

mediated or non-viral means of transfection. Thereafter, the modified T cells can be infused back into the patients as adoptive cell therapy.

In one aspectexample, the disclosure is related to 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. In some embodimentexamples, 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. In some embodimentexamples, the encoded CAR comprises an ALPP antigen binding domain that binds to ALPP expressed in cancer cells.

In some embodimentexamples, the cancer is lung cancer. In some embodimentexamples, the cancer is gastric cancer. In some embodimentexamples, the cancer is pancreatic cancer. In some embodimentexamples, the cancer is head & neck cancer. In some embodimentexamples, the cancer is colorectal cancer. In some embodimentexamples, the cancer is urothelial cancer. In some embodimentexamples, the cancer is renal cancer. In some embodimentexamples, the cancer is cancer of reproductive organs. In some embodimentexamples, the cancer of reproductive organs is ovarian cancer. In some embodimentexamples, the cancer of reproductive organs is endometrial cancer. In some embodimentexamples, the cancer of reproductive organs is cervical cancer. In some embodimentexamples, the cancer of reproductive organs is testicular cancer.

In one aspect, the disclosure refers to use of genetically engineered anti-tumor human T cells in the manufacture of a medicament for treating cancer in a patient in need thereof, wherein the anti-tumor human T cells comprise a chimeric antigen receptor (CAR) comprising a placental alkaline phosphatase (ALPP) antigen binding domain; wherein the antigen binding domain comprises an antibody or antibody fragment thereof; and wherein the antibody or antibody fragment thereof comprises a heavy chain variable region (VH) comprising an amino acid sequence selected from SEQ ID NO: 1 or SEQ ID NO: 3; and a light chain variable region (VL) comprising an amino acid sequence selected from SEQ ID NO: 2 or SEQ ID NO: 4. In one aspectexample, the disclosure is related to 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. In some embodimentexamples, 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. In some embodimentexamples, the encoded CAR comprises an ALPP antigen binding domain. In some embodimentexamples, the CAR-T cell antigen binding domain consists of an antibody or antibody fragment. In some embodimentexamples, 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 NO: 4.

In one aspectexample, the disclosure is related to the method of treating cancer as described herein. In some embodimentexamples, 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.

In one aspectexample, the disclosure is related to the method of treating cancer as described herein. In some embodimentexamples, 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.

In another aspect, the disclosure refers to 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, wherein the antigen-binding domain comprises a heavy chain variable region (VH) and a light chain variable region (VL), and 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 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;

(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 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. In one aspectexample, the disclosure is related to 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.

In some embodimentexamples, the antigen-binding domain comprises a heavy chain variable domain (VH) and a light chain variable domain (VL).

5 In some embodimentexamples, the VH comprises heavy chain complementarity determining regions (CDRs) 1, 2, and 3 and the VL comprises VL CDRs 1, 2, and 3. In some embodimentexamples, 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
10 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;

15 (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
20 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.

In some embodimentexamples, the VH consist of or comprises an amino acid
25 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.

In some embodimentexamples, the VH comprises an amino acid sequence having at
30 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.

In some embodimentexamples, 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.

In some [embodimentexamples](#), 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 In some [embodimentexamples](#), 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.

In some [embodimentexamples](#), the VH comprises an amino acid 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.

In some [embodimentexamples](#), the VH comprises an amino acid 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.

15 In some [embodimentexamples](#), the VH comprises an amino acid 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.

In some [embodimentexamples](#), the antigen-binding domain comprises an scFv.

In some [embodimentexamples](#), the VH region and the VL region are joined by a flexible linker.

20 In some [embodimentexamples](#), the flexible linker comprises the amino acid sequence of EKGRSGGGGSGGGGSGGGGS (SEQ ID NO: 37). In some [embodimentexamples](#), the flexible linker comprises the amino acid sequence of GGGGSGGGGSGGGGS (SEQ ID NO: 87).

25 In some [embodimentexamples](#), the chimeric antigen receptor further comprises a hinge region. In some [embodimentexamples](#), the hinge region comprises a membrane-proximal region from IgG, CD8, or CD28. In some [embodimentexamples](#), the hinge region comprises a CD8 membrane-proximal region. In some [embodimentexamples](#), the transmembrane domain comprises a transmembrane region of CD4, CD8, or CD28. In some [embodimentexamples](#), the transmembrane domain comprises a CD8 transmembrane region.

30 In some [embodimentexamples](#), the hinge region and/or the transmembrane region are from human CD8. In some [embodimentexamples](#), 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.

35 In some [embodimentexamples](#), the intracellular signaling region comprises an activating cytoplasmic signaling domain. In some [embodimentexamples](#), 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). In some [embodimentexamples](#), the intracellular signaling region is or comprises a functional signaling domain of CD3 zeta. In some [embodimentexamples](#), the CD3 zeta is human CD3 zeta. In some [embodimentexamples](#), 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.

In some [embodimentexamples](#), the intracellular signaling region further comprises a costimulatory signaling region. In some [embodimentexamples](#), the costimulatory signaling region is between the transmembrane domain and the intracellular signaling region.

In some [embodimentexamples](#), 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, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, and CD19a.

In some [embodimentexamples](#), the costimulatory signaling region is or comprises a functional signaling domain from OX40, CD28, 4-1BB, ICOS, or a signaling portion thereof. In some [embodimentexamples](#), the costimulatory signaling region comprises an intracellular signaling domain of 4-1BB. In some [embodimentexamples](#), the 4-1BB is human 4-1BB. In some [embodimentexamples](#), the costimulatory signaling region is 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.

In some [embodimentexamples](#), the costimulatory signaling region comprises intracellular signaling domains of CD28 and 4-1BB. In some [embodimentexamples](#), the CD28 is human CD28 and

the 4-1BB is human 4-1BB. In some embodimentexamples, the costimulatory signaling region is 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.

5 In one aspect, the present disclosure refers to a chimeric antigen receptor comprising an amino acid sequence having at least 90% identity to SEQ ID NO: 18, 20, 22, 24, 26, 28, 30, 91, 92, or 93. In one aspectexample, the disclosure is related to 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, 22, 24, 26, 28, 30, 91, 92, or 93.

10 In some embodimentexamples, 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. In some embodimentexamples, 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. In some embodimentexamples, 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. In some embodimentexamples, 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. In some embodimentexamples, 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. In some embodimentexamples, the amino acid sequence is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 28. In some embodimentexamples, the amino acid sequence is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 30. In some embodimentexamples, the amino acid sequence is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 91. In some embodimentexamples, 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. In some embodimentexamples, 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.

25 In one aspect, the disclosure is related to a polynucleotide encoding the chimeric antigen receptor as described herein.

30 In one aspect, the disclosure is related to a vector comprising the polynucleotide as described herein.

In some embodimentexamples, the vector further comprises a nucleic acid encoding an anti-PD-1 antibody or antigen binding fragment thereof. In some embodimentexamples, the vector further comprises a nucleic acid encoding an anti-PD-L1 antibody or antigen binding fragment thereof.

35 In some embodimentexamples, the vector is a viral vector. In some embodimentexamples, the viral

vector is a retroviral vector or a lentiviral vector.

In one aspect, the disclosure is related to an engineered cell, comprising the chimeric antigen receptor as described herein.

In one aspectexample, the disclosure is related to an engineered cell, comprising the polynucleotide or the vector as described herein.

In some embodimentexamples, the engineered cell is a primary cell obtained from a subject (e.g., a human subject). In some embodimentexamples, the engineered cell is a cell line. In some embodimentexamples, the engineered cell is an immune cell. In some embodimentexamples, the immune cell is an NK cell or a T cell. In some embodimentexamples, the engineered cell is a T cell. In some embodimentexamples, the T cell is CD8+. In some embodimentexamples, the T cell is CD4+. In some embodimentexamples, the T cell is isolated from a human subject.

In some embodimentexamples, the engineered cell expresses the chimeric antigen receptor.

In some embodimentexamples, the engineered cell expresses a cytokine and/or a co-stimulatory ligand. In some embodimentexamples, the cytokine and/or the co-stimulatory ligand is membrane tethered. In some embodimentexamples, the cytokine and/or the co-stimulatory ligand is secreted. In some embodimentexamples, the cytokine is IL-2, IL-5, or IL-12. In some embodimentexamples, the co-stimulatory ligand is CD40L (CD154) or 41-BBL (CD137L). In some embodimentexamples, the engineered cell expresses an antibody or antigen-binding fragment thereof (e.g., an scFv).

In some embodimentexamples, the antibody or antigen-binding fragment thereof is an immune checkpoint inhibitor. In some embodimentexamples, the antibody or antigen-binding fragment thereof specifically binds to PD-1, PD-L1, or CTLA-4.

In one aspect, the disclosure is related to a method for producing the engineered cell, comprising introducing a vector as described herein into a cell *in vitro* or *ex vivo*.

In some embodimentexamples, the vector is a viral vector and the introducing is carried out by transduction.

In one aspect, the disclosure is related to a method of generating a population of cells, comprising introducing a nucleic acid into a cell, where the nucleic acid comprises the polynucleotide as described herein, or a nucleic acid encoding the chimeric antigen receptor as described herein.

In another aspect, the disclosure refers to use of the engineered cell as described herein in the manufacture of a medicament for treating an ALPP-associated disease or disorder in a subject. In one aspectexample, the disclosure is related to a method of treating an ALPP-associated disease or disorder in a subject, comprising administering the engineered cell as described herein to the subject.

In some embodimentexamples, the ALPP-associated disease or disorder is a cancer. In some

~~embodiment~~examples, the cancer is testicular cancer, endometrial cancer, ovarian cancer, cervical cancer, uterohelial cancer, pancreatic cancer, liver cancer, or stomach cancer.

In some ~~embodiment~~examples, the method further comprises administering a checkpoint inhibitor to the subject. In some ~~embodiment~~examples, 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.

In yet another aspect, the disclosure refers to 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 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,

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. In one ~~aspect~~example, the disclosure is related to 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 and a light chain variable region (VL) comprising CDRs 1, 2, and 3. In some

~~embodiment~~examples, 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. In some ~~embodiment~~examples, 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. In some ~~embodiment~~examples, 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.

In one aspect, the disclosure refers to 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% identical to SEQ ID NO: 1, 3, 5, 7, 9, 11, or 13, and a light chain variable region (VL) comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 2, 4, 6, 8, 10, 12, and 14. In one aspectexample, the disclosure is related to 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. In some embodimentexamples, 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.

In some embodimentexamples, the antibody or antigen-binding fragment specifically binds to human ALPP.

In some embodimentexamples, the antibody or antigen-binding fragment is a humanized antibody or antigen-binding fragment thereof.

In some embodimentexamples, the antibody or antigen-binding fragment is a single-chain variable fragment (scFv).

In one aspectexample, the disclosure is related to 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 as described herein.

In one aspectexample, the disclosure is related to 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 as described herein.

As used herein, the term “genetically engineered cell” or “genetically modified cell” refers to a cell with a modification of a nucleic acid sequence in the cell, including, but not limited to, a cell having an insertion, deletion, substitution, or modification of one or more nucleotides in its genome, and/or a cell with an exogenous nucleic acid sequence (e.g., a vector), wherein the exogenous nucleic acid sequence is not necessarily integrated into the genome.

As used herein, the term “peripheral blood cells” refers to cells normally found in the peripheral blood including, but is not limited to, eosinophils, neutrophils, T cells, monocytes, K cells, granulocytes, and B cells.

As used herein, the term “cancer” or “cancer cell” refers to the cells dividing in an uncontrolled manner, e.g., forming the solid tumors or the excessive tumor cells in blood. Examples of such cells include cells having an abnormal state or condition characterized by rapidly proliferating cell growth. The term is meant to include cancerous growths, e.g.,

themselves into the genome of a host cell and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" or simply "expression vectors". In some [embodimentexamples](#), the vectors are viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses). In some [embodimentexamples](#), the vector is a retroviral vector. In some [embodimentexamples](#), the vector can be created by removal of the retroviral gag, pol, and env genes and replaced with the gene of interest.

As used herein, a "subject" is a mammal, such as a human or a non-human animal. In some [embodimentexamples](#), the subject, e.g., patient, to whom the cells, cell populations, or compositions are administered is a mammal, typically a primate, such as a human. In some [embodimentexamples](#), the primate is a monkey or an ape. The subject can be male or female and can be any suitable age, including infant, juvenile, adolescent, adult, and geriatric subjects. In some [embodimentexamples](#), the subject is a non-primate mammal, such as a dog, a cat, a horse, a rodent, a rat, or a mouse.

As used herein, the term "T cells" refer to the cells that are a type of lymphocyte which develop in the thymus and play an important role in the immune response. T cells can be distinguished from other lymphocytes by the presence of a T cell receptor on the cell surface.

As used herein, the term "about" refers to a measurable value such as an amount, a time duration, and the like, and encompasses variations of $\pm 20\%$, $\pm 10\%$, $\pm 5\%$, $\pm 1\%$, $\pm 0.5\%$ or $\pm 0.1\%$ from the specified value.

As used herein, the term "antibody" refers to any antigen-binding molecule that contains at least one (e.g., one, two, three, four, five, or six) complementary determining region (CDR) (e.g., any of the three CDRs from an immunoglobulin light chain or any of the three CDRs from an immunoglobulin heavy chain) and is capable of specifically binding to an epitope. Non-limiting examples of antibodies include: monoclonal antibodies, polyclonal antibodies, multi-specific antibodies (e.g., bi-specific antibodies), single-chain antibodies, chimeric antibodies, human antibodies, and humanized antibodies. In some [embodimentexamples](#), an antibody can contain an Fc region of a human antibody. The term antibody also includes derivatives, e.g., bi-specific antibodies, single-chain antibodies, diabodies, linear antibodies, and multi-specific antibodies formed from antibody fragments.

As used herein, the term "antigen-binding fragment" refers to a portion of a full-

length antibody, wherein the portion of the antibody is capable of specifically binding to an antigen. In some embodiment examples, the antigen-binding fragment contains at least one variable domain (e.g., a variable domain of a heavy chain or a variable domain of light chain). Non-limiting examples of antibody fragments include, e.g., Fab, Fab', F(ab')₂, and Fv fragments.

As used herein, the term “humanized antibody” refers to a non-human antibody which contains minimal sequence derived from a non-human (e.g., mouse) immunoglobulin and contains sequences derived from a human immunoglobulin. In non-limiting examples, humanized antibodies are human antibodies (recipient antibody) in which hypervariable (e.g., CDR) region residues of the recipient antibody are replaced by hypervariable (e.g., CDR) region residues from a non-human antibody (e.g., a donor antibody), e.g., a mouse, rat, or rabbit antibody, having the desired specificity, affinity, and capacity. In some embodiment examples, the Fv framework residues of the human immunoglobulin are replaced by corresponding non-human (e.g., mouse) immunoglobulin residues. In some embodiment examples, humanized antibodies may contain residues which are not found in the recipient antibody or in the donor antibody. These modifications can be made to further refine antibody performance. In some embodiment examples, the humanized antibody contains substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops (CDRs) correspond to those of a non-human (e.g., mouse) immunoglobulin and all or substantially all of the framework regions are those of a human immunoglobulin. The humanized antibody can also contain at least a portion of an immunoglobulin constant region (Fc), typically, that of a human immunoglobulin. Humanized antibodies can be produced using molecular biology methods known in the art. Non-limiting examples of methods for generating humanized antibodies are described herein.

As used herein, the term “single-chain antibody” refers to a single polypeptide that contains at least two immunoglobulin variable domains (e.g., a variable domain of a mammalian immunoglobulin heavy chain or light chain) that is capable of specifically binding to an antigen. Non-limiting examples of single-chain antibodies are described herein.

As used herein, when referring to an antibody or antibody related molecule (e.g., CAR), the phrases “specifically binding” and “specifically binds” mean that the antibody interacts with its target molecule (e.g., ALPP) preferably to other molecules, because the interaction is dependent upon the presence of a particular structure (i.e., the antigenic determinant or epitope) on the target molecule; in other words, the reagent is recognizing and binding to molecules that include a specific structure rather than to all molecules in general.

An antibody that specifically binds to the target molecule may be referred to as a target-specific antibody. For example, an antibody that specifically binds to an ALPP molecule may be referred to as an ALPP-specific antibody or an anti-ALPP antibody.

As used herein, the terms “polypeptide,” “peptide,” and “protein” are used interchangeably to refer to polymers of amino acids of any length of at least two amino acids.

As used herein, the terms “polynucleotide,” “nucleic acid molecule,” and “nucleic acid sequence” are used interchangeably herein to refer to polymers of nucleotides of any length of at least two nucleotides, and include, without limitation, DNA, RNA, DNA/RNA hybrids, and modifications thereof.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this ~~invention~~disclosure belongs. Methods and materials are described herein for use in the present ~~invention~~disclosure; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

Other features and advantages of the ~~invention~~disclosure will be apparent from the following detailed description and figures, and from the claims.

DESCRIPTION OF DRAWINGS

Exemplary embodiments are illustrated in referenced figures. It is intended that the embodiments and figures disclosed herein are to be considered illustrative rather than restrictive.

FIG. 1A shows a sequence map for the A02 CAR plasmid.

FIG. 1B shows a sequence map for the A03 CAR plasmid.

FIG. 1C shows a sequence map for the A06 CAR plasmid.

FIG. 1D shows a sequence map for the A02P03 CAR plasmid. T2A encodes a 2A self-cleaving peptide.

FIG. 1E shows a sequence map for the A02PL01 CAR plasmid. T2A encodes a 2A self-cleaving peptide.

FIG. 2 shows A02 and A03 CAR expression in Jurkat T cells. Jurkat cells were untransduced (UT), or transduced to express A02 or A03 CAR. CAR expression levels were