CLAIMS

1. An anti-CC chemokine receptor 4 (CCR4) chimeric antigen receptor (CAR) comprising an anti-CCR4 antigen binding domain, a transmembrane domain, and an intracellular domain.

2. The CAR of claim 1, wherein the anti-CCR4 antigen binding domain comprises at least one heavy chain variable region (HCDR) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, and SEQ ID NO: 6.

3. The CAR of claim 1 or claim 2, wherein the anti-CCR4 antigen binding domain comprises at least one light chain variable region (LCDR) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12.

4. The CAR of any one of claims 1-3, wherein the anti-CCR4 antigen binding domain is selected from the group consisting of a full length antibody or antigen-binding fragment thereof, a Fab, a single-chain variable fragment (scFv), or a single-domain antibody.

5. The CAR of any one of claims 1-4, wherein the anti-CCR4 antigen binding domain is an anti-CCR4 single-chain variable fragment (scFv).

6. The CAR of any one of claims 1-5, wherein the anti-CCR4 antigen binding domain is an anti-CCR4 single-chain variable fragment (scFv) having a heavy chain and a light chain derived from mogamulizumab.

7. The CAR of any one of claims 1-6, wherein the anti-CCR4 antigen binding domain comprises a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 112.

8. The CAR of any one of claims 1-7, wherein the anti-CCR4 antigen binding domain comprises a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 114.

9. The CAR of any one of claims 1-8, wherein the anti-CCR4 antigen binding domain comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 16.

10. The CAR of any one of the preceding claims, wherein the CAR further comprises a CD8 alpha hinge, optionally wherein the CD8 alpha hinge comprises the amino acid sequence set forth in SEQ ID NO: 34.

11. The CAR of any one of the preceding claims, wherein the transmembrane domain comprises a transmembrane domain selected from the group consisting of an artificial hydrophobic sequence, and a transmembrane domain of a type I transmembrane protein, an alpha, beta, or zeta chain of a T cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, OX40 (CD134), 4-1BB (CD137), ICOS, and CD154, or a transmembrane domain derived from a killer immunoglobulin-like receptor (KIR).

12. The CAR of any one of the preceding claims, wherein the transmembrane domain comprises a CD8 alpha transmembrane domain, optionally wherein the CD8 alpha transmembrane domain comprises the amino acid sequence set forth in SEQ ID NO: 36.

13. The CAR of any one of the preceding claims, wherein the intracellular domain comprises a costimulatory domain and an intracellular signaling domain.

14. The CAR of any one of the preceding claims, wherein the intracellular domain comprises a costimulatory domain of a protein selected from the group consisting of proteins in the TNFR superfamily, CD28, 4-1BB (CD137), OX40 (CD134), PD-1, CD7, LIGHT, CD83L, DAP10, DAP12, CD27, CD2, CD5, ICAM-1, LFA-1, Lck, TNFR-I, TNFR-II,

Fas, CD30, CD40, ICOS, NKG2C, and B7-H3 (CD276), or a variant thereof, or an intracellular domain derived from a killer immunoglobulin-like receptor (KIR).

15. The CAR of any one of the preceding claims, wherein the intracellular domain comprises a costimulatory domain of 4-1BB optionally comprising the amino acid sequence set forth in SEQ ID NO: 38.

16. The CAR of any one of the preceding claims, wherein the intracellular domain comprises an intracellular signaling domain of a protein selected from the group consisting of a human CD3 zeta chain (CD3ζ), FcγRIII, FcsRI, a cytoplasmic tail of an Fc receptor, an immunoreceptor tyrosine-based activation motif (ITAM) bearing cytoplasmic receptor, TCR zeta, FcR gamma, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, and CD66d, or a variant thereof.

17. The CAR of any one of the preceding claims, wherein the intracellular signaling domain comprises an intracellular signaling domain of CD3ζ or a variant thereof, optionally comprising the amino acid sequence set forth in SEQ ID NO: 40.

18. The CAR of any one of the preceding claims, wherein the CAR comprises an anti-CCR4 antigen binding domain comprising an anti-CCR4 scFv, a CD8 alpha hinge, a CD8 alpha transmembrane domain, an intracellular domain comprising a 4-1BB costimulatory domain and a CD3ζ intracellular signaling domain.

19. The CAR of claim 18, wherein the CAR further comprises a CD8 leader sequence, optionally comprising the amino acid sequence set forth in SEQ ID NO: 32.

20. The CAR of any one of the preceding claims, wherein the CAR comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID NOs: 30, 54, 62, 70, 82, 90, and 102.

21. The CAR of any one of the preceding claims, wherein the CAR is encoded by a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID NOs: 29, 53, 61, 69, 81, 89, and 101.

22. The CAR of any one of the preceding claims, wherein the CAR is linked to a safety switch via a self-cleavable linker, and wherein the safety switch binds a safety switch agent.

23. The CAR of claim 22, wherein the safety switch is selected from:
    (a) truncated EGFR (EGFRt), wherein the safety switch agent is an anti-EFGR antibody, optionally cetuximab;
    (b) CD20, wherein the safety switch agent is an anti-CD20 antibody, optionally rituximab;
    (c) inducible caspase 9 (iCasp9), wherein the safety switch agent is AP1903; and
    (d) Herpes simplex virus-1 thymidine kinase (HSVTK), wherein the safety switch agent is ganciclovir (GCV).

24. The CAR of claim 22 or claim 23, wherein the CAR is linked to a dominant negative TGFb receptor type II (dnTGFbRII) via a self-cleavable linker.

25. The CAR of claim 22 or claim 23, wherein the safety switch is linked to a dominant negative TGFb receptor type II (dnTGFbRII) via a self-cleavable linker.

26. A nucleic acid comprising a polynucleotide sequence encoding an anti-CCR4 CAR, wherein the CAR comprises an anti-CCR4 antigen binding domain, a transmembrane domain, and an intracellular domain.

27. The nucleic acid of claim 26, wherein the anti-CCR4 antigen binding domain comprises at least one heavy chain variable region (HCDR) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, and SEQ ID NO: 6.

28. The nucleic acid of claim 25 or claim 27, wherein the anti-CCR4 antigen binding domain comprises at least one light chain variable region (LCDR) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12.

29. The nucleic acid of any one of claims 26-28, wherein the anti-CCR4 antigen binding domain is selected from the group consisting of a full length antibody or antigen-binding fragment thereof, a Fab, a single-chain variable fragment (scFv), or a single-domain antibody.

30. The nucleic acid of any one of claims 26-29, wherein the anti-CCR4 antigen binding domain is an anti-CCR4 single-chain variable fragment (scFv).

31. The nucleic acid of any one of claims 26-30, wherein the anti-CCR4 antigen binding domain is an anti-CCR4 single-chain variable fragment (scFv) having a heavy chain and a light chain derived from mogamulizumab.

32. The nucleic acid of any one of claims 26-31, wherein the anti-CCR4 antigen binding domain comprises a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 112.

33. The nucleic acid of any one of claims 26-32, wherein the anti-CCR4 antigen binding domain comprises a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 114.

34. The nucleic acid of any one of claims 26-33, wherein the anti-CCR4 antigen binding domain comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 16.

35. The nucleic acid of any one of the preceding claims, wherein the CAR further comprises a CD8 alpha hinge, optionally wherein the CD8 alpha hinge comprises the amino acid sequence set forth in SEQ ID NO: 34.

36. The nucleic acid of any one of the preceding claims, wherein the transmembrane domain comprises a transmembrane domain selected from the group consisting of an artificial hydrophobic sequence, and a transmembrane domain of a type I transmembrane protein, an alpha, beta, or zeta chain of a T cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, OX40 (CD134), 4-1BB (CD137), ICOS, and CD154, or a transmembrane domain derived from a killer immunoglobulin-like receptor (KIR).

37. The nucleic acid of any one of the preceding claims, wherein the transmembrane domain comprises a CD8 alpha transmembrane domain, optionally wherein the CD8 alpha transmembrane domain comprises the amino acid sequence set forth in SEQ ID NO: 36.

38. The nucleic acid of any one of the preceding claims, wherein the intracellular domain comprises a costimulatory domain and an intracellular signaling domain.

39. The nucleic acid of any one of the preceding claims, wherein the intracellular domain comprises a costimulatory domain of a protein selected from the group consisting of proteins in the TNFR superfamily, CD28, 4-1BB (CD137), OX40 (CD134), PD-1, CD7, LIGHT, CD83L, DAP10, DAP12, CD27, CD2, CD5, ICAM-1, LFA-1, Lck, TNFR-I, TNFR-II, Fas, CD30, CD40, ICOS, NKG2C, and B7-H3 (CD276), or a variant thereof, or an intracellular domain derived from a killer immunoglobulin-like receptor (KIR).

40. The nucleic acid of any one of the preceding claims, wherein the intracellular domain comprises a costimulatory domain of 4-1BB optionally comprising the amino acid sequence set forth in SEQ ID NO: 38.

41. The nucleic acid of any one of the preceding claims, wherein the intracellular domain comprises an intracellular signaling domain of a protein selected from the group consisting of a human CD3 zeta chain (CD3ζ), FcγRIII, FcsRI, a cytoplasmic tail of an Fc receptor, an immunoreceptor tyrosine-based activation motif (ITAM) bearing cytoplasmic receptor, TCR zeta, FcR gamma, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, and CD66d, or a variant thereof.

42. The nucleic acid of any one of the preceding claims, wherein the intracellular signaling domain comprises an intracellular signaling domain of CD3ζ or a variant thereof, optionally comprising the amino acid sequence set forth in SEQ ID NO: 40.

43. The nucleic acid of any one of the preceding claims, wherein the CAR comprises an anti-CCR4 antigen binding domain comprising an anti-CCR4 scFv, a CD8 alpha hinge, a CD8 alpha transmembrane domain, an intracellular domain comprising a 4-1BB costimulatory domain and a CD3ζ intracellular signaling domain.

44. The nucleic acid of claim 43, wherein the CAR further comprises a CD8 leader sequence, optionally comprising the amino acid sequence set forth in SEQ ID NO: 32.

45. The nucleic acid of any one of the preceding claims, wherein the CAR comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID NOs: 30, 54, 62, 70, 82, 90, and 102.

46. The nucleic acid of any one of the preceding claims, wherein the CAR is encoded by a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID NOs: 29, 53, 61, 69, 81, 89, and 101.

47. The nucleic acid of any one of the preceding claims, wherein the CAR is linked to a safety switch via a self-cleavable linker, and wherein the safety switch binds a safety switch agent.

48. The nucleic acid of claim 47, wherein the safety switch is selected from:
    (a) truncated EGFR (EGFRt), wherein the safety switch agent is an anti-EFGR antibody, optionally cetuximab;
    (b) CD20, wherein the safety switch agent is an anti-CD20 antibody, optionally rituximab;
    (c) inducible caspase 9 (iCasp9), wherein the safety switch agent is AP1903; and
    (d) Herpes simplex virus-1 thymidine kinase (HSVTK), wherein the safety switch agent is ganciclovir (GCV).

49. The nucleic acid of claim 47 or claim 48, wherein the CAR is linked to a dominant negative TGFb receptor type II (dnTGFbRII) via a self-cleavable linker.

50. The nucleic acid of claim 47 or claim 48, wherein the safety switch is linked to a dominant negative TGFb receptor type II (dnTGFbRII) via a self-cleavable linker.

51. A vector comprising the nucleic acid of any one of claims 26-50.

52. The vector of claim 51, wherein the vector is a viral vector selected from the group consisting of a retroviral vector, a lentiviral vector, an adenoviral vector, and an adeno-associated viral vector.

53. The vector of claim 52, wherein the viral vector is a lentiviral vector.

54. A modified immune cell or precursor cell thereof, comprising the CAR of any one of claims 1-25, the nucleic acid of any one of claims 26-50, and/or the vector of any one of claims 51-53.

55. The modified immune cell or precursor cell thereof of claim 54, further comprising one or more of the following;
    (a) a CCR4 null knockout allele;
    (b) suppressed CCR4 gene expression; and

(c) a fusion protein comprising an anti-CCR4 scFv and a KDEL motif and/or a nucleic acid encoding the fusion protein.

56. The modified immune cell or precursor cell thereof of claim 55, wherein the CCR4 null knockout allele or suppressed CCR4 gene expression is obtained via a genetic engineering technique comprising a nuclease selected from the group consisting of a clustered regularly interspaced short palindromic repeats (CRISPR) associated nuclease, a transcription activator-like effector nuclease (TALEN), and a zinc-finger nuclease.

57. The modified immune cell or precursor cell thereof of claim 55 or 56, further comprising an inhibitory RNA molecule which suppresses CCR4 gene expression.

58. The modified immune cell or precursor cell thereof of claim 57, wherein the inhibitory RNA molecule is selected from the group consisting of: an RNA interference (RNAi) RNA, a short hairpin RNA (shRNA), a small interfering RNA (siRNA), a trans-acting siRNA (tasiRNA), a micro RNA (miRNA), an antisense RNA (asRNA), a long noncoding RNA (lncRNA), a CRISPR RNA (crRNA), a trans-activating crRNA (tracrRNA), a guide RNA (gRNA), a single guide RNA (sgRNA), a double-stranded RNA (dsRNA), a ribozyme, and any combination thereof.

59. The modified immune cell or precursor cell thereof of any one of claims 54-58, wherein the modified cell is an autologous cell.

60. The modified immune cell or precursor cell thereof of any one of claims 54-59, wherein the modified immune cell is derived from peripheral blood mononuclear cells (PBMCs).

61. The modified immune cell or precursor cell thereof of claim 60, wherein the PBMCs comprise tumor cells.

62. The modified immune cell or precursor cell thereof of any one of claims 54-61, wherein the modified immune cell or precursor cell thereof is a cell isolated from a human subject.

63. The modified immune cell or precursor cell thereof of any one of claims 54-62, wherein the modified immune cell is a modified T cell.

64. A method for generating a modified immune cell or precursor cell thereof, comprising introducing into the immune or precursor cell thereof the nucleic acid of any one of claims 26-50 or the vector of any one of claim 51-53.

65. The method of claim 58, wherein the vector is introduced via viral transduction.

66. The modified immune or precursor cell thereof of any one of claims 54-63, or a modified immune or precursor cell thereof generated by the method of claim 64 or claim 65, for use in treating cancer in a subject in need thereof.

67. The modified immune or precursor cell thereof of claim 66, wherein the modified cell depletes CCR4-positive T cells and not CCR4-negative T cells in the subject, thereby treating the cancer.

68. The modified immune or precursor cell thereof of claim 66 or 67, wherein the subject has previously been administered mogamulizumab.

69. The modified immune or precursor cell thereof of any one of claims 66-68, wherein the cancer is refractory to mogamulizumab.

70. A modified T cell comprising an anti-CCR4 CAR, wherein the CAR comprises an anti-CCR4 antigen binding domain, a transmembrane domain, and an intracellular domain, for use in treating cancer in a subject in need thereof.

71. The modified T cell of claim 70, wherein the anti-CCR4 antigen binding domain comprises at least one heavy chain variable region (HCDR) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, and SEQ ID NO: 6.

72. The modified T cell of claim 70 or claim 71, wherein the anti-CCR4 antigen binding domain comprises at least one light chain variable region (LCDR) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12.

73. The modified T cell of any one of claims 70-72, wherein the anti-CCR4 antigen binding domain is selected from the group consisting of a full length antibody or antigen-binding fragment thereof, a Fab, a single-chain variable fragment (scFv), or a single-domain antibody.

74. The modified T cell of any one of claims 70-73, wherein the anti-CCR4 antigen binding domain is an anti-CCR4 single-chain variable fragment (scFv).

75. The modified T cell of any one of claims 70-74, wherein the anti-CCR4 antigen binding domain is an anti-CCR4 single-chain variable fragment (scFv) having a heavy chain and a light chain derived from mogamulizumab.

76. The modified T cell of any one of claims 70-75, wherein the anti-CCR4 antigen binding domain comprises a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 112.

77. The modified T cell of any one of claims 70-76, wherein the anti-CCR4 antigen binding domain comprises a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 114.

78. The modified T cell of any one of claims 70-77, wherein the anti-CCR4 antigen binding domain comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 16.

79. The modified T cell of any one of the preceding claims, wherein the CAR further comprises a CD8 alpha hinge, optionally wherein the CD8 alpha hinge comprises the amino acid sequence set forth in SEQ ID NO: 34.

80. The modified T cell of any one of the preceding claims, wherein the transmembrane domain comprises a transmembrane domain selected from the group consisting of an artificial hydrophobic sequence, and a transmembrane domain of a type I transmembrane protein, an alpha, beta, or zeta chain of a T cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, OX40 (CD134), 4-1BB (CD137), ICOS, and CD154, or a transmembrane domain derived from a killer immunoglobulin-like receptor (KIR).

81. The modified T cell of any one of the preceding claims, wherein the transmembrane domain comprises a CD8 alpha transmembrane domain, optionally wherein the CD8 alpha transmembrane domain comprises the amino acid sequence set forth in SEQ ID NO: 36.

82. The modified T cell of any one of the preceding claims, wherein the intracellular domain comprises a costimulatory domain and an intracellular signaling domain.

83. The modified T cell of any one of the preceding claims, wherein the intracellular domain comprises a costimulatory domain of a protein selected from the group consisting of proteins in the TNFR superfamily, CD28, 4-1BB (CD137), OX40 (CD134), PD-1, CD7, LIGHT, CD83L, DAP10, DAP12, CD27, CD2, CD5, ICAM-1, LFA-1, Lck, TNFR-I, TNFR-II, Fas, CD30, CD40, ICOS, NKG2C, and B7-H3 (CD276), or a variant thereof, or an intracellular domain derived from a killer immunoglobulin-like receptor (KIR).

84. The modified T cell of any one of the preceding claims, wherein the intracellular domain comprises a costimulatory domain of 4-1BB optionally comprising the amino acid sequence set forth in SEQ ID NO: 38.

85. The modified T cell of any one of the preceding claims, wherein the intracellular domain comprises an intracellular signaling domain of a protein selected from the group consisting of a human CD3 zeta chain (CD3ζ), FcγRIII, FcsRI, a cytoplasmic tail of an Fc receptor, an immunoreceptor tyrosine-based activation motif (ITAM) bearing cytoplasmic receptor, TCR zeta, FcR gamma, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, and CD66d, or a variant thereof.

86. The modified T cell of any one of the preceding claims, wherein the intracellular signaling domain comprises an intracellular signaling domain of CD3ζ or a variant thereof, optionally comprising the amino acid sequence set forth in SEQ ID NO: 40.

87. The modified T cell of any one of the preceding claims, wherein the CAR comprises an anti-CCR4 antigen binding domain comprising an anti-CCR4 scFv, a CD8 alpha hinge, a CD8 alpha transmembrane domain, an intracellular domain comprising a 4-1BB costimulatory domain and a CD3ζ intracellular signaling domain.

88. The modified T cell of claim 87, wherein the CAR further comprises a CD8 leader sequence, optionally comprising the amino acid sequence set forth in SEQ ID NO: 32.

89. The modified T cell of any one of the preceding claims, wherein the CAR comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID NOs: 30, 54, 62, 70, 82, 90, and 102.

90. The modified T cell of any one of the preceding claims, wherein the CAR is encoded by a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID NOs: 29, 53, 61, 69, 81, 89, and 101.

91. The modified T cell of any one of the preceding claims, wherein the CAR is linked to a safety switch via a self-cleavable linker, and wherein the safety switch binds a safety switch agent.

92. The modified T cell of claim 91, wherein the safety switch is selected from:
    (a) truncated EGFR (EGFRt), wherein the safety switch agent is an anti-EFGR antibody, optionally cetuximab;
    (b) CD20, wherein the safety switch agent is an anti-CD20 antibody, optionally rituximab;
    (c) inducible caspase 9 (iCasp9), wherein the safety switch agent is AP1903; and
    (d) Herpes simplex virus-1 thymidine kinase (HSVTK), wherein the safety switch agent is ganciclovir (GCV).

93. The modified T cell of claim 91 or claim 92, wherein the CAR is linked to a dominant negative TGFb receptor type II (dnTGFbRII) via a self-cleavable linker.

94. The modified T cell of claim 91 or claim 92, wherein the safety switch is linked to a dominant negative TGFb receptor type II (dnTGFbRII) via a self-cleavable linker.

95. The modified T cell of any one of claims 91-94, wherein the use further comprises use of the safety switch agent.

96. The modified T cell of any one of claims 70-95, wherein the modified T cell depletes CCR4-positive T cells and not CCR4-negative T cells in the subject, thereby treating the cancer.

97. The modified T cell of any one of claims 70-96, wherein the modified T cell is derived from PBMCs.

98. The modified T cell of claim 97, wherein the PBMCs comprise tumor cells.

99. The modified T cell of any one of claims 70-98, wherein the modified T cell is a human T cell.

100. The modified T cell of any one of claims 70-99, wherein the modified T cell is autologous.

101. The modified T cell of any one of claims 70-100, wherein the subject is human.

102. The modified T cell of any one of claims 70-101, wherein the subject has previously been administered mogamulizumab.

103. The modified T cell of any one of claims 70-102, wherein the cancer is refractory to mogamulizumab.