US005859205A

United States Patent [19]

Adair et al.

[11] **Patent Number: 5,859,205**

[45] **Date of Patent: Jan. 12, 1999**

[54] **HUMANISED ANTIBODIES**

[75] Inventors: **John Robert Adair**, High Wycombe; **Diljeet Singh Athwal**, London; **John Spencer Emtage**, Marlow, all of United Kingdom

[73] Assignee: **Celltech Limited**, Berkshire, United Kingdom

[21] Appl. No.: **303,569**

[22] Filed: **Sep. 7, 1994**

Related U.S. Application Data

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

[30] **Foreign Application Priority Data**

Dec. 21, 1989 [GB] United Kingdom 8928874

[51] **Int. Cl.**[6] **A61K 39/395**

[52] **U.S. Cl.** **530/387.3**; 530/387.1

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

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,348,376	9/1982	Goldenberg .

FOREIGN PATENT DOCUMENTS

0239400 A2	3/1987	European Pat. Off. .
A1 0323806	7/1989	European Pat. Off. .
0 328 404 A1	8/1989	European Pat. Off. .
0 365 209 A2	4/1990	European Pat. Off. .
0 403 156 A1	12/1990	European Pat. Off. .
WO 89/07452	8/1989	WIPO .
WO 90/07861	7/1990	WIPO .
WO 92/04381	3/1992	WIPO .
WO 92/11018	7/1992	WIPO .
WO 92/15683	9/1992	WIPO .
WO 92/16553	10/1992	WIPO .

OTHER PUBLICATIONS

Chothia, Cyrus et al (Dec. 1989) *Nature*, "Conformations of Immunoglobulin Hypervariable Regions", vol. 342, pp. 877–883.

Queen, C. et al (Dec. 1989) Proceedings of the National Academy of Sciences, "A Humanized Antibody That Binds to Interleukin 2 Receptor" vol. 86, pp. 10029–10033.

Riechmann et al (Mar. 1988) Nature, "Reshaping Human Antibodies for Therapy," vol. 332, pp. 323–327.

Roberts et al, "Generation of Antibody with Enhanced Affinity and Specificity for its Antigen by Protein Engineering" Nature, 328(20):731–734, Aug., 1987.

Verhoeyen et al, "Reshaping Human Antibodies: Grafting an Antilysozyme Activity", Science, 239:1534–36 Mar. 25, 1988.

Jones et al., "Replacing the complementarity–Determining Regions in a Human Antibody with those from a Mouse", Nature, 321:522–525, 1986.

Ward et al., "Binding activities of a Repertoire of Single Immunoglobulin Variable Domains Secreted from *Escherichia Coli*", Nature, 341:544–546, 1989.

Primary Examiner—Donald E. Adams

Attorney, Agent, or Firm—Woodcock Washburn Kurtz Mackiewicz & Norris

[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

```
  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  accattcac 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
(SEQ ID NO:4)
```

FIG. 1a

```
  1  MDFQVQIFSF 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* (SEQ ID NO:5)
```

FIG. 1b

```
   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  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 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
 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 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 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* (SEQ ID NO:7)
```

FIG. 2b

```
            1                       23                      42
            NN      N                 N         N     N
RES TYPE    SBspSPESssBSbSsSssPSPSPsPSsse*s*p*Pi^ISsSe
Okt3vl      QIVLTQSPAIMSASPGEKVTMTCSASS.SVSYMNWYQQKSGT
REI         DIQMTQSPSSLSASVGDRVTITCQASQDIIKYLNWYQQIPGK
            ? ?
              CDR1   (LOOP)           *******
              CDR1   (KABAT)        **********


                        56                                  85
            N  NN
RES TYPE    *IsiPpIeesesssSBEsePsPSBSSEsPspsPsseesSPePb
Okt3vl      SPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAAT
REI         APKLLIYEASNLQAGVPSRFSGSGSGTDYTFTISSLQPEDIAT (SEQ
ID NO:8)
            ?  ??                           ?  ?
                  *******  CDR2 (LOOP/KABAT)

                        102   108
RES TYPE    PiPIPies**iPIIsPPSPSPSS
Okt3vl      YYCQQWSSNPFTFGSGTKLEINR (SEQ ID NO:29)
REIvl       YYCQQYQSLPYTFGQGTKLQITR (SEQ ID NO:9)
                        ?    ?
                 ******       CDR3 (LOOP)
               *********      CRD3(KABAT)
```

FIG. 3

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.