor residues and amino acid residues 23, 24, 31 to 35, 49 to 58, 71, 73, 78 and 95 to 102 at least are donor residues.

2. A tumour-specific antibody molecule having affinity for a predetermined tumour antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain comprising acceptor antibody heavy chain framework residues and donor antibody heavy chain antigen-binding residues, said donor antibody having affinity for said predetermined tumour antigen, wherein, according to the Kabat numbering system, in said composite heavy chain, amino acid residues 5, 8, 10, 12 to 17, 19, 21, 22, 40, 42 to 44, 66, 68, 70, 74, 77, 79, 81, 83 to 85, 90, 92, 105, 109, 111 and 113 at least are acceptor residues and amino acid residues 23, 24, 31 to 35, 49 to 58 and 95 to 102 at least are donor residues.

3. An interleukin-specific antibody molecule having affinity for a predetermined interleukin and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain comprising acceptor antibody heavy chain framework residues and donor antibody heavy chain antigen-binding residues, said donor antibody having affinity for said predetermined interleukin wherein, according to the Kabat numbering system, in said