



[54] **HUMANISED ANTIBODIES**

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**Related U.S. Application Data**

[63] Continuation of Ser. No. 743,329, Sep. 17, 1991, abandoned.

[30] **Foreign Application Priority Data**

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[58] **Field of Search** ..... **530/387.1, 387.3, 530/388.22, 867, 864**

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[57] **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.

**8 Claims, 18 Drawing Sheets**

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1 GAATTCCCAA AGACAAAatg gattttcaag tgcagathtt cagcttcctg
51 ctaatcagtg cctcagtcac aatatccaga ggacaaattg ttctcaccca
101 gtctccagca atcatgtctg catctccagg ggagaaggte accatgacct
151 gcagtgccag ctcaagtgtg agttacatga actggtacca gcagaagtca
201 ggcacctccc ccaaaagatg gatttatgac acatccaaac tggcttctgg
251 agtccctgct cacttcaggg gcagtgggte tgggacctct tactctctca
301 caatcagcgg catggaggct gaagatgctg ccacttatta ctgccagcag
351 tggagtagta accattcac gttcggctcg gggacaaagt tggaaaataa
401 ccgggctgat actgcaccaa ctgtatccat cttcccacca tccagtgagc
451 agttaacatc tggagggtgcc 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 aggaaatgagt gtTAGAGACA AAGGTCCTGA GACGCCACCA
751 CCAGCTCCCA GCTCCATCCT ATCTTCCCTT CTAAGGTCTT GGAGGCTTCC
801 CCACAAGCGC tTACCACTGT TGCGGTGCTC TAAACCTCCT CCCACCTCCT
851 TCTCCTCCTC CTCCTTTTCC TTGGCTTTTA TCATGCTAAT ATTTGCAGAA
901 AATATTCAAT AAAGTGAGTC TTTGCCTTGA AAAAAAAAAA AAA
(SEQ ID NO:4)

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*FIG. 1a*

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1 MDFQVQIFSF LLISASVIIS RGDQIVLTQSP AIMSASPGEK VTMTCSASSS
51 VSYMNWYQQK SGTSPKRWIY DTSKLAGSVP AHFRGSGSGT SYSLTISGME
101 AEDAATYYCQ QWSSNPFTFG SGTKLEINRA DTAPTVSIFP PSSEQLTSGG
151 ASVVCFLNNF YPKDINVKWK IDGSERQNGV LNSWTDQDSK DSTYSMSSTL
201 TLTKDEYERH NSYTCEATHK TSTSPIVKSF NRNEC* (SEQ ID NO:5)

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*FIG. 1b*

1 GAATTCCCCT CTCCACAGAC ACTGAAACT 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 ATTCCTAGCC 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 CAAGGGTAT  
551 TTCCCTGAGC CAGTGACCTT GACCTGGAAC TCTGGATCCC TGTCCAGTGG  
601 TGTGCACACC TTCCCAGCTG TCCTGCAGTC TGACCTCTAC ACCCTCAGCA  
651 GCTCAGTGAC TGTAACCTCG AGCACCTGGC CCAGCCAGTC CATCACCTGC  
701 AATGTGGCCC ACCCGGCAAG CAGCACCAAG GTGGACAAGA AAATTGAGCC  
801 ACCTCTTGGG TGGACCATCC GTCTTCATCT TCCCTCCAAA GATCAAGGAT  
851 GTACTIONATGA 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 GTCTTGCCCTC CACCAGAAGA AGAGATGACT AAGAAACAGG TCACTCTGAC  
1201 CTGCATGGTC ACAGACTTCA TGCTGAAGA CATTACGTG GAGTGGACCA  
1251 ACAACGGGAA AACAGAGCTA AACTACAAGA AACTGAACC AGTCCTGGAC  
1301 TCTGATGGTT CTTACTTCAT GTACAGCAAG CTGAGAGTGG AAAAGAAGAA  
1351 CTGGGTGGAA AGAAATAGCT ACTCCTGTTC AGTGGTCCAC GAGGGTCTGC  
1401 ACAATCACCA CACGACTAAG AGCTTCTCCC GGACTCCGGG TAAATGAGCT  
1451 CAGCACCCAC AAAACTCTCA GGTCCAAAGA GAGACCCACA CTCATCTCCA  
1501 TGCTTCCCTT GTATAAATAA AGCACCCAGC AATGCCTGGG ACCATGTAAA  
1551 AAAAAAAAAA AAAGGAATTC (SEQ ID NO:6)

*FIG. 2a*

## 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 VTLGCLVKG Y 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 NYKNTEPVL D SDGSYFMYSK LRVEKKNWVE RNSYSCSVVH  
451 EGLHNHHTTK SFSRTPGK\* (SEQ ID NO: 7)

*FIG. 2b*

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1                23                42
NN      N                N      N      N
RES TYPE SBspSPESsSBSbSsSsPSPSPsPSsse*s*p*Pi^ISsSe
Dkt3vl  QIVLTQSPAIMSASPGEKVTMTCSASS.SVSYMNWYQQKSGT
REI     DIQMTQSPSSLSASVGDRVITCQASQDIIKYLNWYQQIPGK
? ?
      CDR1 (LOOP)          *****
      CDR1 (KABAT)        *****

                    56                85
N  NN
RES TYPE *IsiPpIeesesssSBEsePpPSBSSEsPspPssseesSPePb
Dkt3vl  SPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAAT
REI     APKLLIYEASNLQAGVPSRFSGSGSGTDYTETISSLQPEDIAT (SEQ
ID NO:8)
? ??                ? ?
      ***** CDR2 (LOOP/KABAT)

                    102  108
RES TYPE PiPIPIes**iPIIsPPSPSPSS
Dkt3vl  YYCQWSSNPFTFGSGTKLEINR (SEQ ID NO:29)
REIvl   YYCQYQSLPYTFGQGTKLQITR (SEQ ID NO:9)
? ?
      ***** CDR3 (LOOP)
      ***** CRD3(KABAT)

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FIG. 3

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