CLAIMS

1. An isolated nucleic acid molecule encoding a chimeric antigen receptor (CAR), wherein the CAR comprises an antibody or antibody fragment which includes a humanized anti-CD19 binding domain, a transmembrane domain, and an intracellular signaling domain comprising a stimulatory domain, and wherein said anti-CD19 binding domain comprises a light chain variable region and a heavy chain variable region from any one of SEQ ID NO:2, SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12.

2. The isolated nucleic acid molecule of claim 1, wherein the CD19 binding domain is a scFv.

3. The isolated nucleic acid molecule of any of the preceding claims, wherein the anti-CD19 binding domain comprises an amino acid sequence selected from a group consisting of SEQ ID NO:2, SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12, or an amino acid sequence with at least 95% identity thereof.

4. The isolated nucleic acid molecule of any of the preceding claims, wherein the anti-CD19 binding domain comprises an amino acid sequence selected from a group consisting of SEQ ID NO:2, SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12.

5. The isolated nucleic acid molecule of any of the preceding claims, wherein the nucleic acid sequence encoding the anti-CD19 binding domain comprises a nucleic acid sequence selected from a group consisting of SEQ ID NO:62, SEQ ID NO:61, 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:70, SEQ ID NO:71 and SEQ ID NO:72, or a nucleic acid sequence with at least 95% identity thereof.

6. The isolated nucleic acid molecule of any of the preceding claims, which comprises a nucleic acid sequence selected from a group consisting of SEQ ID NO: 86, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, and SEQ ID NO: 96, or a nucleic acid sequence with at least 95% identity thereof.

7. The isolated nucleic acid molecule of any of the preceding claims, wherein the encoded CAR includes a transmembrane domain that comprises a transmembrane domain of a protein selected from the group consisting of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154.

8. The isolated nucleic acid molecule of any of the preceding claims, wherein the encoded CAR includes a transmembrane domain that comprises a transmembrane domain of the alpha chain of CD8.

9. The isolated nucleic acid molecule of any of the preceding claims, wherein the encoded transmembrane domain comprises the amino acid sequence of SEQ ID NO: 15, or an amino acid sequence with at least 95% identity to the amino acid sequence of SEQ ID NO:15 or the nucleic acid sequence encoding the transmembrane domain comprises the amino acid sequence of SEQ ID NO:56, or a sequence with at least 95% identity thereof.

10. The isolated nucleic acid molecule of any of the preceding claims, wherein the encoded anti-CD19 binding domain is connected to the transmembrane domain by a hinge region, wherein the hinge region is optionally a CD8 alpha hinge.

11. The isolated nucleic acid molecule of claim 10, wherein the encoded hinge region comprises the amino acid sequence of SEQ ID NO:14, or an amino acid sequence with at least 95% identity thereof or wherein the nucleic acid sequence encoding the hinge region comprises the nucleic acid sequence of SEQ ID NO: 55, or a nucleic acid sequence with at least 95% identity thereof.

12. The isolated nucleic acid molecule of any of the preceding claims, wherein the intracellular signaling domain comprises a costimulatory domain.

13. The isolated nucleic acid molecule of claim 12, wherein the costimulatory domain is a functional signaling domain obtained from a protein selected from the group consisting of OX40, CD2, CD27, CD28, CDS, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), and 4-1BB (CD137).

14. The isolated nucleic acid molecule of claim 12 or 13, wherein the encoded costimulatory domain comprises the amino acid sequence of SEQ ID NO:16 or an amino acid sequence with at least 95% identity to the amino acid sequence of SEQ ID NO:16 or the nucleic acid sequence encoding the costimulatory domain comprises the nucleic acid sequence of SEQ ID NO:60, or a nucleic acid sequence with at least 95% identity thereof.

15. The isolated nucleic acid molecule of any of the preceding claims, wherein the intracellular signaling domain comprises a primary signaling domain.

16. The isolated nucleic acid molecule of any of the preceding claims, wherein the encoded intracellular signaling domain comprises a functional signaling domain of 4-1BB and/or a functional signaling domain of CD3 zeta.

17. The isolated nucleic acid molecule of any of the preceding claims, wherein the encoded intracellular signaling domain (i) comprises the amino acid sequence of SEQ ID NO: 16 and/or the amino acid sequence of SEQ ID NO:17 or SEQ ID NO:43, or (ii) an amino acid sequence with at least 95% identity to the amino acid sequence of SEQ ID NO:16 and/or an amino acid sequence of SEQ ID NO:17 or SEQ ID NO:43.

18. The isolated nucleic acid molecule of any of the preceding claims, wherein the encoded intracellular signaling domain comprises the amino acid sequence of SEQ ID NO:16 and the amino acid sequence of SEQ ID NO:17 or SEQ ID NO:43, wherein the sequences comprising the intracellular signaling domain are expressed in the same frame and as a single polypeptide chain.

19. The isolated nucleic acid molecule of any of the preceding claims, wherein the nucleic acid sequence encoding the intracellular signaling domain comprises the nucleic acid sequence of SEQ ID NO:60, or a nucleic acid sequence with at least 95% identity thereof, and/or a nucleic acid sequence of SEQ ID NO:101 or SEQ ID NO:44, or a nucleic acid sequence with at least 95% identity thereof.

20. The isolated nucleic acid molecule of any of the preceding claims, further comprising a leader sequence.

21. The isolated nucleic acid molecule of claim 20, wherein the leader sequence encodes the amino acid sequence of SEQ ID NO: 13, or a sequence with at least 95% identity to the amino acid sequence of SEQ ID NO: 13.

22. An isolated polypeptide molecule encoded by the nucleic acid molecule of any one of the preceding claims.

23. The isolated polypeptide of claim 22, comprising an amino acid sequence selected from the group consisting of SEQ ID NO:32, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, and SEQ ID NO:42.

24. An isolated chimeric antigen receptor (CAR) molecule comprising: (i) an antibody or antibody fragment which includes a humanized anti-CD19 binding domain, (ii) a transmembrane domain, and (iii) an intracellular signaling domain, wherein the humanized anti-CD19 binding domain comprises a light chain variable region and a heavy chain variable region from any one of SEQ ID NO:2, SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12.

25. The isolated CAR molecule of claim 24, wherein the anti-CD19 binding domain comprises an amino acid sequence selected from a group consisting of SEQ ID NO:2, SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO: 4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ

ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11 and SEQ ID NO:12, or an amino acid sequence with at least 95% identity thereof.

26. The isolated CAR molecule of claim 24 or 25, wherein the anti-CD19 binding domain comprises an amino acid sequence selected from a group consisting of SEQ ID NO:2, SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO: 4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11 and SEQ ID NO:12.

27. The isolated CAR molecule of any of claims 24-26, which comprises an amino acid sequence selected from a group consisting of SEQ ID NO:32, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, and SEQ ID NO:42, or an amino acid sequence with at least 95% identity thereof.

28. The isolated CAR molecule of any of claims 24-27, which comprises an amino acid sequence selected from a group consisting of SEQ ID NO:32, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, and SEQ ID NO:42.

29. The isolated CAR molecule of any of claims 24-28, wherein the transmembrane domain comprises a transmembrane domain of a protein selected from the group consisting of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154.

30. The isolated CAR molecule of any of claims 24-29, wherein the transmembrane domain comprises a transmembrane domain of the alpha chain of CD8.

31. The isolated CAR molecule of claim 29 or 30, wherein the transmembrane domain comprises: (i) an amino acid sequence of SEQ ID NO: 15, or (ii) a sequence with at least 95% identity to the amino acid sequence of SEQ ID NO: 15.

32. The isolated CAR molecule of any one of claims 24-31, wherein the humanized anti-CD19 binding domain is connected to the transmembrane domain by a hinge region, wherein optionally the hinge region is a CD8 alpha hinge.

33. The isolated CAR molecule of claim 32, wherein the encoded hinge region comprises the amino acid sequence of SEQ ID NO:14 or SEQ ID NO:102, or a sequence with at least 95% identity thereof.

34. The isolated CAR molecule of any of claims 24-33, wherein the intracellular signaling domain comprises a costimulatory domain.

35. The isolated CAR molecule of claim 34, wherein the costimulatory domain comprises a functional signaling domain of a protein selected from the group consisting of OX40, CD2, CD27, CD28, CDS, ICAM-1, LFA-1 (CD11a/CD18) and 4-1BB (CD137).

36. The isolated CAR molecule of claim 34 or 35, wherein the costimulatory domain comprises the amino acid sequence of SEQ ID NO: 16, or (ii) an amino acid sequence with at least 95% identity to the amino acid sequence of SEQ ID NO: 16.

37. The isolated CAR molecule of any one of claims 24-36, wherein the intracellular signaling domain comprises a primary signaling domain.

38. The isolated CAR molecule of claim 37, wherein the intracellular signaling domain comprises a functional signaling domain of 4-1BB and/or a functional signaling domain of CD3 zeta.

39. The isolated CAR molecule of claim 37 or 38, wherein the intracellular signaling domain comprises: (i) the amino acid sequence of SEQ ID NO: 16 and/or the amino acid sequence of SEQ ID NO:17, or the amino acid sequence of SEQ ID NO:43, or (ii) the amino acid sequence of SEQ ID NO:17 or SEQ ID NO:43, or an amino acid sequence with at least 95% identity to the amino acid sequence of SEQ ID NO:16 and/or the amino acid sequence of SEQ ID NO:17 or SEQ ID NO:43.

40. The isolated CAR molecule of any of claims 37-39, wherein the intracellular signaling domain comprises the amino acid sequence of SEQ ID NO: 16 and the amino acid sequence of SEQ ID NO: 17 or SEQ ID NO:43, wherein the sequences comprising the intracellular signaling domain are expressed in the same frame and as a single polypeptide chain.

41. The isolated CAR molecule of any of claims 24-40, further comprising a leader sequence.

42. The isolated CAR molecule of claim 41, wherein the leader sequence comprises the amino acid sequence of SEQ ID NO: 13, or a sequence with at least 95% identity to the amino acid sequence of SEQ ID NO:13.

43. A humanized anti-CD19 binding domain comprising a light chain variable region and a heavy chain variable region from any one of SEQ ID NO:2, SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12.

44. The humanized anti-CD19 binding domain of claim 43, which comprises an amino acid sequence selected from a group consisting of SEQ ID NO:2, SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12.

45. A vector comprising a nucleic acid molecule encoding a CAR of any of claims 24-42.

46. The vector of claim 45, wherein the vector is selected from the group consisting of a DNA, a RNA, a plasmid, a viral vector, a lentivirus vector, adenoviral vector, or a retrovirus vector.

47. The vector of claim 45 or 46, further comprises a promoter.

48. The vector of claim 47, wherein the promoter is an EF-1 promoter.

49. The vector of claim 48, wherein the EF-1 promoter comprises the nucleic acid sequence of SEQ ID NO: 100.

50. The vector of any of claims 45-49, wherein the vector is an in vitro transcribed vector.

51. The vector of any of claims 45-50, wherein the nucleic acid sequence in the vector further comprises a poly(A) tail.

52. The vector of any of claims 45-51, wherein the nucleic acid sequence in the vector further comprises a 3'UTR.

53. A cell comprising the vector of any of claims 45-52.

54. A cell comprising a nucleic acid of any of claims 1-23.

55. A cell expressing a CAR molecule of any of claims 24-42.

56. The cell of any of claims 53-55, wherein the cell is a human T cell.

57. The cell of any of claims 53-55, wherein the T cell is a CD8+ T cell.

58. The cell of any of claims 53-57, further expressing an inhibitory polypeptide that comprises a first polypeptide that comprises at least a portion of an inhibitory molecule, associated with a second polypeptide that comprises a positive signal from an intracellular signaling domain.

59. The cell of claim 58, wherein the inhibitory polypeptide comprise first polypeptide that comprises at least a portion of PD1 and a second polypeptide comprising a costimulatory domain and primary signaling domain.

60. A method of making a cell comprising transducing a T cell with a vector of any of claims 45-52 or a nucleic acid of any of claims 1-23.

61. A method of generating a population of RNA-engineered cells comprising introducing an in vitro transcribed RNA or synthetic RNA into a cell, where the RNA comprises a nucleic acid encoding a CAR molecule of any of the claims 24-42.

62. A cell of any of claims 53-59, a nucleic acid of any of claims 1-23, or CAR molecule of any of claims 24-42, for use in providing an anti-tumor immunity.

63. Use of a cell of any of claims 53-59, a nucleic acid of any of claims 1-23, or CAR molecule of any of claims 24-42, in the manufacture of a medicament for the provision of an anti-tumor immunity.

64. The cell, nucleic acid, or CAR molecule of claim 62 or the use of claim 63, wherein the cell is an autologous T cell.

65. The cell, nucleic acid, or CAR molecule of claim 62 or 64, or the use of claim 63 or 64, wherein the cell is an allogeneic T cell.

66. The cell, nucleic acid, or CAR molecule of any of claims 62, 64, or 65, or the use of any of claims 63-65 wherein the anti-tumor immunity is provided in a human.

67. A cell of any of claims 53-59, a nucleic acid of any of claims 1-23, or a CAR molecule of any of claims 24-42, for use in treating a proliferative disease.

68. Use of a cell of any of claims 53-59, a nucleic acid of any of claims 1-23, or a CAR molecule of any of claims 24-42, in the manufacture of a medicament for the treatment of a proliferative disease.

69. The cell, nucleic acid, or CAR molecule of claim 67, or the use of claim 68 wherein the proliferative disease is a cancer or malignancy or a precancerous condition such as a myelodysplasia, a myelodysplastic syndrome or a preleukemia.

70. The cell, nucleic acid, or CAR molecule of claim 67 or 69, or the use of claim 68 or 69, wherein the proliferative disease is a proliferative disease in which CD19 is expressed.

71. The cell, nucleic acid, or CAR molecule of any of claims 67, 69, or 70, or the use of any of claims 68-70 wherein the disease is a hematologic cancer selected from the group consisting of one or more acute leukemias including but not limited to B-cell acute lymphoid leukemia ("BALL"), T-cell acute lymphoid leukemia ("TALL"), acute lymphoid leukemia (ALL); one or more chronic leukemias including but not limited to chronic myelogenous leukemia (CML), chronic lymphocytic leukemia (CLL); additional hematologic cancers or hematologic conditions including, but not limited to B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma, follicular lymphoma, hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma, mantle cell lymphoma, Marginal zone lymphoma, multiple myeloma, myelodysplasia and myelodysplastic syndrome, non-Hodgkin's lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, and preleukemia; and combinations thereof.

72. The cell, nucleic acid, or CAR molecule of any of claims 67 or 69-71, or the use of any of claims 68-71, wherein the cell, nucleic acid, or CAR molecule, or the medicament in the use is to be administered in combination with an agent that increases the efficacy of a cell expressing a CAR molecule, an agent that ameliorates one or more side effects associated with administration of a cell expressing a CAR molecule, or an agent that treats the proliferative disease.

73. The cell, nucleic acid, or CAR molecule of any of claims 67 or 69-72, or the use of any of claims 68-72, wherein the cell, nucleic acid, or CAR molecule, or the medicament in the use is to be administered in combination with a chemotherapeutic agent, autologous stem cell transplantation, or a lymphodepleting therapy such as melphalan.

74. The cell, nucleic acid, or CAR molecule of any of claims 67 or 69-73, or the use of any of claims 68-73, wherein the cell, nucleic acid, or CAR molecule, or the medicament in the use is to be administered with vincristine, dexamethasone, doxorubicin, methotrexate and 6-mercaptopurine.

75. The cell, nucleic acid, or CAR molecule of any of claims 67 or 69-74, or the use of any of claims 68-74, wherein the cell, nucleic acid, or CAR molecule, or the medicament in the use is to be administered in combination with a chemotherapeutic drug, immunosuppressive agent, therapy for CRS (cytokine release syndrome), or agent which inhibits PD1, PD-L1, CTLA4, TIM3, LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, or 2B4.

76. The cell, nucleic acid, or CAR molecule of any of claims 67 or 69-75, or the use of any of claims 68-75, wherein the cell, nucleic acid, or CAR molecule, or the medicament is to be administered at a dose of 10^4 to 10^9 cells/kg body weight, 10^5 to 10^6 cells/kg body weight, 1.4 x 10^7 to 1.1 x 10^9 cells per dose, up to 5 x10^7, 1 x 10^7 - 5 x 10^8, or 1 x 10^8 -5 x 10^8 cells per dose.

77. The cell, nucleic acid, or CAR molecule of any of claims 67 or 69-76, or the use of any of claims 68-76, wherein the cell, nucleic acid, or CAR molecule is to be administered in combination with, or the use comprises, one or more subsequent administrations the cell, nucleic acid, or CAR molecule.

78. The cell, nucleic acid, or CAR molecule of any of claims 67 or 69-77, or the use of any of claims 68-77, wherein the cell, nucleic acid, or CAR molecule is to be administered as, or the use comprises, more than one administration (e.g., 2, 3, or 4 administrations) in a week, of the cell, nucleic acid, or CAR molecule.