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] 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: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.

[0019b] In one aspect, there is provided a humanized Type-II anti-CD20 antigen binding molecule comprising a heavy chain and a light chain variable region having the specificity determining residues of the heavy chain and the light chain variable regions 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 and the light chain variable regions of the antigen binding molecule as defined herein.

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

[0021] In a further embodiment, the disclosure is directed to an isolated polynucleotide comprising SEQ ID No.:3. In another embodiment, the disclosure is directed to an isolated polynucleotide comprising SEQ ID No.:4. In a further embodiment, the disclosure is directed to an isolated polynucleotide comprising a sequence selected from the group consisting of SEQ ID No:29; SEQ ID No:31; SEQ ID No:33; SEQ ID No:35; SEQ ID No:37; SEQ ID No:39; SEQ ID No:41; SEQ ID No:43; SEQ ID No:45; SEQ ID No:47; SEQ ID No:49; SEQ ID No:51; SEQ ID No:53; SEQ ID No:55; SEQ ID No:57; SEQ ID No:59; SEQ ID No:61; SEQ ID No:63; SEQ ID No:65; SEQ ID No:67; SEQ ID No:69; and SEQ ID No:71. In another embodiment, the disclosure is directed to an isolated polynucleotide comprising SEQ ID No.:75. In one embodiment, such polynucleotides encode fusion polypeptides.

[0021a] In one aspect, there is provided a humanized Type-II anti-CD20 antigen binding molecule comprising the heavy chain variable region encoded by the polynucleotide sequence as defined herein 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.

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

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.

[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, 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.

[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 lymphoma.

[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, wherein said disorder is a haematological malignancy or an autoimmune disease.

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

[0038] In yet another embodiment, the disclosure is related to a host cell engineered to express at least one nucleic acid encoding a polypeptide having GnTIII activity in an amount sufficient to modify the oligosaccharides in the Fc region of produced by the host cell, wherein the ABM is capable of competing with the murine B-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.

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 KVl light chain variable region of SEQ ID NO:76.

2. A humanized Type-II anti-CD20 antigen binding molecule comprising a heavy chain and a light chain variable region having the specificity determining residues of the heavy chain and the light chain variable regions of the antigen binding molecule of claim 1, 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 IgGl antibody with an identical sequence to rituximab.

3. An isolated polynucleotide encoding the heavy chain and the light chain variable regions of the antigen binding molecule of claim 1.

4. An isolated polynucleotide encoding the heavy chain and the light chain variable regions of the antigen binding molecule of claim 2.

5. A humanized Type-II anti-CD20 antigen binding molecule comprising the heavy chain variable region encoded by the polynucleotide sequence of claim 3 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.

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

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

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

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

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

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

12. Use of the antigen binding molecule of any one of claims 1, 2, 5 and 9, or produced by the method of claim 8, for the manufacture of a medicament to treat a disorder treatable by B-cell depletion, wherein said disorder is a haematological malignancy or an autoimmune disease.

13. The use of claim 12, wherein said haematological malignancy is B-cell lymphoma, non-Hodgkin's lymphoma, or B-cell chronic lymphocytic leukaemia.

14. The use of claim 12, wherein said autoimmune disease is rheumatoid arthritis or lupus.

15. The antigen binding molecule of any one of claims 1, 2, 5 and 9, or produced by the method of claim 8, wherein said antigen binding molecule comprises a glycoengineered Fc region.

16. The antigen binding molecule of claim 15, wherein said antigen binding molecule has an increase in the fraction of non-fucosylated oligosaccharides attached to said glycoengineered Fc region.

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

18. The antigen binding molecule of claim 15, wherein said antigen binding molecule has significantly higher levels of binding to human FcgammaRIII receptors relative to the non-glycoengineered antigen binding molecule.

19. The antigen binding molecule of claim 15, wherein said antigen binding molecule has significantly higher levels of antibody-dependent cell-mediated cytotoxicity (ADCC) activity relative to the non-glycoengineered antigen binding molecule.