

- (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)
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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	0 519 596 A1	12/1992	

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	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.

82 Claims, 9 Drawing Sheets

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).
- Hieter 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).
- Ohtomo 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).
- Tramontano, "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* 45:4559s-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 Apr. 22-27, 1990) 199(1-2):BIOT 60 (Apr. 1990).
- 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. L. et al., "Production of functional chimaeric 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).
- Cheetham, 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 REI refined at 2.0-Å 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* 50:1550-1558 (1990).

- Furey et al., "Structure of a novel Bence-Jones protein (Rhe) 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).
- Hale 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 Anti-proliferative 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 (1983).
- 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. Natl. Acad. Sci. USA* 66:975-82 (Jul. 1970).

- 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 chimaeric 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).
- Verhoeyen, 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-erb-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" *Bio/Technology* 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 Monovalent 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 α/β 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" *Bio/Technology* 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 Clin. Immunol.* 100:110-121 (1997).
- 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" *Ann. Rev. Immunol.* 1:87-117 (1983).

- 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'y 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).
- Hieter 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 0 (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 Minigenen Hypothesis" *J. of Immunology* 125(3):961-969 (Sep. 1980).
- 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).
- Lesk & Chothia, "Evolution of Proteins Formed by β-Sheets" *J. Mol. Biol.* 160:325-342 (1982).
- Lesk & 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" *Bio/Technology* 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 cytolysis 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).
- O'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* XLI: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 preotin Rei); isolation & characterization of the tryptic peptides: . . ." *Hoppes-Seyler's Z. Physiol. Chem.* 356:167-191 (Feb. 1975).

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