In one aspect, the present disclosure provides a genetically engineered hematopoietic cell, which has a modulated Krebs cycle as compared with a native hematopoietic cell of the same type; wherein the genetically engineered hematopoietic cell expresses or overly expresses: (i) a Krebs cycle modulating polypeptide; wherein the Krebs cycle modulating polypeptide is glutamic-oxaloacetic transaminase (GOT), and (ii) a chimeric receptor polypeptide, wherein the chimeric receptor polypeptide comprises: (a) an extracellular target binding domain; (b) a transmembrane domain; and (c) a cytoplasmic signaling domain; wherein the GOT is encoded by an exogenous nucleic acid.

In some examples, the Krebs cycle modulating polypeptide may be an enzyme that catalyzes a reaction of the Krebs cycle. Examples include, but are not limited to, isocitrate dehydrogenase (IDH) such as IDH1 or IDH2, malate dehydrogenase (MDH) such as MDH1 or MDH2, or phosphoglycerate dehydrogenase (PHGDH). In other examples, the Krebs cycle modulating polypeptide is an enzyme that uses a Krebs cycle metabolite as a substrate. Examples include, but are not limited to, a glutamic-oxaloacetic transaminase (GOT) such as GOT1 or GOT2 (also known as aspartate transaminase or aspartate aminotransferase) or phosphoenolpyruvate carboxykinase 1 (PCK1). In yet other examples, the Krebs cycle modulating polypeptide is an enzyme that converts a precursor to a Krebs cycle metabolite. Examples include, but are not limited to, a phosphoserine aminotransferase (PSAT1), a glutamate dehydrogenase (GDH1), a glutamic-pyruvate transaminase 1 (GPT1), or a glutaminase (GLS).

The modified immune cells may further express a chimeric receptor polypeptide, which may comprise (a) an extracellular target binding domain; (b) a transmembrane domain; and (c) a cytoplasmic signaling domain (*e.g.*, a cytoplasmic domain that comprises an immunoreceptor tyrosine-based activation motif (ITAM)). In some examples, the chimeric receptor polypeptide is an antibody-coupled T cell receptor (ACTR), which comprises an extracellular Fc-binding domain (a). In other examples, the chimeric receptor is a chimeric antigen receptor (CAR), which comprises an extracellular antigen binding domain (a). In some examples, (c) is located at the C-terminus of the chimeric receptor polypeptide. In some instances, the chimeric polypeptide may further comprise at least one co-stimulatory signaling domain. In other instances, the chimeric receptor polypeptide may be free of co-stimulatory signaling domains.

Any of the chimeric receptor polypeptides described herein (*e.g.*, an ACTR polypeptide or a CAR polypeptide) may further comprise a hinge domain, which is located at the C-terminus of (a) and the N-terminus of (b). In other examples, the chimeric receptor

polypeptide may be free of any hinge domain. In yet other examples, the chimeric receptor polypeptide, for example, an ACTR polypeptide, may be free of a hinge domain from any non-CD16A receptor. Alternatively or in addition, the chimeric receptor polypeptide further comprises a signal peptide at its N-terminus.

In some examples, the chimeric receptor polypeptide disclosed herein may be an ACTR polypeptide comprising an Fc binding domain (a). In some examples, the Fc binding

domain of (a) can be an extracellular ligand-binding domain of an Fc-receptor, for example, an extracellular ligand-binding domain of an Fc-gamma receptor, an Fc-alpha receptor, or an Fc-epsilon receptor. In particular examples, the Fc binding domain is an extracellular ligand-binding domain of CD16A (*e.g.*, F158 CD16A or V158 CD16A), CD32A, or CD64A. In other examples, the Fc binding domain of (a) can be an antibody fragment that binds the Fc portion of an immunoglobulin. For example, the antibody fragment can be a single chain variable fragment (ScFv), a single domain antibody, (*e.g.*, a nanobody). Additionally, the Fc binding domain of (a) can be a naturally-occurring protein that binds the Fc portion of an immunoglobulin or an Fc-binding fragment thereof. For example, the Fc binding domain can be Protein A or Protein G, or an Fc-binding fragment thereof. In further examples, the Fc binding domain of (a) can be a synthetic polypeptide that binds the Fc portion of an immunoglobulin. Examples include, but are not limited to, a Kunitz peptide, a SMIP, an avimer, an affibody, a DARPin, or an anticalin.

In some examples, the chimeric receptor polypeptide disclosed herein can be a CAR polypeptide comprising an extracellular antigen binding domain (a). In some examples, the extracellular antigen binding domain of (a) is a single chain antibody fragment that binds to a tumor antigen, a pathogenic antigen, or an immune cell specific to an autoantigen. In certain examples, the tumor antigen is associated with a hematologic tumor. Examples include, but are not limited to, CD19, CD20, CD22, Kappa-chain, CD30, CD123, CD33, LeY, CD138, CD5, BCMA, CD7, CD40, and IL-1RAP. In certain examples, the tumor antigen is associated with a solid tumor. Examples include, but are not limited to, GD2, GPC3, FOLR (*e.g.*, FOLR1 or FOLR2), HER2, EphA2, EFGRVIII, IL13RA2, VEGFR2, ROR1, NKG2D, EpCAM, CEA, Mesothelin, MUC1, CLDN18.2, CD171, CD133, PSCA, cMET, EGFR, PSMA, FAP, CD70, MUC16, L1-CAM, B7H3, and CAIX. In certain examples, the pathogenic antigen is a bacterial antigen, a viral antigen, or a fungal antigen, for example, those described herein.

In some examples, the transmembrane domain of (b) in any of the chimeric receptor polypeptide (*e.g.*, ACTR or CAR polypeptide) can be of a single-pass membrane protein, *e.g.*, CD8α, CD8β, 4-1BB, CD28, CD34, CD4, FcεRIγ, CD16A, OX40, CD3ζ, CD3ε, CD3γ, CD3δ, TCRα, CD32, CD64, VEGFR2, FAS, and FGFR2B. Alternatively, the transmembrane domain of (b) can be a non-naturally occurring hydrophobic protein segment.

In some examples, the at least one co-stimulatory signaling domain of the chimeric receptor polypeptides described herein (*e.g.*, ACTR or CAR polypeptides), if

applicable, can be of a co-stimulatory molecule, which can be 4-1BB, CD28, $CD28_{LL\rightarrow GG}$ variant, OX40, ICOS, CD27, GITR, ICOS, HVEM, TIM1, LFA1, and CD2. In some examples, the at least one co-stimulatory signaling domains is a CD28 co-stimulatory signaling domain or a 4-1BB co-stimulatory signaling domain. In some instances, the ACTR polypeptide may comprise two co-stimulatory signaling domains. In some instances, one of the co-stimulatory signaling domains is a CD28 co-stimulatory signaling domain; and the other co-stimulatory domain can be a 4-1BB co-stimulatory signaling domain, an OX40 co-stimulatory signaling domain, a CD27 co-stimulatory signaling domain, or an ICOS co-stimulatory signaling domain. Specific examples include, but are not limited to, CD28 and 4-1BB; or $CD28_{LL\rightarrow GG}$ variant and 4-1BB. Alternatively, any of the chimeric receptor polypeptie may be free of any co-stimulatory signaling domain.

In some examples, the cytoplasmic signaling domain of (c) in any of the chimeric receptor polypeptides described herein (*e.g.*, ACTR or CAR polypeptides) can be a cytoplasmic domain of CD3ζ or FcεR1γ.

In some examples, the hinge domain of any of the chimeric polypeptides described herein (*e.g.*, ACTR or CAR polypeptides), when applicable, can be of CD28, CD16A, CD8α, or IgG. In other examples, the hinge domain is a non-naturally occurring peptide. For example, the non-naturally occurring peptide may be an extended recombinant polypeptide (XTEN) or a $(Gly_4Ser)_n$ polypeptide, in which n is an integer of 3-12, inclusive. In some examples, the hinge domain is a short segment, which may contain up to 60 amino acid residues.

In specific examples, an ACTR polypeptide as described herein may comprise (i) a CD28 co-stimulatory domain; and (ii) a CD28 transmembrane domain, a CD28 hinge domain, or a combination thereof. For example, the ACTR polypeptide comprises components (a)-(e) as shown in Table 3. In particular examples, the ACTR polypeptide comprises the amino acid sequence selected from SEQ ID NOs: 1-80.

In specific examples, a CAR polypeptide described herein may comprise (i) a CD28 co-stimulatory domain or a 4-1BB co-stimulatory domain; and (ii) a CD28 transmembrane domain, a CD28 hinge domain, or a combination thereof. In further specific examples, a CAR polypeptide described herein may comprise (i) a CD28 co-stimulatory domain or a 4-1BB co-stimulatory domain, (ii) a CD8 transmembrane domain, a CD8 hinge domain, or a combination thereof. For example, the CAR polypeptide may comprise an amino acid sequence selected from SEQ ID NOs: 104 and 105.

The hematopoietic cells described herein, expressing the Krebs cycle modulating polypeptide and optionally the chimeric receptor polypeptide, may be a hematopoietic stem cell or a progeny thereof. In some examples, the hematopoietic cells can be immune cells such as natural killer cell, monocyte/macrophage, neutrophil, eosinophil, or T cell. The immune cells can be derived from peripheral blood mononuclear cells (PBMC), hematopoietic stem cells (HSCs), or induced pluripotent stem cells (iPSCs). In some examples, the immune cell is a T cell, in which the expression of an endogenous T cell receptor, an endogenous major histocompatibility complex, an endogenous beta-2-microglobulin, or a combination thereof has been inhibited or eliminated.

In a further aspect, the present disclosure provides a nucleic acid or nucleic acid set, which comprises both of: (A) a first nucleotide sequence encoding a chimeric receptor polypeptide set forth herein; and (B) a second nucleotide sequence encoding a Krebs cycle modulating polypeptide set forth herein; wherein the nucleic acid or nucleic acid set is to be introduced into a population of hematopoietic cells wherein the encoded chimeric receptor polypeptide and the Kreb cycle modulating polypeptide are to be expressed. Any of the hematopoietic cells (*e.g.*, HSCs or immune cells) described herein may comprise a nucleic acid or a nucleic acid set, which collectively comprises: (a) a first nucleotide sequence encoding the Krebs cycle modulating polypeptide; and optionally (b) a second nucleotide sequence encoding the chimeric antigen receptor (CAR) polypeptide. The nucleic acid or the nucleic acid set is an RNA molecule or a set of RNA molecules. In some instances, the immune cell comprises the nucleic acid, which comprises both the first nucleotide sequence and the second nucleotide sequence. In some examples, the coding sequence of the Krebs cycle modulating polypeptide is upstream of that of the CAR polypeptide. In some examples, the coding sequence of the CAR polypeptide is upstream of that of the Krebs cycle modulating polypeptide. Such a nucleic acid may further comprise a third nucleotide sequence located between the first nucleotide sequence and the second nucleotide sequence, wherein the third nucleotide sequence encodes a ribosomal skipping site (*e.g.*, a P2A peptide), an internal ribosome entry site (IRES), or a second promoter.

In some examples, the nucleic acid or the nucleic acid set is comprised within a vector or a set of vectors, which can be an expression vector or a set of expression vectors (*e.g.*, viral vectors such as lentiviral vectors or retroviral vectors). A nucleic acid set or a vector set refers to a group of two or more nucleic acid molecules or two or more vectors, each encoding one of the polypeptides of interest (*i.e.,* the Krebs cycle modulating polypeptide and

the CAR polypeptide). Any of the nucleic acids described herein is also within the scope of the present disclosure.

In another aspect, the present disclosure provides a pharmaceutical composition, comprising a genetically engineered hematopoietic cell as described herein, and a pharmaceutically acceptable carrier. In another example, the present disclosure provides a pharmaceutical composition, comprising any of the immune cells described herein and a pharmaceutically acceptable carrier.

In another aspect, the present disclosure provides a use of a pharmaceutical composition comprising genetically engineered hematopoietic cells in the manufacture of a medicament for inhibiting cells expressing a target antigen in a subject, wherein the genetically engineered hematopoietic cells comprise the genetically engineered hematopoietic cells set forth herein; wherein the genetically engineered hematopoietic cells express a CAR polypeptide, which comprises an extracelluar antigen binding domain specific to the target antigen; and wherein the target antigen is a tumor antigen, a pathogenic antigen, or an immune cell specific to an autoantigen.

or an infectious disorder, and uses thereof for manufacturing a medicament for the intended medical treatment.

The details of one or more examples of the disclosure are set forth in the description below. Other features or advantages of the present disclosure will be apparent from the detailed description of several examples and also from the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present disclosure, which can be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

Figure 1 is a diagram showing proliferation of T cells expressing a GPC3-targeting CAR (SEQ ID NO: 104) or "mock" untransduced T cells in the presence of GPC3-expressing JHH7 or Hep3B target cells and varying concentrations of glucose. Proliferation of CAR-T cells varies as a function of glucose concentration.

Figure 2A and *Figure 2B* are diagrams that show proliferation of T cells expressing a GPC3-targeting CAR (SEQ ID NO: 104) or co-expressing the GPC3-targeting CAR and GOT1 (SEQ ID NO: 87) in the presence of GPC3-expressing JHH7 target cells. *Figure 2A*: 1.25 mM glucose. *Figure 2B*: 10 mM glucose. Proliferation of CAR-T cells co-expressing GOT1 is enhanced relative to cells expressing CAR-T alone at both 1.25 mM and 10 mM glucose.

Figure 3A and *Figure 3B* are diagrams that show proliferation of T cells expressing a GPC3-targeting CAR (SEQ ID NO: 104) or co-expressing the GPC3-targeting CAR and GOT2 (SEQ ID NO: 88) in the presence of GPC3-expressing JHH7 target cells. *Figure 3A*: 1.25 mM glucose. *Figure 3B*: 10 mM glucose. Proliferation of CAR-T cells co-expressing GOT2 is enhanced relative to cells expressing CAR-T alone at both 1.25 mM and 10 mM glucose.

Figure 4 is a chart showing JHH7 xenograft tumor inhibition activity of T cells expressing a GPC3-targeting CAR and T cells co-expressing the GPC3-targeting CAR (SEQ ID NO:104) and GOT2 (SEQ ID NO:88).

WHAT IS CLAIMED IS:

1. A genetically engineered hematopoietic cell, which has a modulated Krebs cycle as compared with a native hematopoietic cell of the same type; wherein the genetically engineered hematopoietic cell expresses or overly expresses:

(i) a Krebs cycle modulating polypeptide; wherein the Krebs cycle modulating polypeptide is glutamic-oxaloacetic transaminase (GOT), and

(ii) a chimeric receptor polypeptide, wherein the chimeric receptor polypeptide comprises:

(a) an extracellular target binding domain;

(b) a transmembrane domain; and

(c) a cytoplasmic signaling domain;

wherein the GOT is encoded by an exogenous nucleic acid.

2. The genetically engineered hematopoietic cell of claim 1, wherein the GOT is GOT1 or GOT2.

3. The genetically engineered hematopoietic cell of claim 1, wherein the Krebs cycle modulating polypeptide is GOT2.

4. The genetically engineered hematopoietic cell of any one of claims 1-3, wherein the chimeric receptor polypeptide is:

(1) an antibody-coupled T cell receptor (ACTR) polypeptide, in which (a) is an extracellular Fc binding domain; or

(2) a chimeric receptor antigen (CAR) polypeptide, in which (a) is an extracellular antigen binding domain.

5. The genetically engineered hematopoietic cell of any one of claims 1-4, wherein the chimeric receptor polypeptide is a CAR polypeptide comprising one or more of the following features:

(i) further comprises at least one co-stimulatory signaling domain;

(ii) is free of co-stimulatory signaling domain; and

(iii) the cytoplasmic signaling domain comprises an immunoreceptor tyrosine-based activation motif (ITAM).

6. The genetically engineered hematopoietic cell of claim 5, wherein the CAR polypeptide further comprises a hinge domain, which is located at the C-terminus of (a) and the N-terminus of (b); and/or wherein the chimeric receptor polypeptide further comprises a signal peptide at its N-terminus.

7. The genetically engineered hematopoietic cell of any one of claims 1-4, wherein the chimeric receptor polypeptide is a CAR polypeptide, in which the extracellular target binding domain of (a) is an antigen binding domain, and wherein the antigen binding domain is a single chain antibody fragment (scFv) that binds to a tumor antigen, a pathogenic antigen, or an immune cell specific to an autoantigen.

8. The genetically engineered hematopoietic cell of claim 7, wherein the antigen binding domain binds the tumor antigen, which is associated with a hematologic tumor, or a solid tumor.

9. The genetically engineered hematopoietic cell of claim 8, wherein:

the tumor antigen associated with the hematologic tumor is selected from the group consisting of CD19, CD20, CD22, Kappa-chain, CD30, CD123, CD33, LeY, CD138, CD5, BCMA, CD7, CD40, and IL-1RAP; or

the tumor antigen associated with the solid tumor is selected from the group consisting of GD2, GPC3, FOLR, HER2, EphA2, EFGRVIII, IL13RA2, VEGFR2, ROR1, NKG2D, EpCAM, CEA, Mesothelin, MUC1, CLDN18.2, CD171, CD133, PSCA, cMET, EGFR, PSMA, FAP, CD70, MUC16, L1-CAM, and CAIX.

10. The genetically engineered hematopoietic cell of any one of claims 1-9, wherein the transmembrane domain is of a membrane protein selected from the group consisting of CD8α, CD8β, 4-1BB, CD28, CD34, CD4, FcεRIγ, CD16A, OX40, CD3ζ, CD3ε, CD3γ, CD3δ, TCRα, CD32, CD64, VEGFR2, FAS, and FGFR2B.

11. The genetically engineered hematopoietic cell of claim 5, wherein the CAR polypeptide comprises the at least one co-stimulatory signaling domain, which is of a co-stimulatory molecule selected from the group consisting of 4-1BB, CD28, $CD28_{LL\rightarrow GG}$ variant, OX40, ICOS, CD27, GITR, ICOS, HVEM, TIM1, LFA1, and CD2.

12. The genetically engineered hematopoietic cell of any one of claims 5-9 and 11, wherein the CAR polypeptide comprises two co-stimulatory signaling domains.

13. The genetically engineered hematopoietic cell of claim 12, wherein:

one of the co-stimulatory signaling domains is a CD28 co-stimulatory signaling domain; and wherein the other co-stimulatory signaling domain is selected from the group consisting of a 4-1BB co-stimulatory signaling domain, an OX40 co-stimulatory signaling domain, a CD27 co-stimulatory signaling domain, and an ICOS co-stimulatory signaling domain; or

wherein the two co-stimulatory signaling domains are: (i) 4-1BB and CD28; or (ii) 4-1BB and $CD28_{LL \rightarrow GG}$ variant.

14. The genetically engineered hematopoietic cell of any one of claims 1-13, wherein the cytoplasmic signaling domain of (c) is a cytoplasmic domain of CD3ζ or FcεR1γ.

15. The genetically engineered hematopoietic cell of claim 5, wherein the CAR polypeptidecomprises (i) a CD28 co-stimulatory domain in combination with a CD28 transmembrane domain, a CD28 hinge domain, or a combination thereof, or (ii) a 4-1BB co-stimulatory domain in combination with a CD8 transmembrane domain, a CD8 hinge domain, or a combination thereof.

16. The genetically engineered hematopoietic cell of claim 5, wherein the CAR polypeptide comprises the amino acid sequence of SEQ ID NOs: 104 or 105.

17. The genetically engineered hematopoietic cell of any one of claims 1-16, wherein the hematopoietic cell is a hematopoietic stem cell or an immune cell.

18. The genetically engineered hematopoietic cell of claim 17, wherein the hematopoietic cell is the immune cell, which is a natural killer cell, macrophage, neutrophil, eosinophil, or T cell.

19. The genetically engineered hematopoietic cell of claim 18, wherein the immune cell is a T cell in which the expression of an endogenous T cell receptor, an endogenous major

histocompatibility complex, an endogenous beta-2-microglobulin, or a combination thereof has been inhibited or eliminated.

20. The genetically engineered hematopoietic cell of any one of claims 1-19, wherein the hematopoietic cell comprises a nucleic acid or nucleic acid set, which comprises both of:

(A) a first nucleotide sequence encoding the Krebs cycle metabolite modulating polypeptide; and

(B) a second nucleotide sequence encoding the chimeric receptor polypeptide.

21. The genetically engineered hematopoietic cell of claim 20, wherein the hematopoietic cell comprises the nucleic acid, which comprises both the first nucleotide sequence and the second nucleotide sequence.

22. The genetically engineered hematopoietic cell of claim 21, wherein the nucleic acid further comprises a third nucleotide sequence located between the first nucleotide sequence and the second nucleotide sequence, wherein the third nucleotide sequence encodes a ribosomal skipping site, an internal ribosome entry site (IRES), or a second promoter.

23. The genetically engineered hematopoietic cell of claim 22, wherein the third nucleotide sequence encodes a ribosomal skipping site, which is a P2A peptide.

24. The genetically engineered hematopoietic cell of any one of claims 20-23, wherein the nucleic acid or the nucleic acid set is comprised within a vector or a set of vectors, which are viral vectors.

25. A pharmaceutical composition, comprising a genetically engineered hematopoietic cell of any one of claims 1-24, and a pharmaceutically acceptable carrier.

26. Use of a pharmaceutical composition comprising genetically engineered hematopoietic cells in the manufacture of a medicament for inhibiting cells expressing a target antigen in a subject, wherein the genetically engineered hematopoietic cells comprise the genetically engineered hematopoietic cells set forth in any one of claims 1-24;
wherein the genetically engineered hematopoietic cells express a CAR polypeptide, which comprises an extracelluar antigen binding domain specific to the target antigen; and

wherein the target antigen is a tumor antigen, a pathogenic antigen, or an immune cell specific to an autoantigen.

27. The use of claim 26, wherein at least some of the cells expressing the target antigen are located in a low-glucose environment.

28. The use of claim 26 or claim 27, wherein the genetically engineered hematopoietic cells are autologous.

29. The use of any one of claims 74-81, wherein the genetically engineered hematopoietic cells are activated, expanded, or both *ex vivo*.

30. The use of any one of claims 26-29, wherein the subject is a human patient suffering from a cancer and the target antigen is a tumor antigen.

31. The use of claim 30, wherein the cancer is selected from the group consisting of a cancer of B-cell origin, breast cancer, gastric cancer, neuroblastoma, osteosarcoma, lung cancer, skin cancer, prostate cancer, colon cancer, renal cell carcinoma, ovarian cancer, rhabdomyosarcoma, leukemia, mesothelioma, pancreatic cancer, head and neck cancer, retinoblastoma, glioma, glioblastoma, liver cancer, and thyroid cancer.

32. The use of claim 31, wherein the cancer of B-cell origin is selected from the group consisting of B-lineage acute lymphoblastic leukemia, B-cell chronic lymphocytic leukemia, and B-cell non-Hodgkin’s lymphoma.

33. A nucleic acid or nucleic acid set, which comprises both of:

(A) a first nucleotide sequence encoding a chimeric receptor polypeptide set forth in any one of claims 1 and 4-16; and

(B) a second nucleotide sequence encoding a Krebs cycle modulating polypeptide set forth in any one of claims 1-3;

wherein the nucleic acid or nucleic acid set is to be introduced into a population of hematopoietic cells wherein the encoded chimeric receptor polypeptide and the Kreb cycle modulating polypeptide are to be expressed.

34. The nucleic acid or nucleic acid set of claim 33, wherein the nucleic acid or the nucleic acid set is an RNA molecule or a set of RNA molecules.

35. The nucleic acid or nucleic acid set of claim 33 or claim 34, wherein the nucleic acid comprises both the first nucleotide sequence and the second nucleotide sequence, and wherein the nucleic acid further comprises a third nucleotide sequence located between the first nucleotide sequence and the second nucleotide sequence, wherein the third nucleotide sequence encodes a ribosomal skipping site, an internal ribosome entry site (IRES), or a second promoter.

36. The nucleic acid or nucleic acid set of claim 35, wherein the ribosomal skipping site is a P2A peptide.

37. The nucleic acid or nucleic acid set of any one of claims 33-36, wherein the nucleic acid or the nucleic acid set is comprised within a vector or a set of vectors.

38. The nucleic acid or nucleic acid set of claim 37, wherein the vector or set of vectors comprises one or more viral vectors.