~~BRIEF~~ SUMMARY ~~OF THE INVENTION~~

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

[0019a] In one aspect, there is provided a humanized Type II anti-CD20 antigen binding molecule comprising: (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 (b) the KVI light chain variable region of SEQ ID NO:76.

[0019b] In one aspect, there is provided a humanized Type-II anti-CD20 antigen binding molecule comprising a heavy chain variable region having the specificity determining residues of the heavy chain variable region of the antigen binding molecule as defined herein, wherein said antigen binding molecule induces higher level of apoptosis when incubated with CD20-positive human cells relative to a control under identical conditions using the C2B8 chimeric IgG1 antibody with an identical sequence to rituximab.

[0019c] In one aspect, there is provided an isolated polynucleotide encoding the heavy chain variable region as defined herein.

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

[0021] In a further embodimentaspect, the disclosureinvention is directed to an isolated polynucleotide comprising SEQ ID No.:2. In another embodimentaspect, the disclosureinvention is directed to an isolated polynucleotide comprising SEQ ID No.:4. In a further embodimentaspect, the disclosureinvention 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 3D 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 embodimentaspect, the disclosureinvention is directed to an isolated polynucleotide comprising SEQ ID No.:75. In one embodiment, such polynucleotides encode fusion polypeptides.

[0021a] In one aspect, there is provided an isolated polynucleotide encoding a heavy chain variable region having an amino acid sequence at least 80% identical to the heavy chain variable region as defined herein.

[0021b] In one aspect, there is provided a humanized Type-II anti-CD20 antigen binding molecule comprising the heavy chain variable region encoded by the nucleic acid sequence as defined herein and the KVI light chain variable region of SEQ ID NO: 76, wherein said antigen binding molecule induces higher level of apoptosis when incubated with CD20-positive human cells relative to a control under identical conditions using the C2B8 chimeric IgG1 antibody with an identical sequence to rituximab.

[0022] The disclosureinvention is further directed to an isolated polynucleotide comprising a sequence having at least 80% identity to SEQ ID NO:2, wherein said isolated polynucleotide encodes a fusion polypeptide. In an additional embodimentaspect, the disclosureinvention 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~~invention~~ is further directed to an isolated polynucleotide comprising a sequence having at least 80% identity to a sequence selected from the group consisting of SEQ ID No:29; SEQ ID No:31; SEQ ID No:33; SEQ ID No:35; SEQ ID No:37; SEQ ID No:39; SEQ ID No:41; SEQ ID No:43; SEQ ID No:45; SEQ ID No:47; SEQ ID No:49; SEQ ID No:51; SEQ ID No:53; SEQ ID No:55; SEQ ID No:57; SEQ ID No:59; SEQ ID No:61; SEQ ID No:63; SEQ ID No:65; SEQ ID No:67; SEQ ID No:69; and SEQ ID No:71, wherein said isolated polynucleotide encodes a fusion polypeptide. In an additional embodiment~~aspect~~, the disclosure~~invention~~ 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~~invention~~ 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~~invention~~ 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~~invention~~ 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~~invention~~ 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 H)No:70; and SBQE>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 embodimentaspect, the disclosureinvention is directed to an isolated polynucleotide encoding a chimeric polypeptide having the sequence of SEQ ID No.:3. In one embodiment, the polynucleotide comprises a sequence encoding a polypeptide having the sequence of SEQ ID No.:3; and a sequence encoding a polypeptide having the sequence of an antibody Fc region, or a fragment thereof, from a species other than mouse. In yet another embodimentaspect, the disclosureinvention is directed to an isolated polynucleotide encoding a chimeric polypeptide having the sequence of SEQ ID No.:76. In one embodiment the polynucleotide comprises a sequence encoding a polypeptide having the sequence of SEQ ID No.:76; and a sequence encoding a polypeptide having the sequence of an antibody Fc region, or a fragment thereof, from a species other than mouse.

[0026] The disclosureinvention 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 embodimentaspect, the disclosureinvention 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.

[0026a] In one aspect, there is provided an expression vector comprising the polynucleotide as defined herein.

[0026b] In one aspect, there is provided an isolated host cell comprising the expression vector as defined herein.

[0027] The disclosureinvention 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 embodimentaspect, the disclosureinvention is directed to a host cell comprising any of the isolated polynucleotides described above.

[0028] In one embodimentaspect, the disclosureinvention 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 embodimentaspect, the disclosureinvention 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 disclosureinvention is also directed to a chimeric polypeptide comprising the sequence of SEQ ID NO.:1 or a variant thereof. The disclosureinvention is further directed to a chimeric polypeptide comprising the sequence of SEQ ID NO.:3 or a variant thereof. In one embodiment, any one of these polypeptides further comprises a human Fc region. The disclosureinvention 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; SBQ 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 disclosureinvention is farther directed to a chimeric polypeptide comprising the sequence of SEQ ID NO.:76 or a variant thereof. In one embodiment, any one of these polypeptides further comprises a human Fc region.

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

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

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

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

[0033] The disclosure~~invention~~ is further related to an ABM of the present disclosure~~invention~~, said ABM having modified oligosaccharides. In one embodiment the modified oligosaccharides have reduced fucosylation as compared to non-modified oligosaccharides. In other embodiments, the modified oligosaccharides are hybrid or complex. In a further embodiment, the ABM has an increased proportion of nonfucosylated oligosaccharides or bisected, nonfucosylated oligosaccharides in the Fc region of said molecule. In one embodiment, the bisected, nonfucosylated oligosaccharides are hybrid. In a further embodiment, the bisected, nonfucosylated oligosaccharides are complex. In a one embodiment, at least 20% of the oligosaccharides in the Fc region of said polypeptide are nonfucosylated or bisected, nonfucosylated. In more preferred embodiments, at least 30%, 35%, 40%, 45%, 50%$_5$ 55%, 60%, 65%, 70% or 75% or more of the oligosaccharides are nonfucosylated or bisected., nonfucosylated.

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

[0034a] In one aspect, there is provided a method for the production of the humanized Type-II anti-CD20 antigen binding molecule as defined herein comprising culturing the host cell as defined herein under conditions that allow for the production of said antigen binding molecule and recovering said antigen binding molecule from said culture.

[0034b] In one aspect, there is provided a humanized Type II anti-CD20 antigen binding molecule, wherein the antigen binding molecule is produced by the host cell as defined herein.

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

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

[0035a] In one aspect, there is provided a pharmaceutical composition comprising the antigen binding molecule as defined herein, or produced by the method as defined herein, and a pharmaceutically acceptable carrier.

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

[0036a] In one aspect, there is provided the use of the antigen binding molecule as defined herein, or produced by the method as defined herein, for the manufacture of a medicament to treat a B-cell disorder.

[0036b] In one aspect, there is provided the use of the antigen binding molecule as defined herein, or produced by the method as defined herein, for the manufacture of a medicament to treat a disorder treatable by B-cell depletion.

[0037] In a further embodiment~~aspect~~, the disclosure~~invention~~ 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~~invention~~ to a human subject in need thereof. In a preferred embodiment, the disease is treated by administering an ABM that is a chimeric antibody, or a chimeric fragment of an antibody.

[0038] In yet another embodiment~~aspect~~, the disclosure~~invention~~ 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 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.

[0038a] In one aspect, there is provided the antigen binding molecule as defined herein, or produced by the method as defined herein, wherein said antigen binding molecule comprises a glycoengineered Fc region.

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

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

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

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

[0042] The host cell of the present disclosure~~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 PER.C6 cell or a hybridoma cell. In one embodiment, the host cell of the disclosure~~invention~~ 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 embodiment, the host cell of the disclosure~~invention~~ 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 embodiment~~aspect~~, the disclosure~~invention~~ 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 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 embodiment, the host cell of the present disclosure~~invention~~ comprises at least one nucleic acid encoding a polypeptide having GnTIII activity that is operably linked to a constitutive promoter element.

[0045] In another embodiment~~aspect~~, the disclosure~~invention~~ 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 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 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 embodimentaspect, the disclosureinvention 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 disclosureinvention. In one embodiment, the ABM is an antibody or a fragment thereof; preferably comprising the Fc region of an IgG. Alternatively, the polypeptide is a fusion protein that includes a region equivalent to the Fc region of a human IgG.

[0047] In one embodimentaspect, the disclosureinvention 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 embodimentaspect, the present disclosureinvention is directed to a method of modifying the glycosylation of the recombinant antibody or a fragment thereof of the disclosureinvention by using a fusion polypeptide having GnTIII activity and comprising the Golgi localization domain of a heterologous Golgi resident polypeptide. In one embodiment, the fusion polypeptides of the disclosureinvention comprise the catalytic domain of GnTIII. In another embodiment, the Golgi localization domain is selected from the group consisting of: the localization domain of mannosidase II, the localization domain of GnTI, the localization domain of mannosidase I, the localization domain of GnTII and the localization domain of α1-6 core fucosyltransferase. Preferably, the Golgi localization domain is from mannosidase II or GnTI.

[0049] In one embodiment, the method of the disclosureinvention 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~~invention~~, these modified oligosaccharides may be hybrid or complex. In another embodiment, the method of the disclosure~~invention~~ is directed towards producing a recombinant, chimeric antibody or a fragment thereof having an increased proportion of bisected, nonfucosylated oligosaccharides in the Fc region of said polypeptide. In one embodiment, the bisected, nonfucosylated oligosaccharides are hybrid. In another embodiment, the bisected, nonfucosylated oligosaccharides are complex. In a further embodiment, the method of the disclosure~~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.

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

[0051] In another embodiment~~aspect~~, the disclosure~~invention~~ 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~~invention~~.

[0052] In another embodiment~~aspect~~, the present disclosure~~invention~~ 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~~invention~~.

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

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

[0055] The disclosure~~invention~~ 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~~invention~~, to a human subject in need thereof.

BRIEF DESCRIPTION OF THE FIGURES

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

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

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

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

WHAT IS CLAIMED IS:

~~1. A humanized Type II anti-CD20 antigen binding molecule comprising:~~

~~(a) a heavy chain variable region selected from SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO. 42, SEQ ID NO:44, and SEQ ID NO:46; and~~

~~(b) the KV1 light chain variable region of SEQ ID NO:76.~~

~~2.~~1. A humanized Type II anti-CD20 antigen binding molecule comprising:

(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

(b) the KV1 light chain variable region of SEQ ID NO:76.

~~3.~~2. A humanized Type-II anti-CD20 antigen binding molecule comprising a heavy chain variable region having the specificity determining residues of the heavy chain variable region of the antigen binding molecule of claim 1 ~~or 2~~, wherein said antigen binding molecule induces higher level of apoptosis when incubated with CD20-positive human cells relative to a control under identical conditions using the C2B8 chimeric IgG1 antibody with an identical sequence to rituximab.

~~4.~~3. An isolated polynucleotide encoding the heavy chain variable region of ~~any one of~~ claim~~s~~ 1 ~~to 3~~or 2.

~~5.~~4. An isolated polynucleotide encoding a heavy chain variable region having an amino acid sequence at least 80% identical to the heavy chain variable region of ~~any one of~~ claim~~s~~ 1 ~~to 3~~or 2.

~~6.~~5. A humanized Type-II anti-CD20 antigen binding molecule comprising the heavy chain variable region encoded by the nucleic acid sequence of claim ~~5~~4 and the KV1 light chain variable region of SEQ ID NO: 76, wherein said antigen binding molecule induces higher level

of apoptosis when incubated with CD20-positive human cells relative to a control under identical conditions using the C2B8 chimeric IgG1 antibody with an identical sequence to rituximab.

~~7.~~6. An expression vector comprising the polynucleotide of claim ~~4~~3 or ~~5~~4.

~~8.~~7. An isolated host cell comprising the expression vector of claim ~~7~~6.

~~9.~~8. A method for the production of the humanized Type-II anti-CD20 antigen binding molecule of any one of claims 1, 2 ~~to 3~~ and ~~6~~5 comprising culturing the host cell of claim ~~8~~7 under conditions that allow for the production of said antigen binding molecule and recovering said antigen binding molecule from said culture.

~~10.~~9. A humanized Type II anti-CD20 antigen binding molecule, wherein the antigen binding molecule is produced by the host cell of claim ~~8~~7.

~~11.~~10. A pharmaceutical composition comprising the antigen binding molecule of any one of claims 1, 2~~to 3~~, ~~6~~5 and ~~10~~9, or produced by the method of claim ~~9~~8, and a pharmaceutically acceptable carrier.

~~12.~~11. Use of the antigen binding molecule of any one of claims 1, 2~~to 3~~, ~~6~~5 and ~~10~~9, or produced by the method of claim ~~9~~8, for the manufacture of a medicament to treat a B-cell disorder.

~~13.~~12. The use of claim ~~12~~11, wherein said B-cell disorder is a B-cell lymphoma.

~~14.~~13. Use of the antigen binding molecule of any one of claims 1, 2~~-to 3~~, ~~6~~5 and ~~10~~9, or produced by the method of claim ~~9~~8, for the manufacture of a medicament to treat a

disorder treatable by B-cell depletion.

~~15.~~14. The use of claim ~~14~~13, wherein said disorder is a haematological malignancy or an autoimmune disease.

~~16.~~15. The use of claim ~~15~~14, wherein said haematological malignancy is B-cell lymphoma, non-Hodgkin's lymphoma, or B-cell chronic lymphocytic leukaemia.

~~17.~~16. The use of claim ~~15~~14, wherein said autoimmune disease is rheumatoid arthritis or lupus.

~~18.~~17. The antigen binding molecule of any one of claims 1, ~~2 to 3~~, ~~6~~5 and ~~10~~9, or produced by the method of claim ~~9~~8, wherein said antigen binding molecule comprises a glycoengineered Fc region.

~~19.~~18. The antigen binding molecule of claim ~~18~~17, wherein said antigen binding molecule has an increase in the fraction of non-fucosylated oligosaccharides attached to said glycoengineered Fc region.

~~20.~~19. The antigen binding molecule of claim ~~18~~17, wherein said antigen binding molecule has an increase in the fraction of bisected, non-fucosylated oligosaccharides attached to said glycoengineered Fc region.

~~21.~~20. The antigen binding molecule of claim ~~18~~17, wherein said antigen binding molecule has significantly higher levels of binding to human FcgammaRIII receptors relative to the non-glycoengineered antigen binding molecule.

~~22.~~21. The antigen binding molecule of claim ~~18~~17, wherein said antigen binding