ANTIGEN BINDING MOLECULES WITH INCREASED Fc RECEPTOR BINDING AFFINITY AND EFFECTOR FUNCTION

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] The present invention relates to antigen binding molecules (ABMs). In particular embodiments, the present invention relates to recombinant monoclonal antibodies, including chimeric, primatized or humanized antibodies specific for human CD20. In addition, the present invention relates to nucleic acid molecules encoding such ABMs, and vectors and host cells comprising such nucleic acid molecules. The invention further relates to methods for producing the ABMs of the invention, and to methods of using these ABMs in treatment of disease. In addition, the present invention relates to ABMs with modified glycosylation having improved therapeutic properties, including antibodies with increased Fc receptor binding and increased effector function.

Background Art

The Immune System and Anti-CD20 Antibodies

[0002] The immune system of vertebrates, including humans, consists of a number of organs and cell types, which have evolved to accurately and specifically recognize, bind and destroy invading foreign microorganisms ("antigens"). Lymphocytes are critical for the proper function of the immune system. These cells are produced in the thymus, spleen and bone marrow (adult) and represent about 30% of the total white blood cells present in the circulatory system of adult humans. There are two major sub-populations of lymphocytes: T cells and B cells. T cells are responsible for cell mediated immunity, while B cells are responsible for antibody production (humoral immunity). However, in a typical immune response, T cells and B cells function interdependently: T cells are activated when the T cell receptor binds to fragments of an antigen that are bound to major histocompatability complex ("MHC") glycoproteins on the surface of an antigen presenting cell; such activation causes release of biological

mediators ("interleukins"), which stimulate B cells to differentiate and produce antibodies ("immunoglobulins") against the antigen.

[0003] Each B cell within the host expresses an antibody of one particular type and specificity, and different B cells express antibodies specific for different antigens. B cell proliferation and antibody production spike as a reaction to a foreign antigen, and both typically cease (or substantially decrease) once the foreign antigen has been neutralized. Occasionally, however, proliferation of a particular B cell will continue unabated; such proliferation can result in a cancer referred to as "B cell lymphoma."

[0004] T cells and B cells both comprise cell surface proteins which can be utilized as "markers" for differentiation and identification. One such human B cell marker is the human B lymphocyte-restricted differentiation antigen Bp35, referred to as "CD20." CD20 is expressed during early pre-B cell development and remains until plasma cell differentiation. Specifically, the CD20 molecule may regulate a step in the activation process that is required for cell cycle initiation and differentiation and is usually expressed at very high levels on neoplastic ("tumor") B cells. Because CD20 is present at high levels on "malignant" B cells, *i.e.*, those B cells whose unabated proliferation can lead to B cell lymphoma, the CD20 surface antigen has the potential of serving as a candidate for "targeting" of B cell lymphomas.

[0005] In essence, such targeting can be generalized as follows: antibodies specific to the CD20 surface antigen of B cells are introduced into a patient, by injection, for example. These anti-CD20 antibodies specifically bind to the CD20 cell surface antigen of (ostensibly) both normal and malignant B cells; the anti-CD20 antibody bound to the CD20 surface antigen may lead to the destruction and depletion of neoplastic B cells. Additionally, chemical agents or radioactive labels having the potential to destroy the tumor can be conjugated to the anti-CD20 antibody such that the agent is specifically "delivered" to e.g., the neoplastic B cells. Irrespective of the approach, a primary goal is to destroy the tumor: the specific approach can be determined by the particular anti-CD20 antibody which is utilized and, thus, the available approaches to targeting the CD20 antigen can vary considerably.

[0006] Unconjugated monoclonal antibodies (mAbs) can be useful medicines for the treatment of cancer, as demonstrated by the U.S. Food and Drug Administration's approval of Rituximab (Rituxan™; IDEC Pharmaceuticals, San Diego, CA, and Genentech Inc., San Francisco, CA), for the treatment of CD20 positive B-cell, low-grade or follicular Non-Hodgkin's lymphoma, Trastuzumab (Herceptin™; Genentech Inc,) for the treatment of advanced breast cancer (Grillo-Lopez, A.-J., *et al., Semin. Oncol. 26*:66-73 (1999); Goldenberg, M. M., *Clin. Ther. 21*:309-18 (1999)), Gemtuzumab (Mylotarg™, Celltech/Wyeth-Ayerst) for the treatment of relapsed acute myeloid leukemia, and Alemtuzumab (CAMPATH™, Millenium Pharmaceuticals/Schering AG) for the treatment of B cell chronic lymphocytic leukemia. The success of these products relies not only on their efficacy but also on their outstanding safety profiles (Grillo-Lopez, A.-J., *et al., Semin. Oncol. 26*:66-73 (1999); Goldenberg, M. M., *Clin. Ther. 21*:309-18 (1999)). In spite of the achievements of these drugs, there is currently a large interest in obtaining higher specific antibody activity than what is typically afforded by unconjugated mAb therapy. The murine monoclonal antibody, B-Ly1, is another antibody known to be specific to human CD20. (Poppema, S. and Visser, L., *Biotest Bulletin 3*: 131-139 (1987)).

[0007] The results of a number of studies suggest that Fc-receptor-dependent mechanisms contribute substantially to the action of cytotoxic antibodies against tumors and indicate that an optimal antibody against tumors would bind preferentially to activation Fc receptors and minimally to the inhibitory partner FcγRIIB. (Clynes, R. A., *et al., Nature Medicine 6(4)*:443-446 (2000); Kalergis, A.M., and Ravetch, J. V., *J. Exp. Med. 195(12)*:1653-1659 (June 2002). For example, the results of at least one study suggest that the FcγRIIIa receptor in particular is strongly associated with the efficacy of antibody therapy. (Cartron, G., *et al., Blood 99(3)*:754-757 (February 2002)). That study showed that patients homozygous for FcγRIIIa have a better response to Rituximab than heterozygous patients. The authors concluded that the superior response was due to better in vivo binding of the antibody to FcγRIIIa, which resulted in better ADCC activity against lymphoma cells. (Cartron, G., *et al., Blood 99(3)*:754-757 (February 2002)).

[0008] Various attempts to target the CD20 surface antigen have been reported. Murine (mouse) monoclonal antibody 1F5 (an anti-CD20 antibody) was reportedly administered by continuous intravenous infusion to B cell lymphoma patients. Extremely high levels (>2 grams) of 1F5 were reportedly required to deplete circulating tumor cells, and the results were described as being "transient." Press et al., "Monoclonal Antibody 1F5 (Anti-CD20) Serotherapy of Human B-Cell Lymphomas." *Blood* 69/2:584-591 (1987). A potential problem with this approach is that non-human monoclonal antibodies (e.g., murine monoclonal antibodies) typically lack human effector functionality, i.e., they are unable to, *inter alia*, mediate complement dependent lysis or lyse human target cells through antibody dependent cellular toxicity or Fc-receptor mediated phagocytosis. Furthermore, non-human monoclonal antibodies can be recognized by the human host as a foreign protein; therefore, repeated injections of such foreign antibodies can lead to the induction of immune responses leading to harmful hypersensitivity reactions. For murine-based monoclonal antibodies, this is often referred to as a Human Anti-Mouse Antibody response, or "HAMA" response. Additionally, these "foreign" antibodies can be attacked by the immune system of the host such that they are, in effect, neutralized before they reach their target site.

[0009] Another reported approach at improving the ability of murine monoclonal antibodies to be effective in the treatment of B-cell disorders has been to conjugate a radioactive label or toxin to the antibody such that the label or toxin is localized at the tumor site. For example, the above-referenced 1F5 antibody has been "labeled" with iodine-131 ("^{131}I") and was reportedly evaluated for biodistribution in two patients. See Eary, J. F. et al., "Imaging and Treatment of B-Cell Lymphoma" J. Nuc. Med. 31/8:1257-1268 (1990); see also, Press, O. W. et al., "Treatment of Refractory Non-Hodgkin's Lymphoma with Radiolabeled MB-1 (Anti-CD37) Antibody" J. Clin. Onc. 7/8:1027-1038 (1989) (indication that one patient treated with ^{131}I-labeled IF-5 achieved a "partial response"); Goldenberg, D. M. et al., "Targeting, Dosimetry and Radioimmunotherapy of B-Cell Lymphomas with Iodine-131-Labeled LL2 Monoclonal Antibody" *J. Clin. Onc.* 9/4:548-564 (1991) (three of eight patients receiving multiple injections

reported to have developed a HAMA response); Appelbaum, F. R. "Radiolabeled Monoclonal Antibodies in the Treatment of Non-Hodgkin's Lymphoma" *Hem./Onc. Clinics of N. A.* 5/5:1013-1025 (1991) (review article); Press, O. W. et al "Radiolabeled-Antibody Therapy of B-Cell Lymphoma with Autologous Bone Marrow Support. "*New England J. Med.* 329/17: 1219-1223 (1993) (iodine-131 labeled anti-CD20 antibody IF5 and B1); and Kaminski, M. G. et al "Radioimmunotherapy of B-Cell Lymphoma with ^{131}I Anti-B1 (Anti-CD20) Antibody". *New England J. Med.* 329/7(1993) (iodine-131 labeled anti-CD20 antibody B1; hereinafter "Kaminski"). Toxins (i.e., chemotherapeutic agents such as doxorubicin or mitomycin C) have also been conjugated to antibodies. See, for example, PCT published application WO 92/07466 (published May 14, 1992).

[0010] Chimeric antibodies comprising portions of antibodies from two or more different species (e.g., mouse and human) have been developed as an alternative to "conjugated" antibodies. For example, Liu, A. Y. et al, "Production of a Mouse-Human Chimeric Monoclonal Antibody to CD20 with Potent Fc-Dependent Biologic Activity" J. Immun. 139/10:3521-3526 (1987), describes a mouse/human chimeric antibody directed against the CD20 antigen. See also, PCT Publication No. WO 88/04936. For example, rituximab (RITUXAN®), a chimeric anti-CD20, antibody has been approved for the treatment of non-Hodgkins lymphoma.

[0011] Given the expression of CD20 by B cell lymphomas, this antigen can serve as a candidate for "targeting" of such lymphomas. In essence, such targeting can be generalized as follows: antibodies specific for CD20 surface antigen on B cells are administered to a patient. These anti-CD20 antibodies specifically bind to the CD20 antigen of (ostensibly) both normal and malignant B cells, and the antibody bound to the CD20 on the cell surface results in the destruction and depletion of tumorigenic B cells. Additionally, chemical agents, cytotoxins or radioactive agents may be directly or indirectly attached to the anti-CD20 antibody such that the agent is selectively "delivered" to the CD20 antigen expressing B cells. With both of these approaches, the primary goal is to destroy the tumor. The specific approach will depend upon the particular anti-CD20

antibody that is utilized. Thus, it is apparent that the various approaches for targeting the CD20 antigen can vary considerably.

[0012] The rituximab (RITUXAN®) antibody is a genetically engineered chimeric human gamma 1 murine constant domain containing monoclonal antibody directed against the human CD20 antigen. This chimeric antibody contains human gamma 1 constant domains and is identified by the name "C2B8" in U.S. Pat. No. 5,736,137 (Andersen et. al.) issued on April 17, 1998, assigned to IDEC Pharmaceuticals Corporation. RITUXAN® is approved for the treatment of patients with relapsed or refracting low-grade or follicular, CD20 positive, B cell non-Hodgkin's lymphoma. In vitro mechanism of action studies have shown that RITUXAN® exhibits human complement--dependent cytotoxicity (CDC) (Reff et. al, *Blood* 83(2): 435-445 (1994)). Additionally, it exhibits significant activity in assays that measure antibody--dependent cellular cytotoxicity (ADCC). RITUXAN® has been shown to possess anti-proliferative activity in thymidine incorporation assays and a limited ability to induce apoptosis directly, whereas CD20 antibodies do not (Maloney *et. al*, *Blood* 88 (10): 637a (1996)).

Antibody Glycosylation

[0013] The oligosaccharide component can significantly affect properties relevant to the efficacy of a therapeutic glycoprotein, including physical stability, resistance to protease attack, interactions with the immune system, pharmacokinetics, and specific biological activity. Such properties may depend not only on the presence or absence, but also on the specific structures, of oligosaccharides. Some generalizations between oligosaccharide structure and glycoprotein function can be made. For example, certain oligosaccharide structures mediate rapid clearance of the glycoprotein from the bloodstream through interactions with specific carbohydrate binding proteins, while others can be bound by antibodies and trigger undesired immune reactions. (Jenkins *et al.*, *Nature Biotechnol.* 14:975-81 (1996)).

[0014] Mammalian cells are the preferred hosts for production of therapeutic glycoproteins, due to their capability to glycosylate proteins in the most compatible form for human application. (Cumming *et al.*, *Glycobiology 1*:115-30

(1991); Jenkins *et al.*, *Nature Biotechnol.* 14:975-81 (1996)). Bacteria very rarely glycosylate proteins, and like other types of common hosts, such as yeasts, filamentous fungi, insect and plant cells, yield glycosylation patterns associated with rapid clearance from the blood stream, undesirable immune interactions, and in some specific cases, reduced biological activity. Among mammalian cells, Chinese hamster ovary (CHO) cells have been most commonly used during the last two decades. In addition to giving suitable glycosylation patterns, these cells allow consistent generation of genetically stable, highly productive clonal cell lines. They can be cultured to high densities in simple bioreactors using serum-free media, and permit the development of safe and reproducible bioprocesses. Other commonly used animal cells include baby hamster kidney (BHK) cells, NS0- and SP2/0-mouse myeloma cells. More recently, production from transgenic animals has also been tested. (Jenkins *et al.*, *Nature Biotechnol.* 14:975-81 (1996)).

[0015] All antibodies contain carbohydrate structures at conserved positions in the heavy chain constant regions, with each isotype possessing a distinct array of N-linked carbohydrate structures, which variably affect protein assembly, secretion or functional activity. (Wright, A., and Morrison, S. L., *Trends Biotech. 15*:26-32 (1997)). The structure of the attached N-linked carbohydrate varies considerably, depending on the degree of processing, and can include high-mannose, multiply-branched as well as biantennary complex oligosaccharides. (Wright, A., and Morrison, S. L., *Trends Biotech. 15*:26-32 (1997)). Typically, there is heterogeneous processing of the core oligosaccharide structures attached at a particular glycosylation site such that even monoclonal antibodies exist as multiple glycoforms. Likewise, it has been shown that major differences in antibody glycosylation occur between cell lines, and even minor differences are seen for a given cell line grown under different culture conditions. (Lifely, M. R. *et al.*, *Glycobiology 5(8)*:813-22 (1995)).

[0016] One way to obtain large increases in potency, while maintaining a simple production process and potentially avoiding significant, undesirable side effects, is to enhance the natural, cell-mediated effector functions of monoclonal antibodies by engineering their oligosaccharide component as described in

Umaña, P. *et al., Nature Biotechnol. 17*:176-180 (1999) and U.S. Pat. No. 6,602,684, the entire contents of which are hereby incorporated by reference in their entirety. IgGl type antibodies, the most commonly used antibodies in cancer immunotherapy, are glycoproteins that have a conserved N-linked glycosylation site at Asn297 in each CH2 domain. The two complex biantennary oligosaccharides attached to Asn297 are buried between the CH2 domains, forming extensive contacts with the polypeptide backbone, and their presence is essential for the antibody to mediate effector functions such as antibody dependent cellular cytotoxicity (ADCC) (Lifely, M. R., *et al., Glycobiology 5*:813-822 (1995); Jefferis, R., *et al., Immunol Rev. 163*:59-76 (1998); Wright, A. and Morrison, S. L., *Trends Biotechnol. 15*:26-32 (1997)).

[0017] The present inventors showed previously that overexpression in Chinese hamster ovary (CHO) cells of β(1,4)-N-acetylglucosaminyltransferase III ("GnTIII"), a glycosyltransferase catalyzing the formation of bisected oligosaccharides, significantly increases the *in vitro* ADCC activity of an anti-neuroblastoma chimeric monoclonal antibody (chCE7) produced by the engineered CHO cells. (*See* Umaña, P. *et al., Nature Biotechnol. 17*:176-180 (1999);and International Publication No. WO 99/54342, the entire contents of which are hereby incorporated by reference). The antibody chCE7 belongs to a large class of unconjugated mAbs which have high tumor affinity and specificity, but have too little potency to be clinically useful when produced in standard industrial cell lines lacking the GnTIII enzyme (Umana, P., *et al., Nature Biotechnol. 17*:176-180 (1999)). That study was the first to show that large increases of ADCC activity could be obtained by engineering the antibody-producing cells to express GnTIII, which also led to an increase in the proportion of constant region (Fc)-associated, bisected oligosaccharides, including bisected, nonfucosylated oligosaccharides, above the levels found in naturally-occurring antibodies.

[0018] There remains a need for enhanced therapeutic approaches targeting the CD20 antigen for the treatment of B cell lymphomas in primates, including, but not limited to, humans.

BRIEF SUMMARY OF THE INVENTION

[0019] Recognizing the tremendous therapeutic potential of antigen binding molecules (ABMs) that have the binding specificity of the murine B-Ly1 antibody and that have been glycoengineered to enhance Fc receptor binding affinity and effector function, the present inventors developed a method for producing such ABMs. *Inter alia*, this method involves producing recombinant, chimeric antibodies or chimeric fragments thereof. The efficacy of these ABMs is further enhanced by engineering the glycosylation profile of the antibody Fc region.

[0019a] In a first asepct, there is provided

an engineered Type II anti-CD20 antigen binding molecule having increased ADCC as a result of said engineering without substantial loss of ability to induce apoptosis of target cells, wherein said antigen binding molecule comprises a complementarity determining region (CDR) of the murine B-Ly1 antibody selected from the group consisting of: the heavy chain CDR1 of SEQ ID NO:15, SEQ ID NO:16, or SEQ ID NO:17; the heavy chain CDR2 of SEQ ID NO:25, SEQ ID NO:26, or SEQ ID NO:27; and the heavy chain CDR3 of SEQ ID NO:28; the light chain CDR1 of SEQ ID NO:18; the light chain CDR2 of SEQ ID NO:19; and the light chain CDR3 of SEQ ID NO:20.

[0019b] In a second aspect, there is provided

a host cell expressing at least one nucleic acid encoding a polypeptide having ß(1,4)-N-acetylglucosaminyltransferase III activity in an amount sufficient to engineer the Fc region of a polypeptide produced by said host cell, wherein said polypeptide is the antigen binding molecule as defined above.

[0019c] In a third aspect, there is provided

an isolated polynucleotide comprising a sequence that encodes the heavy chain variable region of claim 6, wherein said polynucleotide comprises a sequence having at least 80% identity to a sequence selected from the group consisting of: SEQ ID NO:29, SEQ ID

NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, and SEQ ID NO:71.

[0019d] In a fourth aspect, there is provided an isolated polynucleotide comprising a sequence that encodes the light chain variable region as defined above, wherein said polynucleotide comprises the sequence having at least 80% identity to SEQ ID NO: 75.

[0019e] In a fifth aspect, there is provided an expression vector comprising the polynucleotide as defined above.

[0019f] In a sixth aspect, there is provided the use of the antigen binding molecule as defined above for the manufacture of a medicament to treat a disorder treatable by B-cell depletion.

[0019g] In a seventh aspect, there is provided

a humanized Type II anti-CD20 antigen binding molecule comprising a heavy chain variable region comprising the B-Ly1 heavy chain CDR1 of SEQ ID NO:15, SEQ ID NO:16, or SEQ ID NO:17; heavy chain CDR2 of SEQ ID NO:25, SEQ ID NO:26, or SEQ ID NO:27; and heavy chain CDR3 of SEQ ID NO:28, wherein said antigen binding molecule induces higher levels of apoptosis when incubated with CD20-positive human cells relative to a control under identical conditions using the C2B8 chimeric IgG1 antibody with an identical sequence to rituximab.

[0019h] In an eighth aspect, there is provided an isolated polynucleotide comprising a sequence encodes the heavy chain variable region as defined above, wherein said polynucleotide comprises a sequence having at least

80% identity to a sequence selected from the group consisting of: SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, and SEQ ID NO:71.

[0019i] In a ninth aspect, there is provided an isolated polynucleotide comprising a sequence that encodes the light chain variable region as defined above, wherein said polynucleotide comprises the sequence having at least 80% identity to SEQ ID NO: 75.

[0019j] In a tenth aspect, there is provided an expression vector comprising the polynucleotide as defined above.

[0019k] In an eleventh aspect, there is provided a host cell comprising the expression vector of the tenth aspect.

[0019l] In a twelve aspect, there is provided the use of the antigen binding molecule as defined for the manufacture of a medicament to treat a disorder treatable by B-cell depletion.

]0019m] In a thirteeenth aspect, there is provided

a method for producing in a host cell an anti-CD20 antigen binding molecule having an Fc region with modified oligosaccharides and engineered to have increased effector function, said method comprising:

a. culturing a host cell engineered to express at least one nucleic acid encoding a polypeptide having ß(1,4)-N-acetylglucosaminyltransferase III activity under conditions

which permit the production of said antigen binding molecule, and which permit the modification of the oligosaccharides present on the Fc region of said antigen binding molecule; and

b. isolating said antibody;

wherein said antibody is a fusion protein that includes a polypeptide having a variable region sequence selected from the group consisting of SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, and SEQ ID NO:76, and a region equivalent to the Fc region of an immunoglobulin.

[0019n] In a fourteenth aspect, there is provided a pharmaceutical composition comprising the antigen binding molecule as defined above and a pharmaceutically acceptable carrier.

[0019o] In a fifteenth aspect, there is provided an antigen binding molecule as defined above for use as a medicament to treat a B-cell discorder.

[0020] In one embodiment, the disclosure is directed to an isolated polynucleotide comprising: (a) a sequence selected from a group consisting of : SEQ ID NO.:5, SEQ ID NO.: 6 and SEQ ID NO.: 7. (CDRs V_{H-1}) ; (b) a sequence selected from a group consisting of: SEQ ID NO. :21, SEQ ID NO.:22 and SEQ ID NO.:23. (CDRs V_{H-2}) ; and SEQ ID NO:24. In another embodiment, the disclosure is directed to an isolated polynucleotide comprising SEQ ID NO.:8, SEQ ID NO.:9 and SEQ ID NO.: 10. (CDRs V_L). In one embodiment, any of these polynucleotides encodes a fusion polypeptide.

[0021] In a further embodiment, the disclosure is directed to an isolated polynucleotide comprising SEQ ID No.:2. In another embodiment,

the disclosure is directed to an isolated polynucleotide comprising SEQ ID No.:4. In a further embodiment, the disclosure is directed to an isolated polynucleotide comprising a sequence selected from the group consisting of SEQ ID No:29; SEQ ID No:31 ; SEQ ID No:33; SEQ ID No:35; SEQ ID No:37; SEQ ID No:39; SEQ ID No:41 ; SEQ ID No:43; SEQ ID No:45; SEQ ID No:47; SEQ ID No:49; SEQ ID No:51 ; SEQ ID No:53; SEQ ID No:55; SEQ ID No:57; SEQ ID No:59; SEQ ID No:61 ; SEQ ID No:63; SEQ ID No:65; SEQ ID No:67; SEQ ID No:69; and SEQ ID No:71. In another embodiment, the disclosure is directed to an isolated polynucleotide comprising SEQ ID No.:75. In one embodiment, such polynucleotides encode fusion polypeptides.

[0022] The disclosure is further directed to an isolated polynucleotide comprising a sequence having at least 80% identity to SEQ ID NO:2, wherein said isolated polynucleotide encodes a fusion polypeptide. In an additional aspect, the disclosure is directed to an isolated polynucleotide comprising a sequence having

at least 80% identity to SEQ ID NO : 4, wherein said isolated polynucleotide encodes a fusion polypeptide. The disclosure is further directed to an isolated polynucleotide comprising a sequence having at least 80% identity to a sequence selected from the group consisting of SEQ ID No: 29; SEQ ID No: 31 ; SEQ ID No: 33; SEQ ID No: 35; SEQ ID No: 37; SEQ ID No: 39; SEQ ID No: 41 ; SEQ ID No: 43; SEQ ID No: 45; SEQ ID No: 47; SEQ ID No: 49; SEQ ID No: 51 ; SEQ ID No: 53; SEQ ID No: 55; SEQ ID No: 57; SEQ ID No: 59; SEQ ID No: 61 ; SEQ ID No: 63; SEQ ID No: 65; SEQ ID No: 67; SEQ ID No: 69; and SEQ ID No: 71, wherein said isolated polynucleotide encodes a fusion polypeptide. In an additional embodiment, the disclosure is directed to an isolated polynucleotide comprising a sequence having at least 80% identity to SEQ ID NO : 75, wherein said isolated polynucleotide encodes a fusion polypeptide.

[0023] The disclosure is further directed to a polynucleotide comprising SEQ ID NO: 11 (whole heavy chain), or to polynucleotides having 80%, 85%, 90%, 95% or 99% identity to SEQ ID NO: 11. The disclosure is also directed to a polynucleotide comprising SEQ ID NO:12 (whole light chain), or to polynucleotides having 80%, 85%, 90%, 95% or 99% identity SEQ ID NO:12.

[0024] The disclosure is also directed to an isolated polynucleotide encoding a chimeric polypeptide having the sequence of SEQ ID No.:1. In one embodiment, the polynucleotide comprises a sequence encoding a polypeptide having the sequence of SEQ ID No.:l; and a sequence encoding a polypeptide having the sequence of an antibody Fc region, or a fragment thereof, from a species other than mouse. The disclosure is also directed to an isolated polynucleotide encoding a chimeric polypeptide having a sequence selected from the group consisting of SEQ ID No: 30; SEQ ID No: 32; SEQ ID No: 34; SEQ ID No: 36; SEQ ID No: 38; SEQ ID No: 40; SEQ ID No: 42; SEQ ID No: 44; SEQ ID No : 46; SEQ ID No: 48 ; SEQ ID No: 50; SEQ ID No: 52; SEQ ID No: 54; SEQ ID No: 56; SEQ ID No: 58; SEQ ID No: 60; SEQ ID No: 62; SEQ ID No: 64; SEQ ID No: 66; SEQ ID No : 68; SEQ ID No: 70; and SEQ ID No: 72. In one embodiment, the polynucleotide comprises a sequence encoding a polypeptide having a sequence selected from the group consisting of SEQ ID No: 30; SEQ ID No: 32; SEQ ID No: 34; SEQ ID No: 36; SEQ ID No: 38; SEQ ID No: 40; SEQ ID No: 42; SEQ ID

No:44; SEQ ID No:46; SEQ ID No:48; SEQ ID No:50; SEQ ID No:52; SEQ ID No:54; SEQ ID No:56; SEQ ID No:58; SEQ ID No:60; SEQ ID No:62; SEQ ID No:64; SEQ ID No:66; SEQ ID No:68; SEQ ID No:70; and SEQ ID No:72; and a sequence encoding a polypeptide having the sequence of an antibody Fc region, or a fragment thereof, from a species other than mouse.

[0025] In yet another embodiment, the disclosure is directed to an isolated polynucleotide encoding a chimeric polypeptide having the sequence of SEQ ID No.:1. In one embodiment, the polynucleotide comprises a sequence encoding a polypeptide having the sequence of SEQ ID No.:3; and a sequence encoding a polypeptide having the sequence of an antibody Fc region, or a fragment thereof, from a species other than mouse. In yet another embodiment, the disclosure is directed to an isolated polynucleotide encoding a chimeric polypeptide having the sequence of SEQ ID No.:76. In one embodiment, the polynucleotide comprises a sequence encoding a polypeptide having the sequence of SEQ ID No.:76; and a sequence encoding a polypeptide having the sequence of an antibody Fc region, or a fragment thereof, from a species other than mouse.

[0026] The disclosure is also directed to an isolated polynucleotide comprising a sequence encoding a polypeptide having the V_H region of the murine B-Ly1 antibody, or functional variants thereof, and a sequence encoding a polypeptide having the sequence of an antibody Fc region, or a fragment thereof, from a species other than mouse. In another embodiment, the disclosure is directed to an isolated polynucleotide comprising a sequence encoding a polypeptide having the V_L region of the murine B-Lyl antibody, or functional variants thereof, and a sequence encoding a polypeptide having the sequence of an antibody Fc region, or a fragment thereof, from a species other than mouse.

[0027] The disclosure is further directed to an expression vector comprising any of the isolated polynucleotides described above, and to a host cell that comprises such an expression vector. In a further embodiment, the disclosure is directed to a host cell comprising any of the isolated polynucleotides described above.

[0028] In one embodiment, the disclosure is directed to an isolated polypeptide comprising: (a) a sequence selected from a group consisting of: SEQ ID NO.:15, SEQ ID NO.:16 and SEQ ID NO.:17. (CDRs V_{H-1}) ; (b) a sequence selected from

a group consisting of: SEQ ID NO.:25, SEQ ID NO.:26 and SEQ ID NO.:27 (CDRs V_{H-2}) : and SEQ ID NO:28, wherein said polypeptide is a fusion polypeptide. In another embodiment, the disclosure is directed to an isolated polypeptide comprising SEQ ID NO.:18, SEQ ID NO.:19 and SEQ ID NO.:20.

(CDRs V_L), wherein said polypeptide is a fusion polypeptide.

[0029] The disclosure is also directed to a chimeric polypeptide comprising the sequence of SEQ ID NO.:1 or a variant thereof. The disclosure is further directed to a chimeric polypeptide comprising the sequence of SEQ ID NO.:3 or a variant thereof. In one embodiment, any one of these polypeptides further comprises a human Fc region. The disclosure is also directed to a chimeric polypeptide comprising a sequence selected from the group consisting of SEQ ID No:30; SEQ ID No:32; SEQ ID No:34; SEQ ID No:36; SEQ ID No:38; SEQ ID No:40; SEQ ID No:42; SEQ ID No:44; SEQ ID No:46; SEQ ID No:48; SEQ ID No:50; SEQ ID No:52; SEQ ID No:54; SEQ ID No:56; SEQ ID No:58; SEQ ID No:60; SEQ ID No:62; SEQ ID No:64; SEQ ID No:66; SEQ ID No:68; SEQ ID No:70; and SEQ ID No:72, or a variant thereof. The disclosure is further directed to a chimeric polypeptide comprising the sequence of SEQ ID NO.:76 or a variant thereof. In one embodiment, any one of these polypeptides further comprises a human Fc region.

[0030] In another embodiment the disclosure is directed to a polypeptide comprising a sequence derived from the murine B-Lyl antibody and a sequence derived from a heterologous polypeptide and to an antigen-binding molecule comprising such a polypeptide. In one embodiment the antigen-binding molecule is an antibody. In a preferred embodiment, the antibody is chimeric. In another preferred embodiment, the antibody is humanized or primatized.

[0031] In an additional embodiment, the disclosure is directed to an isolated polypeptide comprising SEQ ID NO:13 or a variant thereof. In another embodiment, the disclosure is directed to an isolated polypeptide

comprising SEQ ID NO:14.

[0032] In another embodiment, the disclosure is directed to an ABM, which is capable of competing with the murine B-Lyl antibody for binding to CD20 and which is chimeric. In one embodiment, the ABM is an antibody or a fragment thereof. In a further embodiment, the ABM is a recombinant antibody comprising a V_H

region having an amino acid sequence selected from the group consisting of SEQ ID NO.:1 ; SEQ ID No:30; SEQ ID No:32; SEQ ID No:34; SEQ ID No:36; SEQ ID No:38; SEQ ID No:40; SEQ ID No:42; SEQ ID No:44; SEQ ID No:46; SEQ ID No:48; SEQ ID No:50; SEQ ID No:52; SEQ ID No:54; SEQ ID No:56; SEQ ID No:58 ; SEQ ID No:60; SEQ ID No:62; SEQ ID No:64; SEQ ID No:66; SEQ ID No:68 ; SEQ ID No:70; and SEQ ID No:72. In another embodiment, the ABM is a recombinant antibody comprising a V_L region having an amino acid sequence selected from the group consisting of SEQ ID NO.:3 and SEQ ID NO:76. In a further embodiment the ABM is a recombinant antibody that is primatized. In yet a further embodiment the ABM is a recombinant antibody that is humanized. In another embodiment, the ABM is a recombinant antibody comprising a human Fc region. In a further embodiment, any of the ABMs discussed above may be conjugated to a moiety such as a toxin or a radiolabel.

[0033] The disclosure is further related to an ABM of the present disclosure, said ABM having modified oligosaccharides. In one embodiment the modified oligosaccharides have reduced fucosylation as compared to non-modified oligosaccharides. In other embodiments, the modified oligosaccharides are hybrid or complex. In a further embodiment, the ABM has an increased proportion of nonfucosylated oligosaccharides or bisected, nonfucosylated oligosaccharides in the Fc region of said molecule. In one embodiment, the bisected, nonfucosylated oligosaccharides are hybrid. In a further embodiment, the bisected, nonfucosylated oligosaccharides are complex. In a one embodiment, at least 20% of the oligosacchardies in the Fc region of said polypeptide are nonfucosylated or bisected, nonfucosylated. In more preferred embodiments, at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70% or 75% or more of the oligosaccharides are nonfucosylated or bisected, nonfucosylated.

[0034] The disclosure is further related to a polynucleotide encoding any of the ABMs discussed above, and to expression vectors and cells comprising such a polynucleotide.

[0035] The disclosure is further related to a method of producing an ABM, which is capable of competing with the murine B-Lyl antibody for binding to CD20 and wherein said ABM is chimeric ; said method comprising: (a) culturing a host cell

comprising a polynucleotide that encodes an ABM of the present disclosure in a medium under conditions allowing the expression of said polynucleotide encoding said ABM; and (b) recovering said ABM from the resultant culture.

[0036] In another embodiment, the disclosure is related to a pharmaceutical composition comprising the ABM of the invention. It is contemplated that the pharmaceutical composition may further comprise a pharmaceutically acceptable carrier, an adjuvant or a combination thereof.

[0037] In a further embodiment, the disclosure is related to a method of treating a disease treatable by B-cell depletion. The method comprises administering a therapeutically effective amount of the ABM of the present disclosure to a human subject in need thereof. In a preferred embodiment, the disease is treated by administering an ABM that is a chimeric antibody, or a chimeric fragment of an antibody.

[0038] In yet another embodiment, the disclosure is related to a host cell engineered to express at least one nucleic acid encoding a polypeptide having GnTIII activity in an amount sufficient to modify the oligosaccharides in the Fc region of produced by the host cell, wherein the ABM is capable of competing with the murine B- Lyl antibody for binding to CD20 and wherein the ABM is chimeric. In one embodiment, the polypeptide having GnTIII activity is a fusion polypeptide. In another embodiment, the ABM produced by the host cell is an antibody or an antibody fragment. In a further embodiment, the ABM comprises a region equivalent to the Fc region of a human IgG.

[0039] The disclosure is also directed to an isolated polynucleotide comprising at least one complementarity determining region of the murine B-Lyl antibody, or a variant or truncated form thereof containing at least the specificity-determining residues for said complementarity determining region, wherein said isolated polynucleotide encodes a fusion polypeptide. Preferably, such isolated polynucleotides encode a fusion polypeptide that is an antigen binding molecule.

In one embodiment, the polynucleotide comprises three complementarity determining regions of the murine B-Lyl antibody, or variants or truncated forms thereof containing at least the specificity-determining residues for each of said three complementarity determining regions. In another embodiment, the

polynucleotide encodes the entire variable region of the light or heavy chain of a chimeric (e. g. , humanized) antibody. The disclosure is further directed to the polypeptides encoded by such polynucleotides.

[0040] In another embodiment, the disclosure is directed to an antigen combining molecule comprising at least one complementarity determining region of the murine B-Lyl antibody, or a variant or truncated form thereof containing at lest the specificity-determining residues for said complementarity determining region, and comprising a sequence derived from a heterologous polypeptide. In one embodiment, the antigen binding molecule comprises three complementarity determining regions of the murine B-Lyl antibody, or variants or truncated forms thereof containing at least the specificity-determining residues for each of said three complementarity determining regions. In another embodiment, the antigen binding molecule comprises the variable region of an antibody light or heavy chain. In one particularly useful embodiment, the antigen binding molecule is a chimeric, e.g., humanized, antibody. The disclosure is also directed to methods of making such antigen binding molecules, and the use of same in the treatment of disease, including B cell lymphomas.

[0041] The present disclosure is the first known instance in which a Type II anti-CD20 antibody has been engineered to have increases effector functions such as ADCC, while still retaining potent apoptosis ability. Accordingly, the present disclosure is directed to an engineered Type II anti-CD20 antibody having increased ADCC as a result of said engineering and without loss of substantial ability to induces apoptosis. In one embodiment, the Type II anti-CD20 antibodies have been engineered to have an altered pattern of glycosylation in the Fc region. In a particular embodiment, the altered glycosylation comprises an increased level of bisected complex residues in the Fc region. In another particular embodiment, the altered glycosylation comprises and reduced level of fucose residues in the Fc region. In another embodiment, the Type II anti-CD20 antibodies have undergone polypeptide engineering. The disclosure is further directed to methods of making such engineered Type II antibodies and to methods of using such antibodies in the treatment of various B cell disorders, including B cell lymphomas.

polypeptide, preferably comprising the catalytic domain of GnTIII and the Golgi localization domain of a heterologous Golgi resident polypeptide selected from the group consisting of the localization domain of mannosidase II, the localization domain of ß(1,2)-N-acetylglucosaminyltransferase I ("GnTI"), the localization domain of mannosidase I, the localization domain of ß(1,2)-N- acetylglucosaminyltransferase II ("GnTII"), and the localization domain of αl-6 core fucosyltransferase. Preferably, the Golgi localization domain is from mannosidase II or GnTI.

[0046] In a further embodiment, the disclosure is directed to a method for modifying the glycosylation profile of an anti-CD20 ABM produced by a host cell comprising introducing into the host cell at least one nucleic acid or expression vector of the invention. In one embodiment, the ABM is an antibody or a fragment thereof; preferably comprising the Fc region of an IgG. Alternatively, the polypeptide is a fusion protein that includes a region equivalent to the Fc region of a human IgG.

[0047] In one embodiment, the disclosure is related to a recombinant, chimeric antibody, or a fragment thereof, capable of competing with the murine B-Lyl antibody for binding to CD20 and having reduced fucosylation.

[0048] In another embodiment, the present disclosure is directed to a method of modifying the glycosylation of the recombinant antibody or a fragment thereof of the invention by using a fusion polypeptide having GnTIII activity and comprising the Golgi localization domain of a heterologous Golgi resident polypeptide. In one embodiment, the fusion polypeptides of the invention comprise the catalytic domain of GnTIII. In another embodiment, the Golgi localization domain is selected from the group consisting of: the localization domain of mannosidase II, the localization domain of GnTI, the localization domain of mannosidase I, the localization domain of GnTII and the localization domain of αl-6 core fucosyltransferase. Preferably, the Golgi localization domain is from mannosidase II or GnTI.

[0049] In one embodiment, the method of the disclosure is directed towards producing a recombinant, chimeric antibody or a fragment thereof, with modified oligosaccharides wherein said modified oligosaccharides have reduced fucosylation as compared to non-modified oligosaccharides. According to the

present disclosure, these modified oligosaccharides may be hybrid or complex. In another embodiment, the method of the disclosure is directed towards producing a recombinant, chimeric antibody or a fragment thereof having an increased proportion of bisected, nonfucosylated oligosaccharides in the Fc region of said polypeptide. In one embodiment, the bisected, nonfucosylated oligosaccharides are hybrid. In another embodiment, the bisected, nonfucosylated oligosaccharides are complex. In a further embodiment, the method of the disclosure is directed towards producing a recombinant, chimeric antibody or a fragment thereof having at least 20% of the oligosaccharides in the Fc region of said polypeptide that are bisected, nonfucosylated. In a preferred embodiment, at least 30% of the oligosaccharides in the Fc region of said polypeptide are bisected, nonfucosylated. In another preferred embodiment, wherein at least 35% of the oligosaccharides in the Fc region of said polypeptide are bisected, nonfucosylated.

[0050] In a further embodiment, the disclosure is directed to a recombinant, chimeric antibody or a fragment thereof, that exhibits increased Fc receptor binding affinity and/or increased effector function as a result of the modification of its oligosaccharides. In one embodiment, the increased binding affinity is to an Fc activating receptor. In a further embodiment, the Fc receptor is Fcγ activating receptor, particularly, the FcγRIIIA receptor. The effector function contemplated herein may be selected from the group that includes, but is not limited to, increased Fc-mediated cellular cytotoxicity; increased binding to NK cells; increased binding to macrophages; increased binding to polymorphonuclear cells; increased binding to monocytes; increased direct signaling inducing apoptosis; increased dendritic cell maturation; and increased T cell priming.

[0051] In another embodiment, the disclosure is directed to a recombinant, chimeric antibody fragment, having the binding specificity of the murine B-Lyl antibody and containing the Fc region, that is engineered to have increased effector function produced by any of the methods of the present disclosure.

[0052] In another embodiment, the present disclosure is directed to a fusion protein that includes a polypeptide having the sequence of SEQ ID NO:1 and a region equivalent to the Fc region of an immunoglobulin and engineered to have

increased effector function produced by any of the methods of the present invention.

[0053] In another embodiment, the present disclosure is directed to a fusion protein that includes a polypeptide having the sequence of SEQ ID NO:3 and a region equivalent to the Fc region of an immunoglobulin and engineered to have increased effector function produced by any of the methods of the present disclosure.

[0054] In one embodiment, the present disclosure is directed to a pharmaceutical composition comprising a recombinant, chimeric antibody, produced by any of the methods of the present invention, and a pharmaceutically acceptable carrier. In another embodiment, the present disclosure is directed to a pharmaceutical composition comprising a recombinant, chimeric antibody fragment produced by any of the methods of the present disclosure, and a pharmaceutically acceptable carrier. In another embodiment, the present disclosure is directed to a pharmaceutical composition comprising a fusion protein produced by any of the methods of the present disclosure, and a pharmaceutically acceptable carrier.

[0055] The disclosure is further directed to a method of treating a disease treatable by B-cell depletion comprising administering a therapeutically effective amount of the recombinant, chimeric, antibody or fragment thereof, produced by any of the methods of the present disclosure, to a human subject in need thereof.

In another embodiment, there is provided a host cell comprising the expression vector of the disclosure, wherein said host cell is selected from the group consisting of: a cell in culture, a cell in a cultured animal tissue culture, a cell in a cultured plant tissue, and cell in transgenic plant.

In another emnodiment, there is provided a host cell comprising an isolated polynucleotide according to the disclosure, wherein said host is selected from the group consisting of: a cell in culture, a cell in a cultured plant tissue, and cell in a transgenic plant.

In another embodiment, there is provided a chimeric polypeptide comprising: a. a polypeptide having a sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:16 and SEQ ID Noz: 17; and b. a polypeptide having a sequence selected from the group consisting a SEQ ID NO:25, SEQ ID NO:26 and SEQ ID NO:27; and c. SEQ ID NO:28.

In another embodiment, there is provided a chimeric polypeptide comprising SEQ ID NO:18, SEQ ID NO:19 and SEQ ID NO:20.

In another embodiment, there is provided a host cell engineered to express at least one nucleic acid encoding a polypeptide having β(1,4)-N-acetylglucosaminyltransferase III activity in an amount sufficient to modify the oligosaccharides in the Fc region of a polypeptide produced by said host cell, wherein said polypeptide is an antigen binding molecule according to the disclosure, wherein said host cell is selected from the group consisting of: a cell culture, a cell in a cultured animal tissue culture, a cell in a cultured plant tissue, and cell in a transgenic plant.

In another embodiment, there is provided an antigen binding molecule according to the disclosure for use in the manufacture of a medicament for treating a disorder treatable by B-cell depletion.

In another embodiment, there is provided use of an antigen binding molecule according to the disclosure in the manufacture of a medicament for treatment of a B-cell lymphoma.

BRIEF DESCRIPTION OF THE FIGURES

[0056] FIG 1. Nucleotide (SEQ ID NO :2) and amino acid sequence (SEQ ID NO: 1) of the V_H region of the murine B-Lyl.

[0057] FIG 2. Nucleotide (SEQ ID NO : 4) and amino acid sequence (SEQ ID NO:3) of the VL region of the murine B-Lyl.

[0058] FIG 3. Binding of Rituximab® (O) and ch-B_Lyl (Δ) to CD20 on Raji B-lymphoma cells.

[0059] FIG 4. B-Cell depletion by Rituximab® (O) and ch-B_Lyl (Δ) in whole blood of the three different classes of FcγRIIIa-158V/F genotype: (A) whole blood from a F/F donor, homozygous for the lower affinity receptor; (B) whole

blood from a F/V donor, heterozygous for the affinity receptor; and (C) whole blood from a V/V donor, homozygous for the higher affinity receptor.

[0060] FIG 5. Nucleotide (SEQ ID NO:11) and amino acid sequence (SEQ ID NO:13) of the heavy chain of a chimeric, anti-CD20 antibody.

[0061] FIG 6. Nucleotide (SEQ ID NO:12) and amino acid sequence (SEQ ID NO:14) of the light chain of a chimeric, anti-CD20 antibody.

[0062] FIG 7. Nucleotide and amino acid sequences of the murine B-Ly1 antibody CDRs. (A) Predicted CDRs for the V_H region. (B) Predicted CDRs for the V_L region.

[0063] FIG 8. MALDI-TOF profile of a glycoengineered, chimeric B-Ly1 antibody. (A) Table detailing the percentages of specific peaks; (B) Spectrum for glycoengineered chimeric B-Ly1; (C) Spectrum for glycoengineered chimeric B-Ly1 treated with Endo-H.

[0064] FIG 9. Binding of different humanized anti-CD20 antibodies to Raji B-cells. The differences between the B-HH2 construct and the B-HL8 and B-HL11 constructs are located in the framework 1 and 2 regions with all three CDRs being identical. B-HL8 and B-HL11 have their FR1 and FR2 sequences derived from the human VH3 class, whereas the complete B-HH2 framework is human VH1 derived. B-HL11 is a derivative of B-HL8 with the single mutation Glu1Gln, with Gln being the amino acid residue in the B-HH2 construct. This means that the Glu1Gln exchange does not alter binding affinity or intensity. The other differences between B-HH2 and B-HL8 are 14 FR residues, from which one or more will influence the antigen binding behavior of this antibody.

[0065] FIG 10. Binding of the humanized anti-CD20 antibody BHL4-KV1 on Raji target cells. The B-HL4 construct is derived from the B-HH2 antibody by replacing the FR1 of the B-HH2 with that of the human germ line sequence VH1_45. This construct shows greatly diminished antigen binding capacity, despite of having different amino acids at only three positions within FR1. These residues are located at positions 2, 14, and 30 according to Kabat numbering. Of these, position 30 seems to be the most influential position, since it is part of the Chothia definition of CDR1.

[0066] FIG 11. Comparison of the binding behavior between B-HH1, B-HH2, B-HH3, and the parental antibody B-ly1. The data show that all Abs show a similar EC50 value, but the B-HH1 construct binds with a lower intensity/stoichiometry than the the variants B-HH2 and B-HH3. B-HH1 can be distinguished from B-HH2 and B-HH3 by its partially human CDR1 and CDR2 regions (Kabat definition), as well as the Ala/Thr polymorphism at position 28 (Kabat numbering). This indicates that either position 28, the complete CDR1, and/or the complete CDR2 is important for antibody/antigen interaction.

[0067] FIG 12. The comparison of B-HL1, B-HH1, and the B-ly1 parental antibody. The datashowed absence of any binding activity in the B-HL1 construct, and about half of the binding intensity /stoichiometry of B-HH1 compared to B-ly1. Both B-HL1, as well as B-HH1, are designed based on acceptor frameworks derived from the human VH1 class. Among other differences, position 71 (Kabat numbering) of the B-HL1 construct is a striking difference, indicating its putative importance for antigen binding.

[0068] FIG 13. Fluorocytometric analysis of the capaicty of the anti-CD20 antibody to its antigen. The data showed that the B-HL2 and B-HL3 constructs do not display CD-20 binding activity.

[0069] FIG 14. Apoptosis of anti-CD20 antibodies on Z-138 MCL cells.

[0070] FIG 15. Apoptosis by anti-CD20 antibodies. Assay details: 5 x 105 cells/well were seeded in 24-well plates (5 x 105 cells/ml) in culture medium. 10 mg of the respective Ab, PBS for the negative control or 5mM Camptothecin (CPT) positive control were added to the wells. Samples were incubated o/n (16 h), stained with AnnV-FITC and analysed by FACS. Assay was done in triplicates.(*): Signal for PBS alone subtracted (PBS alone gave 8% and 2% AnnV+ for PR-1 and Z-138 cells respectively). Antibodies used were: C2B8 (chimeric, non-glycoengineered); BHH2-KV1 (humanized, non-glycoengineered). Note: this assay does not involve any additional effector cells, just targets plus antibody or controls.

[0071] FIG 16. Target-cell killing by anti-CD20 antibodies with immune effector cells. Assay details: B-cell depletion in normal whole blood overnight incubation and analysis for CD19+/CD3+ by FACS. ADCC using PBMCs as effectors, 4 h

incubation, 25:1 effector:target ratio, target-killing measured by Calcein-retention relative to detergent-lysis (100%) and to lysis without Ab (0%). Antibodies used: C2B8 (chimeric, non-glycoengineered form); BHH2-KV1-wt (humanized, non-glycoengineered form of BHH2-KV1); BHH2-KV1-GE (humanized, glycoengineered form of BHH2-KV1).

[0072] FIG 17. MALDI/TOF-MS profile of PNGaseF-released Fc-oligosaccharides of unmodified, nonglycoengineered BHH2-KV1 humanized IgG1 B-ly1 anti-human CD20 antibody.

[0073] FIG 18. MALDI/TOF-MS profile of PNGaseF-released Fc-oligosaccharides of glycoengineered BHH2-KV1g1 humanized IgG1 B-ly1 anti-human CD20 antibody. Glycoengineering done by co-expression in host cells of antibody genes and gene encoding enzyme with β-1,4-N-acetylglucosaminyltransferase III (GnT-III) catalytic activity.

[0074] FIG 19. MALDI/TOF-MS profile of PNGaseF-released Fc-oligosaccharides of glycoengineered BHH2-KV1g2 humanized IgG1 B-ly1 anti-human CD20 antibody. Glycoengineering done by co-expression in host cells of antibody genes and genes encoding enzyme with β-1,4-N-acetylglucosaminyltransferase III (GnT-III) catalytic activity and encoding enzyme with Golgi α-mannosidase II catalytic activity.

[0075] FIG 20. Binding of non-glycoengineered and glycoengineered antibodies to human FcgammaRIIIa receptor displayed on the surface of recombinant CHO-CD16 cells.

[0076] FIG 21. Apoptosis of non-Fc engineered and Fc-engineered anti-CD20 antibodies on Z-138 MCL cells. Assay details: 5 x 105 cells/well were seeded in 24-well plates (5 x 105 cells/ml) in culture medium. 10 mg of the respective Ab, PBS for the negative control were added to the wells. Samples were incubated o/n (16 h), stained with AnnV-FITC and analysed by FACS. Assay was done in triplicates. Abs used: C2B8 = rituximab (chimeric, non-glycoengineered form, same as commercial form); BHH2-KV1 (humanized, non-glycoengineered-see Figure 6 for glycosylation profile); BHH2-KV1g1 (humanized, glycoengineered – see Fig.7 for glycosylation profile); BHH2-KV1g2 (humanized, glycoengineered – see Fig 8 for glycosylation profile). Note: this assay does not

involve any additional effector cells, just targets plus antibody or controls. (*): Signal for PBS alone subtracted.

DETAILED DESCRIPTION OF THE INVENTION

[0077] Terms are used herein as generally used in the art, unless otherwise defined as follows.

[0078] As used herein, the term *antibody* is intended to include whole antibody molecules, including monoclonal, polyclonal and multispecific (*e.g.*, bispecific) antibodies, as well as antibody fragments having the Fc region and retaining binding specificity, and fusion proteins that include a region equivalent to the Fc region of an immunoglobulin and that retain binding specificity. Also encompassed are humanized, primatized and chimeric antibodies.

[0079] As used herein, the term *Fc region* is intended to refer to a C-terminal region of an IgG heavy chain. Although the boundaries of the Fc region of an IgG heavy chain might vary slightly, the human IgG heavy chain Fc region is usually defined to stretch from the amino acid residue at position Cys226 to the carboxyl-terminus.

[0080] As used herein, the term *region equivalent to the Fc region of an immunoglobulin* is intended to include naturally occurring allelic variants of the Fc region of an immunoglobulin as well as variants having alterations which produce substitutions, additions, or deletions but which do not decrease substantially the ability of the immunoglobulin to mediate effector functions (such as antibody dependent cellular cytotoxicity). For example, one or more amino acids can be deleted from the N-terminus or C-terminus of the Fc region of an immunoglobulin without substantial loss of biological function. Such variants can be selected according to general rules known in the art so as to have minimal effect on activity. (*See, e.g.*, Bowie, J. U. *et al.*, *Science 247*:1306-10 (1990).

[0081] As used herein, the term *antigen binding molecule* refers in its broadest sense to a molecule that specifically binds an antigenic determinant. More specifically, an *antigen binding molecule that binds CD20* is a molecule which specifically binds to a cell surface non-glycosylated phosphoprotein of 35,000

Daltons, typically designated as the human B lymphocyte restricted differentiation antigen Bp35, commonly referred to as CD20. By "specifically binds" is meant that the binding is selective for the antigen and can be discriminated from unwanted or nonspecific interactions.

[0082] As used herein, the terms *fusion* and *chimeric*, when used in reference to polypeptides such as ABMs refer to polypeptides comprising amino acid sequences derived from two or more heterologous polypeptides, such as portions of antibodies from different species. For chimeric ABMs, for example, the non-antigen binding components may be derived from a wide variety of species, including primates such as chimpanzees and humans. The constant region of the chimeric ABM is most preferably substantially identical to the constant region of a natural human antibody; the variable region of the chimeric antibody is most preferably substantially identical to that of a recombinant antiCD-20 antibody having the amino acid sequence of the murine B-Ly1 variable region. Humanized antibodies are a particularly preferred form of fusion or chimeric antibody.

[0083] As used herein, *a polypeptide having "GnTIII activity"* refers to polypeptides that are able to catalyze the addition of a N-acetylglucosamine (GlcNAc) residue in β-1-4 linkage to the β-linked mannoside of the trimannosyl core of N-linked oligosaccharides. This includes fusion polypeptides exhibiting enzymatic activity similar to, but not necessarily identical to, an activity of β(1,4)-N-acetylglucosaminyltransferase III, also known as β-1,4-mannosyl-glycoprotein 4-beta-N-acetylglucosaminyl-transferase (EC 2.4.1.144), according to the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB), as measured in a particular biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of GnTIII, but rather substantially similar to the dose-dependence in a given activity as compared to the GnTIII (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the GnTIII.)

[0084] As used herein, the term *variant* (or *analog*) refers to a polypeptide differing from a specifically recited polypeptide of the invention by amino acid insertions, deletions, and substitutions, created using, *e g.*, recombinant DNA techniques. Variants of the ABMs of the present invention include chimeric, primatized or humanized antigen binding molecules wherein one or several of the amino acid residues are modified by substitution, addition and/or deletion in such manner that does not substantially affect antigen (e.g., CD20) binding affinity. Guidance in determining which amino acid residues may be replaced, added or deleted without abolishing activities of interest, may be found by comparing the sequence of the particular polypeptide with that of homologous peptides and minimizing the number of amino acid sequence changes made in regions of high homology (conserved regions) or by replacing amino acids with consensus sequence.

[0085] Alternatively, recombinant variants encoding these same or similar polypeptides may be synthesized or selected by making use of the "redundancy" in the genetic code. Various codon substitutions, such as the silent changes which produce various restriction sites, may be introduced to optimize cloning into a plasmid or viral vector or expression in a particular prokaryotic or eukaryotic system. Mutations in the polynucleotide sequence may be reflected in the polypeptide or domains of other peptides added to the polypeptide to modify the properties of any part of the polypeptide, to change characteristics such as ligand-binding affinities, interchain affinities, or degradation/turnover rate.

[0086] Preferably, amino acid "substitutions" are the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, i.e., conservative amino acid replacements. "Conservative" amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively charged (acidic) amino

acids include aspartic acid and glutamic acid. "Insertions" or "deletions" are preferably in the range of about 1 to 20 amino acids, more preferably 1 to 10 amino acids. The variation allowed may be experimentally determined by systematically making insertions, deletions, or substitutions of amino acids in a polypeptide molecule using recombinant DNA techniques and assaying the resulting recombinant variants for activity.

[0087] As used herein, the term *humanized* is used to refer to an antigen –binding molecule derived from a non-human antigen-binding molecule, for example, a murine antibody, that retains or substantially retains the antigen-binding properties of the parent molecule but which is less immunogenic in humans. This may be achieved by various methods including (a) grafting the entire non-human variable domains onto human constant regions to generate chimeric antibodies, (b) grafting only the non-human CDRs onto human framework and constant regions with or without retention of critical framework residues (e.g., those that are important for retaining good antigen binding affinity or antibody functions), or (c) transplanting the entire non-human variable domains, but "cloaking" them with a human-like section by replacement of surface residues. Such methods are disclosed in Jones et al., Morrison et al., *Proc. Natl. Acad. Sci.*, 81:6851-6855 (1984); Morrison and Oi, *Adv. Immunol.*, 44:65-92 (1988); Verhoeyen et al., Science, 239:1534-1536 (1988); Padlan, *Molec. Immun.*, 28:489-498 (1991); Padlan, *Molec. Immun.*, 31(3):169-217 (1994), all of which are incorporated by reference in their entirety herein. There are generally 3 complementarity determining regions, or CDRs, (CDR1, CDR2 and CDR3) in each of the heavy and light chain variable domains of an antibody, which are flanked by four framework subregions (i.e., FR1, FR2, FR3, and FR4) in each of the heavy and light chain variable domains of an antibody: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4. A discussion of humanized antibodies can be found, *inter alia*, in U.S. Patent No. 6,632,927, and in published U.S. Application No. 2003/0175269, both of which are incorporated herein by reference in their entirety.

[0088] Similarly, as used herein, the term *primatized* is used to refer to an antigen-binding molecule derived from a non-primate antigen-binding molecule, for example, a murine antibody, that retains or substantially retains the antigen-

binding properties of the parent molecule but which is less immunogenic in primates.

[0089] In the case where there are two or more definitions of a term which is used and/or accepted within the art, the definition of the term as used herein is intended to include all such meanings unless explicitly stated to the contrary. A specific example is the use of the term "complementarity determining region" ("CDR") to describe the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. This particular region has been described by Kabat et al., U.S. Dept. of Health and Human Services, "Sequences of Proteins of Immunological Interest" (1983) and by Chothia et al., *J. Mol. Biol.* 196:901-917 (1987), which are incorporated herein by reference, where the definitions include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or variants thereof is intended to be within the scope of the term as defined and used herein. The appropriate amino acid residues which encompass the CDRs as defined by each of the above cited references are set forth below in Table I as a comparison. The exact residue numbers which encompass a particular CDR will vary depending on the sequence and size of the CDR. Those skilled in the art can routinely determine which residues comprise a particular CDR given the variable region amino acid sequence of the antibody.

TABLE 1. CDR Definitions[1]

	Kabat	**Chothia**	**AbM**
V_H CDR1	31-35	26-32	26-35
V_H CDR2	50-65	52-58	50-58
V_H CDR3	95-102	95-102	95-102
V_L CDR1	24-34	26-32	
V_L CDR2	50-56	50-52	
V_L CDR3	89-97	91-96	

[1]Numbering of all CDR definitions in Table 1 is according to the numbering conventions set forth by Kabat et al. (see below).

[0090] Kabat et al. also defined a numbering system for variable domain sequences that is applicable to any antibody. One of ordinary skill in the art can unambigously assign this system of "Kabat numbering" to any variable domain

sequence, without reliance on any experimental data beyond the sequence itself. As used herein, "Kabat numbering" refers to the numbering system set forth by Kabat et al., U.S. Dept. of Health and Human Services, "Sequence of Proteins of Immunological Interest" (1983). Unless otherwise specified, references to the numbering of specific amino acid residue positions in an ABM are according to the Kabat numbering system. The sequences of the sequence listing (i.e., SEQ ID NO:1 to SEQ ID NO:78) are not numbered according to the Kabat numbering system.

[0091] By a nucleic acid or polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. The query sequence may be the entire sequence shown in either FIG. 24 or FIG. 25.

[0092] As a practical matter, whether any particular nucleic acid molecule or polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence or polypeptide sequence of the present invention can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag *et al.*, *Comp. App. Biosci. 6*:237-245 (1990). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting U's to T's. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are:

Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter.

[0093] If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of the present invention. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score.

[0094] For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually

corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to made for the purposes of the present invention.

[0095] By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, or substituted with another amino acid. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

[0096] As a practical matter, whether any particular polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a reference polypeptide can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag *et al.*, *Comp. App. Biosci. 6*:237-245 (1990). In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

[0097] If the subject sequence is shorter than the query sequence due to N– or C-terminal deletions, not because of internal deletions, a manual correction must

be made to the results. This is because the FASTDB program does not account for N– and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N– and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N– and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N– and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N– and C-terminal residues of the subject sequence.

[0098] For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N– and C- termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N– or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N– and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the

query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

[0099] As used herein, a nucleic acid that "*hybridizes under stringent conditions*" to a nucleic acid sequence of the invention, refers to a polynucleotide that hybridizes in an overnight incubation at 42° C in a solution comprising 50% formamide, 5x SSC (750 mM NaCl, 75 mM sodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 μg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

[0100] As used herein, the term *Golgi localization domain* refers to the amino acid sequence of a Golgi resident polypeptide which is responsible for anchoring the polypeptide in location within the Golgi complex. Generally, localization domains comprise amino terminal "tails" of an enzyme.

[0101] As used herein, the term *effector function* refers to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody. Examples of antibody effector functions include, but are not limited to, Fc receptor binding affinity, antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), cytokine secretion, immune-complex-mediated antigen uptake by antigen-presenting cells, down-regulation of cell surface receptors, etc.

[0102] As used herein, the terms *engineer, engineered, engineering and glycosylation engineering* are considered to include any manipulation of the glycosylation pattern of a naturally occurring or recombinant polypeptide or fragment thereof. Glycosylation engineering includes metabolic engineering of the glycosylation machinery of a cell, including genetic manipulations of the oligosaccharide synthesis pathways to achieve altered glycosylation of glycoproteins expressed in cells. Furthermore, glycosylation engineering includes the effects of mutations and cell environment on glycosylation.

[0103] As used herein, the term *host cell* covers any kind of cellular system which can be engineered to generate the polypeptides and antigen-binding molecules of the present invention. In one embodiment, the host cell is engineered to allow the production of an antigen binding molecule with modified

glycoforms. In a preferred embodiment, the antigen binding molecule is an antibody, antibody fragment, or fusion protein. In certain embodiments, the host cells have been further manipulated to express increased levels of one or more polypeptides having GnTIII activity. Host cells include cultured cells, *e.g.*, mammalian cultured cells, such as CHO cells, BHK cells, NS0 cells, SP2/0 cells, YO myeloma cells, P3X63 mouse myeloma cells, PER cells, PER.C6 cells or hybridoma cells, yeast cells, insect cells, and plant cells, to name only a few, but also cells comprised within a transgenic animal, transgenic plant or cultured plant or animal tissue.

[0104] As used herein, the term *Fc-mediated cellular cytotoxicity* includes antibody-dependent cellular cytotoxicity and cellular cytotoxicity mediated by a soluble Fc-fusion protein containing a human Fc-region. It is an immune mechanism leading to the lysis of "antibody-targeted cells" by "human immune effector cells", wherein:

[0105] The *human immune effector cells* are a population of leukocytes that display Fc receptors on their surface through which they bind to the Fc-region of antibodies or of Fc-fusion proteins and perform effector functions. Such a population may include, but is not limited to, peripheral blood mononuclear cells (PBMC) and/or natural killer (NK) cells.

[0106] The *antibody-targeted cells* are cells bound by the antibodies or Fc-fusion proteins. The antibodies or Fc fusion-proteins bind to target cells via the protein part N-terminal to the Fc region.

[0107] As used herein, the term *increased Fc-mediated cellular cytotoxicity* is defined as either an increase in the number of "antibody-targeted cells" that are lysed in a given time, at a given concentration of antibody, or of Fc-fusion protein, in the medium surrounding the target cells, by the mechanism of Fc-mediated cellular cytotoxicity defined above, and/or a reduction in the concentration of antibody, or of Fc-fusion protein, in the medium surrounding the target cells, required to achieve the lysis of a given number of "antibody-targeted cells", in a given time, by the mechanism of Fc -mediated cellular cytotoxicity. The increase in Fc-mediated cellular cytotoxicity is relative to the cellular cytotoxicity mediated by the same antibody, or Fc-fusion protein, produced by

the same type of host cells, using the same standard production, purification, formulation and storage methods, which are known to those skilled in the art, but that has not been produced by host cells engineered to express the glycosyltransferase GnTIII by the methods described herein.

[0108] By *antibody having increased antibody dependent cellular cytotoxicity (ADCC)* is meant an antibody, as that term is defined herein, having increased ADCC as determined by any suitable method known to those of ordinary skill in the art. One accepted *in vitro* ADCC assay is as follows:

1) the assay uses target cells that are known to express the target antigen recognized by the antigen-binding region of the antibody;

2) the assay uses human peripheral blood mononuclear cells (PBMCs), isolated from blood of a randomly chosen healthy donor, as effector cells;

3) the assay is carried out according to following protocol:

i) the PBMCs are isolated using standard density centrifugation procedures and are suspended at 5 x 10^6 cells/ml in RPMI cell culture medium;

ii) the target cells are grown by standard tissue culture methods, harvested from the exponential growth phase with a viability higher than 90%, washed in RPMI cell culture medium, labeled with 100 micro-Curies of ^{51}Cr, washed twice with cell culture medium, and resuspended in cell culture medium at a density of 10^5 cells/ml;

iii) 100 microliters of the final target cell suspension above are transferred to each well of a 96-well microtiter plate;

iv) the antibody is serially-diluted from 4000 ng/ml to 0.04 ng/ml in cell culture medium and 50 microliters of the resulting antibody solutions are added to the target cells in the 96-well microtiter plate, testing in triplicate various antibody concentrations covering the whole concentration range above;

v) for the maximum release (MR) controls, 3 additional wells in the plate containing the labeled target cells, receive 50 microliters of a 2%

(V/V) aqueous solution of non-ionic detergent (Nonidet, Sigma, St. Louis), instead of the antibody solution (point iv above);

vi) for the spontaneous release (SR) controls, 3 additional wells in the plate containing the labeled target cells, receive 50 microliters of RPMI cell culture medium instead of the antibody solution (point iv above);

vii) the 96-well microtiter plate is then centrifuged at 50 x g for 1 minute and incubated for 1 hour at 4°C;

viii) 50 microliters of the PBMC suspension (point i above) are added to each well to yield an effector:target cell ratio of 25:1 and the plates are placed in an incubator under 5% CO_2 atmosphere at 37°C for 4 hours;

ix) the cell-free supernatant from each well is harvested and the experimentally released radioactivity (ER) is quantified using a gamma counter;

x) the percentage of specific lysis is calculated for each antibody concentration according to the formula (ER-MR)/(MR-SR) x 100, where ER is the average radioactivity quantified (see point ix above) for that antibody concentration, MR is the average radioactivity quantified (see point ix above) for the MR controls (see point v above), and SR is the average radioactivity quantified (see point ix above) for the SR controls (see point vi above);

4) "increased ADCC" is defined as either an increase in the maximum percentage of specific lysis observed within the antibody concentration range tested above, and/or a reduction in the concentration of antibody required to achieve one half of the maximum percentage of specific lysis observed within the antibody concentration range tested above. The increase in ADCC is relative to the ADCC, measured with the above assay, mediated by the same antibody, produced by the same type of host cells, using the same standard production, purification, formulation and storage methods, which are known to those skilled in the art, but that has not been produced by host cells engineered to overexpress GnTIII.

[0109] In one aspect, the present invention is related to antigen binding molecules having the binding specificity of the murine B-Ly1 antibody, and to

the discovery that their effector functions can be enhanced by altered glycosylation. In one embodiment, the antigen binding molecule is a chimeric antibody. In a preferred embodiment, the invention is directed to a chimeric antibody, or a fragment thereof, comprising the CDRs shown in Figure 7. Specifically, in a preferred embodiment, the invention is directed to an isolated polynucleotide comprising: (a) a sequence selected from a group consisting of: SEQ ID NO.:5, SEQ ID NO.: 6 and SEQ ID NO.:7. (CDRs V_{H-1}); and (b) a sequence selected from a group consisting of: SEQ ID NO.:21, SEQ ID NO.:22 and SEQ ID NO.:23. (CDRs V_{H-2}); and SEQ ID NO:24. In another preferred embodiment, the invention is directed to an isolated polynucleotide comprising SEQ ID NO.:8, SEQ ID NO.: 9 and SEQ ID NO.:10. (CDRs V_L). In one embodiment, any of these polynucleotides encodes a fusion polypeptide.

[0110] In another embodiment, the antigen binding molecule comprises the V_H domain of the murine B-Ly1 antibody shown in Figure 1, or a variant thereof; and a non-murine polypeptide. In another preferred embodiment, the invention is directed to an antigen binding molecule comprising the V_L domain of the murine B-Ly1 antibody shown in Figure 2, or a variant thereof; and a non-murine polypeptide.

[0111] In another aspect, the invention is directed to antigen binding molecules comprising one or more truncated CDRs of BLy-1. Such truncated CDRs will contain, at a minimum, the specificity-determining amino acid residues for the given CDR. By "specificity-determining residue" is meant those residues that are directly involved in the interaction with the antigen. In general, only about one-fifth to one-third of the residues in a given CDR participate in binding to antigen. The specificity-determining residues in a particular CDR can be identified by, for example, computation of interatomic contacts from three-dimensional modeling and determination of the sequence variability at a given residue position in accordance with the methods described in Padlan *et al.*, *FASEB J. 9(1)*:133-139 (1995), the contents of which are hereby incorporated by reference in their entirety.

[0112] Accordingly, the invention is also directed to an isolated polynucleotide comprising at least one complementarity determining region of the murine B-Ly1

antibody, or a variant or truncated form thereof containing at least the specificity-determining residues for said complementarity determining region, wherein said isolated polynucleotide encodes a fusion polypeptide. Preferably, such isolated polynucleotides encode a fusion polypeptide that is an antigen binding molecule. In one embodiment, the polynucleotide comprises three complementarity determining regions of the murine B-Ly1 antibody, or variants or truncated forms thereof containing at least the specificity-determining residues for each of said three complementarity determining regions. In another embodiment, the polynucleotide encodes the entire variable region of the light or heavy chain of a chimeric (e.g., humanized) antibody. The invention is further directed to the polypeptides encoded by such polynucleotides.

[0113] In another embodiment, the invention is directed to an antigen combining molecule comprising at least one complementarity determining region of the murine B-Ly1 antibody, or a variant or truncated form thereof containing at lest the specificity-determining residues for said complementarity determining region, and comprising a sequence derived from a heterologous polypeptide. In one embodiment, the antigen binding molecule comprises three complementarity determining regions of the murine B-Ly1 antibody, or variants or truncated forms thereof containing at least the specificity-determining residues for each of said three complementarity determining regions. In another aspect, the antigen binding molecule comprises the variable region of an antibody light or heavy chain. In one particularly useful embodiment, the antigen binding molecule is a chimeric, e.g., humanized, antibody. The invention is also directed to methods of making such antigen binding molecules, and the use of same in the treatment of disease, including B cell lymphomas.

[0114] It is known that several mechanism are involved in the therapeutic efficacy of anti-CD20 antibodies, including antibody dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and the induction of growth arrest or apoptosis. For example, the majority of experimental evidence indicates that rituximab operates through conventional effector mechanisms measured by CDC and ADCC assays. Similarly, it has been shown that the resistance of different lymphoma cells to rituximab in vivo is a

function of their sensitivity to CDC in vitro. In contrast, the mode of action in vivo of another antibody that has been approved for therapeutic use, B1, requires neither complement nor natural killer (NK) cell activity. Rather, the efficacy of B1 in vivo is due to its ability to induce potent apoptosis.

[0115] In general, anti-CD20 monoclonal antibodies fall into two distinct categories based on their mechanism of action in eradicating lymphoma cells. Type I anti-CD20 antibodies primarily utilize complement to kill target cells, while Type II antibodies operate by different mechanisms, primarily apoptosis. Rituximab and 1F5 are examples of Type I anti-CD20 antibodies, whereas B1 is an example of a Type II antibody. See, e.g., Cragg, M.S. and Glennie, M.J., Blood 103(7):2738-2743 (April 2004); Teeling, J.L. et al., Blood 104(6):1793-1800 (September 2004), the entire contents of which are hereby incorporated by reference.

[0116] The present invention is the first known instance in which a Type II anti-CD20 antibody has been engineered to have increases effector functions such as ADCC, while still retaining potent apoptosis ability. Accordingly, the present invention is directed to an engineered Type II anti-CD20 antibody having increased ADCC as a result of said engineering and without loss of substantial ability to induces apoptosis. In one embodiment, the Type II anti-CD20 antibodies have been engineered to have an altered pattern of glycosylation in the Fc region. In a particular embodiment, the altered glycosylation comprises an increased level of bisected complex residues in the Fc region. In another particular embodiment, the altered glycosylation comprises and reduced level of fucose residues in the Fc region. See U.S. Pat. Appl. Pub. No. 2004 0093621 to Shitara *et al.*, the entire contents of which is incorporated by reference. In another embodiment, the Type II anti-CD20 antibodies have undergone polypeptide engineering as taught in U.S. Pat. No. 6,737,056 to Presta or U.S. Pat. Appl. Pub. No. 2004 0185045 (Macrogenics) or U.S. Pat. Appl. Pub. No. 2004 0132101 (Xencor), the entire contents of each of which are incorporated by reference. The invention is further directed to methods of making such engineered Type II antibodies and to methods of using such antibodies in the treatment of various B cell disorders, including B cell lymphomas.

[0117] Chimeric mouse/human antibodies have been described. See, for example, Morrison, S. L. et al., *PNAS* 11:6851-6854 (November 1984); European Patent Publication No. 173494; *Boulianna*, G. L, at al., *Nature* 312:642 (December 1984); Neubeiger, M. S. et al., *Nature* 314:268 (March 1985); European Patent Publication No. 125023; Tan et al., *J. Immunol.* 135:8564 (November 1985); Sun, L. K et al., *Hybridoma* 5(1):517 (1986); Sahagan et al., *J. Immunol.* 137:1066-1074 (1986). See generally, Muron, *Nature* 312:597 (December 1984); Dickson, *Genetic Engineering News* 5(3) (March 1985); Marx, *Science* 229:455 (August 1985); and Morrison, *Science* 229:1202-1207 (September 1985). Robinson *et al.*, in PCT Publication Number WO/88104936 describe a chimeric antibody with human constant region and murine variable region, having specificity to an epitope of CD20; the murine portion of the chimeric antibody of the Robinson references is derived from the 2H7 mouse monoclonal antibody (gamma 2b, kappa). While the reference notes that the described chimeric antibody is a "prime candidate" for the treatment of B cell disorders, this statement can be viewed as no more than a suggestion to those in the art to determine whether or not this suggestion is accurate for this particular antibody, particularly because the reference lacks any data to support an assertion of therapeutic effectiveness, and importantly, data using higher order mammals such as primates or humans.

[0118] Methodologies for generating chimeric antibodies are available to those in the art. For example, the light and heavy chains can be expressed separately, using, for example, immunoglobulin light chain and immunoglobulin heavy chains in separate plasmids, or on a single (e.g., polycistronic) vector. These can then be purified and assembled in vitro into complete antibodies; methodologies for accomplishing such assembly have been described. See, for example, Scharff, M., Harvey Lectures 69:125 (1974). In vitro reaction parameters for the formation of IgG antibodies from reduced isolated light and heavy chains have also been described. See, for example, Sears *et al.*, *Biochem.* 16(9):2016-25 (1977).

[0119] In a particularly preferred embodiment, the chimeric ABM of the present invention is a humanized antibody. Methods for humanizing non-human antibodies are known in the art. For example, humanized ABMs of the present

invention can be prepared according to the methods of U.S. Pat. No. 5,225,539 to Winter, U.S. Pat. No. 6,180,370 to Queen *et al.*, or U.S. Pat. No. 6,632,927 to Adair *et al.*, the entire contents of each of which is hereby incorporated by reference. Preferably, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones et al., *Nature*, 321:522-525 (1986); Riechmann et al., *Nature*, 332:323-327 (1988); Verhoeyen et al., *Science*, 239:1534-1536 (1988)), by substituting hypervariable region sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Pat. No. 4,816,567) wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some hypervariable region residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies. The subject humanized anti-CD20 antibodies will comprise constant regions of human immunoglobulin.

[0120] The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework region (FR) for the humanized antibody (Sims et al., *J. Immunol.*, 151:2296 (1993); Chothia et al., *J. Mol. Biol.*, 196:901 (1987)). Another method of selecting the human framework sequence is to compare the sequence of each individual subregion of the full rodent framework (i.e., FR1, FR2, FR3, and FR4) or some combination of the individual subregions (e.g., FR1 and FR2) against a library of known human variable region sequences that correspond to that framework subregion (e.g., as determined by Kabat numbering), and choose the human sequence for each subregion or combination that is the closest to that of the rodent (Leung U.S. Patent

Application Publication No. 2003/0040606A1, published Feb. 27, 2003). Another method uses a particular framework region derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (Carter et al., *Proc. Natl. Acad. Sci.* USA, 89:4285 (1992); Presta et al., *J. Immunol.*, 151:2623 (1993)).

[0121] It is further important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the hypervariable region residues are directly and most substantially involved in influencing antigen binding.

[0122] In another embodiment, the antigen binding molecules of the present invention are engineered to have enhanced binding affinity according to, for example, the methods disclosed in U.S. Pat. Appl. Pub. No. 2004/0132066 to Balint *et al.*, the entire contents of which are hereby incorporated by reference.

[0123] In one embodiment, the antigen binding molecule of the present invention is conjugated to an additional moiety, such as a radiolabel or a toxin. Such conjugated ABMs can be produced by numerous methods that are well known in the art.

[0124] A variety of radionuclides are applicable to the present invention and those skilled in the art are credited with the ability to readily determine which

radionuclide is most appropriate under a variety of circumstances. For example, ^{131}iodine is a well known radionuclide used for targeted immunotherapy. However, the clinical usefulness of ^{131}iodine can be limited by several factors including: eight-day physical half-life; dehalogenation of iodinated antibody both in the blood and at tumor sites; and emission characteristics (eg, large gamma component) which can be suboptimal for localized dose deposition in tumor. With the advent of superior chelating agents, the opportunity for attaching metal chelating groups to proteins has increased the opportunities to utilize other radionuclides such as ^{111}indium and ^{90}yttrium. ^{90}Yttrium provides several benefits for utilization in radioimmunotherapeutic applications: the 64 hour half-life of ^{90}yttrium is long enough to allow antibody accumulation by tumor and, unlike eg, ^{131}iodine, ^{90}yttrium is a pure beta emitter of high energy with no accompanying gamma irradiation in its decay, with a range in tissue of 100 to 1000 cell diameters. Furthermore, the minimal amount of penetrating radiation allows for outpatient administration of ^{90}yttrium-labeled antibodies. Additionally, internalization of labeled antibody is not required for cell killing, and the local emission of ionizing radiation should be lethal for adjacent tumor cells lacking the target antigen.

[0125] Effective single treatment dosages (i.e., therapeutically effective amounts) of ^{90}yttrium labeled anti-CD20 antibodies range from between about 5 and about 75 mCi, more preferably between about 10 and about 40 mCi. Effective single treatment non-marrow ablative dosages of ^{131}iodine labeled anti-CD20 antibodies range from between about 5 and about 70 mCi, more preferably between about 5 and about 40 mCi. Effective single treatment ablative dosages (ie, may require autologous bone marrow transplantation) of ^{131}iodine labeled anti-CD20 antibodies range from between about 30 and about 600 mCi, more preferably between about 50 and less than about 500 mCi. In conjunction with a chimeric anti-CD20 antibody, owing to the longer circulating half life vis-a-vis murine antibodies, an effective single treatment non-marrow ablative dosages of ^{131}iodine labeled chimeric anti-CD20 antibodies range from between about 5 and about 40 mCi, more preferably less than about 30 mCi. Imaging criteria for, e.g., the ^{111}indium label, are typically less than about 5 mCi.

[0126] With respect to radiolabeled anti-CD20 antibodies, therapy therewith can also occur using a single therapy treatment or using multiple treatments. Because of the radionuclide component, it is preferred that prior to treatment, peripheral stem cells ("PSC") or bone marrow ("BM") be "harvested" for patients experiencing potentially fatal bone marrow toxicity resulting from radiation. BM and/or PSC are harvested using standard techniques, and then purged and frozen for possible reinfusion. Additionally, it is most preferred that prior to treatment a diagnostic dosimetry study using a diagnostic labeled antibody (eg, using ^{111}indium) be conducted on the patient, a purpose of which is to ensure that the therapeutically labeled antibody (eg, using ^{90}yttrium) will not become unnecessarily "concentrated" in any normal organ or tissue.

[0127] In a preferred embodiment, the present invention is directed to an isolated polynucleotide comprising a sequence that encodes a polypeptide having an amino acid sequence as shown in Table 3 below. The invention is further directed to an isolated nucleic acid comprising a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence shown in Table 2 below. In another embodiment, the invention is directed to an isolated nucleic acid comprising a sequence that encodes a polypeptide having an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence in Table 3. The invention also encompasses an isolated nucleic acid comprising a sequence that encodes a polypeptide having the amino acid sequence of any of the constructs in Table 3 with conservative amino acid substitutions.

TABLE 2

CONSTRUCT	NUCLEOTIDE SEQUENCE	SEQ ID NO

CONSTRUCT	NUCLEOTIDE SEQUENCE	SEQ ID NO
B-HH1	CAGGTGCAATTGGTGCAGTCTGGCGCTGAAGTTAA GAAGCCTGGGAGTTCAGTGAAGGTCTCCTGCAAGG CTTCCGGATACACCTTCAGCTATTCTTGGATGAGCT GGGTGCGGCAGGCCCCTGGACAAGGGCTCGAGTGG ATGGGACGGATCTTTCCCGGCGATGGGGATACTGA CTACGCACAGAAATTCCAAGGAAGAGTCACAATTA CCGCCGACAAATCCACTAGCACAGCCTATATGGAG CTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTA TTACTGTGCAAGAAATGTCTTTGATGGTTACTGGCT TGTTTACTGGGGCCAGGGAACCCTGGTCACCGTCT CCTCA	29
B-HH2	CAGGTGCAATTGGTGCAGTCTGGCGCTGAAGTTAA GAAGCCTGGGAGTTCAGTGAAGGTCTCCTGCAAGG CTTCCGGATACGCCTTCAGCTATTCTTGGATGAACT GGGTGCGGCAGGCCCCTGGACAAGGGCTCGAGTGG ATGGGACGGATCTTTCCCGGCGATGGGGATACTGA CTACAATGGGAAATTCAAGGGCAGAGTCACAATTA CCGCCGACAAATCCACTAGCACAGCCTATATGGAG CTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTA TTACTGTGCAAGAAATGTCTTTGATGGTTACTGGCT TGTTTACTGGGGCCAGGGAACCCTGGTCACCGTCT CCTCA	31
B-HH3	CAGGTGCAATTGGTGCAGTCTGGCGCTGAAGTTAA GAAGCCTGGGAGTTCAGTGAAGGTCTCCTGCAAGG CTTCCGGATACGCCTTCAGCTATTCTTGGATGAACT GGGTGCGGCAGGCCCCTGGACAAGGGCTCGAGTGG ATGGGACGGATCTTTCCCGGCGATGGGGATACTGA CTACAATGGGAAATTCAAGGGCAGAGTCACAATTA CCGCCGACAAATCCACTAGCACAGCCTATATGGAG CTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTA TCTGTGTGCAAGAAATGTCTTTGATGGTTACTGGCT TGTTTACTGGGGCCAGGGAACCCTGGTCACCGTCT CCTCAGCTAGCACC	33
B-HH4	CAGGTGCAATTGGTGCAGTCTGGCGCTGAAGTTAA GAAGCCTGGAGCTTCAGTGAAGGTCTCCTGCAAGG TCTCCGGATACGCGTTCAGCTATTCTTGGATGAACT GGGTGCGGCAGGCCCCTGGACAAGGGCTCGAGTGG ATGGGACGGATCTTTCCCGGCGATGGGGATACTGA CTACAATGGGAAATTCAAGGGCAGAGTCACAATTA CCGCCGACAAATCCACTAGCACAGCCTATATGGAG CTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTA TTACTGTGCAAGAAATGTCTTTGATGGTTACTGGCT TGTTTACTGGGGCCAGGGAACCCTGGTCACCGTCT CCTCA	35

CONSTRUCT	NUCLEOTIDE SEQUENCE	SEQ ID NO
B-HH5	CAGGTGCAATTGGTGCAGTCTGGCGCTGAAGTTAA GAAGCCTGGGAGTTCAGTGAAGGTCTCCTGCAAGG CTTCCGGATACGCGTTCAGCTATTCTTGGATGAGCT GGGTGCGGCAGGCGCCTGGACAAGGGCTCGAGTG GATGGGACGGATCTTTCCCGGCGATGGGGATACTG ACTACAATGGGAAATTCAAGGGCAGAGTCACAATT ACCGCCGACAAATCCACTAGCACAGCCTATATGGA GCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGT ATTACTGTGCAAGAAATGTCTTTGATGGTTACTGGC TTGTTTACTGGGGCCAGGGAACCCTGGTCACCGTCT CCTCA	37
B-HH6	CAGGTGCAATTGGTGCAGTCTGGCGCTGAAGTTAA GAAGCCTGGGAGTTCAGTGAAGGTCTCCTGCAAGG CTTCCGGATACGCCTTCAGCTATTCTTGGATCAATT GGGTGCGGCAGGCGCCTGGACAAGGGCTCGAGTG GATGGGACGGATCTTTCCCGGCGATGGGGATACTG ACTACAATGGGAAATTCAAGGGCAGAGTCACAATT ACCGCCGACAAATCCACTAGCACAGCCTATATGGA GCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGT ATTACTGTGCAAGAAATGTCTTTGATGGTTACTGGC TTGTTTACTGGGGCCAGGGAACCCTGGTCACCGTCT CCTCA	39
B-HH7	CAGGTGCAATTGGTGCAGTCTGGCGCTGAAGTTAA GAAGCCTGGGAGTTCAGTGAAGGTCTCCTGCAAGG CTTCCGGATACGCCTTCAGCTATTCTTGGATCTCGT GGGTGCGGCAGGCGCCTGGACAAGGGCTCGAGTG GATGGGACGGATCTTTCCCGGCGATGGGGATACTG ACTACAATGGGAAATTCAAGGGCAGAGTCACAATT ACCGCCGACAAATCCACTAGCACAGCCTATATGGA GCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGT ATTACTGTGCAAGAAATGTCTTTGATGGTTACTGGC TTGTTTACTGGGGCCAGGGAACCCTGGTCACCGTCT CCTCA	41
B-HH8	CAGGTGCAATTGGTGCAGTCTGGCGCTGAAGTTAA GAAGCCTGGCGCCTCAGTGAAGGTCTCCTGCAAGG CTTCCGGATACACCTTCACATACAGCTGGATGAAC TGGGTGCGGCAGGCCCCTGGACAAGGGCTCGAGTG GATGGGACGGATCTTTCCCGGCGATGGGGATACTG ACTACAATGGGAAATTCAAGGGCAGAGTCACAATT ACCGCCGACAAATCCACTAGCACAGCCTATATGGA GCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGT ATTACTGTGCAAGAAATGTCTTTGATGGTTACTGGC TTGTTTACTGGGGCCAGGGAACCCTGGTCACCGTCT CCTCA	43

CONSTRUCT	NUCLEOTIDE SEQUENCE	SEQ ID NO
B-HH9	CAGGTGCAATTGGTGCAGTCTGGCGCTGAAGTTAA GAAGCCTGGCGCCTCAGTGAAGGTCTCCTGCAAGG CTTCCGGATACACCTTCAGCTATTCTTGGATGAACT GGGTGCGGCAGGCCCCTGGACAAGGGCTCGAGTGG ATGGGACGGATCTTTCCCGGCGATGGGGATACTGA CTACAATGGGAAATTCAAGGGCAGAGTCACAATTA CCGCCGACAAATCCACTAGCACAGCCTATATGGAG CTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTA TTACTGTGCAAGAAATGTCTTTGATGGTTACTGGCT TGTTTACTGGGGCCAGGGAACCCTGGTCACCGTCT CCTCA	45
B-HL1	CAGGTGCAATTGGTGCAGTCTGGCGCTGAAGTTAA GAAGCCTGGGGCCTCAGTGAAGGTCTCCTGCAAGG CTTCCGGATACACCTTCACCTATTCTTGGATGCACT GGGTGCGGCAGGCCCCTGGACAAGGGCTCGAGTGG ATGGGACGGATCTTTCCCGGCGATGGGGATACTGA CTACGCACAGAAATTCCAAGGAAGAGTCACAATGA CACGGGACACGTCCACTTCCACCGTCTATATGGAG CTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTA TTACTGTGCAAGAAATGTCTTTGATGGTTACTGGCT TGTTTACTGGGGCCAGGGAACCCTGGTCACCGTCT CCTCA	47
B-HL2	GAGGTGCAATTGGTGCAGTCTGGCGCTGAAGTTAA GAAGCCTGGGGCCACCGTGAAGATCTCCTGCAAGG TGTCCGGATACACCTTCACCTATTCTTGGATGCACT GGGTGCAGCAGGCCCCTGGAAAGGGGCTCGAGTG GATGGGACGGATCTTTCCCGGCGATGGGGATACTG ACTACGCAGAGAAATTCCAAGGAAGAGTCACAATC ACAGCCGACACGTCCACTGACACCGCCTATATGGA GCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGT ATTACTGTGCAACCAATGTCTTTGATGGTTACTGGC TTGTTTACTGGGGCCAGGGAACCCTGGTCACCGTCT CCTCA	49
B-HL3	GAGGTGCAATTGGTGCAGTCTGGCGCTGAAGTTAA GAAGCCTGGGGCCACCGTGAAGATCTCCTGCAAGG TGTCCGGATACACCTTCACCTATTCTTGGATGAACT GGGTGCAGCAGGCCCCTGGAAAGGGGCTCGAGTG GATGGGACGGATCTTTCCCGGCGATGGGGATACTG ACTACAATGGGAAATTCAAGGGAAGAGTCACAATC ACAGCCGACACGTCCACTGACACCGCCTATATGGA GCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGT ATTACTGTGCAACCAATGTCTTTGATGGTTACTGGC TTGTTTACTGGGGCCAGGGAACCCTGGTCACCGTCT CCTCA	51

CONSTRUCT	NUCLEOTIDE SEQUENCE	SEQ ID NO
B-HL4	CAGATGCAATTGGTGCAGTCTGGCGCTGAAGTTAA GAAGACCGGGAGTTCAGTGAAGGTCTCCTGCAAGG CTTCCGGATACACCTTCACCTATTCTTGGATGAGCT GGGTGCGGCAGGCCCCTGGACAAGGGCTCGAGTGG ATGGGACGGATCTTTCCCGGCGATGGGGATACTGA CTACGCACAGAAATTCCAAGGAAGAGTCACAATTA CCGCCGACAAATCCACTAGCACAGCCTATATGGAG CTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTA TTACTGTGCAAGAAATGTCTTTGATGGTTACTGGCT TGTTTACTGGGGCCAGGGAACCCTGGTCACCGTCT CCTCAGCTAGCACC	53
B-HL8	GAAGTGCAGCTGGTGGAGTCTGGAGGAGGCTTGGT CAAGCCTGGCGGGTCCCTGCGGCTCTCCTGTGCAG CCTCTGGATTCACATTTAGCTATTCTTGGATGAACT GGGTGCGGCAGGCTCCTGGAAAGGGCCTCGAGTGG GTGGGACGGATCTTTCCCGGCGATGGGGATACTGA CTACAATGGGAAATTCAAGGGCAGAGTCACAATTA CCGCCGACAAATCCACTAGCACAGCCTATATGGAG CTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTA TTACTGTGCAAGAAATGTCTTTGATGGTTACTGGCT TGTTTACTGGGGCCAGGGAACCCTGGTCACCGTCT CCTCA	55
B-HL10	CGGAATTCGGCCCACCGGTGGCCACCATGGACTGG ACCTGGAGGATCCTCTTCTTGGTGGCAGCAGCCAC AGGAGCCCACTCCGAAGTGCAGCTGGTGGAGTCTG GAGGAGGCTTGGTCAAGCCTGGCGGGTCCCTGCGG CTCTCCTGTGCAGCCTCTGGATTCGCATTCAGCTAT TCTTGGATGAACTGGGTGCGGCAGGCTCCTGGAAA GGGCCTCGAGTGGGTGGGACGGATCTTTCCCGGCG ATGGGGATACTGACTACAATGGGAAATTCAAGGGC AGAGTCACAATTACCGCCGACAAATCCACTAGCAC AGCCTATATGGAGCTGAGCAGCCTGAGATCTGAGG ACACGGCCGTGTATTACTGTGCAAGAAATGTCTTT GATGGTTACTGGCTTGTTTACTGGGGCCAGGGAAC CCTGGTCACCGTCTCCTCAGCTAGCGAATTCTCGA	57
B-HL11	CAGGTGCAGCTGGTGGAGTCTGGAGGAGGCTTGGT CAAGCCTGGCGGGTCCCTGCGGCTCTCCTGTGCAG CCTCTGGATTCACATTTAGCTATTCTTGGATGAACT GGGTGCGGCAGGCTCCTGGAAAGGGCCTCGAGTGG GTGGGACGGATCTTTCCCGGCGATGGGGATACTGA CTACAATGGGAAATTCAAGGGCAGAGTCACAATTA CCGCCGACAAATCCACTAGCACAGCCTATATGGAG CTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTA TTACTGTGCAAGAAATGTCTTTGATGGTTACTGGCT TGTTTACTGGGGCCAGGGAACCCTGGTCACCGTCT CCTCA	59

CONSTRUCT	NUCLEOTIDE SEQUENCE	SEQ ID NO
B-HL12	CGGAATTCGGCCCACCGGTGGCCACCATGGACTGG ACCTGGAGGATCCTCTTCTTGGTGGCAGCAGCCAC AGGAGCTCACTCCGAAGTGCAGCTCGTGGAGTCTG GAGCAGGCTTGGTCAAGCCTGGCGGGTCCCTGCGG CTCTCCTGCGCAGCCTCTGGATTCACATTTAGCTAT TCTTGGATGAACTGGGTGCGGCAGGCTCCTGGAAA GGGCCTCGAGTGGGTGGGACGGATCTTTCCCGGCG ATGGGGATACTGACTACAATGGGAAATTCAAGGGC AGAGTCACAATTACCGCCGACAAATCCACTAGCAC AGCCTATATGGAGCTGAGCAGCCTGAGATCTGAGG ACACGGCCGTGTATTACTGTGCAAGAAATGTCTTT GATGGTTACTGGCTTGTTTACTGGGGCCAGGGAAC CCTGGTCACCGTCTCCTCAGCTAGCGAATTCTCGA	61
B-HL13	CGGAATTCGGCCCACCGGTGGCCACCATGGACTGG ACCTGGAGGATCCTCTTCTTGGTGGCAGCAGCCAC AGGAGCTCACTCCGAAGTGCAGCTCGTCGAGTCTG GAGGAGGCGTGGTCAAGCCTGGCGGGTCCCTGCGG CTCTCCTGCGCAGCCTCTGGATTCACATTTAGCTAT TCTTGGATGAACTGGGTGCGGCAGGCTCCTGGAAA GGGCCTCGAGTGGGTGGGACGGATCTTTCCCGGCG ATGGGGATACTGACTACAATGGGAAATTCAAGGGC AGAGTCACAATTACCGCCGACAAATCCACTAGCAC AGCCTATATGGAGCTGAGCAGCCTGAGATCTGAGG ACACGGCCGTGTATTACTGTGCAAGAAATGTCTTT GATGGTTACTGGCTTGTTTACTGGGGCCAGGGAAC CCTGGTCACCGTCTCCTCAGCTAGCGAATTCTCGA	63
B-HL14	CGGAATTCGGCCCACCGGTGGCCACCATGGACTGG ACCTGGAGGATCCTCTTCTTGGTGGCAGCAGCCAC AGGAGCTCACTCCGAAGTGCAGCTGGTCGAGTCCG GAGGAGGCTTGAAGAAGCCTGGCGGGTCCCTGCGG CTCTCCTGCGCAGCCTCTGGATTCACATTTAGCTAT TCTTGGATGAACTGGGTGCGGCAGGCTCCTGGAAA GGGCCTCGAGTGGGTGGGACGGATCTTTCCCGGCG ATGGGGATACTGACTACAATGGGAAATTCAAGGGC AGAGTCACAATTACCGCCGACAAATCCACTAGCAC AGCCTATATGGAGCTGAGCAGCCTGAGATCTGAGG ACACGGCCGTGTATTACTGTGCAAGAAATGTCTTT GATGGTTACTGGCTTGTTTACTGGGGCCAGGGAAC CCTGGTCACCGTCTCCTCAGCTAGCGAATTCTCGA	65

CONSTRUCT	NUCLEOTIDE SEQUENCE	SEQ ID NO
B-HL15	CGGAATTCGGCCCACCGGTGGCCACCATGGACTGG ACCTGGAGGATCCTCTTCTTGGTGGCAGCAGCCAC AGGAGCCCACTCCGAAGTGCAGCTGGTGGAGTCTG GAGGAGGCTTGGTCAAGCCTGGCTCTTCCCTGCGG CTCTCCTGCGCAGCCTCTGGATTCACATTTAGCTAT TCTTGGATGAACTGGGTGCGGCAGGCTCCTGGAAA GGGCCTCGAGTGGGTGGGACGGATCTTTCCCGGCG ATGGGGATACTGACTACAATGGGAAATTCAAGGGC AGAGTCACAATTACCGCCGACAAATCCACTAGCAC AGCCTATATGGAGCTGAGCAGCCTGAGATCTGAGG ACACGGCCGTGTATTACTGTGCAAGAAATGTCTTT GATGGTTACTGGCTTGTTTACTGGGGCCAGGGAAC CCTGGTCACCGTCTCCTCAGCTAGCGAATTCTCGA	67
B-HL16	CGGAATTCGGCCCACCGGTGGCCACCATGGACTGG ACCTGGAGGATCCTCTTCTTGGTGGCAGCAGCCAC AGGAGCCCACTCCGAAGTGCAGCTGGTGGAGTCTG GAGGAGGCTTGGTCAAGCCTGGCGGGTCCCTGCGG GTCAGCTGCGCAGCCTCTGGATTCACATTTAGCTAT TCTTGGATGAACTGGGTGCGGCAGGCTCCTGGAAA GGGCCTCGAGTGGGTGGGACGGATCTTTCCCGGCG ATGGGGATACTGACTACAATGGGAAATTCAAGGGC AGAGTCACAATTACCGCCGACAAATCCACTAGCAC AGCCTATATGGAGCTGAGCAGCCTGAGATCTGAGG ACACGGCCGTGTATTACTGTGCAAGAAATGTCTTT GATGGTTACTGGCTTGTTTACTGGGGCCAGGGAAC CCTGGTCACCGTCTCCTCAGCTAGCGAATTCTCGA	69
B-HL17	CGGAATTCGGCCCACCGGTGGCCACCATGGACTGG ACCTGGAGGATCCTCTTCTTGGTGGCAGCAGCCAC AGGAGCCCACTCCGAAGTGCAGCTGGTGGAGTCTG GAGGAGGCTTGGTCAAGCCTGGCGGGTCCCTGCGG CTCTCCTGCGCAGCCTCTGGATTCACATTTAGCTAT TCTTGGATGAACTGGGTGCGGCAGGCTCCTGGAAA GGGCCTCGAGTGGGTGGGACGGATCTTTCCCGGCG ATGGGGATACTGACTACAATGGGAAATTCAAGGGC AGAGTCACAATTACCGCCGACAAATCCACTAGCAC AGCCTATATGGAGCTGAGCAGCCTGAGATCTGAGG ACACGGCCGTGTATTACTGTGCAAGAAATGTCTTT GATGGTTACTGGCTTGTTTACTGGGGCCAGGGAAC CCTGGTCACCGTCTCCTCAGCTAGCGAATTCTCGA	71
VH Signal Sequence	ATGGACTGGACCTGGAGGATCCTCTTCTTGGTGGC AGCAGCCACAGGAGCCCACTCC	73
B-KV1	GATATCGTGATGACCCAGACTCCACTCTCCCTGCCC GTCACCCCTGGAGAGCCCGCCAGCATTAGCTGCAG GTCTAGCAAGAGCCTCTTGCACAGCAATGGCATCA CTTATTTGTATTGGTACCTGCAAAAGCCAGGGCAG TCTCCACAGCTCCTGATTTATCAAATGTCCAACCTT GTCTCTGGCGTCCCTGACCGGTTCTCCGGATCCGGG TCAGGCACTGATTTCACACTGAAAATCAGCAGGGT GGAGGCTGAGGATGTTGGAGTTTATTACTGCGCTC AGAATCTAGAACTTCCTTACACCTTCGGCGGAGGG ACCAAGGTGGAGATCAAACGTACGGTG	75

CONSTRUCT	NUCLEOTIDE SEQUENCE	SEQ ID NO
VL Signal Sequence	ATGGACATGAGGGTCCCCGCTCAGCTCCTGGGCCT CCTGCTGCTCTGGTTCCCAGGTGCCAGGTGT	77

TABLE 3

CONSTRUCT	AMINO ACID SEQUENCE	SEQ ID NO
B-HH1	QVQLVQSGAEVKKPGSSVKVSCKASGYTFSYSWMSWVR QAPGQGLEWMGRIFPGDGDTDYAQKFQGRVTITADKSTS TAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQGTL VTVSS	30
B-HH2	QVQLVQSGAEVKKPGSSVKVSCKASGYAFSYSWMNWV RQAPGQGLEWMGRIFPGDGDTDYNGKFKGRVTITADKS TSTAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQG TLVTVSS	32
B-HH3	QVQLVQSGAEVKKPGSSVKVSCKASGYAFSYSWMNWV RQAPGQGLEWMGRIFPGDGDTDYNGKFKGRVTITADKS TSTAYMELSSLRSEDTAVYLCARNVFDGYWLVYWGQGT LVTVSS	34
B-HH4	QVQLVQSGAEVKKPGASVKVSCKVSGYAFSYSWMNWV RQAPGQGLEWMGRIFPGDGDTDYNGKFKGRVTITADKS TSTAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQG TLVTVSS	36
B-HH5	QVQLVQSGAEVKKPGSSVKVSCKASGYAFSYSWMSWV RQAPGQGLEWMGRIFPGDGDTDYNGKFKGRVTITADKS TSTAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQG TLVTVSS	38
B-HH6	QVQLVQSGAEVKKPGSSVKVSCKASGYAFSYSWINWVR QAPGQGLEWMGRIFPGDGDTDYNGKFKGRVTITADKSTS TAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQGTL VTVSS	40
B-HH7	QVQLVQSGAEVKKPGSSVKVSCKASGYAFSYSWISWVR QAPGQGLEWMGRIFPGDGDTDYNGKFKGRVTITADKSTS TAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQGTL VTVSS	42
B-HH8	QVQLVQSGAEVKKPGASVKVSCKASGYTFTYSWMNWV RQAPGQGLEWMGRIFPGDGDTDYNGKFKGRVTITADKS TSTAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQG TLVTVSS	44
B-HH9	QVQLVQSGAEVKKPGASVKVSCKASGYTFSYSWMNWV RQAPGQGLEWMGRIFPGDGDTDYNGKFKGRVTITADKS TSTAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQG TLVTVSS	46
B-HL1	QVQLVQSGAEVKKPGASVKVSCKASGYTFTYSWMHWV RQAPGQGLEWMGRIFPGDGDTDYAQKFQGRVTMTRDT STSTVYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQ GTLVTVSS	48

CONSTRUCT	AMINO ACID SEQUENCE	SEQ ID NO
B-HL2	EVQLVQSGAEVKKPGATVKISCKVSGYTFTYSWMHWV QQAPGKGLEWMGRIFPGDGDTDYAEKFQGRVTITADTS TDTAYMELSSLRSEDTAVYYCATNVFDGYWLVYWGQG TLVTVSS	50
B-HL3	EVQLVQSGAEVKKPGATVKISCKVSGYTFTYSWMNWV QQAPGKGLEWMGRIFPGDGDTDYNGKFKGRVTITADTS TDTAYMELSSLRSEDTAVYYCATNVFDGYWLVYWGQG TLVTVSS	52
B-HL4	QMQLVQSGAEVKKTGSSVKVSCKASGYTFTYSWMSWV RQAPGQGLEWMGRIFPGDGDTDYAQKFQGRVTITADKS TSTAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQG TLVTVSS	54
B-HL8	EVQLVESGGGLVKPGGSLRLSCAASGFTFSYSWMNWVR QAPGKGLEWVGRIFPGDGDTDYNGKFKGRVTITADKST STAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQGT LVTVSS	56
B-HL10	EVQLVESGGGLVKPGGSLRLSCAASGFAFSYSWMNWVR QAPGKGLEWVGRIFPGDGDTDYNGKFKGRVTITADKSTS TAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQGTL VTVSS	58
B-HL11	QVQLVESGGGLVKPGGSLRLSCAASGFTFSYSWMNWVR QAPGKGLEWVGRIFPGDGDTDYNGKFKGRVTITADKST STAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQGT LVTVSS	60
B-HL12	EVQLVESGAGLVKPGGSLRLSCAASGFTFSYSWMNWVR QAPGKGLEWMGRIFPGDGDTDYNGKFKGRVTITADKST STAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQGT LVTVSS	62
B-HL13	EVQLVESGGGVVKPGGSLRLSCAASGFTFSYSWMNWVR QAPGKGLEWMGRIFPGDGDTDYNGKFKGRVTITADKSTS TAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQGTL VTVSS	64
B-HL14	EVQLVESGGGLKKPGGSLRLSCAASGFTFSYSWMNWVR QAPGKGLEWMGRIFPGDGDTDYNGKFKGRVTITADKSTS TAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQGTL VTVSS	66
B-HL15	EVQLVESGGGLVKPGSSLRLSCAASGFTFSYSWMNWVR QAPGKGLEWMGRIFPGDGDTDYNGKFKGRVTITADKST STAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQGT LVTVSS	68
B-HL16	EVQLVESGGGLVKPGGSLRVSCAASGFTFSYSWMNWVR QAPGKGLEWMGRIFPGDGDTDYNGKFKGRVTITADKSTS TAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQGTL VTVSS	70
B-HL17	EVQLVESGGGLVKPGGSLRLSCAASGFTFSYSWMNWVR QAPGKGLEWMGRIFPGDGDTDYNGKFKGRVTITADKST STAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQGT LVTVSS	72
VH Signal Sequence	MDWTWRILFLVAAATGAHS	74

CONSTRUCT	AMINO ACID SEQUENCE	SEQ ID NO
B-KV1	DIVMTQTPLSLPVTPGEPASISCRSSKSLLHSNGITYLYWY LQKPGQSPQLLIYQMSNLVSGVPDRFSGSGSGTDFTLKIS RVEAEDVGVYYCAQNLELPYTFGGGTKVEIKRTV	76
VL Signal Sequence	MDMRVPAQLLGLLLLWFPGARC	78

[0128] In another preferred embodiment, the present invention is directed to an isolated polynucleotide comprising a sequence that encodes a polypeptide having the amino acid sequence shown in FIG. 1 or FIG. 2. The invention is further directed to an isolated nucleic acid comprising a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleotide sequence shown in FIG. 5 or FIG. 6. In another embodiment, the invention is directed to an isolated nucleic acid comprising a sequence that encodes a polypeptide having an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence FIG. 5 or FIG. 6. The invention also encompasses an isolated nucleic acid comprising a sequence that encodes a polypeptide having the amino acid sequence of any of FIG. 1, FIG. 2, FIG. 5 or FIG. 6 with conservative amino acid substitutions.

[0129] In another embodiment, the present invention is directed to an expression vector and/or a host cell which comprise one or more isolated polynucleotides of the present invention.

[0130] Generally, any type of cultured cell line can be used to express the ABM of the present invention. In a preferred embodiment, CHO cells, BHK cells, NS0 cells, SP2/0 cells, YO myeloma cells, P3X63 mouse myeloma cells, PER cells, PER.C6 cells or hybridoma cells, other mammalian cells, yeast cells, insect cells, or plant cells are used as the background cell line to generate the engineered host cells of the invention.

[0131] The therapeutic efficacy of the ABMs of the present invention can be enhanced by producing them in a host cell that further expresses a polynucleotide encoding a polypeptide having GnTIII activity. In a preferred embodiment, the polypeptide having GnTIII activity is a fusion polypeptide comprising the Golgi localization domain of a Golgi resident polypeptide. In another preferred embodiment, the expression of the ABMs of the present invention in a host cell

that expresses a polynucleotide encoding a polypeptide having GnTIII activity results in ABMs with increased Fc receptor binding affinity and increased effector function. Accordingly, in one embodiment, the present invention is directed to a host cell comprising (a) an isolated nucleic acid comprising a sequence encoding a polypeptide having GnTIII activity; and (b) an isolated polynucleotide encoding an ABM of the present invention, such as a chimeric, primatized or humanized antibody that binds human CD20. In a preferred embodiment, the polypeptide having GnTIII activity is a fusion polypeptide comprising the catalytic domain of GnTIII and the Golgi localization domain is the localization domain of mannosidase II. Methods for generating such fusion polypeptides and using them to produce antibodies with increased effector functions are disclosed in U.S. Provisional Pat. Appl. No. 60/495,142, the entire contents of which are expressly incorporated herein by reference. In another preferred embodiment, the chimeric ABM is a chimeric antibody or a fragment thereof, having the binding specificity of the murine B-LY1 antibody. In a particularly preferred embodiment, the chimeric antibody comprises a human Fc. In another preferred embodiment, the antibody is primatized or humanized.

[0132] In one embodiment, one or several polynucleotides encoding an ABM of the present invention may be expressed under the control of a constitutive promoter or, alternately, a regulated expression system. Suitable regulated expression systems include, but are not limited to, a tetracycline-regulated expression system, an ecdysone-inducible expression system, a lac-switch expression system, a glucocorticoid-inducible expression system, a temperature-inducible promoter system, and a metallothionein metal-inducible expression system. If several different nucleic acids encoding an ABM of the present invention are comprised within the host cell system, some of them may be expressed under the control of a constitutive promoter, while others are expressed under the control of a regulated promoter. The maximal expression level is considered to be the highest possible level of stable polypeptide expression that does not have a significant adverse effect on cell growth rate, and will be determined using routine experimentation. Expression levels are determined by methods generally known in the art, including Western blot analysis using an

antibody specific for the ABM or an antibody specific for a peptide tag fused to the ABM; and Northern blot analysis. In a further alternative, the polynucleotide may be operatively linked to a reporter gene; the expression levels of a chimeric ABM having substantially the same binding specificity of the murine B-Ly1 antibody are determined by measuring a signal correlated with the expression level of the reporter gene. The reporter gene may be transcribed together with the nucleic acid(s) encoding said fusion polypeptide as a single mRNA molecule; their respective coding sequences may be linked either by an internal ribosome entry site (IRES) or by a cap-independent translation enhancer (CITE). The reporter gene may be translated together with at least one nucleic acid encoding a chimeric ABM having substantially the same binding specificity of the murine B-Ly1 antibody such that a single polypeptide chain is formed. The nucleic acids encoding the AMBs of the present invention may be operatively linked to the reporter gene under the control of a single promoter, such that the nucleic acid encoding the fusion polypeptide and the reporter gene are transcribed into an RNA molecule which is alternatively spliced into two separate messenger RNA (mRNA) molecules; one of the resulting mRNAs is translated into said reporter protein, and the other is translated into said fusion polypeptide.

[0133] Methods which are well known to those skilled in the art can be used to construct expression vectors containing the coding sequence of an ABM having substantially the same binding specificity of the murine B-Ly1 antibody along with appropriate transcriptional/translational control signals. These methods include *in vitro* recombinant DNA techniques, synthetic techniques and *in vivo* recombination/genetic recombination. *See*, for example, the techniques described in Maniatis *et al.*, MOLECULAR CLONING A LABORATORY MANUAL, Cold Spring Harbor Laboratory, N.Y. (1989) and Ausubel *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Greene Publishing Associates and Wiley Interscience, N.Y (1989).

[0134] A variety of host-expression vector systems may be utilized to express the coding sequence of the ABMs of the present invention. Preferably, mammalian cells are used as host cell systems transfected with recombinant plasmid DNA or cosmid DNA expression vectors containing the coding sequence of the protein of

interest and the coding sequence of the fusion polypeptide. Most preferably, CHO cells, BHK cells, NS0 cells, SP2/0 cells, YO myeloma cells, P3X63 mouse myeloma cells, PER cells, PER.C6 cells or hybridoma cells, other mammalian cells, yeast cells, insect cells, or plant cells are used as host cell system. Some examples of expression systems and selection methods are described in the following references, and references therein: Borth *et al.*, *Biotechnol. Bioen.* 71(4):266-73 (2000-2001), in Werner *et al.*, *Arzneimittelforschung/Drug Res.* 48(8):870-80 (1998), in Andersen and Krummen, *Curr. Op. Biotechnol.* 13:117-123 (2002), in Chadd and Chamow, *Curr. Op. Biotechnol.* 12:188-194 (2001), and in Giddings, *Curr. Op. Biotechnol.* 12: 450-454 (2001). In alternate embodiments, other eukaryotic host cell systems may be contemplated, including yeast cells transformed with recombinant yeast expression vectors containing the coding sequence of an ABM of the present invention; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing the coding sequence of a chimeric ABM having substantially the same binding specificity of the murine B-Ly1 antibody; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing the coding sequence of the ABM of the invention; or animal cell systems infected with recombinant virus expression vectors (*e.g.*, adenovirus, vaccinia virus) including cell lines engineered to contain multiple copies of the DNA encoding a chimeric ABM having substantially the same binding specificity of the murine B-Ly1 antibody either stably amplified (CHO/dhfr) or unstably amplified in double-minute chromosomes (*e.g.*, murine cell lines). In one embodiment, the vector comprising the polynucleotide(s) encoding the ABM of the invention is polycistronic. Also, in one embodiment the ABM discussed above is an antibody or a fragment thereof. In a preferred embodiment, the ABM is a humanized antibody.

[0135] For the methods of this invention, stable expression is generally preferred to transient expression because it typically achieves more reproducible results and also is more amenable to large-scale production. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed

with the respective coding nucleic acids controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows selection of cells which have stably integrated the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines.

[0136] A number of selection systems may be used, including, but not limited to, the herpes simplex virus thymidine kinase (Wigler *et al.*, *Cell 11*:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, *Proc. Natl. Acad. Sci. USA 48*:2026 (1962)), and adenine phosphoribosyltransferase (Lowy *et al.*, *Cell 22*:817 (1980)) genes, which can be employed in tk^-, $hgprt^-$ or $aprt^-$ cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for dhfr, which confers resistance to methotrexate (Wigler *et al.*, *Natl. Acad. Sci. USA 77*:3567 (1989); O'Hare *et al.*, *Proc. Natl. Acad. Sci. USA 78*:1527 (1981)); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, *Proc. Natl. Acad. Sci. USA 78*:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin *et al.*, *J. Mol. Biol. 150*:1 (1981)); and hygro, which confers resistance to hygromycin (Santerre *et al.*, *Gene 30*:147 (1984) genes. Recently, additional selectable genes have been described, namely trpB, which allows cells to utilize indole in place of tryptophan; hisD, which allows cells to utilize histinol in place of histidine (Hartman & Mulligan, *Proc. Natl. Acad. Sci. USA 85*:8047 (1988)); the glutamine synthase system; and ODC (ornithine decarboxylase) which confers resistance to the ornithine decarboxylase inhibitor, 2-(difluoromethyl)-DL-ornithine, DFMO (McConlogue, *in*: *Current Communications in Molecular Biology*, Cold Spring Harbor Laboratory ed. (1987)).

[0137] The present invention is further directed to a method for modifying the glycosylation profile of the ABMs of the present invention that are produced by a host cell, comprising expressing in said host cell a nucleic acid encoding an ABM

of the invention and a nucleic acid encoding a polypeptide with GnTIII activity, or a vector comprising such nucleic acids. Preferably, the modified polypeptide is IgG or a fragment thereof comprising the Fc region. In a particularly preferred embodiment the ABM is a humanized antibody or a fragment thereof.

[0138] The modified ABMs produced by the host cells of the invention exhibit increased Fc receptor binding affinity and/or increased effector function as a result of the modification. In a particularly preferred embodiment the ABM is a humanized antibody or a fragment thereof containing the Fc region. Preferably, the increased Fc receptor binding affinity is increased binding to a Fcγ activating receptor, such as the FcγRIIIa receptor. The increased effector function is preferably an increase in one or more of the following: increased antibody-dependent cellular cytotoxicity, increased antibody-dependent cellular phagocytosis (ADCP), increased cytokine secretion, increased immune-complex-mediated antigen uptake by antigen-presenting cells, increased Fc-mediated cellular cytotoxicity, increased binding to NK cells, increased binding to macrophages, increased binding to polymorphonuclear cells (PMNs), increased binding to monocytes, increased crosslinking of target-bound antibodies, increased direct signaling inducing apoptosis, increased dendritic cell maturation, and increased T cell priming.

[0139] The present invention is also directed to a method for producing an ABM of the present invention, having modified oligosaccharides in a host cell comprising (a) culturing a host cell engineered to express at least one nucleic acid encoding a polypeptide having GnTIII activity under conditions which permit the production of an ABM according to the present invention, wherein said polypeptide having GnTIII activity is expressed in an amount sufficient to modify the oligosaccharides in the Fc region of said ABM produced by said host cell; and (b) isolating said ABM. In a preferred embodiment, the polypeptide having GnTIII activity is a fusion polypeptide comprising the catalytic domain of GnTIII. In a particularly preferred embodiment, the fusion polypeptide further comprises the Golgi localization domain of a Golgi resident polypeptide.

[0140] Preferably, the Golgi localization domain is the localization domain of mannosidase II or GnTI. Alternatively, the Golgi localization domain is selected

from the group consisting of: the localization domain of mannosidase I, the localization domain of GnTII, and the localization domain of α 1-6 core fucosyltransferase. The ABMs produced by the methods of the present invention have increased Fc receptor binding affinity and/or increased effector function. Preferably, the increased effector function is one or more of the following: increased Fc-mediated cellular cytotoxicity (including increased antibody-dependent cellular cytotoxicity), increased antibody-dependent cellular phagocytosis (ADCP), increased cytokine secretion, increased immune-complex-mediated antigen uptake by antigen-presenting cells, increased binding to NK cells, increased binding to macrophages, increased binding to monocytes, increased binding to polymorphonuclear cells, increased direct signaling inducing apoptosis, increased crosslinking of target-bound antibodies, increased dendritic cell maturation, or increased T cell priming. The increased Fc receptor binding affinity is preferably increased binding to Fc activating receptors such as FcγRIIIa. In a particularly preferred embodiment the ABM is a humanized antibody or a fragment thereof.

[0141] In another embodiment, the present invention is directed to a chimeric ABM having substantially the same binding specificity of the murine B-Ly1 antibody produced by the methods of the invention which has an increased proportion of bisected oligosaccharides in the Fc region of said polypeptide. It is contemplated that such an ABM encompasses antibodies and fragments thereof comprising the Fc region. In a preferred embodiment, the ABM is a humanized antibody. In one embodiment, the percentage of bisected oligosaccharides in the Fc region of the ABM is at least 50%, more preferably, at least 60%, at least 70%, at least 80%, or at least 90%, and most preferably at least 90-95% of the total oligosaccharides. In yet another embodiment, the ABM produced by the methods of the invention has an increased proportion of nonfucosylated oligosaccharides in the Fc region as a result of the modification of its oligosaccharides by the methods of the present invention. In one embodiment, the percentage of nonfucosylated oligosaccharides is at least 50%, preferably, at least 60% to 70%, most preferably at least 75%. The nonfucosylated oligosaccharides may be of the hybrid or complex type. In a particularly

preferred embodiment, the ABM produced by the host cells and methods of the invention has an increased proportion of bisected, nonfucosylated oligosaccharides in the Fc region. The bisected, nonfucosylated oligosaccharides may be either hybrid or complex. Specifically, the methods of the present invention may be used to produce ABMs in which at least 15%, more preferably at least 20%, more preferably at least 25%, more preferably at least 30%, more preferably at least 35% of the oligosaccharides in the Fc region of the ABM are bisected, nonfucosylated. The methods of the present invention may also be used to produce polypeptides in which at least 15%, more preferably at least 20%, more preferably at least 25%, more preferably at least 30%, more preferably at least 35% of the oligosaccharides in the Fc region of the polypeptide are bisected hybrid nonfucosylated.

[0142] In another embodiment, the present invention is directed to a chimeric ABM having substantially the same binding specificity of the murine B-Ly1 antibody engineered to have increased effector function and/or increased Fc receptor binding affinity, produced by the methods of the invention. Preferably, the increased effector function is one or more of the following: increased Fc-mediated cellular cytotoxicity (including increased antibody-dependent cellular cytotoxicity), increased antibody-dependent cellular phagocytosis (ADCP), increased cytokine secretion, increased immune-complex-mediated antigen uptake by antigen-presenting cells, increased binding to NK cells, increased binding to macrophages, increased binding to monocytes, increased binding to polymorphonuclear cells, increased direct signaling inducing apoptosis, increased crosslinking of target-bound antibodies, increased dendritic cell maturation, or increased T cell priming. In a preferred embodiment, the increased Fc receptor binding affinity is increased binding to a Fc activating receptor, most preferably FcγRIIIa. In one embodiment, the ABM is an antibody, an antibody fragment containing the Fc region, or a fusion protein that includes a region equivalent to the Fc region of an immunoglobulin. In a particularly preferred embodiment, the ABM is a humanized antibody.

[0143] The present invention is further directed to pharmaceutical compositions comprising the ABMs of the present invention and a pharmaceutically acceptable carrier.

[0144] The present invention is further directed to the use of such pharmaceutical compositions in the method of treatment of cancer. Specifically, the present invention is directed to a method for the treatment of cancer comprising administering a therapeutically effective amount of the pharmaceutical composition of the invention.

[0145] The present invention further provides methods for the generation and use of host cell systems for the production of glycoforms of the ABMs of the present invention, having increased Fc receptor binding affinity, preferably increased binding to Fc activating receptors, and/or having increased effector functions, including antibody-dependent cellular cytotoxicity. The glycoengineering methodology that can be used with the ABMs of the present invention has been described in greater detail in U.S. Pat. No. 6,602,684 and Provisional U.S. Patent Application No. 60/441,307 and WO 2004/065540, the entire contents of each of which is incorporated herein by reference in its entirety. The ABMs of the present invention can alternatively be glycoengineered to have reduced fucose residues in the Fc region according to the techniques disclosed in EP 1 176 195 A1, the entire contents of which is incorporated by reference herein.

. Generation Of Cell Lines For The Production Of Proteins With Altered Glycosylation Pattern

[0146] The present invention provides host cell expression systems for the generation of the ABMs of the present invention having modified glycosylation patterns. In particular, the present invention provides host cell systems for the generation of glycoforms of the ABMs of the present invention having an improved therapeutic value. Therefore, the invention provides host cell expression systems selected or engineered to express a polypeptide having GnTIII activity. In one embodiment, the polypeptide having GnTIII activity is a fusion polypeptide comprising the Golgi localization domain of a heterologous Golgi resident polypeptide. Specifically, such host cell expression systems may

be engineered to comprise a recombinant nucleic acid molecule encoding a polypeptide having GnTIII, operatively linked to a constitutive or regulated promoter system.

[0147] In one specific embodiment, the present invention provides a host cell that has been engineered to express at least one nucleic acid encoding a fusion polypeptide having GnTIII activity and comprising the Golgi localization domain of a heterologous Golgi resident polypeptide. In one aspect, the host cell is engineered with a nucleic acid molecule comprising at least one gene encoding a fusion polypeptide having GnTIII activity and comprising the Golgi localization domain of a heterologous Golgi resident polypeptide.

[0148] Generally, any type of cultured cell line, including the cell lines discussed above, can be used as a background to engineer the host cell lines of the present invention. In a preferred embodiment, CHO cells, BHK cells, NS0 cells, SP2/0 cells, YO myeloma cells, P3X63 mouse myeloma cells, PER cells, PER.C6 cells or hybridoma cells, other mammalian cells, yeast cells, insect cells, or plant cells are used as the background cell line to generate the engineered host cells of the invention.

[0149] The invention is contemplated to encompass any engineered host cells expressing a polypeptide having GnTIII activity, including a fusion polypeptide that comprises the Golgi localization domain of a heterologous Golgi resident polypeptide as defined herein.

[0150] One or several nucleic acids encoding a polypeptide having GnTIII activity may be expressed under the control of a constitutive promoter or, alternately, a regulated expression system. Such systems are well known in the art, and include the systems discussed above. If several different nucleic acids encoding fusion polypeptides having GnTIII activity and comprising the Golgi localization domain of a heterologous Golgi resident polypeptide are comprised within the host cell system, some of them may be expressed under the control of a constitutive promoter, while others are expressed under the control of a regulated promoter. Expression levels of the fusion polypeptides having GnTIII activity are determined by methods generally known in the art, including Western blot analysis, Northern blot analysis, reporter gene expression analysis or

measurement of GnTIII activity. Alternatively, a lectin may be employed which binds to biosynthetic products of the GnTIII, for example, E_4-PHA lectin. Alternatively, a functional assay which measures the increased Fc receptor binding or increased effector function mediated by antibodies produced by the cells engineered with the nucleic acid encoding a polypeptide with GnTIII activity may be used.

Identification Of Transfectants Or Transformants That Express The Protein Having A Modified Glycosylation Pattern

[0151] The host cells which contain the coding sequence of a chimeric ABM having substantially the same binding specificity of the murine B-Ly1 antibody and which express the biologically active gene products may be identified by at least four general approaches; (a) DNA-DNA or DNA-RNA hybridization; (b) the presence or absence of "marker" gene functions; (c) assessing the level of transcription as measured by the expression of the respective mRNA transcripts in the host cell; and (d) detection of the gene product as measured by immunoassay or by its biological activity.

[0152] In the first approach, the presence of the coding sequence of a chimeric ABM having substantially the same binding specificity of the murine B-Ly1 antibody and the coding sequence of the polypeptide having GnTIII activity can be detected by DNA-DNA or DNA-RNA hybridization using probes comprising nucleotide sequences that are homologous to the respective coding sequences, respectively, or portions or derivatives thereof.

[0153] In the second approach, the recombinant expression vector/host system can be identified and selected based upon the presence or absence of certain "marker" gene functions (*e.g.*, thymidine kinase activity, resistance to antibiotics, resistance to methotrexate, transformation phenotype, occlusion body formation in baculovirus, etc.). For example, if the coding sequence of the ABM of the invention, or a fragment thereof, and the coding sequence of the polypeptide having GnTIII activity are inserted within a marker gene sequence of the vector, recombinants containing the respective coding sequences can be identified by the absence of the marker gene function. Alternatively, a marker gene can be placed

in tandem with the coding sequences under the control of the same or different promoter used to control the expression of the coding sequences. Expression of the marker in response to induction or selection indicates expression of the coding sequence of the ABM of the invention and the coding sequence of the polypeptide having GnTIII activity.

[0154] In the third approach, transcriptional activity for the coding region of the ABM of the invention, or a fragment thereof, and the coding sequence of the polypeptide having GnTIII activity can be assessed by hybridization assays. For example, RNA can be isolated and analyzed by Northern blot using a probe homologous to the coding sequences of the ABM of the invention, or a fragment thereof, and the coding sequence of the polypeptide having GnTIII activity or particular portions thereof. Alternatively, total nucleic acids of the host cell may be extracted and assayed for hybridization to such probes.

[0155] In the fourth approach, the expression of the protein products can be assessed immunologically, for example by Western blots, immunoassays such as radioimmuno-precipitation, enzyme-linked immunoassays and the like. The ultimate test of the success of the expression system, however, involves the detection of the biologically active gene products.

Generation And Use Of ABMs Having Increased Effector Function Including Antibody-Dependent Cellular Cytotoxicity

[0156] In preferred embodiments, the present invention provides glycoforms of chimeric ABMs having substantially the same binding specificity of the murine B-Ly1 antibody and having increased effector function including antibody-dependent cellular cytotoxicity. Glycosylation engineering of antibodies has been previously described. See, *e.g.*, U.S. Patent No. 6,602,684, incorporated herein by reference in its entirety.

[0157] Clinical trials of unconjugated monoclonal antibodies (mAbs) for the treatment of some types of cancer have recently yielded encouraging results. Dillman, *Cancer Biother. & Radiopharm. 12*:223-25 (1997); Deo *et al.*, *Immunology Today 18*:127 (1997). A chimeric, unconjugated IgG1 has been approved for low-grade or follicular B-cell non-Hodgkin's lymphoma. Dillman,

Cancer Biother. & Radiopharm. 12:223-25 (1997), while another unconjugated mAb, a humanized IgG1 targeting solid breast tumors, has also been showing promising results in phase III clinical trials. Deo *et al.*, *Immunology Today 18*:127 (1997). The antigens of these two mAbs are highly expressed in their respective tumor cells and the antibodies mediate potent tumor destruction by effector cells *in vitro* and *in vivo*. In contrast, many other unconjugated mAbs with fine tumor specificities cannot trigger effector functions of sufficient potency to be clinically useful. Frost *et al.*, *Cancer 80*:317-33 (1997); Surfus *et al.*, *J. Immunother. 19*:184-91 (1996). For some of these weaker mAbs, adjunct cytokine therapy is currently being tested. Addition of cytokines can stimulate antibody-dependent cellular cytotoxicity (ADCC) by increasing the activity and number of circulating lymphocytes. Frost *et al.*, *Cancer 80*:317-33 (1997); Surfus *et al.*, *J. Immunother. 19*:184-91 (1996). ADCC, a lytic attack on antibody-targeted cells, is triggered upon binding of leukocyte receptors to the constant region (Fc) of antibodies. Deo *et al.*, *Immunology Today 18*:127 (1997).

[0158] A different, but complementary, approach to increase ADCC activity of unconjugated IgG1s is to engineer the Fc region of the antibody. Protein engineering studies have shown that FcγRs interact with the lower hinge region of the IgG CH2 domain. Lund *et al.*, *J. Immunol. 157*:4963-69 (1996). However, FcγR binding also requires the presence of oligosaccharides covalently attached at the conserved Asn 297 in the CH2 region. Lund *et al.*, *J. Immunol. 157*:4963-69 (1996); Wright and Morrison, *Trends Biotech. 15*:26-31 (1997), suggesting that either oligosaccharide and polypeptide both directly contribute to the interaction site or that the oligosaccharide is required to maintain an active CH2 polypeptide conformation. Modification of the oligosaccharide structure can therefore be explored as a means to increase the affinity of the interaction.

[0159] An IgG molecule carries two N-linked oligosaccharides in its Fc region, one on each heavy chain. As any glycoprotein, an antibody is produced as a population of glycoforms which share the same polypeptide backbone but have different oligosaccharides attached to the glycosylation sites. The oligosaccharides normally found in the Fc region of serum IgG are of complex bi-antennary type (Wormald *et al.*, *Biochemistry 36*:130-38 (1997), with a low

level of terminal sialic acid and bisecting N-acetylglucosamine (GlcNAc), and a variable degree of terminal galactosylation and core fucosylation. Some studies suggest that the minimal carbohydrate structure required for FcγR binding lies within the oligosaccharide core. Lund *et al., J. Immunol. 157*:4963-69 (1996)

[0160] The mouse- or hamster-derived cell lines used in industry and academia for production of unconjugated therapeutic mAbs normally attach the required oligosaccharide determinants to Fc sites. IgGs expressed in these cell lines lack, however, the bisecting GlcNAc found in low amounts in serum IgGs. Lifely *et al., Glycobiology 318*:813-22 (1995). In contrast, it was recently observed that a rat myeloma-produced, humanized IgG1 (CAMPATH-1H) carried a bisecting GlcNAc in some of its glycoforms. Lifely *et al., Glycobiology 318*:813-22 (1995). The rat cell-derived antibody reached a similar maximal *in vitro* ADCC activity as CAMPATH-1H antibodies produced in standard cell lines, but at significantly lower antibody concentrations.

[0161] The CAMPATH antigen is normally present at high levels on lymphoma cells, and this chimeric mAb has high ADCC activity in the absence of a bisecting GlcNAc. Lifely *et al., Glycobiology 318*:813-22 (1995). In the N-linked glycosylation pathway, a bisecting GlcNAc is added by GnTIII. Schachter, *Biochem. Cell Biol. 64*:163-81 (1986).

[0162] Previous studies used a single antibody-producing CHO cell line, that was previously engineered to express, in an externally-regulated fashion, different levels of a cloned GnT III gene enzyme (Umana, P., *et al., Nature Biotechnol. 17*:176-180 (1999)). This approach established for the first time a rigorous correlation between expression of GnTIII and the ADCC activity of the modified antibody. Thus, the invention contemplates a recombinant, chimeric antibody or a fragment thereof with the binding specificity of the murine B-Ly1 antibody, having altered glycosylation resulting from increased GnTIII activity. The increased GnTIII activity results in an increase in the percentage of bisected oligosaccharides, as well as a decrease in the percentage of fucose residues, in the Fc region of the ABM. This antibody, or fragment thereof, has increased Fc receptor binding affinity and increased effector function. In addition, the

invention is directed to antibody fragment and fusion proteins comprising a region that is equivalent to the Fc region of immunoglobulins.

Therapeutic Applications of ABMs Produced According to the Methods of the Invention.

[0163] The ABMs of the present can be used alone to target and kill tumor cells in vivo. The ABMs can also be used in conjunction with an appropriate therapeutic agent to treat human carcinoma. For example, the ABMs can be used in combination with standard or conventional treatment methods such as chemotherapy, radiation therapy or can be conjugated or linked to a therapeutic drug, or toxin, as well as to a lymphokine or a tumor-inhibitory growth factor, for delivery of the therapeutic agent to the site of the carcinoma. The conjugates of the ABMs of this invention that are of prime importance are (1) immunotoxins (conjugates of the ABM and a cytotoxic moiety) and (2) labeled (e.g. radiolabeled, enzyme-labeled, or fluorochrome-labeled) ABMs in which the label provides a means for identifying immune complexes that include the labeled ABM. The ABMs can also be used to induce lysis through the natural complement process, and to interact with antibody dependent cytotoxic cells normally present.

[0164] The cytotoxic moiety of the immunotoxin may be a cytotoxic drug or an enzymatically active toxin of bacterial or plant origin, or an enzymatically active fragment ("A chain") of such a toxin. Enzymatically active toxins and fragments thereof used are diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolacca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, and enomycin. In another embodiment, the ABMs are conjugated to small molecule anticancer drugs. Conjugates of the ABM and such cytotoxic moieties are made using a variety of bifunctional protein coupling agents. Examples of such reagents are SPDP, IT, bifunctional derivatives of imidoesters such a dimethyl adipimidate HCl, active esters such as

disuccinimidyl suberate, aldehydes such as glutaraldehyde, bis-azido compounds such as bis (p-azidobenzoyl) hexanediamine, bis-diazonium derivatives such as bis-(p-diazoniumbenzoyl)-ethylenediamine, diisocyanates such as tolylene 2,6-diisocyanate, and bis-active fluorine compounds such as 1,5-difluoro-2,4-dinitrobenzene. The lysing portion of a toxin may be joined to the Fab fragment of the ABMs. Additional appropriate toxins are known in the art, as evidenced in e.g., published U.S. Patent Application No. 2002/0128448, incorporated herein by reference in its entirety.

[0165] In one embodiment, a chimeric, glycoengineered ABM having substantially the same binding specificity of the murine B-Ly1 antibody, is conjugated to ricin A chain. Most advantageously, the ricin A chain is deglycosylated and produced through recombinant means. An advantageous method of making the ricin immunotoxin is described in Vitetta et al., *Science* 238, 1098 (1987), hereby incorporated by reference.

[0166] When used to kill human cancer cells in vitro for diagnostic purposes, the conjugates will typically be added to the cell culture medium at a concentration of at least about 10 nM. The formulation and mode of administration for in vitro use are not critical. Aqueous formulations that are compatible with the culture or perfusion medium will normally be used. Cytotoxicity may be read by conventional techniques to determine the presence or degree of cancer.

[0167] As discussed above, a cytotoxic radiopharmaceutical for treating cancer may be made by conjugating a radioactive isotope (e.g., I, Y, Pr) to a chimeric, glycoengineered ABM having substantially the same binding specificity of the murine B-Ly1 antibody. The term "cytotoxic moiety" as used herein is intended to include such isotopes.

[0168] In another embodiment, liposomes are filled with a cytotoxic drug and the liposomes are coated with the ABMs of the present invention. Because there are many CD20 molecules on the surface of the malignant B-cell, this method permits delivery of large amounts of drug to the correct cell type.

[0169] Techniques for conjugating such therapeutic agents to antibodies are well known (see, e.g., Arnon et al., "Monoclonal Antibodies for Immunotargeting of Drugs in Cancer Therapy", in *Monoclonal Antibodies and Cancer Therapy*,

Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera et al. (eds.), pp. 475-506 (1985); and Thorpe *et al.*, "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.*, 62:119-58 (1982)).

[0170] Still other therapeutic applications for the ABMs of the invention include conjugation or linkage, e.g., by recombinant DNA techniques, to an enzyme capable of converting a prodrug into a cytotoxic drug and the use of that antibody-enzyme conjugate in combination with the prodrug to convert the prodrug to a cytotoxic agent at the tumor site (see, e.g., Senter et al., "Anti-Tumor Effects of Antibody-alkaline Phosphatase", *Proc. Natl. Acad. Sci. USA* *85*:4842-46 (1988); "Enhancement of the in vitro and in vivo Antitumor Activites of Phosphorylated Mitocycin C and Etoposide Derivatives by Monoclonal Antibody-Alkaline Phosphatase Conjugates", Cancer Research 49:5789-5792 (1989); and Senter, "Activation of Prodrugs by Antibody-Enzyme Conjugates: A New Approach to Cancer Therapy," *FASEB J. 4*:188-193 (1990)).

[0171] Still another therapeutic use for the ABMs of the invention involves use, either unconjugated, in the presence of complement, or as part of an antibody-drug or antibody-toxin conjugate, to remove tumor cells from the bone marrow of cancer patients. According to this approach, autologous bone marrow may be purged ex vivo by treatment with the antibody and the marrow infused back into the patient [see, e.g., Ramsay et al., "Bone Marrow Purging Using Monoclonal Antibodies", *J. Clin. Immunol.*, 8(2):81-88 (1988)].

[0172] Furthermore, it is contemplated that the invention comprises a single-chain immunotoxin comprising antigen binding domains that allow substantially the same specificity of binding as the murine B-Ly1 antibody (e.g., polypeptides comprising the CDRs of the murine B-Ly1 antibody) and further comprising a toxin polypeptide. The single-chain immunotoxins of the invention may be used to treat human carcinoma *in vivo*.

[0173] Similarly, a fusion protein comprising at least the antigen-binding region of an ABM of the invention joined to at least a functionally active portion of a second protein having anti-tumor acitivty, *e.g.,* a lymphokine or oncostatin, can be used to treat human carcinoma in vivo.

[0174] The present invention provides a method for selectively killing tumor cells expressing CD20. This method comprises reacting the immunoconjugate (e.g., the immunotoxin) of the invention with said tumor cells. These tumor cells may be from a human carcinoma.

[0175] Additionally, this invention provides a method of treating carcinomas (for example, human carcinomas) *in vivo*. This method comprises administering to a subject a pharmaceutically effective amount of a composition containing at least one of the immunoconjugates (e.g., the immunotoxin) of the invention.

[0176] In a further aspect, the invention is directed to an improved method for treating B-cell proliferative disorders including B-cell lymphoma, as well as an autoimmune disease produced in whole or in part by pathogenic autoantibodies, based on B-cell depletion comprising administering a therapeutically effective amount of an ABM of the present invention to a human subject in need thereof. In a preferred embodiment, the ABM is a glycoengineered anti-CD20 antibody with a binding specificity substantially the same as that of the murine B-Ly1 antibody. In another preferred embodiment the antibody is humanized. Examples of autoimmune diseases or disorders include, but are not limited to, immune-mediated thrombocytopenias, such as acute idiopathic thrombocytopenic purpurea and chronic idiopathic thrombocytopenic purpurea, dermatomyositis, Sydenham's chorea, lupus nephritis, rheumatic fever, polyglandular syndromes, Henoch-Schonlein purpura, post-streptococcal nephritis, erythema nodosum, Takayasu's arteritis, Addison's disease, erythema multiforme, polyarteritis nodosa, ankylosing spondylitis, Goodpasture's syndrome, thromboangitis ubiterans, primary biliary cirrhosis, Hashimoto's thyroiditis, thyrotoxicosis, chronic active hepatitis, polymyositis/dermatomyositis, polychondritis, pamphigus vulgaris, Wegener's granulomatosis, membranous nephropathy, amyotrophic lateral sclerosis, tabes dorsalis, polymyaglia, pernicious anemia, rapidly progressive glomerulonephritis and fibrosing alveolitis, inflammatory

responses such as inflammatory skin diseases including psoriasis and dermatitis (*e.g.* atopic dermatitis); systemic scleroderma and sclerosis; responses associated with inflammatory bowel disease (such as Crohn's disease and ulcerative colitis); respiratory distress syndrome (including adult respiratory distress syndrome; ARDS); dermatitis; meningitis; encephalitis; uveitis; colitis; glomerulonephritis; allergic conditions such as eczema and asthma and other conditions involving infiltration of T cells and chronic inflammatory responses; atherosclerosis; leukocyte adhesion deficiency; rheumatoid arthritis; systemic lupus erythematosus (SLE); diabetes mellitus (*e.g.* Type 1 diabetes mellitus or insulin dependent diabetes mellitus); multiple sclerosis; Reynaud's syndrome; autoimmune thyroiditis; allergic encephalomyelitis; Sjorgen's syndrome; juvenile onset diabetes; and immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes typically found in tuberculosis, sarcoidosis, polymyositis, granulomatosis and vasculitis; pernicious amenia (Addison's disease); diseases involving leukocyte diapedesis; central nervous system (CNS) inflammatory disorder; multiple organ injury syndrome; hemolytic anemia (including, but not limited to cryoglobinemia or Coombs positive anemia); myasthenia gravis; antigen-antibody complex mediated diseases; anti-glomerular basement membrane disease; antiphospholipid syndrome; allergic neuritis; Graves' disease; Lambert-Eaton myasthenic syndrome; pemphigoid bullous; pemphigus; autoimmune polyendocrinopathies; Reiter's disease; stiff-man syndrome; Behcet disease; giant cell arteritis; immune complex nephritis; IgA nephropathy; IgM polyneuropathies; immune thrombocytopenic purpura (ITP) or autoimmune thrombocytopenia etc. In this aspect of the invention, the ABMs of the invention are used to deplete the blood of normal B-cells for an extended period.

[0177] In accordance with the practice of this invention, the subject may be a human, equine, porcine, bovine, murine, canine, feline, and avian subjects. Other warm blooded animals are also included in this invention.

[0178] The subject invention further provides methods for inhibiting the growth of human tumor cells, treating a tumor in a subject, and treating a proliferative

type disease in a subject. These methods comprise administering to the subject an effective amount of the composition of the invention.

[0179] It is apparent, therefore, that the present invention encompasses pharmaceutical compositions, combinations and methods for treating human carcinomas, such as a B cell lymphoma. For example, the invention includes pharmaceutical compositions for use in the treatment of human carcinomas comprising a pharmaceutically effective amount of an antibody of the present invention and a pharmaceutically acceptable carrier.

[0180] The ABM compositions of the invention can be administered using conventional modes of administration including, but not limited to, intravenous, intraperitoneal, oral, intralymphatic or administration directly into the tumor. Intravenous administration is preferred.

[0181] In one aspect of the invention, therapeutic formulations containing the ABMs of the invention are prepared for storage by mixing an antibody having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes);

and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

[0182] Exemplary anti-CD20 ABM formulations are described in WO98/56418, expressly incorporated herein by reference. This publication describes a liquid multidose formulation comprising 40 mg/mL rituximab, 25 mM acetate, 150 mM trehalose, 0.9% benzyl alcohol, 0.02% polysorbate 20 at pH 5.0 that has a minimum shelf life of two years storage at 2-8°C. Another anti-CD20 formulation of interest comprises 10 mg/mL rituximab in 9.0 mg/mL sodium chloride, 7.35 mg/mL sodium citrate dihydrate, 0.7 mg/mL polysorbate 80, and Sterile Water for Injection, pH6.5. In the present invention, RITUXAN® will be substituted by an ABM of the present invention.

[0183] Lyophilized formulations adapted for subcutaneous administration are described in WO97/04801. Such lyophihized formulations may be reconstituted with a suitable diluent to a high protein concentration and the reconstituted formulation may be administered subcutaneously to the mammal to be treated herein.

[0184] The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, it may be desirable to further provide a cytotoxic agent, chemotherapeutic agent, cytokine or immunosuppressive agent (e.g. one which acts on T cells, such as cyclosporin or an antibody that binds T cells, e.g., one which binds LFA-1). The effective amount of such other agents depends on the amount of antagonist present in the formulation, the type of disease or disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as used hereinbefore or about from 1 to 99% of the heretofore employed dosages.

[0185] The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-

particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

[0186] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antagonist, which matrices are in the form of shaped articles, e.g., films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid.

[0187] The formulations to be used for in vivo administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

[0188] The compositions of the invention may be in a variety of dosage forms which include, but are not limited to, liquid solutions or suspension, tablets, pills, powders, suppositories, polymeric microcapsules or microvesicles, liposomes, and injectable or infusible solutions. The preferred form depends upon the mode of administration and the therapeutic application.

[0189] The compositions of the invention also preferably include conventional pharmaceutically acceptable carriers and adjuvants known in the art such as human serum albumin, ion exchangers, alumina, lecithin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, and salts or electrolytes such as protamine sulfate.

[0190] The most effective mode of administration and dosage regimen for the pharmaceutical compositions of this invention depends upon the severity and course of the disease, the patient's health and response to treatment and the judgment of the treating physician. Accordingly, the dosages of the compositions should be titrated to the individual patient. Nevertheless, an effective dose of the compositions of this invention will generally be in the range of from about 0.01 to about 2000 mg/kg.

[0191] The molecules described herein may be in a variety of dosage forms which include, but are not limited to, liquid solutions or suspensions, tablets, pills, powders, suppositories, polymeric microcapsules or microvesicles, liposomes, and injectable or infusible solutions. The preferred form depends upon the mode of administration and the therapeutic application.

[0192] The composition comprising an ABM of the present invention will be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disease or disorder being treated, the particular mammal being treated, the clinic condition of the individual patient, the cause of the disease or disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The therapeutically effective amount of the antagonist to be administered will be governed by such considerations.

[0193] As a general proposition, the therapeutically effective amount of the antibody administered parenterally per dose will be in the range of about 0.1 to 20 mg/kg of patient body weight per day, with the typical initial range of antagonist used being in the range of about 2 to 10 mg/kg.

[0194] In a preferred embodiment, the ABM is an antibody, preferably a humanized antibody Suitable dosages for such an unconjugated antibody are, for example, in the range from about 20 mg/m^2 to about 1000 mg/m^2. In one embodiment, the dosage of the antibody differs from that presently recommended for RITUXAN®. For example, one may administer to the patient one or more doses of substantially less than 375 mg/m^2 of the antibody, e.g., where the dose is in the range from about 20 mg/m^2 to about 250 mg/m^2, for example from about 50 mg/m^2 to about 200 mg/m^2.

[0195] Moreover, one may administer one or more initial dose(s) of the antibody followed by one or more subsequent dose(s), wherein the mg/m^2 dose of the antibody in the subsequent dose(s) exceeds the mg/m^2 dose of the antibody in the initial dose(s). For example, the initial dose may be in the range from about 20 mg/m^2 to about 250 mg/m^2 (e.g., from about 50 mg/m^2 to about 200mg/m^2) and

the subsequent dose may be in the range from about 250 mg/m^2 to about 1000 mg/m^2.

[0196] As noted above, however, these suggested amounts of ABM are subject to a great deal of therapeutic discretion. The key factor in selecting an appropriate dose and scheduling is the result obtained, as indicated above. For example, relatively higher doses may be needed initially for the treatment of ongoing and acute diseases. To obtain the most efficacious results, depending on the disease or disorder, the antagonist is administered as close to the first sign, diagnosis, appearance, or occurrence of the disease or disorder as possible or during remissions of the disease or disorder.

[0197] The ABM of the present invention is administered by any suitable means, including parenteral, subcutaneous, intraperitoneal, intrapulinonary, and intranasal, and, if desired for local immunosuppressive treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. In addition, the antagonist may suitably be administered by pulse infusion, e.g., with declining doses of the antagonist. Preferably the dosing is given by injections, most preferably intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic.

[0198] One may administer other compounds, such as cytotoxic agents, chemotherapeutic agents, immunosuppressive agents and/or cytokines with the antagonists herein. The combined administration includes coadministration, using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein preferably there is a time period while both (or all) active agents simultaneously exert their biological activities.

[0199] It would be clear that the dose of the composition of the invention required to achieve cures may be further reduced with schedule optimization.

[0200] In accordance with the practice of the invention, the pharmaceutical carrier may be a lipid carrier. The lipid carrier may be a phospholipid. Further, the lipid carrier may be a fatty acid. Also, the lipid carrier may be a detergent. As used herein, a detergent is any substance that alters the surface tension of a liquid, generally lowering it.

[0201] In one example of the invention, the detergent may be a nonionic detergent. Examples of nonionic detergents include, but are not limited to, polysorbate 80 (also known as Tween 80 or (polyoxyethylenesorbitan monooleate), Brij, and Triton (for example Triton WR-1339 and Triton A-20).

[0202] Alternatively, the detergent may be an ionic detergent. An example of an ionic detergent includes, but is not limited to, alkyltrimethylammonium bromide.

[0203] Additionally, in accordance with the invention, the lipid carrier may be a liposome. As used in this application, a "liposome" is any membrane bound vesicle which contains any molecules of the invention or combinations thereof.

[0204] The examples below explain the invention in more detail. The following preparations and examples are given to enable those skilled in the art to more clearly understand and to practice the present invention. The present invention, however, is not limited in scope by the exemplified embodiments, which are intended as illustrations of single aspects of the invention only, and methods which are functionally equivalent are within the scope of the invention. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

EXAMPLES

[NOTE: Unless otherwise specified, references to the numbering of specific amino acid residue positions in the following Examples are according to the Kabat numbering system.]

EXAMPLE 1

Materials and Methods

Cloning and Expression of Recombinant Antibody B-Ly1

[0205] B-Ly1 expressing hybridoma cells were grown in RPMI containing 10% FBS and 4 mM L-glutamine. 6 x 10^6 cells with a viability > 90% were harvested and total RNA was isolated using a Qiagen RNAeasy midi kit. cDNAs encoding the variable light and heavy chains of B-Ly1 were amplified by RT-PCR. The RT-PCR reaction was performed using the following conditions: 30 min 50 °C for the first strand cDNA synthesis; 15 min 95 °C initial denaturation; 30 cycles of 1 min 94 °C, 1 min 45 °C, 1.5 min 72 °C; and a final elongation step for 10 min at 72 °C. The expected size of the PCR products was confirmed by gel electrophoresis. The PCR products were cloned into suitable E. coli vectors and DNA sequencing confirmed that the variable light and heavy chain encoding genes were isolated.

[0206] For construction of chimeric B-Ly1 expression vectors, synthetic signal sequences and appropriate restriction sites were fused to the variable chains by additional PCR reactions. After a final confirmation of the correct DNA sequence of the variable chains, they were combined with the corresponding human IgG1 constant regions. Once the genes were constructed, they were cloned under control of the MPSV promoter and upstream of a synthetic polyA site, using two separate vectors, one for each chain, resulting in the plasmids pETR1808 (heavy chain expression vector) and pETR1813 (light chain expression vector). Each vector carried an EBV OriP sequence.

[0207] Chimeric B-Ly1 was produced by co-transfecting HEK293-EBNA cells with vectors pETR1808 and pETR1813 using a calcium phosphate-transfection approach. Exponentially growing HEK293-EBNA cells were transfected by the calcium phosphate method. Cells were grown as adherent monolayer cultures in T flasks using DMEM culture medium supplemented with 10% FCS, and were transfected when they were between 50 and 80% confluent. For the transfection of a T75 flask, 8 million cells were seeded 24 hours before transfection in 14 ml DMEM culture medium supplemented with FCS (at 10% V/V final), 250 µg/ml neomycin, and cells were placed at 37°C in an incubator with a 5% CO_2 atmosphere overnight. For each T75 flask to be transfected, a solution of DNA, $CaCl_2$ and water was prepared by mixing 47 µg total plasmid vector DNA divided equally between the light and heavy chain expression vectors, 235 µl of a 1M $CaCl_2$ solution, and adding water to a final volume of 469 µl. To this solution, 469 µl of a 50mM HEPES, 280 mM NaCl, 1.5 mM Na_2HPO_4 solution at pH 7.05 were added, mixed immediately for 10 sec and left to stand at room temperature for 20 sec. The suspension was diluted with 12 ml of DMEM supplemented with 2% FCS, and added to the T75 in place of the existing medium. The cells were incubated at 37°C, 5% CO_2 for about 17 to 20 hours, then medium was replaced with 12 ml DMEM, 10% FCS. For the production of unmodified antibody "chB-Ly1", the cells were transfected only with antibody expression vectors pETR1808 and pETR1813 in a 1:1 ratio. For the production of the glycoengineered antibody "chB-Ly1-ge", the cells were co-transfected with four plasmids, two for antibody expression (pETR1808 and pETR1813), one for a fusion GnTIII polypeptide expression (pETR1519), and one for mannosidase II expression (pCLF9) at a ratio of 4:4:1:1, respectively. At day 5 post-transfection, supernatant was harvested, centrifuged for 5 min at 1200 rpm, followed by a second centrifugation for 10 min at 4000 rpm and kept at 4°C.

[0208] chB-Ly1 and chB-Ly1-ge were purified from culture supernatant using three sequential chromatographic steps, Protein A chromatography, cation exchange chromatography, and a size exclusion chromatography step on a Superdex 200 column (Amersham Pharmacia) exchanging the buffer to phosphate buffer saline and collecting the monomeric antibody peak from this

last step. Antibody concentration was estimated using a spectrophotometer from the absorbance at 280 nm.

Oligosaccharide Analysis

[0209] Oligosaccharides were enzymatically released from the antibodies by PNGaseF digestion, with the antibodies being either immobilized on a PVDF membrane or in solution.

[0210] The resulting digest solution containing the released oligosaccharides either prepared directly for MALDI/TOF-MS analysis or was further digested with EndoH glycosidase prior to sample preparation for MALDI/TOF-MS analysis.

Oligosaccharide release method for PVDF membrane-immobilized antibodies

[0211] The wells of a 96-well plate made with a PVDF (Immobilon P, Millipore, Bedford, Massachusetts) membrane were wetted with 100 µl methanol and the liquid was drawn through the PVDF membrane using vacuum applied to the Multiscreen vacuum manifold (Millipore, Bedford, Massachusetts). The PVDF membranes were washed three times with 300 µl of water. The wells were then washed with 50 µl RCM buffer (8M Urea, 360mM Tris, 3.2mM EDTA, pH 8.6). Between 30-40 µg antibody was loaded in a well containing 10 µl RCM buffer. The liquid in the well was drawn through the membrane by applying vacuum, and the membrane was subsequently washed twice with 50 µl RCM buffer. The reduction of disulfide bridges was performed by addition of 50 µl of 0.1M dithiothreitol in RCM and incubation at 37°C for 1 h.

[0212] Following reduction, a vacuum was applied to remove the dithiothreitol solution from the well. The wells were washed three times with 300 µl water before performing the carboxymethylation of the cysteine residues by addition of 50 µl 0.1M iodoacetic acid in RCM buffer and incubation at room temperature in the dark for 30 min.

[0213] After carboxymethylation, the wells were drawn with vacuum and subsequently washed three times with 300 µl water. The PVDF membrane was

then blocked, to prevent adsorption of the endoglycosidase, by incubating 100 µl of a 1% aqueous solution of polyvinylpyrrolidone 360 at room temperature for 1 hour. The blocking reagent was then removed by gentle vacuum followed by three washes with 300 µl water.

[0214] N-linked oligosaccharides were released by addition of 2.5 mU peptide-N-glycosydase F (recombinat N-Glycanase, GLYKO, Novato, CA) and 0.1 mU Sialidase (GLYKO, Novato, CA), to remove any potential charged monosaccharide residues, in a final volume of 25 µl in 20mM $NaHCO_3$, pH7.0). Digestion was performed for 3 hours at 37°C.

Oligosaccharide release method for antibodies in solution

[0215] Between 40 and 50 µg of antibody were mixed with 2.5 mU of PNGaseF (Glyko, U.S.A.) in 2 mM Tris, pH7.0 in a final volume of 25 microliters, and the mix was incubated for 3 hours at 37°C.

Use of Endoglycosidase H digestion of PNGaseF-released oligosaccharides for the assignment of hybrid bisected oligosaccharide structures to MALDI/TOF-MS neutral oligosaccharide peaks

[0216] The PNGaseF released oligosaccharides were subsequently digested with Endoglycosidase H (EC 3.2.1.96). For the EndoH digestion, 15 mU of EndoH (Roche, Switzerland) were added to the PNGaseF digest (antibody in solution method above) to give a final volume of 30 microliters, and the mix was incubated for 3 hours at 37°C. EndoH cleaves between the N-acetylglucosamine residues of the chitobiose core of N-linked oligosaccharides. The enzyme can only digest oligomannose and most hybrid type glycans, whereas complex type oligosaccharides are not hydrolyzed.

Sample preparation for MALDI/TOF-MS

[0217] The enzymatic digests containing the released oligosaccharides were incubated for a further 3 h at room after the addition of acetic acid to a final concentration of 150 mM, and were subsequently passed through 0.6 ml of cation

exchange resin (AG50W-X8 resin, hydrogen form, 100-200 mesh, BioRad, Switzerland) packed into a micro-bio-spin chromatography column (BioRad, Switzerland) to remove cations and proteins. One microliter of the resulting sample was applied to a stainless steel target plate, and mixed on the plate with 1 µl of sDHB matrix. sDHB matrix was prepared by dissolving 2 mg of 2,5-dihydroxybenzoic acid plus 0.1 mg of 5-methoxysalicylic acid in 1 ml of ethanol/10 mM aqueous sodium chloride 1:1 (v/v). The samples were air dried, 0.2 µl ethanol was applied, and the samples were finally allowed to re-crystallize under air.

MALDI/TOF-MS

[0218] The MALDI-TOF mass spectrometer used to acquire the mass spectra was a Voyager Elite (Perspective Biosystems). The instrument was operated in the linear configuration, with an acceleration of 20kV and 80 ns delay. External calibration using oligosaccharide standards was used for mass assignment of the ions. The spectra from 200 laser shots were summed to obtain the final spectrum.

Whole blood B Cell Depletion

[0219] 495 µl heparinized blood from a healthy donor was aliquoted into 5 ml polystyrene tubes, 5 µl 100-fold concentrated antibody samples (1-1000 ng/ml final concentration) or PBS only were added and the tubes were incubated at 37°. After 24h 50 µl blood was transferred to a fresh tube and stained with anti-CD3-FITC, anti-CD19-PE and anti-CD45-CyChrome (Becton-Dickinson) for 15 min at room temperature in the dark. Before analysis, 500 µl FACS buffer (PBS containing 2% FCS and 5mM EDTA) was added to the tubes. The CD3-FITC and CD19-PE fluorescence of the blood samples were flowcytometrically analyzed by setting a threshold on CD45-CyChrome. B cell-depletion was determined by plotting the ratio of $CD19^+$ B cells to $CD3^+$ T cells.

Binding of anti-CD20 Antibodies to Raji Cells

[0220] 500.000 in 180 µl FACS buffer (PBS containing 2% FCS and 5mM EDTA) were transferred to 5 ml polystyrene tubes and 20 ul 10 fold concentrated anti-CD20 antibody samples (1-5000 ng/ml final concentration) or PBS only were added and the tubes were incubated at 4°C for 30min. Subsequently, samples were washed twice with FACS buffer and pelleted at 300 x g for 3min. Supernatant was aspirated off and cells were taken up in 100 Φl FACS buffer and 1 µl anti-Fc-specific F(ab')2-FITC fragments (Jackson Immuno Research Laboratories, USA) was added and the tubes were incubated at 4°C for 30min. Samples were washed twice with FACS buffer and taken up in 500 µl of FACS buffer containing 0.5 µg/ml PI for analysis by Flow Cytometry. Binding was determined by plotting the geometric mean fluorescence against the antibody concentrations.

EXAMPLE 2

High Homology Acceptor Approach

[0221] The high homology antibody acceptor framework search was performed by aligning the mouse B-ly1 protein sequence to a collection of human germ-line sequences and picking that human sequence that showed the highest sequence identity. Here, the sequence VH1_10 from the VBase database was chosen as the heavy chain framework acceptor sequence, and the VK_2_40 sequence was chosen to be the framework acceptor for the light chain. Onto these two acceptor frameworks, the three complementary determining regions (CDRs) of the mouse heavy and light variable domains were grafted. Since the framework 4 region is not part of the variable region of the germ line V gene, the alignment for that position was done individually. The JH4 region was chosen for the heavy chain, and the JK4 region was chosen for the light chain. Molecular modelling of the designed immunoglobulin domain revealed one spot potentially requiring the murine amino acid residues instead of the human ones outside of the CDR. Re-introducing murine amino acid residues into the human framework would

generate the so-called back mutations. For example, the human acceptor amino acid residue at Kabat position 27 was back mutated to a tyrosine residue. Humanized antibody variants were designed that either included or omitted the back mutations. The humanized antibody light chain did not require any back mutations. After having designed the protein sequences, DNA sequences encoding these proteins were synthesized as detailed below.

Mixed Framework Approach

[0222] In order to avoid introducing back mutations at critical amino acid residue positions (critical to retain good antigen binding affinity or antibody functions) of the human acceptor framework, it was investigated whether either the whole framework region 1 (FR1), or framework regions 1 (FR1) and 2 (FR2) together, could be replaced by human antibody sequences already having donor residues, or functionally equivalent ones, at those important positions in the natural human germline sequence. For this purpose, the VH frameworks 1 and 2 of the mouse Bly1 sequence were aligned individually to human germ-line sequences. Here, highest sequence identity was not important, and was not used, for choosing acceptor frameworks, but instead matching of several critical residues was assumed to be more important. Those critical residues comprise residues 24, 71, and 94 (Kabat numbering), and also those residues at position 27, 28, and 30 (Kabat numbering), which lie outside of the CDR1 definition by Kabat, but often are involved in antigen binding. The IMGT sequence VH_3_15 was chosen as a suitable one. After having designed the protein sequences, DNA sequences encoding these proteins were synthesized as detailed below. Using this approach no back mutations were required either for the light or heavy chain, in order to retain good levels of antigen binding.

Synthesis of the antibody genes

[0223] After having designed the amino acid sequence of the humanized antibody V region, the DNA sequence had to be generated. The DNA sequence data of the individual framework regions was found in the databases for human

germ line sequences. The DNA sequence of the CDR regions was taken from the corresponding murine cDNA data. With these sequences, the whole DNA sequence was virtually assembled. Having this DNA sequence data, diagnostic restriction sites were introduced in the virtual sequence, by introducing silent mutations, creating recognition sites for restriction endonucleases. To obtain the physical DNA chain, gene synthesis was performed (e.g., Wheeler et al. 1995). In this method, oligonucleotides are designed from the genes of interest, such, that a series of oligonucleotides is derived from the coding strand, and one other series is from the non-coding strand. The 3' and 5' ends of each oligonucleotide (except the very first and last in the row) always show complementary sequences to two primers derived from the opposite strand. When putting these oligonucleotides into a reaction buffer suitable for any heat stable polymerase, and adding Mg^{2+}, dNTPs and a DNA polymerase, each oligonucleotide is extended from its 3' end. The newly formed 3' end of one primer then anneals with the next primer of the opposite strand, and extending its sequence further under conditions suitable for template dependant DNA chain elongation. The final product was cloned into a conventional vector for propagation in E. coli.

Antibody production

[0224] Human heavy and light chain leader sequences (for secretion) were added upstream of the above variable region sequences and these were then joined upstream of human IgG1 kappa constant heavy and light chain sequences, respectively, using standard molecular biology techniques. The resulting full antibody heavy and light chain DNA sequences were subcloned into mammalian expression vectors (one for the light chain and one for the heavy chain) under the control of the MPSV promoter and upstream of a synthetic polyA site, each vector carrying an EBV OriP sequence, as described in Example 1 above. Antibodies were produced as described in Example 1 above, namely by co-transfecting HEK293-EBNA with the mammalian antibody heavy and light chain expression vectors, harvesting the conditioned culture medium 5 to 7 days post-transfection, and purifying the secreted antibodies by Protein A affinity chromatography, followed by cation exchange chromatography and a final size

exclusion chromatographic step to isolate pure monomeric IgG1 antibodies. The antibodies were formulated in a 25 mM potassium phosphate, 125 mM sodium chloride, 100 mM glycine solution of pH 6.7. Glycoengineered variants of the humanized antibody variants were produced by co-transfection of the antibody expression vectors together with a GnT-III glycosyltransferase expression vectors, or together with a GnT-III expression vector plus a Golgi mannosidase II expression vector, as described for the chimeric antibody in Example 1 above. Glycoengineered antibodies were purified and formulated as described above for the non-glycoengineered antibodies. The oligosaccharides attached to the Fc region of the antibodies was analysed by MALDI/TOF-MS as described below.

Oligossacharide analysis

[0225] Oligosaccharide release method for antibodies in solution

Between 40 and 50 µg of antibody were mixed with 2.5 mU of PNGaseF (Glyko, U.S.A.) in 2 mM Tris, pH7.0 in a final volume of 25 microliters, and the mix was incubated for 3 hours at 37°C.

Sample preparation for MALDI/TOF-MS

[0226] The enzymatic digests containing the released oligosaccharides were incubated for a further 3 h at room temperature after the addition of acetic acid to a final concentration of 150 mM, and were subsequently passed through 0.6 ml of cation exchange resin (AG50W-X8 resin, hydrogen form, 100-200 mesh, BioRad, Switzerland) packed into a micro-bio-spin chromatography column (BioRad, Switzerland) to remove cations and proteins. One microliter of the resulting sample was applied to a stainless steel target plate, and mixed on the plate with 1 µl of sDHB matrix. sDHB matrix was prepared by dissolving 2 mg of 2,5-dihydroxybenzoic acid plus 0.1 mg of 5-methoxysalicylic acid in 1 ml of ethanol/10 mM aqueous sodium chloride 1:1 (v/v). The samples were air dried, 0.2 µl ethanol was applied, and the samples were finally allowed to re-crystallize under air.

MALDI/TOF-MS

[0227] The MALDI-TOF mass spectrometer used to acquire the mass spectra was a Voyager Elite (Perspective Biosystems). The instrument was operated in the linear configuration, with an acceleration of 20kV and 80 ns delay. External calibration using oligosaccharide standards was used for mass assignment of the ions. The spectra from 200 laser shots were summed to obtain the final spectrum.

Antigen binding assay

[0228] The purified, monomeric humanized antibody variants were tested for binding to human CD20 on Raji B-cell lymphoma target cells using a flow cytometry-based assay, as described for the chimeric B-ly1 antibody in Example 1 above.

Binding of monomeric IgG1 glycovariants to NK cells and FCγ RIIIA-expressing CHO cell line

[0229] Human NK cells were isolated from freshly isolated peripheral blood mononuclear cells (PBMC) applying a negative selection enriching for CD16- and CD56-positive cells (MACS system, Miltenyi Biotec GmbH, Bergisch Gladbach/Germany). The purity determined by CD56 expression was between 88-95 %. Freshly isolated NK cells were incubated in PBS without calcium and magnesium ions (3 x 10^5 cells/ml) for 20 minutes at 37°C to remove NK cell-associated IgG. Cells were incubated at 10^6 cells/ml at different concentrations of anti-CD20 antibody (0, 0.1, 0.3, 1, 3, 10 µg/ml) in PBS, 0.1% BSA. After several washes antibody binding was detected by incubating with 1:200 FITC-conjugated $F(ab')_2$ goat anti-human, F(ab')2 specific IgG (Jackson ImmunoReasearch, West Grove, PA/USA) and anti-human CD56-PE (BD Biosciences, Allschwil/Switzerland). The anti-FcgammaRIIIA 3G8 F(ab')2 fragments (Ancell, Bayport, MN/USA) were added at a concentration of 10 µg/ml to compete binding of antibody glycovariants (3 µg/ml). The fluorescence intensity referring to the bound antibody variants was determined for CD56-positive cells on a

FACSCalibur (BD Biosciences, Allschwil /Switzerland). CHO cells were transfected by electroporation (280 V, 950 µF, 0.4 cm) with an expression vector coding for theFcgammaRIIIA-Val158 α-chain and the γ-chain. Transfectants were selected by addition of 6 µg/ml puromycin and stable clones were analyzed by FACS using 10 µl FITC-conjugated-anti-FcgammaRIII 3G8 monoclonal antibody (BD Biosciences, Allschwil/Switzerland) for 10^6 cells. Binding of IgG1 to FcgammaRIIIA-Val158-expressing CHO cells was performed analogously to the NK cell binding described above.

ADCC assay

[0230] Human peripheral blood mononuclear cells (PBMC) were used as effector cells and were prepared using Histopaque-1077 (Sigma Diagnostics Inc., St. Louis, MO63178 USA) and following essentially the manufacturer's instructions. In brief, venous blood was taken with heparinized syringes from volunteers. The blood was diluted 1:0.75-1.3 with PBS (not containing Ca++ or Mg++) and layered on Histopaque-1077. The gradient was centrifuged at 400 x g for 30 min at room temperature (RT) without breaks. The interphase containing the PBMC was collected and washed with PBS (50 ml per cells from two gradients) and harvested by centrifugation at 300 x g for 10 minutes at RT. After resuspension of the pellet with PBS, the PBMC were counted and washed a second time by centrifugation at 200 x g for 10 minutes at RT. The cells were then resuspended in the appropriate medium for the subsequent procedures.

[0231] The effector to target ratio used for the ADCC assays was 25:1 and 10:1 for PBMC and NK cells, respectively. The effector cells were prepared in AIM-V medium at the appropriate concentration in order to add 50 µl per well of round bottom 96 well plates. Target cells were human B lymphoma cells (e.g., Raji cells) grown in DMEM containing 10% FCS. Target cells were washed in PBS, counted and resuspended in AIM-V at 0.3 million per ml in order to add 30'000 cells in 100 µl per microwell. Antibodies were diluted in AIM-V, added in 50 µl to the pre-plated target cells and allowed to bind to the targets for 10 minutes at RT. Then the effector cells were added and the plate was incubated for 4 hours at 37°C in a humified atmoshpere containing 5% CO_2. Killing of target cells was

assessed by measurement of lactate dehydrogenase (LDH) release from damaged cells using the Cytotoxicity Detection kit (Roche Diagnostics, Rotkreuz, Switzerland). After the 4-hour incubation the plates were centrifuged at 800 x g. 100 µl supernatant from each well was transferred to a new transparent flat bottom 96 well plate. 100 µl color substrate buffer from the kit were added per well. The Vmax values of the color reaction were determined in an ELISA reader at 490 nm for at least 10 min using SOFTmax PRO software (Molecular Devices, Sunnyvale, CA94089, USA). Spontaneous LDH release was measured from wells containing only target and effector cells but no antibodies. Maximal release was determined from wells containing only target cells and 1% Triton X-100. Percentage of specific antibody-mediated killing was calculated as follows: ((x – SR)/(MR – SR)*100, where x is the mean of Vmax at a specific antibody concentration, SR is the mean of Vmax of the spontaneous release and MR is the mean of Vmax of the maximal release.

Complement dependent cytotoxicity assay

[0232] Target cells were counted, washed with PBS, resuspended in AIM-V (Invitrogen) at 1 million cells per ml. 50 µl cells were plated per well in a flat bottom 96 well plate. Antibody dilutions were prepared in AIM-V and added in 50 µl to the cells. Antibodies were allowed to bind to the cells for 10 minutes at room temperature. Human serum complement (Quidel) was freshly thawed, diluted 3-fold with AIM-V and added in 50 µl to the wells. Rabbit complement (Cedarlane Laboratories) was prepared as described by the manufacturer, diluted 3-fold with AIM-V and added in 50 µl to the wells. As a control, complement sources were heated for 30 min at 56°C before addition to the assay.

The assay plates were incubated for 2h at 37°C. Killing of cells was determined by measuring LDH release. Briefly, the plates were centrifuged at 300 x g for 3 min. 50 µl supernatant per well were transferred to a new 96 well plate and 50 µl of the assay reagent from the Cytotoxicity Kit (Roche) were added. A kinetic measurement with the ELISA reader determined the Vmax corresponding with

LDH concentration in the supernatant. Maximal release was determined by incubating the cells in presence of 1% Trition X-100.

Whole blood B-cell depletion assay

[0233] Normal B-cell depletion in whole blood by the anti-CD20 antibodies was carried out as described in Example 1 above.

Apoptosis Assay

[0234] The apoptotic potency of the antibodies was assayed by incubating the antibody at 10µg/ml (saturating conditions in respect to antigen binding) with the target cells (at a target cell concentration of 5×10^5 cells/ml) overnight (16-24 h). Samples were stained with AnnV-FITC and analyzed by FACS. Assay was done in triplicates.

[0235] Detection is performed by flow cytometry by following the appearance of apoptotic markers like annexin V and phosphatidy serine. Negative control (no apoptosis induced) does not contain any antibody, but only phosphate buffered saline. Positive control (maximal apoptosis) contains 5 micromolar of the strong apoptosis inducer Camptothecin (CPT).

Results and Discussion

[0236] Comparison of the binding to human CD20 antigen of antibody variants B-HH1, B-HH2, B-HH3, either complexed with the chimeric B-ly1 light chain (mVL, as described in Example 1 above) or with the humanized B-ly1 light chain (KV1), and the parental, chimeric antibody chB-ly1 (described in Example 1 above) shows that all antibodies have a similar EC50 value, but the B-HH1 construct binds with a lower intensity/stoichiometry than the variants B-HH2 and B-HH3 (Figure 11). B-HH1 can be distinguished from B-HH2 and B-HH3 by its partially human CDR1 and CDR2 regions (Kabat definition), as well as the Ala/Thr polymorphism at position 28 (Kabat numbering). This indicates that either position 28, the complete CDR1, and/or the complete CDR2 are important for antibody/antigen interaction.

[0237] The comparison of the B-HL1, B-HH1, and the chimeric chB-ly1 parental antibody showed absence of any binding activity in the B-HL1 construct, and about half of the binding intensity /stoichiometry of the B-HH1 compared to B-ly1 (Figure 12). Both the B-HL1 as well as the B-HH1 are designed based on acceptor frameworks derived from the human VH1 class. Among other differences, position 71 (Kabat numbering; Kabat position 71 corresponds to position 72 of SEQ ID NO:48) of the B-HL1 construct is a striking difference, indicating its putative importance for antigen binding.

[0238] When comparing the antigen binding data of Figures 9 to 13, the BHH2-KV1, BHL8-KV1, and BHL11-KV1 variants show the best binding affinity, among the different humanized antibody variants tested, to human CD20 on the surface of human cells.. The differences between B-HH2, on one hand, and B-HL8 and B-HL11 on the other hand are located in the FR1 and FR2 regions only, with all three CDRs being identical (compare, e.g., SEQ ID NOs: 32, 56, and 60, which are not numbered according to Kabat, but whose Kabat numbering can be readily determined by one of ordinary skill). B-HL8 and B-HL11 have their FR1 and FR2 sequences derived from the human VH3 class, whereas the complete B-HH2 framework is human VH1 derived. B-HL11 is a derivative of B-HL8 with the single mutation Glu1Gln (position 1 is the same in both Kabat numbering and the conventional numbering system used in the sequence listing), with Gln being the amino acid residue in the B-HH2 construct. This means that Glu1Gln exchange does not alter binding affinity nor intensity. The other differences between B-HH2 and B-HL8 are 14 framework residues, of which one or more will influence the antigen binding behavior of this antibody.

[0239] The B-HL4 construct is derived from the B-HH2 antibody by replacing the FR1 of the B-HH2 with that of the human germ line sequence VH1_45. This construct shows greatly diminished antigen binding capacity, despite having different amino acids at only three positions within FR1. These residues are located at positions 2, 14, and 30 (Kabat numbering). Of these, position 30 could be an influential position, since it is part of the Chothia definition of CDR1. Overall analysis of all the binding curves from Figures 9 to 13 indicates that the following humanized B-ly1 heavy chain residues (Kabat numbering) are

important for binding to CD20: N35 (end of Kabat CDR1), full Kabat CDR1, full Kabat CDR2 and full Kabat CDR3, residues A71 and R94 (in this case R94 cannot be replaced by a threonine) and Y27. A28 and S30 also contribute to a lesser extent. In addition, Kabat CDR3 and all canonical residues are important for antigen binding. No back mutations were introduced in the humanized light chain, which had the full Kabat CDR1, CDR2 and CDR3 grafted. In induction of apoptosis (Figures 14, 15 and 21), the most potent variant was humanized B-ly1 variant BHH2-KV1 (even more potent than the original chB-ly1 and a lot more potent than an antibody with a sequence identical to rituximab, C2B8). Other humanized variants (derivatives of BHL8) that can recover the increased apoptosis are: B-HL12 to B-HL17 (see Table) and BHH8 (mixed frameworks) and BHH9 ("mixed frameworks" with one back mutation, S30T). Positions 9 and 48 (Kabat numbering) can contact the antigen. Variants BHH4 to BHH7 are other humanized B-ly1 variants that do not introduce additional non-human sequences.

[0240] Important properties of the humanized B-ly1 antibody are that it is a type II anti-CD20 antibody as defined in Cragg, M.S. and Glennie, M.J., *Blood 103(7)*:2738-2743 (April 2004); . It therefore did not induce, upon binding to CD20, any significant resistance to non-ionic detergent extraction of CD20 from the surface of CD20+ human cells, using the assay described for this purposes in Polyak, M.J. and Deans, J.P., *Blood 99(9)*:3256-3262 (2002). It certainly induced significantly less resistance to non-ionic detergent extraction of CD20 than the C2B8 antibody does (another anti-CD20 antibody with identical sequence to rituximab,(*See* U.S. Pat. Pub. No. 2003 0003097 to Reff). As expected of a type II anti-CD20 antibody, the humanized B-ly1 did not have any significant complement mediated lysis activity and certainly a lot complement mediated lysis activity than the anti-CD20 antibody C2B8 (chimeric IgG1 with identical sequence to rituximab). Another important property of the humanized B-ly1 antibody was that it was very potent in the homotypic aggregation assay. In this assay CD20-positive human cells, Daudi cells, were incubated in cell culture medium for up to 24 hours at 37°C in a 5%CO2 atmosphere in a mammalian cell incubator as described in detail in (Deans reference), with the antibody at a concentration of 1 microgram per ml and in parallel at a concentration of 5

micrograms per ml. As a comparison, control, parallel incubation of the cells were done under identical conditions but using the anti-CD20 antibody C2B8. At different time points, including 8 hours and 24 hours of incubation, the cells were inspected visually using a microscope. It was found that the humanized B-ly1 antibody led to strong homotypic aggregation, with aggregates being significantly larger that those induced by addition of the C2B8 control antibody. In addition, and consistent with the antibody being anti-CD20 type II, it induced higher levels of apoptosis when CD20-positive human cells were incubated with the humanized B-ly1 antibody, relative to a control under identical conditions using the C2B8 chimeric IgG1 antibody with identical sequence to rituximab.

[0241] Glycoengineered variants of the humanized antibodies were produced by co-expression of GnTIII glycosyltransferase, together with the antibody genes, in mammalian cells. This led to an increase in the fraction of non-fucosylated oligosaccharides attached to the Fc region of the antibodies, including bisected non-fucosylated oligosaccharides, as has been described in WO 2004/065540 (Figures 17-19). The glycoengineered antibodies had significantly higher levels of binding to human FcgammaRIII receptors (Figure 20) and ADCC activity as well (Figure 16), relative to the non-glycoengineered antibody and relative to the C2B8 antibody. The humanized B-ly1 antibody was also more potent at inducing human B-cell depletion in a whole blood assay (Figure 16) than the control C2B8 antibody. This was true both for the non-glycoengineered B-ly1 antibody and for the glycoengineered version of it. The glycoengineered antibody was approximately 1000-fold more potent than the C2B8 control anti-CD20 antibody in depleting B-cells in the whole blood assay. This comparison is important both for the non-glycoengineered and for the glycoengineered humanized forms of B-ly1 antibody, because it showed that in assays that combined Fc receptor-dependent activities, such as ADCC, plus complement mediated lysis, plus induction of apoptosis, that both forms of B-ly1 were significantly more potent than C2B8, although both forms of B-lyl have dramatically lower complement mediated lysis activity. The ADCC, Fc receptor-dependent cell killing activities and apoptosis induction were present in this superior activity of the humanized B-ly1 antibody variants. Furthermore, in the apoptosis assay, both the

glycoengineered and non-glycoengineered forms of this type II anti-CD20 antibody were potent, with the Fc-engineered variants with increased binding affinity to Fcgamma receptors being even more potent in apoptosis induction than the non-Fc-engineered variant, and with all variants being significantly more potent than the control antibody C2B8. The exact mechanism for enhanced homotypic aggregation and induction of apoptopsis mediated by type II anti-CD20 antibodies is not known and concomitant binding to other molecules on the surface of CD20-positive cells, such as Fc gamma receptors, can influence this important property. It was therefore important to demonstrate that anti-CD20 antibodies of type II that have been engineered in their Fc region for increased binding affinity to Fc gamma receptors, including FcgammaRIII and with an associated increase in ADCC activity, were still able to induce strong apoptosis, even higher than the non-Fc-engineered, and homotypic aggregation. Apoptopsis induction is important as in vivo, as there are locations in the body where the target CD20-positive cells can be found, but were access to FcgammaRIII-positive cells is more difficult than in blood, such locations are, for example, lymph nodes. In those locations, the induction of apoptosis by the anti-CD20 antibody itself can be crucial for good efficacy of the anti-CD20 antibody therapy in humans, both for the treatment of haematological malignancies such as non-Hodgkins lymphomas and B-cell chronic lymphocytic leukaemia, and for the treatment of autoimmune diseases such as rheumatoid arthritis and lupus via a B-cell depletion approach. The increased binding affinity to FcgammaRIII and higher ADCC of the humanized, Fc-engineered type II anti-CD20 antibody can also be a very important attribute for such therapies. Finally, the reduced or negligible complement mediated lysis activity of this type II anti-CD20 antibodies, including humanized and Fc-engineered variants, can also be important higher complement activation by anti-CD20 antibodies has been correlated with increased, undesirable side-effects

CLAIMS

1. An engineered Type II anti-CD20 antigen binding molecule having increased ADCC as a result of said engineering without substantial loss of ability to induce apoptosis of target cells, wherein said antigen binding molecule comprises a complementarity determining region (CDR) of the murine B-Ly1 antibody selected from the group consisting of: the heavy chain CDR1 of SEQ ID NO:15, SEQ ID NO:16, or SEQ ID NO:17; the heavy chain CDR2 of SEQ ID NO:25, SEQ ID NO:26, or SEQ ID NO:27; and the heavy chain CDR3 of SEQ ID NO:28; the light chain CDR1 of SEQ ID NO:18; the light chain CDR2 of SEQ ID NO:19; and the light chain CDR3 of SEQ ID NO:20.

2. The antigen binding molecule of claim 1, wherein said antigen binding molecule comprises a heavy chain variable region comprising the B-Ly1 heavy chain CDR1 of SEQ ID NO:15, SEQ ID NO:16, or SEQ ID NO:17; heavy chain CDR2 of SEQ ID NO:25, SEQ ID NO:26, or SEQ ID NO:27; and heavy chain CDR3 of SEQ ID NO:28.

3. The antigen binding molecule of claim 2, further comprising a light chain variable region comprising the B-Ly1 light chain CDR1, CDR2, and CDR3 represented by SEQ ID NO:18, SEQ ID NO:19, and SEQ ID NO:20, respectively.

4. The antigen binding molecule of claim 1, wherein said antigen binding molecule has been engineered to have an increase in the amount of bisected complex oligosaccharides.

5. The antigen binding molecule of claim 1, wherein said antigen binding molecule has been engineered to have a decrease in the amount of fucose residues.

6. The antigen binding molecule of claim 1, wherein said antigen binding molecule comprises an isolated polypeptide comprising a heavy chain variable region sequence selected from the group consisting of: SEQ ID NO:30; SEQ ID NO:32; SEQ ID NO:34; SEQ ID NO:36; SEQ ID NO:38; SEQ ID NO:40; SEQ ID NO:42; SEQ ID NO:44; SEQ ID NO:46; SEQ ID NO:48; SEQ ID NO:50; SEQ ID NO:52; SEQ ID NO:54; SEQ ID

NO:56; SEQ ID NO:58; SEQ ID NO:60; SEQ ID NO:62; SEQ ID NO:64; SEQ ID NO:66; SEQ ID NO:68; SEQ ID NO:70; and SEQ ID NO:72.

7. The antigen binding molecule of claim 3, wherein said antigen binding molecule comprises an isolated polypeptide comprising a light chain variable region sequence of SEQ ID NO:76.

8. The antigen binding molecule of claim 1, wherein said antigen binding molecule is a humanized antigen binding molecule comprising a polypeptide comprising:

a. a heavy chain CDR1 sequence selected from the group consisting of: SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17;
b. a heavy chain CDR2 sequence selected from the group consisting of: SEQ ID NO:25, SEQ ID NO:26, and SEQ ID NO:27; and
c. the heavy chain CDR3 of SEQ ID NO:28.

9. The antigen binding molecule of claim 1, wherein said antigen binding molecule is a humanized antigen binding molecule comprising a polypeptide comprising: the light chain CDR1 of SEQ ID NO:18, the light chain CDR2 of SEQ ID NO:19, and the light chain CDR3 of SEQ ID NO: 20.

10. The antigen binding molecule of claim 1, wherein said antigen binding molecule comprises a first isolated polypeptide comprising a heavy chain variable region sequence of SEQ ID NO: 40 and a second isolated polypeptide comprising a light chain variable region sequence of SEQ ID NO: 76.

11. The antigen binding molecule of any of claims 1-10, said antigen binding molecule having an Fc region with modified oligosaccharides.

12. The antigen binding molecule of claim 11, wherein at least 20% of the oligosaccharides in the Fc region of said polypeptide are bisected, nonfucosylated.

13. The antigen binding molecule of claim 11, wherein at least 50% of the oligosaccharides in the Fc region are nonfucosylated.

14. A host cell expressing at least one nucleic acid encoding a polypeptide having ß(1,4)-N-acetylglucosaminyltransferase III activity in an amount sufficient to engineer the Fc region of a polypeptide produced by said host cell, wherein said polypeptide is the antigen binding molecule of any of claims 6-10.

15. The host cell of claim 14, wherein said antigen binding molecule produced by said host cell exhibits increased Fc receptor binding affinity as a result of said engineering.

16. A host cell according to claim 15, wherein said Fc receptor is FcγRIIIA receptor.

17. The host cell of claim 14, wherein said antigen binding molecule produced by said host cell exhibits increased effector function as a result of said engineering.

18. The host cell of claim 17, wherein said increased effector function is increased direct signaling inducing apoptosis.

19. The host cell of claim 14, further comprising at least one transfected polynucleotide encoding a polypeptide according to any of claims 6-10, and wherein said nucleic acid comprises a sequence encoding a region equivalent to the Fc region of a human immunoglobulin.

20. An isolated polynucleotide comprising a sequence that encodes the heavy chain variable region of claim 6, wherein said polynucleotide comprises a sequence having at least 80% identity to a sequence selected from the group consisting of: SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID

NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, and SEQ ID NO:71.

21. The polynucleotide of claim 20, wherein said polynucleotide comprises a sequence selected from the group consisting of: SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, and SEQ ID NO:71.

22. The polynucleotide of claim 20, wherein said polynucleotide comprises a sequence that encodes a polypeptide having an amino acid sequence selected from the group consisting of: SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, and SEQ ID NO:72, wherein said polypeptide has conservative amino acid substitutions.

23. An isolated polynucleotide comprising a sequence that encodes the light chain variable region of claim 7, wherein said polynucleotide comprises the sequence having at least 80% identity to SEQ ID NO:75.

24. The polynucleotide of claim 23, wherein said polynucleotide comprises SEQ ID NO:75.

25. The polynucleotide of claim 23, wherein said polynucleotide comprises a sequence that encodes a polypeptide having the amino acid sequence of SEQ ID NO:76, wherein said polypeptide has conservative amino acid substitutions.

26. An expression vector comprising the polynucleotide of any one of claims 20-25.

27. Use of the antigen binding molecule of any one of claims 1-10 for the manufacture of a medicament to treat a disorder treatable by B-cell depletion.

28. Use of the antigen binding molecule according to claim 27, wherein said disorder is a haematological malignancy or an autoimmune disease.

29. Use of the antigen binding molecule according to claim 28, wherein said haematological malignancy is B-cell lymphoma, non-Hodgkin's lymphoma, or B-cell chronic lymphocytic leukaemia.

30. Use of the antigen binding molecule according to claim 28, wherein said autoimmune disease is rheumatoid arthritis or lupus.

31. A humanized Type II anti-CD20 antigen binding molecule comprising a heavy chain variable region comprising the B-Ly1 heavy chain CDR1 of SEQ ID NO:15, SEQ ID NO:16, or SEQ ID NO:17; heavy chain CDR2 of SEQ ID NO:25, SEQ ID NO:26, or SEQ ID NO:27; and heavy chain CDR3 of SEQ ID NO:28, wherein said antigen binding molecule induces higher levels of apoptosis when incubated with CD20-positive human cells relative to a control under identical conditions using the C2B8 chimeric IgG1 antibody with an identical sequence to rituximab.

32. The antigen binding molecule of claim 31, wherein said antigen binding molecule comprises a heavy chain variable region comprising the B-Ly1 heavy chain CDR1, CDR2, and CDR3 represented by SEQ ID NO:16, SEQ ID NO:26, and SEQ ID NO:28, respectively.

33. The antigen binding molecule of claim 31, further comprising a light chain variable region comprising the B-Ly1 light chain CDR1, CDR2, and CDR3 represented by SEQ ID NO:18, SEQ ID NO:19, and SEQ ID NO:20, respectively.

34. The antigen binding molecule of claim 31, wherein said antibody comprises the heavy chain variable region selected from the group consisting of: SEQ ID NO:30; SEQ ID NO:32; SEQ ID NO:34; SEQ ID NO:36; SEQ ID NO:38; SEQ ID NO:40; SEQ ID NO:42; SEQ ID NO:44; SEQ ID NO:46; SEQ ID NO:48; SEQ ID NO:50; SEQ ID NO:52; SEQ ID NO:54; SEQ ID NO:56; SEQ ID NO:58; SEQ ID NO:60; SEQ ID NO:62; SEQ ID NO:64; SEQ ID NO:66; SEQ ID NO:68; SEQ ID NO:70; and SEQ ID NO:72.

35. The antigen binding molecule of claim 31, wherein said antigen binding molecule comprises the heavy chain variable region of SEQ ID NO:40.

36. The antigen binding molecule of claim 33, wherein said antigen binding molecule comprises the KV1 light chain variable region of SEQ ID NO:76.

37. The antigen binding molecule of claim 31, wherein said antigen binding molecule comprises a first isolated polypeptide comprising a heavy chain variable region sequence of SEQ ID NO:40 and a second isolated polypeptide comprising a light chain variable region sequence of SEQ ID NO:76.

38. An isolated polynucleotide comprising a sequence that encodes the heavy chain variable region of claim 34, wherein said polynucleotide comprises a sequence having at least 80% identity to a sequence selected from the group consisting of: SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, and SEQ ID NO:71.

39. The polynucleotide of claim 38, wherein said polynucleotide comprises a sequence selected from the group consisting of: SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ

ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, and SEQ ID NO:71.

40. The polynucleotide of claim 38, wherein said polynucleotide comprises a sequence that encodes a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:30; SEQ ID NO:32; SEQ ID NO:34; SEQ ID NO:36; SEQ ID NO:38; SEQ ID NO:40; SEQ ID NO:42; SEQ ID NO:44; SEQ ID NO:46; SEQ ID NO:48; SEQ ID NO:50; SEQ ID NO:52; SEQ ID NO:54; SEQ ID NO:56; SEQ ID NO:58; SEQ ID NO:60; SEQ ID NO:62; SEQ ID NO:64; SEQ ID NO:66; SEQ ID NO:68; SEQ ID NO:70; and SEQ ID NO:72, wherein said polypeptide has conservative amino acid substitutions.

41. An isolated polynucleotide comprising a sequence that encodes the light chain variable region of claim 36, wherein said polynucleotide comprises the sequence having at least 80% identity to SEQ ID NO:75.

42. The polynucleotide of claim 41, wherein said polynucleotide comprises SEQ ID NO:75.

43. The polynucleotide of claim 41, wherein said polynucleotide comprises a sequence that encodes a polypeptide having the amino acid sequence of SEQ ID NO:76, wherein said polypeptide has conservative amino acid substitutions.

44. An expression vector comprising the polynucleotide of any one of claims 38-43.

45. A host cell comprising the expression vector of claim 44.

46. Use of the antigen binding molecule of any one of claims 31-37 for the manufacture of a medicament to treat a disorder treatable by B-cell depletion.

47. Use of the antigen binding molecule according to claim 46, wherein said disorder is a haematological malignancy or an autoimmune disease.

48. Use of the antigen binding molecule according to claim 46, wherein said haematological malignancy is B-cell lymphoma, non-Hodgkin's lymphoma, or B-cell chronic lymphocytic leukaemia.

49. Use of the antigen binding molecule according to claim 47, wherein said autoimmune disease is rheumatoid arthritis or lupus.

50. The antigen binding molecule of any of claim 31-37, wherein said antigen binding molecule comprises a glycoengineered Fc region.

51. The antigen binding molecule of claim 50, wherein said antigen binding molecule has an increase in the fraction of non-fucosylated oligosaccharides attached to said glycoengineered Fc region.

52. The antigen binding molecule of claim 50, wherein said antigen binding molecule has an increase in the fraction of bisected, non-fucosylated oligosaccharides attached to said glycoengineered Fc region.

53. The antigen binding molecule of claim 50, wherein said antigen binding molecule has significantly higher levels of binding to human FcgammaRIII receptors relative to the non-glycoengineered antibody.

54. The antigen binding molecule of claim 50, wherein said antigen binding molecule has significantly higher levels of ADCC activity relative to the non-glycoengineered antibody.

55. A method for producing in a host cell an anti-CD20 antigen binding molecule having an Fc region with modified oligosaccharides and engineered to have increased effector function, said method comprising:

a. culturing a host cell engineered to express at least one nucleic acid encoding a polypeptide having ß(1,4)-N-acetylglucosaminyltransferase III activity under conditions

which permit the production of said antigen binding molecule, and which permit the modification of the oligosaccharides present on the Fc region of said antigen binding molecule; and

b. isolating said antibody;

wherein said antibody is a fusion protein that includes a polypeptide having a variable region sequence selected from the group consisting of SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, and SEQ ID NO:76, and a region equivalent to the Fc region of an immunoglobulin.

56. A method according to claim 55, wherein said modified oligosaccharides have reduced fucosylation as compared to non-modified oligosaccharides.

57. A method according to claim 55, wherein said antigen binding molecule produced by said host cell has an increased proportion of bisected, nonfucosylated oligosaccharides in the Fc region of said polypeptide.

58. A pharmaceutical composition comprising the antigen binding molecule of any one of claims 1-10 or claims 31-37 and a pharmaceutically acceptable carrier.

59. An antigen binding molecule according to any one of claims 1-10 or claims 31-37 for use as a medicament to treat a B cell disorder.

60. The antigen binding molecule or antigen binding molecule of claim 59, wherein said disorder is a B cell lymphoma.

SEQUENCE LISTING

<110> GlycArt Biotechnology AG
Umana, Pablo
Bruenker, Peter
Suter, Tobias
Puentener, Ursula
Moessner, Ekkehard
Ferrara, Claudia

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<160> 78

<170> PatentIn version 3.3

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Thr Leu Arg Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr
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Cys Ala Gln Asn Leu Glu Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys
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Leu Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro
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Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
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Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
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Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
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Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
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Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Leu Cys
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cctggacaag ggctcgagtg gatgggacgg atctttcccg gcgatgggga tactgactac 180

aatgggaaat tcaagggcag agtcacaatt accgccgaca aatccactag cacagcctat 240

atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc aagaaatgtc 300

tttgatggtt actggcttgt ttactggggc cagggaaccc tggtcaccgt ctcctca 357

<210> 38
<211> 119
<212> PRT
<213> Artificial

<220>
<223> Mouse-human chimeric polypeptide

<400> 38

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Tyr Ser
20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50 55 60

Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 39
<211> 357
<212> DNA
<213> Artificial

<220>
<223> Mouse-human chimeric DNA

<400> 39
caggtgcaat tggtgcagtc tggcgctgaa gttaagaagc ctgggagttc agtgaaggtc 60

tcctgcaagg cttccggata cgccttcagc tattcttgga tcaattgggt gcggcaggcg 120

cctggacaag ggctcgagtg gatgggacgg atctttcccg gcgatgggga tactgactac 180

aatgggaaat tcaagggcag agtcacaatt accgccgaca aatccactag cacagcctat 240

atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc aagaaatgtc 300

tttgatggtt actggcttgt ttactggggc cagggaaccc tggtcaccgt ctcctca 357

<210> 40
<211> 119
<212> PRT
<213> Artificial

<220>
<223> Mouse-human chimeric polypeptide

<400> 40

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Tyr Ser
20 25 30

Trp Ile Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50 55 60

Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 41
<211> 357
<212> DNA
<213> Artificial

<220>
<223> Mouse-human chimeric DNA

<400> 41
caggtgcaat tggtgcagtc tggcgctgaa gttaagaagc ctgggagttc agtgaaggtc 60

tcctgcaagg cttccggata cgccttcagc tattcttgga tctcgtgggt gcggcaggcg 120

cctggacaag ggctcgagtg gatgggacgg atctttcccg gcgatgggga tactgactac 180

aatgggaaat tcaagggcag agtcacaatt accgccgaca aatccactag cacagcctat 240

atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc aagaaatgtc 300

tttgatggtt actggcttgt ttactggggc cagggaaccc tggtcaccgt ctcctca 357

<210> 42
<211> 119
<212> PRT
<213> Artificial

<220>

<223> Mouse-human chimeric polypeptide

<400> 42

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Tyr Ser
20 25 30

Trp Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50 55 60

Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 43
<211> 357
<212> DNA
<213> Artificial

<220>
<223> Mouse-human chimeric DNA

<400> 43
caggtgcaat tggtgcagtc tggcgctgaa gttaagaagc ctggcgcctc agtgaaggtc 60

tcctgcaagg cttccggata caccttcaca tacagctgga tgaactgggt gcggcaggcc 120

cctggacaag ggctcgagtg gatgggacgg atctttcccg gcgatgggga tactgactac 180

aatgggaaat tcaagggcag agtcacaatt accgccgaca aatccactag cacagcctat 240

atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc aagaaatgtc 300

tttgatggtt actggcttgt ttactggggc cagggaaccc tggtcaccgt ctcctca 357

<210> 44
<211> 119
<212> PRT

<213> Artificial

<220>
<223> Mouse-human chimeric polypeptide

<400> 44

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Tyr Ser
20 25 30

Trp Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50 55 60

Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 45
<211> 357
<212> DNA
<213> Artificial

<220>
<223> Mouse-human chimeric DNA

<400> 45
caggtgcaat tggtgcagtc tggcgctgaa gttaagaagc ctggcgcctc agtgaaggtc 60

tcctgcaagg cttccggata caccttcagc tattcttgga tgaactgggt gcggcaggcc 120

cctggacaag ggctcgagtg gatgggacgg atctttcccg gcgatgggga tactgactac 180

aatgggaaat tcaagggcag agtcacaatt accgccgaca aatccactag cacagcctat 240

atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc aagaaatgtc 300

tttgatggtt actggcttgt ttactggggc cagggaaccc tggtcaccgt ctcctca 357

<210> 46
<211> 119
<212> PRT
<213> Artificial

<220>
<223> Mouse-human chimeric polypeptide

<400> 46

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ser Tyr Ser
20 25 30

Trp Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50 55 60

Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 47
<211> 357
<212> DNA
<213> Artificial

<220>
<223> Mouse-human chimeric DNA

<400> 47
caggtgcaat tggtgcagtc tggcgctgaa gttaagaagc ctggggcctc agtgaaggtc 60

tcctgcaagg cttccggata caccttcacc tattcttgga tgcactgggt gcggcaggcc 120

cctggacaag ggctcgagtg gatgggacgg atctttcccg gcgatgggga tactgactac 180

gcacagaaat tccaaggaag agtcacaatg acacgggaca cgtccacttc caccgtctat 240

atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc aagaaatgtc 300

tttgatggtt actggcttgt ttactggggc cagggaaccc tggtcaccgt ctcctca 357

<210> 48
<211> 119
<212> PRT
<213> Artificial

<220>
<223> Mouse-human chimeric polypeptide

<400> 48

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Tyr Ser
20 25 30

Trp Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 49
<211> 357
<212> DNA
<213> Artificial

<220>
<223> Mouse-human chimeric DNA

<400> 49
gaggtgcaat tggtgcagtc tggcgctgaa gttaagaagc ctggggccac cgtgaagatc 60

tcctgcaagg tgtccggata caccttcacc tattcttgga tgcactgggt gcagcaggcc 120

cctggaaagg ggctcgagtg gatgggacgg atctttcccg gcgatgggga tactgactac 180

gcagagaaat tccaaggaag agtcacaatc acagccgaca cgtccactga caccgcctat 240

atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc aaccaatgtc 300

tttgatggtt actggcttgt ttactggggc cagggaaccc tggtcaccgt ctcctca 357

<210> 50
<211> 119
<212> PRT
<213> Artificial

<220>
<223> Mouse-human chimeric polypeptide

<400> 50

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Thr Val Lys Ile Ser Cys Lys Val Ser Gly Tyr Thr Phe Thr Tyr Ser
20 25 30

Trp Met His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Ala Glu Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Asp Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Thr Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 51
<211> 357
<212> DNA
<213> Artificial

<220>
<223> Mouse-human chimeric DNA

<400> 51
gaggtgcaat tggtgcagtc tggcgctgaa gttaagaagc ctggggccac cgtgaagatc 60

tcctgcaagg tgtccggata caccttcacc tattcttgga tgaactgggt gcagcaggcc 120

cctggaaagg ggctcgagtg gatgggacgg atctttcccg gcgatgggga tactgactac 180

aatgggaaat tcaagggaag agtcacaatc acagccgaca cgtccactga caccgcctat 240

atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc aaccaatgtc 300

tttgatggtt actggcttgt ttactggggc cagggaaccc tggtcaccgt ctcctca 357

<210> 52
<211> 119
<212> PRT
<213> Artificial

<220>
<223> Mouse-human chimeric polypeptide

<400> 52

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Thr Val Lys Ile Ser Cys Lys Val Ser Gly Tyr Thr Phe Thr Tyr Ser
20 25 30

Trp Met Asn Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50 55 60

Lys Gly Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Asp Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Thr Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 53
<211> 366
<212> DNA
<213> Artificial

<220>
<223> Mouse-human chimeric DNA

<400> 53
cagatgcaat tggtgcagtc tggcgctgaa gttaagaaga ccgggagttc agtgaaggtc 60

tcctgcaagg cttccggata caccttcacc tattcttgga tgagctgggt gcggcaggcc 120

cctggacaag ggctcgagtg gatgggacgg atctttcccg gcgatgggga tactgactac 180

gcacagaaat tccaaggaag agtcacaatt accgccgaca aatccactag cacagcctat 240

atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc aagaaatgtc 300

tttgatggtt actggcttgt ttactggggc cagggaaccc tggtcaccgt ctcctcagct 360

agcacc 366

<210> 54
<211> 119
<212> PRT
<213> Artificial

<220>
<223> Mouse-human chimeric polypeptide

<400> 54

Gln Met Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Thr Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Tyr Ser
20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 55
<211> 357
<212> DNA
<213> Artificial

<220>
<223> Mouse-human chimeric DNA

<400> 55
gaagtgcagc tggtggagtc tggaggaggc ttggtcaagc ctggcgggtc cctgcggctc 60

tcctgtgcag cctctggatt cacatttagc tattcttgga tgaactgggt gcggcaggct 120

cctggaaagg gcctcgagtg ggtgggacgg atctttcccg gcgatgggga tactgactac 180

aatgggaaat tcaagggcag agtcacaatt accgccgaca aatccactag cacagcctat 240

atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc aagaaatgtc 300

tttgatggtt actggcttgt ttactggggc cagggaaccc tggtcaccgt ctcctca 357

<210> 56
<211> 119
<212> PRT
<213> Artificial

<220>
<223> Mouse-human chimeric polypeptide

<400> 56

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Tyr Ser
20 25 30

Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50 55 60

Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 57
<211> 456

<212> DNA
<213> Artificial

<220>
<223> Mouse-human chimeric DNA

<400> 57
cggaattcgg cccaccggtg gccaccatgg actggacctg gaggatcctc ttcttggtgg 60

cagcagccac aggagcccac tccgaagtgc agctggtgga gtctggagga ggcttggtca 120

agcctggcgg gtccctgcgg ctctcctgtg cagcctctgg attcgcattc agctattctt 180

ggatgaactg ggtgcggcag gctcctggaa agggcctcga gtgggtggga cggatctttc 240

ccggcgatgg ggatactgac tacaatggga aattcaaggg cagagtcaca attaccgccg 300

acaaatccac tagcacagcc tatatggagc tgagcagcct gagatctgag gacacggccg 360

tgtattactg tgcaagaaat gtctttgatg gttactggct tgtttactgg ggccagggaa 420

ccctggtcac cgtctcctca gctagcgaat tctcga 456

<210> 58
<211> 119
<212> PRT
<213> Artificial

<220>
<223> Mouse-human chimeric polypeptide

<400> 58

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ala Phe Ser Tyr Ser
20 25 30

Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50 55 60

Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 59
<211> 357
<212> DNA
<213> Artificial

<220>
<223> Mouse-human chimeric DNA

<400> 59
caggtgcagc tggtggagtc tggaggaggc ttggtcaagc ctggcgggtc cctgcggctc 60

tcctgtgcag cctctggatt cacatttagc tattcttgga tgaactgggt gcggcaggct 120

cctggaaagg gcctcgagtg ggtgggacgg atctttcccg gcgatgggga tactgactac 180

aatgggaaat tcaagggcag agtcacaatt accgccgaca aatccactag cacagcctat 240

atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc aagaaatgtc 300

tttgatggtt actggcttgt ttactggggc cagggaaccc tggtcaccgt ctcctca 357

<210> 60
<211> 119
<212> PRT
<213> Artificial

<220>
<223> Mouse-human chimeric polypeptide

<400> 60

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Tyr Ser
20 25 30

Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50 55 60

Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 61
<211> 456
<212> DNA
<213> Artificial

<220>
<223> Mouse-human chimeric DNA

<400> 61
cggaattcgg cccaccggtg gccaccatgg actggacctg gaggatcctc ttcttggtgg 60

cagcagccac aggagctcac tccgaagtgc agctcgtgga gtctggagca ggcttggtca 120

agcctggcgg gtccctgcgg ctctcctgcg cagcctctgg attcacattt agctattctt 180

ggatgaactg ggtgcggcag gctcctggaa agggcctcga gtgggtggga cggatctttc 240

ccggcgatgg ggatactgac tacaatggga aattcaaggg cagagtcaca attaccgccg 300

acaaatccac tagcacagcc tatatggagc tgagcagcct gagatctgag gacacggccg 360

tgtattactg tgcaagaaat gtctttgatg gttactggct tgtttactgg ggccagggaa 420

ccctggtcac cgtctcctca gctagcgaat tctcga 456

<210> 62
<211> 119
<212> PRT
<213> Artificial

<220>
<223> Mouse-human chimeric polypeptide

<400> 62

Glu Val Gln Leu Val Glu Ser Gly Ala Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Tyr Ser
20 25 30

Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50 55 60

Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr

65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 63
<211> 456
<212> DNA
<213> Artificial

<220>
<223> Mouse-human chimeric DNA

<400> 63
cggaattcgg cccaccggtg gccaccatgg actggacctg gaggatcctc ttcttggtgg 60

cagcagccac aggagctcac tccgaagtgc agctcgtcga gtctggagga ggcgtggtca 120

agcctggcgg gtccctgcgg ctctcctgcg cagcctctgg attcacattt agctattctt 180

ggatgaactg ggtgcggcag gctcctggaa agggcctcga gtgggtggga cggatctttc 240

ccggcgatgg ggatactgac tacaatggga aattcaaggg cagagtcaca attaccgccg 300

acaaatccac tagcacagcc tatatggagc tgagcagcct gagatctgag gacacggccg 360

tgtattactg tgcaagaaat gtctttgatg gttactggct tgtttactgg ggccagggaa 420

ccctggtcac cgtctcctca gctagcgaat tctcga 456

<210> 64
<211> 119
<212> PRT
<213> Artificial

<220>
<223> Mouse-human chimeric polypeptide

<400> 64

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Tyr Ser
20 25 30

Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50 55 60

Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 65
<211> 456
<212> DNA
<213> Artificial

<220>
<223> Mouse-human chimeric DNA

<400> 65
cggaattcgg cccaccggtg gccaccatgg actggacctg gaggatcctc ttcttggtgg 60

cagcagccac aggagctcac tccgaagtgc agctggtcga gtccggagga ggcttgaaga 120

agcctggcgg gtccctgcgg ctctcctgcg cagcctctgg attcacattt agctattctt 180

ggatgaactg ggtgcggcag gctcctggaa agggcctcga gtgggtggga cggatctttc 240

ccggcgatgg ggatactgac tacaatggga aattcaaggg cagagtcaca attaccgccg 300

acaaatccac tagcacagcc tatatggagc tgagcagcct gagatctgag gacacggccg 360

tgtattactg tgcaagaaat gtctttgatg gttactggct tgtttactgg ggccagggaa 420

ccctggtcac cgtctcctca gctagcgaat tctcga 456

<210> 66
<211> 119
<212> PRT
<213> Artificial

<220>
<223> Mouse-human chimeric polypeptide

<400> 66

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Lys Lys Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Tyr Ser
20 25 30

Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50 55 60

Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 67
<211> 456
<212> DNA
<213> Artificial

<220>
<223> Mouse-human chimeric DNA

<400> 67
cggaattcgg cccaccggtg gccaccatgg actggacctg gaggatcctc ttcttggtgg 60

cagcagccac aggagcccac tccgaagtgc agctggtgga gtctggagga ggcttggtca 120

agcctggctc ttccctgcgg ctctcctgcg cagcctctgg attcacattt agctattctt 180

ggatgaactg ggtgcggcag gctcctggaa agggcctcga gtgggtggga cggatctttc 240

ccggcgatgg ggatactgac tacaatggga aattcaaggg cagagtcaca attaccgccg 300

acaaatccac tagcacagcc tatatggagc tgagcagcct gagatctgag gacacggccg 360

tgtattactg tgcaagaaat gtctttgatg gttactggct tgtttactgg ggccagggaa 420

ccctggtcac cgtctcctca gctagcgaat tctcga 456

<210> 68
<211> 119
<212> PRT
<213> Artificial

<220>

<223> Mouse-human chimeric polypeptide

<400> 68

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Ser
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Tyr Ser
20 25 30

Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50 55 60

Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 69
<211> 456
<212> DNA
<213> Artificial

<220>
<223> Mouse-human chimeric DNA

<400> 69
cggaattcgg cccaccggtg gccaccatgg actggacctg gaggatcctc ttcttggtgg 60

cagcagccac aggagcccac tccgaagtgc agctggtgga gtctggagga ggcttggtca 120

agcctggcgg gtccctgcgg gtcagctgcg cagcctctgg attcacattt agctattctt 180

ggatgaactg ggtgcggcag gctcctggaa agggcctcga gtgggtggga cggatctttc 240

ccggcgatgg ggatactgac tacaatggga aattcaaggg cagagtcaca attaccgccg 300

acaaatccac tagcacagcc tatatggagc tgagcagcct gagatctgag gacacggccg 360

tgtattactg tgcaagaaat gtctttgatg gttactggct tgtttactgg ggccagggaa 420

ccctggtcac cgtctcctca gctagcgaat tctcga 456

<210> 70
<211> 119
<212> PRT
<213> Artificial

<220>
<223> Mouse-human chimeric polypeptide

<400> 70

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Arg Val Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Tyr Ser
20 25 30

Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50 55 60

Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 71
<211> 456
<212> DNA
<213> Artificial

<220>
<223> Mouse-human chimeric DNA

<400> 71
cggaattcgg cccaccggtg gccaccatgg actggacctg gaggatcctc ttcttggtgg 60

cagcagccac aggagcccac tccgaagtgc agctggtgga gtctggagga ggcttggtca 120

agcctggcgg gtccctgcgg ctctcctgcg cagcctctgg attcacattt agctattctt 180

ggatgaactg ggtgcggcag gctcctggaa agggcctcga gtgggtggga cggatctttc 240

ccggcgatgg ggatactgac tacaatggga aattcaaggg cagagtcaca attaccgccg 300

acaaatccac tagcacagcc tatatggagc tgagcagcct gagatctgag gacacggccg 360

tgtattactg tgcaagaaat gtctttgatg gttactggct tgtttactgg ggccagggaa 420

ccctggtcac cgtctcctca gctagcgaat tctcga 456

<210> 72
<211> 119
<212> PRT
<213> Artificial

<220>
<223> Mouse-human chimeric polypeptide

<400> 72

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Tyr Ser
20 25 30

Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50 55 60

Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 73
<211> 57
<212> DNA
<213> Homo sapiens

<400> 73
atggactgga cctggaggat cctcttcttg gtggcagcag ccacaggagc ccactcc 57

<210> 74
<211> 19

<212> PRT
<213> Homo sapiens

<400> 74

Met Asp Trp Thr Trp Arg Ile Leu Phe Leu Val Ala Ala Ala Thr Gly
1 5 10 15

Ala His Ser

<210> 75
<211> 345
<212> DNA
<213> Artificial

<220>
<223> Mouse-human chimeric DNA

<400> 75
gatatcgtga tgacccagac tccactctcc ctgcccgtca cccctggaga gcccgccagc 60

attagctgca ggtctagcaa gagcctcttg cacagcaatg gcatcactta tttgtattgg 120

tacctgcaaa agccagggca gtctccacag ctcctgattt atcaaatgtc caaccttgtc 180

tctggcgtcc ctgaccggtt ctccggatcc gggtcaggca ctgatttcac actgaaaatc 240

agcagggtgg aggctgagga tgttggagtt tattactgcg ctcagaatct agaacttcct 300

tacaccttcg gcggagggac caaggtggag atcaaacgta cggtg 345

<210> 76
<211> 115
<212> PRT
<213> Artificial

<220>
<223> Mouse-human chimeric polypeptide

<400> 76

Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
20 25 30

Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Leu Val Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ala Gln Asn
85 90 95

Leu Glu Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg Thr Val
115

<210> 77
<211> 66
<212> DNA
<213> Homo sapiens

<400> 77
atggacatga gggtccccgc tcagctcctg ggcctcctgc tgctctggtt cccaggtgcc 60

aggtgt 66

<210> 78
<211> 22
<212> PRT
<213> Homo sapiens

<400> 78

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1 5 10 15

Phe Pro Gly Ala Arg Cys
20