



US006407213B1

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

(10) **Patent No.:** **US 6,407,213 B1**
(45) **Date of Patent:** **Jun. 18, 2002**

- (54) **METHOD FOR MAKING HUMANIZED ANTIBODIES**
- (75) Inventors: **Paul J. Carter; Leonard G. Presta,**
both of San Francisco, 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.: **08/146,206**
- (22) PCT Filed: **Jun. 15, 1992**
- (86) PCT No.: **PCT/US92/05126**
§ 371 (c)(1),
(2), (4) Date: **Nov. 17, 1993**

Related U.S. Application Data

- (63) Continuation-in-part of application No. 07/715,272, filed on
Jun. 14, 1991, now abandoned.
- (51) **Int. Cl.⁷** **C07K 16/00**
- (52) **U.S. Cl.** **530/387.3; 435/69.6; 435/69.7;**
435/70.21; 435/91; 536/23.53; 424/133.1
- (58) **Field of Search** **435/69.6, 69.7,**
435/70.21, 91, 172.2, 240.1, 240.27, 252.3,
320.1, 328; 536/23.53; 424/133.1; 530/387.3

References Cited

U.S. PATENT DOCUMENTS

4,816,567 A	3/1989	Cabilly et al.	
4,845,198 A	7/1989	Urdal et al.	530/388.22
5,132,405 A	7/1992	Huston et al.	530/387.3
5,225,539 A	7/1993	Winter	530/389.3
5,530,101 A	6/1996	Queen et al.	530/387.3
5,558,864 A	9/1996	Bendig et al.	424/133.1
5,585,089 A	12/1996	Queen et al.	424/133.1
5,677,171 A	10/1997	Hudziak et al.	435/7.23
5,693,762 A	* 12/1997	Queen et al.	530/387.2
5,714,350 A	2/1998	Co et al.	435/69.6
5,772,997 A	6/1998	Hudziak et al.	424/130.1
5,821,337 A	10/1998	Carter et al.	530/387.3
5,834,598 A	11/1998	Lowman et al.	530/399
5,859,205 A	1/1999	Adair et al.	530/387.3

FOREIGN PATENT DOCUMENTS

AU	85058/91	3/1992 C07K/15/12
EP	120694	10/1984	
EP	125023 A1	11/1984	
EP	0 239 400	* 9/1987 C12N/15/00
EP	323806 A1	7/1989	
EP	328404 A1	8/1989 A61K/39/395
EP	338745 A1	10/1989	
EP	365209 A2	4/1990	
EP	365997 A2	5/1990	
EP	368684	5/1990	
EP	403156 A1	12/1990	
EP	438310 A2	7/1991	
EP	438312 A2	7/1991	
EP	440351 A2	8/1991	
EP	0 460 167 B1	12/1991	

EP	0 592 106 A1	4/1994	
EP	0 620 276	10/1994	
EP	682040 A1	11/1995	
EP	451216 B1	1/1996 C12P/21/08
EP	432249 B1	9/1996	
GB	2 188941	10/1987	
WO	WO 87/02671	5/1987	
WO	WO 88/09344	12/1988	
WO	WO 89/01783	3/1989	
WO	WO 89/06692	7/1989	
WO	WO 89/09622	10/1989	
WO	WO 90/07861	7/1990	
WO	90/07861	* 7/1990 C12P/21/00
WO	WO 91/07492	5/1991	
WO	WO 91/07500	5/1991	
WO	WO 91/09966	7/1991 C12P/21/08
WO	WO 91/09968	7/1991 C12P/21/08
WO	WO 91/09967	11/1991	
WO	WO 92/01047	1/1992	
WO	WO 92/04380	3/1992	
WO	WO 92/04381	3/1992	
WO	WO 92/05274	4/1992	
WO	WO 92/11383	7/1992	
WO	WO 92/11018	9/1992 A61K/35/14
WO	WO 92/15683	9/1992	
WO	WO 92/16562	10/1992	
WO	WO 92/22653	12/1992	
WO	WO 93/02191	2/1993	
WO	94/11509	5/1994	
WO	94/12214	6/1994	

OTHER PUBLICATIONS

- Riechmann et al. [Nature 332:323-327 (1988)].*
- Queen et al. [Proc. Natl. Acad. Sci. 86:10029-10033 (1989)].*
- Roitt [Immunology, published 1985, by Gower Medical Publishing Ltd. (London, England) p. 5.5].*
- Tramontano et al. [J. Mol. Biol. 215:175-182 (1990)].*
- "Biosym Technologies" in New Products, Chemical Design Automation 3 (Dec. 1988).
- "Polygen Corporation" in New Products, Chemical Design Automation 3 (Nov. 1988).
- Adair et al., "Humanization of the murine anti-human CD3 monoclonal antibody OKT3" *Hum. Antibod. Hybridomas* 5:41-47 (1994).
- Chothia et al., "Principles of protein-protein recognition" *Nature* 256:705-708 (1975).
- Chothia et al., "Transmission of conformational change in insulin" *Nature* 302:500-505 (1983).
- Corti et al., "Idiotope Determining Regions of a Mouse Monoclonal Antibody and Its Humanized Versions" *J. Mol. Biol.* 235:53-60 (1994).

(List continued on next page.)

Primary Examiner—Anthony C. Caputa
Assistant Examiner—Minh-Tam Davis
(74) *Attorney, Agent, or Firm*—Wendy M. Lee

(57) **ABSTRACT**

Variant immunoglobulins, particularly humanized antibody polypeptides are provided, along with methods for their preparation and use. Consensus immunoglobulin sequences and structural models are also provided.

OTHER PUBLICATIONS

- Couto et al., "Anti-BA46 Monoclonal Antibody Mc3 Humanization Using a Novel Positional Consensus and in Vivo and in Vitro Characterization" *Cancer Research Supplement* 55:1717-1722 (1995).
- Couto et al., "Humanization of KC4G3, an Anti-Human Carcinoma Antibody" *Hybridoma* 13:215-219 (1994).
- Ellis et al., "Engineered Anti-CD38 Monoclonal Antibodies for Immunotherapy of Multiple Myeloma" *The Journal of Immunology* pp. 925-937 (1995).
- Hicter et al., "Evolution of Human Immunoglobulin K J Region Genes" *The Journal of Biological Chemistry* 257:1516-1522 (1982).
- Lesk, Arthur M., "How Different Amino Acid Sequences Determine Similar Protein Structures: The Structure and Evolutionary Dynamics of the Globins" *J. Mol. Biol.* 135:225-270 (1980).
- Matsumura et al., "Hydrophobic stabilization in T4 lysozyme determined directly by multiple substitutions of Ile 3" *Nature* 334:406-410 (1988).
- Morrison, S. L., "Transfectomas Provide Novel Chimeric Antibodies" *Science* 229:1202-1207 (Sep. 20, 1985).
- Nakatani et al., "Humanization of mouse anti-human IL-2 receptor antibody B-B10" *Protein Engineering* 7:435-443 (1994).
- Ohmoto et al., "Humanization of Mouse ONS-M21 Antibody with the Aid of Hybrid Variable Regions" *Molecular Immunology* 32:407-416 (1995).
- Rodrigues et al., "Engineering a humanized bispecific F(ab)₂ fragment for improved binding to T cells" *Int. J. Cancer (Suppl.)* 7:45-50 (1992).
- Sha et al., "A Heavy-Chain Grafted Antibody that Recognizes the Tumor-Associated TAG72 Antigen" *Cancer Biotherapy* 9:341-349 (1994).
- Tempest et al., "Identification of framework residues required to restore antigen binding during reshaping of a monoclonal antibody against the glycoprotein gB of human cytomegalovirus" *Int. J. Biol. Macromol.* 17:37-42 (1995).
- Tromontano, "Structural Determinants of the Conformations of Medium-Sized Loops in Proteins" *Proteins* 6:382-394 (1989).
- Uchiyama et al., "A Monoclonal Antibody (Anti-Tac) Reactive with Activated and Functionally Mature Human T Cells" *Journal of Immunology* 126:1393-1397 (1981).
- Vincenti et al., "Interleukin-2-Receptor Blockade with Daclizumab to Prevent Acute Rejection in Renal Transplantation" *New Engl. J. Med.* 338:161-165 (1998).
- Vitetta et al., "Redesigning Nature's Poisons to Create Anti-Tumor Reagents" *Science* 238:1098-1104 (1987).
- Waldmann et al., "Interleukin 2 Receptor (Tac Antigen) Expression in HTLV-1-associated Adult T-Cell Leukemia" *Cancer Research* 45:4555s-4562s (1985).
- Waldmann, Thomas A., "The Structure, Function, and Expression of Interleukin-2 Receptors on Normal and Malignant Lymphocytes" *Science* 232:727-732 (1986).
- Wu et al., "An Analysis of the Sequences of the Variable Regions of Bence Jones Proteins and Myeloma Light Chains and Their Implications for Antibody Complementarity" *Journal of Experimental Medicine* 132:211-250 (1970).
- Rhodes, P., "Recombinant antibodies from CHO cells" *Abstr Pap Am Chem Soc* (Abstract No. 60 from the 199th American Chemical Society National Meeting held in Boston, MA Amzel and Poljak, "Three-dimensional structure of immunoglobulins" *Ann. Rev. Biochem.* 48:961-967 (1979).
- Bindon et al., "Human monoclonal IgG isotypes differ in complement activating function at the level of C4 as well as C1q" *Journal of Experimental Medicine* 168(1):127-142 (Jul. 1988).
- Boulianne, G. I. et al., "Production of functional chimeric mouse/human antibody" *Nature* 312(5995):643-646 (Dec. 1984).
- Brown et al., "Anti-Tac-H, a humanized antibody to the interleukin 2 receptor, prolongs primate cardiac allograft survival" *Proc. Natl. Acad. Sci. USA* 88:2663-2667 (1991).
- Bruccoleri, "Structure of antibody hypervariable loops reproduced by a conformational search algorithm" *Nature* (erratum to article in *Nature* 335(6190):564-568 and) 336:266 (1988).
- Bruggemann, M. et al., "Comparison of the effector functions of human immunoglobulins using a matched set of chimeric antibodies" *Journal of Experimental Medicine* 166:1351-1361 (1987).
- Burgess et al., "Possible Dissociation of the Heparin-binding and Mitogenic Activities of Heparin-binding (Acidic Fibroblast) Growth Factor-1 from Its Receptor-binding Activities by Site-directed Mutagenesis of a Single Lysine Residue" *Journal of Cell Biology* 111:2129-2138 (1990).
- Carter et al., "Humanization of an anti-p185^{HER2} antibody for human cancer therapy" *Proc. Natl. Acad. Sci.* 89:4285-4289 (1992).
- Cheatham, J., "Reshaping the antibody combining site by CDR replacement-tailoring or tinkering to fit?" *Protein Engineering* 2(3):170-172 (1988).
- Chothia and Lesk, "Canonical Structures for the Hypervariable Regions" *J. Mol. Biol.* 196:901-917 (1987).
- Chothia et al., "The predicted structure of immunoglobulin D1.3 and its comparison with the crystal structure" *Science* 233:755-758 (Aug. 15, 1986).
- Chothia, C. et al., "Conformations of immunoglobulin hypervariable regions" *Nature* 342(6252):877-883 (1989).
- Chothia, Cyrus, "Domain association in immunoglobulin molecules: The packing of variable domains" *J. Mol. Biol.* 186:651-663 (1985).
- Clark et al., "The improved lytic function and in vivo efficacy of monovalent monoclonal CD3 antibodies" *European Journal of Immunology* 19:381-388 (1989).
- Co et al., "Humanized antibodies for antiviral therapy" *Proc. Natl. Acad. Sci. USA* 88:2869-2873 (1991).
- Coussens et al., "Tyrosine Kinase Receptor with Extensive Homology to EGF Receptor Shares Chromosomal Location with neu Oncogene" *Science* 230:1132-1139 (1985).
- Daugherty, BL et al., "Polymerase chain reaction facilitates the cloning, CDR-grafting, and rapid expression of a murine monoclonal antibody directed against the CD18 component of leukocyte integrins" *Nucleic Acids Research* 19(9):2471-2476 (May 11, 1991).
- Davies, D. R. et al., "Antibody-Antigen Complexes" *Ann. Rev. Biochem.* 59:439-473 (1990).
- Epp et al., "The molecular structure of a dimer composed of the variable portions of the Bence-Jones protein RE1 refined at 2.0-A resolution" *Biochemistry* 14(22):4943-4952 (1975).
- Fendly et al., "Characterization of murine monoclonal antibodies reactive to either the human epidermal growth factor receptor or HER2/neu gene product" *Cancer Research*

- Furey et al., "Structure of a novel Bence-Jones protein (Rhc) fragment at 1.6 Å resolution" *J. Mol. Biol.* 167(3):661-692 (Jul. 5, 1983).
- Gorman, SD et al., "Reshaping a therapeutic CD4 antibody" *Proc. Natl. Acad. Sci. USA* 88(10):4181-4185 (May 15, 1991).
- Gregory et al., "The solution conformations of the subclasses of human IgG deduced from sedimentation and small angle X-ray scattering studies" *Molecular Immunology* 24(8):821-829 (Aug. 1987).
- Halc et al., "Remission induction in non-hodgkin lymphoma with reshaped human monoclonal antibody campath-1H" *Lancet* 1:1394-1399 (1988).
- Harris and Emery, "Therapeutic antibodies—the coming of age" *Tibtech* 11:42-44 (Feb. 1993).
- Huber et al., "Crystallographic structure studies of an IgG molecule and an Fc fragment" *Nature* 264:415-420 (Dec. 2, 1976).
- Hudziak et al., "p185^{HER2} Monoclonal Antibody Has Antiproliferative Effects In Vitro and Sensitizes Human Breast Tumor Cells to Tumor Necrosis Factor" *Molecular & Cellular Biology* 9(3):1165-1172 (1989).
- Jaffers, G. J. et al., "Monoclonal antibody therapy. Anti-idiotypic and non-anti-idiotypic antibodies to OKT3 arising despite intense immunosuppression" *Transplantation* 41(5):572-578 (May 1986).
- Jones, P. T. et al., "Replacing the complementarity-determining regions in a human antibody with those from a mouse" *Nature* 321(6069):522-525 (1986).
- Junghans et al., "Anti-Tac-H, a humanized antibody to the interleukin 2 receptor with new features for immunotherapy in malignant and immune disorders" *Cancer Research* 50(5):1495-1502 (Mar. 1, 1990).
- Kabat et al. *Sequences of Proteins of Immunological Interest*, Bethesda, MD:National Institutes of Health pp. iii-xxvii, 41-176 (1987).
- King et al., "Amplification of a Novel v-erbB-Related Gene in a Human Mammary Carcinoma" *Science* 229:974-976 (1985).
- Lazar et al., "Transforming Growth Factor α : Mutation of Aspartic Acid 47 and Leucine 48 Results in Different Biological Activities" *Molecular & Cellular Biology* 8(3):1247-1252 (1988).
- Love et al., "Recombinant antibodies possessing novel effector functions" *Methods in Enzymology* 178:515-527 (1989).
- Lupu et al., "Direct interaction of a ligand for the erbB2 oncogene product with the EGF receptor and p185^{erbB2}" *Science* 249:1552-1555 (1990).
- Margni RA and Binaghi RA, "Nonprecipitating asymmetric antibodies" *Ann. Rev. Immunol.* 6:535-554 (1988).
- Margolies et al., "Diversity of light chain variable region sequences among rabbit antibodies elicited by the same antigens." *Proc. Natl. Acad. Sci. USA* 72:2180-84 (Jun. 1975).
- Marquart et al., "Crystallographic refinement and atomic models of the intact immunoglobulin molecule Kol and its antigen-binding fragment at 3.0 Å and 1.0 Å resolution" *J. Mol. Biol.* 141(4):369-391 (Aug. 25, 1980).
- Mian, IS et al., "Structure, function and properties of antibody binding sites" *J. Mol. Biol.* 217(1):133-151 (Jan. 5, 1991).
- Miller, R. et al., "Monoclonal antibody therapeutic trials in seven patients with T-cell lymphoma" *Blood* 62:988-995
- Morrison, S. L. et al., "Chimeric human antibody molecules: mouse antigen-binding domains with human constant region domains" *Proc. Natl. Acad. Sci. USA* 81(21):6851-6855 (Nov. 1984).
- Neuberger et al., "Recombinant antibodies possessing novel effector functions" *Nature* 312(5995):604-608 (Dec. 1984).
- Neuberger, M. S. et al., "A hapten-specific chimaeric IgE antibody with human physiological effector function" *Nature* 314(6008):268-270 (Mar. 1985).
- Novotny 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(14):4592-4596 (Jul. 1985).
- Pluckthun, Andreas, "Antibody engineering: advances from the use of *Escherichia coli* expression systems" *Biotechnology* 9:545-51 (1991).
- Queen, M. et al., "A humanized antibody that binds to the interleukin 2 receptor" *Proc. Natl. Acad. Sci. USA* 86:10029-10033 (1989).
- Riechmann, L. et al., "Reshaping human antibodies for therapy" *Nature* 332:323-327 (1988).
- Roitt et al. *Immunology* (Gower Medical Publishing Ltd., London, England) pp. 5.5 (1985).
- Saul et al., "Preliminary refinement and structural analysis of the Fab fragment from human immunoglobulin new at 2.0 Å resolution" *Journal of Biological Chemistry* 253(2):585-597 (Jan. 25, 1978).
- Schroff, R. et al., "Human anti-murine immunoglobulin responses in patients receiving monoclonal antibody therapy" *Cancer Research* 45:879-885 (1985).
- Segal et al., "The three-dimensional structure of a phosphorylcholine-binding mouse immunoglobulin Fab and the nature of the antigen binding site" *Proc. Natl. Acad. Sci. USA* 71(11):4298-4302 (Nov. 1974).
- Shalaby et al., "Development of humanized bispecific antibodies reactive with cytotoxic lymphocytes and tumor cells overexpressing the HER2 protooncogene" *Journal of Experimental Medicine* 175(1):217-225 (Jan. 1, 1992).
- Shepard and Lewis, "Resistance of tumor cells to tumor necrosis factor" *J. Clin. Immunol.* 8(5):333-395 (1988).
- Sheriff et al., "Three-dimensional structure of an antibody-antigen complex" *Proc. Natl. Acad. Sci. USA* 84(22):8075-8079 (Nov. 1987).
- Sherman et al., "Haloperidol binding to monoclonal antibodies" *Journal of Biological Chemistry* 263:4064-4074 (1988).
- Silverton et al., "Three-dimensional structure of an intact human immunoglobulin" *Proc. Natl. Acad. Sci. USA* 74:5140-5144 (1977).
- Slamon et al., "Human Breast Cancer: Correlation of Relapse and Survival with Amplification of the HER-2/neu Oncogene" *Science* 235:177-182 (1987).
- Slamon et al., "Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer" *Science* 244:707-712 (1989).
- Snow and Amzel, "Calculating three-dimensional changes in protein structure due to amino-acid substitutions: the variable region of immunoglobulins" *Protein: Structure, Function, and Genetics*, Alan R. Liss, Inc. vol. 1:267-279 (1986).
- Sox et al., "Attachment of carbohydrate to the variable region of myeloma immunoglobulin light chains" *Proc.*

- Spiegelberg et al., "Localization of the carbohydrate within the variable region of light and heavy chains of human γ G myeloma proteins" *Biochemistry* 9:4217-23 (Oct. 1970).
- Takeda et al., "Construction of chimeric processed immunoglobulin genes containing mouse variable and human constant region sequences" *Nature* 314(6010):452-454 (Apr. 1985).
- Tao et al., "Role of Carbohydrate in the Structure and Effector Functions Mediated by the Human IgG Constant Region" *J. Immunol.* 143(8):2595-2601 (1989).
- Tramontano et al., "Framework residue 71 is a major determinant of the position and conformation of the second hypervariable region in the VH domains of immunoglobulins" *J-Mol-Biol* 215(1):175-182 (Sep. 5, 1990).
- Verhocyen, M. et al., "Reshaping human antibodies: grafting an antilysozyme activity" *Science* 239(4847):1534-1536 (Mar. 25, 1988).
- Waldmann, T., "Monoclonal antibodies in diagnosis and therapy" *Science* 252:1657-1662 (1991).
- Wallick et al., "Glycosylation of a VH residue of a monoclonal antibody against alpha (1-6) dextran increases its affinity for antigen" *Journal of Experimental Medicine* 168(3):1099-1109 (Sep. 1988).
- Winter and Milstein, "Man-made antibodies" *Nature* 349(6307):293-299 (Jan. 24, 1991).
- Yamamoto et al., "Similarity of protein encoded by the human c-crb-B-2 gene to epidermal growth factor receptor" *Nature* 319:230-34 (1986).
- Carter et al., "High level *Escherichia coli* expression and production of a bivalent humanized antibody fragment" *BioTechnology* 10:163-167 (1992).
- Foote et al., "Antibody Framework Residues Affecting the Conformation of the Hypervariable Loops" *J. Mol. Biol.* 224:487-499 (1992).
- Foote, J., "Humanized Antibodies" *Nova acta Leopoldina* 61(269):103-110 (1989).
- Kabat et al., "Sequences of Proteins of Immunological Interest", Bethesda, MD:National Institute of Health pp. 14-32 (1983).
- 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).
- Maeda et al., "Construction of Reshaped Human Antibodies with HIV-neutralizing Activity" *Hum. Antibod. Hybridomas* 2:124-134 (Jul. 1991).
- Riechmann et al., "Expression of an Antibody Fv Fragment in Myeloma Cells" *J. Mol. Biol.* 203:825-828 (1988).
- Routledge et al., "A Humanized Monoclonal CD3 Antibody which Can Activate Homologous Complement" *European Journal of Immunology* 21:2717-2725 (1991).
- Shearman et al., "Construction, Expression and Characterization of Humanized Antibodies Directed Against the Human $\alpha\beta$ T Cell Receptor" *J. Immunol.* 147(12):4366-4373 (Dec. 15, 1991).
- Tempest et al., "Reshaping a Human Monoclonal Antibody to Inhibit Human Respiratory Syncytial Virus Infection In Vivo" *BioTechnology* 9:266-271 (Mar. 1991).
- Brown, Jr. et al., "Anti-Tac-H, a humanized antibody to the interleukin 2 receptor, prolongs primate cardiac allograft survival" *Proc. Natl. Acad. Sci. USA* 88:2663-2667 (1991).
- Casale et al., "Use of an anti-IgE humanized monoclonal antibody in ragweed-induced allergic rhinitis" *J. Allergy*
- Fahy et al., "The Effect of an Anti-IgE Monoclonal Antibody on the Early- and Late-Phase Responses to Allergen Inhalation in Asthmatic Subjects" *Am J. Respir. Crit. Care Med* 155:1828-1834 (1997).
- Mathieson et al., "Monoclonal-Antibody Therapy in Systemic Vasculitis" *New England J. of Medicine* pp. 250-254 (Jul. 1990).
- 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).
- Amit et al., "Three-Dimensional Structure of an Antigen-Antibody Complex at 2.8 Å Resolution" *Science* 233:747-753 (Aug. 1986).
- Amzel et al., "The Three Dimensional Structure of a Combining Region-Ligand Complex of Immunoglobulin New at 3.5-Å Resolution" *Proc. Natl. Acad. Sci. USA* 71(4):1427-1430 (Apr. 1974).
- Baselga et al., "Phase II Study of Weekly Intravenous Recombinant Humanized Anti-p185/HER2 Monoclonal Antibody in Patients With HER2/neu-Overexpressing Metastatic Breast Cancer" *J. Clin. Oncol.* 14(3):737-744 (1996).
- Beverley & Callard, "Distinctive functional characteristics of human "T" lymphocytes defined by E rosetting or a monoclonal anti-T cell antibody" *European Journal of Immunology* 11:329-334 (1981).
- Bird et al., "Single-chain antigen-binding proteins" *Science* 242:423-426 (Oct. 1988).
- Brennan et al., "Preparation of bispecific antibodies by chemical recombination of monoclonal immunoglobulin G₁ fragments" *Science* 229:81-83 (Jul. 1985).
- Bruccoleri et al., "Structure of antibody hypervariable loops reproduced by a conformational search algorithm" *Nature* 335:564-568 (Oct. 1988).
- Caron et al., "Biological and Immunological Features of Humanized M195 (Anti-CD33) Monoclonal Antibodies" *Cancer Research* 52:6761-6767 (Dec. 1992).
- Chothia & Lesk, "The relation between the divergence of sequence and structure in proteins" *EMBO Journal* 5(4):823-826 (1986).
- Co & Queen, "Humanized antibodies for therapy" *Nature* 351:501-502 (Jun. 1991).
- Co et al., "Chimeric and Humanized Antibodies with Specificity for the CD33 Antigen" *J. of Immunology* 148(4):1149-1154 (Feb. 1992).
- Co et al., "Humanized Anti-Lewis Y Antibodies: In Vitro Properties and Pharmacokinetics in Rhesus Monkeys" *Cancer Research* 56:1118-1125 (Mar. 1996).
- Colman et al., "Crystal and Molecular Structure of the Dimer of Variable Domains of the Bence-Jones Protein ROY" *J. Mol. Biol.* 116:73-79 (1977).
- Colman et al., "Three-dimensional structure of a complex of antibody with influenza virus neuraminidase" *Nature* 326:358-363 (Mar. 1987).
- Cook et al., "A map of the human immunoglobulin V_H locus completed by analysis of the telomeric region of chromosome 14q" *Nature Genetics* 7:162-168 (Jun. 1994).
- Darsley & Rees, "Nucleotide sequences of five anti-lysozyme monoclonal antibodies" *EMBO Journal* 4(2):393-398 (1985).
- Davies & Metzger, "Structural Basis of Antibody Function"

- Davies et al., "Antibody-Antigen Complexes" *Journal of Biological Chemistry* 263(22):10541-10544 (Aug. 1988).
- 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_H Residues 59 to 65" *Proteins* 18:49-62 (1994).
- Eigenbrot et al., "X-ray structures of the antigen-binding domains from three variants of humanized anti-p185HER2 antibody 4D5 and comparison with molecular modeling" *J. Mol. Biol.* 229:969-995 (1993).
- Ellison et al., "The nucleotide sequence of a human immunoglobulin C_{γ1} gene" *Nucleic Acids Research* 10(13):4071-4079 (1982).
- Emery & Adair, "Humanised monoclonal antibodies for therapeutic applications" *Exp. Opin. Invest. Drugs* 3(3):241-251 (1994).
- Epp et al., "Crystal and Molecular Structure of a Dimer Composed of the Variable Portions of the Bence-Jones Protein REI" *European Journal of Biochemistry* 45:513-524 (1974).
- Fanger et al., "Bispecific antibodies and targeted cellular cytotoxicity" *Immunology Today* 12(2):51-54 (1991).
- Fanger et al., "Cytotoxicity mediated by human Fc receptors for IgG" *Immunology Today* 10(3):92-99 (1989).
- Feldmann et al., "A Hypothetical Space-Filling Model of the V-Regions of the Galactan-Binding Myeloma Immunoglobulin J539" *Molecular Immunology* 18(8):683-698 (1981).
- Fendley et al., "The Extracellular Domain of HER2/neu Is a Potential Immunogen for Active Specific Immunotherapy of Breast Cancer" *J. Biol. Resp. Mod.* 9:449-455 (1990).
- Glennie et al., "Preparation and Performance of Bispecific F(ab')₂ Antibody Containing Thioether-Linked Fab' Fragments" *J. Immunol.* 139(7):2367-2375 (Oct. 1, 1987).
- Gonzalez et al., "Humanization of Murine 6G425: An Anti-IL8 Monoclonal Antibody Which Blocks Binding of IL8 to Human Neutrophils" *1996 Keystone Symposia on Exploring and Exploiting Antibody and Ig Superfamily Combining Sites* (Poster) pp. 1-21 (Feb. 1996).
- Gussow & Seemann, "Humanization of Monoclonal Antibodies" *Meth. Enzymology*, Academic Press, Inc. vol. 203:99-121 (1991).
- Hicter et al., "Cloned human and mouse kappa immunoglobulin constant and J region genes conserve homology in functional segments" *Cell* 22 (Part 1):197-207 (1980).
- Houghton, A., "Building a better monoclonal antibody" *Immunology Today* 9(9):265-267 (1988).
- Huston et al., "Protein engineering of antibody binding sites: Recovery of specific activity in an anti-digoxin single-chain Fv analogue produced in *Escherichia coli*" *Proc. Natl. Acad. Sci. USA* 85:5879-5883 (Aug. 1988).
- Isaacs et al., "Humanised Monoclonal Antibody Therapy for Rheumatoid Arthritis" *Lancet* 340:748-752 (Sep. 26, 1992).
- Johnson et al., "Biological and Molecular Modeling Studies Comparing Murine Monoclonal Antibodies with Their Engineered Chimeric and Humanized Counterparts" *J. Cell. Biochem. Suppl* 13 (Part A) (18th Ann. UCLA Symp on Mol. & Cell. Biol., Park City, UT Jan. 17-22, 1989) pp. 87 (1989).
- Kabat E., "Origins of Antibody Complementarity and Specificity—Hypervariable Regions and the Minigenes Hypoth-
- 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).
- Kindt & Capra *The Antibody Enigma*, New York:Plenum Press pp. 79-86 (1984).
- Lcsk & Chothia, "Evolution of Proteins Formed by β-Sheets" *J. Mol. Biol.* 160:325-342 (1982).
- Lcsk & Chothia, "The response of protein structures to amino-acid sequence changes" *Phil. Trans. R. Soc. Lond. A* 317:345-356 (1986).
- Mariuzza et al., "The Structure Basis of Antigen-Antibody Recognition" *Ann. Rev. Biophys. Biophys. Chem.* 16:139-159 (1987).
- Nadler et al., "Immunogenicity of Humanized and Human Monoclonal Antibodies" *Clin. Pharmacology & Therapeutics* pp. 180 (Feb. 1994).
- Nelson, H., "Targeted Cellular Immunotherapy with Bifunctional Antibodies" *Cancer Cells* 3:163-172 (1991).
- Neuberger et al., "Antibody Engineering" *Proceedings 8th Intl. Biotech. Symp.*, Paris II:792-799 (1988).
- Newmark, P., "Making Chimeric Antibodies Even More Human" *BioTechnology* 6:468 (May 1988).
- Nishimura et al., "Human c-erbB-2 Proto-Oncogene Product as a Target for Bispecific-Antibody-Directed Adoptive Tumor Immunotherapy" *Int. J. Cancer* 50:800-804 (1992).
- Nitta et al., "Preliminary trial of specific targeting therapy against malignant glioma" *Lancet* 335(8686):368-371 (Feb. 17, 1990).
- Nitta, T. et al., "Bispecific F(ab')₂ monomer prepared with anti-CD3 and anti-tumor monoclonal antibodies is most potent in induction of cytotoxicity of human T cells" *European Journal of Immunology* 19:1437-1441 (1989).
- Nolan et al., "Bifunctional antibodies: concept, production and applications" *Biochimica et Biophysica Acta* 1040:1-11 (1990).
- Connor et al., "Calcium Dependence of an Anti-Protein C Humanized Antibody Involves Framework Residues" (manuscript).
- Orlandi et al., "Cloning Immunoglobulin Variable Domains for Expression by the Polymerase Chain Reaction" *Proc. Natl. Acad. Sci. USA* 86:3833-3837 (May 1989).
- Orlandi et al., "Cloning of cDNA Corresponding to Heavy and Light Chain Immunoglobulin Variable Domains" *Protein and Pharmaceutical Engineering* pp. 90 (1989).
- Ostberg & Queen, "Human and humanized monoclonal antibodies: preclinical studies and clinical experience" *Biochem. Soc. Transactions* pp. 1038-1043 (1995).
- Pedlan et al., "Model-building Studies of Antigen-binding Sites: The Hapten-binding Site of MOPC-315" *Cold Springs Harbor Symposia On Quantitative Biology* XI:627-637 (1977).
- Padlan, E., "Anatomy of the Antibody Molecule" *Molecular Immunology* 31(3):169-217 (1994).
- Padlan, E., "Evaluation of the Structural Variation Among Light Chain Variable Domains" *Molecular Immunology* 16:287-296 (1979).
- Palm & Hilschmann, "Primary structure of a crystalline monoclonal immunoglobulin K-type L-chain, subgroup I (Bence-Jones protein Re1): isolation & characterization of the tryptic peptides: . . ." *Hoppes-Seyler's Z. Physiol.*

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.