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(54) **METHODS OF PRODUCING
IMMUNOGLOBULINS, VECTORS AND
TRANSFORMED HOST CELLS FOR USE
THEREIN**

37723 10/1981 (EP).
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041767 12/1981 (EP).
41313 12/1981 (EP).
41767 12/1981 (EP).
044722 1/1982 (EP).
055945 7/1982 (EP).
57107 8/1982 (EP).
0068763 1/1983 (EP).
68763 1/1983 (EP).
0057107 3/1983 (EP).
0073656 3/1983 (EP).
075444 3/1983 (EP).
73656 3/1983 (EP).
75444 3/1983 (EP).
A-073656 3/1983 (EP).
088994 9/1983 (EP).
88994 9/1983 (EP).
093619 11/1983 (EP).
0120694 10/1984 (EP).
0125023 11/1984 (EP).
194276 9/1986 (EP).
196864 10/1986 (EP).
234592 9/1987 (EP).
255694 2/1988 (EP).
324162 7/1989 (EP).
550400 7/1993 (EP).
08235 3/1987 (GB).
62 201 581 9/1987 (JP).
WO 86/01533 3/1986 (WO).

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(56) **References Cited**

U.S. PATENT DOCUMENTS

4,179,337	12/1979	Davis et al. .
4,237,224	12/1980	Cohen .
4,338,397	7/1982	Gilbert et al. .
4,342,832	8/1982	Goeddel et al. .
4,403,036	9/1983	Hartley et al. .
4,444,878	4/1984	Paulus .
4,510,244	4/1985	Parks et al. .
4,512,922	4/1985	Jones et al. .
4,518,584	5/1985	Mark et al. .
4,642,334	2/1987	Moore et al. .
4,704,362	11/1987	Itakura et al. .
4,816,397	3/1989	Boss et al. .
4,816,567	3/1989	Cabilly et al. .
5,225,539	7/1993	Winter .
5,545,403	8/1996	Page .
5,545,404	8/1996	Page .
5,545,405	8/1996	Page .

FOREIGN PATENT DOCUMENTS

194982	2/1983	(AU) .
12417/83	9/1983	(AU) .
B-26429/84	10/1984	(AU) .
46556/85	3/1986	(AU) .
65981/86	5/1987	(AU) .
0037723	10/1981	(EP) .

OTHER PUBLICATIONS

Dolby et al. *Proc. Natl. Acad. Sci.* 77(10):6027–6031 (1980).
Rice et al. *Proc. Natl. Acad. Sci.* 77:7862–7865 (1982).
Accolla et al. *Proc. Natl. Acad. Sci.* 77(1):563–566 (1980).
Raso et al. *Cancer Res.* 41:2073–2078 (1981).
Nisonoff et al. *Arch. Biochem. Biophys.* 93:460–462 (1960).
Glennie et al. *Nature* 295:712–714 (1982).
Eisen *Immunology* Harper & Row, Publishers, pp. 415 and 428–436 (1974).
Hozumi et al. *Nuc. Acids. Res.* 5(6):1779–1799 (1978).
Wetzel et al. *Gene* 16:63–71 (1981).
Williams et al. *Science* 215:687–689 (1982).
Falkner et al. *Nature* 298:286–288 (1982).
Boss et al. *Gene Expressions—Proc. Cetus—UCLA Symposium* pp. 513–522, Mar. 26–Apr. 1, 1983.

(List continued on next page.)

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(57) **ABSTRACT**

The invention relates to processes for producing an immunoglobulin or an immunologically functional immunoglobulin fragment containing at least the variable domains of the immunoglobulin heavy and light chains. The processes can use one or more vectors which produce both the heavy and light chains or fragments thereof in a single cell. The invention also relates to the vectors used to produce the immunoglobulin or fragment, and to cells transformed with the vectors.

36 Claims, 19 Drawing Sheets

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OTHER PUBLICATIONS

- Amster et al. *Nucleic Acid Research* 8(9):2055–2065 (1980).
- DeBoer et al., Rodriguez et al. (Ed.) *Promoters* 462–481 (1982).
- Gough *Tibs* 6(8):203–205 (Aug., 1981).
- Morrison *J. of Immunology* 123(2):793–800 (Aug., 1979).
- Kohler *Proc. Natl. Acad. Sci.* 77(4):2197–2199 (Apr., 1980).
- Roberts *Promoters* 452–461 (1982).
- Kemp et al. *Proc. Natl. Acad. Sci.* 78(7):4520–4524 (Jul., 1981).
- Valle et al. *Nature* 300:71–74 (Nov. 4, 1982).
- Microbiology* 3rd edition, Harper Int. Ed. 338–379 (1980).
- Hitzeman et al. *Science* 219:620–625 (1983).
- Mercreau-Pujalon et al. in *Expression of Eukaryotic Viral and Cellular Genes*, Pettersson et al. (ED) 295–303 (1981) Academic Press.
- Pettersson et al. (Ed.) 295–303 (1981) Academic Pr.
- Keslhet et al. *Nucleic Acids Res.* 9(1):19–30 (1981).
- Taniguchi et al. *Proc. Natl. Acad. Sci.* 77(9):5230–5233 (1980).
- Ohsuye et al. *Nucleic Acids Res.* 11(5):1283–1295 (1983).
- Kadonaga et al. *J. Biol. Chem.* 259(4):2149–2154 (1984).
- Maniatis *Molecular Cloning* p. 433 (Sep., 1985).
- Fujisawa et al. *Nucleic Acids Res.* 11(11):3581–3591 (1983).
- Roberts *Promoters Structures and Function* Rodriguez, R. L. (Ed.) 452–461 (1982).
- Adams et al. *Biochemistry* 19:2711–2719 (1980).
- Haley et al. *DNA* 1:155–162 (1982).
- Gough et al. *Biochemistry* 19:2702–2710 (1980).
- Iserentant et al. *Gene* 9:1–12 (1980).
- Seidman et al. *Nature* 271:582–585 (1978).
- Stevens et al. *J. Mol. Biol.* 78:517–525 (1973).
- Deacon et al. *Biochem. Soc. Trans.* 4:818–820 (1976).
- Colman et al. *Cell* 17:517–526 (1979).
- Valle et al. *Nature* 291:338–340 (1981).
- Colman et al. *J. Mol. Biol.* 160:459–474 (Sep., 1982).
- Cowan et al. *J. Mol. Biol.* 90:691–701 (1974).
- Morrison et al. *J. Immunol.* 114:655–659 (1975).
- Mosmann et al. *J. Immunol.* 115:955–962 (1975).
- Levy et al. *Proc. Nat. Acad. Sci.* 75:2411–2415 (1978).
- Robertson et al. *Nature* 287:390–392 (1980).
- Mosmann et al. *Cell* 20:283–292 (1980).
- Wilde et al. *Eur. J. Immunol.* 10:462–467 (1980).
- Ochi et al. *Nature* 302:340–342 (Mar. 24, 1983).
- Kemp et al. in Nagley et al., Ed., *Manipulation and Expression of Genes in Eukaryotes* Proceedings of an International Conference, 12th International Congress of Biochemistry, in Australia Aug. 9–13, 1982 (1983).
- Picard et al. *Proc. Nat. Acad. Sci.* 80:417–421 (Jan., 1983).
- Oi et al. *Proc. Nat. Acad. Sci.* 80:825–829 (Feb., 1983).
- Hawley et al. *Proc. Nat. Acad. Sci.* 79:7425–7429 (Dec., 1982).
- Boss et al. *Immunology Today* 6(1):12–13 (1985).
- Lewin, Ed. *Genes* 3rd Edition, 359–360 (1987).
- Skerra et al. *Science* 240:1038–1040 (1988).
- Kohler et al. *Eur. J. Immunol.* 6:292–295 (1976).
- Adaiz et al., “Engineering Antibodies for Therapy” *Immunological Reviews* 130:5–40 (1992).
- Adelman et al., “In Vitro Deletional Mutagenesis for Bacterial Production of the 20,000-Dalton Form of Human Pituitary Growth Hormone” *DNA* 2(3):183–193 (1983).
- Adetugbo, K., “Spontaneous Somatic Mutations” *Journal of Biological Chemistry* 253(17):6076–6080 (1978).
- Alt et al., “Activity of Multiple Light Chain Genes in Murine Myeloma Cells Producing a Single, Functional Light Chain” *Cell* 21:1–12 (Aug. 1980).
- Alt et al., “Joining of immunoglobulin heavy chain gene segments: Implications from a chromosome with evidence of three D–J_H fusions” *Proc. Natl. Acad. Sci. USA* 79:4118–4122 (Jul. 1982).
- Alt et al., “Multiple Immunoglobulin Heavy-Chain Gene Transcripts in Abelson Murine Leukemia Virus-Transformed Lymphoid Cell Lines” *Molecular & Cellular Biology* 2(4):386–400 (Apr. 1982).
- Alt et al., “Organization and Reorganization of Immunoglobulin Genes in A-MuLV-Transformed Cells: Rearrangement of Heavy but Not Light Chain Genes” *Cell* 27:381–390 (Dec. 1981).
- Altenburger et al., “Functional and non-functional joining in immunoglobulin light chain genes of a mouse myeloma” *Nature* 287:603–607 (Oct. 16, 1980).
- Amzel and Poljak, “Three-dimensional structure of immunoglobulins” *Ann. Rev. Biochem.* 48:961–997 (1979).
- Astaldi et al., “Increase of hybridoma formation by human lymphocytes after stimulation in vitro; effect of antigen, endothelial cells, and PWM” *J. Immunol.* 128(6):2539–2542 (1982).
- August, “Monoclonal Antibodies—I: Discussion” *Cell Fusion: Gene Transfer and Transformation*, Beers et al. pp. 345–351 (1984).
- Barnett-Foster and Painter, “The interaction of the Fab fragment of rabbit anti-sheep red cell IgG with guinea pig macrophages, and human monocytes and granulocytes” *Molecular Immunology* 19(2):247–252 (1982).
- Bernard and Gough, “Nucleotide sequence of immunoglobulin heavy chain joining segments between translocated V_H and μ constant region genes” *Proc. Natl. Acad. Sci. USA* 77(6):3630–3634 (1980).
- Bernard et al., “Plasmacytomas with more than one immunoglobulin κ mRNA: Implications for allelic exclusion” *Proc. Natl. Acad. Sci. USA* 78(9):5812–5816 (Sep. 1981).
- Bernstein et al., “Monoclonal Antibody Therapy of Mouse Leukemia” *Monoclonal Antibodies*, Kennett et al., Plenum Press pp. 275–291 (1980).
- Better and Horwitz, “Expression of engineered antibodies and antibody fragments in microorganisms” *Methods in Enzymology* 178:476–496 (1989).
- Better et al., “Escherichia coli secretion of an active chimeric antibody fragment” *Science* 240:1041–1043 (1988).
- Bevan et al., “Biosynthesis of immunoglobulins” *Progress In Biophysics and Molecular Biology*, Butler and Noble, Pergamon Press pp. 133–162 (1972).
- Birshtein et al., “Effects of immunoglobulin structure on Fc receptor binding: a mouse myeloma variant immunoglobulin with a γ 2b– γ 2a hybrid heavy chain having a complete γ 2a Fc region fails to bind a γ 2a Fc receptors on mouse macrophages” *J. Immunol.* 129(2):610–614 (1982).
- Blythman et al., “Immunotoxins: hybrid molecules of monoclonal antibodies and a toxin subunit specifically kill tumour cells” *Nature* 290:145–146 (1981).
- Bobrzcka et al., “The method of controlled rearrangement of protein disulphides and its use for synthesis of chimeric immunoglobulin G” *Immunology Letters* 2:151–155 (1980).
- Bock et al., “Cloning and expression of the cDNA for human antithrombin III” *Nucleic Acids Research* 10(24):8113–8125 (1982).

Sanofi/Regeneron Ex. 1001, pg 2

- Bock et al., "Hybridization-selected translation of *Bombyx mori* high-cysteine chorion proteins in *Xenopus laevis* oocytes" *Proc. Natl. Acad. Sci. USA* 79:1032–1036 (1982).
- Boss et al., "Assembly of functional antibodies from immunoglobulin heavy and light chains synthesised in *E. coli*" *Nucleic Acids Research* 12(9):3791–3806 (1984).
- Boss et al., "Production of immunoglobulin molecules in *Escherichia coli*" *J. Cell. Biochem.* (Abstract Only) Supplement 7A:140 (0364) (1983).
- Bothwell et al., "Dual expression of λ genes in the MOPC-315 plasmacytoma" *Nature* 290:65–67 (1981).
- Bothwell et al., "Heavy chain variable region contribution to the NP^b family of antibodies: somatic mutation evident in a $\gamma 2a$ variable region" *Cell* 24:625–637 (1981).
- Bothwell et al., "Somatic variants of murine immunoglobulin α light chains" *Nature* 298:380–382 (Jul. 22, 1982).
- Boulian et al., "Production of functional chimaeric mouse/human antibody" *Nature* 312:643–646 (Dec. 13, 1984).
- Boulian et al., "The production of chimeric mouse/human antibodies" *Abstracts of papers presented at the meeting on Cellular and Molecular Biology of Neoplasia* (Abstract only) pp. #25 (1983).
- Boyd et al., "Human monoclonal antibodies—production and potential" *Trends in Biotechnology* 2(3):70–77 (1984).
- Boyden, Alan, "Homology and Analogy" *Science* 164:455–456 (Apr. 1969).
- Boylston et al., "Production of human IgM anti-D in tissue culture by EB-virus-transformed lymphocytes" *Scand. J. Immunol.* 12:355–358 (1980).
- Bruggemann et al., "Immunoglobulin V region variants in hybridoma cells. I. Isolation of a variant with altered idiotypic and antigen binding specificity" *EMBO Journal* 1(5):629–634 (1982).
- Buchner et al., "Renaturation, purification and characterization of recombinant Fab-fragments produced in *Escherichia coli*" *Bio/Technology* 9:157–162 (1991).
- Burrows et al., "Evidence that murine pre-B cells synthesize σ heavy chains but no light chains" *Nature* 280:838–841 (Aug. 30, 1979).
- Cabilly et al., "Generation of Antibody Activity from Immunoglobulin Polypeptide Chains Produced in *Escherichia coli*" *Proc. Natl. Acad. Sci. USA* 81:3273–3277 (1984).
- Chang et al., "Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid" *J. Bacteriol.* 134(3):1141–1156 (1978).
- Cheng et al., "Effect of deglycosylation on the binding and immunoreactivity of human thyroxine-binding globulin" *Journal of Biological Chemistry* 254(18):8830–8835 (Sep. 25, 1979).
- Choi et al., "RNA splicing generates a variant light chain from an aberrantly rearranged κ gene" *Nature* 286:776–779 (Aug. 21, 1980).
- Coffino and Laskov, "Immunoglobulin production: method for quantitatively detecting variant myeloma cells" *Science* 167:186–188 (1970).
- Coffino et al., "Suppression of Immunoglobulin Synthesis by Cellular Hybridization" *Nature New Biology* 231:87–90 (May 19, 1971).
- Cook and Scharff, "Antigen-binding mutants of mouse myeloma cells" *Proc. Natl. Acad. Sci. USA* 7(12):5687–5691 (1977).
- Cook et al., "Somatic mutation in a cultured mouse myeloma cell affects antigen binding" *Proc. Natl. Acad. Sci. USA* 79:1240–1244 (1982).
- Cotton and Milstein, "Fusion of two immunoglobulin-producing myeloma cells" *Nature* 244:42–43 (Jul. 6, 1973).
- Crews et al., "A Single VH Gene Segment Encodes the Immune Response to Phosphorylcholine: Somatic Mutation Is Correlated with the Class of the Antibody" *Cell* 25:59–66 (1981).
- Croce et al., "Production of human hybridomas secreting antibodies to measles virus" *Nature* 288:488–489 (1980).
- Dangl et al., "Segmental flexibility and complement fixation of genetically engineered chimeric human, rabbit and mouse antibodies" *The EMBO Journal* 7(7):1989–1994 (1988).
- Dangl, "Rapid isolation of cloned isotype switch variants using fluorescence activated cell sorting" *Cytometry* 2(6):395–401 (1982).
- De Bernardez-Clark and Georgiou, "Inclusion bodies and recovery of proteins from the aggregated state" *Protein Refolding* Chapter 1:1–20 (1991).
- De Boer et al., "Construction of a Tandem trp-lac Promoter and a Hybrid trp-lac Promoter for Efficient and Controlled Expression of the Human Growth hormone Gene in *Escherichia coli*" *Promoters, Structure and Function* (Praeger Publishers, R. Rodriguez and M. Chamberline, eds.) pp. 462–481 (1982).
- Dildrop et al., "Immunoglobulin V region variants in hybridoma cells. II. Recombination between V genes" *EMBO Journal* 1(5):635–640 (1982).
- Dover, Gabby, "Nonhomologous Views of a Terminology Muddle" *Cell* 51:515–516 (Nov. 20, 1987).
- Duyvesteyn and De Waard, "A new sequence-specific endonuclease from a thermophilic cyanobacterium, *mastigocladus laminosus*" *FEBS Letters* 111(2):423–426 (1980).
- Duyvesteyn et al., "Sequence-specific endonucleases in strains of *anabaena* and *nostoc*" *Arch. Microbiol.* 134:276–281 (1983).
- Early and Hood, "Mouse immunoglobulin genes" *Genetic Engineering, Principles and Methods*, Setlow and Hollaender, N.Y. and London, UK:Plenum Press vol. 3:157–188 (1981).
- Early et al., "Allelic Exclusion and Nonproductive Immunoglobulin Gene Rearrangements" *Cell* 24:1–3 (Apr. 1981).
- Early et al., "An immunoglobulin heavy chain variable region gene is generated from three segments of DNA: V_H D and J_H" *Cell* 19:981–992 (1980).
- Edelman, G., "Antibody structure and molecular immunology" *Annals of the New York Academy of Sciences* 190:5–25 (1971).
- Edwards et al., "A human-human hybridoma system based on a fast-growing mutant of the ARH-77 plasma cell leukemia-derived line" *European J. Immunol.* 12:641–648 (1982).
- Ellison et al., "Linkage and sequence homology of two human immunoglobulin gamma heavy chain constant region genes" *Proc. Natl. Acad. Sci. USA* 79:1984–1988 (Mar. 1982).
- Ellison et al., "Nucleotide sequence of a human immunoglobulin C_{v4} gene" *DNA* 1(1):11–18 (1981).
- Ellison et al., "The nucleotide sequence of a human immunoglobulin C_{v1} gene" *Nucleic Acids Research* 10(13):4071–4079 (1982).

- Eshhar et al., "Induction of secretion of IgM from cells of the B cell line 38C-13 by somatic cell hybridization" *J. Immunol.* 122(6):2430-2434 (1979).
- Feiss et al., "Separate sites for binding and nicking of bacteriophage λ DNA by terminase" *Proc. Natl. Acad. Sci. USA* 80:955-959 (1983).
- Fiers et al., "Complete Nucleotide Sequence of SV40 DNA" *Nature* 273:113-120 (May 11, 1978).
- Gillies et al., "A tissue-specific transcription enhancer element is located in the major intron of a rearranged immunoglobulin heavy chain gene" *Cell* 33:717-728 (1983).
- Gillies et al., "Antigen Binding and Biological Activities of Engineered Mutant Chimeric Antibodies with Human Tumor Specificities" *Hum. Antibod. Hybridomas* 1(1):47-54 (1990).
- Givol et al., "Diversity of germ-line immunoglobulin V_H genes" *Nature* 292:426-430 (1981).
- Gluzman, Yakov, "SV40-Transformed Simian Cells Support the Replication of Early SV40 Mutants" *Cell* 23:175-182 (Jan. 1981).
- Goldsby et al., "Hybrid cell lines with T-cell characteristics" *Nature* 267:707-708 (Jun. 23, 1977).
- Graham and van der Eb, "A New Technique for the Assay of Infectivity of Human Adenovirus 5 DNA" *Virology* 52:456-467 (1973).
- Gritz and Davies, "Plasmid-encoded hygromycin B resistance: the sequence of hygromycin B phosphotransferase gene and its expression in *Escherichia coli* and *Saccharomyces cerevisiae*" *Gene* 25:179-188 (1983).
- Gupta et al., "General Orientation of Human Lymphocyte Subpopulations" *Clinical Immunobiol.*, Bach and Good, Academic Press vol. 4:1-18 (1980).
- Hedin et al., "Specificities and Binding Properties of Eight Monoclonal Antibodies Against Carcinoembryonic Antigen" *Molecular Immunology* 19:1641-1648 (1982).
- Herlyn et al., "Inhibition of Growth of Colorectal Carcinoma in Nude Mice by Monoclonal Antibody" *Cancer Research* 40:717-721 (Mar. 1980).
- Herzenberg et al., "Hybridoma Variants Affecting Isotype, Antigen Binding, and Idiotype" *Biotechnology in Diagnostics*, Koprowski et al. vol. 21:3-16 (1985).
- 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).
- Honjo et al., "Cloning and complete nucleotide sequence of mouse immunoglobulin $\gamma 1$ Chain Gene" *Cell* 18:559-568 (1979).
- Honjo et al., "Rearrangements of Immunoglobulin Genes during Differentiation and Evolution" *Immunological Rev.* 59:33-67 (1981).
- Hood et al., "Antibodies" *Immunology*, Forkner and Moore, Philippines: The Benjamin/Cummings Publishing Co., Inc., Chapter 3, pp. 199-221 (1978).
- Horwitz et al., "Secretion of functional antibody and Fab fragment from yeast cells" *Proc. Natl. Acad. Sci. USA* 85:8678-8682 (1988).
- Houghton et al., "Detection of cell surface and intracellular antigens by human monoclonal antibodies" *Journal of Experimental Medicine* 158:53-65 (1983).
- Howard et al., "A Rapid Method for the Detection of Antibodies to Cell Surface Antigens: A Solid Phase Radioimmunoassay Using Cell Membranes" *Journal of Immunological Methods* 38:75-84 (1980).
- Howard et al., "Long-term culture of normal mouse B lymphocytes" *Proc. Natl. Acad. Sci. USA* 78(9):5788-5792 (Sep. 1981).
- Hughes and Murray, "The nucleotide sequences recognized by endonucleases AvI and AvII from *Anabaena variabilis*" *Biochemical Journal* 185:65-75 (1980).
- Hunkapiller et al., "The growing immunoglobulin gene superfamily" *Nature* 323:15-16 (1986).
- "Immunoglobulin molecules and genes" *Microbiology Including Immunology and Molecular Genetics*, Third edition, Harper International Edition vol. Chapter 17:338-379.
- Inouye et al., "Signal Sequence of Alkaline Phosphatase of *Escherichia coli*" *J. Bacteriol.* 149:434 (1982).
- Isenman et al., "The structure and function of immunoglobulin domains" *J. Immunol.* 114(6):1726-1929 (1975).
- Itakura and Riggs, "Chemical DNA synthesis and recombinant DNA studies" *Science* 209:1401-1405 (1980).
- Jaton et al., "Conformational changes induced in a homogeneous anti-type III pneumococcal antibody by oligosaccharides of increasing size" *Biochemistry* 14(24):5312-5315 (1975).
- Johnson et al., "The Complete V Domain Amino Acid Sequences of Two Myeloma Inulin-Binding Proteins" *Journal of Immunology* 128:302-307 (1982).
- Jones, P.T. et al., "Replacing the Complementarity-determining Regions in a Human Antibody with Those From a Mouse" *Nature* 321:522-525 (May 29, 1986).
- Kabat, E., "Activation of the complement system and its effect on cells" *Structural Concepts in Immunology and Immunoochemistry*, Second edition, Holt, Rinehart and Winston vol. Chapter 13:413-435 (1976).
- Kaivarainen et al., "Hapten-induced changes in pig anti-Dansyl antibodies revealed by EPR spectra of spin-labelled antibodies" *Immunol. Letters* 3:5-11 (1981).
- Kalderon et al., "Deletion loop mutagenesis: a novel method for the construction of point mutations using deletion mutants" *Nucl. Acids Res.* 10:5161-5168 (1982).
- Keshet et al., "Cloning of bovine growth hormone gene and its expression in bacteria" *Nucleic Acids Research* 9:19-30 (1981).
- Kipps et al., "Allotype Switch Variants in Cultured Monoclonal Producing Hybridomas" *Journal of Cellular Biochemistry* (abstract only) pp. 163 (-1984).
- Kitai et al., "Extracellular production of human immunoglobulin G FC region" *Microbiol. Biotechnol.* 28(1):52-56 (1988).
- Klein et al., "Expression of biological effector functions by immunoglobulin G molecules lacking the hinge region" *Proc. Natl. Acad. Sci. USA* 78(1):524-528 (1981).
- Kohl and Moore, "Human antibody-dependent cellular cytotoxicity and natural killer cytotoxicity to herpes simplex virus-infected autologous and allogeneic cells" *Immunology* 48:187-193 (1983).
- Kohler et al., "Derivation of Specific Antibody-producing Tissue Culture and Tumor Lines by Cell Fusion" *European Journal of Immunology* 6:511-519 (1976).
- Kohler, G. and Milstein, C., "Continuous Cultures of Fused Cells Secreting Antibody of Predefined Specificity" *Nature* 256:495-497 (Aug. 7, 1975).
- Konieczny et al., "The combination of IgM subunits and proteolytic IgG fragments by controlled formation of inter-chain disulphides" *Haematologia* 14(1):95-99 (1981).

Sanofi/Regeneron Ex. 1001, pg 4

- Koskimies, S., "Human lymphoblastoid cell line producing specific antibody against Rh-antigen D" *Scand. J. Immunol.* 11:73-77 (1980).
- Kuehl, W.M., "Light chain fragments: aberrant expression of immunoglobulin genes" *TIBS* pp. 206-208 (Aug. 1981).
- Kupchik et al., "Monoclonal Antibodies to Carcinoembryonic Antigen Produced by Somatic Cell Fusion" *Cancer Research* 41:3306-3310 (Sep. 1981).
- Kwan, "Two Kappa Immunoglobulin Genes Are Expressed in the Myeloma S107" *Cell* 26:57-66 (Oct. 1981).
- Larson et al., "Saccharomyces cerevisiae actin-Escherichia coli lacZ gene fusions: synthetic-oligonucleotide-mediated deletion of the 309 base pair intervening sequence in the actin gene" *Gene* 22:31-39 (1983).
- Laskov and Scharff, "Synthesis, assembly, and secretion of gamma globulin by mouse myeloma cells" *Journal of Experimental Medicine* 131(3):515-541 (1970).
- Laskov et al., "Induction of amplified synthesis and secretion of IgM by fusion of murine B Lymphoma with myeloma cells" *Proc. Natl. Acad. Sci. USA* 76(2):915-919 (Feb. 1979).
- Lau and Doolittle, "Aqu I: a more easily purified isoschizomer of AVA I" *FEBS Letters* 121(2):200-202 (1980).
- Leder, P., "The genetics of antibody diversity" *Scientific America* 246:72-83 (1982).
- Lee et al., "Characterization of the Gene Encoding Heat-Stable Toxin II and Preliminary Molecule Epidemiological Studies of Enterotoxigenic *Escherichia coli* Heat-Stable Toxin II Producers" *Infection and Immunity* 42:264-268 (Oct. 1983).
- Letherbarrow et al., "Effector functions of a monoclonal aglycosylated mouse IgG_{2a}:binding and activation of complement component C1 and interaction with human monocyte Fc receptor" *Molec. Immunol.* 22(4):407-415 (1985).
- Levy and Miller, "Tumor therapy with monoclonal antibodies" *Fed. Proc.* 42:2650-2656 (1983).
- Lewin, Roger, "When Does Homology Mean Something Else?" *Science* 237:1570 (1987).
- Liu et al., "Chimeric mouse-human IgG1 antibody that can mediate lysis of cancer cells" *Proc. Natl. Acad. Sci. USA* 84:3439-3443 (May 1987).
- Liu, Pinghui V., "Pseudomonas Toxins" *J. Infect. Dis.* 130:S94-S99 (1974).
- Maniatis et al. *Molecular Cloning: A Laboratory Manual*, 1st edition, New York: Cold Spring Harbor Lab Press, Chapter 12, pps. selected pages (1982).
- Maniatis, T., "Recombinant DNA procedures in the study of eukaryotic genes" *Cell Biol.* 3:563-608 (1980).
- Margulies et al., "Regulation of immunoglobulin expression in mouse myeloma cells" *Immunoglobulin Expression* pp. 781-791 (1977).
- Margulies et al., "Somatic Cell Hybridization of Mouse Myeloma" *Cell* 8:405-415 (Jul. 1976).
- Martinis et al., "Monoclonal antibodies with dual antigen specificity" *Oncology* pp. 311-316.
- Mather et al., "Transcriptional regulation of immunoglobulin V genes" *Nucleic Acids Research* 9(24):6855-6867 (1981).
- Matsuuchi and Morrison, "Antigen binding variants of mouse plasmacytoma J558" *Fed. Proc.* (Abstract only) 37:1763 (2703) (1978).
- Max et al., "Variation in the Crossover Point of Kappa Immunoglobulin Gene V-J Recombination: Evidence from a Cryptic Gene" *Cell* 21:793-799 (Oct. 1980).
- McCutchan et al., "Enhancement of the Infectivity of Simian Virus 40 Deoxyribonucleic Acid with Diethylaminoethyl-Dextran" *J. Natl. Cancer Institute* 41:351-356 (1968).
- Melchers, F., "Biosynthesis of the carbohydrate portion of immunoglobulin radiochemical and chemical analysis of the carbohydrate moieties of two myeloma proteins purified from different subcellular fractions of plasma cells" *Biochemistry* 10(4):653-659 (1971).
- Mertz et al., "Defective Simian Virus 40 Genomes: Isolation and Growth of Individual Clones" *Virology* 62:112-124 (1974).
- Messing et al., "A System for Shotgun DNA Sequencing" *Nucleic Acids Research* 9(2):309-321 (1981).
- Milstein et al., "Clonal Variants of Myeloma Cells" *Progress in Immunology II* 1:157-168 (1974).
- Mohit and Fan, "Hybrid Cell Line from a Cloned Immunoglobulin-Producing Mouse Myeloma and a Nonproducing Mouse Lymphoma" *Science* 171:75-77 (Jan. 8, 1971).
- Morrison and Scharff, "Mutational events in mouse myeloma cells" *Critical Reviews in Immunology* 3(1):1-22 (1981).
- Morrison et al., "Chimeric Human Antibody Molecules: Mouse Antigen-binding Domains with Human Constant Region Domains" *Proc. Natl. Acad. Sci. USA* 81:6851-6855 (Nov. 1984).
- Morrison et al., "Genetically Engineered Antibody Molecules" *Adv. Immunol.* 44:65-92 (1989).
- Morrison et al., "Transfectedomas Provide Antibodies With Novel Structures and Functions" *Antibodies: Structure, Synthesis, Function, and Immunologic Intervention in Disease*, Szentivanyi et al. pp. 167-178 (1987).
- Morrison et al., "Transfer and expression of immunoglobulin genes" *Annual Review of Immunology* 2:239-256 (1984).
- Morrison, S. L., "Transfectedomas Provide Novel Chimeric Antibodies" *Science* 229:1202-1207 (Sep. 20, 1985).
- Movva et al., "Amino Acid Sequence of the Signal Peptide of ompA Protein, a Major Outer Membrane Protein of *Escherichia coli*" *The Journal of Biological Chemistry* 255:27-29 (Jan. 10, 1980).
- Nakabayashi et al., "The transforming function of bovine papillomavirus DNA" *Proc. Natl. Acad. Sci. USA* 80:5832-5836 (1983).
- Neuberger et al., "A hapten-specific chimaeric IgE antibody with human physiological effector function" *Nature* 314:268-270 (Mar. 21, 1985).
- Neuberger et al., "Recombinant Antibodies Possessing Novel Effector Functions" *Nature* 312:604-608 (Dec. 13, 1984).
- Neuberger, "Switch from hapten-specific immunoglobulin M to immunoglobulin D secretion in a hybrid mouse cell line" *Proc. Natl. Acad. Sci. USA* 78(2):1138-1142 (1981).
- Ochi et al., "Functional immunoglobulin M production after transfection of cloned immunoglobulin heavy and light chain genes into lymphoid cells" *Proc. Natl. Acad. Sci. USA* 80:6351-6355 (1983).
- Oi et al., "Correlation between segmental flexibility and effector function of antibodies" *Nature* 307:136-140 (1984).
- Oi et al., "Hybridoma Antibody-Producing Switch Variants: A Variant Lacking the CH1 Domain" *Cell Fusion: Gene Transfer and Transformation*, R.F. Beers, Jr. and E.G. Bassett, Raven Press, New York pp. 281-287 (1984).

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