CLAIMS

1. An antigen binding fragment of a humanized antibody that binds human CD20, wherein the humanized antibody comprises the VH sequence of the amino acid sequence of SEQ ID NO. 8 and the VL sequence of the amino acid sequence of SEQ ID NO. 2.

2. The antigen binding fragment of claim 1 conjugated to a cytotoxic agent.

3. The antigen binding fragment of claim 2, wherein the cytotoxic agent is a radioactive isotope or a toxin.

4. A composition comprising the antigen binding fragment of any one of claims 1-3 and a pharmaceutically acceptable carrier.

5. An article of manufacture comprising a container and a composition contained therein, wherein the composition comprises the antigen binding fragment of any one of claims 1-3.

6. The article of manufacture of claim 5, further comprising a package insert indicating that the composition can be used to treat a CD20 positive cancer and/or an autoimmune disease.

7. The article of manufacture of claim 6, wherein the autoimmune disease is multiple sclerosis.

8. An isolated nucleic acid that encodes the antigen binding fragment of claim 1.

9. An expression vector comprising the nucleic acid of claim 8.

10. A host cell comprising the nucleic acid of claim 8.

11. The host cell of claim 10, which is a CHO cell.

12 A method of producing an antigen binding fragment of a humanized antibody, comprising culturing a host cell that produces the antigen binding fragment of claim 1 and recovering the antigen binding fragment produced by the cell.

13. An antigen binding fragment produced by a method comprising expressing a nucleic acid encoding an antigen binding fragment of an antibody comprising the VH and VL sequences of SEQ ID NO. 8 and 2, respectively in a host cell, and recovering the antigen binding fragment produced by the host cell.

14. Use of the antigen binding fragment of any one of claims 1-3 in the manufacture of a medicament for depleting B cells in a mammal.

15. The use of claim 14, wherein the mammal is a human.

16. The use of claim 14, wherein peripheral B cells are depleted in the mammal.

17. The use of claim 14, wherein the peripheral B cells are partially depleted in the mammal.

18. Use of the antigen binding fragment of any one of claims 1-3 in the manufacture of a medicament for treating a CD20 positive cancer, comprising administering to a patient suffering from the cancer, a therapeutically effective amount of the antigen binding fragment.

19. The use of claim 18, wherein the CD20 positive cancer is a B cell lymphoma or leukemia.

20. The use of claim 19, wherein the CD20 positive cancer is non-Hodgkin's lymphoma (NHL) or lymphocyte predominant Hodgkin's disease (LPHD).

21. The use of claim 18, wherein the CD20 positive cancer is chronic lymphocytic leukemia (CLL) or small lymphocytic lymphoma (SLL).

22. The use of claim 19, wherein the antigen binding fragment is to be administered at a dosage range of about 250 mg/m^2 to about 500 mg/m^2.

23. The use of claim 19, wherein the antigen binding fragment is to be administered at a dosage range of about 275 mg/m^2 to about 375 mg/m^2.

24. The use of claim 19, wherein the patient is to be administered at least two doses of the antigen binding fragment at 375 mg/m^2 per dose.

25. The use of claim 24, wherein the two doses are administered two weeks apart.

26. The use of claim 19, wherein the patient is to be further administered with at least one chemotherapeutic agent.

27. The use of claim 26, wherein the cancer is non-Hodgkin's lymphoma (NHL) and the chemotherapeutic agent is selected from the group consisting of doxorubicin, cyclophosphamide, vincristine, prednisolone, and CHOP.

28. The use of claim 18, wherein the antigen binding fragment is administered by intravenous administration or by subcutaneous administration.

29. Use of the antigen binding fragment of any one of claims 1-3 in the manufacture of a medicament for treating an autoimmune disease, comprising administering to a patient suffering from the autoimmune disease, a therapeutically effective amount of the antigen binding fragment.

30. The use of claim 29, wherein the autoimmune disease is selected from the group consisting of rheumatoid arthritis, juvenile rheumatoid arthritis, systemic lupus erythematosus (SLE), lupus nephritis, ulcerative colitis, Wegener's disease, inflammatory bowel disease, idiopathic thrombocytopenic purpura (ITP), thrombotic thrombocytopenic purpura (TTP), autoimmune thrombocytopenia, multiple sclerosis, psoriasis, IgA nephropathy, IgM polyneuropathies, myasthenia gravis, vasculitis, ANCA vasculitis, solid organ transplant rejection, graft versus host disease, diabetes mellitus, Reynaud's syndrome, Sjorgen's syndrome and glomerulonephritis.

31. The use of claim 29, wherein the antigen binding fragment is administered by intravenous administration or by subcutaneous administration.

32. The use of claim 30, wherein the autoimmune disease is rheumatoid arthritis.

33. The use of claim 32, wherein the patient is suffering from moderate to severe rheumatoid arthritis and has failed treatment with at least one disease-modifying antirheumatic drug.

34. The use of claim 32, wherein the patient is to be administered with a second therapeutic agent.

35. The use of claim 34, wherein the second therapeutic agent is an immunosuppressive agent.

36. The use of claim 35, wherein the immunosuppressive agent is methotrexate.

37. The use of claim 32, wherein the antigen binding fragment is to be administered at a dosage selected from 2x10mg, 2x50mg, 2x 200mg, and 2x500mg.

38. The use of claim 37, wherein the antigen binding fragment is to be administered by intravenous infusion.

39. Use of the antigen binding fragment of any one of claims 1-3 in the manufacture of a medicament for treating rheumatoid arthritis (RA) in a human subject, wherein the antigen binding fragment is to be administered at a dosage selected from the group consisting of 2x10 mg, 2x50 mg, 2x200 mg, and 2x500 mg.

40. The use of claim 39, wherein the rheumatoid arthritis is moderate rheumatoid arthritis to severe rheumatoid arthritis.

~~WHAT IS CLAIMED IS:~~

1. A humanized antibody that binds human CD20, or an antigen-binding fragment thereof, wherein the antibody is effective to deplete primate B cells in vivo, the antibody comprising in the H chain Variable region (V_H) at least a CDR3 sequence of SEQ ID NO. 12 from an anti-human CD20 antibody and substantially the human consensus framework (FR) residues of human heavy chain subgroup III (V_HIII).

2. The antibody of claim 1, further comprising the H chain CDR1 sequence of SEQ ID NO. 10 and CDR2 sequence of SEQ ID NO. 11.

3. The antibody of claim 2, further comprising the L chain CDR1 sequence of SEQ ID NO. 4, CDR2 sequence of SEQ ID NO. 5, CDR3 sequence of SEQ ID NO. 6 and substantially the human consensus framework (FR) residues of human light chain κ subgroup I (V_KI).

4. The antibody of the preceding claims, comprising the V_H sequence of SEQ ID NO.8.

5. The antibody of claim 4, further comprising the V_L sequence of SEQ ID NO.2.

6. The antibody of claim 3, wherein the V_H region is joined to a human IgG chain constant region.

7. The antibody of claim 6, wherein the human IgG is IgG1 or IgG3.

8. The antibody of claim 1, wherein the antibody is 2H7.v16 having the light and heavy chain amino acid sequence of SEQ ID NO. 21 and 22, respectively.

9. The antibody of claim 1, wherein the antibody is 2H7.v31 having the light and heavy chain amino acid sequence of SEQ ID NO. 21 and 23, respectively.

10. The antibody of claim 5, but with the amino acid substitutions of D56A and N100A in the H chain and S92A in the L chain.

11. The antibody of any of the preceding claims, further comprising at least one amino acid substitution in the Fc region that improves ADCC and/or CDC activity.

12. The antibody of claim 11, wherein the amino acid substitutions are S298A/E333A/K334A.

13. The antibody of claim 12, wherein the antibody is 2H7.v31 having the heavy chain amino acid sequence of SEQ ID NO. 23.

14. The antibody of any of claims 1-10, further comprising at least one amino acid substitution in the Fc region that decreases CDC activity.

15. The antibody of any of claim 14, comprising at least the substitution K322A.

16. The antibody of claim 1-10 wherein the antibody is 2H7.v114 or 2H7.v115 having at least 10-fold improved ADCC activity as compared to Rituxan.

17. The antibody of claim 1 wherein the primate B cells are from human and Cynomolgus monkey.

18. The antibody of any of the preceding claims conjugated to a cytotoxic agent.

19. The antibody of claim 18 wherein the cytotoxic agent is a radioactive isotope or a toxin.

20. The antibody of any of the preceding claims, which antibody is produced in CHO cells.

21. An isolated nucleic acid that encodes the antibody of any one of the preceding claims.

22. An expression vector encoding the antibody of any of the preceding claims.

23. A host cell comprising a nucleic acid of claim 21.

24. The host cell of claim 23 that produces the antibody of any one of preceding claims.

25. The host cell of claim 24 which is a CHO cell.

~~26. A method of producing the antibody of any one of the preceding claims, comprising culturing the~~ cell that produces the antibody of claim 24 and recovering the antibody from the cell culture.

27. A composition comprising the antibody of claim 1 and a carrier.

28. The composition of claim 27 wherein the antibody is 2H7.v16 and the carrier is a pharmaceutically acceptable carrier.

29. An article of manufacture comprising a container and a composition contained therein, wherein the composition comprises an antibody of any of the preceding claims.

30. The article of manufacture of claim 29, further comprising a package insert indicating that the composition can be used to treat non-Hodgkin's lymphoma.

31. A method of inducing apoptosis in B cells in vivo, comprising contacting B cells with the antibody of any of the preceding claims, thereby killing the B cells.

32. A method of treating a CD20 positive cancer, comprising administering to a patient suffering from the cancer, a therapeutically effective amount of the humanized CD20 binding antibody of any of the preceding claims.

33. The method of claim 32 wherein the CD20 positive cancer is a B cell lymphoma or leukemia.

34. The method of claim 33 wherein CD20 positive cancer is non-Hodgkin's lymphoma (NHL) or lymphocyte predominant Hodgkin's disease (LPHD).

35. The method of claim 32 wherein the cancer is chronic lymphocytic leukemia or SLL.

36. The method of claims 34 or 35 wherein the antibody is selected from the group consisting of 2H7.v16, v31.v96, v114, v115 having the respective amino acid sequences as shown in the figures and tables.

37. The method of claim 34 or 35 wherein the antibody is 2H7.v16 having the light and heavy chain amino acid sequence of SEQ ID NO. 21 and 22, respectively.

38. The method of claim 33, wherein the antibody is administered at a dosage range of about 275-375mg/m^2.

39. The method of claim 32, further comprising administering to the patient at least one chemotherapeutic agent.

40. The method of claim 39, wherein the cancer is non-Hodgkin's lymphoma (NHL) and the chemotherapeutic agent is selected from the group consisting of doxorubicin, cyclophosphamide, vincristine and prednisolone.

41. A method of treating an autoimmune disease, comprising administering to a patient suffering from the autoimmune disease, a therapeutically effective amount of the humanized CD20 binding antibody of any one of the preceding claims.

42. The method of claim 41, wherein the autoimmune disease is selected from the group consisting of rheumatoid arthritis, juvenile rheumatoid arthritis, systemic lupus erythematosus (SLE), Wegener's disease, inflammatory bowel disease, idiopathic thrombocytopenic purpura (ITP), thrombotic thrombocytopenic purpura (TTP), autoimmune thrombocytopenia, multiple sclerosis, psoriasis, IgA nephropathy, IgM polyneuropathies, myasthenia gravis, vasculitis, diabetes mellitus, Reynaud's syndrome, Sjorgen's syndrome and glomerulonephritis.

43. The method of claim 42, wherein the autoimmune disease is rheumatoid arthritis.

44. The method of claim 43, further comprising administering to the patient a second therapeutic agent.

45. The method of claim 44, wherein the second therapeutic agent is an immunosuppressive agent.

46. The method of claim 45, wherein the immunosuppressive agent is methotrexate.

47. A method of treating an autoimmune disease selected from the group consisting of Dermatomyositis, Wegner's granulomatosis, ANCA (included under vasculitis), Aplastic anemia, Autoimmune hemolytic anemia (AIHA), factor VIII deficiency, hemophilia A, Autoimmune neutropenia, Castleman's syndrome, Goodpasture's syndrome, solid organ transplant rejection, graft versus host disease (GVHD), IgM mediated, thrombotic thrombocytopenic purpura (TTP), Hashimoto's Thyroiditis, autoimmune hepatitis, lymphoid interstitial pneumonitis (HIV), bronchiolitis obliterans (non-transplant) vs. NSIP, Guillain-Barre Syndrome, large vessel vasculitis, giant cell (Takayasu's) arteritis, medium vessel vasculitis, Kawasaki's Disease, and polyarteritis nodosa, comprising administering to a patient suffering from the disease, a therapeutically effective amount of a CD20 binding antibody or functional fragment thereof.

48. The method of claim 47 wherein the CD20 binding antibody is Rituxan®.

49. An isolated nucleic acid comprising the nucleotide sequence of SEQ ID NO.: 24 of the Cynomolgus monkey CD20, or a degenerate variant of this sequence.

50. An isolated nucleic acid comprising a sequence that encodes a polypeptide with the amino acid sequence of SEQ ID NO. 25, or SEQ ID NO. 25 with conservative amino acid substitutions.

51. A vector comprising the nucleic acid of claim 50.

52. The vector of claim 51 which is an expression vector comprising the nucleic acid of claim 49 operably lined to an expression control sequence.

53. A host cell comprising the nucleic acid of claim 50.

54. An isolated polypeptide comprising the amino acid sequence of SEQ ID NO. 25 of the Cynomolgus monkey CD20.

55. A liquid formulation comprising a humanized 2H7 antibody at 20mg/mL, 10mM histidine sulfate at pH5.8, 60mg/ml sucrose, 0.2 mg/ml polysorbate 20.