

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

(10) **Patent No.:** **US 7,060,269 B1**  
(45) **Date of Patent:** **Jun. 13, 2006**

(54) **ANTI-VEGF ANTIBODIES**

(75) Inventors: **Manuel Baca**, Foster City, CA (US);  
**James A. Wells**, Burlingame, CA (US);  
**Leonard G. Presta**, San Francisco, CA  
(US); **Henry B. Lowman**, El Granada,  
CA (US); **Yvonne Man-yea Chen**, San  
Mateo, 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 697 days.

(21) Appl. No.: **09/723,752**

(22) Filed: **Nov. 27, 2000**

**Related U.S. Application Data**

(62) Division of application No. 08/908,469, filed on Aug.  
6, 1997, now Pat. No. 6,884,879.

(51) **Int. Cl.**  
**A61K 39/395** (2006.01)

(52) **U.S. Cl.** ..... **424/133.1**; 424/156.1;  
530/387.3; 530/388.85

(58) **Field of Classification Search** ..... 424/130.1,  
424/133.1, 135.1, 141.1, 155.1, 156.1; 530/387.1,  
530/387.3, 388.1, 388.24, 388.8, 388.85  
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,816,567	A	3/1989	Cabilly et al.
5,530,101	A	6/1996	Queen et al.
5,558,864	A *	9/1996	Bendig et al.
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	0451216	1/1996
GB	2188638	10/1987
GB	2268744	12/1994
WO	WO 91/09967	* 7/1991
WO	WO 92/22653	12/1992
WO	WO 94/04679	3/1994
WO	WO 94/10202	* 5/1994
WO	WO 96/30046	10/1996
WO	WO 98/45332	10/1998
WO	WO 98/45331	10/1999

OTHER PUBLICATIONS

Lopez et al Invest. Opthal. and Visual Science 37:855,  
1996.\*  
Rudikoff et al., PNAS 79:1979, 1982.\*  
Yelton et al., J. of Immunol 155:1994-2004, 1995.\*  
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).

Adamis et al., "Inhibition of Vascular Endothelial Growth  
Factor Prevents Retinal Ischemia-Associated Iris  
Neovascularization in a Nonhuman Primate" *Arch Ophthalmol*  
114(1):66-71 (1996).

Aiello et al., "Vascular endothelial growth factor in ocular  
fluid of patients with diabetic retinopathy and other retinal  
disorders" *New England J. of Medicine* 331(22):1480-1487  
(1994).

Alberts et al., "Molecular Biology of the Cell", 3rd edition,  
Garland Publishing pp. 1154 (1994).

Allen et al., "Specificity of the T cell Receptor: Two Dif-  
ferent Determinants are Generated by the Same Peptide and  
the I A<sup>k</sup> Molecule" *J. Immunol.* 135(1):368-373 (Jul. 1985).  
Baca et al., "Antibody Humanization Using Monovalent  
Phage Display" *Journal of Biological Chemistry*  
272(16):10678-10684 (1997).

Bass et al., "Hormone Phage: An Enrichment Method for  
Variant Proteins with Altered Binding Properties" *Proteins:  
Structure, Function, and Genetics* 8(4):309-314 (1990).

Bendig, M. W., "Humanization of Rodent Monoclonal Anti-  
bodies" *Methods: A Companion to Methods in Enzymology*  
8:83-93 (1994).

Berkman et al., "Expression of the vascular permeability  
factor/vascular endothelial growth factor gene in central  
nervous system neoplasms" *J. Clin. Invest.* 91(1):153-159  
(1993).

Borgstrom 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 Research*  
56(17):4032-4039 (1996).

Brown et al., "Expression of vascular permeability factor  
(vascular endothelial growth factor) and its receptors in  
adenocarcinomas of the gastrointestinal tract" *Cancer  
Research* 53(19):4727-4735 (1993).

Brown et al., "Expression of vascular permeability factor  
(vascular endothelial growth factor) and its receptors in  
breast cancer" *Human Pathology* 26(1):86-91 (1995).

Carter et al., "Humanization of an anti-p185<sup>HER2</sup> antibody  
for human cancer therapy" *Proc. Natl. Acad. Sci.* 89:4285-  
4289 (1992).

Chang et al., "High-level secretion of human growth hor-  
mone by *Escherichia coli*" *Gene* 55:189-196 (1987).

Chisholm, "High Efficiency Gene Transfer into Mammalian  
Cells" *DNA Cloning 4, Mammalian Systems* pp. 1-41  
(1995).

(Continued)

*Primary Examiner*—Larry R. Helms

(74) *Attorney, Agent, or Firm*—Steven X. Cui; 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.

**2 Claims, 16 Drawing Sheets**

## OTHER PUBLICATIONS

- Chothia et al., "Domain Association in Immunoglobulin Molecules. The Packing of Variable Domains" *Journal of Molecular Biology* 186:651-663 (1985).
- Clapp et al., "The 16-kilodalton N-terminal fragment of human prolactin is a potent inhibitor of angiogenesis" *Endocrinology* 133(3):1292-1299 (1993).
- Cunningham et al., "Production of an Atrial Natriuretic Peptide Variant that is Specific for Type A Receptor" *EMBO Journal* 13(11):2508-2515 (1994).
- de Vries et al., "The fms-like tyrosine kinase, a receptor for vascular endothelial growth factor" *Science* 255:989-991 (1992).
- Dvorak et al., "Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis" *American Journal of Pathology* 146(5):1029-1039 (1995).
- Eaton et al., "Construction and characterization of an active factor VIII variant lacking the central one-third of the molecule" *Biochemistry* 25:8343-8347 (1986).
- Eigenbrot et al., "X-Ray Structures of Fragments From Binding and Nonbinding Versions of a Humanized Anti-CD18 Antibody: Structural Indications of the Key Role of V<sub>H</sub> Residues 59 to 65" *Proteins: Structure, Function, and Genetics* 18:49-62 (1994).
- Eigenbrot et al., "X-ray structures of the antigen-binding domains from three variants of humanized anti-p185<sup>HER2</sup> antibody 4D5 and comparison with molecular modeling" *J. Mol. Biol.* 229:969-995 (1993).
- Ferrara and Davis-Smyth, "The Biology of vascular endothelial growth factor" *Endocrine Reviews* 18(1):4-25 (1997).
- Folkman and Shing, "Angiogenesis" *Journal of Biological Chemistry* 267:10931-10934 (1992).
- Foote et al., "Antibody Framework Residues Affecting the Conformation of the Hypervariable Loops" *J. Mol. Biol.* 224:487-499 (1992).
- Garner, A., "Vascular Diseases" *Pathobiology of Ocular Disease, A Dynamic Approach*, Garner, A., Klintworth GK Eds., 2nd edition, NY:Marcel Dekker pp. 1625-1710 (1994).
- Garrard et al., "Fab assembly and enrichment in a monovalent phage display system" *Bio/technology* 9:1373-1377 (1991).
- 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(17):6624-6628 (1990).
- Gorman et al., "Transient Production of Proteins Using an Adenovirus Transformed Cell Line" *DNA Prot. Eng. Tech.* 2(1):3-10 (1990).
- Graham et al., "Characteristics of a Human Cell Line Transformed by DNA from Human Adenovirus Type 5" *J. Gen. Virol.* 36:59-74 (1977).
- Hawkins et al., "Selection of Phage Antibodies by Binding Affinity Mimicking Affinity Maturation" *J. Mol. Biol.* 226:889-896 (1992).
- Horak et al., "Angiogenesis, assessed by platelet/endothelial cell adhesion molecule antibodies, as indicator of node metastases and survival in breast cancer" *Lancet* 340(8828):1120-1124 (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).
- Karlsson et al., "Kinetic analysis of monoclonal antibody-antigen interactions with a new biosensor based analytical system" *J. Immun. Methods* 145:229-240 (1991).
- Karlsson et al., "Kinetic and Concentration Analysis Using BIA Technology" *Methods: A Comparison to Methods in Enzymology* 6:99-110 (1994).
- Kettleborough et al., "Humanization of a Mouse Monoclonal Antibody by CDR-grafting: the Importance of Framework Residues on Loop Conformation" *Protein Engineering* 4(7):773-783 (1991).
- Kim et al., "Inhibition of Vascular Endothelial Growth Factor-Induced Angiogenesis Suppresses Tumour Growth in vivo" *Nature* 362:841-844 (1993).
- Kim et al., "The Vascular Endothelial Growth Factor Proteins: Identification of Biologically Relevant Regions by Neutralizing Monoclonal Antibodies" *Growth Factors* 7(1):53-64 (1992).
- Klagsbrun and D'Amore, "Regulators of angiogenesis" *Ann. Rev. Physiol.* 53:217-239 (1991).
- Kunkel et al., "Efficient site-directed mutagenesis using uracil-containing DNA" *Methods in Enzymology* 204:125-139 (1991).
- Kunkel, T., "Rapid and Efficient Site-Specific Mutagenesis Without Phenotypic Selection" *Proc. Natl. Acad. Sci.* 82:488-492 (1985).
- Leung et al., "Vascular Endothelial Growth Factor is a Secreted Angiogenic Mitogen" *Science* 246:1306-1309 (1989).
- 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).
- Lowman et al., "Selecting High-Affinity Binding Proteins by Monovalent Phage Display" *Biochemistry* 30(45):10832-10838 (1991).
- Lucas et al., "High-level production of recombinant proteins in CHO cells using a dicistronic DHFR intron expression vector" *Nucleic Acids Research* 24(9):1774-1779 (1996).
- Macchiarini et al., "Relation of neovascularization to metastasis of non-small-cell lung cancer" *Lancet* 340(8812):145-146 (1992).
- 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(7):931-934 (1996).
- Melnyk et al., "Vascular endothelial growth factor promotes tumor dissemination by a mechanism distinct from its effect on primary tumor growth" *Cancer Research* 56(4):921-924 (1996).
- Novotny et al., "Structural invariants of antigen binding: comparison of immunoglobulin V<sub>L</sub>-V<sub>H</sub> and V<sub>L</sub>-V<sub>L</sub> domain dimers" *Proc. Natl. Acad. Sci. USA* 82(14):4592-4596 (Jul. 1985).
- O'Reilly et al., "Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma" *Cell* 79(2):315-328 (1994).
- O'Reilly et al., "Endostatin: an endogenous inhibitor of angiogenesis and tumor growth" *Cell* 88(2):277-285 (1997).
- Padlan, E., "A Possible Procedure for Reducing the Immunogenicity of Antibody Variable Domains While Preserving Their Ligand-Binding Properties" *Molecular Immunology* 28(4/4):489-498 (1991).

- Park et al., "Placenta growth factor, Potentiation of vascular endothelial growth factor bioactivity, in vitro and in vivo, and high affinity binding to F1t-1 but not to Flk-1/KDR" *Journal of biological Chemistry* 269(41):25646-25654 (1994).
- Presta et al., "Humanization of an Antibody Directed Against IgE" *J. Immunol.* 151(5):2623-2632.
- Queen et al., "A humanized antibody that binds to the interleukin 2 receptor" *Proc. Natl. Acad. Sci. USA* 86(24):10029-10033 (Dec. 1989).
- Roguska et al., "Humanization of murine monoclonal antibodies through variable domain resurfacing" *Proc. Natl. Acad. Sci. USA* 91:969-973 (Feb. 1994).
- Rosok et al., "A Combinatorial Library Strategy for the Rapid Humanization of Anticarcinoma BR96 Fab" *Journal of Biological Chemistry* 271(37):22611-22618 (Sep. 13, 1996).
- Sanger et al., "DNA Sequencing with Chain-terminating Inhibitors" *Proc. Natl. Acad. Sci. USA* 74(12):5463-5467 (Dec. 1977).
- Shalaby et al., "Development of Humanized Bispecific Antibodies Reactive with Cytotoxic Lymphocytes and Tumor Cells Overexpressing the HER2 Protooncogene" *Journal of Experimental Medicine* 175:217-225 (Jan. 1, 1992).
- Studnicka et al., "Human-engineered monoclonal antibodies retain full specific binding activity by preserving non-CDR complementarity-modulating residues" *Protein Eng.* 7(6):805-814 (1994).
- Tempest et al., "Reshaping a Human Monoclonal Antibody to Inhibit Human Respiratory Syncytial Virus Infection In Vitro" *Bio/Technology* 9:266-271 (Mar. 1991).
- Vieira et al., "Production of Single-stranded Plasmid DNA" *Methods in Enzymology* 151:3-11 (1987).
- Warren et al., "Regulation by vascular endothelial growth factor of human colon cancer tumorigenesis in a mouse model of experimental liver metastasis" *J. Clin. Invest.* 95(4):1789-1797 (1995).
- Weidner et al., "Tumor angiogenesis and metastasis—correlation in invasive breast carcinoma" *New England J. of Medicine* 324(1):1-8 (1991).
- 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 LPA-1" *J. of Immunology* 157:4986-4995 (1996).
- Winter et al., "Making antibodies by phage display technology" *Annual Review of Immunology* 12:433-455 (1994).
- Yang et al., "CDR walking mutagenesis for the affinity maturation of a potent human anti-HIV-1 antibody into the picomolar range" *Journal of molecular Biology* 254(3):392-403 (Dec. 1, 1995).

\* cited by examiner

Variable Heavy

A4.6.1 EIQLVQSGPELKQPGETVRISCKASGYTETNYGMNWVKQAPGKGLKWMG  
 \* \* \*\* \* \*\*\* \* \* \* \* \* \* \*  
 F(ab)-12 EVQLVESGGGLVQPGGSLRSLCAASGYTETNYGMNWVRQAPGKGLEWVG  
 \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \*  
 humIII EVQLVESGGGLVQPGGSLRSLCAASGFTFSSYAMSWVRQAPGKGLEWVS  
 1 10 20 30 40

A4.6.1 WINTYTGEPITYAADEKRRFTFSLETSASTAYLQISNLKNDTATYFCAK  
 \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \*  
 F(ab)-12 WINTYTGEPITYAADEKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAK  
 \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \*  
 humIII VISGDGGSTYYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAR  
 50 a 60 70 80 abc 90

Fig. 1A

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

Variable Light

A4.6.1 DIQMTQTTSSLSASLGDRVIISCSASODISNYLNWYQQKPDGTVKVLIIY  
 \*\* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \*  
 F(ab)-12 DIQMTQSPSSLSASVGDVRTITCSASODISNYLNWYQQKPGKAPKVLIIY  
 \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \*  
 humKI DIQMTQSPSSLSASVGDVRTITCRASQISNYLAWYQQKPGKAPKLLIIY  
 1 10 20 30 40

Fig. 1B

A4.6.1 FTSSLHSGVPSRFSGSGSGTDYSLTISNLEPEDIATYYCQOYSTVPWTF  
 \*\* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \*  
 F(ab)-12 FTSSLHSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQOYSTVPWTF  
 \*\* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \*  
 humKI AASSLESGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQOYNSLPWTF  
 50 60 70 80 90

A4.6.1 GGGKLEIKR (SEQ ID NO: 10)  
 \* \*  
 F(ab)-12 GQGTKVEIKR (SEQ ID NO: 8)  
 humKI GQGTKVEIKR (SEQ ID NO: 12)  
 100

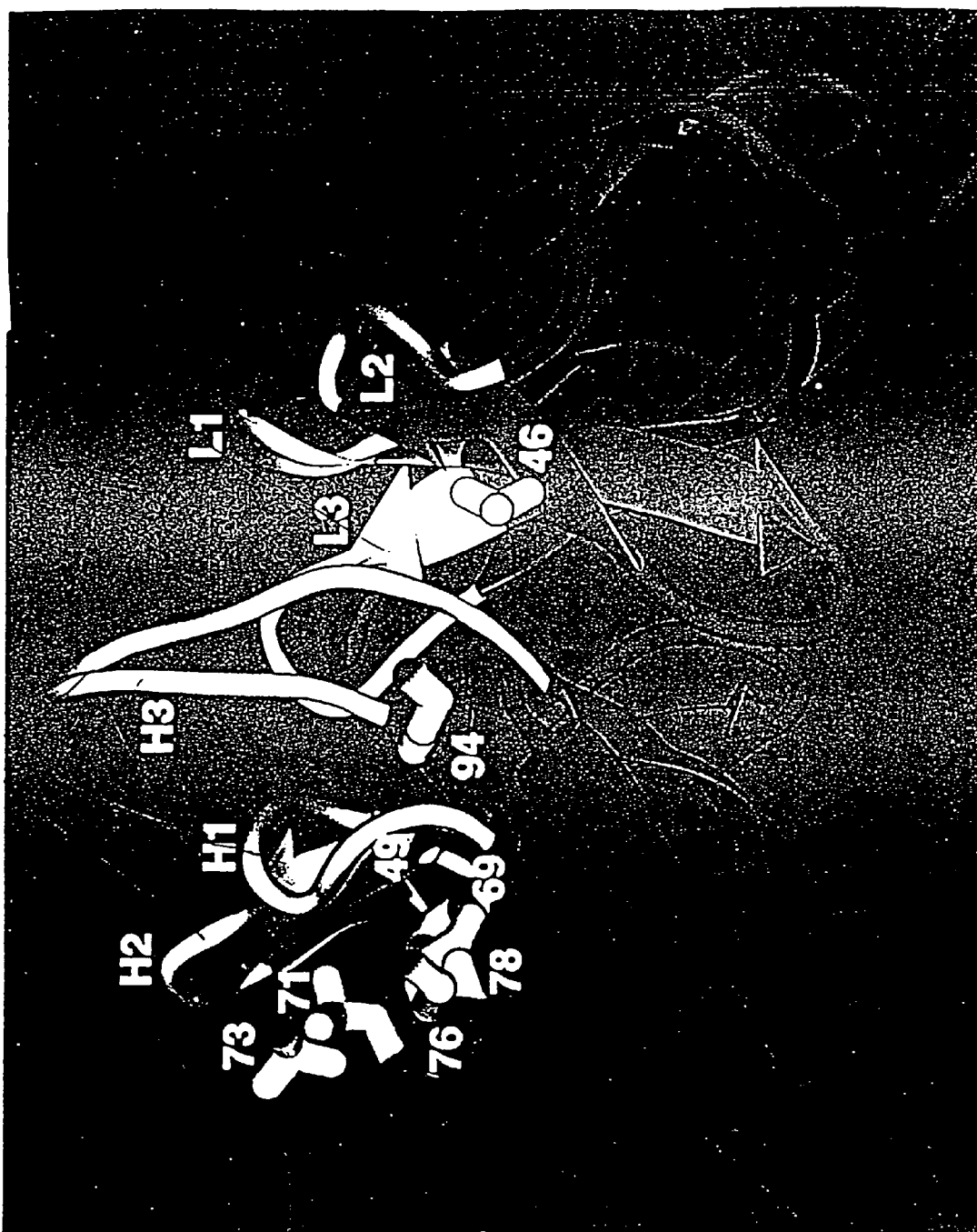


Fig. 2

# 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.