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(54) Benævnelse: **Antigenbindende molekyler med øget Fc-receptorbindingsaffinitet og effektorfunktion**

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DESCRIPTION

BACKGROUND OF THE INVENTION

Field of the Invention

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

The invention is defined in the claims. The invention relates to a humanized Type II anti-CD20 antibody comprising

1. (a) a heavy chain variable region selected from 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
2. (b) the KV1 light chain variable region of SEQ ID NO:76.

Furthermore, the present invention relates to a humanized Type II anti-CD20 antibody comprising a heavy chain variable region that is encoded by a nucleic acid which is 80% identical to SEQ ID NOs: 61, 63, 65, 67, 67, 69 or 71 and a light chain variable region that is encoded by a light chain variable region that is encoded by a nucleic acid sequence which is at least 80% identical to SEQ ID NO: 75, wherein said antibody 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 a sequence identical to rituximab.

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 histocompatibility 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-Lyl, is another antibody known to be specific to human CD20 (Poppema, S. and Visser, L., Biostest Bulletin 3: 131-139 (1987)). Polyak, M.J. and Deans J.P., Blood 99:3256-3262 (2002) describes the murine B-Lyl antibody as one of 16 anti-CD20 antibodies. However, none of the individually disclosed antibodies has been completely structurally characterized and none of them has been deposited.

[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 FcyRIIB. (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 FcyRIIIa 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 FcyRIIIa 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 FcyRIIIa, 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 ("¹³¹I") 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. Oncol. 7/8:1027-1038 (1989) (indication that one patient treated with ¹³¹I-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: 1219-12223 (1993)(iodine-131 labeled anti-CD20 antibody IF5 and B1); and Kaminski, M. G. et al "Radioimmunotherapy of B-Cell Lymphoma with ¹³¹IAnti-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 Umana, P. et al., *Nature Biotechnol.* 17:176-180 (1999) and U.S. Pat. No. 6,602,684.

[0017] IgG1 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)).

[0018] It was shown 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 Umafia, P. et al., *Nature Biotechnol.* 17:176-180 (1999); and International Publication No. WO 99/54342).

[0019] 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.

[0020] 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

[0021] Recognizing the tremendous therapeutic potential of antigen binding molecules (ABMs) that have the binding specificity of the murine B-Lyl antibody and that have been glycoengineered to enhance Fc receptor binding affinity and effector function, it was 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.

[0022] Accordingly, in one aspect, 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 aspect, 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.

[0023] In a further aspect, the disclosure is directed to an isolated polynucleotide comprising SEQ ID No.:3. In another aspect, the disclosure is directed to an isolated polynucleotide comprising SEQ ID No.:4. In a further aspect, 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 aspect, the invention is directed to an isolated polynucleotide comprising SEQ ID No.:75. In one embodiment, such polynucleotides encode fusion polypeptides.

[0024] The disclosure is further directed to an isolated polynucleotide comprising a sequence having at least 80% identity to SEQ ID NO:3, 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 aspect, 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.

[0025] 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 to SEQ ID NO:12.

[0026] 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.:1; 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.

[0027] In yet another aspect, the disclosure is directed to an isolated polynucleotide encoding a chimeric polypeptide having the sequence of SEQ ID No.:2. In one embodiment, the polynucleotide comprises a sequence encoding a polypeptide having the sequence of SEQ ID No.:2; 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 aspect, 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.

[0028] The disclosure is also directed to an isolated polynucleotide comprising a sequence encoding a polypeptide having the V_H 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. In another aspect, 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.

[0029] 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 aspect, the disclosure is directed to a host cell comprising any of the isolated polynucleotides described above.

[0030] In one aspect, 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 aspect, 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.

[0031] 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.:2 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.

[0032] In another aspect the disclosure is directed to a polypeptide comprising a sequence derived from the murine B-Ly1 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.

[0033] In an additional aspect, the disclosure is directed to an isolated polypeptide comprising SEQ ID NO: 13 or a variant thereof. In another aspect, the disclosure is directed to an isolated polypeptide comprising SEQ ID NO: 14.

[0034] In another aspect, the disclosure is directed to an ABM, which is capable of competing with the murine B-Ly1 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.: 2 and SEQ ID NO:76. In a further embodiment the ABM is a recombinant antibody that is primateized. 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.

[0035] The disclosure is further related to an ABM as described herein, 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 one embodiment, at least 20% of the oligosaccharides 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.

[0036] 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.

[0037] 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 invention in a medium under conditions allowing the expression of said polynucleotide encoding said ABM; and (b) recovering said ABM from the resultant culture.

[0038] In another aspect, the disclosure is related to a pharmaceutical composition comprising the ABM described herein. It is contemplated that the pharmaceutical composition may further comprise a pharmaceutically acceptable carrier, an adjuvant or a combination thereof.

[0039] In a further aspect, 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 described herein 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.

[0040] In yet another aspect, 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.

[0041] 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.

[0042] 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 least 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 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 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.

[0043] The present disclosure is the first known instance in which a Type II anti-CD20 antibody has been engineered to have increased 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 induce 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 a 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.

[0044] The host cell of the present invention may be selected from the group that includes, but is not limited to, a CHO cell, a BHK cell, a NSO cell, a SP2/0 cell, a YO myeloma cell, a P3X63 mouse myeloma cell, a PER cell, a PERC6 cell or a hybridoma cell. In one embodiment, the host cell of the disclosure further comprises a transfected polynucleotide comprising a polynucleotide encoding the V_L region of the murine B-Lyl antibody or variants thereof and a sequence encoding a region equivalent to the Fc region of a human immunoglobulin. In another embodiment, the host cell of the disclosure further comprises a transfected polynucleotide comprising a polynucleotide encoding the V_H region of the murine B-Lyl antibody or variants thereof and a sequence encoding a region equivalent to the Fc region of a human immunoglobulin.

[0045] In a further aspect, the disclosure is directed to a host cell that produces an ABM 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 receptor, particularly, the Fc γ RIIIA 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.

[0046] In a further embodiment, the host cell as described herein comprises at least one nucleic acid encoding a polypeptide having GnTIII activity that is operably linked to a constitutive promoter element.

[0047] In another aspect, the disclosure is directed to a method for producing an ABM in a host cell, comprising: (a) culturing a host cell engineered to express at least one polynucleotide encoding a fusion polypeptide having GnTIII activity under conditions which permit the production of said ABM and which permit the modification of the oligosaccharides present on the Fc region of said ABM; and (b) isolating said ABM; wherein said ABM is capable of competing with the murine B-Lyl antibody for binding to CD20 and wherein said ABM is chimeric. In one embodiment, the polypeptide having GnTIII activity is a fusion 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 β (1,2)-N-acetylglucosaminyltransferase 1 ("GnT1"), the localization domain of mannosidase I, the localization domain of β (1,2)-N-acetylglucosaminyltransferase II ("GnTII"), and the localization domain of α 1-6 core fucosyltransferase. Preferably, the Golgi localization domain is from mannosidase II or GnT1.

[0048] In a further aspect, 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.

[0049] In one aspect, the disclosure is related to a recombinant, chimeric antibody, or a fragment thereof, capable of competing with the murine B-Ly1 antibody for binding to CD20 and having reduced fucosylation.

[0050] In another aspect, 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 described herein 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 GnT1, the localization domain of mannosidase I, the localization domain of GnTII and the localization domain of α 1-6 core fucosyltransferase. Preferably, the Golgi localization domain is from mannosidase II or GnT1.

[0051] In one embodiment, the method described herein 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 described herein 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 invention 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.

[0052] In a further aspect, 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 Fc γ activating receptor, particularly, the Fc γ RIIIA 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.

[0053] In another aspect, 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 invention.

[0054] In another aspect, 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 described herein.

[0055] In another aspect, the present disclosure is directed to a fusion protein that includes a polypeptide having the sequence of SEQ ID NO:2 and a region equivalent to the Fc region of an immunoglobulin and engineered to have increased effector function produced by any of the methods described herein.

[0056] In one aspect, 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 aspect, the present disclosure is directed to a pharmaceutical composition comprising a recombinant, chimeric antibody fragment produced by any of the methods of the present invention, and a pharmaceutically acceptable carrier. In another aspect, the present disclosure is directed to a pharmaceutical composition comprising a fusion protein produced by any of the methods described herein, and a pharmaceutically acceptable carrier.

[0057] 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 described herein, to a human subject in need thereof.

BRIEF DESCRIPTION OF THE FIGURES

[0058]

FIG 1. Nucleotide (SEQ ID NO:3) and amino acid sequence (SEQ ID NO:1) of the V_H region of the murine B-Lyl.

FIG 2. Nucleotide (SEQ ID NO:4) and amino acid sequence (SEQ ID NO:2) of the V_L region of the murine B-Ly1.

FIG 3. Binding of Rituximab® (O) and ch-B_Ly1 (Δ) to CD20 on Raji B-lymphoma cells.

FIG 4. B-Cell depletion by Rituximab® (O) and ch-B_Ly1 (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.

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.

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.

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

FIG 8. MALDI-TOF profile of a glycoengineered, chimeric B-Lyl 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.

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-HL1 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 14FR residues, from which one or more will influence the antigen binding behavior of this antibody.

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.

FIG 11. Comparison of the binding behavior between B-HH1, B-HH2, B-HH3, and the parental antibody B-lyl. The data show that all Abs show a similar EC50 value, but the B-HH1 construct binds with a lower intensity/stoichiometry than 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.

FIG 12. The comparison of B-HH, B-HH1, and the B-ly1 parental antibody. The data showed 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.

FIG 13. Fluorocytometric analysis of the capacity 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.

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

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.

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 CD 19+/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).

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

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.

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.

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

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

[0059] Terms are used herein as generally used in the art, unless otherwise defined as follows.

[0060] 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.

[0061] 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-term inus.

[0062] 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).

[0063] 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.

[0064] 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.

[0065] 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 mannose 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.)

[0066] 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.

[0067] 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.

[0068] 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.

[0069] 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); Verhoeven et al., Science, 239:1534-1536 (1988); Padlan, Molec. Immun., 28:489-498 (1991); Padlan, Molec. Immun., 31(3):169-217 (1994).

[0070] 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.

[0071] 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.

[0072] 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),

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 Definitions¹

	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	

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

[0073] 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 unambiguously 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.

[0074] By a nucleic acid or polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present disclosure, 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.

[0075] 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 disclosure 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.

[0076] 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.

[0077] 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 be made for the purposes of the present disclosure.

[0078] By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a query amino acid sequence described herein, 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.

[0079] 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.

[0080] 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.

[0081] 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.

[0082] 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.

[0083] 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.

[0084] 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.

[0085] 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.

[0086] 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.

[0087] 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:

[0088] 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.

[0089] 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.

[0090] 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.

[0091] 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. 1) the assay uses target cells that are known to express the target antigen recognized by the antigen-binding region of the antibody;
2. 2) the assay uses human peripheral blood mononuclear cells (PBMCs), isolated from blood of a randomly chosen healthy donor, as effector cells;
3. 3) the assay is carried out according to following protocol:
 1. i) the PBMCs are isolated using standard density centrifugation procedures and are suspended at 5×10^6 cells/ml in RPMI cell culture medium;
 2. 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;
 3. iii) 100 microliters of the final target cell suspension above are transferred to each well of a 96-well microtiter plate;
 4. 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;
 5. 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);
 6. 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);
 7. vii) the 96-well microtiter plate is then centrifuged at $50 \times g$ for 1 minute and incubated for 1 hour at 4°C ;
 8. 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;
 9. ix) the cell-free supernatant from each well is harvested and the experimentally released radioactivity (ER) is quantified using a gamma counter;
 10. x) the percentage of specific lysis is calculated for each antibody concentration according to the formula $(\text{ER}-\text{MR})/(\text{MR}-\text{SR}) \times 100$, 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. 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.

[0092] In one aspect, the present disclosure is related to antigen binding molecules having the binding specificity of the murine B-Lyl 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 disclosure is directed to a chimeric antibody, or a fragment thereof, comprising the CDRs shown in Figure 7. Specifically, in a preferred 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); 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 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.

[0093] In another embodiment, the antigen binding molecule comprises the V_H domain of the murine B-Lyl 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-Lyl antibody shown in Figure 2, or a variant thereof; and a non-murine polypeptide.

[0094] In another aspect, the disclosure 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).

[0095] Accordingly, 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.

[0096] 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 least 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 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 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.

[0097] It is known that several mechanisms 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.

[0098] 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).

[0099] The present disclosure is the first known instance in which a Type II anti-CD20 antibody has been engineered to have increased 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 induce 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 a reduced level of fucose residues in the Fc region. See U.S. Pat. Appl. Pub. No. 2004 0093621 to Shitara et al.. 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. 20040132101 (Xencor).

[0100] The disclosure is further directed to methods of making such engineered Type 11 antibodies and to methods of using such antibodies in the treatment of various B cell disorders, including B cell lymphomas.

[0101] 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. et 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.

[0102] 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).

[0103] In a particularly preferred embodiment, the chimeric ABM described herein is a humanized antibody. Methods for humanizing non-human antibodies are known in the art. For example, humanized ABMs described herein 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..

[0104] 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); Verhoeven 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.

[0105] 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)).

[0106] 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.

[0107] In another embodiment, the antigen binding molecules described herein 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..

[0108] In one embodiment, the antigen binding molecule described herein 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.

[0109] A variety of radionuclides are applicable to the present disclosure 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, ¹³¹iodine is a well known radionuclide used for targeted immunotherapy. However, the clinical usefulness of ¹³¹iodine 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 ¹¹¹indium and ⁹⁰yttrium. ⁹⁰Yttrium provides several benefits for utilization in radioimmunotherapeutic applications: the 64 hour half-life of ⁹⁰yttrium is long enough to allow antibody accumulation by tumor and, unlike eg, ¹³¹iodine, ⁹⁰yttrium 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 ⁹⁰yttrium-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.

[0110] Effective single treatment dosages (i.e., therapeutically effective amounts) of ⁹⁰yttrium 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 ¹³¹iodine 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 ¹³¹iodine 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 ¹³¹iodine 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 ¹¹¹indium label, are typically less than about 5 mCi.

[0111] 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 ¹¹¹indium) be conducted on the patient, a purpose of which is to ensure that the therapeutically labeled antibody (eg, using ⁹⁰yttrium) will not become unnecessarily "concentrated" in any normal organ or tissue.

[0112] In a preferred embodiment, the present disclosure 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 disclosure 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 disclosure 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 disclosure 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
B-HH1	<u>CAGGTGCAATTGGTGCAGTCTGGCGCTGAAGTTAA</u> <u>GAAGCCTGGGAGTTCACTGAAGGTCTCTGCAAGG</u> <u>CTTCCGGATAACACCTTCAGCTATTCTTGGATGAGCT</u> <u>GGGTGCGGCAGGGCCCTGGACAAGGGCTCGAGTGG</u> <u>ATGGGACGGATCTTCCCAGCGATGGGGATACTGA</u> <u>CTACGCACAGAAATCCAAGGAAGAGTCACAATTAA</u> <u>CCGCCGACAAATCCACTAGCACAGCCTATATGGAG</u> <u>CTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTA</u> <u>TTACTGTGCAAGAAAATGTCTTGATGGTAACTGGCT</u> <u>TGTTTACTGGGGCCAGGGAACCTGGTCACCGTCT</u> <u>CCTCA</u>	29
B-HH2	<u>CAGGTGCAATTGGTGCAGTCTGGCGCTGAAGTTAA</u> <u>GAAGCCTGGGAGTTCACTGAAGGTCTCTGCAAGG</u> <u>CTTCCGGATAACACCTTCAGCTATTCTTGGATGAACT</u> <u>GGGTGCGGCAGGGCCCTGGACAAGGGCTCGAGTGG</u> <u>ATGGGACGGATCTTCCCAGCGATGGGGATACTGA</u> <u>CTACAAATGGGAAATCAAGGGCAGAGTCACAATTAA</u> <u>CCGCCGACAAATCCACTAGCACAGCCTATATGGAG</u> <u>CTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTA</u> <u>TTACTGTGCAAGAAAATGTCTTGATGGTAACTGGCT</u> <u>TGTTTACTGGGGCCAGGGAACCTGGTCACCGTCT</u> <u>CCTCA</u>	31
B-HH3	<u>CAGGTGCAATTGGTGCAGTCTGGCGCTGAAGTTAA</u> <u>GAAGCCTGGGAGTTCACTGAAGGTCTCTGCAAGG</u> <u>CTTCCGGATAACACCTTCAGCTATTCTTGGATGAACT</u> <u>GGGTGCGGCAGGGCCCTGGACAAGGGCTCGAGTGG</u>	33

CONSTRUCT	NUCLEOTIDE SEQUENCE	SEQ ID NO
	<code>ATGGGACGGATCTTCCCCGGCGATGGGGATACTGA CTACAATGGGAAATTCAAGGGCAGAGTCACAATT CCGCGACAAATCCACTAGCACAGCCTATATGGAG CTGAGCAGCCTGAGATCTGAGGACACGGCCGTGA TCTGTGTGCAAGAAATGTCTTGATGGTTACTGGCT TGTTTACTGGGGCCAGGGAACCTGGTCACCGTCT CCTCAGCTAGCACC</code>	
B-HH4	<code>CAGGTGCAATTGGTGCAGTCTGGCGCTGAAGTAA GAAGCCTGGAGCTTCAGTGAAGGTCTCTGCAAGG CTTCCGGATAACGGCTTCAGCTATTCTGGATGAGCT GGGTGCGGCAGGGCCCTGGACAAGGGCTCGAGTG ATGGGACGGATCTTCCCCGGCGATGGGGATACTGA CTACAATGGGAAATTCAAGGGCAGAGTCACAATT CCGCGACAAATCCACTAGCACAGCCTATATGGAG CTGAGCAGCCTGAGATCTGAGGACACGGCCGTGA TTACGTGCAAGAAATGTCTTGATGGTTACTGGCT TGTTTACTGGGGCCAGGGAACCTGGTCACCGTCT CCTCA</code>	35
B-HH5	<code>CAGGTGCAATTGGTGCAGTCTGGCGCTGAAGTAA GAAGCCTGGGAGTTCACTGAAGGTCTCTGCAAGG CTTCCGGATAACGGCTTCAGCTATTCTGGATGAGCT GGGTGCGGCAGGGCCCTGGACAAGGGCTCGAGTG GATGGGACGGATCTTCCCCGGCGATGGGGATACTG ACTACAATGGGAAATTCAAGGGCAGAGTCACAATT ACCGCCGACAAATCCACTAGCACAGCCTATATGGAG GCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGT ATTACTGTGCAAGAAATGTCTTGATGGTTACTGGC TGTGTTACTGGGGCCAGGGAACCTGGTCACCGTCT CCTCA</code>	37
B-HH6	<code>CAGGTGCAATTGGTGCAGTCTGGCGCTGAAGTAA GAAGCCTGGGAGTTCACTGAAGGTCTCTGCAAGG CTTCCGGATAACGGCTTCAGCTATTCTGGATGAGCT GGGTGCGGCAGGGCCCTGGACAAGGGCTCGAGTG GATGGGACGGATCTTCCCCGGCGATGGGGATACTG ACTACAATGGGAAATTCAAGGGCAGAGTCACAATT ACCGCCGACAAATCCACTAGCACAGCCTATATGGAG GCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGT ATTACTGTGCAAGAAATGTCTTGATGGTTACTGGC TGTGTTACTGGGGCCAGGGAACCTGGTCACCGTCT CCTCA</code>	39
B-HH7	<code>CAGGTGCAATTGGTGCAGTCTGGCGCTGAAGTAA GAAGCCTGGGAGTTCACTGAAGGTCTCTGCAAGG CTTCCGGATAACGGCTTCAGCTATTCTGGATGAGCT GGGTGCGGCAGGGCCCTGGACAAGGGCTCGAGTG GATGGGACGGATCTTCCCCGGCGATGGGGATACTG ACTACAATGGGAAATTCAAGGGCAGAGTCACAATT ACCGCCGACAAATCCACTAGCACAGCCTATATGGAG GCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGT ATTACTGTGCAAGAAATGTCTTGATGGTTACTGGC TGTGTTACTGGGGCCAGGGAACCTGGTCACCGTCT CCTCA</code>	41

CONSTRUCT	NUCLEOTIDE SEQUENCE	SEQ ID NO
B-HH8	<u>CAGGTGCAATTGGTGCAGTCTGGCGCTGAAGTTAA</u> <u>GAAGCCTGGCGCCTCAGTGAAGGTCTCTGCAAGG</u> <u>CTTCCGGATAACACCTCACATACAGCTGGATGAAC</u> <u>TGGGTGCGGCAGGCCCCCTGGACAAGGGCTCGAGTG</u> <u>GATGGGACGGATCTTCCCGCGATGGGATACTG</u> <u>ACTACAATGGGAAATTCAAGGGCAGAGTCACAATT</u> <u>ACCGCCGACAAATCCACTAGCACAGCCTATATGGA</u> <u>GCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGT</u> <u>ATTACTGTGCAAGAAATGTCTTGATGGTTACTGGC</u> <u>TTGTTTACTGGGCCAGGGAACCCCTGGTCACCGTCT</u> <u>CCTCA</u>	43
B-HH9	<u>CAGGTGCAATTGGTGCAGTCTGGCGCTGAAGTTAA</u> <u>GAAGCCTGGCGCCTCAGTGAAGGTCTCTGCAAGG</u> <u>CTTCCGGATAACACCTCAGCTATTCTTGGATGAAC</u> <u>GGGTGCGGCAGGCCCCCTGGACAAGGGCTCGAGTG</u> <u>ATGGGACGGATCTTCCCGCGATGGGATACTGA</u> <u>CTACAATGGGAAATTCAAGGGCAGAGTCACAATT</u> <u>CCGCCGACAAATCCACTAGCACAGCCTATATGGAG</u> <u>CTGAGCAGCCTGAGATCTGAGGACACGGCCGTGT</u> <u>TTACTGTGCAAGAAATGTCTTGATGGTTACTGGC</u> <u>TGTTTACTGGGCCAGGGAACCCCTGGTCACCGTCT</u> <u>CCTCA</u>	45
B-HL1	<u>CAGGTGCAATTGGTGCAGTCTGGCGCTGAAGTTAA</u> <u>GAAGCCTGGGCCCTCAGTGAAGGTCTCTGCAAGG</u> <u>CTTCCGGATAACACCTCACCTATTCTTGGATGCACT</u> <u>GGGTGCGGCAGGCCCCCTGGACAAGGGCTCGAGTG</u> <u>ATGGGACGGATCTTCCCGCGATGGGATACTGA</u> <u>CTACGACACAGAAATTCCAAGGAAGAGTCACAATGA</u> <u>CACGGGACACGTCCACTCCACCGCTATATGGAG</u> <u>CTGAGCAGCCTGAGATCTGAGGACACGGCCGTGT</u> <u>TTACTGTGCAAGAAATGTCTTGATGGTTACTGGC</u> <u>TGTTTACTGGGCCAGGGAACCCCTGGTCACCGTCT</u> <u>CCTCA</u>	47
B-HL2	<u>GAGGTGCAATTGGTGCAGTCTGGCGCTGAAGTTAA</u> <u>GAAGCCTGGGCCACCGTGAAGATCTCTGCAAGG</u> <u>TGTCCGGATAACACCTCACCTATTCTTGGATGCACT</u> <u>GGGTGCGCAGGCCCCCTGGAAAGGGCTCGAGTG</u> <u>GATGGGACGGATCTTCCCGCGATGGGATACTG</u> <u>ACTACGGCAGAGAAATTCCAAGGAAGAGTCACAATC</u> <u>ACAGCCGACACGTCCACTGACACCGCTATATGGA</u> <u>GCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGT</u> <u>ATTACTGTGCAACCAATGTCTTGATGGTTACTGGC</u> <u>TTGTTTACTGGGCCAGGGAACCCCTGGTCACCGTCT</u> <u>CCTCA</u>	49
B-HL3	<u>GAGGTGCAATTGGTGCAGTCTGGCGCTGAAGTTAA</u> <u>GAAGCCTGGGCCACCGTGAAGATCTCTGCAAGG</u> <u>TGTCCGGATAACACCTCACCTATTCTTGGATGAACT</u> <u>GGGTGCGCAGGCCCCCTGGAAAGGGCTCGAGTG</u> <u>GATGGGACGGATCTTCCCGCGATGGGATACTG</u> <u>ACTACAATGGGAAATTCAAGGGAAGAGTCACAATC</u> <u>ACAGCCGACACGTCCACTGACACCGCTATATGGA</u> <u>GCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGT</u> <u>ATTACTGTGCAACCAATGTCTTGATGGTTACTGGC</u> <u>TTGTTTACTGGGCCAGGGAACCCCTGGTCACCGTCT</u> <u>CCTCA</u>	51

CONSTRUCT	NUCLEOTIDE SEQUENCE	SEQ ID NO
	<u>ACAUCCCCAACCTTCCACTGACACCGGCTATATGAA</u> <u>GCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGT</u> <u>ATTACTGTGCAACCAATGTCTTGATGGTTACTGGC</u> <u>TGTTTACTGGGCCAGGGAACCCCTGGTCACCGTCT</u> <u>CCTCA</u>	
B-HL4	<u>CAGATGCAATTGGTGCAGTCGGCGCTGAAGTAA</u> <u>GAAGACCGGGAGTTCACTGAAGGTCTCTGCAAGG</u> <u>CTTCCGGATACACCTTCACCTATTCTTGGATGAGCT</u> <u>GGGTGCGGCAGGGCCCTGGACAAGGGCTCGAGTGG</u> <u>ATGGGACGGATCTTCCCGCGATGGGGATACTGA</u> <u>CTACGCACAGAAATTCCAAGGAAGAGTCACAATT</u> <u>CCGCCACAAATCCACTAGCACAGCCTATATGGAG</u> <u>CTGAGCAGCCTGAGATCTGAGGACACGGCCGTGT</u> <u>TTACTGTGCAAGAAATGTCTTGATGGTTACTGGCT</u> <u>TGTTTACTGGGCCAGGGAACCCCTGGTCACCGTCT</u> <u>CCTCAGCTAGCACC</u>	53
B-HL8	<u>GAAGTGCAGCTGGTGGAGTCTGGAGGGAGGCTTGGT</u> <u>CAAGCCTGGCGGGTCCCTGGCGCTCTCTGTGCAG</u> <u>CCTCTGGATTACACATTAGCTAATTCTTGGATGAAC</u> <u>GGGTGCGGCAGGGCTCTGGAAAGGGCCTCGAGTGG</u> <u>GTGGGACGGATCTTCCCGCGATGGGGATACTGA</u> <u>CTACAATGGGAAATTCAAGGGCAGAGTCACAATT</u> <u>CCGCCACAAATCCACTAGCACAGCCTATATGGAG</u> <u>CTGAGCAGCCTGAGATCTGAGGACACGGCCGTGT</u> <u>TTACTGTGCAAGAAATGTCTTGATGGTTACTGGCT</u> <u>TGTTTACTGGGCCAGGGAACCCCTGGTCACCGTCT</u> <u>CCTCA</u>	55
B-HL10	<u>CGGAATTGGCCCCACCGGTGGCCACCATGGACTGG</u> <u>ACCTGGAGGATCCTCTTGGTGGCAGCAGCCAC</u> <u>AGGAGCCCACCTCGAACGTGAGCTGCTGGAGTCTG</u> <u>GAGGAGGCTTGGTCAAGCCTGGCGGGTCCCTGCGG</u> <u>CTCTCCTGTCAGCCTCTGGATTTCGAAATTAGCTAT</u> <u>TCTTGGATGAACCTGGGTGCGGCAGGCTCTGGAAA</u> <u>GGGCCTCGAGTGGGTGGGACGGATCTTCCCGCGG</u> <u>ATGGGGATACTGACTACAATGGGAAATTCAAGGGC</u> <u>AGAGTCACAATTACCGCCGACAAATCCACTAGCAC</u> <u>AGCCTATATGGACCTGAGCAGCCTGAGATCTGAGG</u> <u>ACACGGCCGTGTATTACTGTGCAAGAAATGTCTT</u> <u>GATGGTTACTGGCTTGTATTACTGGGCCAGGGAAC</u> <u>CCTGTCACCGTCTCCTCAGCTAGCGAATTCTCGA</u>	57
B-HL11	<u>CAGGTGCAGCTGGTGGAGTCTGGAGGGAGGCTTGGT</u> <u>CAAGCCTGGCGGGTCCCTGGCGCTCTCTGTGCAG</u> <u>CCTCTGGATTACACATTAGCTATTCTTGGATGAAC</u> <u>GGGTGCGGCAGGGCTCTGGAAAGGGCCTCGAGTGG</u> <u>GTGGGACGGATCTTCCCGCGATGGGGATACTGA</u> <u>CTACAATGGGAAATTCAAGGGCAGAGTCACAATT</u> <u>CCGCCACAAATCCACTAGCACAGCCTATATGGAG</u> <u>CTGAGCAGCCTGAGATCTGAGGACACGGCCGTGT</u> <u>TTACTGTGCAAGAAATGTCTTGATGGTTACTGGCT</u> <u>TGTTTACTGGGCCAGGGAACCCCTGGTCACCGTCT</u> <u>CCTCA</u>	59

CONSTRUCT	NUCLEOTIDE SEQUENCE	SEQ ID NO
B-HL12	<u>CGGAATT</u> <u>CGGCC</u> <u>ACCGG</u> <u>TGGC</u> <u>ACCAT</u> <u>GGACT</u> <u>GG</u> <u>ACCTG</u> <u>GAGG</u> <u>ATC</u> <u>CTT</u> <u>CTT</u> <u>GGT</u> <u>GG</u> <u>CAG</u> <u>CAG</u> <u>CCAC</u> <u>AGGAG</u> <u>GCT</u> <u>ACT</u> <u>CCG</u> <u>AAG</u> <u>TG</u> <u>CAG</u> <u>CTG</u> <u>CG</u> <u>GG</u> <u>AG</u> <u>TCTG</u> <u>GAG</u> <u>CAGG</u> <u>CT</u> <u>GGT</u> <u>CA</u> <u>AG</u> <u>CCT</u> <u>GG</u> <u>GG</u> <u>TC</u> <u>CC</u> <u>TG</u> <u>CG</u> <u>CT</u> <u>CT</u> <u>GG</u> <u>CG</u> <u>C</u> <u>AG</u> <u>CCT</u> <u>CT</u> <u>GG</u> <u>AT</u> <u>T</u> <u>CAC</u> <u>AT</u> <u>TT</u> <u>AG</u> <u>CT</u> <u>T</u> <u>CT</u> <u>GG</u> <u>AT</u> <u>G</u> <u>T</u> <u>A</u> <u>CT</u> <u>GG</u> <u>GT</u> <u>GG</u> <u>CG</u> <u>GG</u> <u>AG</u> <u>G</u> <u>CT</u> <u>CT</u> <u>GG</u> <u>AA</u> <u>GGG</u> <u>C</u> <u>CT</u> <u>CG</u> <u>A</u> <u>G</u> <u>T</u> <u>GG</u> <u>GT</u> <u>GG</u> <u>AC</u> <u>GG</u> <u>AT</u> <u>CT</u> <u>TC</u> <u>CC</u> <u>GG</u> <u>AT</u> <u>GGG</u> <u>GG</u> <u>A</u> <u>T</u> <u>C</u> <u>G</u> <u>A</u> <u>T</u> <u>C</u> <u>A</u> <u>T</u> <u>GG</u> <u>AA</u> <u>A</u> <u>T</u> <u>T</u> <u>CA</u> <u>AG</u> <u>GG</u> <u>AG</u> <u>AG</u> <u>T</u> <u>CAC</u> <u>A</u> <u>TT</u> <u>A</u> <u>CC</u> <u>GG</u> <u>CC</u> <u>G</u> <u>A</u> <u>CA</u> <u>A</u> <u>AT</u> <u>CC</u> <u>A</u> <u>T</u> <u>AG</u> <u>GC</u> <u>AC</u> <u>AG</u> <u>C</u> <u>C</u> <u>T</u> <u>A</u> <u>T</u> <u>A</u> <u>T</u> <u>GG</u> <u>AG</u> <u>G</u> <u>T</u> <u>A</u> <u>G</u> <u>C</u> <u>A</u> <u>G</u> <u>C</u> <u>CT</u> <u>G</u> <u>A</u> <u>G</u> <u>AT</u> <u>CT</u> <u>G</u> <u>AG</u> <u>AC</u> <u>AC</u> <u>GG</u> <u>CC</u> <u>GT</u> <u>GT</u> <u>A</u> <u>T</u> <u>T</u> <u>A</u> <u>T</u> <u>CT</u> <u>GT</u> <u>CA</u> <u>AG</u> <u>AA</u> <u>A</u> <u>T</u> <u>G</u> <u>T</u> <u>CT</u> <u>G</u> <u>A</u> <u>T</u> <u>GG</u> <u>TT</u> <u>A</u> <u>T</u> <u>C</u> <u>G</u> <u>CT</u> <u>GT</u> <u>TT</u> <u>A</u> <u>T</u> <u>CT</u> <u>GG</u> <u>GG</u> <u>CC</u> <u>A</u> <u>GG</u> <u>GA</u> <u>C</u> <u>C</u> <u>T</u> <u>GG</u> <u>TC</u> <u>A</u> <u>C</u> <u>C</u> <u>GT</u> <u>TC</u> <u>CT</u> <u>TC</u> <u>AG</u> <u>C</u> <u>T</u> <u>AG</u> <u>CG</u> <u>GA</u> <u>A</u> <u>TT</u> <u>CT</u> <u>CG</u>	61
B-HL13	<u>CGGAATT</u> <u>CGGCC</u> <u>ACCGG</u> <u>TGGC</u> <u>ACCAT</u> <u>GGACT</u> <u>GG</u> <u>ACCTG</u> <u>GAGG</u> <u>ATC</u> <u>CTT</u> <u>CTT</u> <u>GGT</u> <u>GG</u> <u>CAG</u> <u>CAG</u> <u>CCAC</u> <u>AGGAG</u> <u>GCT</u> <u>ACT</u> <u>CCG</u> <u>AAG</u> <u>TG</u> <u>CAG</u> <u>CTG</u> <u>CG</u> <u>AG</u> <u>TCTG</u> <u>GAG</u> <u>GAGG</u> <u>CT</u> <u>GGT</u> <u>CA</u> <u>AG</u> <u>CCT</u> <u>GG</u> <u>GG</u> <u>TC</u> <u>CC</u> <u>TG</u> <u>CG</u> <u>CT</u> <u>CT</u> <u>GG</u> <u>CG</u> <u>C</u> <u>AG</u> <u>CCT</u> <u>CT</u> <u>GG</u> <u>AT</u> <u>T</u> <u>CAC</u> <u>AT</u> <u>TT</u> <u>AG</u> <u>CT</u> <u>T</u> <u>CT</u> <u>GG</u> <u>AT</u> <u>G</u> <u>T</u> <u>A</u> <u>CT</u> <u>GG</u> <u>GT</u> <u>GG</u> <u>CG</u> <u>GG</u> <u>AG</u> <u>G</u> <u>CT</u> <u>CT</u> <u>GG</u> <u>AA</u> <u>GGG</u> <u>C</u> <u>CT</u> <u>CG</u> <u>A</u> <u>G</u> <u>T</u> <u>GG</u> <u>GT</u> <u>GG</u> <u>AC</u> <u>GG</u> <u>AT</u> <u>CT</u> <u>TC</u> <u>CC</u> <u>GG</u> <u>AT</u> <u>GGG</u> <u>GG</u> <u>A</u> <u>T</u> <u>C</u> <u>G</u> <u>A</u> <u>T</u> <u>C</u> <u>A</u> <u>T</u> <u>GG</u> <u>AA</u> <u>A</u> <u>T</u> <u>T</u> <u>CA</u> <u>AG</u> <u>GG</u> <u>AG</u> <u>AG</u> <u>T</u> <u>CAC</u> <u>A</u> <u>TT</u> <u>A</u> <u>CC</u> <u>GG</u> <u>CC</u> <u>G</u> <u>A</u> <u>CA</u> <u>A</u> <u>AT</u> <u>CC</u> <u>A</u> <u>T</u> <u>AG</u> <u>GC</u> <u>AC</u> <u>AG</u> <u>C</u> <u>C</u> <u>T</u> <u>A</u> <u>T</u> <u>A</u> <u>T</u> <u>GG</u> <u>AG</u> <u>G</u> <u>T</u> <u>A</u> <u>G</u> <u>C</u> <u>A</u> <u>G</u> <u>C</u> <u>CT</u> <u>G</u> <u>A</u> <u>G</u> <u>AT</u> <u>CT</u> <u>G</u> <u>AG</u> <u>AC</u> <u>AC</u> <u>GG</u> <u>CC</u> <u>GT</u> <u>GT</u> <u>A</u> <u>T</u> <u>T</u> <u>A</u> <u>T</u> <u>CT</u> <u>GT</u> <u>CA</u> <u>AG</u> <u>AA</u> <u>A</u> <u>T</u> <u>G</u> <u>T</u> <u>CT</u> <u>G</u> <u>A</u> <u>T</u> <u>GG</u> <u>TT</u> <u>A</u> <u>T</u> <u>C</u> <u>G</u> <u>CT</u> <u>GT</u> <u>TT</u> <u>A</u> <u>T</u> <u>CT</u> <u>GG</u> <u>GG</u> <u>CC</u> <u>A</u> <u>GG</u> <u>GA</u> <u>C</u> <u>C</u> <u>T</u> <u>GG</u> <u>TC</u> <u>A</u> <u>C</u> <u>C</u> <u>GT</u> <u>TC</u> <u>CT</u> <u>TC</u> <u>AG</u> <u>C</u> <u>T</u> <u>AG</u> <u>CG</u> <u>GA</u> <u>A</u> <u>TT</u> <u>CT</u> <u>CG</u>	63
B-HL14	<u>CGGAATT</u> <u>CGGCC</u> <u>ACCGG</u> <u>TGGC</u> <u>ACCAT</u> <u>GGACT</u> <u>GG</u> <u>ACCTG</u> <u>GAGG</u> <u>ATC</u> <u>CTT</u> <u>CTT</u> <u>GGT</u> <u>GG</u> <u>CAG</u> <u>CAG</u> <u>CCAC</u> <u>AGGAG</u> <u>GCT</u> <u>ACT</u> <u>CCG</u> <u>AAG</u> <u>TG</u> <u>CAG</u> <u>CTG</u> <u>CG</u> <u>AG</u> <u>TCTG</u> <u>GAG</u> <u>GAGG</u> <u>CT</u> <u>GGT</u> <u>GA</u> <u>AG</u> <u>A</u> <u>G</u> <u>C</u> <u>C</u> <u>T</u> <u>GG</u> <u>GG</u> <u>TC</u> <u>CC</u> <u>TG</u> <u>CG</u> <u>CT</u> <u>CT</u> <u>GG</u> <u>CG</u> <u>C</u> <u>AG</u> <u>CCT</u> <u>CT</u> <u>GG</u> <u>AT</u> <u>T</u> <u>CAC</u> <u>AT</u> <u>TT</u> <u>AG</u> <u>CT</u> <u>T</u> <u>CT</u> <u>GG</u> <u>AT</u> <u>G</u> <u>T</u> <u>A</u> <u>CT</u> <u>GG</u> <u>GT</u> <u>GG</u> <u>CG</u> <u>GG</u> <u>AG</u> <u>G</u> <u>CT</u> <u>CT</u> <u>GG</u> <u>AA</u> <u>GGG</u> <u>C</u> <u>CT</u> <u>CG</u> <u>A</u> <u>G</u> <u>T</u> <u>GG</u> <u>GT</u> <u>GG</u> <u>AC</u> <u>GG</u> <u>AT</u> <u>CT</u> <u>TC</u> <u>CC</u> <u>GG</u> <u>AT</u> <u>GGG</u> <u>GG</u> <u>A</u> <u>T</u> <u>C</u> <u>G</u> <u>A</u> <u>T</u> <u>C</u> <u>A</u> <u>T</u> <u>GG</u> <u>AA</u> <u>A</u> <u>T</u> <u>T</u> <u>CA</u> <u>AG</u> <u>GG</u> <u>AG</u> <u>AG</u> <u>T</u> <u>CAC</u> <u>A</u> <u>TT</u> <u>A</u> <u>CC</u> <u>GG</u> <u>CC</u> <u>G</u> <u>A</u> <u>CA</u> <u>A</u> <u>AT</u> <u>CC</u> <u>A</u> <u>T</u> <u>AG</u> <u>GC</u> <u>AC</u> <u>AG</u> <u>C</u> <u>C</u> <u>T</u> <u>A</u> <u>T</u> <u>A</u> <u>T</u> <u>GG</u> <u>AG</u> <u>G</u> <u>T</u> <u>A</u> <u>G</u> <u>C</u> <u>A</u> <u>G</u> <u>C</u> <u>CT</u> <u>G</u> <u>A</u> <u>G</u> <u>AT</u> <u>CT</u> <u>G</u> <u>AG</u> <u>AC</u> <u>AC</u> <u>GG</u> <u>CC</u> <u>GT</u> <u>GT</u> <u>A</u> <u>T</u> <u>T</u> <u>A</u> <u>T</u> <u>CT</u> <u>GT</u> <u>CA</u> <u>AG</u> <u>AA</u> <u>A</u> <u>T</u> <u>G</u> <u>T</u> <u>CT</u> <u>G</u> <u>A</u> <u>T</u> <u>GG</u> <u>TT</u> <u>A</u> <u>T</u> <u>C</u> <u>G</u> <u>CT</u> <u>GT</u> <u>TT</u> <u>A</u> <u>T</u> <u>CT</u> <u>GG</u> <u>GG</u> <u>CC</u> <u>A</u> <u>GG</u> <u>GA</u> <u>C</u> <u>C</u> <u>T</u> <u>GG</u> <u>TC</u> <u>A</u> <u>C</u> <u>C</u> <u>GT</u> <u>TC</u> <u>CT</u> <u>TC</u> <u>AG</u> <u>C</u> <u>T</u> <u>AG</u> <u>CG</u> <u>GA</u> <u>A</u> <u>TT</u> <u>CT</u> <u>CG</u>	65
B-HL15	<u>CGGAATT</u> <u>CGGCC</u> <u>ACCGG</u> <u>TGGC</u> <u>ACCAT</u> <u>GGACT</u> <u>GG</u> <u>ACCTG</u> <u>GAGG</u> <u>ATC</u> <u>CTT</u> <u>CTT</u> <u>GGT</u> <u>GG</u> <u>CAG</u> <u>CAG</u> <u>CCAC</u> <u>AGGAG</u> <u>CCC</u> <u>ACT</u> <u>CCG</u> <u>AAG</u> <u>TG</u> <u>CAG</u> <u>CTG</u> <u>GG</u> <u>AG</u> <u>TCTG</u> <u>GAG</u> <u>GAGG</u> <u>CT</u> <u>GGT</u> <u>CA</u> <u>AG</u> <u>C</u> <u>C</u> <u>T</u> <u>GG</u> <u>CT</u> <u>TC</u> <u>CC</u> <u>TG</u> <u>CG</u> <u>CT</u> <u>CT</u> <u>GG</u> <u>CG</u> <u>C</u> <u>AG</u> <u>CCT</u> <u>CT</u> <u>GG</u> <u>AT</u> <u>T</u> <u>CAC</u> <u>AT</u> <u>TT</u> <u>AG</u> <u>CT</u> <u>T</u> <u>CT</u> <u>GG</u> <u>AT</u> <u>G</u> <u>T</u> <u>A</u> <u>CT</u> <u>GG</u> <u>GT</u> <u>GG</u> <u>CG</u> <u>GG</u> <u>AG</u> <u>G</u> <u>CT</u> <u>CT</u> <u>GG</u> <u>AA</u> <u>GGG</u> <u>C</u> <u>CT</u> <u>CG</u> <u>A</u> <u>G</u> <u>T</u> <u>GG</u> <u>GT</u> <u>GG</u> <u>AC</u> <u>GG</u> <u>AT</u> <u>CT</u> <u>TC</u> <u>CC</u> <u>GG</u> <u>AT</u> <u>GGG</u> <u>GG</u> <u>A</u> <u>T</u> <u>C</u> <u>G</u> <u>A</u> <u>T</u> <u>C</u> <u>A</u> <u>T</u> <u>GG</u> <u>AA</u> <u>A</u> <u>T</u> <u>T</u> <u>CA</u> <u>AG</u> <u>GG</u> <u>AG</u> <u>AG</u> <u>T</u> <u>CAC</u> <u>A</u> <u>TT</u> <u>A</u> <u>CC</u> <u>GG</u> <u>CC</u> <u>G</u> <u>A</u> <u>CA</u> <u>A</u> <u>AT</u> <u>CC</u> <u>A</u> <u>T</u> <u>AG</u> <u>GC</u> <u>AC</u> <u>AG</u> <u>C</u> <u>C</u> <u>T</u> <u>A</u> <u>T</u> <u>A</u> <u>T</u> <u>GG</u> <u>AG</u> <u>G</u> <u>T</u> <u>A</u> <u>G</u> <u>C</u> <u>A</u> <u>G</u> <u>C</u> <u>CT</u> <u>G</u> <u>A</u> <u>G</u> <u>AT</u> <u>CT</u> <u>G</u> <u>AG</u> <u>AC</u> <u>AC</u> <u>GG</u> <u>CC</u> <u>GT</u> <u>GT</u> <u>A</u> <u>T</u> <u>T</u> <u>A</u> <u>T</u> <u>CT</u> <u>GT</u> <u>CA</u> <u>AG</u> <u>AA</u> <u>A</u> <u>T</u> <u>G</u> <u>T</u> <u>CT</u> <u>G</u> <u>A</u> <u>T</u> <u>GG</u> <u>TT</u> <u>A</u> <u>T</u> <u>C</u> <u>G</u> <u>CT</u> <u>GT</u> <u>TT</u> <u>A</u> <u>T</u> <u>CT</u> <u>GG</u> <u>GG</u> <u>CC</u> <u>A</u> <u>GG</u> <u>GA</u> <u>C</u> <u>C</u> <u>T</u> <u>GG</u> <u>TC</u> <u>A</u> <u>C</u> <u>C</u> <u>GT</u> <u>TC</u> <u>CT</u> <u>TC</u> <u>AG</u> <u>C</u> <u>T</u> <u>AG</u> <u>CG</u> <u>GA</u> <u>A</u> <u>TT</u> <u>CT</u> <u>CG</u>	67

CONSTRUCT	NUCLEOTIDE SEQUENCE	SEQ ID NO
B-HL16	<u>CGGAATTGGCCCCACCGGTGGCCACCATGGACTGG</u> <u>ACCTGGAGGATCCTCTTCTTGGTGGCAGCAGCCAC</u> <u>AGGAGCCCACCTCGAACGTGCAGCTGGGGAGTCG</u> <u>GAGGAGGCTTGGTCAAGCCTGGCGGGTCCCTGCGG</u> <u>GTCAGCTGCGCAGCCTCTGGATTACACATTAGCTAT</u> <u>TCTTGGATGAACCTGGGTGCGGCAGGCTCTGGAAA</u> <u>GGGCCTCGAGTGGGTGGGACGGATCTTCCCGCG</u> <u>ATGGGGATACTGACTACAATGGGAAATTCAAGGGC</u> <u>AGAGTCACAATTACCGCCGACAAATCCACTAGCAC</u> <u>AGCCTATATGGAGCTGAGCAGCCTGAGATCTGAGG</u> <u>ACACGGCCGTGTATTACTGTGCAAGAAATGTCTT</u> <u>GATGGTTACTGGCTTGTACTGGGGCCAGGGAAC</u> <u>CCTGGTCACCGTCTCCTCAGCTAGCGAATTCTCGA</u>	69
B-HL17	<u>CGGAATTGGCCCCACCGGTGGCCACCATGGACTGG</u> <u>ACCTGGAGGATCCTCTTCTTGGTGGCAGCAGCCAC</u> <u>AGGAGCCCACCTCGAACGTGCAGCTGGGGAGTCG</u> <u>GAGGAGGCTTGGTCAAGCCTGGCGGGTCCCTGCGG</u> <u>CTCTCCTGCGCAGCCTCTGGATTACACATTAGCTAT</u> <u>TCTTGGATGAACCTGGGTGCGGCAGGCTCTGGAAA</u> <u>GGGCCTCGAGTGGGTGGGACGGATCTTCCCGCG</u> <u>ATGGGGATACTGACTACAATGGGAAATTCAAGGGC</u> <u>AGAGTCACAATTACCGCCGACAAATCCACTAGCAC</u> <u>AGCCTATATGGAGCTGAGCAGCCTGAGATCTGAGG</u> <u>ACACGGCCGTGTATTACTGTGCAAGAAATGTCTT</u> <u>GATGGTTACTGGCTTGTACTGGGGCCAGGGAAC</u> <u>CCTGGTCACCGTCTCCTCAGCTAGCGAATTCTCGA</u>	71
VH Signal Sequence	<u>ATGGACTGGACCTGGAGGATCCTCTTGGTGGC</u> <u>AGCAGCCCACAGGAGCCCACCTCC</u>	73
B-KV1	<u>GATATCGTGATGACCCAGACTCCACTCTCCCTGCC</u> <u>GTCACCCCTGGAGAGCCGCCAGCATTAGCTGCAG</u> <u>GTCTAGCAAGAGCCTCTGCACAGCAATGGCATCA</u> <u>CTTATTGTATTGGTACCTGCAAAAGCCAGGGCAG</u> <u>TCTCCACAGCTCCGTATTATCAAATGTCAAACCTT</u> <u>GTCTCTGGCGTCCCTGACCGGTTCTCCGGATCCGGG</u> <u>TCAGGCAGTATTACACTGAAAATCAGCAGGGT</u> <u>GGAGGCTGAGGATGTTGGAGTTTATTACTGCGCTC</u> <u>AGAATCTAGAACCTCCTTACACCTCAGGGAGGG</u> <u>ACCAAGGTGGAGATCAAACGTACGGTG</u>	75
VL Signal Sequence	<u>ATGGACATGAGGGTCCCCGCTCAGCTCCTGGGCCT</u> <u>CCTGCTGCTCTGGTCCAGGTGCCAGGTGT</u>	77

TABLE 3

<u>CONSTRUCT</u>	<u>AMINO ACID SEQUENCE</u>	<u>SEQ ID NO</u>
B-HH1	<u>QVQLVOSGAEVKKPGSSVKVSCKASGYTFSYSWMSWVR</u> <u>QAPGQGLEWMGRIFPGDGDTDYAQKFQGRVTITADKSTS</u> <u>TAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQTL</u> <u>VTVSS</u>	<u>30</u>
B-HH2	<u>QVQLVOSGAEVKKPGSSVKVSCKASGYAFSYSWMNWV</u> <u>ROAPGQGLEWMGRIFPGDGDTDYNGKFKGRVTITADKS</u> <u>TSTAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQG</u> <u>TLTVSS</u>	<u>32</u>
B-HH3	<u>QVQLVOSGAEVKKPGSSVKVSCKASGYAFSYSWMNWV</u> <u>ROAPGQGLEWMGRIFPGDGDTDYNGKFKGRVTITADKS</u> <u>TSTAYMELSSLRSEDTAVYLCARNVFDGYWLVYWGQGT</u> <u>LTVSS</u>	<u>34</u>
B-HH4	<u>QVQLVOSGAEVKKPGASVKVSCKVSGYAFSYSWMNWV</u> <u>ROAPGQGLEWMGRIFPGDGDTDYNGKFKGRVTITADKS</u> <u>TSTAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQG</u> <u>TLTVSS</u>	<u>36</u>
B-HH5	<u>QVQLVOSGAEVKKPGSSVKVSCKASGYAFSYSWMNWV</u> <u>ROAPGQGLEWMGRIFPGDGDTDYNGKFKGRVTITADKS</u> <u>TSTAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQG</u> <u>TLTVSS</u>	<u>38</u>
B-HH6	<u>QVQLVOSGAEVKKPGSSVKVSCKASGYAFSYSWINWVR</u> <u>QAPGQGLEWMGRIFPGDGDTDYNGKFKGRVTITADKSTS</u> <u>TAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQTL</u> <u>VTVSS</u>	<u>40</u>
B-HH7	<u>QVQLVOSGAEVKKPGSSVKVSCKASGYAFSYSWISWVR</u> <u>QAPGQGLEWMGRIFPGDGDTDYNGKFKGRVTITADKSTS</u> <u>TAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQTL</u> <u>VTVSS</u>	<u>42</u>
B-HH8	<u>QVQLVOSGAEVKKPGASVKVSCKASGYTFTYSWMNWV</u> <u>ROAPGQGLEWMGRIFPGDGDTDYNGKFKGRVTITADKS</u> <u>TSTAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQG</u> <u>TLTVSS</u>	<u>44</u>
B-HH9	<u>QVQLVOSGAEVKKPGASVKVSCKASGYTFSYSWMNWV</u> <u>ROAPGQGLEWMGRIFPGDGDTDYNGKFKGRVTITADKS</u> <u>TSTAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQG</u> <u>TLTVSS</u>	<u>46</u>

<u>CONSTRUCT</u>	<u>AMINO ACID SEQUENCE</u>	<u>SEQ ID NO</u>
B-HL1	<u>EVOLVOSGAEVKKPGASVKVSCKASGYTFTYSWMHWV QAPGKQGLEWMGRIFPGDGDTDYAQKFQGRVTMTRDT STSTVYMEMLSSLRSEDTAVYYCARNVFDGYWLVYWGQ GTLTVSS</u>	<u>48</u>
B-HL2	<u>EVOLVOSGAEVKKPGATVKISCKVSGYTFTYSWMHWV QAPGKQGLEWMGRIFPGDGDTDYAEKFQGRVTITADTS TDTAYMEMLSSLRSEDTAVYYCATNVFDGYWLVYWGQG TLTVSS</u>	<u>50</u>
B-HL3	<u>EVOLVOSGAEVKKPGATVKISCKVSGYTFTYSWMNWV QAPGKQGLEWMGRIFPGDGDTDYNGKFKGRVTITADTS TDTAYMEMLSSLRSEDTAVYYCATNVFDGYWLVYWGQG TLTVSS</u>	<u>52</u>
B-HL4	<u>EVOLVOSGAEVKKTGSSVKVSCKASGYTFTYSWMSWV QAPGKQGLEWMGRIFPGDGDTDYAQKFQGRVTITADKS TSTAYMEMLSSLRSEDTAVYYCARNVFDGYWLVYWGQG TLTVSS</u>	<u>54</u>
B-HL8	<u>EVOLVESGGGLVKPGGSLRLSCAASGFTFSYSWMNWVR QAPGKQGLEWVGRIFPGDGDTDYNGKFKGRVTITADKST STAYMEMLSSLRSEDTAVYYCARNVFDGYWLVYWGQGT LTVSS</u>	<u>56</u>
B-HL10	<u>EVOLVESGGGLVKPGGSLRLSCAASGFAFSYSWMNWVR QAPGKQGLEWVGRIFPGDGDTDYNGKFKGRVTITADKSTS TAYMEMLSSLRSEDTAVYYCARNVFDGYWLVYWGQGT LTVSS</u>	<u>58</u>
B-HL11	<u>EVOLVESGGGLVKPGGSLRLSCAASGFTFSYSWMNWVR QAPGKQGLEWVGRIFPGDGDTDYNGKFKGRVTITADKST STAYMEMLSSLRSEDTAVYYCARNVFDGYWLVYWGQGT LTVSS</u>	<u>60</u>
B-HL12	<u>EVOLVESGAGLVKPGGSLRLSCAASGFTFSYSWMNWVR QAPGKQGLEWMGRIFPGDGDTDYNGKFKGRVTITADKST STAYMEMLSSLRSEDTAVYYCARNVFDGYWLVYWGQGT LTVSS</u>	<u>62</u>
B-HL13	<u>EVOLVESGGGVVKPGGSLRLSCAASGFTFSYSWMNWVR QAPGKQGLEWMGRIFPGDGDTDYNGKFKGRVTITADKSTS TAYMEMLSSLRSEDTAVYYCARNVFDGYWLVYWGQGT LTVSS</u>	<u>64</u>

<u>CONSTRUCT</u>	<u>AMINO ACID SEQUENCE</u>	<u>SEQ ID NO</u>
	<u>TAYMELSSRSEDTAVYYCARNVFDGYWLVYWGQGT</u> <u>VTVSS</u>	
B-HL14	<u>EVOLVESGGGLKPGGSLRLSCAASGFTFSYSWMNWVR</u> <u>QAPGKGLEWMGRIFPGDGDTDYNGKFKGRVTITADKSTS</u> <u>TAYMELSSRSEDTAVYYCARNVFDGYWLVYWGQGT</u> <u>VTVSS</u>	<u>66</u>
B-HL15	<u>EVOLVESGGLVKPGSSLRLSCAASGFTFSYSWMNWVR</u> <u>QAPGKGLEWMGRIFPGDGDTDYNGKFKGRVTITADKST</u> <u>STAYMELSSRSEDTAVYYCARNVFDGYWLVYWGQGT</u> <u>LTVSS</u>	<u>68</u>
B-HL16	<u>EVOLVESGGLVKPGGSLRVSCAASGFTFSYSWMNWVR</u> <u>QAPGKGLEWMGRIFPGDGDTDYNGKFKGRVTITADKSTS</u> <u>TAYMELSSRSEDTAVYYCARNVFDGYWLVYWGQGT</u> <u>VTVSS</u>	<u>70</u>
B-HL17	<u>EVOLVESGGLVKPGGSLRLSCAASGFTFSYSWMNWVR</u> <u>QAPGKGLEWMGRIFPGDGDTDYNGKFKGRVTITADKST</u> <u>STAYMELSSRSEDTAVYYCARNVFDGYWLVYWGQGT</u> <u>LTVSS</u>	<u>72</u>
VH Signal Sequence	<u>MDWTWRLFLVAAATGAHS</u>	<u>74</u>
B-KV1	<u>DIVMTOTPLSLPVTPGEPASISCRSSKSLLHSNGITYLYWY</u> <u>LOKPGOSSPOLIYOMSNLVSGVPDRFSGSGSGTDFTLKIS</u> <u>RVEAEDVGVYYCAONLELPYTFGGGKTKVEIKRTV</u>	<u>76</u>
VL Signal Sequence	<u>MDMRVPAQLLGLLLLWFPGARC</u>	<u>78</u>

[0113] In another preferred embodiment, the present disclosure 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 disclosure 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 disclosure 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 disclosure 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.

[0114] In another embodiment, the present disclosure is directed to an expression vector and/or a host cell which comprise one or more isolated polynucleotides described herein.

[0115] 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 described herein.

[0116] The therapeutic efficacy of the ABMs described herein 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 disclosure 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 described herein, 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.

[0117] 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.

[0118] In one embodiment, one or several polynucleotides encoding an ABM of the present disclosure 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 described herein 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 ABMs described herein 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.

[0119] 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).

[0120] 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 disclosure 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.

[0121] For the methods of this disclosure, 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.

[0122] 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)).

[0123] The present disclosure is further directed to a method for modifying the glycosylation profile of the ABMs described herein that are produced by a host cell, comprising expressing in said host cell a nucleic acid encoding an ABM of the disclosure 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.

[0124] 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.

[0125] The present disclosure is also directed to a method for producing an ABM described herein, 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 disclosure, 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.

[0126] Preferably, the Golgi localization domain is the localization domain of mannosidase II or GnT1. 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 described herein 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.

[0127] In another embodiment, the present disclosure is directed to a chimeric ABM having substantially the same binding specificity of the murine B-Ly1 antibody produced by the methods described herein 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 described herein has an increased proportion of nonfucosylated oligosaccharides in the Fc region as a result of the modification of its oligosaccharides by the methods described herein. 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 described herein 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 described herein 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 described herein 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.

[0128] In another embodiment, the present disclosure 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 described herein. 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.

[0129] The present disclosure is further directed to pharmaceutical compositions comprising the ABMs described herein and a pharmaceutically acceptable carrier.

[0130] The present disclosure is further directed to the use of such pharmaceutical compositions in the method of treatment of cancer. Specifically, the present disclosure is directed to a method for the treatment of cancer comprising administering a therapeutically effective amount of the pharmaceutical composition described herein.

[0131] The present disclosure further provides methods for the generation and use of host cell systems for the production of glycoforms of the ABMs described herein, 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 described herein 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 ABMs of the present disclosure can alternatively be glycoengineered to have reduced fucose residues in the Fc region according to the techniques disclosed in EP 1 176 195 A1.

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

[0132] The present disclosure describes host cell expression systems for the generation of the ABMs described herein having modified glycosylation patterns. In particular, the present disclosure describes host cell systems for the generation of glycoforms of the ABMs described herein having an improved therapeutic value. Therefore, the disclosure describes 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.

[0133] In one specific embodiment, the present disclosure relates to 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.

[0134] 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 described herein. 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 described herein.

[0135] The disclosure 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.

[0136] 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, E₄-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

[0137] 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.

[0138] 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.

[0139] 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 described herein, 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 described herein and the coding sequence of the polypeptide having GnTIII activity.

[0140] 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 described herein, 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.

[0141] In the fourth approach, the expression of the protein products can be assessed immunologically, for example by Western blots, immunoassays such as radioimmuno-precipitation and enzyme-linked immunoassays. 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

[0142] In preferred embodiments, the present disclosure describes 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.

[0143] 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).

[0144] 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 Fc_YRs interact with the lower hinge region of the IgG CH2 domain. Lund et al., J. Immunol. 157:4963-69 (1996). However, Fc_YR 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.

[0145] 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 Fc_YR binding lies within the oligosaccharide core. Lund et al., J. Immunol. 157:4963-69 (1996)

[0146] 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.

[0147] 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).

[0148] 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 disclosure 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 disclosure 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 disclosure

[0149] The ABMs described herein 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 described herein 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

[0150] 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 as 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.

[0151] In one embodiment, a chimeric, glycoengineered ABM having substantially the same

binding specificity of the murine B-Lyl 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).

[0152] 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.

[0153] 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-Lyl antibody. The term "cytotoxic moiety" as used herein is intended to include such isotopes.

[0154] In another embodiment, liposomes are filled with a cytotoxic drug and the liposomes are coated with the ABMs described herein. 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.

[0155] 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)).

[0156] Still other therapeutic applications for the ABM described herein 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 Activities 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)).

[0157] Still another therapeutic use for the ABMs described herein 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)].

[0158] Furthermore, it is contemplated that the disclosure 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*.

[0159] 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 acitivity, e.g., a lymphokine or oncostatin, can be used to treat human carcinoma *in vivo*.

[0160] The present disclosure describes a method for selectively killing tumor cells expressing CD20. This method comprises reacting the immunoconjugate (e.g., the immunotoxin) described herein with said tumor cells. These tumor cells may be from a human carcinoma.

[0161] Additionally, this disclosure describes 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) described herein.

[0162] In a further aspect, the disclosure 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 described herein 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 purpura and chronic idiopathic thrombocytopenic purpura, 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, thromboangiitis obliterans, primary biliary cirrhosis, Hashimoto's thyroiditis, thyrotoxicosis, chronic active hepatitis, polymyositis/dermatomyositis, polychondritis, pemphigus 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 anemia (Addison's disease); diseases involving leukocyte diapedesis; central nervous system (CNS) inflammatory disorder, multiple organ injury syndrome; hemolytic anemia (including, but not limited to cryoglobulinemia 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 disclosure, the ABMs described herein are used to deplete the blood of normal B-cells for an extended period.

[0163] In accordance with the practice of this disclosure, the subject may be a human, equine, porcine, bovine, murine, canine, feline, and avian subjects. Other warm blooded animals are also included herein.

[0164] The disclosure further describes 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 described herein.

[0165] It is apparent, therefore, that the present disclosure encompasses pharmaceutical compositions, combinations and methods for treating human carcinomas, such as a B cell lymphoma. For example, the disclosure includes pharmaceutical compositions for use in the treatment of human carcinomas comprising a pharmaceutically effective amount of an antibody described herein and a pharmaceutically acceptable carrier.

[0166] The ABM compositions described herein 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.

[0167] In one aspect of the disclosure, therapeutic formulations containing the ABMs described herein 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).

[0168] Exemplary anti-CD20 ABM formulations are described in WO98/56418. 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 described herein.

[0169] Lyophilized formulations adapted for subcutaneous administration are described in WO97/04801. Such lyophilized 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.

[0170] 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.

[0171] 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-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nanoparticles and nanocapsules) or in macroemulsions. Such techniques are

disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

[0172] 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-hydroxyethylmethacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and ethyl-L-glutamate, non-degradable ethylenevinyl 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.

[0173] The formulations to be used for in vivo administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

[0174] The compositions described herein 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.

[0175] The compositions described herein 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.

[0176] 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.

[0177] 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.

[0178] 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.

[0179] 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.

[0180] 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/m² to about 1000 mg/m². 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/m² of the antibody, e.g., where the dose is in the range from about 20 mg/m² to about 250 mg/m², for example from about 50 mg/m² to about 200 mg/m².

[0181] Moreover, one may administer one or more initial dose(s) of the antibody followed by one or more subsequent dose(s), wherein the mg/m² dose of the antibody in the subsequent dose(s) exceeds the mg/m² dose of the antibody in the initial dose(s). For example, the initial dose may be in the range from about 20 mg/m² to about 250 mg/m² (e.g., from about 50 mg/m² to about 200 mg/m²) and the subsequent dose may be in the range from about 250 mg/m² to about 1000 mg/m².

[0182] 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.

[0183] The ABM described herein is administered by any suitable means, including parenteral, subcutaneous, intraperitoneal, intrapulmonary, 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.

[0184] 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.

[0185] It would be clear that the dose of the composition described herein required to achieve cures may be further reduced with schedule optimization.

[0186] In accordance with the practice of the disclosure, 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.

[0187] In one example of the disclosure 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).

[0188] Alternatively, the detergent may be an ionic detergent An example of an ionic detergent includes, but is not limited to, alkyltrimethylammonium bromide.

[0189] Additionally, in accordance with the disclosure, 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.

[0190] In the following embodiments of the present disclosure are mentioned which are preferred:

Item 1: An isolated polynucleotide comprising

1. a. a sequence selected from the group consisting of: SEQ ID NO.:5, SEQ ID NO.: 6 and SEQ ID NO:7; and
2. b. a sequence selected from the group consisting of:SEQ ID NO: 21, SEQ ID NO: 22 and SEQ ID NO:23; and
3. c. sequence ID NO:24.

Item 2: An isolated polynucleotide comprising SEQ ID NO.:8, SEQ ID NO.: 9 and SEQ ID NO.:10.

Item 3: An isolated polynucleotide according to item 1 or item 2, which encodes a fusion polypeptide.

Item 4: An isolated polynucleotide comprising SEQ ID No.:3.

Item 5: An isolated polynucleotide comprising SEQ ID No.:4.

Item 6: An isolated polynucleotide according to item 4 or 5, wherein said isolated polynucleotide encodes a fusion polypeptide.

Item 7: An isolated polynucleotide comprising a sequence having at least 80% identity to SEQ ID NO:3, wherein said isolated polynucleotide encodes a fusion polypeptide.

Item 8: An isolated polynucleotide comprising a sequence having at least 80% identity to SEQ ID NO:4, wherein said isolated polynucleotide encodes a fusion polypeptide.

Item 9: An isolated polynucleotide comprising

1. a. a sequence encoding a polypeptide having the sequence of SEQ ID No.:1; and
2. b. a sequence encoding a polypeptide having the sequence of an antibody Fc region, or a fragment thereof, from a species other than mouse.

Item 10: An isolated polynucleotide comprising

1. a. a sequence encoding a polypeptide having the sequence of SEQ ID No.:2; and
2. b. a sequence encoding a polypeptide having the sequence of an antibody Fc region, or a fragment thereof, from a species other than mouse.

Item 11: An isolated polynucleotide encoding a polypeptide having the sequence of SEQ ID No.:1.

Item 12: An isolated polynucleotide encoding a polypeptide having the sequence of SEQ ID No.:2.

Item 13: An isolated polynucleotide comprising SEQ ID NO.: 11.

Item 14: An isolated polynucleotide comprising SEQ ID NO.: 12.

Item 15: An isolated polynucleotide comprising a sequence having at least 80% identity to SEQ ID NO.:11 or to SEQ ID NO.:12.

Item 16: An isolated polynucleotide comprising a sequence having at least 85% identity to SEQ ID NO.:11 or to SEQ ID NO.:12.

Item 17: An isolated polynucleotide comprising a sequence having at least 90% identity to SEQ ID NO.:11 or to SEQ ID NO.: 12.

Item 18: An isolated polynucleotide comprising a sequence having at least 95% identity to SEQ ID NO.:11 or to SEQ ID NO.: 12.

Item 19: An isolated polynucleotide comprising a sequence having at least 99% identity to SEQ ID NO.:11 or to SEQ ID NO.:12.

Item 20: An isolated polynucleotide comprising a sequence that encodes a polypeptide having SEQ ID NO:13 (heavy chain aa).

Item 21: An isolated polynucleotide comprising a sequence that encodes a polypeptide having SEQ ID NO: 14 (light chain aa).

Item 22: An isolated polynucleotide comprising

1. a. a sequence encoding a polypeptide having the V_H region of the murine B-Ly1 antibody, or variants thereof; and
2. b. a sequence encoding a polypeptide having the sequence of an antibody Fc region, or a fragment thereof, from a species other than mouse.

Item 23: An isolated polynucleotide comprising

1. a. a sequence encoding a polypeptide having the V_L region of the murine B-Ly1 antibody, or variants thereof; and
2. b. a sequence encoding a polypeptide having the sequence of an antibody Fc region, or a fragment thereof, from a species other than mouse.

Item 24: An expression vector comprising an isolated polynucleotide according to any one of items 1-23.

Item 25: The vector of item 24, wherein said vector is polycistronic.

Item 26: A host cell comprising the expression vector of item 24.

Item 27: A host cell comprising an isolated polynucleotide according to any one of items 1-23.

Item 28: A polypeptide comprising a sequence derived from the murine B-Ly1 antibody and a sequence derived from a heterologous polypeptide.

Item 29: An antigen-binding molecule comprising the polypeptide of item 28.

Item 30: An antigen-binding molecule according to item 29, which binds human CD20.

Item 31: An antigen-binding molecule according to item 29, which is an antibody.

Item 32: An antigen-binding molecule according to item 31, which is a chimeric antibody.

Item 33: An antigen-binding molecule according to item 31, which is a humanized antibody.

Item 34: An antigen-binding molecule according to item 31, which is a primatized antibody.

Item 35: A chimeric polypeptide comprising the sequence of SEQ ID NO:1 or a variant thereof.

Item 36: A chimeric polypeptide comprising the sequence of SEQ ID NO:2 or a variant thereof.

Item 37: A chimeric polypeptide comprising:

- d. a polypeptide having a sequence selected from the group consisting of: SEQ ID NO: 15, SEQ ID NO: 16 and SEQ ID NO: 17; and
- e. a polypeptide having a sequence selected from the group consisting of: SEQ ID NO:25, SEQ ID NO:26 and SEQ ID NO: 27; and
- f. SEQ ID NO:28.

Item 38: A chimeric polypeptide according to item 37, further comprising a heavy chain variable region framework, wherein said framework comprises an alanine residue at position 71, according to Kabat numbering.

Item 39: A chimeric polypeptide according to item 37, further comprising a heavy chain variable region framework, wherein said framework comprises an arginine residue at position 94, according to Kabat numbering.

Item 40: A chimeric polypeptide comprising SEQ ID NO:18, SEQ ID NO:19 and SEQ ID NO: 20.

Item 41: An isolated polypeptide comprising SEQ ID NO: 13 or a variant thereof.

Item 42: An isolated polypeptide comprising SEQ ID NO:14 or a variant thereof.

Item 43: The isolated polypeptide according to any of items 35-40, wherein said polypeptide is a fusion polypeptide.

Item 44: An antigen binding molecule comprising the isolated polypeptide of item 43.

Item 45: The antigen binding molecule of item 44, wherein said antigen binding molecule is an antibody.

Item 46: The antibody of item 45, wherein said antibody is a primatized antibody.

Item 47: The antibody of item 45, wherein said antibody is a humanized antibody.

Item 48: The antigen binding molecule of item 44, wherein said antigen binding molecule is an antibody fragment.

Item 49: The antibody fragment of item 48, wherein said antibody fragment is primatized.

Item 50: The antigen binding molecule of item 48, wherein said antibody fragment is humanized.

Item 51: An antigen binding molecule, which is capable of competing with the murine B-Lyl antibody for binding to CD20, wherein said antigen binding molecule is chimeric.

Item 52: The antigen binding molecule of item 51, wherein said antigen binding molecule is an antibody.

Item 53: The antigen binding molecule of item 52, wherein said antibody is primatized.

Item 54: The antigen binding molecule of item 51, wherein said recombinant antibody is humanized.

Item 55: The antigen binding molecule of item 51, wherein said recombinant antibody comprises a human Fc region.

Item 56: The antigen binding molecule of item 55, wherein said human Fc region is a human IgG Fc region.

Item 57: An antigen binding molecule of any of items 29-34 or 44-56, said antigen binding molecule having an Fc region with modified oligosaccharides.

Item 58: An antigen binding molecule according to item 57, wherein said Fc region has been modified to have reduced fucose residues as compared to non-modified antigen binding molecule.

Item 59: An antigen binding molecule according to item 57, wherein said Fc region has an increased proportion of bisected oligosaccharides.

Item 60: An antigen binding molecule according to item 59, wherein said modified oligosaccharides are bisected complex.

Item 61: An antigen binding molecule according to item 57, wherein said modified oligosaccharides have an increased proportion of bisected, nonfucosylated oligosaccharides in the Fc region of said antigen binding molecule.

Item 62: An antigen binding molecule according to item 61, wherein said bisected, nonfucosylated oligosaccharides are hybrid.

Item 63: An antigen binding molecule according to item 61, wherein said bisected, nonfucosylated oligosaccharides are complex.

Item 64: An antigen binding molecule according to item 57, wherein at least 20% of the oligosaccharides in the Fc region of said polypeptide are bisected, nonfucosylated.

Item 65: An antigen binding molecule according to item 57, wherein at least 30% of the oligosaccharides in the Fc region of said polypeptide are bisected, nonfucosylated.

Item 66: An antigen binding molecule according to item 57, wherein at least 35% of the oligosaccharides in the Fc region of said polypeptide are bisected, nonfucosylated.

Item 67: An antigen binding molecule according to item 57, wherein at least 70% of the oligosaccharides in the Fc region of said polypeptide are bisected, nonfucosylated.

Item 68: A method of producing an antigen binding molecule, which is capable of competing with the murine B-Ly1 antibody for binding to human CD20, and wherein said antigen binding molecule is chimeric; said method comprising

1. a. culturing the host cell of item 26 or item 27 in a medium under conditions allowing the expression of said polynucleotide encoding said antigen binding molecule; and
2. b. recovering said antigen binding molecule from the resultant culture.

Item 69: The method of item 68, wherein said antigen binding molecule is an antibody.

Item 70: A pharmaceutical composition comprising the antigen binding molecule according to any of items 29-34 or 46-67 and a pharmaceutically acceptable carrier.

Item 71: The pharmaceutical composition of item 70, wherein said composition further

comprises a pharmaceutically acceptable carrier.

Item 72: The pharmaceutical composition of item 70, wherein said composition further comprises an adjuvant.

Item 73: A method of treating a disorder treatable by B-cell depletion comprising administering a therapeutically effective amount of pharmaceutical composition of any of items 70-72 to a human subject in need thereof.

Item 74: A host cell engineered to express at least one nucleic acid encoding a polypeptide having $\beta(1,4)$ -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 any of items 44-67.

Item 75: The host cell of item 74, wherein said polypeptide having $\beta(1,4)$ -N-acetylglucosaminyltransferase III activity is a fusion polypeptide.

Item 76: The host cell of item 74, wherein said antigen binding molecule is an antibody.

Item 77: The host cell of item 74, wherein said antigen binding molecule is an antibody fragment.

Item 78: The host cell of item 74, wherein said antigen binding molecule comprises a region equivalent to the Fc region of a human IgG.

Item 79: The host cell of item 74, wherein said antigen binding molecule produced by said host cell exhibits increased Fc receptor binding affinity as a result of said modification.

Item 80: The host cell of item 74, wherein said antigen binding molecule produced by said host cell exhibits increased effector function as a result of said modification.

Item 81: A host cell according to item 75, wherein said fusion polypeptide comprises the catalytic domain of $\beta(1,4)$ -N-acetylglucosaminyltransferase III.

Item 82: A host cell according to item 75, wherein said fusion polypeptide further comprises the Golgi localization domain of a heterologous Golgi resident polypeptide.

Item 83: A host cell according to item 82, wherein said Golgi localization domain is the localization domain of mannosidase II.

Item 84: A host cell according to item 82, wherein said Golgi localization domain is the localization domain of $\beta(1,2)$ -N-acetylglucosaminyltransferase I.

Item 85: A host cell according to item 82, wherein said Golgi localization domain is the localization domain of $\beta(1,2)$ -N-acetylglucosaminyltransferase II.

Item 86: A host cell according to item 82, wherein said Golgi localization domain is the localization domain of mannosidase I.

Item 87: A host cell according to item 82, wherein said Golgi localization domain is the localization domain of α 1-6 core fucosyltransferase.

Item 88: A host cell according to item 80, wherein said increased effector function is increased Fc-mediated cellular cytotoxicity.

Item 89: A host cell according to item 80, wherein said increased effector function is increased binding to NK cells.

Item 90: A host cell according to item 80, wherein said increased effector function is increased binding to macrophages.

Item 91: A host cell according to item 80, wherein said increased effector function is increased binding to polymorphonuclear cells.

Item 92: A host cell according to item 80, wherein said increased effector function is increased binding to monocytes.

Item 93: A host cell according to item 80, wherein said increased effector function is increased direct signaling inducing apoptosis.

Item 94: A host cell according to item 80, wherein said increased effector function is increased dendritic cell maturation.

Item 95: A host cell according to item 80, wherein said increased effector function is increased T cell priming.

Item 96: A host cell according to item 79, wherein said Fc receptor is Fc γ activating receptor.

Item 97: A host cell according to item 79, wherein said Fc receptor is Fc γ RIIIA receptor.

Item 98: A host cell according to item 74, wherein said host cell is a CHO cell, a BHK cell, a NSO cell, a SP2/0 cell, a YO myeloma cell, a P3X63 mouse myeloma cell, a PER cell, a PER.C6 cell or a hybridoma cell.

Item 99: The host cell of item 74, further comprising at least one transfected polynucleotide encoding a polypeptide according to any of items 28 and 35-41, and wherein said nucleic acid comprises a sequence encoding a region equivalent to the Fc region of a human immunoglobulin.

Item 100: The host cell of item 74, wherein said at least one nucleic acid encoding a polypeptide having β (1,4)N-acetylglucosaminyltransferase III activity is operably linked to a constitutive promoter element.

Item 101: The host cell of item 100, wherein said polypeptide having β (1,4)-N-acetylglucosaminyltransferase III activity is a fusion polypeptide.

Item 102: A method for producing an antigen binding molecule having modified oligosaccharides in a host cell, said method comprising:

1. a. culturing a host cell engineered to express at least one nucleic acid encoding a polypeptide having β (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
2. b. isolating said antigen binding molecule wherein said antigen binding molecule is capable of competing with the murine B-Lyl antibody for binding to CD20 and wherein said antigen binding molecule or fragment thereof is chimeric.

Item 103: A method according to item 102, wherein said modified oligosaccharides have reduced fucosylation as compared to non-modified oligosaccharides.

Item 104: A method according to item 102, wherein said modified oligosaccharides are hybrid.

Item 105: A method according to item 102, wherein said modified oligosaccharides are complex.

Item 106: A method according to item 102, wherein said recombinant antibody or fragment thereof produced by said host cell has an increased proportion of bisected, nonfucosylated oligosaccharides in the Fc region of said polypeptide. Item 107: A method according to item 106, wherein said bisected, nonfucosylated oligosaccharides are hybrid.

Item 108: A method according to item 106, wherein said bisected, nonfucosylated oligosaccharides are complex.

Item 109: A method according to item 102, wherein at least 20% of the oligosaccharides in the Fc region of said polypeptide are bisected, nonfucosylated.

Item 110: A method according to item 102, wherein at least 30% of the oligosaccharides in the Fc region of said polypeptide are bisected, nonfucosylated.

Item 111: A method according to item 102, wherein at least 35% of the oligosaccharides in the Fc region of said polypeptide are bisected, nonfucosylated.

Item 112: An antigen binding molecule engineered to have increased effector function produced by the method according to any one of items 102-111.

Item 113: The antigen binding molecule of item 112, wherein said antigen binding molecule is an antibody.

Item 114: An antibody engineered to have increased Fc receptor binding affinity produced by the method of any one of items 102-111.

Item 115: The antigen binding molecule of item 114, wherein said antigen binding molecule is an antibody.

Item 116: An antigen binding molecule according to item 112, wherein said increased effector function is increased Fc-mediated cellular cytotoxicity.

Item 117: An antigen binding molecule according to item 112, wherein said increased effector function is increased binding to NK cells.

Item 118: An antigen binding molecule according to item 112, wherein said increased effector function is increased binding to macrophages.

Item 119: An antigen binding molecule according to item 112, wherein said increased effector function is increased binding to monocytes.

Item 120: An antigen binding molecule according to item 112, wherein said increased effector function is increased binding to polymorphonuclear cells.

Item 121: An antigen binding molecule according to item 112, wherein said increased effector function is direct signaling inducing apoptosis.

Item 122: An antigen binding molecule according to item 112, wherein said increased effector function is increased dendritic cell maturation.

Item 123: An antigen binding molecule according to item 112, wherein said increased effector function is increased T cell priming.

Item 124: An antigen binding molecule according to item 114, wherein said Fc receptor is Fc activating receptor.

Item 125: An antigen binding molecule according to item 114, wherein said Fc receptor is Fc_YRIIIa receptor.

Item 126: An antigen binding molecule produced by the method of any of items 102 to 111, wherein said antigen binding molecule is an antibody fragment containing the Fc region and engineered to have increased effector function.

Item 127: An antigen binding molecule produced by the method of any of items 102 to 111, wherein said antigen binding molecule is 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.

Item 128: An antigen binding molecule produced by the method of any of items 102 to 111, wherein said antigen binding molecule is a fusion protein that includes 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 region equivalent to the Fc region of an immunoglobulin and engineered to have increased effector function.

Item 129: An antigen binding molecule produced by the method of any of items 102 to 111, wherein said antigen binding molecule is 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.

Item 130: An antigen binding molecule produced by the method of any of items 102 to 111, wherein said antigen binding molecule is a fusion protein that includes a polypeptide having the sequence of SEQ ID NO:76 and a region equivalent to the Fc region of an immunoglobulin and engineered to have increased effector function.

Item 131: A pharmaceutical composition comprising the antigen binding molecule of any of items 112 to 130 and a pharmaceutically acceptable carrier.

Item 132: A pharmaceutical composition comprising the antibody fragment of item 126 and a pharmaceutically acceptable carrier.

Item 133: A pharmaceutical composition comprising the fusion protein of any of items 127 to 130 and a pharmaceutically acceptable carrier.

Item 134: A method of treating a disease treatable by B-cell depletion comprising administering a therapeutically effective amount of the antigen binding according to any of items 112 to 130 to a human subject in need thereof.

Item 135: An isolated polynucleotide according to item 4 or 5 further comprising a sequence encoding a human antibody light or heavy chain constant region.

Item 136: An isolated polynucleotide comprising a sequence with at least 80% identity to SEQ ID NO:24, wherein said isolated polynucleotide encodes a fusion polypeptide.

Item 137: A host cell coexpressing an isolated polynucleotide according to item 136 and a polynucleotide comprising a sequence encoding the variable region of an antibody light chain.

Item 138: An antigen binding molecule according to item 57, wherein at least 50% of the oligosaccharides in the Fc region are bisected.

Item 139: An antigen binding molecule according to item 57, wherein at least 60% of the oligosaccharides in the Fc region are bisected.

Item 140: An antigen binding molecule according to item 57, wherein at least 70% of the oligosaccharides in the Fc region are bisected.

Item 141: An antigen binding molecule according to item 57, wherein at least 80% of the oligosaccharides in the Fc region are bisected.

Item 142: An antigen binding molecule according to item 57, wherein at least 90% of the oligosaccharides in the Fc region are bisected.

Item 143: An antigen binding molecule according to item 57, wherein at least 50% of the oligosaccharides in the Fc region are nonfucosylated.

Item 144: An antigen binding molecule according to item 57, wherein at least 60% of the oligosaccharides in the Fc region are nonfucosylated.

Item 145: An antigen binding molecule according to item 57, wherein at least 70% of the oligosaccharides in the Fc region are nonfucosylated.

Item 146: An antigen binding molecule according to item 57, wherein at least 75% of the oligosaccharides in the Fc region are nonfucosylated.

Item 147: A method according to item 73, wherein said disorder is a B cell lymphoma.

Item 148: 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.

Item 149: An isolated polynucleotide according to item 148, wherein said isolated polynucleotide comprises SEQ ID NO:31.

Item 150: An isolated polynucleotide according to item 148, wherein said isolated polynucleotide comprises SEQ ID NO:55.

Item 151: An isolated polynucleotide according to item 148, wherein said isolated polynucleotide comprises SEQ ID NO:59.

Item 152: An isolated polynucleotide comprising SEQ ID No:75.

Item 153: An isolated polynucleotide according to item 148 or 149, wherein said isolated polynucleotide encodes a fusion polypeptide.

Item 154: An isolated polynucleotide comprising

1. a) a sequence encoding a polypeptide having a sequence selected from the group consisting of SEQ ID NO:32; SEQ ID NO:56; and SEQ ID NO:60; and
2. b) a sequence encoding a polypeptide having the sequence of SEQ ID NO:76

Item 155: 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.

Item 156: An isolated polynucleotide comprising a sequence having at least 80% identity to SEQ ID NO:75, wherein said isolated polynucleotide encodes a fusion polypeptide.

Item 157: An isolated polynucleotide comprising a sequence having at least 85% 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.

Item 158: An isolated polynucleotide comprising a sequence having at least 85% identity to SEQ ID No: 75.

Item 159: An isolated polynucleotide comprising a sequence having at least 90% 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.

Item 160: An isolated polynucleotide comprising a sequence having at least 90% identity to SEQ ID No: 75.

Item 161: An isolated polynucleotide comprising a sequence having at least 95% 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.

Item 162: An isolated polynucleotide comprising a sequence having at least 95% identity to SEQ ID No: 75.

Item 163: An isolated polynucleotide comprising a sequence having at least 99% 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.

Item 164: An isolated polynucleotide comprising a sequence having at least 99% identity to SEQ ID No: 75.

Item 165: An isolated polynucleotide comprising

1. a) 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
2. b) a sequence encoding a polypeptide having the sequence of an antibody Fc region, or a fragment thereof, from a species other than mouse.

Item 166: An isolated polynucleotide comprising

1. a) a sequence encoding a polypeptide having the sequence of SEQ ID No:76; and
2. b) a sequence encoding a polypeptide having the sequence of an antibody Fc region, or a fragment thereof, from a species other than mouse.

Item 167: An isolated polynucleotide 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. Item 168: An isolated polynucleotide encoding a polypeptide having the sequence of SEQ ID No:76.

Item 169: An isolated polynucleotide comprising a sequence that encodes 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.

Item 170: An isolated polynucleotide comprising a sequence that encodes a polypeptide having SEQ ID NO:76.

Item 171: An expression vector comprising an isolated polynucleotide according to any one of items 148-170.

Item 172: The vector of item 171, wherein said vector is polycistronic.

Item 173: A host cell comprising the expression vector of item 171.

Item 174: A host cell comprising an isolated polynucleotide according to any one of items 148-170.

Item 175: A humanized 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. Item 176: A humanized polypeptide comprising the sequence of SEQ ID NO:76, or a variant thereof.

Item 177: An isolated 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.

Item 178: An isolated polypeptide comprising SEQ ID NO:76, or a variant thereof.

Item 179: The isolated polypeptide according to any of items 177-178, wherein said polypeptide is a fusion polypeptide.

Item 180: An antigen binding molecule comprising the isolated polypeptide of item 179.

Item 181: The antigen binding molecule of item 180, wherein said antigen binding molecule is an antibody.

Item 182: The antibody of item 181, wherein said antibody is a humanized antibody.

Item 183: The antigen binding molecule of item 180, wherein said antigen binding molecule is an antibody fragment.

Item 184: The antigen binding molecule of item 183, wherein said antibody fragment is humanized.

Item 185: The antigen binding molecule of item 180, wherein said antigen binding molecule is a recombinant antibody.

Item 186: The antigen binding molecule of item 185, wherein said recombinant antibody is humanized.

Item 187: The antigen binding molecule of item 180, wherein said recombinant antibody comprises a human Fc region.

Item 188: The antigen binding molecule of item 187, wherein said human Fc region is a human IgG Fc region.

Item 189: An antigen binding molecule of any of items 180-188, said antigen binding molecule having an Fc region with modified oligosaccharides.

Item 190: An antigen binding molecule according to item 189, wherein said Fc region has been modified to have reduced fucose residues as compared to non-modified antigen binding molecule.

Item 191: An antigen binding molecule according to item 189, wherein said Fc region has an increased proportion of bisected oligosaccharides.

Item 192: An antigen binding molecule according to item 189, wherein said modified oligosaccharides are bisected complex.

Item 193: An antigen binding molecule according to item 189, wherein said modified oligosaccharides have an increased proportion of bisected, nonfucosylated oligosaccharides in the Fc region of said antigen binding molecule.

Item 194: An antigen binding molecule according to item 193, wherein said bisected, nonfucosylated oligosaccharides are hybrid.

Item 195: An antigen binding molecule according to item 193, wherein said bisected,

nonfucosylated oligosaccharides are complex.

Item 196: An antigen binding molecule according to item 189, wherein at least 20% of the oligosaccharides in the Fc region of said polypeptide are bisected, nonfucosylated.

Item 197: An antigen binding molecule according to item 189, wherein at least 30% of the oligosaccharides in the Fc region of said polypeptide are bisected, nonfucosylated.

Item 198: An antigen binding molecule according to item 189, wherein at least 35% of the oligosaccharides in the Fc region of said polypeptide are bisected, nonfucosylated.

Item 199: An antigen binding molecule according to item 189, wherein at least 70% of the oligosaccharides in the Fc region of said polypeptide are bisected, nonfucosylated.

Item 200: A method of producing an antigen binding molecule, which is capable of competing with the murine B-Ly1 antibody for binding to human CD20, and wherein said antigen binding molecule is chimeric; said method comprising

1. a) culturing the host cell of item 173 or item 174 in a medium under conditions allowing the expression of said polynucleotide encoding said antigen binding molecule; and
2. b) recovering said antigen binding molecule.

Item 201: The method of item 200, wherein said antigen binding molecule is an antibody.

Item 202: The method of item 200, wherein said chimeric antigen binding molecule is humanized.

Item 203: A pharmaceutical composition comprising the antigen binding molecule according to any of items 189-199 and a pharmaceutically acceptable carrier.

Item 204: The pharmaceutical composition of item 203, wherein said composition further comprises a pharmaceutically acceptable carrier.

Item 205: The pharmaceutical composition of item 203, wherein said composition further comprises an adjuvant.

Item 206: A method of treating a disorder treatable by B-cell depletion comprising administering a therapeutically effective amount of pharmaceutical composition of any of items 203-205 to a human subject in need thereof.

Item 207: A host cell engineered to express at least one nucleic acid encoding a polypeptide having $\beta(1,4)$ -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 any of items 180-197.

Item 208: The host cell of item 207, wherein said polypeptide having $\beta(1,4)$ -N-acetylglucosaminyltransferase III activity is a fusion polypeptide.

Item 209: The host cell of item 207, wherein said antigen binding molecule is an antibody.

Item 210: The host cell of item 207, wherein said antigen binding molecule is an antibody fragment.

Item 211: The host cell of item 207, wherein said antigen binding molecule comprises a region equivalent to the Fc region of a human IgG.

Item 212: The host cell of item 207, wherein said antigen binding molecule produced by said host cell exhibits increased Fc receptor binding affinity as a result of said modification.

Item 213: The host cell of item 207, wherein said antigen binding molecule produced by said host cell exhibits increased effector function as a result of said modification.

Item 214: A host cell according to item 208, wherein said fusion polypeptide comprises the catalytic domain of β (1,4)-N-acetylglucosaminyltransferase III.

Item 215: A host cell according to item 208, wherein said fusion polypeptide further comprises the Golgi localization domain of a heterologous Golgi resident polypeptide.

Item 216: A host cell according to item 215, wherein said Golgi localization domain is the localization domain of mannosidase II.

Item 217: A host cell according to item 215, wherein said Golgi localization domain is the localization domain of β (1,2)-N-acetylglucosaminyltransferase I.

Item 218: A host cell according to item 215, wherein said Golgi localization domain is the localization domain of β (1,2)-N-acetylglucosaminyltransferase II.

Item 219: A host cell according to item 215, wherein said Golgi localization domain is the localization domain of mannosidase I.

Item 220: A host cell according to item 215, wherein said Golgi localization domain is the localization domain of α 1-6 core fucosyltransferase.

Item 221: A host cell according to item 213, wherein said increased effector function is increased Fc-mediated cellular cytotoxicity.

Item 222: A host cell according to item 213, wherein said increased effector function is increased binding to NK cells.

Item 223: A host cell according to item 213, wherein said increased effector function is increased binding to macrophages.

Item 224: A host cell according to item 213, wherein said increased effector function is increased binding to polymorphonuclear cells.

Item 225: A host cell according to item 213, wherein said increased effector function is increased binding to monocytes.

Item 226: A host cell according to item 213, wherein said increased effector function is

increased direct signaling inducing apoptosis.

Item 227: A host cell according to item 213, wherein said increased effector function is increased dendritic cell maturation.

Item 228: A host cell according to item 213, wherein said increased effector function is increased T cell priming.

Item 229: A host cell according to item 212, wherein said Fc receptor is Fcγ activating receptor.

Item 230: A host cell according to item 212, wherein said Fc receptor is FcγRIIIA receptor.

Item 231: A host cell according to item 207, wherein said host cell is a CHO cell, a BHK cell, a NSO cell, a SP2/0 cell, a YO myeloma cell, a P3X63 mouse myeloma cell, a PER cell, a PER.C6 cell or a hybridoma cell.

Item 232: The host cell of item 207, further comprising at least one transfected polynucleotide encoding a polypeptide according to any of items 28 and 35-41, and wherein said nucleic acid comprises a sequence encoding a region equivalent to the Fc region of a human immunoglobulin.

Item 233: The host cell of item 207, wherein said at least one nucleic acid encoding a polypeptide having $\beta(1,4)$ -N-acetylglucosaminyltransferase III activity is operably linked to a constitutive promoter element.

Item 234: The host cell of item 233, wherein said polypeptide having $\beta(1,4)$ -N-acetylglucosaminyltransferase III activity is a fusion polypeptide.

Item 235: An antigen binding molecule according to item 189, wherein at least 50% of the oligosaccharides in the Fc region are bisected.

Item 236: An antigen binding molecule according to item 189, wherein at least 60% of the oligosaccharides in the Fc region are bisected.

Item 237: An antigen binding molecule according to item 189, wherein at least 70% of the oligosaccharides in the Fc region are bisected.

Item 238: An antigen binding molecule according to item 189, wherein at least 80% of the oligosaccharides in the Fc region are bisected.

Item 239: An antigen binding molecule according to item 189, wherein at least 90% of the oligosaccharides in the Fc region are bisected.

Item 240: An antigen binding molecule according to item 189, wherein at least 50% of the oligosaccharides in the Fc region are nonfucosylated.

Item 241: An antigen binding molecule according to item 189, wherein at least 60% of the oligosaccharides in the Fc region are nonfucosylated.

Item 242: An antigen binding molecule according to item 189, wherein at least 70% of the oligosaccharides in the Fc region are nonfucosylated.

Item 243: An antigen binding molecule according to item 189, wherein at least 75% of the oligosaccharides in the Fc region are nonfucosylated.

Item 244: A method according to item 206, wherein said disorder is a B cell lymphoma.

Item 245: 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.

Item 246: An isolated polynucleotide according to item 245, wherein said complementarity determining region is selected from the group consisting of : [LIST ALL B-Lyl CDR SEQUENCES].

Item 247: An isolated polynucleotide according to item 245, wherein said isolated polynucleotide encodes an antigen binding molecule.

Item 248: An isolated polynucleotide comprising at least 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, wherein said isolated polynucleotide encodes a fusion polypeptide.

Item 249: An isolated polynucleotide according to item 248, wherein said complementarity determining regions comprise at least one sequence selected from the group consisting of: SEQ ID NO:5, SEQ ID NO:6 and SEQ ID NO:7; and at least one sequence selected from the group consisting of: SEQ ID NO:21, SEQ ID NO:22, and SEQ ID NO:23; and SEQ ID NO:24, or variants or truncated forms of said sequences that contain at least the specificity-determining residues for each of said complementarity determining regions.

Item 250: An isolated polypeptide encoded by the isolated polynucleotides of any of items 245-249.

Item 251: An antigen binding molecule 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, and further comprising a sequence derived from a heterologous polypeptide.

Item 252: An antigen binding molecule according to item 251, wherein said antigen binding molecule comprises at least 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 complementarity determining regions.

Item 253: An antigen binding molecule according to item 252, wherein said antigen binding molecule comprises the variable region of an antibody light or heavy chain.

Item 254: An antigen binding molecule according to item 252, wherein said antigen binding molecule is a chimeric or humanized, antibody.

Item 255: An engineered Type II anti-CD20 antibody having increased ADCC as a result of said engineering without substantial loss of ability to induce apoptosis of target cells.

Item 256: An engineered Type II anti-CD20 antibody according to item 255, wherein said antibody has been engineered to have an altered pattern of glycosylation in the Fc region.

Item 257: An engineered Type II anti-CD20 antibody according to item 256, wherein said altered glycosylation is an increase in the amount of bisected complex oligosaccharides.

Item 258: An engineered Type II anti-CD20 antibody according to item 256, wherein said altered glycosylation is a decrease in the amount of fucose residues.

Item 259: An engineered Type II anti-CD20 antibody according to item 255, wherein said antibody has been engineered to have an altered amino acid sequence in the Fc region.

[0191] 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 invention.

EXAMPLES

[0192] [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-Lyl

[0193] B-Lyl expressing hybridoma cells were grown in RPMI containing 10% FBS and 4 mM L-glutamine. 6×10^6 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-Lyl 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.

[0194] For construction of chimeric B-Lyl 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.

[0195] Chimeric B-Lyl was produced by co-transfected 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% CO₂ atmosphere overnight. For each T75 flask to be transfected, a solution of DNA, CaCl₂ 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 CaCl₂ 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 Na₂HPO₄ 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% CO₂ 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.

[0196] 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

[0197] Oligosaccharides were enzymatically released from the antibodies by PNGaseF digestion, with the antibodies being either immobilized on a PVDF membrane or in solution.

[0198] 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

[0199] The wells of a 96-well plate made with a PVDF (Immobilon P, Millipore, Bedford, Massachusetts) membrane were wetted with 100 μ l 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 μ l of water. The wells were then washed with 50 μ l RCM buffer (8M Urea, 360mM Tris, 3.2mM EDTA, pH 8.6). Between 30-40 μ g antibody was loaded in a well containing 10 μ l RCM buffer. The liquid in the well was drawn through the membrane by applying vacuum, and the membrane was subsequently washed twice with 50 μ l RCM buffer. The reduction of disulfide bridges was performed by addition of 50 μ l of 0.1M dithiothreitol in RCM and incubation at 37°C for 1 h.

[0200] Following reduction, a vacuum was applied to remove the dithiothreitol solution from the well. The wells were washed three times with 300 μ l water before performing the carboxymethylation of the cysteine residues by addition of 50 μ l 0.1M iodoacetic acid in RCM buffer and incubation at room temperature in the dark for 30 min.

[0201] After carboxymethylation, the wells were drawn with vacuum and subsequently washed three times with 300 μ l water. The PVDF membrane was then blocked, to prevent adsorption of the endoglycosidase, by incubating 100 μ l 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 μ l water.

[0202] N-linked oligosaccharides were released by addition of 2.5 mU peptide-N-glycosidase F (recombinant 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 μ l in 20mM NaHCO₃, pH7.0). Digestion was performed for 3 hours at 37°C.

Oligosaccharide release method for antibodies in solution

[0203] 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

[0204] 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

[0205] 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

[0206] 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

[0207] 495 μ l heparinized blood from a healthy donor was aliquoted into 5 ml polystyrene tubes, 5 μ l 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 μ l blood was transferred to a fresh tube and stained with anti-CD3-FITC, anti-CD 19-PE and anti-CD45-CyChrome (Becton-Dickinson) for 15 min at room temperature in the dark. Before analysis, 500 μ l 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

[0208] 500.000 in 180 μ l FACS buffer (PBS containing 2% FCS and 5mM EDTA) were transferred to 5 ml polystyrene tubes and 20 μ l 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 μ l FACS buffer and 1 μ l 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 μ l of FACS buffer containing 0.5 μ g/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

[0209] 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. Reintroducing 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

[0210] 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

[0211] 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

[0212] 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-transfected 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.

Oligosaccharide analysis

[0213] Oligosaccharide release method for antibodies in solution Between 40 and 50 μ g 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

[0214] 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 μ l 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 μ l ethanol was applied, and the samples were finally allowed to re-crystallize under air.

MALDI/TOF-MS

[0215] 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

[0216] 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 Fc γ RIIIA-expressing CHO cell line

[0217] 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 (3×10^5 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 μ g/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 ImmunoResearch, West Grove, PA/USA) and anti-human CD56-PE (BD Biosciences, Allschwil/Switzerland). The anti-Fc γ RIIIA 3G8 F(ab')₂ fragments (Ancell, Bayport, MN/USA) were added at a concentration of 10 μ g/ml to compete binding of antibody glycovariants (3 μ g/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 μ F, 0.4 cm) with an expression vector coding for the Fc γ RIIIA-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

[0218] 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.

[0219] 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 atmosphere containing 5% CO₂. 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

[0220] Target cells were counted, washed with PBS, resuspended in AIM-V (Invitrogen) at 1 million cells per ml. 50 μ l cells were plated per well in a flat bottom 96 well plate. Antibody dilutions were prepared in AIM-V and added in 50 μ l 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 μ l to the wells. Rabbit complement (Cedarlane Laboratories) was prepared as described by the manufacturer, diluted 3-fold with AIM-V and added in 50 μ l 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 μ l supernatant per well were transferred to a new 96 well plate and 50 μ l 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% Triton X-100.

Whole blood B-cell depletion assay

[0221] Normal B-cell depletion in whole blood by the anti-CD20 antibodies was carried out as described in Example 1 above.

Apoptosis Assay

[0222] The apoptotic potency of the antibodies was assayed by incubating the antibody at 10 μ g/ml (saturating conditions in respect to antigen binding) with the target cells (at a target cell concentration of 5 x 10⁵ cells/ml) overnight (16-24 h). Samples were stained with AnnV-FITC and analyzed by FACS. Assay was done in triplicates.

[0223] 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

[0224] 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.

[0225] 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.

[0226] 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 Gin 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.

[0227] 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.

[0228] 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 2001). 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, MJ. 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 than 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.

[0229] 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-ly1 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 apoptosis 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. Apoptosis induction is important as *in vivo*, as there are locations in the body where the target CD20-positive cells can be found, but where 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.

SEQUENCE LISTING

[0230]

<110> GlycArt Biotechnology AG

Umana, Pablo

Bruenker, Peter

Suter, Tobias

Puentener, Ursula

Moessner, Ekkehard

Ferrara, Claudia

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Asp Phe Thr Leu Arg Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val
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Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe

50	55	60			
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<400> 33

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 tccttgcaagg cttccggata cgccttcagc taatatttggta tgaacttgggtt gggccaggcc 120
 cctggacaag ggctcgagtg gatgggacgg atctttcccg gcgatggggta tactgactac 180
 aatggggaaat tcaagggcag agtcacaattt accggccgaca aatccactag cacagccat 240
 atggagctga gcagccctgag atctgaggac acggccgtgtt atctgtgtgc aagaaaatgtc 300
 ttgtatggtt actgggtttgt ttactggggc cagggaaaccc tggtcacccgt ctctctagct 360
 agcacc 366

<210> 34

<211> 119

<212> PRT

<213> Artificial

<220>

<223> Mouse-human chimeric polypeptide

<400> 34

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 Iys Ala Ser Gly Tyr Ala 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 Leu 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> 35

<211> 357

<212> DNA

<213> Artificial

<220>

<223> Mouse-human chimeric DNA

<400> 35

caggtgcata	tgggtgcagtc	tggcgctgaa	gttaagaagc	ctggagctc	agtgaaggc	60
tcgtgcagg	tctcggata	cgcggtcage	tatttttgg	tgaactgggt	gcggcaggcc	120
cctggacaag	ggctcgagtc	gatggacgg	atctttcccg	gcgtatggga	tactgactac	180
aatggaaat	tcaaggggcag	agtcacaat	acggccgaca	aatocactag	cacagccat	240
atggagctga	gcagcgtgag	atcggaggac	acggccgtgt	attactgtgc	aagaaatgtc	300
tttgatggtt	actgggttgt	ttactgggc	cagggtaccc	tggtcacccgt	ctctca	357

<210> 36

<211> 119

<212> PRT

<213> Artificial

<220>

<223> Mouse-human chimeric polypeptide

<400> 36

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 Val Ser Gly Tyr Ala 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> 37

<211> 357

<212> DNA

<213> Artificial

<220>

<223> Mouse-human chimeric DNA

<400> 37

caggtgcaat	tgggtgcagtc	tggcgctgaa	gttaagaaggc	ctggggaggtc	agtggaaaggtc	60
tcctgcagg	cttcggata	cgcggttgc	tatcttttgg	tgagctgggt	ggggcaggcg	120
cctggacaag	ggtcgagtg	gtgggacgg	atctttcccg	gcgcgtggga	tactgactac	180
aatggaaat	tcaagggoag	agtccacaatt	cccgccgaca	aatccactag	cacagcttat	240
atggagctga	gcagccctgag	atctgaggac	ccggccgtgt	atctactgtgc	aagaaatgtc	300
tatgtatgtt	actggccrtgt	ttactggggc	caggaaaccc	tggtcacacgt	ctctca	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

cagggtcaat tgggtgcagtc tggcgctgaa gtcagaagaagc ctggggagttc agtgaaggtc	60
tcctgtcaagg cttcggata cgccttcagc tatttttggta tcaattgggt gggcaggcg	120
ccctggacaag ggctcgagtg gatgggacgg atctttcccg gggatgggta tactgactac	180
aatgggaaat tcggggcag agtcaacaatt accggcggaca aatccactag cacagcctat	240
atggagctga gcagcgtgag atctgaggad accggcgtgtt attactgtgc aagaaatgtc	300
tttggatggtt actgggttgtt ttactgggc caggaaaccc tggtcacggt ctccatca	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	
55 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 Asn Gly Tyr Thr Leu Val Tyr Tyr Gly Gln Glu	
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100	105	110
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Thr Leu Val Thr Val Ser Ser
115

<210> 41

<211> 357

<212> DNA

<213> Artificial

<220>

<223> Mouse-human chimeric DNA

<400> 41

cagggtgcata	tgggtgcagtc	tggcgctgaa	gtcaagaago	ctgggagttc	agtgaaggcc	60
tccctgcaagg	cttccggata	cgccttcage	tatccattgga	tctcggtgggt	ggggcaggcg	120
cctggacaag	ggctcgagtg	gatgggacgg	atcttcccg	gcgatgggga	tactgactac	180
aatgggaaat	tcaagggcag	agtcacaact	accggccaca	aatccactag	cacagccat	240
atggagctga	gcagccctgag	atctgaggac	acggccggtgt	attactgtgc	aagaaatgtc	300
tttgcgtt	actggcttgt	ttactggggc	cagggaaaccc	tggtcacccgt	ctccctca	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	Glu	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

<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 50

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
 cagggtgcata tgggtgcagtc tggagctgaa gtttagaaga gtc ctggcgcc tc agtgaagg tc
 60
 tcctgcataagg ctgcggata cacattcagc tatttttggc tgaactgggc gggcaaggcc
 120
 cctggacaag ggctcgagtg gatgggacgg atcttcccg gcatgggg tactgactac
 180
 aatggaaat tcaagggcag agtcacaatt accggcgaca aatccactag cacagctat
 240
 atggagctga gcagactgag atctgaggac acggcgtgtt attactgtgc aagaaatgtc
 300
 ttgtatggtt actggcttgtt ttactggggc cagggcaccc tggtcacgt ctctca
 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 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	gttaagaaga	ctggggcctc	agtgaaggta	60
tcctgcaagg	cgtccggata	cacccttcacc	tattttttgg	tgcactgggt	goggcaggcc	120
cctggacaaag	ggctcgagtg	gatgggaacgg	atctttcccg	gcatatggga	tactgactac	180
gcacagaaaat	tccaaaggaag	agtcacaatg	acacgggaca	cgtccacttc	cacccgttat	240
atggagctga	gcagccctgag	atotgaggac	acggccgtgt	attactgtgc	aagaaatgtc	300

tttgatggtt	actggcttgt	ttactggggc	caggaaaccc	tggtdaccq	ctactca	357
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<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															
															16

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

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

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

Gln	Gly	Arg	Val	Thr	Met	Thr	Arg	Asp	Thr	Ser	Thr	Ser	Thr	Val	Tyr
															80

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

Ala	Arg	Asn	Val	Phe	Asp	Gly	Tyr	Trp	Leu	Val	Tyr	Trp	Gly	Gln	Gly
															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	ttttaagaaga	ctggggccac	cgtgaagatc	60
tccctgcaagg	tgtcccgata	cacccttccacc	tatttttggc	tgcactgggt	gcagcaggcc	120
cctggaaagg	ggctcgagtg	gatgggacgg	atctttcccg	gatgggaa	tactgactac	180
gcagagaaat	tccaaaggaag	agtccacaatc	acagccgaca	cgtccactga	caccgcctat	240
atggagctga	gcagccctgag	atccggaggac	acggccgtgt	attactgtgc	aaccaatgtc	300
tttgcattt	actggctctt	ttactggggc	cacggaaaccc	tcgtcaccgt	cgtctca	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

gggtgcata	tggtgcatgc	tggcgctgaa	gttaagaago	ctggggccac	cgtaagatc	60
tcctgcaagg	tgtccggata	cacccacc	tatttttgg	tgaactgggt	gcagcaggcc	120
cctggaaagg	ggotcgagtg	gatggacgg	atcttcccg	gcgatgggg	tactgactac	180
aatggaaat	tcaagggaaag	agtccacaatc	acagccgaca	cgtccactga	cacggctat	240
atggagctga	gcagcctgag	atctgaggac	acggccgtgt	attactgtgc	aaccaatgtc	300
tttgcgtt	actggcttgt	ttactggggc	cagggaaaccc	tggtcaccgt	ctccica	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									10						15

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

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

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

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

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

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

Thr	Leu	Val	Thr	Val	Ser	Ser

<210> 53

<211> 366

<212> DNA

<213> Artificial

<220>

<223> Mouse-human chimeric DNA

<400> 53

cagatgcata	tggtgcaagtc	tggcgctgaa	gtttaagaaga	cggggatc	agtgaaggc	60
tcctgcaagg	tttccggata	cacccacc	tatccattgga	ttagatgggt	ggggcaggcc	120
ccctggacaag	ggctcgagtc	atgggacgg	atcttcccg	gatgtggga	tactgactac	180
gcacagaaat	tccaaaggaag	atgcacaatt	accggccgaca	aatccactag	cacagccat	240
atggagctga	gcagcctgag	atctgaggac	acggccgtgt	attactgtgc	aagaaatgtc	300
tttgatgtt	actggcttgt	ttactgggyc	cagggaaaccc	tggtcacogt	ctcttcagct	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

gaagtgcaga	tggtggagtc	tggaggaggc	ttggtcaagc	ctggggggtc	cctgaggata	60
tcctgtgcag	cctctggatt	cacattttgc	tatttttggc	tgtactgggt	ggggcaggct	120
cctggaaaagg	gcctcgagtg	ggtgggacgg	atctttcccg	gdgatgggca	taatgtactac	180
aatgggaaat	tcaagggcag	agtcacaatt	accggccgaca	aatccactag	cacagccat	240
atggagctga	ccagccctgag	atctgaggac	acggccgtgc	attactgtgc	aagaatgtc	300
tttgcgttttgc	actgggtttgt	ttactggggc	caggaaaccc	tggtccacgt	ctactca	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	ccacccggts	gcacccatgg	actggacctg	gaggatcc	ttcttggtgg	60
cagcagccac	aggagcccas	tcogaagtgc	agctggtgg	gtctggagga	ggcttggtca	120
agcctggcgg	gtccctgcgg	ctctctgt	cagcctctgg	attecattt	agctattttt	180
ggatgaactg	ggtgccggd	gtctctggaa	aggccctcga	gtgggtggaa	cgatctttc	240
ccggcgtatgg	ggataactgac	tacaatggaa	aaatccagg	cagagtacaca	attaccggcg	300
acaaatccac	tagcacagcc	tatatggagc	tgacgacoot	gagatctgag	gacaoggcog	360
tgttattactg	tgcaagaaat	gtcttgatg	gttaactggct	tgtttactgg	ggccaggaa	420
ccctggcac	cgtatccatca	gctagcgaac	tctoga			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	Ieu	Val	Lys	Pro	Gly	Gly
1															15

Ser	Ieu	Arg	Ieu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Ala	Phe	Ser	Tyr	Ser
20															30

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

Gly	Arg	Ile	Phe	Pro	Gly	Asp	Gly	Asp	Thr	Asp	Tyr	Asn	Gly	Iys	Phe
50															60

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

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

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

Thr	Ieu	Val	Thr	Val	Ser	Ser									
															115

<210> 59

<211> 357

<212> DNA

<213> Artificial

<220>

<223> Mouse-human chimeric DNA

<400> 59

cagggtgcagc	tcggggagtc	tgaggaggc	ttggtcaagc	ctggcggtc	cctgcggc	tc	50
tccgtgtcag	cctctggatt	cacatccagc	tattcttggc	tgaactgggt	ggggcaggt	tt	120
ccctggaaagg	gcctcgagtg	ggtggacgg	atcttcccg	gcgtatggga	taatgactac	tt	180
aatggaaat	tcaagggcag	agtcacaatt	accgcggaca	aatccactag	cacagcctat	tt	240
atggagctga	gcagcccgag	atctgaggac	acggccgtgt	attactgtgc	aagaaatgtc	tt	300
tttgcgtt	actgggttgt	ttactggggc	cagggaaacc	tggtcaccgt	ctccctca	tt	357

<210> 60

<211> 119

<212> PRT

<213> Artificial

<220>

<223> Mouse-human chimeric polypeptide

<400> 60

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20															30

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

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

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

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

Ala	Arg	Asn	Val	Phe	Asp	Gly	Tyr	Trp	Leu	Val	Tyr	Trp	Gly	Gln	Gly
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ggatgaactg ggtggggcag gctccctggaa aggccatcga gtgggtggaa cggatcttc	240
cggcgatgg ggataactgac tacaatggga aatcaaggg cagagtcaca attaccggcg	300
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<210> 62

<211> 119

<212> PRT

<213> Artificial

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<400> 62

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20	25	30

Trp Met Asn Trp Val Arg Glu 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			
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Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys		
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Thr Leu Val Thr Val Ser Ser	
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<210> 63

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Trp	Met	Asn	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Met
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Gly	Arg	Ile	Phe	Pro	Gly	Asp	Gly	Asp	Thr	Asp	Tyr	Asn	Gly	Lys	Phe
50															60

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

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

Ala	Arg	Asn	Val	Phe	Asp	Gly	Tyr	Trp	Leu	Val	Tyr	Trp	Gly	Gln	Gly
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Thr	Leu	Val	Thr	Val	Ser	Ser									
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<210> 65

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<211> 119

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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
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<210> 67

<211> 456

<212> DNA

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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 ⁷⁰Leu Thr Ala Asp Lys ⁷⁵Ser 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
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115

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acaaatccac tagcacagcc tataatggggc tgaggcagcc gagatctgg gacaoggccg	360
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<211> 119

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Irrp 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

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<211> 57

<212> DNA

<213> Homo sapiens

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<211> 19

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Ala His Ser

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tacctgcaaa agccaggggca gtctccacag ctctgtatcc atcaaatgtc caaccttgtc 180
tctggcgtcc ctgaccggtt ctccggatcc gggtcaggca ctgatttcac actgaaaatc 240
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<211> 115

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<213> Artificial

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

Ash 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 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 Thr Lys Val Glu Ile Lys
 100 105 110

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<210> 77

<211> 66

<212> DNA

<213> Homo sapiens

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<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

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Patentkrav**1. Humaniseret Type II anti-CD20-antistof omfattende**

(a) en tung kæde variabel region valgt fra SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70 og SEQ ID NO: 72, og

5 (b) den KV1-let kæde variable region af SEQ ID NO:76.

2. Humaniseret Type II anti-CD20-antistof omfattende en tung kæde variabel region der kodes af en nukleinsyresekvens der er 80% identisk med SEQ ID NO:

10 61, 63, 65, 67, 69 eller 71 og en let kæde variabel region der kodes af en nukleinsyresekvens der er mindst 80% identisk med SEQ ID NO: 75, hvor

antistoffet inducerer højere apoptosis-niveauer når de inkuberes med CD20-positive humane celler i forhold til en kontrol under identiske betingelser under anvendelse af det C2B8-kimære IgG1-antistof med en sekvens identisk med rituximab.

15

3. Ekspressionsvektor omfattende et polynukleotid der koder for en tung kæde variabel region ifølge krav 1 eller krav 2 og et polynukleotid der koder for en let kæde variabel region ifølge krav 1 eller krav 2.

20 **4. Vektoren ifølge krav 3, der er polycistronisk.**

5. Isoleret værtscelle omfattende ekspressionsvektoren ifølge krav 3 eller krav 4

eller et polynukleotid der koder for en tung kæde variabel region ifølge krav 1

eller krav 2 og et polynukleotid der koder for en let kæde variabel region ifølge

25 krav 1 eller krav 2.

6. Værtscellen ifølge krav 5, hvor værtscellen er bearbejdet til at udtrykke mindst én nukleinsyre der koder for et polypeptid med $\beta(1,4)$ -N-acetylglucosylaminyltransferase III-aktivitet.

30

7. Værtscellen ifølge krav 6, hvor polypeptidet der har $\beta(1,4)$ -N-acetylglucosylaminyltransferase III-aktivitet er et fusionspolypeptid yderligere omfattende Golgi-lokalisationsdomænet af et heterologt Golgi resident polypeptid.

5 **8.** Værtscellen ifølge krav 7, hvor Golgi-lokalisationsdomænet er valgt fra lokalisationsdomænet af mannosidase II, lokalisationsdomænet af $\beta(1,2)$ -N-acetylglucosaminyltransferase I, $\beta(1,2)$ -N-acetylglucosaminyltransferase II, mannosidase I eller $\alpha 1$ -6 kerne-fucosyltransferase.

10 **9.** Fremgangsmåde til fremstillingen af et humaniseret Type-II anti-CD20-antistof ifølge krav 1 eller krav 2 omfattende dyrkning af værtscellen ifølge et hvilket som helst af kravene 5 til 8 under betingelser der tillader fremstilling af nævnte antistof og genvinding af antistoffet fra dyrkningen/kulturen.

15 **10.** Humaniseret Type II anti-CD20-antistof, hvor antistoffet er opnåeligt fra værtscellen ifølge et hvilket som helst af kravene 5 til 8.

20 **11.** Antistoffet ifølge et hvilket som helst af kravene 1, 2 eller 10, eller opnåeligt ved fremgangsmåden ifølge krav 9, hvor antistoffet omfatter en glycobearbejdet Fc-region.

25 **12.** Antistoffet ifølge krav 11, hvor antistoffet har en stigning i fraktionen af ikke-fucosylerede oligosaccharider bundet til den glycobearbejdede Fc-region.

30 **13.** Antistoffet ifølge krav 11, hvor antistoffet har en stigning i fraktionen af bisected, ikke-fucosylerede oligosaccharider bundet til den glycobearbejdede Fc-region.

14. Antistoffet ifølge krav 11, hvor antistoffet har signifikant højere niveauer af binding til humane FcgammaRIII-receptorer i forhold til det ikke-glycobearbejdede antistof.

15. Antistoffet ifølge krav 11, hvor antistoffet har signifikant højere niveauer af ADCC-aktivitet i forhold til det ikke-glycobearbejdede antistof.

16. Farmaceutisk sammensætning omfattende antistoffet ifølge et hvilket som helst af kravene 1, 2, eller 10 til 15, eller opnåelig ved fremgangsmåden ifølge krav 9, og en farmaceutisk acceptabel bærer.

5 **17.** Antistof ifølge et hvilket som helst af kravene 1, 2, eller 10 til 15, eller opnåelig ved fremgangsmåden ifølge krav 9, til anvendelse som et medikament til behandling af en B-celle-sygdom.

18. Antistoffet ifølge anvendelsen ifølge krav 17, hvor B-celle-sygdommen er et
10 B-celle-lymfom.

19. Anvendelse af antistoffet ifølge et hvilket som helst af kravene 1, 2, eller 10 til 15, eller opnåelig ved fremgangsmåden ifølge krav 9, til fremstillingen af et medikament til behandling af en sygdom der kan behandles med B-celle-
15 depletering.

20. Antistof ifølge et hvilket som helst af kravene 1, 2, eller 10 til 15, eller opnåelig ved fremgangsmåden ifølge krav 9, til anvendelse som et medikament til behandling af en sygdom der kan behandles med B-celle-depletering.

20

21. Anvendelsen ifølge krav 19 eller antistoffet ifølge anvendelse ifølge krav 20, hvor sygdommen er en hæmatologisk malignitet eller en autoimmun sygdom.

22. Antistoffet eller anvendelsen ifølge krav 21, hvor den hæmatologiske
25 malignitet er B-celle-lymfom, non-Hodgkin's lymfom, eller B-celle kronisk lymfocytisk leukæmi.

30

DRAWINGS

Linker Start of SEQ ID NO:1 (amino acid) and SEQ ID NO:2 (nucleotide)

1 GAGGTGAGC TGGCGAGTC T GCGCTGAA CTGGTGAGC CTTGGGCTTC AGTGGAGATTC TCCCTGAAAG CTTCCTGCTA CGGATTCGCTA CGGATTCGCTA ATGGAGACCT
 CTCCTGCTTC ACCTGGCTTC GCGGACTTC GACCCCTGGAG TCACCTCTAA AGGAGCTTC GAAAGCTTC ATGGAGACCT
 S V K R T Q Q S C P M I V K P G A S V E I S C K A S G V A T S Y S W
 >>.....B-Lys1 vh.....>

101 TGTACTGCTT GAAACGAGG CCTTGACAGG GTCTTGAGTC GATTTCTTG GAGATGGGGA TACTTGACAC AATGGGAAAT TCAAGGGCAA
 ATTTGACCA CTTTGACTTC GGTGCTTC CAGAATTCAC CTAACCCGG TAAAGGAC CTCACCCCT ATGAGGAGG TTACCCCTTA AGTTCGGTT
 M N W V K L R P S O G L E W T G R I F P B G D G D T D Y N G K F K Q
 >.....3-Lys1 vh.....>

201 GAGCACACTG ACTCTGACA ARTCTCTCAA CACAGCTTAC ATGCACTCA CGAGCTGAC CTCCTGAC CTCCTGAC TCTGCTGAC TCTGCTGAC ATTTGGTC AGAAAGTC
 CGCGCTGAC TGTGACTT TTGAGGTT GTGGGAGG TACCTGGAGC GAGACCTG AGACCTG AGACCTG TGTATACAGG TGTCTTACAG
 R A T L T A D R S S N T A V M Q L T S L T S V D S A V V N C A R N V
 >.....B-Lys1 vh.....>

301 TTTGATGGTT ACGGGTTAGT TGTCTGGGC CAAGGGAGTC TGGTGTAGT CTCTGCA
 AACATACCAA TGTACCAATCA ATGACCAATCA ATGACCCCA GTTCCTGAG ACCAGTGACA GAGCTGCT
 F D G Y W L V Y W G Q G T L V T V S A
 >.....B-Lys1 vh.....>

FIG. 1

Linter	Start of SEQ ID NO:1 (amino acid) and SEQ ID NO:2 (nucleotide)	
MphI		MsII
1.	GACATTCGCTACGAACTACAATTCCA GTCACTCTTGACATCAGCTTCCATATCC TCGAGGCTTA GAAAGAGCT OCTACATAGT AAATGGATCA CTGAACTACG AGTTCAGCTTG ATGT TTAACTT CAGTTGAAAC CTTCTAGTCG AACCTGAGG ACCTCCAGAT CATTCTGAGA GGATGTATCA TAACTGGATGT D T V L P Q T N P V T L G T S A S I S C R S S K S L L H S N G T >>>.....B-LYI v.1.....>	
BstEII		MseI
101	CTTATTTCTA TTGGTATGAC CACAGGCCAG GCGCATGTCCTC TCAGCCCTCTG ATTATTCAGA TGTGCAACCT TCTTCAGGA GTCAGACA GCTTCAGTAG GAATTAACAT AACCTAGACG GCTTCGTCGCT CGCTTCAGAG AATCGAGGAC TAAATAGCTT ACAGGTGGA AGCTAGGCTT CCAAGTCATC T V H V W Y L Q K P G Q S P Q L I V Q M S N L V S G V P D R F S >>>.....B-LYI v.1.....>	
BstS I		AfI YTT
201	CTGGGGCTCA GGAATCTGATT TCAACTCTG AATTCAGCAGA GTCAGAGCTG AGGATGTTGG TGTGTTATTC TGTGCTCAAAT ATCTGAACT TCCGTGACAGC GTCGGCGAT CTCTGACTAA ACTGGTACTC TTAAAGCTCTT CAGCTCCAG TCTTACACCC ACAAAATTAAG ACAGGAGCTT TAGACTTGA AGGCAATTCAC S S Q S Q T D F T L R T S R V E A E D V G V V Y C A Q W L E L P Y T >>>.....B-LYI v.1.....>	
301	TTGGAGGGG GCAACCAAGCT GGAATAAAA CGG AAGCTTCCCG CGCGCTCTCA CCTTATTTT GCG F G G G T K C T L R I K R >>>.....B-LYI v.1.....>	

HIGG

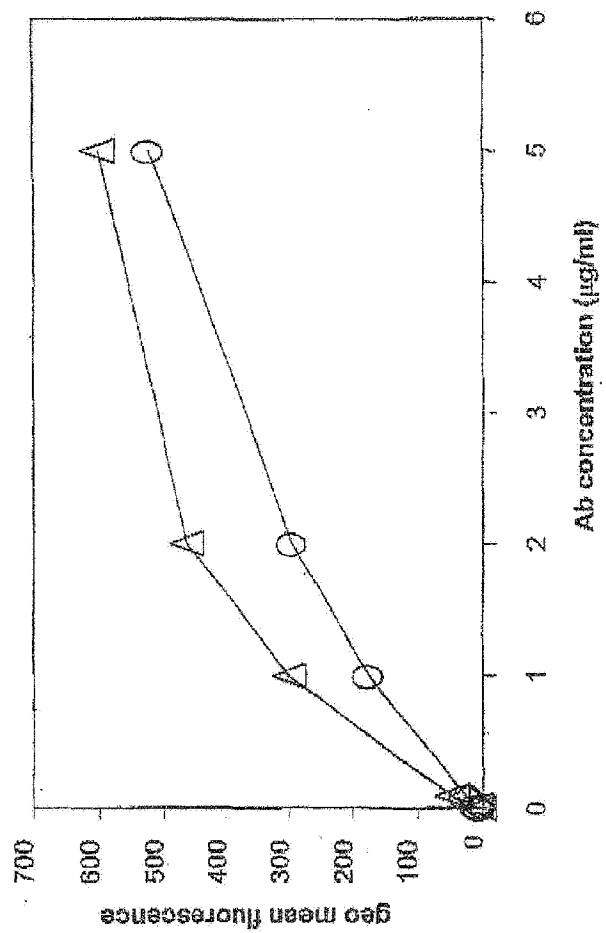


FIG. 3

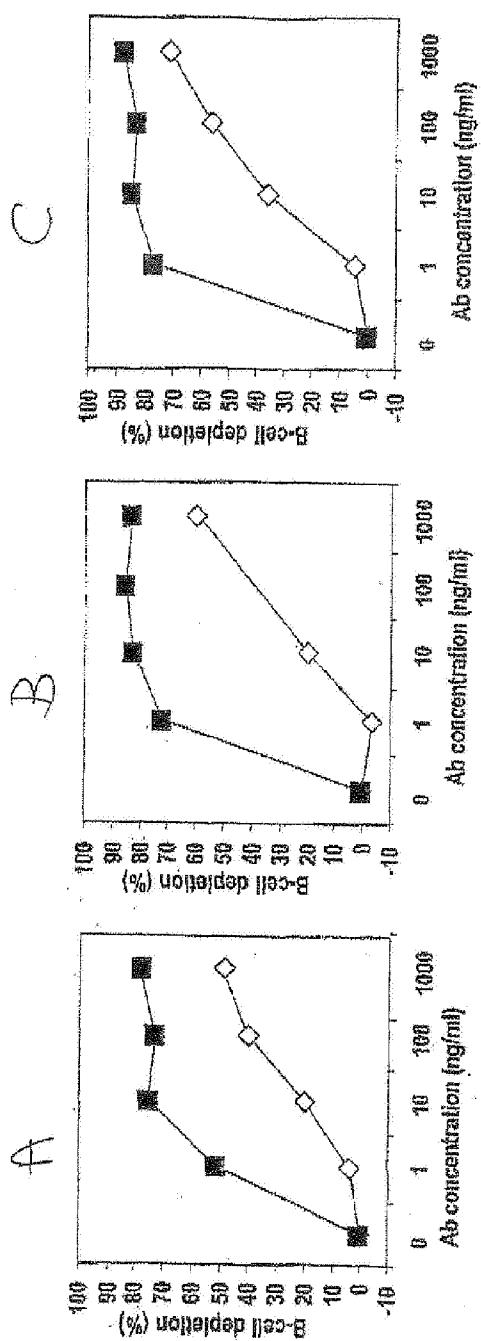


FIG. 4

1 ATGGTGTGAA GCCTCACCTT GTCCTGTTTA CTACCGGTGT CCTGTCAGAG AGCGCTCTGG ACCTGAGCTGG GTGGAGCTGG
TACCGGTGT CCTGTCAGAG AGCGCTCTGG ACCTGAGCTGG GTGGAGCTGG AGCGCTCTGG TACCGGTGT CCTGTCAGAG
H D W S E I L B P E V A V A E R V L S B V R L Q D S G P E L V K P
>>> B-Lys h.c.>

501 GGGCTCACT GAGATCTCC TGTGATCTTC TGTGATCTTC ATTCAGTATCA TGTGATCTTC ATTCAGTATCA
TCCTGAGTCA CCTTAAAGG ATTCAGTATCA GGGCTCACT GAGATCTCC TGTGATCTTC ATTCAGTATCA
G A S V R K T S U E R S G Y A F S Y S W M H W V X L R P G Q E L B R
>>> B-Lys h.c.>

1001 TGTGATCTTC TGTGATCTTC ATTCAGTATCA GGGCTCACT GAGATCTCC TGTGATCTTC ATTCAGTATCA
TCCTGAGTCA CCTTAAAGG ATTCAGTATCA GGGCTCACT GAGATCTCC TGTGATCTTC ATTCAGTATCA
I G R T F P G D G D T D V N G E P K G K A T L T A D K S S W P A V M
>>> B-Lys h.c.>

1501 CAACCTGAGA GGTGACCT TGTGAGCTT GGGCTCTT TGTGAGCTT GGTGAGCTT GGTGAGCTT GGTGAGCTT
GTTGAGCTT GGTGAGCTT GGTGAGCTT GGTGAGCTT GGTGAGCTT GGTGAGCTT GGTGAGCTT GGTGAGCTT
Q L T S E L T S V D S A V Y L C A R N V F D E Y W L V Y W Q Q G T R
>>> B-Lys h.c.>

2001 CAACCTGAGA GGTGACCT TGTGAGCTT GGGCTCTT TGTGAGCTT GGTGAGCTT GGTGAGCTT GGTGAGCTT
GTTGAGCTT GGTGAGCTT GGTGAGCTT GGTGAGCTT GGTGAGCTT GGTGAGCTT GGTGAGCTT GGTGAGCTT
I G R T F P G D G D T D V N G E P K G K A T L T A D K S S W P A V M
>>> B-Lys h.c.>

2501 CAACCTGAGA GGTGACCT TGTGAGCTT GGGCTCTT TGTGAGCTT GGTGAGCTT GGTGAGCTT GGTGAGCTT
GTTGAGCTT GGTGAGCTT GGTGAGCTT GGTGAGCTT GGTGAGCTT GGTGAGCTT GGTGAGCTT GGTGAGCTT
I G R T F P G D G D T D V N G E P K G K A T L T A D K S S W P A V M
>>> B-Lys h.c.>

3001 CAACCTGAGA GGTGACCT TGTGAGCTT GGGCTCTT TGTGAGCTT GGTGAGCTT GGTGAGCTT GGTGAGCTT
GTTGAGCTT GGTGAGCTT GGTGAGCTT GGTGAGCTT GGTGAGCTT GGTGAGCTT GGTGAGCTT GGTGAGCTT
I G R T F P G D G D T D V N G E P K G K A T L T A D K S S W P A V M
>>> B-Lys h.c.>

3501 CAACCTGAGA GGTGACCT TGTGAGCTT GGGCTCTT TGTGAGCTT GGTGAGCTT GGTGAGCTT GGTGAGCTT
GTTGAGCTT GGTGAGCTT GGTGAGCTT GGTGAGCTT GGTGAGCTT GGTGAGCTT GGTGAGCTT GGTGAGCTT
I G R T F P G D G D T D V N G E P K G K A T L T A D K S S W P A V M
>>> B-Lys h.c.>

4001 TGTGATCTTC TGTGATCTTC ATTCAGTATCA GGGCTCACT GAGATCTCC TGTGATCTTC ATTCAGTATCA
TCCTGAGTCA CCTTAAAGG ATTCAGTATCA GGGCTCACT GAGATCTCC TGTGATCTTC ATTCAGTATCA
V T V E A A S E T K G P S V F P E A P S S R S T S G V A A L G C A
>>> B-Lys h.c.>

FIG. 5A

FIG. 5C

1 ATGAGATTC AGCTTCCGAT TATGCCATCA GTCCTCACTA GTCCTCACTA AGAGGAGA CAATGCTACG CCTAACTACA AAATGCTCA
 > M D F Q V Q T I S P L L T S R N S V T M S R Q D E V L T Q T T N P V
 > B-Lys1 1.c.>

101 CTCCTGGAACT ATCTCTCCG ATCTCTGGAA GTCCTGAGA GTCCTGAGA GTCCTGAGA GTCCTGAGA GTCCTGAGA GTCCTGAGA
 GAGAACCTCG TATCTCTGGAA TATCTCTGGAA CTCTGAGCTC GTCATGATCG CTCATGATCG CTCATGATCG CTCATGATCG
 > T M G T S A S T S C R S S K R S V L H W G N G I T Y L Y W Q L O R P G
 > B-Lys1 1.c.>

201 GTCCTGGAG ATCTCTGGAT ATCTCTGGAT ATCTCTGGAT ATCTCTGGAT ATCTCTGGAT ATCTCTGGAT ATCTCTGGAT ATCTCTGGAT
 CAGAGGAGTC GTCATGATCG CTCATGATCG TATCTCTGGAA GTCCTGAGA GTCCTGAGA GTCCTGAGA GTCCTGAGA GTCCTGAGA
 > Q B P Q T L I T Y O M S N L V S G V P D R F S B Q S G T D F T K R I
 > B-Lys1 1.c.>

PstI

301 AGCTTCTGGAG AGCTTCTGGAG TATCTCTGGAT TATCTCTGGAT CTGAACTTC AGCTTCTGGAT TATCTCTGGAT TATCTCTGGAT
 TCTCTCTGAC TCTCTCTGAC AGCTTCTGGAT AGCTTCTGGAT AGCTTCTGGAT AGCTTCTGGAT AGCTTCTGGAT AGCTTCTGGAT
 > S R W H A E B V G V C A Q M P E L P V T P S E G V R K L E I K R
 > B-Lys1 1.c.>

XbaI

401 GCGCTCCGTC ACCCTCTTC TCTCTCTGAG GCGCTCCGTC TCTCTCTGAG TCTCTCTGAG TCTCTCTGAG TCTCTCTGAG
 GCCACCCAGC TCTCTCTGAG TCTCTCTGAG GCGCTCCGTC TCTCTCTGAG TCTCTCTGAG TCTCTCTGAG TCTCTCTGAG
 > T V A A P S V E I F P P S D R Q L K S G T A E V V C L L N N F Y P
 > B-Lys1 1.c.>

501 AGAGGCTCAA GTCAGTGGAA AGCTTCTGGAT GTCCTGAGA TCTCTCTGAG TCTCTCTGAG TCTCTCTGAG TCTCTCTGAG
 TCTCTCTGAG TCTCTCTGAG TCTCTCTGAG TCTCTCTGAG TCTCTCTGAG TCTCTCTGAG TCTCTCTGAG TCTCTCTGAG
 > R R A K V Q W K V D N A B Q S G K S Q E S V T E Q D S K D S T Y S L
 > B-Lys1 1.c.>

HsIVI

XbaI

XbaI

XbaI

FIG. 6A

601	ACUAGACCC' TCAACGCGAG CTAACCCGAC TACCGAACG ACTGAGCTA CGCGCTGAA GTCACCCGAC AGGCGCTTG CTCGCGCTTC AGAA(GAGC') TGTGCTGGG ACTGAGCTC GTTTCGCTG ATTCCTTG TTTCATAT GCGACGCTT GTCGGGTT GTCGGGACTG TTGTTCTCA S S T L T N S K A D Y E R H V V Y A C E V T H Q G R S S P V W K S >.....B-Lys 1.c.....>	SEQ ID NO:12 (nucleic acid)
701	TCAACGGG AGAATGTTAG AGTTGGCCG TCTCAGATA E N R G E CB-Lys 1.c.....>	SEQ ID NO:14 (amino acid)

FIG 6B

A

CDR1(Kabat):
TACTCTGGATGAAC
TyrSerTrpMetAsn

SEQ ID NO: 5
SEQ ID NO: 15

CDR1(Chothia):
GGCTACGCATTCACTTAC
GlyTyrAlaPheSerTyr

SEQ ID NO: 6
SEQ ID NO: 16

CDR1(AbM):
GGCTACGCATTCACTTAC
GlyTyrAlaPheSerTyrSerTrpMetAsn

SEQ ID NO: 7
SEQ ID NO: 17

CDR2(Kabat):
CGGATTTTCCCTGGAGATGGGGATACTGACTACAATGGGAAATTCAAGGGC
ArgIlePheProGlyAspGlyAspThrAspTyrAsnGlyLysPheLysGly

SEQ ID NO: 21
SEQ ID NO: 25

CDR2(Chothia):
TTTCCTGGAGATGGGGATACTGAC
PheProGlyAspGlyAspThrAsp

SEQ ID NO: 22
SEQ ID NO: 26

CDR2(AbM):
CGGATTTTCCCTGGAGATGGGGATACTGAC
ArgIlePheProGlyAspGlyAspThrAsp

SEQ ID NO: 23
SEQ ID NO: 27

CDR3(Kabat, Chothia, AbM):
AATGTCTTTGATGGTTACTGGTTAGTTTAC
AsnValPheAspGlyTyrTrpLeuValTyr

SEQ ID NO: 24
SEQ ID NO: 28

B

CDR1(Kabat):
AGGTCTAGTAAGAGTCTCCTACATAGTAATGGCATCACTTATTGTAT
ArgSerSerLysSerLeuLeuHisSerAsnGlyIleThrTyrLeuTyr

SEQ ID NO: 8
SEQ ID NO: 18

CDR2(Kabat):
CAGATGTCCAACCTTGTCTCA
GlnMetSerAsnLeuValSer

SEQ ID NO: 9
SEQ ID NO: 19

CDR3(Kabat):
GCTAAAATCTAGAACTTCCGTACACG
AlaGlnAsnLeuGluLeuProTyrThr

SEQ ID NO: 10
SEQ ID NO: 20

FIG. 7

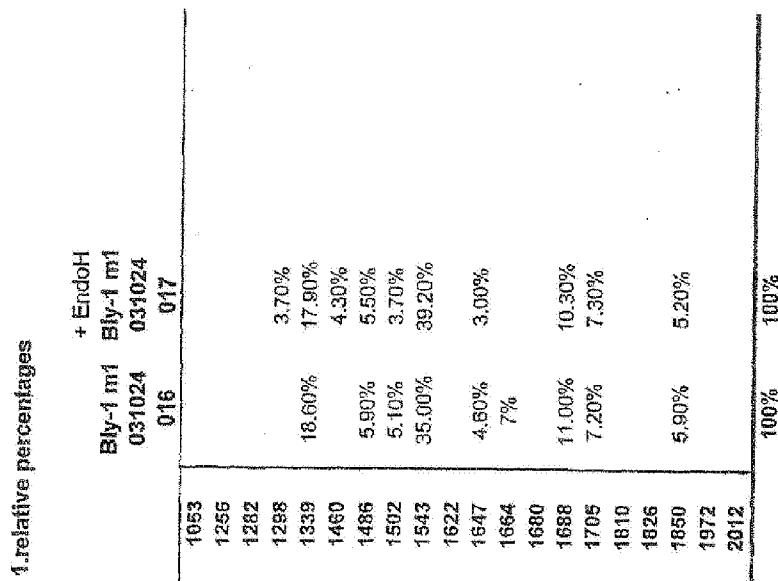
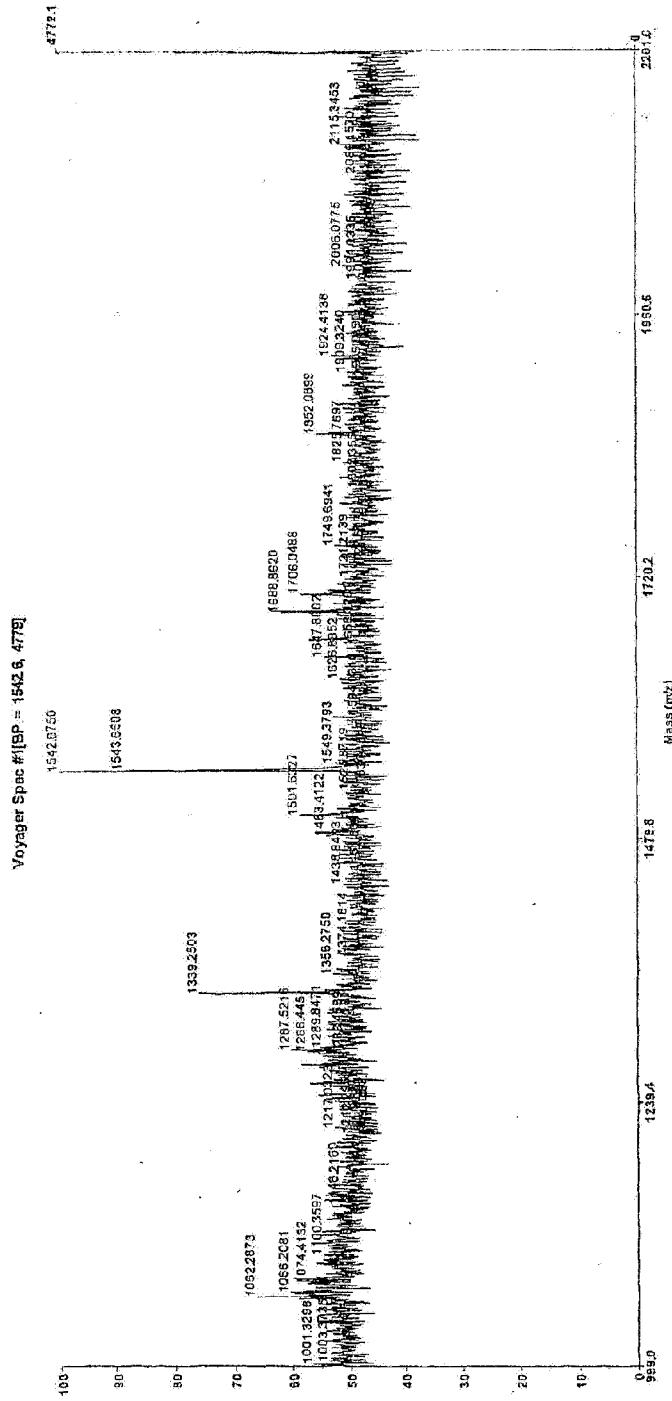
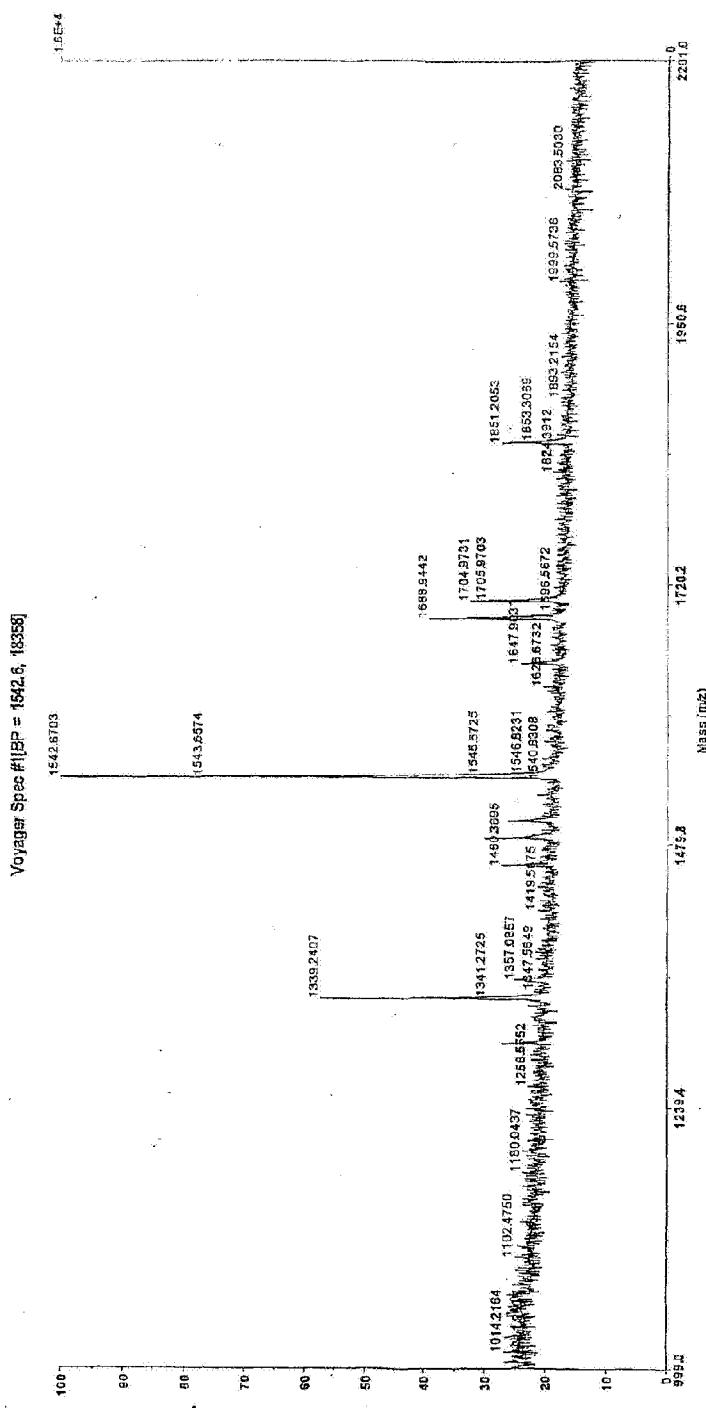


FIG. 8A



Newly filed

FIG. 8B



Newly filed

FIG. 8C

Figure 9

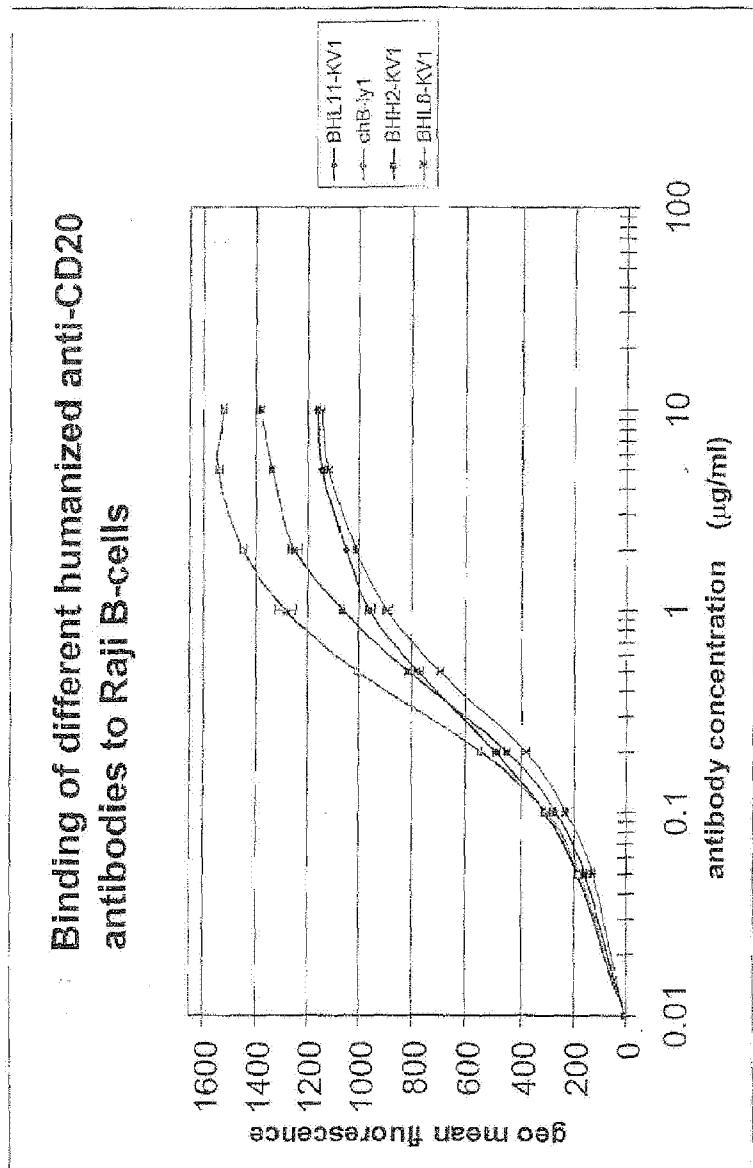


Figure 10

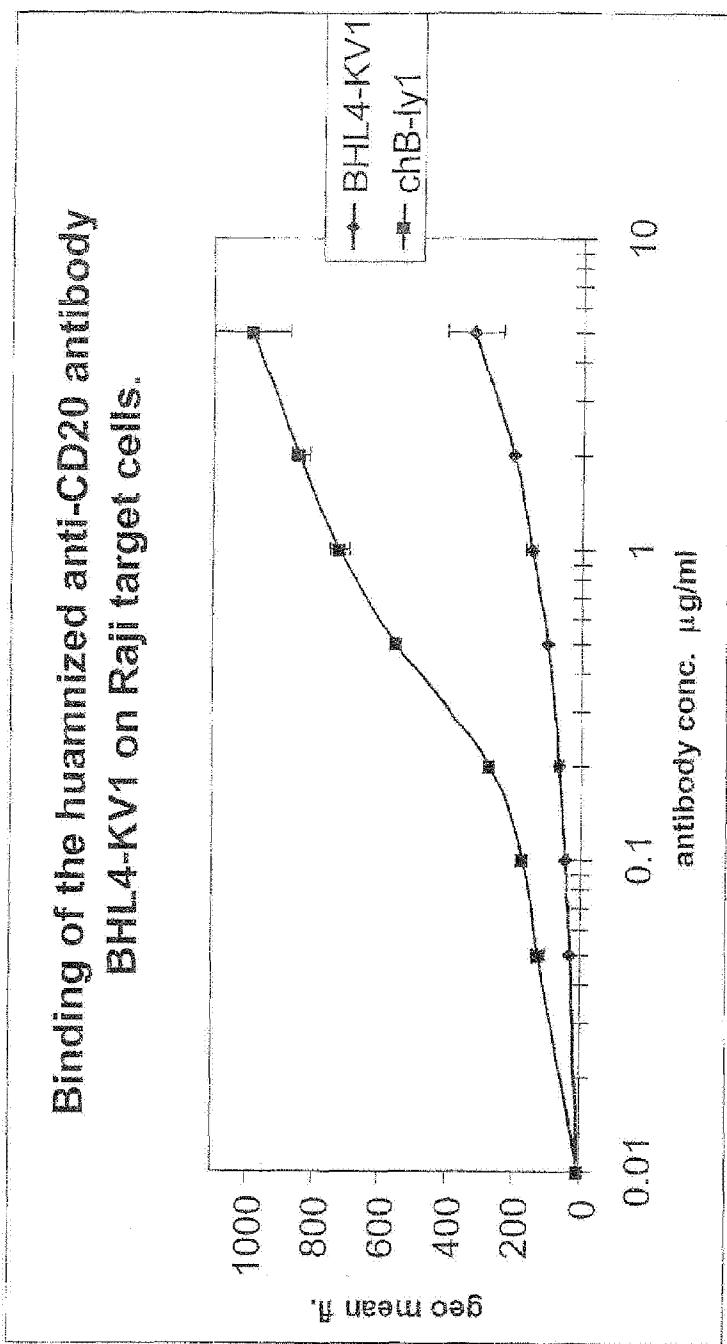


Figure 14

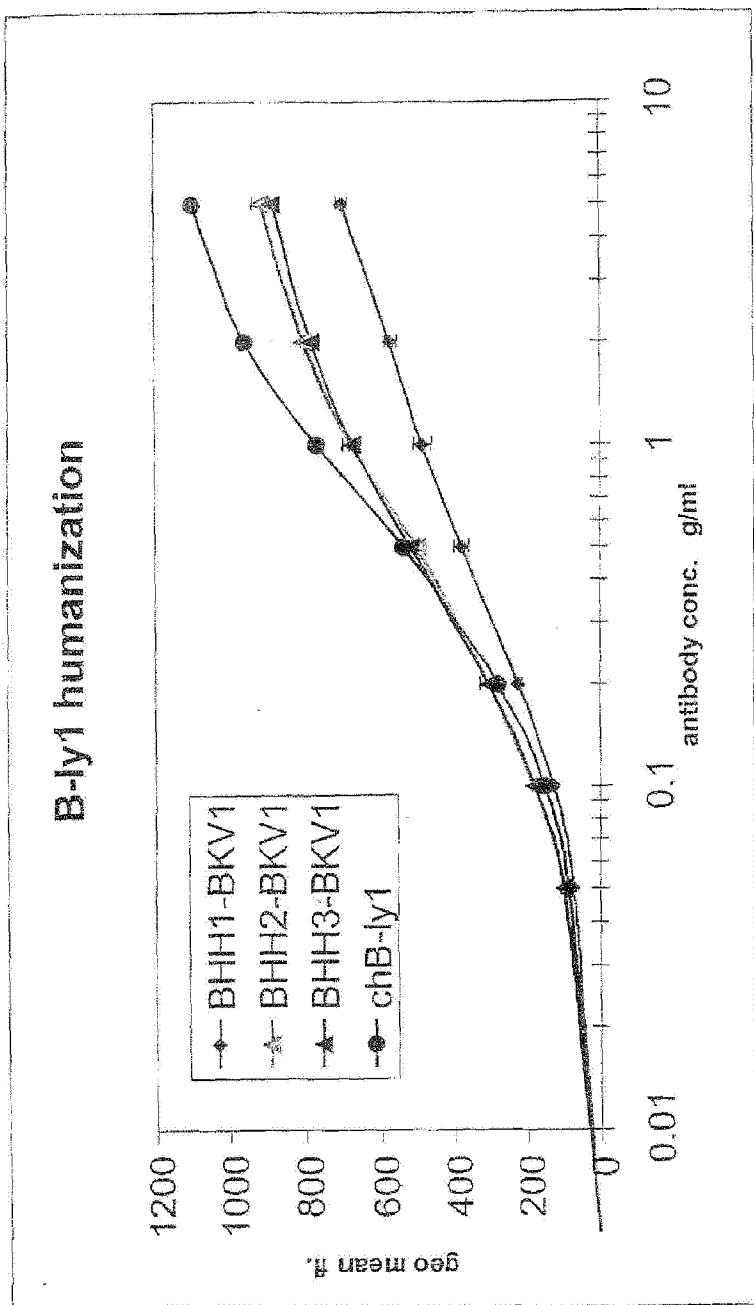


Figure 12

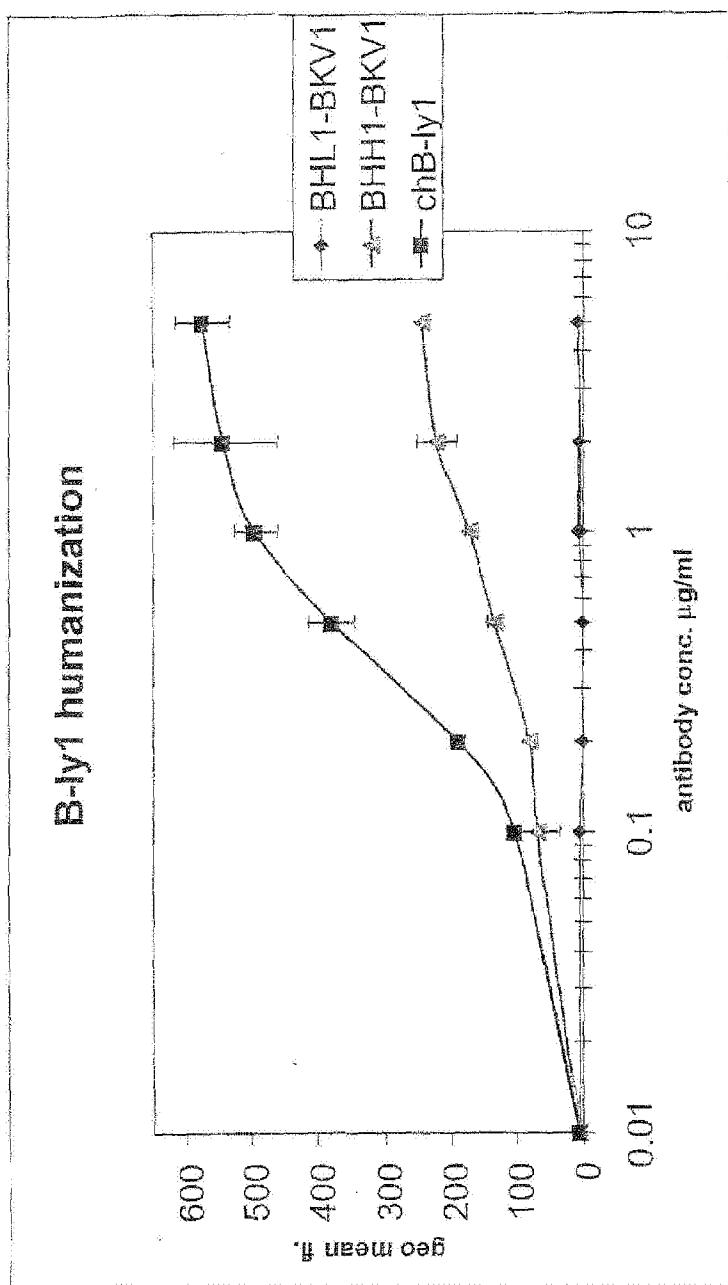


Figure 13

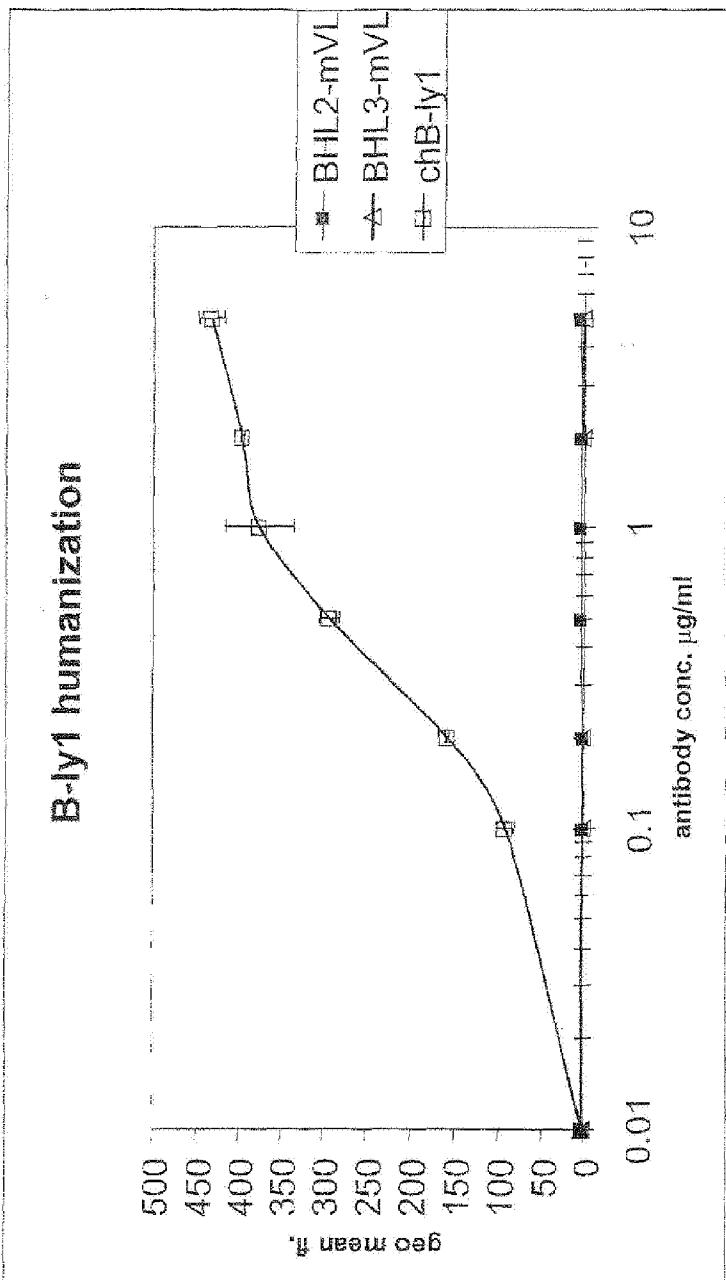


Figure 14

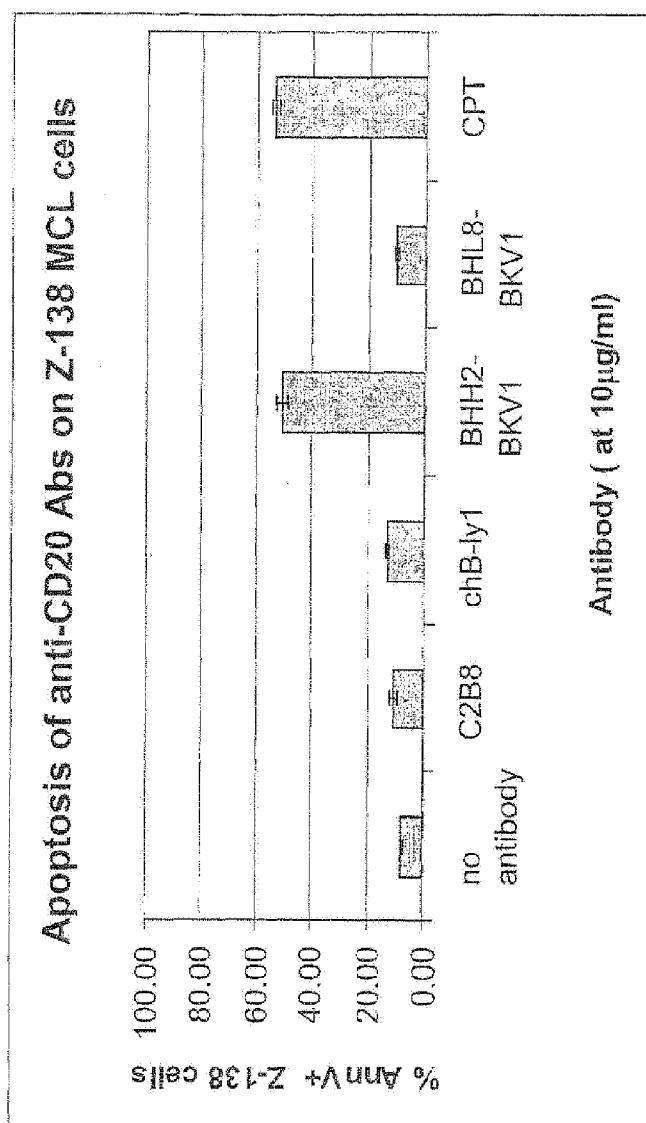
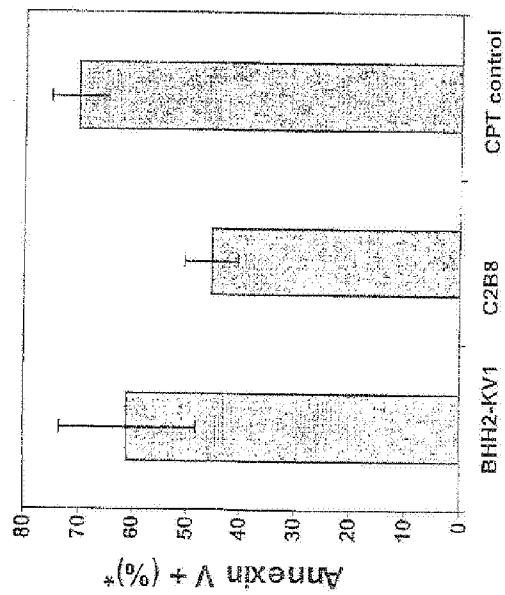


Figure 15

PR-1 (DLBCL cell line)



Z-138 (MCL cell line)

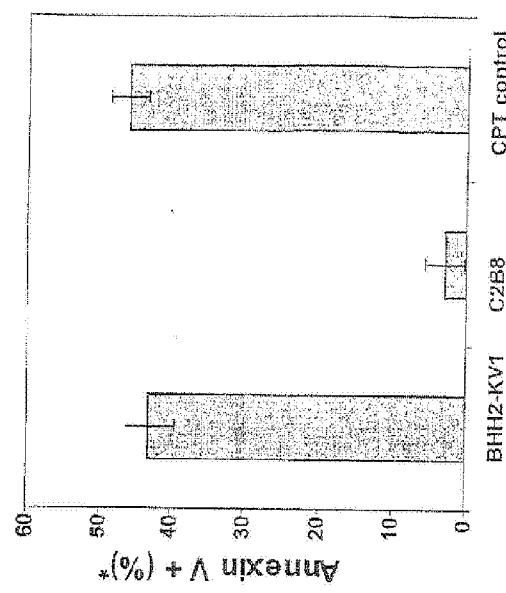


Figure 16

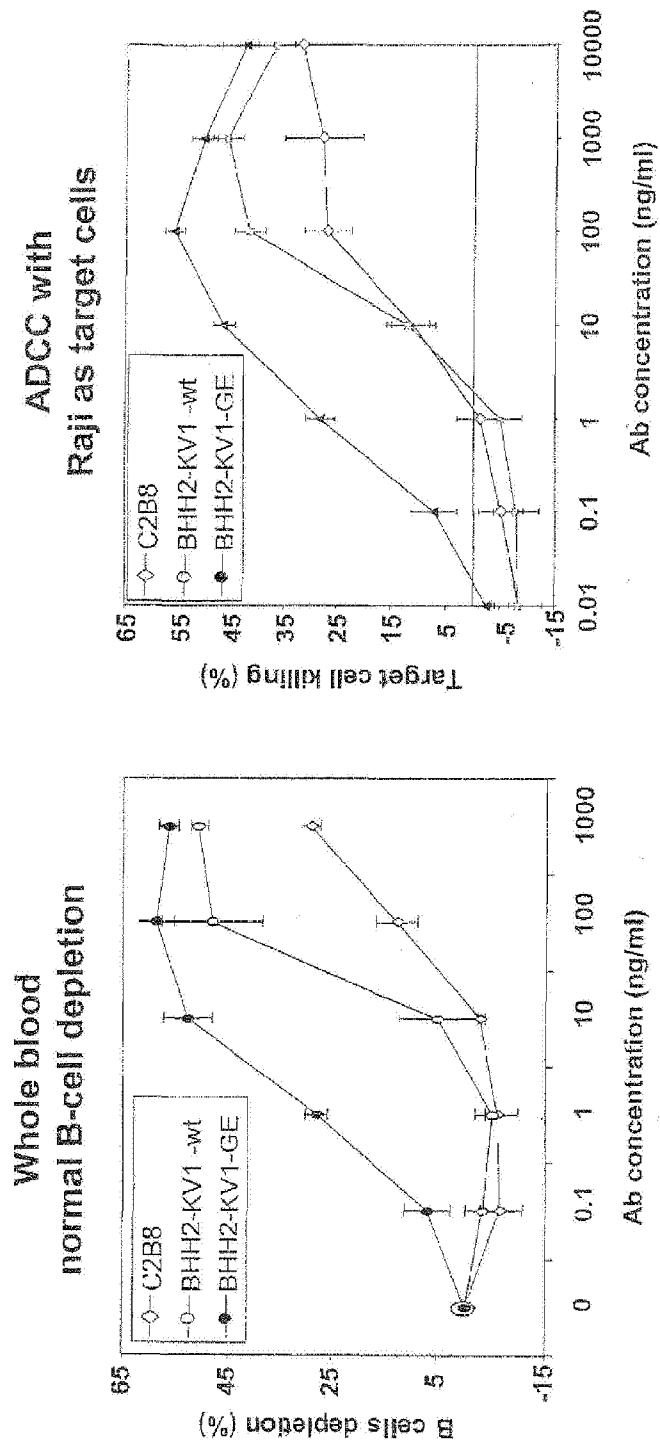
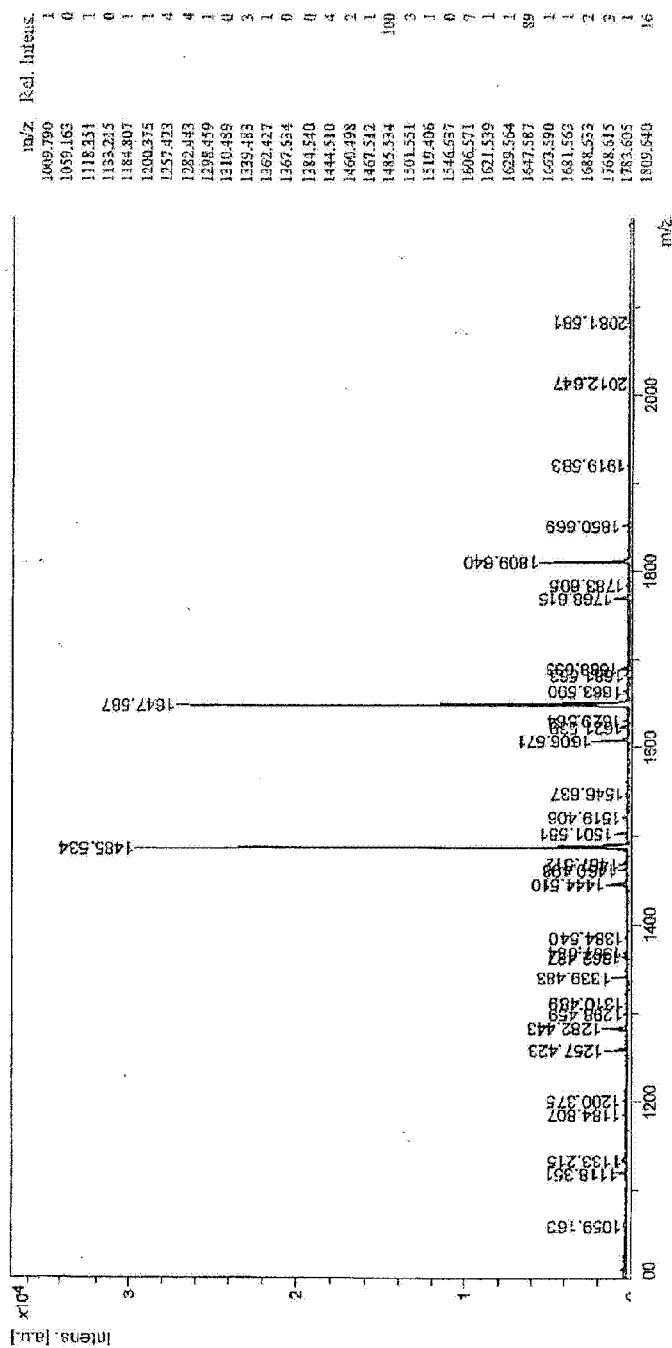
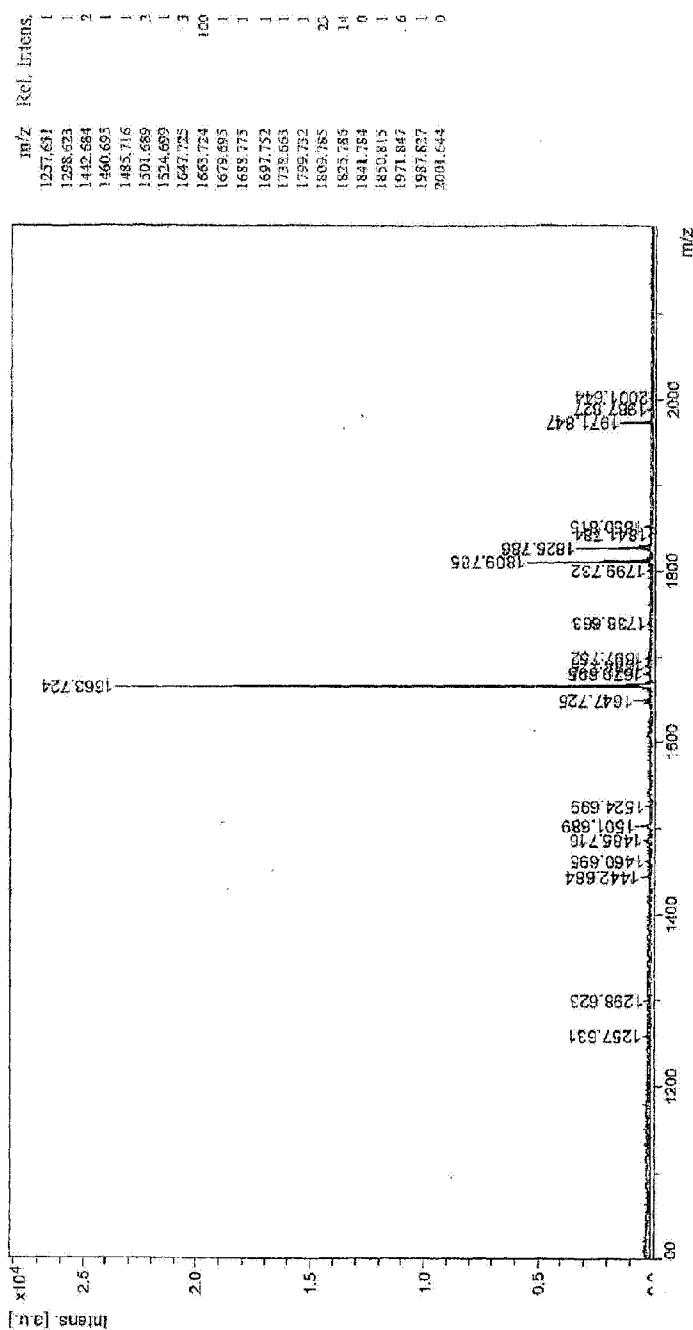


Figure 17



Newly filed

Figure 18



Newly filed

Figure 19

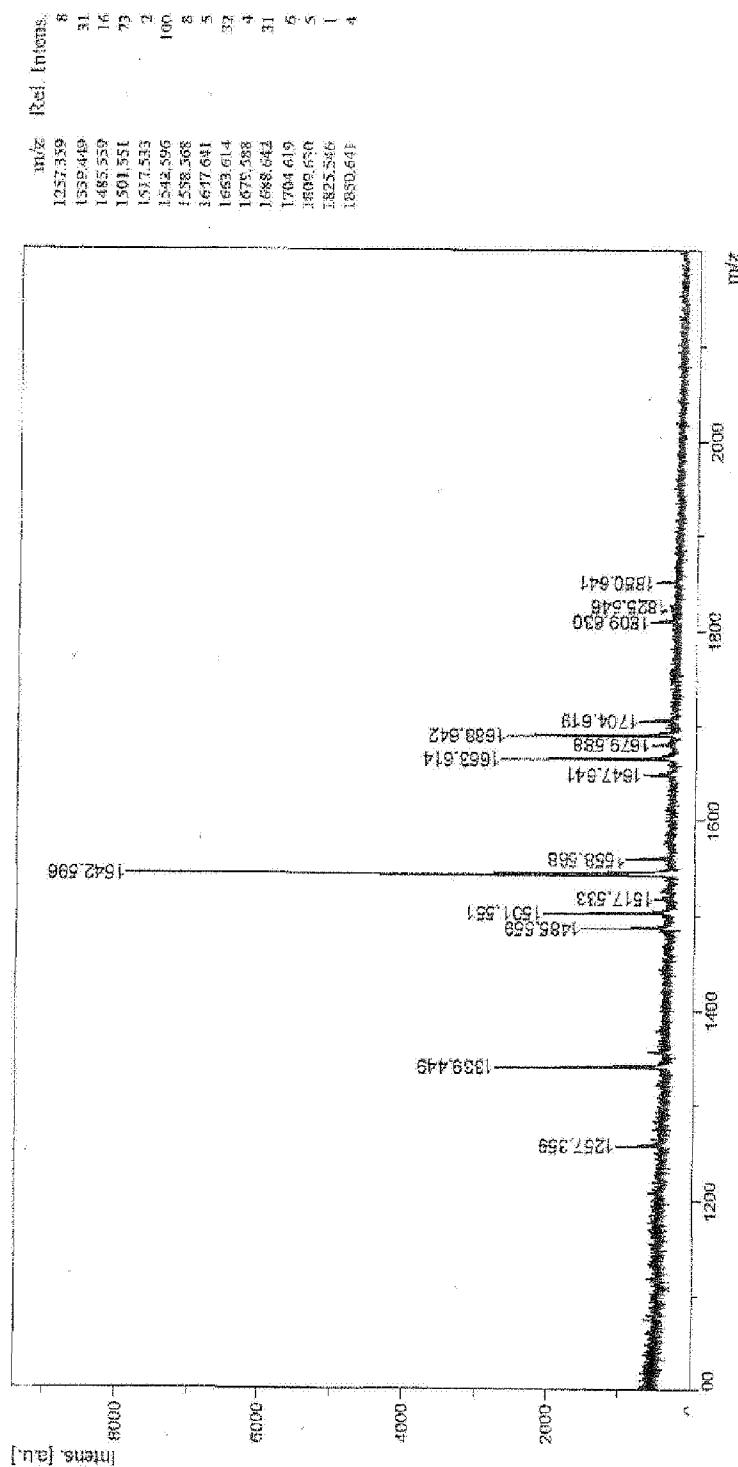


Figure 20

Ab binding to Fc gamma RIII on the surface of CHO-CD16 cells

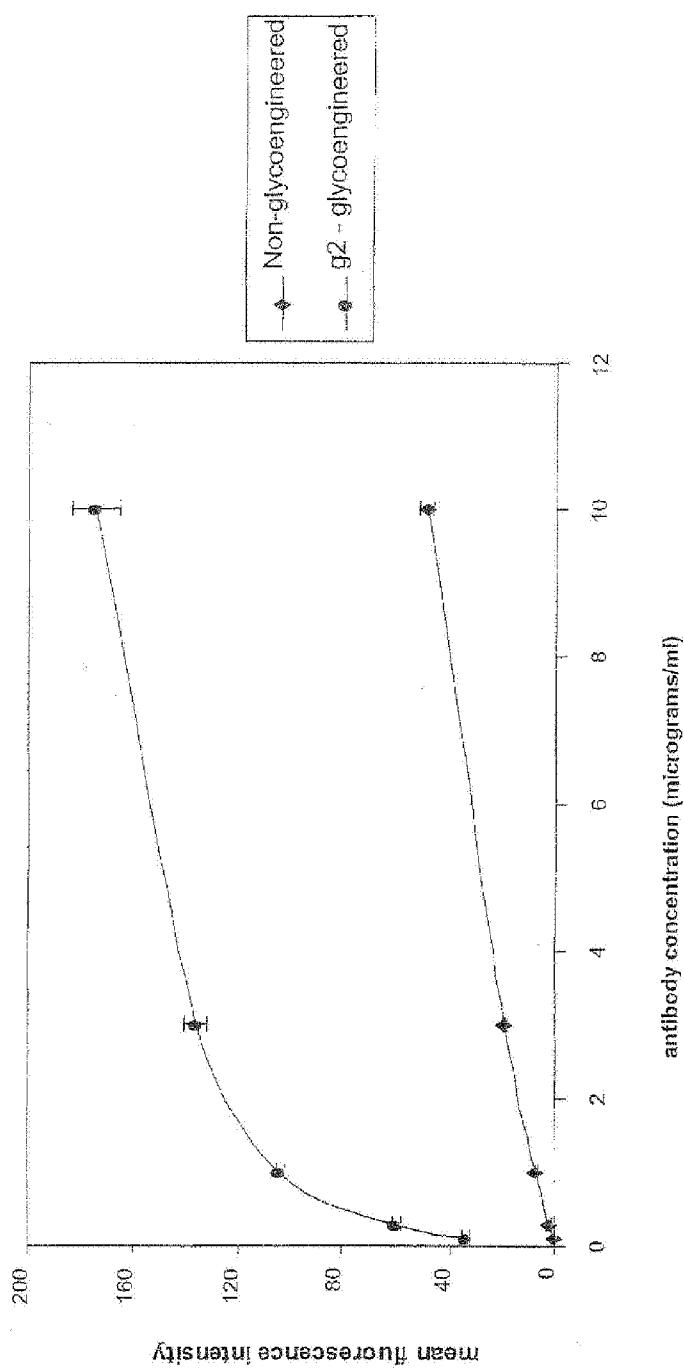


Figure 21

Apoptosis by anti-CD20 Abs

Z-138 mantle cell lymphoma cell line

