BRIEF SUMMARY

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

[0019a] Accordingly, in one aspect, the disclosure is directed to a humanized Type II anti-CD20 antibody comprising (a) a heavy chain variable region selected from the group consisting of SEQ ID NO:32 and SEQ ID NO:40, and (b) the KV1 light chain variable region of SEQ ID NO:76.

[0019b] In another aspect, the disclosure is directed to a humanized Type-II anti-CD20 antibody comprising a heavy chain variable region having the specificity determining residues of the heavy chain variable region of the antibody of the disclosure, 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.

[0019c] In another aspect, the disclosure is directed to a humanized Type-II anti-CD20 antibody comprising a heavy chain variable region that is encoded by a nucleic acid sequence which is at least 80% identical to SEQ ID NO: 31 or SEQ ID NO: 39 and 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.

[0019d] In another aspect, the disclosure is directed to an expression vector comprising a polynucleotide encoding a heavy chain variable region of the disclosure and a polynucleotide encoding a light chain variable region of the disclosure.

[0019e] In another aspect, the disclosure is directed to an isolated host cell comprising the expression vector of the disclosure or a polynucleotide encoding a heavy chain variable region of the disclosure and a polynucleotide encoding a light chain variable region of the disclosure.

[0019f] In another aspect, the disclosure is directed to a method for the production of a humanized Type-II anti-CD20 antibody of the disclosure comprising culturing the host cell of the disclosure under conditions that allow for the production of said antibody and recovering said antibody from said culture.

[0019g] In another aspect, the disclosure is directed to a humanized Type II anti-CD20 antibody, wherein the antibody is produced by the host cell of the disclosure.

[0019h] In another aspect, the disclosure is directed to a pharmaceutical composition comprising the antibody of the disclosure, or produced by the method of the disclosure, and a pharmaceutically acceptable carrier.

[0019i] In another aspect, the disclosure is directed to an antibody of the disclosure, or produced by the method of the disclosure, for use in therapy.

[0019j] In another aspect, the disclosure is directed to a use of antibody of the disclosure, or produced by the method of the disclosure, in the manufacture of a medicament for treating a B-cell disorder.

[0019k] In another aspect, the disclosure is directed to a use of the antibody of the disclosure, or produced by the method of the disclosure, in the manufacture of a medicament to treat a disorder treatable by B-cell depletion.

[0019l] In another aspect, the disclosure is directed to an of the disclosure, or produced by the method of the disclosure, for use in therapy.

[0020] Accordingly, in one example, 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 example, 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 example, any of these polynucleotides encodes a fusion polypeptide.

[0021] In a further example, the disclosure is directed to an isolated polynucleotide comprising SEQ ID No.:3 In another example, the disclosure is directed to an isolated polynucleotide comprising SEQ ID No.:4. In a further example, 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 example, the disclosure is directed to an isolated polynucleotide comprising SEQ ID No.:75. In one example, such polynucleotides encode fusion polypeptides.

[0022] The disclosure is further directed to an isolated polynucleotide comprising a sequence having at least 80% identity to SEQ ID NO:3, wherein said isolated polynucleotide encodes a fusion polypeptide. In an additional example, 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 example, the disclosure is directed to an isolated polynucleotide comprising a sequence having at least 80% identity to SEQ ID NO:75, wherein said isolated polynucleotide encodes a fusion polypeptide.

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

[0024] The disclosure is also directed to an isolated polynucleotide encoding a chimeric polypeptide having the sequence of SEQ ID No.:1. In one example, 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 example, the polynucleotide comprises a sequence encoding a polypeptide having a sequence selected from the group consisting of SEQ ID No:30; SEQ ID No:32; SEQ ID No:34; SEQ ID No:36; SEQ ID No:38; SEQ ID No:40; SEQ ID No:42; SEQ ID

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

[0025] In yet another example, the disclosure is directed to an isolated polynucleotide encoding a chimeric polypeptide having the sequence of SEQ ID No.:2. In one example, 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 example, the disclosure is directed to an isolated polynucleotide encoding a chimeric polypeptide having the sequence of SEQ ID No.:76. In one example, the polynucleotide comprises a sequence encoding a polypeptide having the sequence of SEQ ID No.:76; and a sequence encoding a polypeptide having the sequence of an antibody Fc region, or a fragment thereof, from a species other than mouse.

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

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

[0028] In one example, 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 example, the disclosure is directed to an isolated polypeptide comprising SEQ ID NO.:18, SEQ ID NO.: 19 and SEQ ID NO.:20. (CDRs V_L), wherein said polypeptide is a fusion polypeptide.

[0029] The disclosure is also directed to a chimeric polypeptide comprising the sequence of SEQ ID NO.:1 or a variant thereof. The disclosure is further directed to a chimeric polypeptide comprising the sequence of SEQ ID NO.:2 or a variant thereof. In one example, 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 example, any one of these polypeptides further comprises a human Fc region.

[0030] In another example 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 example the antigen-binding molecule is an antibody. In a preferred example, the antibody is chimeric. In another preferred example, the antibody is humanized or primatized.

[0031] In an additional example, the disclosure is directed to an isolated polypeptide comprising SEQ ID NO: 13 or a variant thereof. In another example, the disclosure is directed to an isolated polypeptide comprising SEQ ID NO: 14.

[0032] In another example, 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 example, the ABM is an antibody or a fragment thereof. In a further example, 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 example, 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 example the ABM is a recombinant antibody that is primatized. In yet a further example the ABM is a recombinant antibody that is humanized. In another example, the ABM is a recombinant antibody comprising a human Fc region. In a further example, any of the ABMs discussed above may be conjugated to a moiety such as a toxin or a radiolabel.

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

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

[0035] The disclosure is further related to a method of producing an ABM, which is capable of competing with the murine B-Ly1 antibody for binding to CD20 and wherein said ABM is chimeric; said method comprising: (a) culturing a host cell

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

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

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

[0038] In yet another example, 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-Ly1 antibody for binding to CD20 and wherein the ABM is chimeric. In one example, the polypeptide having GnTIII activity is a fusion polypeptide. In another example, the ABM produced by the host cell is an antibody or an antibody fragment. In a further example, the ABM comprises a region equivalent to the Fc region of a human IgG.

[0039] The disclosure is also directed to an isolated polynucleotide comprising at least one complementarity determining region of the murine B-Ly1 antibody, or a variant or truncated form thereof containing at least the specificity-determining residues for said complementarity determining region, wherein said isolated polynucleotide encodes a fusion polypeptide. Preferably, such isolated polynucleotides encode a fusion polypeptide that is an antigen binding molecule. In one example, the polynucleotide comprises three complementarity determining regions of the murine B-Ly1 antibody, or variants or truncated forms thereof containing at least the specificity-determining residues for each of said three complementarity determining regions. In another example, the

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

[0040] In another example, the disclosure is directed to an antigen combining molecule comprising at least one complementarity determining region of the murine B-Ly1 antibody, or a variant or truncated form thereof containing at lest the specificity-determining residues for said complementarity determining region, and comprising a sequence derived from a heterologous polypeptide. In one example, the antigen binding molecule comprises three complementarity determining regions of the murine B-Ly1 antibody, or variants or truncated forms thereof containing at least the specificity-determining residues for each of said three complementarity determining regions. In another example, the antigen binding molecule comprises the variable region of an antibody light or heavy chain. In one particularly useful example, the antigen binding molecule is a chimeric, e.g., humanized, antibody. The disclosure is also directed to methods of making such antigen binding molecules, and the use of same in the treatment of disease, including B cell lymphomas.

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

[0042] The host cell of the present disclosure 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 PER.C6 cell or a hybridoma cell. In one example, the host cell of the disclosure further comprises a transfected polynucleotide comprising a polynucleotide encoding the V_L region of the murine B-Ly1 antibody or variants thereof and a sequence encoding a region equivalent to the Fc region of a human immunoglobulin. In another example, the host cell of the disclosure further comprises a transfected polynucleotide comprising a polynucleotide encoding the V_H region of the murine B-Ly1 antibody or variants thereof and a sequence encoding a region equivalent to the Fc region of a human immunoglobulin.

[0043] In a further example, 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 example, the increased binding affinity is to an Fc receptor, particularly, the FcγRIIIA receptor. The effector function contemplated herein may be selected from the group that includes, but is not limited to, increased Fc-mediated cellular cytotoxicity; increased binding to NK cells; increased binding to macrophages; increased binding to polymorphonuclear cells; increased binding to monocytes; increased direct signaling inducing apoptosis; increased dendritic cell maturation; and increased T cell priming.

[0044] In a further example, the host cell of the present disclosure comprises at least one nucleic acid encoding a polypeptide having GnTIII activity that is operably linked to a constitutive promoter element.

[0045] In another example, 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-Ly1 antibody for binding to CD20 and wherein said ABM is chimeric. In one example, 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 I ("GnTI"), the localization domain of mannosidase I, the localization domain of β(1,2)-N-acetylglucosaminyltransferase II ("GnTII"), and the localization domain of α1-6 core fucosyltransferase. Preferably, the Golgi localization domain is from mannosidase II or GnTI.

[0046] In a further example, 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 disclosure. In one example, the ABM is an antibody or a fragment thereof; preferably comprising the Fc region of an IgG. Alternatively, the polypeptide is a fusion protein that includes a region equivalent to the Fc region of a human IgG.

[0047] In one example, 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.

[0048] In another example, the present disclosure is directed to a method of modifying the glycosylation of the recombinant antibody or a fragment thereof of the disclosure by using a fusion polypeptide having GnTIII activity and comprising the Golgi localization domain of a heterologous Golgi resident polypeptide. In one example, the fusion polypeptides of the disclosure comprise the catalytic domain of GnTIII. In another example, the Golgi localization domain is selected from the group consisting of: the localization domain of mannosidase II, the localization domain of GnTI, the localization domain of mannosidase I, the localization domain of GnTII and the localization domain of α1-6 core fucosyltransferase. Preferably, the Golgi localization domain is from mannosidase II or GnTI.

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

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

[0050] In a further example, 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 example, the increased binding affinity is to an Fc activating receptor. In a further example, the Fc receptor is Fcγ activating receptor, particularly, the FcγRIIIA receptor. The effector function contemplated herein may be selected from the group that includes, but is not limited to, increased Fc-mediated cellular cytotoxicity; increased binding to NK cells; increased binding to macrophages; increased binding to polymorphonuclear cells; increased binding to monocytes; increased direct signaling inducing apoptosis; increased dendritic cell maturation; and increased T cell priming.

[0051] In another example, the disclosure is directed to a recombinant, chimeric antibody fragment, having the binding specificity of the murine B-Ly1 antibody and containing the Fc region, that is engineered to have increased effector function produced by any of the methods of the present disclosure.

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

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

[0053] In another example, 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 of the present disclosure.

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

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

BRIEF DESCRIPTION OF THE FIGURES

[0056] FIG 1. Nucleotide (SEQ ID NO:3) and amino acid sequence (SEQ ID NO:1) of the V_H region of the murine B-Ly1.

[0057] 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.

[0058] FIG 3. Binding of Rituximab® (O) and ch-B_Ly1 (Δ) to CD20 on Raji B-lymphoma cells.

[0059] 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

WHAT IS CLAIMED IS:

1. A humanized Type II anti-CD20 antibody comprising
(a) a heavy chain variable region selected from the group consisting of SEQ ID NO:32 and SEQ ID NO:40, and
(b) the KV1 light chain variable region of SEQ ID NO:76.

2. The antibody of claim 1, wherein the heavy chain variable region is SEQ ID NO: 40.

3. A humanized Type-II anti-CD20 antibody comprising a heavy chain variable region having the specificity determining residues of the heavy chain variable region of the antibody of claim 1 or claim 2, 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.

4. A humanized Type-II anti-CD20 antibody comprising a heavy chain variable region that is encoded by a nucleic acid sequence which is at least 80% identical to SEQ ID NO: 31 or SEQ ID NO: 39 and 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.

5. An expression vector comprising a polynucleotide encoding a heavy chain variable region of any one of claims 1 to 4 and a polynucleotide encoding a light chain variable region of any one of claims 1 or 4.

6. The vector of claim 5, which is polycistronic.

7. An isolated host cell comprising the expression vector of claim 5 or claim 6 or a polynucleotide encoding a heavy chain variable region of any one of claims 1 to 4 and a polynucleotide encoding a light chain variable region of any one of claims 1 or 4.

8. The host cell of claim 7, wherein said host cell is engineered to express at least one nucleic acid encoding a polypeptide having β(1,4)-N-acetylglucosylaminyltransferase III activity.

9. The host cell of claim 8, wherein said polypeptide having β(1,4)-N-acetylglucosylaminyltransferase III activity is a fusion polypeptide further comprising the Golgi localization domain of a heterologous Golgi resident polypeptide.

10. The host cell of claim 9, wherein said Golgi localization domain is selected from the localization domain of mannosidase II, the localization domain of β(1,2)-N-acetylglucosaminyltransferase I, β(1,2)-N-acetylglucosaminyltransferase II, mannosidase I or α1-6 core fucosyltransferase.

11. A method for the production of a humanized Type-II anti-CD20 antibody of any one of claims 1 to 4 comprising culturing the host cell of any one of claims 7 to 10 under conditions that allow for the production of said antibody and recovering said antibody from said culture.

12. A humanized Type II anti-CD20 antibody, wherein the antibody is produced by the host cell of any one of claims 7 to 10.

13. The antibody of any one of claims 1 to 4 or 12, or produced by the method of claim 11, wherein said antibody comprises a glycoengineered Fc region.

14. The antibody of claim 13, wherein said antibody has an increase in the fraction of non-fucosylated oligosaccharides attached to said glycoengineered Fc region.

15. The antibody of claim 13, wherein said antibody has an increase in the fraction of bisected, non-fucosylated oligosaccharides attached to said glycoengineered Fc region.

16. The antibody of claim 13, wherein said antibody has significantly higher levels of binding to human FcgammaRIII receptors relative to the non-glycoengineered antibody.

17. The antibody of claim 13, wherein said antibody has significantly higher levels of ADCC activity relative to the non-glycoengineered antibody

18. A pharmaceutical composition comprising the antibody of any one of claims 1 to 4 or 12 to 17, or produced by the method of claim 11, and a pharmaceutically acceptable carrier.

19. An antibody of any one of claims 1 to 4 or 12 to 17, or produced by the method of claim 11, for use in therapy.

20. Use of antibody of any one of claims 1 to 4 or 12 to 17, or produced by the method of claim 11, in the manufacture of a medicament for treating a B-cell disorder.

21. The antibody of claim 20, wherein said B-cell disorder is a B-cell lymphoma.

22. Use of the antibody of any one of claims 1 to 4 or 12 to 17, or produced by the method of claim 11, in the manufacture of a medicament to treat a disorder treatable by B-cell depletion.

23. An antibody of any one of claims 1 to 4 or 12 to 17, or produced by the method of claim 11, for use in therapy.

24. The use of claim 22, wherein said disorder is a haematological malignancy or an autoimmune disease.

25. The use of claim 24, wherein said haematological malignancy is B-cell lymphoma, non-Hodgkin's lymphoma, or B-cell chronic lymphocytic leukaemia.

26. The use of claim 24, wherein said autoimmune disease is rheumatoid arthritis or lupus.