

WHAT IS CLAIMED IS:

1. A method of treating cancer, comprising:
administering an effective amount of genetically engineered anti-tumor human T cells to
a patient to treat cancer of the patient, wherein the anti-tumor human T cells have been
5 obtained by incorporating a recombinant DNA sequence encoding a CAR into T cells
extracted from the patient, wherein the encoded CAR comprises an ALPP antigen binding
domain that binds to ALPP expressed in cancer cells.
2. The method of treating a patient of claim 1, wherein the cancer is lung cancer.
- 10 3. The method of treating a patient of claim 1, wherein the cancer is gastric cancer.
4. The method of treating a patient of claim 1, wherein the cancer is pancreatic cancer.
- 15 5. The method of treating a patient of claim 1, wherein the cancer is head & neck cancer.
6. The method of treating a patient of claim 1, wherein the cancer is colorectal cancer.
7. The method of treating a patient of claim 1, wherein the cancer is urothelial cancer.
- 20 8. The method of treating a patient of claim 1, wherein the cancer is renal cancer.
9. The method of treating a patient of claim 1, wherein the cancer is cancer of reproductive
organs.
- 25 10. The method of treating a patient of claim 8, wherein the cancer of reproductive organs is
ovarian cancer.
11. The method of treating a patient of claim 8, wherein the cancer of reproductive organs is
30 endometrial cancer.
12. The method of treating a patient of claim 8, wherein the cancer of reproductive organs is
cervical cancer.

13. The method of treating a patient of claim 8, wherein the cancer of reproductive organs is testicular cancer.

- 5 14. A method of treating cancer, comprising:
administering an effective amount of genetically engineered anti-tumor human T cells to a
patient to treat cancer of the patient, wherein the anti-tumor human T cells have been
obtained by incorporating a recombinant DNA sequence encoding a CAR into T cells
extracted from the patient, wherein the encoded CAR comprises an ALPP antigen binding
10 domain;
wherein the CAR-T cell antigen binding domain consists of an antibody or antibody
fragment;
wherein the said antibody has a variable heavy chain region selected from SEQ ID NO: 1
or SEQ ID NO: 3; and a variable light chain region selected from SEQ ID NO: 2 or SEQ ID
15 NO: 4.

15. A method of treating cancer of claim 14, wherein the antibody is murine antibody against
ALPP having a variable heavy chain region SEQ ID NO: 1 and variable light chain region
SEQ ID NO: 2.

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16. A method of treating cancer of claim 14, wherein the antibody is humanized antibody
against ALPP having a variable heavy chain region SEQ ID NO: 3 and variable light chain
region SEQ ID NO: 4.

- 25 17. A chimeric antigen receptor comprising: (a) an extracellular antigen-binding domain that
specifically recognizes alkaline phosphatase, placental (ALPP); (b) a transmembrane domain;
and (c) an intracellular signaling region.

18. The chimeric antigen receptor of claim 17, wherein the antigen-binding domain
30 comprises a heavy chain variable domain (VH) and a light chain variable domain (VL).

19. The chimeric antigen receptor of claim 18, wherein the VH comprises heavy chain
complementarity determining regions (CDRs) 1, 2, and 3 and the VL comprises VL CDRs 1,

2, and 3,

wherein the VH CDRs 1, 2, and 3 amino acid sequences and the VL CDRs, 1, 2, and 3 amino acid sequences are one of the following:

(1) the VH CDRs 1, 2, and 3 amino acid sequences are set forth in SEQ ID NOs: 45, 46, and
5 47, respectively, and the VL CDRs 1, 2, and 3 amino acid sequences are set forth in SEQ ID NOs: 48, 49, and 50, respectively;

(2) the VH CDRs 1, 2, and 3 amino acid sequences are set forth in SEQ ID Nos: 51, 52, and 53, respectively, and the VL CDRs 1, 2, and 3 amino acid sequences are set forth in SEQ ID NOs: 54, 55, and 56, respectively;

10 (3) the VH CDRs 1, 2, and 3 amino acid sequences are set forth in SEQ ID NOs: 57, 58, and 59, respectively, and the VL CDRs 1, 2, and 3 amino acid sequences are set forth in SEQ ID NOs: 60, 61, and 62, respectively;

(4) the VH CDRs 1, 2, and 3 amino acid sequences are set forth in SEQ ID NOs: 63, 64, and 65, respectively, and the VL CDRs 1, 2, and 3 amino acid sequences are set forth in SEQ ID
15 NOs: 66, 67, and 68, respectively; and

(5) the VH CDRs 1, 2, and 3 amino acid sequences are set forth in SEQ ID NOs: 81, 82, and 83, respectively, and the VL CDRs 1, 2, and 3 amino acid sequences are set forth in SEQ ID NOs: 84, 85, and 86, respectively.

20 20. The chimeric antigen receptor of claim 18 or 19, wherein the VH consist of or comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NOs: 1, 3, 5, 7, 9, 11, or 13; and the VL consists of or comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NOs: 2, 4, 6, 8, 10, 12, or 14.

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21. The chimeric antigen receptor of claim 20, wherein the VH comprises an amino acid sequence having at least 90% identity to SEQ ID NO: 1 and the VL comprises an amino acid sequence having at least 90% identity to SEQ ID NO: 2.

30 22. The chimeric antigen receptor of claim 20, wherein the VH comprises an amino acid sequence having at least 90% identity to SEQ ID NO: 3 and the VL comprises an amino acid sequence having at least 90% identity to SEQ ID NO: 4.

23. The chimeric antigen receptor of claim 20, wherein the VH comprises an amino acid sequence having at least 90% identity to SEQ ID NO: 5 and the VL comprises an amino acid sequence having at least 90% identity to SEQ ID NO: 6.
- 5 24. The chimeric antigen receptor of claim 20, wherein the VH comprises an amino acid sequence having at least 90% identity to SEQ ID NO: 7 and the VL comprises an amino acid sequence having at least 90% identity to SEQ ID NO: 8.
25. The chimeric antigen receptor of claim 20, wherein the VH comprises an amino acid
10 sequence having at least 90% identity to SEQ ID NO: 9 and the VL comprises an amino acid sequence having at least 90% identity to SEQ ID NO: 10.
26. The chimeric antigen receptor of claim 20, wherein the VH comprises an amino acid
15 sequence having at least 90% identity to SEQ ID NO: 11 and the VL comprises an amino acid sequence having at least 90% identity to SEQ ID NO: 12.
27. The chimeric antigen receptor of claim 20, wherein the VH comprises an amino acid
20 sequence having at least 90% identity to SEQ ID NO: 13 and the VL comprises an amino acid sequence having at least 90% identity to SEQ ID NO: 14.
28. The chimeric antigen receptor of any one of claims 17-27, wherein the antigen-binding domain comprises an scFv.
29. The chimeric antigen receptor of any one of claims 17-28, wherein the VH region and the
25 VL region are joined by a flexible linker.
30. The chimeric antigen receptor of claim 29, wherein the flexible linker comprises the amino acid sequence of EKGRSGGGSGGGSGGGGS (SEQ ID NO: 37).
- 30 31. The chimeric antigen receptor of claim 29, wherein the flexible linker comprises the amino acid sequence of GGGSGGGSGGGGS (SEQ ID NO: 87).
32. The chimeric antigen receptor of any one of claims 17-31, wherein the chimeric antigen

receptor further comprises a hinge region.

33. The chimeric antigen receptor of claim 32, wherein the hinge region comprises a membrane-proximal region from IgG, CD8, or CD28.

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34. The chimeric antigen receptor of claim 33, wherein the hinge region comprises a CD8 membrane-proximal region.

35. The chimeric antigen receptor of any of claims 17-34, wherein the transmembrane domain comprises a transmembrane region of CD4, CD8, or CD28.

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36. The chimeric antigen receptor of claim 35, wherein the transmembrane domain comprises a CD8 transmembrane region.

37. The chimeric antigen receptor of claim 34 or 36, wherein the hinge region and/or the transmembrane region are from human CD8.

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38. The chimeric antigen receptor of any one of claims 17-37, wherein the chimeric antigen receptor comprises an amino acid sequence set forth in SEQ ID NO: 38, or an amino acid sequence that is at least 90% identical to SEQ ID NO: 38.

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39. The chimeric antigen receptor of any of claims 17-38, wherein the intracellular signaling region comprises an activating cytoplasmic signaling domain.

40. The chimeric antigen receptor of claim 39, wherein the activating cytoplasmic signaling domain is capable of inducing a primary activation signal in a T cell, is a T cell receptor (TCR) component, and/or comprises an immunoreceptor tyrosine-based activation motif (ITAM).

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41. The chimeric antigen receptor of claims 17-40, wherein the intracellular signaling region is or comprises a functional signaling domain of CD3 zeta.

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42. The chimeric antigen receptor of claim 41, wherein the CD3 zeta is human CD3 zeta.

43. The chimeric antigen receptor of claim 42, wherein the intracellular signaling region is or comprises the amino acid sequence set forth in SEQ ID NO: 40 or an amino acid sequence that is at least 90% sequence identical to SEQ ID NO: 40.

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44. The chimeric antigen receptor of any of claims 17-43, wherein the intracellular signaling region further comprises a costimulatory signaling region.

45. The chimeric antigen receptor of claims 44, wherein the costimulatory signaling region is between the transmembrane domain and the intracellular signaling region.

46. The chimeric antigen receptor of claim 44 or 45, wherein the costimulatory signaling region comprises a functional signaling domain from a protein selected from the group consisting of a MHC class I molecule, a TNF receptor protein, an Immunoglobulin-like protein, a cytokine receptor, an integrin, a signaling lymphocytic activation molecule (SLAM protein), an activating NK cell receptor, BTLA, a Toll ligand receptor, OX40, CD2, CD7, CD27, CD28, CD30, CD40, CDS, ICAM-1, LFA-1, CD11a/CD18, 4-1BB (CD137), B7-H3, CDS, ICAM-1, ICOS (CD278), GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD 11b, ITGAX, CD 11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, NKG2C, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, and CD19a.

47. The chimeric antigen receptor of claim 46, wherein the costimulatory signaling region is or comprises a functional signaling domain from OX40, CD28, 4-1BB, ICOS, or a signaling portion thereof.

48. The chimeric antigen receptor of claim 47, wherein the costimulatory signaling region comprises an intracellular signaling domain of 4-1BB.

49. The chimeric antigen receptor of claim 48, wherein the 4-1BB is human 4-1BB.
50. The chimeric antigen receptor of claim 49, wherein the costimulatory signaling region is
5 or comprises an amino acid sequence set forth in SEQ ID NO: 39 or an amino acid sequence that is at least 90% identical to SEQ ID NO: 39.
51. The chimeric antigen receptor of claim 47, wherein the costimulatory signaling region comprises intracellular signaling domains of CD28 and 4-1BB.
- 10 52. The chimeric antigen receptor of claim 50, wherein the CD28 is human CD28 and the 4-1BB is human 4-1BB.
53. The chimeric antigen receptor of claim 52, wherein the costimulatory signaling region is
15 or comprises an amino acid sequence set forth in SEQ ID NO: 90 or an amino acid sequence that is at least 90% identical to SEQ ID NO: 90.
54. A chimeric antigen receptor comprising an amino acid sequence having at least 90%,
91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to SEQ ID NO: 18, 20,
20 22, 24, 26, 28, 30, 91, 92, or 93.
55. The chimeric antigen receptor of claim 54, wherein the amino acid sequence is at least
90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:
18.
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56. The chimeric antigen receptor of claim 54, wherein the amino acid sequence is at least
90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:
20.
- 30 57. The chimeric antigen receptor of claim 54, wherein the amino acid sequence is at least
90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:
22.

58. The chimeric antigen receptor of claim 54, wherein the amino acid sequence is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 24.
- 5 59. The chimeric antigen receptor of claim 54, wherein the amino acid sequence is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 26.
60. The chimeric antigen receptor of claim 51, wherein the amino acid sequence is at least
10 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 28.
61. The chimeric antigen receptor of claim 54, wherein the amino acid sequence is at least
15 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 30.
62. The chimeric antigen receptor of claim 54, wherein the amino acid sequence is at least
20 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 91.
63. The chimeric antigen receptor of claim 54, wherein the amino acid sequence is at least
90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 92.
- 25 64. The chimeric antigen receptor of claim 54, wherein the amino acid sequence is at least
90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 93.
65. A polynucleotide encoding the chimeric antigen receptor of any of claims 17-64.
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66. A vector comprising the polynucleotide of claim 65.
67. The vector of claim 66, wherein the vector further comprises a nucleic acid encoding an

anti-PD-1 antibody or antigen binding fragment thereof.

68. The vector of claim 66, wherein the vector further comprises a nucleic acid encoding an anti-PD-L1 antibody or antigen binding fragment thereof.

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69. The vector of any one of claims 66-68, wherein the vector is a viral vector.

70. The vector of claim 69, wherein the viral vector is a retroviral vector or a lentiviral vector.

10 71. An engineered cell, comprising the chimeric antigen receptor of any one of claims 17- 64.

72. An engineered cell, comprising the polynucleotide of claim 65 or the vector of any one of claims 66-70.

15 73. The engineered cell of claim 71 or 72, wherein the engineered cell is a primary cell obtained from a subject (e.g., a human subject).

74. The engineered cell of claim 71 or 72, wherein the engineered cell is a cell line.

20 75. The engineered cell of any one of claims 71-74, wherein the engineered cell is an immune cell.

76. The engineered cell of claim 75, wherein the immune cell is an NK cell or a T cell.

25 77. The engineered cell of any one of claims 71-76, wherein the engineered cell is a T cell.

78. The engineered cell of claim 77, wherein the T cell is CD8+.

79. The engineered cell of claim 77, wherein the T cell is CD4+.

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80. The engineered cell of any one of claims 76-79, wherein the T cell is isolated from a human subject.

81. The engineered cell of any one of claims 71-80, wherein the engineered cell expresses the chimeric antigen receptor.

5 82. The engineered cell of any one of claims 71-81, wherein the engineered cell expresses a cytokine and/or a co-stimulatory ligand.

83. The engineered cell of claim 82, wherein the cytokine and/or the co-stimulatory ligand is membrane tethered.

10 84. The engineered cell of claim 82, wherein the cytokine and/or the co-stimulatory ligand is secreted.

85. The engineered cell of any one of claims 82-84, wherein the cytokine is IL-2, IL-5, or IL-12.

15 86. The engineered cell of any one of claims 82-84, wherein the co-stimulatory ligand is CD40L (CD154) or 41-BBL (CD137L).

20 87. The engineered cell of any one of claims 71-86, wherein the engineered cell expresses an antibody or antigen-binding fragment thereof (e.g., an scFv).

88. The engineered cell of any one of claims 87, wherein the antibody or antigen-binding fragment thereof is an immune checkpoint inhibitor.

25 89. The engineered cell of claim 88, wherein the antibody or antigen-binding fragment thereof specifically binds to PD-1, PD-L1, or CTLA-4.

90. A method for producing the engineered cell, comprising introducing a vector of claims 66-70 into a cell *in vitro* or *ex vivo*.

30 91. The method of claim 90, wherein the vector is a viral vector and the introducing is carried out by transduction.

92. A method of generating a population of cells, comprising introducing a nucleic acid into a cell, where the nucleic acid comprises the polynucleotide of claim 65, or a nucleic acid encoding the chimeric antigen receptor of any one of claims 17-64.

- 5 93. A method of treating an ALPP-associated disease or disorder in a subject, comprising administering the engineered cell of any of claims 71-89 to the subject.

94. The method of claim 93, wherein the ALPP-associated disease or disorder is a cancer.

- 10 95. The method of claim 94, wherein the cancer is testicular cancer, endometrial cancer, ovarian cancer, cervical cancer, urothelial cancer, pancreatic cancer, liver cancer, or stomach cancer.

96. The method of any one of claims 93-95, wherein the method further comprises

- 15 administering a checkpoint inhibitor to the subject.

97. The method of claim 96, wherein the checkpoint inhibitor is an anti-PD-1 antibody or antigen binding fragment thereof, an anti-PD-L1 antibody or antigen binding fragment thereof, or an anti-CTLA-4 antibody or antigen-binding fragment thereof.

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98. An anti-ALPP antibody or antigen-binding fragment thereof comprising:

a heavy chain variable region (VH) comprising complementarity determining regions (CDRs) 1, 2, and 3, wherein the VH CDR1 region comprises an amino acid sequence that is at least 80% identical to a selected VH CDR1 amino acid sequence, the VH CDR2 region comprises an amino acid sequence that is at least 80% identical to a selected VH CDR2 amino acid sequence, and the VH CDR3 region comprises an amino acid sequence that is at least 80% identical to a selected VH CDR3 amino acid sequence; and

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a light chain variable region (VL) comprising CDRs 1, 2, and 3, wherein the VL CDR1 region comprises an amino acid sequence that is at least 80% identical to a selected VL CDR1 amino acid sequence, the VL CDR2 region comprises an amino acid sequence that is at least 80% identical to a selected VL CDR2 amino acid sequence, and the VL CDR3 region comprises an amino acid sequence that is at least 80% identical to a selected VL CDR3 amino acid sequence,

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wherein the selected VH CDRs 1, 2, and 3 amino acid sequences and the selected VL CDRs, 1, 2, and 3 amino acid sequences are one of the following:

- (1) the selected VH CDRs 1, 2, and 3 amino acid sequences are set forth in SEQ ID NOs: 51, 52, and 53, respectively, and the selected VL CDRs 1, 2, and 3 amino acid sequences are set forth in SEQ ID NOs: 54, 55, and 56, respectively;
- (2) the selected VH CDRs 1, 2, and 3 amino acid sequences are set forth in SEQ ID NOs: 57, 58, and 59, respectively, and the selected VL CDRs 1, 2, and 3 amino acid sequences are set forth in SEQ ID NOs: 60, 61, and 62, respectively;
- (3) the selected VH CDRs 1, 2, and 3 amino acid sequences are set forth in SEQ ID NOs: 63, 64, and 65, respectively, and the selected VL CDRs 1, 2, and 3 amino acid sequences are set forth in SEQ ID NOs: 66, 67, and 68, respectively; and
- (4) the selected VH CDRs 1, 2, and 3 amino acid sequences are set forth in SEQ ID NOs: 81, 82, and 83, respectively, and the selected VL CDRs 1, 2, and 3 amino acid sequences are set forth in SEQ ID NOs: 84, 85, and 86, respectively.

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99. An antibody or antigen-binding fragment thereof that binds to ALPP comprising a heavy chain variable region (VH) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a selected VH sequence, and a light chain variable region (VL) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a selected VL sequence, wherein the selected VH sequence is selected from SEQ ID NOs: 1, 3, 5, 7, 9, 11, and 13, and the selected VL sequence is selected from SEQ ID NOs: 2, 4, 6, 8, 10, 12, and 14.

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100. The antibody or antigen-binding fragment thereof of claim 98 or 99, wherein the antibody or antigen-binding fragment specifically binds to human ALPP.

101. The antibody or antigen-binding fragment thereof of any one of claims 98-100, wherein the antibody or antigen-binding fragment is a humanized antibody or antigen-binding fragment thereof.

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102. The antibody or antigen-binding fragment thereof of any one of claims 98-101, wherein the antibody or antigen-binding fragment is a single-chain variable fragment (scFv).

103. An antibody or antigen-binding fragment thereof comprising the VH CDRs 1, 2, and 3, and the VL CDRs 1, 2, and 3 of the antibody or antigen-binding fragment thereof of any one of claims 98-102.

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104. A chimeric antigen receptor comprising the VH CDRs 1, 2, and 3, and the VL CDRs 1, 2, and 3 of the antibody or antigen-binding fragment thereof of any one of claims 98-103.