



US006884879B1

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

(10) **Patent No.:** **US 6,884,879 B1**
(45) **Date of Patent:** **Apr. 26, 2005**

(54) **ANTI-VEGF ANTIBODIES**

(75) Inventors: **Manuel Baca**, Foster City, CA (US);
James A. Wells, Burlingame, CA (US)

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

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

(21) Appl. No.: **08/908,469**

(22) Filed: **Aug. 6, 1997**

Related U.S. Application Data

(63) Continuation-in-part of application No. 08/833,504, filed on
Apr. 7, 1997, now abandoned.

(51) **Int. Cl.**⁷ **C07H 21/04**

(52) **U.S. Cl.** **536/23.53**; 435/320.1;
530/387.3; 530/388.85

(58) **Field of Search** 435/327, 252,
435/1, 320.1, 252.3, 69.1; 536/23.1, 23.53;
530/382.1, 387.3, 388, 388.85

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,816,567	A	3/1989	Cabilly et al.	530/387
5,530,101	A	6/1996	Queen et al.	530/387.3
5,580,723	A	12/1996	Wells et al.	
6,037,454	A	3/2000	Jardieu et al.	
2002/0032315	A1	3/2002	Baca et al.	

FOREIGN PATENT DOCUMENTS

EP	0 451 216	1/1996
GB	2 188 638	10/1987
GB	2 268 744	1/1994
WO	91/09967	7/1991
WO	92/22653	12/1992
WO	94/04679	3/1994
WO	94/10202	* 5/1994
WO	96/30046	10/1996
WO	98/45331	10/1998
WO	98/45332	10/1998

OTHER PUBLICATIONS

Yelton et al., *J of Immunology* 155:1994–2004, 1995.*
 Kim et al., “The Vascular Endothelial Growth Factor Proteins: Identification of Biologically Relevant Regions by Neutralizing Monoclonal Antibodies,” *Growth Factors* 7:53–64 (1992).
 Kim et al., “Inhibition of Vascular Endothelial Growth Factor–Induced Angiogenesis Suppresses Tumor Growth in vivo,” *Nature* 362:841–844 (1993).
 Alberts et al., *Molecular Biology of the Cell*, 3rd Ed., Garland Publishing, p. 1154 (1994).
 Chothia et al., “Domain Association in Immunoglobulin Molecules The Packing of Variable Domains” *J. Mol. Biol.*, 186:651–663 (1985).

Foote et al., “Antibody Framework Residues Affecting the Conformation of the Hypervariable Loops” *J. Mol. Biol.*, 224:487–499 (1992).

Queen et al., “A Humanized Antibody that Binds to the Interleukin 2 Receptor,” *PNAS USA*, 86:10029–10033 (1989).

Kettleborough et al., “Humanization of a Mouse Monoclonal Antibody by the CDR–Grafting: The Importance of Framework Residues on Loop Conformation” *Protein Eng.*, 4(7):773–783 (1991).

Tempest et al., “Reshaping a Human Monoclonal Antibody to Inhibit Human Respiratory Syncytial Virus Infection in vivo” *Biotechnology*, 9:266–271 (1991).

Padlan, “A Possible Procedure for Reducing the Immunogenicity of Antibody Variable Domains While Preserving their Ligand–Binding Properties,” *Mol. Immunol.*, 28(4/5):489–198 (1991).

Roguska et al., “Humanization of Murine Monoclonal Antibodies Through Variable Domain Resurfacing” *PNAS USA*, 91:969–973 (1994).

Studnicka et al., “Human–Engineered Monoclonal Antibodies Retain Full Specific Binding Activity by Preserving non–CDR Complementarity–Modulating Residues” *Protein Eng.*, 7:805 (1994).

Allen et al., “Specificity of the T Cell Receptor: Two Different Determinants are Generated by the Same Peptide and the I–a^k Molecule ^{1,2}” *J. Immunol.*, 135:368–373 (1985).

Carter et al., “Humanization of an anti–p185^{HER2} Antibody for Human Cancer Therapy” *PNAS USA*, 89:4285–4289 (1992).

Presta et al., “Humanization of an Antibody Directed Against IgE,” *J. Immunol.*, 151:2623–2632 (1993).

Eigenbrot et al., X–Ray Structure of Fragments from Binding and Nonbinding Versions of a Humanized Anti–CD18 Antibody: Structural Indications of the Key Role of V_H Residues 59–65” *Proteins*, 18:49–62 (1994).

Shalaby et al., “Development of Humanized Bispecific Antibodies Reactive with Cytotoxic Lymphocytes and Tumor Cells Overexpressing the HER2 Protooncogene” *J. Exp. Med.*, 175:217–225 (1992).

Kabat et al., *Sequences of Proteins of Immunological Interest*, U.S. Dept. Of Health and Human Services, NIH, 5th edition, vol. 1:103–108, 324–331 (1991).

Rosok et al., “A Combinatorial Library Strategy for the Rapid Humanization of Anticarcinoma BR96 Fab” *J. Biol. Chem.*, 271:22611–22618 (1996).

(Continued)

Primary Examiner—Larry R. Helms
(74) *Attorney, Agent, or Firm*—Genentech, Inc.

(57) **ABSTRACT**

Humanized and variant anti-VEGF antibodies and various uses therefor are disclosed. The anti-VEGF antibodies have strong binding affinities for VEGF; inhibit VEGF-induced proliferation of endothelial cells in vitro; and inhibit tumor growth in vivo.

14 Claims, 16 Drawing Sheets

OTHER PUBLICATIONS

- Novotný and Haber, "Structural Invariants of Antigen Binding; Comparison of Immunoglobulin V_L - V_H and V_L - V_L Domain Dimers" *Proc. Natl. Acad. Sci. USA*, 82:4592-4596 (1985).
- Bending, M.M. "Humanization of Rodent Monoclonal Antibodies," *Methods: A Companion to Methods In Enzymology* 8:83-93 (1994).
- Baca et al., "Antibody Humanization Using Monovalent Phage Display," *Journal of Biological Chemistry* 272(16):10678-10684 (1997).
- Garrard et al., "Assembly and Enrichment in a Monovalent Phage Display System," *Biotechnology*, 9:1373-1377 (1991).
- Chang et al., "High-level secretion of human growth hormone by *Escherichia coli*," *Gene*, 55:189-196 (1987).
- Kunkel et al., "Efficient Site-Directed Mutagenesis Using Uracil-Containing DNA," *Methods Enzymol.*, 204:125-139 (1991).
- Winter et al., "Making Antibodies by Phage Display Technology," *Ann. Rev. Immunol.*, 12:433-455 (1994).
- Vieira et al., "Production of Single-Stranded Plasmid DNA," *Methods Enzymol.*, 153:3-11 (1987).
- Karlsson et al., "Kinetic analysis of monoclonal antibody-antigen interactions with a new biosensor based analytical system," *J. Immun. Methods*, 145:229-240 (1991).
- Cunningham et al., "Production of an atrial natriuretic peptide variant that is specific for type a receptor," *EMBO J.* 13(11):2508-2515 (1994).
- Lowman et al., "Selecting High-Affinity Binding Proteins by Monovalent Phage Display," *Biochemistry*, 30:10832-10838 (1991).
- Hawkins et al., "Selection of Phage Antibodies by Binding Affinity Mimicking Affinity Maturation," *J. Mol. Biol.* 226:889-896 (1992).
- Folkman et al., "Angiogenesis," *J. Biol. Chem.*, 267(16):10931-10934 (1992).
- Klagsbrun et al., "Regulators of Angiogenesis," *Annu. Rev. Physiol.*, 53:217-239 (1991).
- Garner, A., "Vascular Diseases", *Pathobiology of Ocular Disease. A Dynamic Approach*. 2nd Ed. (Garner and Klintworth, eds.) Marcel Dekker:New York, pp. 1625-1710 (1994).
- Weidner et al., "Tumor Angiogenesis and Metastasis—Correlation in Invasive Breast Carcinoma," *New Engl. J. Med.*, 324(1):1-8 (1991).
- Horak et al., "Angiogenesis, assessed by platelet/endothelial cell adhesion molecule antibodies, as indicator of node metastases and survival in breast cancer," *Lancet*, 340:1120-1124 (1992).
- Macchiarini et al., "Relation of neovascularisation to metastasis of non-small-cell lung cancer," *Lancet*, 340:145-146 (1992).
- Good et al., "A tumor suppressor-dependent inhibitor of angiogenesis is immunologically and functionally indistinguishable from a fragment of thrombospondin," *Proc. Natl. Acad. Sci. USA*, 87:6624-6628 (1990).
- Clapp et al., "The 16-Kilodalton N-Terminal Fragment of Human Prolactin is a Potent Inhibitor of Angiogenesis," *Endocrinology*, 133(3):1292-1299 (1993).
- O'Reilly et al., "Angiostatin: A Novel Angiogenesis Inhibitor that Mediates the Suppression of Metastases by a Lewis Lung Carcinoma," *Cell*, 79:315-328 (1994).
- O'Reilly et al., "Endostatin: An Endogenous Inhibitor of Angiogenesis and Tumor Growth," *Cell*, 88:277-285 (1997).
- Ferrara et al., "The Biology of Vascular Endothelial Growth Factor," *Endocr. Rev.*, 18(1):4-25 (1997).
- Berkman et al., "Expression of the Vascular Permeability Factor/Vascular Endothelial Growth Factor Gene in Central Nervous System Neoplasms," *J. Clin. Invest.*, 91:153-159 (1993).
- Brown et al., "Expression of Vascular Permeability Factor (Vascular Endothelial Growth Factor) and Its Receptors in Breast Cancer," *Human Pathol.*, 26(1):86-91 (1995).
- Brown et al., "Expression of Vascular Permeability Factor (Vascular Endothelial Growth Factor) and its Receptors in Adenocarcinomas of the Gastrointestinal Tract," *Cancer Res.*, 53:4727-4735 (1993).
- Mattern et al., "Association of vascular endothelial growth factor expression with intratumoral microvessel density and tumour cell proliferation in human epidermoid lung carcinoma," *Brit. J. Cancer*, 73:931-934 (1996).
- Dvorak et al., "Vascular Permeability Factor/Vascular Endothelial Growth Factor, Microvascular Hyperpermeability, and Angiogenesis," *Am. J. Pathol.*, 146(5):1029-1039 (1995).
- Aiello et al., "Vascular Endothelial Growth Factor in Ocular Fluid of Patients with Diabetic Retinal Disorders," *New Engl. J. Med.*, 331:1480-1487 (1994).
- Lopez et al., "Transdifferentiated Retinal Pigment Epithelial Cells Are Immunoreactive for Vascular Endothelial Growth Factor in Surgically Excised Age-Related Macular Degeneration-Related Choroidal Neovascular Membranes," *Invest. Ophthalmol. Vis. Sci.*, 37(5):855-868 (1996).
- Warren et al., "Regulation by Vascular Endothelial Growth Factor of Human Color Cancer Tumorigenesis in a Mouse Model of Experimental Liver Metastasis," *J. Clin. Invest.*, 95:1789-1797 (1995).
- Borgström et al., "Complete Inhibition of Angiogenesis and Growth of Microtumors by Anti-Vascular Endothelial Growth Factor Neutralizing Antibody: Novel Concepts of Angiostatic Therapy from Intravital Videomicroscopy," *Cancer Res.*, 56:4032-4039 (1996).
- Melnyk et al., "Vascular Endothelial Growth Factor Promotes Tumor Dissemination by a Mechanism Distinct from Its Effect on Primary Tumor Growth," *Cancer Res.*, 56:921-924 (1996).
- Adams et al. "Prohibition of Vascular Endothelial Growth Factor Prevents Retinal Ischemia-Associated Iris Neovascularization in a Nonhuman Primate," *Arch. Ophthalmol.*, 114:66-71 (1996).
- De Vries et al., The fms-Like Tyrosine Kinase, a Receptor for Vascular Endothelial Growth Factor, *Science*, 255:989-991 (1992).
- Leung et al., "Vascular Endothelial Growth Factor Is a Secreted Angiogenic Mitogen," *Science*, 246:1306-1309 (1989).
- Sanger et al., "DNA sequencing with chain-terminating inhibitors," *Proc. Natl. Acad. Sci. USA*, 74(12):5463-5467 (1977).
- Graham et al., "Characteristics of a Human Cell Line Transformed by DNA from Human Adenovirus Type 5," *J. Gen. Virol.*, 36:59-72 (1977).

- Werther et al., "Humanization of an Anti-Lymphocyte Function-Associated Antigen (LFA)-1 Monoclonal Antibody and Reengineering of the Humanized Antibody for Binding to Rhesus LFA-1," *J. Immunol.*, 157:4986-4995 (1996).
- Eigenbrot et al., "X-ray Structures of the Antigen-binding Domains from Three Variants of Humanized anti-p185^{HER2}," *J. Mol. Biol.*, 229:969-995 (1993).
- Kunkel et al., "Rapid and efficient site-specific mutagenesis without phenotypic selection," *Proc. Natl. Acad. Sci. USA*, 82:488-492 (1985).
- Eaton et al., "Construction and Characterization of an Active Factor VIII Variant Lacking the Central One-Third of the Molecule," *Biochemistry*, 25(26):8343-8347 (1986).
- Gorman et al., "Transient Production of Proteins Using an Adenovirus Transformed Cell Line," *DNA Prot. Eng. Tech.*, 2:3-10 (1990).
- Lucas et al., "High-level production of recombinant proteins in CHO cells using a dicistronic DHFR intron expression vector," *Nucleic Acid Res.*, 24(9):1774-1779 (1996).
- Chisholm, "High Efficiency Gene Transfer into Mammalian Cells," *DNA Cloning 4. Mammalian Systems*, Glover, D.M., Hames, B.D. eds., Oxford University Press, Oxford, pp. 1-41 (1995).
- Park et al., "Placenta Growth Factor," *J. Biol. Chem.*, 269(41):25646-25654 (1994).
- Karlsson et al., "Kinetic and Concentration Analysis Using BIA Technology," *Methods: A Comparison to Methods in Enzymology*, 6:99-110 (1994).
- Bass et al., "Hormone Phage: An Enrichment Method for Variant Proteins with Altered Binding Properties," *Proteins*, 8:309-314 (1990).
- Yang et al., "CRD Walking Mutagenesis for the Affinity Maturation of a Potent Human Anti-HIV-1 Antibody into the Picomolar Range," *J. Mol. Biol.*, 254:392-403 (1995).
- Chen et al., "Selection and Analysis of an Optimized Anti-VEGF Antibody: Crystal Structure of an Affinity-matured Fab in Complex with Antigen" *Journal of Molecular Biology* 293(4):865-881 (1999).
- Presta et al., "Humanization of an Anti-Vascular Endothelial Growth Factor Monoclonal Antibody for the Therapy of Solid Tumors and Other Disorders" *Cancer Research* 57(20):4593-4599 (Oct. 15, 1997).

* cited by examiner

Variable Heavy

A4.6.1 EIQLVQSGPELKQPGETVRISCKASGYTFETNYGMNWVKQAPGKGLKWMG
 * * * * *
 F(ab)-12 EVQLVESGGGLVQPGGSLRLSCAASGYTFETNYGMNWVRQAPGKGLEWVG
 * * * * *
 humIII EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVS
 1 10 20 30 40

A4.6.1 WINTYTGEPTYAADEFKRRFTFSLETSASTAYLQISNLKNDTATYFCAK
 * * * * *
 F(ab)-12 WINTYTGEPTYAADEFKRRFTFSLDTSKSTAYLQMNLSLRAEDTAVYYCAK
 * * * * *
 humIII VISGDGGSTYYADSVKGRFTISRDNKNTLYLQMNLSLRAEDTAVYYCAR
 50 a 60 70 80 abc 90

Fig. 1A

A4.6.1 YPHYYGSSHWFFDVWGAGTTVTVSS (SEQ ID NO:9)
 * *
 F(ab)-12 YPHYYGSSHWFFDVWGQGLVTVSS (SEQ ID NO:7)
 * *
 humIII G-----FDYWGQGLVTVSS (SEQ ID NO:11)
 110

Variable Light

A4.6.1 DIQMTQTTSSLSASLGDRVIISCSASODISNYLNWYQQKPDGTVKVLII
 ** * * * *
 F(ab)-12 DIQMTQSPSSLSASVGDRTTITCSASODISNYLNWYQQKPKAPKVLII
 * * * * *
 humKI DIQMTQSPSSLSASVGDRTTITCRASQISNYLAWYQQKPKAPKLLII
 1 10 20 30 40

Fig. 1B

A4.6.1 FTSSLHSGVPSRFGSGSGTDYSLTISNLEPEDIATYYCOOYETVPEWTF
 ** * * *
 F(ab)-12 FTESLHSGVPSRFGSGSGTDFTLTISLQPEDFATYYCOOYSTVEWTF
 ** * * *
 humKI AASSLESNVPSRFGSGSGTDFTLTISLQPEDFATYYCOQYNSLPWTF
 50 60 70 80 90

A4.6.1 GGGTKI^{*}EIKR (SEQ ID NO:10)
 * *
 F(ab)-12 GQGTKVEIKR (SEQ ID NO:8)
 humKI GQGTKVEIKR (SEQ ID NO:12)
 100



Fig. 2

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.