CLAIMS

1. A pharmaceutical composition comprising a cell and a pharmaceutically or physiologically acceptable carrier, diluent or excipient, wherein the cell comprises an isolated nucleic acid molecule encoding a chimeric antigen receptor (CAR), wherein the CAR comprises a scFv which comprises a CD19 binding domain, a transmembrane domain, and an intracellular signaling domain comprising a stimulatory domain, wherein said CD19 binding domain comprises:

(i) a light chain complementarity determining region 1 (LC CDR1) comprising the amino acid sequence of SEQ ID NO: 25,

(ii) a light chain complementarity determining region 2 (LC CDR2) comprising the amino acid sequence of SEQ ID NO: 26,

(iii) a light chain complementarity determining region 3 (LC CDR3) comprising the amino acid sequence of SEQ ID NO: 27,

(iv) a heavy chain complementarity determining region 1 (HC CDR1) comprising the amino acid sequence of SEQ ID NO: 19,

(v) a heavy chain complementarity determining region 2 (HC CDR2) comprising the amino acid sequence of any of SEQ ID NOS: 22, 21, or 23, and

(vi) a heavy chain complementarity determining region 3 (HC CDR3) comprising the amino acid sequence of SEQ ID NO: 24.

2. The pharmaceutical composition of claim 1, wherein the HC CDR2 comprises the amino acid sequence of SEQ ID NO: 21.

3. The pharmaceutical composition of claim 1, wherein the HC CDR2 comprises the amino acid sequence of SEQ ID NO: 22.

4. The pharmaceutical composition of claim 1, wherein the HC CDR2 comprises the amino acid sequence of SEQ ID NO: 23.

5. The pharmaceutical composition of claim 1, which encodes any light chain variable region listed in SEQ ID NOs: 32, 31, or 33-42.

6. The pharmaceutical composition of claim 1, which encodes any heavy chain variable region listed in SEQ ID NOs: 32, 31, or 33-42.

7. The pharmaceutical composition of claim 1, which encodes any light chain variable region listed in SEQ ID NOs: 32, 31, or 33-42 and any heavy chain variable region listed SEQ ID NOs: 32, 31, or 33-42.

8. The pharmaceutical composition of any of claims 1-4 or 6, wherein the light chain variable region comprises an amino acid sequence with at least 95% identity to an amino acid sequence provided in SEQ ID NOs: 32, 31, or 33-42.

9. The pharmaceutical composition of any of claims 1-5, wherein the heavy chain variable region comprises an amino acid sequence with at least 95% identity to an amino acid sequence provided in SEQ ID NOs: 32, 31, or 33-42.

10. The pharmaceutical composition of any of the preceding claims, wherein the CD19 binding domain comprises an amino acid sequence selected from the 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.

11. The pharmaceutical composition of any of the preceding claims, wherein the nucleic acid sequence encoding the CD19 binding domain comprises a nucleic acid sequence selected from the 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: 69, SEQ ID NO:70, SEQ ID NO:71 and SEQ ID NO:72, or a nucleic acid sequence with at least 95% identity thereof.

12. The pharmaceutical composition of any of the preceding claims, wherein the cell comprises a nucleic acid sequence selected from the 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.

13. The pharmaceutical composition of any of the preceding claims, wherein the encoded CAR comprises 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 or the encoded CAR comprises a transmembrane domain that comprises a transmembrane domain of the alpha chain of CD8.

14. The pharmaceutical composition 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 nucleic acid sequence of SEQ ID NO:56, or a sequence with at least 95% identity thereof.

15. The pharmaceutical composition of any of the preceding claims, wherein the encoded CD19 binding domain is connected to the transmembrane domain by a hinge region, wherein the hinge region is optionally a CD8 alpha hinge.

16. The pharmaceutical composition of claim 15, 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.

17. The pharmaceutical composition of any of the preceding claims, wherein the intracellular signaling domain comprises a costimulatory domain.

18. The pharmaceutical composition of claim 17, 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).

19. The pharmaceutical composition of claim 17 or 18, wherein the encoded costimulatory domain comprises the amino acid sequence of SEQ ID NO:16or 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.

20. The pharmaceutical composition of any of the preceding claims, wherein the intracellular signaling domain comprises a primary signaling domain.

21. The pharmaceutical composition 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.

22. The pharmaceutical composition of any of the preceding claims, wherein the encoded 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 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 the amino acid sequence of SEQ ID NO:17 or SEQ ID NO:43.

23. The pharmaceutical composition 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.

24. The pharmaceutical composition 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 the nucleic acid sequence of SEQ ID NO:101 or SEQ ID NO:44, or a nucleic acid sequence with at least 95% identity thereof.

25. The pharmaceutical composition of any of the preceding claims, wherein the isolated nucleic acid molecule further comprises a leader sequence.

26. The pharmaceutical composition of claim 25, 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.

27. A pharmaceutical composition comprising a cell and a pharmaceutically or physiologically acceptable carrier, diluent or excipient, wherein the cell comprises an isolated chimeric antigen receptor (CAR) molecule comprising: (i) a scFv which comprises a humanized CD19 binding domain, (ii) a transmembrane domain, and (iii) an intracellular signaling domain, wherein the humanized CD19 binding domain comprises:

(i) a light chain complementarity determining region 1 (LC CDR1) comprising the amino acid sequence of SEQ ID NO: 25,

(ii) a light chain complementarity determining region 2 (LC CDR2) comprising the amino acid sequence of SEQ ID NO: 26,

(iii) a light chain complementarity determining region 3 (LC CDR3) comprising the amino acid sequence of SEQ ID NO: 27,

(iv) a heavy chain complementarity determining region 1 (HC CDR1) comprising the amino acid sequence of SEQ ID NO: 19,

(v) a heavy chain complementarity determining region 2 (HC CDR2) comprising the amino acid sequence of any of SEQ ID NOS: 22, 21, or 23, and

(vi) a heavy chain complementarity determining region 3 (HC CDR3) comprising the amino acid sequence of SEQ ID NO: 24.

28. The pharmaceutical composition of claim 27, wherein the HC CDR2 comprises the amino acid sequence of SEQ ID NO: 21.

29. The pharmaceutical composition of claim 27, wherein the HC CDR2 comprises the amino acid sequence of SEQ ID NO: 22.

30. The pharmaceutical composition of claim 27, wherein the HC CDR2 comprises the amino acid sequence of SEQ ID NO: 23.

31. The pharmaceutical composition of any of claims 27-30, wherein the CD19 binding domain comprises a light chain and a heavy chain of an amino acid sequence listed in SEQ ID NOs: 32, 31, or 33-42.

32. The pharmaceutical composition of any of claims 28-30, wherein the CD19 binding domain comprises: (i) a light chain variable region listed in SEQ ID NOs: 32, 31, or 33-42, or (ii) an amino acid

sequence with at least 95% identity with a light chain variable region provided in SEQ ID NOs: 32, 31, or 33-42.

33. The pharmaceutical composition of any of claims 27-30 or 32, wherein the CD19 binding domain comprises: (i) a heavy chain variable region listed in SEQ ID NOs: 32, 31, or 33-42, or (ii) an amino acid sequence with at least 95% identity to a heavy chain variable region provided in SEQ ID NOs: 32, 31, or 33-42.

34. The pharmaceutical composition of any of claims 27-33, wherein the CD19 binding domain comprises an amino acid sequence selected from the 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, or

wherein the CD19 binding domain comprises 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, or an amino acid sequence with at least 95% identity thereof.

35. The pharmaceutical composition of any of claims 27-34, 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, or the transmembrane domain comprises a transmembrane domain of the alpha chain of CD8.

36. The pharmaceutical composition of claim 35, wherein the transmembrane domain comprises: (i) the 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.

37. The pharmaceutical composition of any one of claims 27-36, wherein the humanized CD19 binding domain is connected to the transmembrane domain by a hinge region, wherein optionally the hinge region is a CD8 alpha hinge.

38. The pharmaceutical composition of claim 37, 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.

39. The pharmaceutical composition of any of claims 27-38, wherein the intracellular signaling domain comprises a costimulatory domain.

40. The pharmaceutical composition of claim 39, 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).

41. The pharmaceutical composition of claim 39 or 40, wherein the costimulatory domain comprises: (i) 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.

42. The pharmaceutical composition of any one of claims 27-41, wherein the intracellular signaling domain comprises a primary signaling domain.

43. The pharmaceutical composition of claim 42, wherein the intracellular signaling domain comprises a functional signaling domain of 4-1BB and/or a functional signaling domain of CD3 zeta.

44. The pharmaceutical composition of claim 42 or 43 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.

45. The pharmaceutical composition of any of claims 42-44, 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.

46. The pharmaceutical composition of any of claims 27-45, wherein the CAR further comprises a leader sequence.

47. The pharmaceutical composition of claim 46, 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.

48. A pharmaceutical composition comprising a cell and a pharmaceutically or physiologically acceptable carrier, diluent or excipient, wherein the cell comprises a scFv comprising a humanized CD19 binding domain comprising:

(i) a light chain complementarity determining region 1 (LC CDR1) comprising the amino acid sequence of SEQ ID NO: 25,

(ii) a light chain complementarity determining region 2 (LC CDR2) comprising the amino acid sequence of SEQ ID NO: 26,

(iii) a light chain complementarity determining region 3 (LC CDR3) comprising the amino acid sequence of SEQ ID NO: 27,

(iv) a heavy chain complementarity determining region 1 (HC CDR1) comprising the amino acid sequence of SEQ ID NO: 19,

(v) a heavy chain complementarity determining region 2 (HC CDR2) comprising the amino acid sequence of any of SEQ ID NOS: 22, 21, or 23, and

(vi) a heavy chain complementarity determining region 3 (HC CDR3) comprising the amino acid sequence of SEQ ID NO: 24.

49. The pharmaceutical composition of claim 48, wherein the HC CDR2 comprises the amino acid sequence of SEQ ID NO: 21.

50. The pharmaceutical composition of claim 48, wherein the HC CDR2 comprises the amino acid sequence of SEQ ID NO: 22.

51. The pharmaceutical composition of claim 48, wherein the HC CDR2 comprises the amino acid sequence of SEQ ID NO: 23.

52. The pharmaceutical composition of any of claims 48-51, wherein the humanized CD19 binding domain comprises:

(i) a light chain variable region and a heavy chain variable region of an amino acid sequence of any of SEQ ID NOs: 32, 31, or 33-42;

(ii) a light chain variable region comprising an amino acid sequence with at least 95% identity to an amino acid sequence of a light chain variable region provided in any of SEQ ID NOs: 32, 31, or 33-42; and/or

(iii) a heavy chain variable region comprising an amino acid sequence with at least 95% identity to an amino acid sequence of a heavy chain variable region provided in any of SEQ ID NOs: 32, 31, or 33-42.

53. A humanized CD19 binding domain comprising:

(i) a light chain complementarity determining region 1 (LC CDR1) comprising the amino acid sequence of SEQ ID NO: 25,

(ii) a light chain complementarity determining region 2 (LC CDR2) comprising the amino acid sequence of SEQ ID NO: 26,

(iii) a light chain complementarity determining region 3 (LC CDR3) comprising the amino acid sequence of SEQ ID NO: 27,

(iv) a heavy chain complementarity determining region 1 (HC CDR1) comprising the amino acid sequence of SEQ ID NO: 19,

(v) a heavy chain complementarity determining region 2 (HC CDR2) comprising the amino acid sequence of any of SEQ ID NOS: 22, 21, or 23, and

(vi) a heavy chain complementarity determining region 3 (HC CDR3) comprising the amino acid sequence of SEQ ID NO: 24;

wherein the CD19 binding domain:

(i) retains the affinity for human CD19 of an scFv having the amino acid sequence of SEQ ID NO:59.

54. An isolated chimeric antigen receptor (CAR) molecule comprising a humanized CD19 binding domain as defined in claim 53, a transmembrane domain, and an intracellular signaling domain.

55. An isolated nucleic acid molecule encoding a CD19 binding domain as defined in claim 53 or a CAR molecule as defined in claim 54.

56. A vector comprising a nucleic acid molecule encoding the CAR molecule as defined in any one of any of claims 27-47 or 54.

57. The vector of claim 56, wherein the vector is selected from the group consisting of a DNA, an RNA, a plasmid, a viral vector, a lentivirus vector, an adenoviral vector, and a retrovirus vector.

58. The vector of claim 56 or 57, further comprises a promoter.

59. The vector of claim 58, wherein the promoter is an EF-1 promoter.

60. The vector of claim 59, wherein the EF-1 promoter comprises the nucleic acid sequence of SEQ ID NO: 100.

61. The vector of any of claims 56-60, wherein the vector is an in vitro transcribed vector.

62. The vector of any of claims 56-61, wherein the nucleic acid sequence in the vector further comprises a poly(A) tail.

63. The vector of any of claims 56-62, wherein the nucleic acid sequence in the vector further comprises a 3'UTR.

64. A cell comprising the vector of any of claims 56-63.

65. A cell comprising the nucleic acid molecule of claim 55.

66. A cell expressing the CAR molecule of claim 54.

67. The cell of any of claims 64-66, wherein the cell is a human T cell.

68. The cell of any of claims 64-66, wherein the cell is a CD8+ T cell.

69. A method of making a cell comprising transducing a T cell with the vector of any of claims 56-63 or the nucleic acid molecule of claim 55.

70. 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 the CAR molecule of claim 55.

71. The pharmaceutical composition of any one of claims 1-47, for use in providing an anti-tumor immunity to a subject having a hematological cancer that is positive for CD19, wherein the cell within the pharmaceutical composition is a T cell or an NK cell.

72. Use of the pharmaceutical composition of any one of claims 1-47, in the manufacture of a medicament for the provision of an anti-tumor immunity to a subject having a hematological cancer that is positive for CD19, wherein the cell within the pharmaceutical composition is a T cell or an NK cell.

73. The pharmaceutical composition for use of claim 71, or the use of claim 72, wherein the cell is an autologous T cell.

74. The pharmaceutical composition for use of claim 71, or the use of claim 72, wherein the T cell is an allogeneic T cell.

75. The pharmaceutical composition for use of claim 71, or the use of claim 72, wherein the anti-tumor immunity is provided in a human.

76. The pharmaceutical composition of any one of claims 1-47, for use in treating a hematological cancer that is positive for CD19, wherein the cell within the pharmaceutical composition is a T cell or an NK cell.

77. Use of the pharmaceutical composition of any one of claims 1-47, in the manufacture of a medicament for the treatment of a hematological cancer that is positive for CD19, wherein the cell within the pharmaceutical composition is a T cell or an NK cell.

78. The pharmaceutical composition for use of any one of claims 71 or 73-76 or the use of any one of claims 72-75 or 77, wherein the hematologic cancer that is positive for CD19 is selected from the group consisting of B-cell acute lymphoblastic leukemia (“BALL”), T-cell acute lymphoblastic leukemia

("TALL"), acute lymphoblastic leukemia (ALL); chronic myelogenous leukemia (CML), chronic lymphocytic leukemia (CLL); B cell prolymphocytic leukemia Burkitt's lymphoma, diffuse large B cell lymphoma, follicular lymphoma, hairy cell leukemia, small cell- or a large cell-follicular lymphoma, MALT lymphoma, mantle cell lymphoma, Marginal zone lymphoma, myelodysplasia and myelodysplastic syndrome, non-Hodgkin's lymphoma, plasmablastic lymphoma, Waldenstrom macroglobulinemia; and combinations thereof.

79. The pharmaceutical composition for use of any one of claims 71, 73-76, or 78, or the use of any one of claims 72-75, 77, or 78, which 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 hematological cancer that is positive for CD19.

80. The pharmaceutical composition for use of any one of claims 71, 73-76, 78, or 79, or the use of any one of claims 72-75 or 77-79, which is to be administered in combination with a chemotherapeutic drug, immunosuppressive agent, therapy for CRS (cytokine release syndrome), or agent which inhibits PD-1, PD-L1, CTLA-4, TIM-3, LAG-3, VISTA, BTLA, TIGIT, LAIR1, CD160, or 2B4.

81. The pharmaceutical for use of any one of claims 71, 73-76, or 78-80, or the use of any one of claims 72-75 or 77-80, wherein:

the cell expressing a CAR molecule is to be administered at a dose of 10^4 to 109 cells/kg body weight, 10^5 to 10^6 cells/kg body weight, 1.4 x $l0^7$ to 1.1 x $l0^9$ cells per dose, up to 5 x10^7, 1 x 10^7 - 5 x 10^8, or l x 10^8 -5 x 10^8 cells per dose;

the use comprises one or more subsequent administrations of the cell, nucleic acid molecule, or CAR molecule; or

the use comprises more than one administration (e.g., 2, 3, or 4 administrations) in a week, of the cell, nucleic acid molecule, or CAR molecule.