CLAIMS

1. A modified anti-CD20 antibody, wherein said modified antibody induces higher levels of apoptosis when complexed with CD20 as compared to when the chimeric B-Ly1 antibody is complexed with CD20, and wherein said modified antibody comprises:

   a first polypeptide comprising a heavy chain variable region sequence of SEQ ID NO: 128 and a second polypeptide comprising a light chain variable region sequence of SEQ ID NO: 48; or

   a first polypeptide comprising a heavy chain variable region sequence of SEQ ID NO: 129 and a second polypeptide comprising a light chain variable region sequence of SEQ ID NO: 48.

2. The antibody of claim 1, wherein said antibody comprises an Fc region that has been glycoengineered to have an increase in the amount of bisected complex oligosaccharides.

3. The antibody of claim 1 or 2, wherein said antibody comprises an Fc region that has been glycoengineered to have a decrease in the amount of fucose residues.

4. The antibody of any of claims 1-3, said antibody having an Fc region with modified oligosaccharides.

5. The antibody of claim 4, wherein at least 20% of the oligosaccharides in the Fc region are bisected, nonfucosylated.

6. The antibody of claim 4, wherein at least 50% of the oligosaccharides in the Fc region are nonfucosylated.

7. A host cell expressing at least one nucleic acid encoding a polypeptide having β(1,4)-N-acetylglucosaminyltransferase III activity in an amount sufficient to glycoengineer the Fc

region of a polypeptide produced by said host cell, wherein said polypeptide is the antibody of any one of claims 1-6.

8. The host cell of claim 7, wherein said host cell further expresses a nucleic acid encoding a polypeptide having mannosidase II activity.

9. The host cell of claim 7 or 8, wherein said antibody produced by said host cell exhibits increased Fc receptor binding affinity as a result of said glycoengineering.

10. The host cell of claim 9, wherein said Fc receptor is FcγRIIIA receptor.

11. The host cell of claim 7 or 8, wherein said antibody produced by said host cell exhibits increased effector function as a result of said glycoengineering.

12. The host cell of claim 11, wherein said increased effector function is increased Fc-mediated cellular cytotoxicity.

13. The host cell of claim 11 or 12, wherein said increased effector function is increased antibody-dependent cellular cytotoxicity.

14. The host cell of claim 7 or 8, further comprising at least one transfected polynucleotide encoding the first and second polypeptides according to claim 1, and wherein said polynucleotide further comprises a sequence encoding a region equivalent to the Fc region of a human immunoglobulin.

15. A pharmaceutical composition comprising the antibody of any one of claims 1-6 and a pharmaceutically acceptable carrier.

16. Use of the antibody of any one of claims 1-6 for the manufacture of a medicament to treat a disorder treatable by B-cell depletion.

17. Use of the antibody according to claim 16, wherein said disorder is a haematological malignancy or an autoimmune disease.

18. Use of the antibody according to claim 17, wherein said haematological malignancy is B-cell lymphoma, non-Hodgkin's lymphoma, or B-cell chronic lymphocytic leukaemia.

19. Use of the antibody according to claim 17, wherein said autoimmune disease is rheumatoid arthritis or lupus.

20. Use of the antibody according to claim 17, wherein said haematological malignancy is B-cell lymphoma.

21. An antibody according to any one of claims 1-6 for use as a medicament to treat B-cell lymphoma.

22. An isolated polynucleotide comprising a sequence that encodes a light chain variable region sequence of SEQ ID NO: 48.

23. The isolated polynucleotide of claim 22, wherein said isolated polynucleotide further comprises a sequence that encodes a heavy chain variable region sequence of SEQ ID NO: 128.

24. The isolated polynucleotide of claim 22, wherein said isolated polynucleotide further comprises a sequence that encodes a heavy chain variable region sequence of SEQ ID NO: 129.

25. An expression vector comprising the polynucleotide of any one of claims 22-24.

26. A host cell comprising the expression vector of claim 25.

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

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

conditions which permit the production of said antibody, and which permit the modification of the oligosaccharides present on the Fc region of said antibody; and

b. isolating said antibody;

wherein said antibody is the modified antibody of any one of claims 1-6.

28. The method of claim 27, wherein said host cell is further engineered to express a nucleic acid encoding a polypeptide having mannosidase II activity.

29. The method of claim 27 or 28, wherein said modified oligosaccharides have reduced fucosylation as compared to non-modified oligosaccharides.

30. The method of any one of claims 27-29, wherein said antibody produced by said host cell has an increased proportion of bisected, non-fucosylated oligosaccharides in the Fc region of said polypeptide.

WHAT IS CLAIMED IS:

1. A modified antigen binding molecule comprising a heavy chain or light chain variable region comprising at least one amino acid residue substitution in at least one framework region of said heavy chain or light chain variable region as compared to the heavy chain or light chain variable region of a parent antigen binding molecule, wherein said substitution results in altered cell signaling activity of a target antigen when said modified antigen binding molecule is complexed with said target antigen.

2. A modified antigen binding molecule comprising a heavy chain or light chain variable region comprising at least one amino acid residue substitution in at least one framework region of said heavy chain or light chain variable region as compared to the heavy chain or light chain variable region of a parent antigen binding molecule, wherein said modified antigen binding molecule has altered ability to mediate cross-linking of one or more target antigens as a result of said substitution.

3. The modified antigen binding molecule of claim 1 or 2, wherein said substitution is in said heavy chain variable region.

4. The modified antigen binding molecule of claim 3, wherein said substitution is in FR1 of said heavy chain variable region.

5. The modified antigen binding molecule of claim 3, wherein the entire FR1 of said heavy chain variable region is replaced by a germline VH FR1.

6. The modified antigen binding molecule of claim 5, wherein the germline VH FR1 comprises an amino acid sequence at Kabat positions 8 to 13 selected from the group consisting of SEQ ID NO:63, SEQ ID NO:64,

SEQ ID NO: 65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:101, SEQ ID NO:102, SEQ ID NO:103, SEQ ID NO:104, and SEQ ID NO:105.

7. The modified antigen binding molecule of claim 4, wherein said substitution in FR1 of said heavy chain variable region comprises a replacement of an amino acid residue at one or more of Kabat positions 8, 9, 10, 11, 12, or 13.

8. The modified antigen binding molecule of claim 7, wherein said substitution in FR1 of said heavy chain variable region comprises a replacement of the amino acid residue at Kabat position 11.

9. The modified antigen binding molecule of claim 8, wherein said substitution comprises a replacement of the amino acid residue at Kabat position 11 with a valine.

10. The modified antigen binding molecule of claim 7, wherein said substitution in FR1 of said heavy chain variable region comprises a replacement of the amino acid residue at Kabat position 12.

11. The modified antigen binding molecule of claim 10, wherein said substitution comprises a replacement of the amino acid residue at Kabat position 12 with a lysine.

12. The modified antigen binding molecule of claim 7, wherein said substitution in FR1 of said heavy chain variable region comprises a replacement of the amino acid residues at Kabat positions 11 and 12.

13. The modified antigen binding molecule of claim 12, wherein said substitution comprises a replacement of the amino acid residue at Kabat position 11 with a valine and at Kabat position 12 with a lysine.

14. The modified antigen binding molecule of claim 12, wherein said substitution comprises a replacement of the amino acid residue at Kabat position 11 with a leucine and at Kabat position 12 with a valine.

15. The modified antigen binding molecule of claim 3, wherein said substitution comprises replacement of at least one amino acid residue in FR4 of said heavy chain variable region.

16. The modified antigen binding molecule of claim 15, wherein said substitution in FR4 of said heavy chain variable region comprises a replacement of an amino acid residue at one or more of Kabat positions 110 or 112.

17. The modified antigen binding molecule of claim 1 or 2, wherein said substitution is in said light chain variable region.

18. The modified antigen binding molecule of claim 17, wherein said substitution in said light chain variable region comprises a replacement of an amino acid residue at one or more of Kabat positions 40, 80, 83, 105, or 106.

19. The modified antigen binding molecule of claim 1, wherein said altered cell signaling activity is increased agonist activity.

20. The modified antigen binding molecule of claim 19, wherein said increased agonist activity is induction of apoptosis.

21. The modified antigen binding molecule of claim 1, wherein said altered cell signaling activity is increased antagonist activity.

22. The modified antigen binding molecule of claim 1 or 2, wherein said modified antigen binding molecule binds specifically to human CD20.

23. The modified antigen binding molecule of claim 1 or 2, wherein said modified antigen binding molecule binds specifically to a receptor tyrosine kinase.

24. The modified antigen binding molecule of claim 23, wherein said receptor tyrosine kinase is selected from the group consisting of HER1 (EGFR1), HER2/neu, HER3, HER4, IGF-1R, FGFR, PDGFR, VEGFR1, VEGFR2, and VEGFR3.

25. The modified antigen binding molecule of claim 1 or 2, wherein said parent antigen binding molecule comprises a heavy chain variable region selected from the group consisting of SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, and SEQ ID NO:62.

26. The modified antigen binding molecule of claim 1 or 2, further comprising a human Fc region.

27. The modified antigen binding molecule of claim 26, wherein said Fc region has been modified to have altered oligosaccharides.

28. The modified antigen binding molecule of claim 27, wherein said Fc region has been modified to have a decreased proportion of fucose residues compared to a non-modified Fc region.

29. The modified antigen binding molecule of claim 27, wherein said Fc region has an increased ratio of GlcNAc residues to fucose residues in the modified Fc region compared to a non-modified Fc region.

30. A modified antigen binding molecule comprising a CH1 domain comprising at least one amino acid residue substitution as compared to the CH1 domain of a parent polypeptide, wherein said substitution results in either altered cell signaling activity of a target antigen when said modified antigen binding molecule is complexed with said target antigen or altered ability to mediate cross-linking of one or more target antigens as a result of said substitution.

31. The modified antigen binding molecule of claim 30, wherein said substitution in CH1 comprises a replacement of an amino acid residue at one or more of positions 148, 149 or 150.

32. An isolated polynucleotide encoding a polypeptide comprising a heavy chain or light chain variable region, wherein said heavy chain or light chain variable region comprises at least one amino acid residue substitution in at least one framework region as compared to a parent heavy chain or light chain variable region, and wherein said substitution results in either altered cell signaling activity of a target antigen when said polypeptide is complexed with said target antigen or altered ability to mediate cross-linking of one or more target antigens as a result of said substitution.

33. A vector comprising the polynucleotide of claim 32.

34. A host cell comprising the vector of claim 33.

35. A host cell engineered to express at least one nucleic acid encoding a polypeptide having β(1,4)-N-acetylglucosaminyltransferase III activity in an amount sufficient to modify the oligosaccharides in the Fc region of a

polypeptide produced by said host cell, wherein said polypeptide is a modified antigen binding molecule according to claim 1 or 2.

36. The host cell of claim 35, wherein said antigen binding molecule produced by said host cell exhibits increased effector function as a result of said oligosaccharide modification.

37. A host cell according to claim 36, wherein said increased effector function is increased Fc-mediated cellular cytotoxicity.

38. A host cell according to claim 37, wherein said increased effector function is increased direct signaling inducing apoptosis.

39. A method for producing a modified antigen binding molecule comprising a heavy chain or light chain variable region comprising at least one amino acid residue substitution in at least one framework region of said heavy chain or light chain variable region as compared to the heavy chain or light chain variable region of a parent antigen binding molecule, wherein said substitution results in altered cell signaling activity of a target antigen when said modified antigen binding molecule is complexed with said target antigen, said method comprising:

(i) culturing the host cell of claim 34 under conditions permitting the expression of said polynucleotide; and

(ii) recovering said modified antigen binding molecule from the culture medium.

40. A method for producing a modified antigen binding molecule comprising a heavy chain or light chain variable region comprising at least one amino acid residue substitution in at least one framework region of said heavy chain or light chain variable region as compared to the heavy chain or light chain variable region of a parent antigen binding molecule, wherein said substitution results in altered ability to mediate cross-linking as a result of said substitution, said method comprising:

(i) culturing the host cell of claim 34 under conditions permitting the expression of said polynucleotide; and

(ii) recovering said modified antigen binding molecule from the culture medium.

41. A pharmaceutical composition comprising the modified antigen binding molecule of any one of claims 1, 2, or 30 and a pharmaceutically acceptable carrier.

42. Use of the antigen binding molecule according to claim 1 or 2 for the manufacture of a medicament for the treatment or prophylaxis of cancer or a precancerous condition or lesion.

43. The use according to claim 42, wherein said cancer is selected from the group consisting of B-cell lymphoma, breast cancer, bladder cancer, head and neck cancer, skin cancer, pancreatic cancer, lung cancer, ovarian cancer, colon cancer, prostate cancer, kidney cancer, and brain cancer.

44. The use according to claim 42, wherein said precancerous condition or lesion is selected from the group consisting of oral leukoplakia, actinic keratosis (solar keratosis), precancerous polyps of the colon or rectum, gastric epithelial dysplasia, adenomatous dysplasia, hereditary nonpolyposis colon cancer syndrome (HNPCC), Barrett's esophagus, bladder dysplasia, and precancerous cervical conditions.

45. The use according to claim 42, wherein said antigen binding molecule is used in a therapeutically effective amount from about 1.0 mg/kg to about 15 mg/kg.

46. A modified antigen binding molecule according to claim 1 or 2 for use as a medicament in the treatment or prophylaxis of a disorder that is related to altered cell signaling activity and/or altered cross-linking of one or more target antigens.

47. The modified antigen binding molecule according to claim 46, wherein said altered cell signaling activity is increased apoptosis.

48. The modified antigen binding molecule according to claim 46, wherein said disorder is a B cell disorder.

49. The modified antigen binding molecule according to claim 46, wherein said target antigen is CD20.

50. Use of a modified antigen binding molecule comprising a heavy chain or light chain variable region for the preparation of a medicament for inducing apoptosis in a cell, wherein said heavy chain or light chain variable region comprises at least one amino acid residue substitution in at least one framework region of said heavy chain or light chain variable region as compared to the heavy chain or light chain variable region of a parent antigen binding molecule, and wherein said modified antigen binding molecule has increased ability to induce apoptosis compared to said parent polypeptide.

51. Use of a modified antigen binding molecule for the preparation of a medicament for the treatment of a disease or disorder that is treatable by altered cell signaling activity of a target antigen, wherein said modified antigen binding molecule comprises a heavy chain or light chain variable region comprising at least one amino acid substitution in at least one framework region of said heavy chain or light chain variable region compared to the heavy chain or light chain variable region of a parent antigen binding molecule, and wherein said substitution results in altered cell signaling activity of a target antigen when said modified antigen binding molecule is complexed with said target antigen.

52. The use of claim 51, wherein said altered cell signaling activity is increased induction of apoptosis.

53. Use of a modified antigen binding molecule for the preparation of a medicament for the treatment of a disease or disorder that is treatable by altered ability to mediate cross-linking of one or more target antigens, wherein said modified antigen binding molecule comprises a heavy chain or light chain variable region comprising at least one amino acid substitution in at least one framework region of said heavy chain or light chain variable region compared to the heavy chain or light chain variable region of a parent antigen binding molecule, and wherein said modified antigen binding molecule has altered ability to mediate cross-linking of one or more target antigens as a result of said substitution.

54. The use of claim 51 or 53, wherein said modified antigen binding molecule comprises said heavy chain variable region.

55. The use of claim 54, wherein said modified antigen binding molecule comprises a heavy chain variable region selected from the group consisting of SEQ ID NO:4, SEQ ID NO:36, and SEQ ID NO:38.

56. The use of claim 51 or 53, wherein said disease or disorder is a cell proliferation disorder.

57. The use of claim 56, wherein said cell proliferation disorder is cancer.

58. The use of claim 57, wherein said cancer is a B cell lymphoma.