

What Is Claimed Is:

1. A chimeric antigen receptor (CAR) comprising:
 - (a) an extracellular antigen binding domain;
 - 5 (b) a 4-1BB co-stimulatory domain;
 - (c) an IL-2R β cytoplasmic signaling domain; and
 - (d) a CD3 ζ signaling domain.

2. The CAR of claim 1, wherein the 4-1BB co-stimulatory signaling domain

10 comprises the amino acid sequence set forth in

KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFEEEEGGCEL (SEQ ID NO: 1).

3. The CAR of claim 1 or 2, wherein the IL-2R β cytoplasmic signaling domain

comprises the amino acid sequence set forth in

15 NCRNTGPWLKKVLKCNTDPDSKFFSQLSSEHGGDVQKWLSSPFPSSSFSPGGLAPE

ISPLEVLERDKVTQLLPLNTDAYLSLQELQGQDPHTLV (SEQ ID NO: 2).

4. The CAR of any one of claims 1-3, wherein the CD3 ζ signaling domain

comprises the amino acid sequence set forth

20 inRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPGEMGGKPRRK

NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH

MQALPPR (SEQ ID NO: 3).

5. The CAR of any one of claims 1-4, further comprising a transmembrane

25 domain, which is C-terminal to the extracellular antigen binding domain and N-terminal to

the 4-1BB co-stimulatory domain.

6. The CAR of claim 5, wherein the transmembrane domain is derived from a cell

surface receptor selected from the group consisting of the alpha, beta or zeta chain of the

30 T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33,

CD37, CD64, CD80, CD86, CD134, CD137, CD154, CD271, TNFRSF19 and Killer Cell

Immunoglobulin-Like Receptor (KIR), or any combination thereof.

7. The CAR of any one of claims 1-6, further comprising a hinge domain linked to the C-terminus of the extracellular antigen binding domain and to the N-terminus of the transmembrane domain.

5 8. The CAR of claim 7, wherein the hinge domain is of CD28, CD8, or an IgG, which optionally is IgG1 or IgG4.

9. The CAR of any one of claims 1-8, further comprising a STAT3 binding motif, which is located at the C-terminal of the CD3 ζ signaling domain.

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10. The CAR of claim 9, wherein the STAT3 binding motif comprises the amino sequence set forth in YX₁X₂Q, wherein X₁ and X₂ are each independently an amino acid.

11. The CAR of claim 10, wherein the STAT3 binding motif comprises the amino acid sequence set forth in YRHQ (SEQ ID NO: 4).

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12. The CAR of any one of claims 9-11, which comprises a C-terminus fragment comprising the CD3 ζ signaling domain and the STAT3 binding motif, and wherein the C-terminus fragment comprises the amino acid sequence set forth
20 inRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK
NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAYR
HQALPPR (SEQ ID NO: 5).

13. The CAR of any one of claims 1-12, wherein the extracellular antigen binding domain binds a tumor associated antigen, which optionally is selected from the group consisting of 5T4, CD2, CD5, CD3, CD 7, CD19, CD20, CD22, CD30, CD33, CD38, CD70, CD123, CD133, CD171, CEA, CS1, Claudin 18.2, BCMA, BAFF-R, PSMA, PSCA, desmoglein (Dsg3), HER-2, FAP, FSHR, NKG2D, GD2, EGFRVIII, mesothelin, ROR1, MAGE, MUC1, MUC16, GPC3, Lewis Y, and VEGFR11.

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14. The CAR of any one of claims 1-13, wherein the extracellular antigen binding domain is a single-chain antibody fragment (scFv).

15. The CAR of claim 14, wherein the scFv binds CD19 and comprises the amino acid sequence set forth in SEQ ID NO: 6, 39, 40 or 41.

5 16. The CAR of claim 14, wherein the scFv binds BCMA and comprises the amino acid sequence set forth in SEQ ID NO: 7.

17. The CAR of any one of claims 1-16, further comprising a signal peptide located at the N-terminus of the CAR.

10 18. A population of immune cells, comprising a first plurality of immune cells that express the CAR of any one of claims 1-17.

15 19. The population of immune cells of claim 18, further comprising a second plurality of immune cells that express an antibody specific to interleukin-6 (IL-6) or IL-6 receptor (IL-6R).

20 20. The population of immune cells of claim 19, wherein the antibody comprises the same heavy chain complementarity determining domains (CDRs) and the same light chain CDRs as a reference antibody, and wherein the reference antibody comprises (a) a heavy chain variable domain (V_H) amino acid sequence set forth in SEQ ID NO: 14, 16, 18, 20, 22, or 24 and a light chain variable domain (V_L) amino acid sequence set forth in SEQ ID NO: 15, 17, 19, 21, 23, or 25.

25 21. The population of immune cells of claim 20, wherein the antibody specific to IL-6 or IL-6R comprises the same V_H and the same V_L as the reference antibody.

22. The population of immune cells of any one of claims 19-21, wherein the antibody specific to IL-6 or IL-6R is a scFv.

30 23. The population of immune cells of claim 22, wherein the scFv comprises the amino acid sequence set forth in SEQ ID NO: 8, 9, 26, or 27.

24. The population of immune cells of any one of claims 18-23, further comprising a third plurality of immune cells that express an IL-1 antagonist.

25. The population of immune cells of claim 24, wherein the IL-1 antagonist is IL-1RA.

5 26. The population of immune cells of any one of claims 19-25, wherein at least two of the first plurality of immune cells, the second plurality of immune cells, and the third plurality of immune cells comprise common members.

10 27. The population of immune cells of claim 26, wherein at least 10% of the immune cells therein express the CAR, the antibody specific to IL-6 or IL-6R, and the IL-1 antagonist.

15 28. The population of immune cells of claim 27, wherein about 50-70% of the cells express the CAR, the antibody specific to IL-6 or IL-6R, and the IL-1 antagonist.

20 29. The population of immune cells of any one of claims 18-28, wherein the immune cells are T-cells, Natural Killer (NK) cells, tumor infiltrating lymphocytes, dendritic cells, macrophages, B cells, neutrophils, eosinophils, basophils, mast cells, myeloid-derived suppressor cells, mesenchymal stem cells, precursors thereof, or a combination thereof.

25 30. The population of immune cells of any one of claims 18-29, wherein the immune cells are T cells, and wherein at least a portion of the T cells do not express one or more of an endogenous T cell receptor, CD52, interferon gamma (IFN- γ), beta-2 microglobulin (B2M), and granulocyte macrophage-colony stimulating factor (GM-CSF).

31. The population of immune cells of claim 30, wherein the portion of the T cells do not express IFN- γ .

30 32. A method of producing a population of modified immune cells, the method comprising:

(a) providing a population of immune cells; and

(b) introducing into the immune cells a first nucleic acid coding for the CAR of any one of claims 1-17.

33. The method of claim 32, further comprising introducing into the immune cells a second nucleic acid coding for an antibody specific to interleukin-6 (IL-6) or IL-6 receptor (IL-6R), wherein the antibody is set forth in any one of claims 19-23.

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34. The method of claim 33, wherein the first nucleic acid and the second nucleic acid are located in the same vector.

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35. The method of claim 33, wherein the first nucleic acid and the second nucleic acid are located in different vectors

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36. The method of any one of claims 32-35, further comprising introducing into the immune cells a third nucleic acid encoding an IL-1 antagonist, which optionally is IL-1RA.

37. The method of claim 36, wherein the first nucleic acid and the third nucleic acid are located in the same vector.

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38. The method of claim 36, wherein the second nucleic acid and the third nucleic acid are located in the same vector.

39. The method of claim 36, wherein the first nucleic acid, the second nucleic acid, and the third nucleic acid are located in different vectors.

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40. The method of any one of claims 32-39, wherein the immune cells are T cells, Natural Killer (NK) cells, tumor infiltrating lymphocytes, dendritic cells, macrophages, B cells, neutrophils, eosinophils, basophils, mast cells, myeloid-derived suppressor cells, mesenchymal stem cells, precursors thereof, or a combination thereof; optionally wherein the immune cell is a human immune cell.

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41. A cell therapy-based method of treating a disease, comprising administering to a subject in need thereof the population of immune cells of any one of claims 18-31.

42. The method of claim 41, wherein the subject is a human patient.

43. The method of claim 41 or 42, wherein the disease is a cancer, an infectious disease, or an immune disorder.

5 44. The method of claim 42, wherein the disease is a cancer, and wherein prior to the cell therapy, the human patient received a therapy against the cancer to reduce tumor burden.

10 45. The method of claim 44, wherein the therapy is a chemotherapy, an immunotherapy, a radiotherapy, or a surgery.

46. The method of any one of claims 41-45, wherein prior to the cell therapy, the subject received a lymphodepleting treatment to condition the subject for the cell therapy.

15 47. The method of claim 46, wherein the lymphodepleting treatment comprises administering to the subject one or more of fludarabine and cyclophosphamide.

20 48. The method of any one of claims 41-47, wherein the CAR binds CD19 and the subject is a human patient having lymphoblastic leukemia, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, mantle cell lymphoma, large B-cell lymphoma, or non-Hodgkin's lymphoma.

25 49. The method of any one of claims 41-47, wherein the CAR binds BCMA and the subject is a human patient having multiple myeloma, relapsed multiple myeloma, or refractory multiple myeloma.

50. A population of immune cells comprising a first plurality of genetically engineered immune cells, wherein the genetically engineered immune cells

- 30 (a) comprise a disrupted endogenous interferon gamma (IFN γ) gene or IFN γ receptor (IFN γ R) gene; and/or
- (b) express an IFN γ antagonist.

51. The population of immune cells of claim 50, wherein the genetically engineered immune cells comprise the disrupted endogenous IFN γ or IFN γ R gene.

5 52. The population of immune cells of claim 51, wherein the disrupted endogenous IFN γ or IFN γ R gene is produced by gene editing, optionally wherein the gene editing is mediated by a CRISPR/Cas gene editing system.

53. The population of immune cells of any one of claims 50- 52, wherein the genetically engineered cells express the IFN γ antagonist.

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54. The population of immune cells of any one of claim 53, wherein the genetically engineered cells secretes the IFN γ antagonist.

15 55. The population of immune cells of any one of claims 50-54, wherein the IFN γ antagonist is selected from the group consisting of an anti-IFN γ antibody; a secreted IFN γ receptor; and an anti-IFN γ R antibody; optionally wherein the anti- IFN γ antibody and/or the anti-IFN γ R antibody is a single chain variable fragment (scFv).

20 56. The population of immune cells of claim 55, wherein the IFN γ antagonist is an anti-IFN γ scFv.

25 57. The population of immune cells of claim 56, wherein the anti-IFN γ scFv comprises (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 53 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 52; (b) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 56 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 55; or (c) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 59 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 58.

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58. The population of immune cells of claim 57, wherein the anti-IFN γ scFv comprises the amino acid sequence of SEQ. ID. NO: 54; 57, or 60.

59. The population of immune cells of claim 55, wherein the IFN γ antagonist is a secreted IFN γ R.

5 60. The population of immune cells of any one of claims 50-59, further comprises a chimeric antigen receptor (CAR).

61. The population of immune cells of claim 60, wherein the CAR comprises:

- (a) an extracellular antigen binding domain;
- (b) a co-stimulatory domain;
- 10 (c) a cytoplasmic signaling domain; and optionally
- (d) a transmembrane domain.

62. The population of immune cells of claim 61, wherein the extracellular antigen binding domain comprises a single chain variable fragment (scFv).

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63. The population of immune cells of claim 61 or 62 wherein the extracellular antigen binding domain binds a tumor associated antigen, which optionally is selected from the group consisting of 5T4, CD2, CD5, CD3, CD 7, CD19, CD20, CD22, CD30, CD33, CD38, CD70, CD123, CD133, CD171, CEA, CS1, Claudin 18.2, BCMA, BAFF-R, PSMA, PSCA, desmoglein (Dsg3), HER-2, FAP, FSHR, NKG2D, GD2, EGFRVIII, mesothelin, ROR1, MAGE, MUC1, MUC16, GPC3, Lewis Y, and VEGFR1L.

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64. The population of immune cells of claim 63, wherein the tumor associated antigen is CD19 and the extracellular antigen binding domain comprises a scFv that binds CD19.

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65. The population of immune cells of claim 64, wherein the scFv that binds CD19 comprises the amino acid sequence of SEQ. ID. NO: 6, 39, 40, or 41.

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66. The population of immune cells of claim 63, wherein the tumor associated antigen is B cell maturation antigen (BCMA) and the extracellular antigen binding domain comprises a scFv that binds BCMA.

67. The population of immune cells of claim 66, wherein the scFv that binds BCMA comprises the amino acid sequence of SEQ. ID. NO: 7.

5 68. The population of immune cells of any one of claims 61-67, wherein the co-stimulatory domain is a co-stimulatory domain from 4-1BB (CD137), OX40, CD70, CD27, CD28, CD5, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), DAP10, and DAP12, or any combination thereof.

10 69. The population of immune cells of any one of claims 61-68, wherein the cytoplasmic signaling domain is a CD3zeta (CD3 ζ) signaling domain, an interleukin 2 receptor beta subunit (IL-2R β) cytoplasmic signaling domain, or a combination thereof.

15 70. The population of immune cells of any one of claims 61-69, wherein the transmembrane domain is from a cell surface receptor 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, CD154, CD271, TNFRSF19 and Killer Cell Immunoglobulin-Like Receptor (KIR), or any combination thereof.

20 71. The population of immune cells of any one of claims 61-70, wherein the CAR further comprises a hinge or a spacer or a combination of both to connect the functional domains of (a)-(d).

25 72. The population of immune cells of claim 71, wherein the CAR comprises a hinge domain linked to the C-terminus of the extracellular antigen binding domain and to the N-terminus of the transmembrane domain.

30 73. The population of immune cells of claim 71 or 72, wherein the hinge domain is of CD28, CD8, or an IgG, which optionally is IgG1 or IgG4.

 74. The population of immune cells of any one of claims 61-73, wherein the CAR further comprises a STAT3 binding motif, which is located at the C-terminal of the cytoplasmic signaling domain.

75. The population of immune cells of claim 74, wherein the STAT3 binding motif comprises the amino sequence set forth in YX₁X₂Q, wherein X₁ and X₂ are each independently an amino acid, optionally, wherein the STAT3 binding motif comprises the amino acid sequence set forth in YRHQ (SEQ ID NO: 4).

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76. The population of immune cells of any one of claims 50-75, wherein the IFN γ antagonist further comprising a signal peptide located at the N-terminus, optionally the signal peptide is selected from a signal peptide derived from albumin, CD8, a growth hormone, IL-2, an antibody light chain; and a *Gaussia* luciferase.

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77. The population of immune cells of any one of claims 50-76, wherein the immune cells comprise T cells, Natural Killer (NK) cells, tumor infiltrating lymphocytes, dendritic cells, macrophages, neutrophils, eosinophils, basophils, mast cells, myeloid-derived suppressor cells, mesenchymal stem cells or precursors thereof, or a combination thereof; optionally wherein the immune cells are human immune cells.

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78. The population of immune cells of any one of claims 50-77, further comprising a second plurality of immune cells that express an antibody specific to interleukin-6 (IL-6) or IL-6 receptor (IL-6R).

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79. The population of immune cells of claim 78, wherein the antibody specific to IL-6 or IL-6R is a scFv.

80. The population of immune cells of any one of claims 50-79, further comprising a third plurality of immune cells that express an IL-1 antagonist.

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81. The population of immune cells of claim 80, wherein the IL-1 antagonist is IL-1RA.

82. A pharmaceutical composition comprising the population of immune cells of any one of claims 50-81 and a pharmaceutically acceptable carrier.

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83. A method for reducing or eliminating undesired cells in a subject, the method comprising administering to a subject in need thereof a therapeutically effective amount of the population of immune cells of any one of claims 50-81 or the pharmaceutical composition of claim 82.

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84. The method of claim 83, wherein the subject is a human cancer patient and the genetically engineered immune cell expresses the CAR, which is specific to a tumor associated antigen, optionally wherein the tumor associated antigen is selected from the group consisting of 5T4, CD2, CD5, CD3, CD7, CD19, CD20, CD22, CD30, CD33, CD38, CD70, CD123, CD133, CD171, CEA, CS1, Claudin 18.2, BCMA, BAFF-R, PSMA, PSCA, desmoglein (Dsg3), HER-2, FAP, FSHR, NKG2D, GD2, EGFRVIII, mesothelin, ROR1, MAGE, MUC1, MUC16, GPC3, Lewis Y, and VEGFR11.

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85. The method of claim 84, wherein the cancer is a solid tumor cancer.

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86. The method of claim 85, wherein the cancer is selected from the group consisting of breast cancer, lung cancer, pancreatic cancer, liver cancer, glioblastoma (GBM), prostate cancer, ovarian cancer, mesothelioma, colon cancer, and stomach cancer.

87. The method of claim 84, wherein the cancer is a hematological cancer.

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88. The method of claim 87, wherein the hematological cancer is leukemia, lymphoma, or multiple myeloma, optionally wherein the leukemia is chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), or chronic myelogenous leukemia (CML), optionally wherein the lymphoma is mantle cell lymphoma, non-Hodgkin's lymphoma or Hodgkin's lymphoma.

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89. The method of claim 88, wherein the CAR binds CD19 and the subject is a human patient having lymphoblastic leukemia, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, mantle cell lymphoma, large B-cell lymphoma, or non-Hodgkin's lymphoma.

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90. The method of 88, wherein the CAR binds BCMA and the subject is a human patient having multiple myeloma, relapsed multiple myeloma, or refractory multiple myeloma.

5 91. The method of any one of claims 83-90, wherein prior to the cell therapy, the subject received a lymphodepleting treatment to condition the subject for the cell therapy.

92. The method of claim 91, wherein the lymphodepleting treatment comprises administering to the subject one or more of fludarabine and cyclophosphamide.

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