

SUMMARY

[0008] The disclosure is directed, at least in part, to T-cells expressing an amino acid transporter protein, for example, an arginine transporter protein, and a CAR that specifically binds a cell surface antigen on a target cell. Such CAR-T cells are useful for the treatment of malignancies such as cancer. Genetically modified T-cells and expression vectors described herein may have enhanced robustness and/or survival in a tumor microenvironment and resource-depleted, for example, arginine-depleted, microenvironments, as compared to, for example, T-cell populations not subject to genetic modification. The described genetically modified T-cells and expression vectors are useful for treating cancers and other diseases that require targeting of T-cells to a specific cell population.

[0008a] In one aspect, the present disclosure refers to a genetically modified T-cell genetically modified to express: a) a recombinant arginine transporter selected from the group consisting of (i) CAT-1, (ii) CAT-2, (iii) CAT-3, (iv) CAT-4, (v) y⁺LAT1 and 4F2hc, (vi) y⁺LAT2 and 4F2hc, (vii) b^{0,+}AT and rBAT, and (viii) ATB^{0,+}, and b) a chimeric antigen receptor (CAR) having at least one antigen-specific targeting region that specifically binds a cell surface antigen present on a target cell population, a transmembrane domain, and an intracellular signaling domain, wherein the genetically modified T-cell comprises an intracellular arginine level that allows the genetically modified T-cell to survive in an arginine-depleted environment.

[0009] In another example, the disclosure is directed, to genetically modified T-cells expressing an amino acid transporter, for example, an arginine transporter. The amino acid transporter can be the product of a recombinant amino acid transporter nucleotide sequence. T-cells described herein that are genetically modified to express or overexpress an amino acid transporter may have enhanced robustness and/or survival in a tumor microenvironment and resource-depleted, for example, arginine-depleted, microenvironments, as compared to, for example, T-cell populations not genetically modified to express or overexpress the amino acid transporter. The described genetically modified T-cells and expression vectors are useful for treating cancers and other diseases that require targeting of T-cells to a specific cell population or which require enhanced

robustness of T-cells in order to survive in a biological environment depleted of one or more amino acids, for example, arginine.

[0010] In one example, disclosed herein is a genetically modified T-cell that is genetically modified to express an arginine transporter and a chimeric antigen receptor (CAR). In some examples, the CAR has at least one antigen-specific targeting region that specifically binds a cell surface antigen present on a target cell population, a transmembrane domain, and an intracellular signaling domain. In some examples, the CAR has at least an antigen-specific targeting region that specifically binds a cell surface antigen present on a target cell population, a transmembrane domain, at least one co-stimulatory domain, and an intracellular signaling domain.

[0011] Also described herein are expression vectors that include nucleotide sequences encoding a CAR and/or an amino acid transporter, for example, an arginine transporter. In some examples, transcription of expression vectors described herein results in producing a ribonucleic acid (RNA), for example a messenger RNA (mRNA) sequence encoding a CAR and/or an amino acid transporter, for example, an arginine transporter nucleotide sequence. In some examples, expression vectors described herein are capable of expressing a CAR and/or an amino acid transporter, for example, an arginine transporter. In another example, described herein is an expression vector comprising an isolated nucleic acid sequence encoding an antigen-specific targeting region, a transmembrane domain, optionally, at least one co-stimulatory domain, an intracellular signaling domain, and an arginine transporter. In some examples described herein is an expression vector comprising an isolated nucleic acid sequence encoding an antigen-specific targeting region, a transmembrane domain, optionally, at least one co-stimulatory domain, and an intracellular signaling domain. In some examples described herein is an expression vector comprising an isolated nucleic acid sequence encoding an arginine transporter. In some examples described herein is an expression vector comprising an isolated nucleic acid sequence encoding an antigen-specific targeting region, a transmembrane domain, optionally at least one co-stimulatory domain, an intracellular signaling domain, and an amino acid transporter. In some examples described herein is an expression vector comprising an isolated nucleic acid sequence encoding an amino acid transporter.

[0012] Also described herein are expression vectors that include nucleotide sequences encoding an amino acid transporter, for example, an arginine transporter. In some examples, transcription of expression vectors described herein results in producing a ribonucleic acid (RNA), for example a messenger RNA (mRNA) sequence encoding an amino acid transporter, for example, an arginine transporter nucleotide sequence. In some examples, expression vectors described herein are capable of expressing an amino acid transporter, for example, an arginine transporter. In some examples described herein is an expression vector comprising an isolated nucleic acid sequence encoding an arginine transporter. In some examples described herein is an expression vector comprising an isolated nucleic acid sequence encoding an arginine transporter. In some examples described herein is an expression vector comprising an isolated nucleic acid sequence

encoding an amino acid transporter. In some examples described herein is an expression vector comprising an isolated nucleic acid sequence encoding an amino acid transporter. In examples described herein, a nucleic acid sequence can be, for example, a ribonucleic acid (RNA) sequence, a deoxyribonucleic acid (DNA) sequence, or a mixed DNA and RNA sequence.

[0013] In some examples, an expression vector described herein comprises a nucleotide sequence encoding a CAR and an amino acid transporter, wherein the CAR and the amino acid transporter nucleotide sequences are transcribed into separate mRNA transcripts. In some examples, an expression vector described herein comprises a nucleotide sequence encoding a CAR and an amino acid transporter, wherein the CAR and the amino acid transporter nucleotide sequences are transcribed together into a single mRNA transcript. In examples wherein the CAR and the amino acid transporter nucleotide sequences are transcribed together into a single mRNA transcript, the expression vector nucleotide sequence encoding the CAR and the amino acid transporter mRNA transcript can include an internal ribosome entry sequence (IRES). In some examples, the IRES is disposed between the portion of the nucleotide sequence encoding the CAR and the portion of the nucleotide sequence encoding the amino acid transporter. Thus, in examples, a CAR nucleotide sequence and an amino acid transporter nucleotide sequence are separated by an IRES sequence. In examples wherein the CAR and the amino acid transporter nucleotide sequences are transcribed together into a single mRNA transcript, the expression vector nucleotide sequence encoding the CAR and the amino acid transporter mRNA transcript may include a 2A self-cleavage sequence disposed between the portion of the nucleotide sequence encoding the CAR and the portion of the nucleotide sequence encoding the amino acid transporter. Thus, in some examples, a CAR nucleotide sequence and an amino acid transporter nucleotide sequence are separated by a 2A self-cleavage sequence. In some examples, a peptide translated from an mRNA that includes a CAR nucleotide sequence, a 2A self-cleavage sequence, and an amino acid transporter nucleotide sequence, is cleaved after translation at the 2A self-cleavage site.

[0014] In another example, also described herein is a genetically modified T-cell modified to express a CAR encoded by an expression vector. Also described herein is a genetically modified T-cell modified to express a CAR and an amino acid transporter, for example, an arginine transporter, encoded by an expression vector. Also described herein is a genetically modified T-cell modified to express an

identity to one of SEQ ID NO: 180, 184-188, 204, 205, 210, 214, 215, 220-222, 227-230, 234-236, 242, and 246.

[0023] In another aspect, described herein is a pharmaceutically acceptable composition comprising a genetically modified T-cell described herein and a pharmaceutically acceptable excipient.

[0024] Also described herein is a priming medium comprising L-arginine for priming a genetically modified T-cell, for example, a genetically modified T-cell described herein. A priming medium described herein can increase intracellular arginine concentration in a genetically modified T-cell, for example, a genetically modified T-cell expressing an arginine transporter. A priming medium described herein can prime genetically modified T-cells for treatment. For example, a priming medium described herein can increase intracellular arginine concentration in genetically modified T-cells prior to administration of the genetically modified T-cells to a patient in need thereof, for example, a patient in need of treatment of a cancer. In another aspect, described herein is a priming medium comprising a genetically modified T-cell described herein and L-arginine.

[0025] Also described herein are pharmaceutical compositions comprising CAR-T cells. In a further example, described herein is a pharmaceutical composition comprising a CAR-T cell which expresses a recombinant arginine transporter and a chimeric antigen receptor protein. In some examples, a pharmaceutical composition of the disclosure comprises a CAR-T cell, wherein the CAR-T cell comprises one or more expression vectors that comprise a nucleic acid sequence encoding an arginine transporter and/or a chimeric antigen receptor protein. In some examples, a pharmaceutical composition described herein comprises a CAR-T cell which expresses an arginine transporter. In some examples, a pharmaceutical composition of the disclosure comprises a CAR-T cell, wherein the CAR-T cell comprises one or more recombinant nucleic acid sequences encoding an arginine transporter and/or a chimeric antigen receptor protein. In some examples, a pharmaceutical composition described herein comprises a CAR-T cell which expresses an arginine transporter, for example, a recombinant protein arginine transporter. In various examples, the arginine transporter is selected from the group consisting of CAT-1,

cells. In some examples, a kit described herein can include agents for producing CAR-T cells, including expression vectors, viral constructs, cells, transfection reagents and media, agents for cell selection (for example, antibodies), and/or growth media.

[0026a] In another aspect, the present disclosure refers to use of the pharmaceutical composition described herein in the manufacture of a medicament for treating a cancer in a patient in need thereof. In a further aspect, the present disclosure refers to use of the pharmaceutical composition as described herein, wherein the CAR binds a solid tumor cancer cell surface antigen, in the manufacture of a medicament for treating a solid tumor cancer in a patient in need thereof. In a further aspect, the present disclosure refers to use of the pharmaceutical composition as described herein, wherein the CAR binds a hematological cancer cell surface antigen, in the manufacture of a medicament for treating a hematological cancer in a patient in need thereof. In a further example, the present disclosure refers to use of the pharmaceutical composition as described herein in the manufacture of a medicament for modulating intracellular arginine levels to effect a T cell-mediated immune response in a patient in need thereof. In a further example, the present disclosure refers to use of composition comprising a chimeric antigen receptor T cell (CAR-T cell) which expresses a recombinant arginine transporter and a chimeric antigen receptor protein in the manufacture of a medicament for treating a condition in a human patient in need thereof. In a further example, the present disclosure refers to use of T-cells that are a) genetically modified to express a chimeric antigen receptor wherein the chimeric antigen receptor comprises: at least one antigen-specific targeting region that specifically binds the cell surface antigen present on the target cell population, a transmembrane domain, an intracellular signaling domain, and b) genetically modified to express a recombinant arginine transporter in the manufacture of a medicament for modulating a T cell-mediated immune response to a target cell population expressing a cell surface antigen in a patient in need thereof. In a further example, the present disclosure refers to use of a composition comprising a T cell which expresses a recombinant arginine transporter in the manufacture of a medicament for treating a condition in a human patient in need thereof.

[0027] Also described herein are methods of treating cancer using a pharmaceutical composition described herein. For example, described herein is a method of treating a solid tumor

cancer in a patient in need thereof, the method comprising administering to the patient an effective amount of a pharmaceutical composition described herein. For example, described herein is a method of treating a solid tumor cancer in a patient in need thereof, the method comprising administering to the patient an effective amount of a pharmaceutical composition comprising a genetically modified T-cell described herein (for example, a CAR-T cell or a T-cell genetically modified to express an amino acid transporter) and a pharmaceutically acceptable excipient.

[0028] Also described herein are methods of treating a hematological cancer using a pharmaceutical composition described herein. For example, described herein is a method of treating a hematological cancer in a patient in need thereof, the method comprising administering to the patient an effective amount of a pharmaceutical composition described herein. For example, described herein is a method of treating a hematological cancer in a patient in need thereof, the method comprising administering to the patient an effective amount of a pharmaceutical composition comprising a genetically modified T-cell described herein (for example, a CAR-T cell or a T-cell genetically modified to express an amino acid transporter) and a pharmaceutically acceptable excipient.

[0029] Also described herein are methods of modulating intracellular arginine levels (for example, intracellular T-cell arginine levels) to effect a T cell-mediated immune response in a patient in need of treatment. For example, described herein is a method of modulating intracellular arginine levels to effect a T cell-mediated immune response in a patient in need thereof, the method comprising modulating intracellular arginine levels of a genetically modified T-cell. In some examples, the method of modulating intracellular arginine levels to effect a T cell-mediated immune response in a patient in need thereof further comprises administering to the patient an effective amount of a pharmaceutical composition described herein (*e.g.*, a pharmaceutical

patient in need thereof, the method comprising administering to the human patient a therapeutically effective amount of a composition comprising a CAR-T cell once every week, once every 2 weeks, once every 3 weeks, or once every 4 weeks. Also described herein is a method for modulating a T cell-mediated immune response to a target cell population expressing a cell surface antigen in a patient in need thereof, the method comprising administering to the patient a therapeutically effective amount of genetically modified T-cells once every week, once every 2 weeks, once every 3 weeks, or once every 4 weeks.

[0038] In some examples, the methods described herein comprise administering a specified number of CAR-T cells based on the weight of the patient or a specified range of CAR-T cells based on the weight of the patient. In some examples, a method described herein comprises administering about 10^2 , about 10^3 , about 10^4 , about 10^5 , about 10^6 , about 10^7 , about 10^8 , about 10^9 , about 10^{10} , about 10^{11} , about 10^{12} , about 10^{13} , about 10^{14} , about 10^{15} , about 10^{16} , about 10^{17} , about 10^{18} , about 10^{19} , about 10^{20} , about 10^{25} , about 10^{30} , about 10^{35} , about 10^{40} , about 10^{45} , or about 10^{50} CAR-T cells per kilogram of the patient. In some examples, a method described herein comprises administering about 10^2 to 10^7 , about 10^2 to 10^{10} , about 10^3 to 10^{10} , about 10^4 to 10^{10} , about 10^5 to 10^{10} , about 10^6 to 10^{10} , about 10^7 to 10^{10} , about 10^8 to 10^{11} , about 10^9 to 10^{12} , about 10^{10} to 10^{13} , about 10^7 to 10^{15} , about 10^5 to 10^{15} , about 10^{10} to 10^{20} , about 10^{10} to 10^{25} , about 10^{10} to 10^{30} , about 10^7 to 10^{20} , about 10^7 to 10^{25} , about 10^{10} to 10^{50} , or about 10^7 to 10^{50} CAR-T cells per kilogram of the patient. For example, in some examples, a method described herein comprises administering about 10^7 to 10^{10} CAR-T cells per kilogram of the patient.

[0039] Another aspect of the disclosure includes a method of making a genetically modified CAR-T cell that expresses a recombinant arginine transporter selected from the group consisting of: (i) CAT-1, (ii) CAT-2, (iii) CAT-3, (iv) CAT-4, (v) y^+LAT1 and 4F2hc, (vi) y^+LAT2 and 4F2hc, (vii) $b^{0,+}AT$ and rBAT, and (viii) $ATB^{0,+}$, the method comprising: transfecting a T-cell with a DNA construct comprising a nucleotide sequence for a specific chimeric antigen receptor and for an arginine transporter thereby producing a genetically modified CAR-T cell that expresses both the chimeric antigen receptor and the arginine transporter; and culturing the genetically modified CAR-T cell in a culture medium comprising arginine wherein said culturing comprises culturing the genetically modified CAR-T cell in the culture medium until the genetically modified CAR-T cell comprises an intracellular arginine level that allows the genetically modified CAR-T-cell to survive

in an arginine-depleted environment. Examples described herein also include a method of making a genetically modified CAR-T cell that expresses an arginine transporter, the method comprising: transducing a T-cell with a virus that includes a nucleotide construct comprising a nucleotide sequence for a specific chimeric antigen receptor and for an arginine

transporter thereby producing a genetically modified CAR-T cell that expresses both the chimeric antigen receptor and the arginine transporter; and culturing the genetically modified CAR-T cell in a culture medium comprising arginine. In yet another aspect, the present disclosure refers to a genetically modified T-cell genetically modified to express a recombinant arginine transporter selected from the group consisting of (i) CAT-1, (ii) CAT-2, (iii) CAT-3, (iv) CAT-4, (v) y^+LAT1 and 4F2hc, (vi) y^+LAT2 and 4F2hc, (vii) $b^{0,+}AT$ and rBAT, and (viii) $ATB^{0,+}$, wherein the genetically modified T-cell comprises an intracellular arginine level that allows the genetically modified T-cell to survive in an arginine-depleted environment. In yet another aspect, the present disclosure refers to a pharmaceutical composition comprising a T cell which expresses a recombinant arginine transporter protein. In another aspect, the present disclosure refers to a priming medium comprising the genetically modified T-cell as described herein, and L-arginine. In some examples, the genetically modified CAR-T cell expresses a recombinant arginine transporter nucleotide sequence. In some examples, the genetically modified CAR-T cell expresses a recombinant arginine transporter protein. In some examples, culturing comprises culturing the genetically modified CAR-T cell in the culture medium until the intracellular arginine level of the CAR-T cell accumulates to a certain level. In some examples, the intracellular arginine level of the CAR-T cell is an intracellular arginine level that allows the CAR-T cell to survive in a tumor microenvironment. For example, in some examples, culturing comprises culturing the genetically modified CAR-T cell in the culture medium until the intracellular arginine level of the CAR-T cell is about 500 μM , about 600 μM , about 700 μM , about 800 μM , about 900 μM , about 1,000 μM , about 1,100 μM , about 1,200 μM , about 1,300 μM , about 1,400 μM , about 1,500 μM , about 1,600 μM , about 1,700 μM , about 1,800 μM , about 1,900 μM , about 2,000 μM , about 2,500 μM , about 3,000 μM , about 3,500 μM , or about 4,000 μM . In some examples, culturing comprises culturing the genetically modified CAR-T cell in the culture medium until the intracellular arginine level of the CAR-T cell is about 500 μM to about 1,000 μM , about 800 μM to about 1,200 μM , about 1,000 μM to about 1,500 μM , about 1,000 μM to about 2,000 μM , about 1,500 μM to about 2,000 μM , about 700 μM to about 900 μM , about 900 μM to about 1,100 μM , about 900 μM to about 1,200 μM , or about 1,300 μM to about 1,500 μM .

[0040] Examples described herein also include a method of making a genetically modified T cell that expresses an arginine transporter, the method comprising: transfecting a T-cell with a DNA construct comprising a nucleotide sequence for an arginine transporter thereby producing a

genetically modified T cell that expresses the arginine transporter; and culturing the genetically modified T cell in a culture medium comprising arginine. Examples described herein also include a method of making a genetically modified T cell that expresses an arginine transporter, the method comprising: transducing a T-cell with a virus that includes a nucleotide construct comprising a nucleotide sequence for an arginine transporter thereby producing a genetically modified T cell that expresses the arginine transporter; and culturing the genetically modified T cell

in a culture medium comprising arginine. In some examples, culturing comprises culturing the genetically modified T cell in the culture medium until the intracellular arginine level of the T cell accumulates to a certain level. In some examples, the intracellular arginine level of the T cell is an intracellular arginine level that allows the T cell to survive in a tumor microenvironment or an arginine-depleted environment. For example, in some examples, culturing comprises culturing the genetically modified T cell in the culture medium until the intracellular arginine level of the T cell is about 500 μ M, about 600 μ M, about 700 μ M, about 800 μ M, about 900 μ M, about 1,000 μ M, about 1,100 μ M, about 1,200 μ M, about 1,300 μ M, about 1,400 μ M, about 1,500 μ M, about 1,600 μ M, about 1,700 μ M, about 1,800 μ M, about 1,900 μ M, about 2,000 μ M, about 2,500 μ M, about 3,000 μ M, about 3,500 μ M, or about 4,000 μ M. In some examples, culturing comprises culturing the genetically modified T cell in the culture medium until the intracellular arginine level of the T cell is about 500 μ M to about 1,000 μ M, about 800 μ M to about 1,200 μ M, about 1,000 μ M to about 1,500 μ M, about 1,000 μ M to about 2,000 μ M, about 1,500 μ M to about 2,000 μ M, about 700 μ M to about 900 μ M, about 900 μ M to about 1,100 μ M, about 900 μ M to about 1,200 μ M, or about 1,300 μ M to about 1,500 μ M.

[0040a] In yet another aspect, the present disclosure refers to a method of making a genetically modified T cell that expresses a recombinant arginine transporter selected from the group consisting of: (i) CAT-1, (ii) CAT-2, (iii) CAT-3, (iv) CAT-4, (v) y^+ LAT1 and 4F2hc, (vi) y^+ LAT2 and 4F2hc, (vii) $b^{0,+}$ AT and rBAT, and (viii) ATB^{0,+}, the method comprising: transfecting a T-cell with a DNA construct comprising a nucleotide sequence for an arginine transporter thereby producing a genetically modified T cell that expresses the arginine transporter; and culturing the genetically modified T cell in a culture medium comprising arginine, wherein said culturing comprises culturing the genetically modified T cell in the culture medium until the genetically modified T-cell comprises an intracellular arginine level that allows the genetically modified T-cell to survive in an arginine-depleted environment.

[0040b] In yet another aspect, the present disclosure refers to an *in vitro* method of increasing T cell survival in a low arginine environment, the method comprising: administering a genetically modified T cell comprising a recombinant arginine transporter to a low arginine environment, wherein the recombinant arginine transporter is selected from the group consisting of: (i) CAT-1, (ii) CAT-2, (iii) CAT-3, (iv) CAT-4, (v) y^+ LAT1 and 4F2hc, (vi) y^+ LAT2 and 4F2hc, (vii) $b^{0,+}$ AT and rBAT, and (viii) ATB^{0,+}, wherein the genetically modified T-cell comprises an

intracellular arginine level that allows the genetically modified T-cell to survive in an arginine-depleted environment.

[0040c] In yet another example, the present disclosure refers to use of a T cell comprising a recombinant arginine transporter in the manufacture of a medicament for increasing T cell survival in a low arginine environment, comprising that the T cell is to be administered to a low arginine environment.

BRIEF DESCRIPTION OF THE FIGURES

[0041] **FIG. 1** is a map of the pBCTex01G expression vector. FIG. 1 discloses "(G4S)³" as SEQ ID NO: 30.

[0042] **FIG. 2** is a map of the pBCTex02mini expression vector.

[0043] **FIG. 3A** is a schematic showing transfection and arginine depletion steps of the experiment described in Example 1.

[0044] **FIG. 3B** is a schematic showing cell filtering and counting steps of the experiment described in Example 1.

[0045] **FIG. 3C** is a set of graphs showing the estimated change in percent of cells transfected with an expression construct (Control, CAT, or ASS) after 72 hours in an arginine-rich (left) or

WHAT IS CLAIMED IS:

1. A genetically modified T-cell genetically modified to express:
 - a) a recombinant arginine transporter selected from the group consisting of:
 - (i) CAT-1,
 - (ii) CAT-2,
 - (iii) CAT-3,
 - (iv) CAT-4,
 - (v) y⁺LAT1 and 4F2hc,
 - (vi) y⁺LAT2 and 4F2hc,
 - (vii) b⁰⁺AT and rBAT, and
 - (viii) ATB⁰⁺, and
 - b) a chimeric antigen receptor (CAR) having at least one antigen-specific targeting region that specifically binds a cancer cell surface antigen present on a target cell population, a transmembrane domain, and an intracellular signaling domain, wherein the genetically modified T-cell comprises an intracellular arginine level that allows the genetically modified T-cell to survive in an arginine-depleted environment.
2. The genetically modified T-cell of claim 1, wherein the genetically modified T-cell comprises a nucleic acid sequence having at least 90%, 95%, or 99% identity to a nucleic acid sequence selected from the group consisting of: SEQ ID NO:180, 184-188, 196-197, 201-203, 204, 205, 210, 214, 215, 220-222, 227-230, 234-236, 242, and 246.
3. The genetically modified T-cell of claim 2, wherein the genetically modified T-cell comprises a nucleic acid expressing a sequence selected from the group consisting of SEQ ID NO: 180, 184-188, 196-197, 201-203, 204, 205, 210, 214, 215, 220-222, 227-230, 234-236, 242, and 246.
4. A pharmaceutically acceptable composition comprising the genetically modified T-cell of any one of claims 1-3, and a pharmaceutically acceptable excipient.
5. A priming medium comprising the genetically modified T-cell of any one of claims 1-3, and L-arginine.

6. The pharmaceutical composition of claim 4, wherein the arginine transporter is CAT-1.
7. The pharmaceutical composition of claim 4, wherein the arginine transporter is CAT-2.
8. The pharmaceutical composition of claim 4, wherein the arginine transporter is CAT-3.
9. The pharmaceutical composition of claim 4, wherein the arginine transporter is CAT-4.
10. The pharmaceutical composition of claim 4, wherein the arginine transporter is y^+LAT1 and 4F2hc.
11. The pharmaceutical composition of claim 4, wherein the arginine transporter is y^+LAT2 and 4F2hc.
12. The pharmaceutical composition of claim 4, wherein the arginine transporter is $b^{0,+}AT$ and rBAT.
13. The pharmaceutical composition of claim 4, wherein the arginine transporter is $ATB^{0,+}$.
14. The pharmaceutical composition of claim 4, wherein the pharmaceutical composition is packaged as a kit.
15. Use of the pharmaceutical composition of any one of claims 4 or 6-14 in the manufacture of a medicament for treating a cancer in a patient in need thereof.
16. Use of the pharmaceutical composition of any one of claims 4 or 6-14, wherein the CAR binds a solid tumor cancer cell surface antigen, in the manufacture of a medicament for treating a solid tumor cancer in a patient in need thereof.
17. Use of the pharmaceutical composition of any one of claims 4 or 6-14, wherein the CAR binds a hematological cancer cell surface antigen, in the manufacture of a medicament for treating a hematological cancer in a patient in need thereof.
18. The use of any one of claims 15-17, wherein the genetically modified T-cells are cultured in a culture medium comprising arginine before they are to be administered.

19. The use of any one of claims 15-18, comprising that the genetically modified T-cell of any one of claims 1-3 is to be administered.
20. The use according to claim 19, further comprising that a second therapeutic agent is to be administered to the patient.
21. The use according to claim 20, wherein the second therapeutic agent is an anti-PD-1, anti-PD-L1, or an anti-CTLA-4 antibody.
22. The use according to claim 20 or 21, wherein the second therapeutic agent is to be administered before, during or after the composition comprising the CAR-T cell.
23. The use according to claim 20 or 21, wherein the second therapeutic agent is to be administered before, during or after the T-cells.
24. The use according to any one of claims 15-23, wherein the composition comprising the CAR-T cells is to be administered to the patient once every week, once every 2 weeks, once every 3 weeks, or once every 4 weeks.
25. The use according to any one of claims 15-23, comprising that 10^7 to 10^{10} CAR-T cells are to be administered per kilogram of the patient.
26. A method of making a genetically modified CAR-T cell that expresses a recombinant arginine transporter selected from the group consisting of:
- (i) CAT-1,
 - (ii) CAT-2,
 - (iii) CAT-3,
 - (iv) CAT-4,
 - (v) y^+LAT1 and 4F2hc,
 - (vi) y^+LAT2 and 4F2hc,
 - (vii) $b^{0,+}AT$ and rBAT, and
 - (viii) $ATB^{0,+}$,
- the method comprising:

transfecting a T-cell with a DNA construct comprising a nucleotide sequence for a specific chimeric antigen receptor and for an arginine transporter thereby producing a genetically modified CAR-T cell that expresses both the chimeric antigen receptor and the arginine transporter; and

culturing the genetically modified CAR-T cell in a culture medium comprising arginine, wherein said culturing comprises culturing the genetically modified CAR-T cell in the culture medium until the genetically modified CAR-T cell comprises an intracellular arginine level that allows the genetically modified CAR-T-cell to survive in an arginine-depleted environment.

27. A genetically modified T-cell genetically modified to express a recombinant arginine transporter selected from the group consisting of:

- (i) CAT-1,
- (ii) CAT-2,
- (iii) CAT-3,
- (iv) CAT-4,
- (v) y^+ LAT1 and 4F2hc,
- (vi) y^+ LAT2 and 4F2hc,
- (vii) $b^{0,+}$ AT and rBAT, and
- (viii) ATB $^{0,+}$,

wherein the genetically modified T-cell comprises an intracellular arginine level that allows the genetically modified T-cell to survive in an arginine-depleted environment.

28. The genetically modified T-cell of claim 27, wherein the genetically modified T-cell comprises a nucleic acid sequence having at least 90%, 95%, or 99% identity to a nucleic acid sequence selected from the group consisting of: SEQ ID NO:180, 184-188, 196-197, 201-203, 204, 205, 210, 214, 215, 220-222, 227-230, 234-236, 242, and 246.

29. The genetically modified T-cell of claim 27, wherein the genetically modified T-cell comprises a nucleic acid expressing a sequence selected from the group consisting of SEQ ID NO: 180, 184-188, 196-197, 201-203, 204, 205, 210, 214, 215, 220-222, 227-230, 234-236, 242, and 246.

30. A pharmaceutically acceptable composition comprising the genetically modified T-cell of any one of claims 27-29, and a pharmaceutically acceptable excipient.

31. A priming medium comprising the genetically modified T-cell of any one of claims 27-29, and L-arginine.
32. The pharmaceutical composition of claim 30, wherein the arginine transporter is CAT-1.
33. The pharmaceutical composition of claim 30, wherein the arginine transporter is CAT-2.
34. The pharmaceutical composition of claim 30, wherein the arginine transporter is CAT-3.
35. The pharmaceutical composition of claim 30, wherein the arginine transporter is CAT-4.
36. The pharmaceutical composition of claim 30, wherein the arginine transporter is y^+LAT1 and 4F2hc.
37. The pharmaceutical composition of claim 30, wherein the arginine transporter is y^+LAT2 and 4F2hc.
38. The pharmaceutical composition of claim 30, wherein the arginine transporter is $b^{0,+}AT$ and rBAT.
39. The pharmaceutical composition of claim 30, wherein the arginine transporter is $ATB^{0,+}$.
40. The pharmaceutical composition of claim 30, wherein the pharmaceutical composition is packaged as a kit.
41. A method of making a genetically modified T cell that expresses a recombinant arginine transporter selected from the group consisting of:
- (i) CAT-1,
 - (ii) CAT-2,
 - (iii) CAT-3,
 - (iv) CAT-4,
 - (v) y^+LAT1 and 4F2hc,
 - (vi) y^+LAT2 and 4F2hc,
 - (vii) $b^{0,+}AT$ and rBAT, and

(viii) ATB^{0,+},

the method comprising:

transfecting a T-cell with a DNA construct comprising a nucleotide sequence for an arginine transporter thereby producing a genetically modified T cell that expresses the arginine transporter; and

culturing the genetically modified T cell in a culture medium comprising arginine, wherein said culturing comprises culturing the genetically modified T cell in the culture medium until the genetically modified T-cell comprises an intracellular arginine level that allows the genetically modified T-cell to survive in an arginine-depleted environment.

42. An *in vitro* method of increasing T cell survival in a low arginine environment, the method comprising: administering a genetically modified T cell comprising a recombinant arginine transporter to a low arginine environment, wherein the recombinant arginine transporter is selected from the group consisting of:

- (i) CAT-1,
- (ii) CAT-2,
- (iii) CAT-3,
- (iv) CAT-4,
- (v) y⁺LAT1 and 4F2hc,
- (vi) y⁺LAT2 and 4F2hc,
- (vii) b^{0,+}AT and rBAT, and
- (viii) ATB^{0,+}, and

wherein the genetically modified T-cell comprises an intracellular arginine level that allows the genetically modified T-cell to survive in an arginine-depleted environment.

43. The method of claim 42, wherein prior to the administration, the genetically modified T cell is produced by transfecting a T cell with a DNA construct comprising a nucleotide sequence encoding the recombinant arginine transporter.

44. The method of claim 42 or 43, wherein the genetically modified T-cell comprises a chimeric antigen receptor and/or a DNA construct comprising a nucleotide sequence encoding a chimeric antigen receptor.

45. The method of any one of claims 42-44, wherein prior to the administration, the method comprises culturing the genetically modified T cell in a culture medium comprising arginine.
46. The method of any one of claims 43-45, wherein the DNA construct comprising a nucleotide sequence encoding the recombinant arginine transporter comprises a nucleic acid sequence having at least 90%, 95%, or 99% identity to a nucleic acid sequence selected from the group consisting of: SEQ ID NO:180, 184-188, 196-197, 201-203, 204, 205, 210, 214, 215, 220-222, 227-230, 234-236, 242, and 246.
47. The method of any one of claims 43-45, wherein the DNA construct comprising a nucleotide sequence encoding the recombinant arginine transporter comprises a nucleic acid expressing a sequence selected from the group consisting of SEQ ID NO: 180, 184-188, 196-197, 201-203, 204, 205, 210, 214, 215, 220-222, 227-230, 234-236, 242, and 246.
48. The method of any one of claims 42-47, wherein the low arginine environment is a cell culture medium.
49. The use of any one of claims 42-47, wherein the low arginine environment is a tumor microenvironment.