

GENETICALLY MODIFIED IMMUNE CELLS EXPRESSING A CHIMERIC ANTIGEN RECEPTOR AND HAVING REDUCED PROINFLAMMATORY CYTOKINE SIGNALING

5 CROSS REFERENCE TO RELATED APPLICATION

This application claims benefit under 35 U.S.C. § 119(e) of the U.S. provisional application No. 63/005,684 filed April 6, 2020, the contents of which are incorporated herein by reference in their entirety.

10 SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on April 5, 2021, is named 112126-0025-70003WO00_SEQ.txt and is 59,251 bytes in size.

15 BACKGROUND OF THE INVENTION

Adoptive cell transfer therapy is a type of immunotherapy that involves *ex vivo* expansion of autologous or allogeneic immune cells and subsequent infusion into a patient. The immune cells may be modified *ex vivo* to specifically target malignant cells. Modifications include engineering of T cells to express chimeric antigen receptors (CARs). The promise of
20 adoptive cell transfer therapy, such as CAR T-cell (CAR-T) therapy is often limited by toxicity (*e.g.*, cytokine-associated toxicity). For example, adoptive cell transfer immunotherapy may trigger non-physiologic elevation of cytokine levels (cytokine release syndrome), which could lead to death of recipients (see, *e.g.*, Morgan et al., *Molecular Therapy* 18(4): 843-851, 2010).
25 In addition, modified immune cells may not expand well in patients.

It is therefore of great interest to develop approaches to improve the proliferation of modified immune cells and reduce toxicity associated with CAR-T therapy, while maintaining or enhancing therapeutic efficacy.

30 SUMMARY ~~OF THE INVENTION~~

The present disclosure is based, at least in part, on the development of a chimeric antigen receptor (CAR) comprising an IL-2R β cytoplasmic signaling domain in combination with co-stimulatory signaling and cytoplasmic signaling domains. CAR-T cells expressing

such a CAR construct, and optionally having a knock-out of an endogenous interferon γ gene, are expected to show more effective proliferation of T cells upon activation by tumor target cells, and/or reduced cytokine toxicity, thereby enhancing CAR-T therapeutic efficacy, safety, or a combination thereof.

Accordingly, one aspect of the present disclosure features a chimeric antigen receptor (CAR) comprising: (a) an extracellular antigen binding domain; (b) a co-stimulatory domain such as a 4-1BB co-stimulatory domain; (c) an IL-2R β cytoplasmic signaling domain; and (d) a CD3 ζ signaling domain.

In some embodiment examples, the 4-1BB co-stimulatory signaling domain comprises the amino acid sequence set forth in

KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL (SEQ ID NO: 1).

Alternatively or in addition, the IL-2R β cytoplasmic signaling domain may comprise the amino acid sequence set forth in

NCRNTGPWLKKVLKCNTDPDSKFFSQLSSEHGGDVQKWLSSFPSSSFSPGGLAPEISP

LEVLERDKVTQLPLNTDAYLSLQELQGQDPHTLV (SEQ ID NO: 2). Alternatively or in addition, the CD3 ζ signaling domain comprises the amino acid sequence set forth

in RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDRGRDPEMGGKPRRKNPQ
EGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPP
R (SEQ ID NO: 3).

In some embodiment examples, any of the CAR disclosed herein may further comprise a transmembrane domain, which is C-terminal to the extracellular antigen binding domain and N-terminal to the 4-1BB co-stimulatory domain. Such a transmembrane domain may be derived from a cell surface receptor, which can be the alpha, beta or zeta chain of a T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, CD271, TNFRSF19 and Killer Cell Immunoglobulin-Like Receptor (KIR), or any combination thereof.

In some embodiment examples, the CAR disclosed herein may further comprise a hinge domain, which may be linked to the C-terminus of the extracellular antigen binding domain and to the N-terminus of the transmembrane domain. Exemplary hinge domains may be of CD28, CD8, or an IgG, which optionally is IgG1 or IgG4.

In some embodiment examples, the CAR disclosed herein may further comprise a STAT3 binding motif, which can be located at the C-terminal of the CD3 ζ signaling domain. In one example, the STAT3 binding motif comprises the amino sequence set forth in YX₁X₂Q, wherein X₁ and X₂ are each independently an amino acid. For example, the STAT3 binding

motif comprises the amino acid sequence set forth in YRHQ (SEQ ID NO: 4). In one example, the CAR disclosed herein may comprise a C-terminus fragment comprising the CD3 ζ signaling domain and the STAT3 binding motif, and wherein the C-terminus fragment comprises the amino acid sequence set forth in

RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQE
GLYNELQKDKMAEAYSEIGMKGERRRRGKGHDGLYQGLSTATKDTYDAYRHQALPPR
(SEQ ID NO: 5).

The extracellular antigen binding domain in the CAR disclosed herein may bind a tumor associated antigen. Examples include, but are not limited to, 5T4, CD2, CD5, CD3, CD7, CD19, CD20, CD22, CD30, CD33, CD38, CD70, CD123, CD133, CD171, CEA, CS1, Claudin 18.2, BCMA, BAFF-R, PSMA, PSCA, desmoglein (Dsg3), HER-2, FAP, FSHR, NKG2D, GD2, EGFRVIII, mesothelin, ROR1, MAGE, MUC1, MUC16, GPC3, Lewis Y, and VEGFRII. In some embodiment examples, the extracellular antigen binding domain is a single-chain antibody fragment (scFv). In some examples, the extracellular antigen binding domain in the CAR is an scFv that binds CD19 (anti-CD19 scFv). Such an anti-CD19 scFv may comprise the amino acid sequence of SEQ ID NO: 6. Alternatively, the anti-CD19 scFv may comprise the amino acid sequence of SEQ ID NO:39. In another example, the anti-CD19 scFv may comprise the amino acid sequence of SEQ ID NO:40. In yet another example, the anti-CD19 scFv may comprise the amino acid sequence of SEQ ID NO:41. In other examples, the extracellular antigen binding domain in the CAR is an scFv that binds BCMA (anti-BCMA scFv). In one example, the anti-BCMA scFv may comprise the amino acid sequence set forth in SEQ ID NO: 7.

Any of the CAR disclosed herein may further comprise a signal peptide located at the N-terminus of the CAR.

In another aspect, provided herein is a population of immune cells, comprising a first plurality of immune cells that express any of the CARs disclosed herein. Such a population of immune cells may further comprise a second plurality of immune cells that expresses an antibody specific to interleukin-6 (IL-6) or IL-6 receptor (IL-6R). In some embodiment examples, the anti-IL6 or anti-IL6R antibody may comprise the same heavy chain complementarity determining domains (CDRs) and the same light chain CDRs as a reference antibody. The reference antibody can be one of the following:

(a) comprising a heavy chain variable domain (V_H) having the amino acid sequence set forth in SEQ ID NO: 14 and a light chain variable domain (V_L) having the amino acid sequence set forth in SEQ ID NO: 15;

(b) comprising a heavy chain variable domain (V_H) having the amino acid sequence set forth in SEQ ID NO: 16 and a light chain variable domain (V_L) having the amino acid sequence set forth in SEQ ID NO: 17;

5 (c) comprising a heavy chain variable domain (V_H) having the amino acid sequence set forth in SEQ ID NO: 18 and a light chain variable domain (V_L) having the amino acid sequence set forth in SEQ ID NO: 19;

(d) comprising a heavy chain variable domain (V_H) having the amino acid sequence set forth in SEQ ID NO: 20 and a light chain variable domain (V_L) having the amino acid sequence set forth in SEQ ID NO: 21;

10 (e) comprising a heavy chain variable domain (V_H) having the amino acid sequence set forth in SEQ ID NO: 22 and a light chain variable domain (V_L) having the amino acid sequence set forth in SEQ ID NO: 23; and

(f) comprising a heavy chain variable domain (V_H) having the amino acid sequence set forth in SEQ ID NO: 24 and a light chain variable domain (V_L) having the amino acid
15 sequence set forth in SEQ ID NO: 25.

In some instances, the antibody specific to IL-6 or IL-6R comprises the same V_H and the same V_L as the reference antibody. Any of the antibodies specific to IL-6 or IL-6R may be a scFv. In one example, the scFv may comprise the amino acid sequence of SEQ ID NO:8. In another example, the scFv may comprise the amino acid sequence of SEQ ID NO:9. In yet
20 another example, the scFv may comprise the amino acid sequence of SEQ ID NO:26. In yet another example, the scFv may comprise the amino acid sequence of SEQ ID NO: 27.

The population of immune cells disclosed herein may further comprise a third plurality of immune cells that express an IL-1 antagonist. In some examples, the IL-1 antagonist is IL-1RA.

In some instances, at least two of the first plurality of immune cells, the second plurality of
25 immune cells, and the third plurality of immune cells in the immune cell population comprise common members. For example, at least 10% of the immune cells therein express the CAR, the antibody specific to IL-6 or IL-6R, and the IL-1 antagonist. In another example, about 50-70% of the cells express the CAR, the antibody specific to IL-6 or IL-6R, and the IL-1 antagonist.

The immune cells may be T-cells, Natural Killer (NK) cells, tumor infiltrating lymphocytes,
30 dendritic cells, macrophages, B cells, neutrophils, eosinophils, basophils, mast cells, myeloid-derived suppressor cells, mesenchymal stem cells, precursors thereof, or a combination thereof. In some instances, the immune cells are T cells. In some examples, at

least a portion of the T cells (*e.g.*, at least 30%, at least 40%, at least 50%, at least 60%, at least 70% or above) do not express one or more of an endogenous T cell receptor, CD52, interferon gamma (IFN- γ), beta-2 microglobulin (B2M), and granulocyte macrophage-colony stimulating factor (GM-CSF). In one specific example, at least a portion of the T cells (*e.g.*, at least 30%,
5 at least 40%, at least 50%, at least 60%, at least 70% or above) do not express IFN- γ . A cell that does not express a protein of interest means that expression of the protein cannot be detected or only background level of expression can be detected by a conventional method (*e.g.*, ELISA or FACS).

In additiona further aspect, provided herein is a method for producing a population of
10 modified immune cells, the method comprising: (a) providing a population of immune cells (*e.g.*, those disclosed herein); and (b) introducing into the immune cells a first nucleic acid coding for a CAR such as those disclosed herein. Such a method may further comprise (c) introducing into the immune cells a second nucleic acid coding for an antibody specific to interleukin-6 (IL-6) or IL-6 receptor (IL-6R), *e.g.*, those disclosed herein. In some instances,
15 the first nucleic acid and the second nucleic acid are located in the same vector. In other instances, the first nucleic acid and the second nucleic acid are located in different vectors.

In some embodimentexamples, the method may further comprise introducing into the immune cells a third nucleic acid encoding an IL-1 antagonist, for example, IL-1RA. In some instances, the first nucleic acid and the third nucleic acid are located in the same vector. In
20 other instances, the second nucleic acid and the third nucleic acid are located in the same vector. Alternatively, the first nucleic acid, the second nucleic acid, and the third nucleic acid are located in different vectors.

Additionally, the present disclosure is based, at least in part, on the discovery that, unexpectedly, genetically engineered immune cells having reduced IFN γ signaling (*e.g.*, by
25 knocking out endogenous IFNG gene or expressing an IFN γ antagonist) maintained robust T cell cytotoxicity and also significantly reduced cytokine release syndrome (CRS) in patients receiving the CAR-T cell therapy. In certain embodimentexamples, the reduced expression of the endogenous IFNG gene ranges from 5%-70% compared to the same type of immune cells having a wild-type IFNG gene.

AccordinglyIn one aspect, the present disclosure also provides a population of immune
30 cells comprising a first plurality of genetically engineered immune cells that (a) comprise a disrupted endogenous interferon gamma (IFN γ) gene or IFN γ receptor (IFN γ R) gene; and/or (b) express an IFN γ antagonist.

In some embodiment examples, the genetically engineered immune cells comprise the disrupted endogenous IFN γ or IFN γ R gene. In some examples, the disrupted endogenous IFN γ or IFN γ R gene is produced by gene editing, for example, gene editing is mediated by a CRISPR/Cas gene editing system.

5 In some embodiment examples, the genetically engineered cells express the IFN γ antagonist. For example, the genetically engineered cells secretes the IFN γ antagonist. In some instances, the IFN γ antagonist can be anti-IFN γ antibody, a secreted IFN γ receptor, or an anti-IFN γ R antibody. In some examples, the IFN γ antagonist can be anti-IFN γ antibody or anti-IFN γ R antibody. In specific examples, the antibody is a single chain variable fragment (scFv).

10 In some examples, the IFN γ antagonist is an anti-IFN γ scFv. In one example, the anti-IFN γ scFv comprises: a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 53, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 52. In another example, the anti-IFN γ scFv comprises: a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 56, and a light chain variable region
15 comprising the amino acid sequence of SEQ ID NO: 55. In yet another example, the anti-IFN γ scFv comprises: a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 59, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 58. In specific examples, the anti-IFN γ scFv comprises the amino acid sequence of SEQ ID NO: 54. In another specific example, the anti-IFN γ scFv comprises the amino acid sequence of
20 SEQ ID NO: 57. In yet another specific example, the anti-IFN γ scFv comprises the amino acid sequence of SEQ ID NO: 60.

In some embodiment examples, the population of immune cells disclosed herein may express a chimeric antigen receptor (CAR), which may comprise (a) an extracellular antigen binding domain, (b) a co-stimulatory domain (*e.g.*, a 4-1BB or CD28 co-stimulatory domain),
25 (c) a cytoplasmic signaling domain, and optionally (d) a transmembrane and/or hinge domain. In some examples, the extracellular antigen binding domain may comprise a single chain variable fragment (scFv), which may binds a tumor associated antigen, *e.g.*, those disclosed herein. In some examples, the tumor associated antigen is CD19 and the extracellular antigen binding domain comprises a scFv that binds CD19, for example, any of the anti-CD19 scFv
30 antibodies disclosed herein. In other examples, the tumor associated antigen is B cell maturation antigen (BCMA) and the extracellular antigen binding domain comprises a scFv that binds BCMA, for example, any of the anti-BCMA scFv antibody disclosed herein.

The co-stimulatory domain used in the CAR disclosed herein may be from 4-1BB (CD137), OX40, CD70, CD27, CD28, CD5, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), DAP10, and DAP12, or any combination thereof. Alternatively or in addition, the cytoplasmic signaling domain may comprise a CD3zeta (CD3ζ) signaling domain, an interleukin 2 receptor beta subunit (IL-2Rβ) cytoplasmic signaling domain, or a combination thereof. Alternatively or in addition, the transmembrane domain, if any, may be from a cell surface receptor, which can be the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, CD271, TNFRSF19 and Killer Cell Immunoglobulin-Like Receptor (KIR), or any combination thereof. In some instances, the CAR may further comprises a hinge or a spacer or a combination of both to connect the functional domains of (a)-(d).

Alternatively or in addition, the CAR may comprise a hinge domain linked to the C-terminus of the extracellular antigen binding domain and to the N-terminus of the transmembrane domain. Examples include a hinge domain from CD28, CD8, or an IgG, which optionally is IgG1 or IgG4.

In some instances, the CAR may be any of the CARs disclosed herein that comprise an IL-2Rβ) cytoplasmic signaling domain. Such a CAR may further comprises a signal peptide located at the N-terminus. Examples include, but are not limited to, a signal peptide derived from albumin, CD8, a growth hormone, IL-2, an antibody light chain, or a *Gaussia* luciferase.

The population of immune cells may further comprises a second plurality of immune cells that express an antibody specific to interleukin-6 (IL-6) or IL-6 receptor (IL-6R), and/or a third plurality of immune cells that express an IL-1 antagonist, such as those disclosed herein. In some instances, the first plurality, the second plurality, and/or the third plurality of immune cells comprise common members. In some examples, the population of immune cells comprise genetically engineered cells (e.g., at least 20%, at least 30%, at least 40%, at least 50% or above) having reduced IFNγ signaling, expressing a CAR, expressing an IL-6 antagonist, and expressing an IL-1 antagonist. In other examples, the population of immune cells comprise genetically engineered cells that exhibit two or more of the just-noted genetic modifications.

Also within the scope of the present disclosure is a pharmaceutical composition comprising any of the immune cell populations disclosed herein comprising one or more of the genetic modifications as also disclosed herein, e.g., expressing a CAR, having reduced IFNγ signaling, expressing an IL-6 antagonist, and/or expressing an IL-1 antagonist, and a pharmaceutically acceptable carrier.

In another aspect, the present disclosure provides a pharmaceutical composition comprising the population of immune cells as described herein and a pharmaceutically acceptable carrier. In yet another aspect, the present disclosure provides a use of the population of immune cells as described herein or the pharmaceutical composition as described herein in the manufacture of a medicament for reducing or eliminating undesired cells in a subject. In yet another aspect, the present disclosure provides a use of the population of immune cells as described herein in the manufacture of a cell therapy-based medicament for treating a cancer, an infectious disease, or an immune disorder. In a further aspect, the present disclosure provides the population of immune cells as described herein for use in therapy.

In addition, provided herein is a method for reducing or eliminating undesired cells in a subject, the method comprising administering to a subject in need thereof a therapeutically effective amount of the population of immune cells or the pharmaceutical composition as disclosed herein. In some ~~embodiment~~examples, the subject is a human cancer patient. The genetically engineered immune cell expresses a CAR that is specific to a tumor associated antigen. Examples include, but are not limited to, 5T4, CD2, CD5, CD3, CD7, CD19, CD20, CD22, CD30, CD33, CD38, CD70, CD123, CD133, CD171, CEA, CS1, Claudin 18.2, BCMA, BAFF-R, PSMA, PSCA, desmoglein (Dsg3), HER-2, FAP, FSHR, NKG2D, GD2, EGFRVIII, mesothelin, ROR1, MAGE, MUC1, MUC16, GPC3, Lewis Y, and VEGFR11.

In some instances, the cancer is a solid tumor cancer. Examples include, but are not limited to, breast cancer, lung cancer, pancreatic cancer, liver cancer, glioblastoma (GBM), prostate cancer, ovarian cancer, mesothelioma, colon cancer, and stomach cancer. In some instances, the cancer is a hematological cancer. Examples include, but are not limited to, leukemia, lymphoma, or multiple myeloma. Exemplary leukemia includes chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), or chronic myelogenous leukemia (CML). Exemplary lymphoma includes mantle cell lymphoma, non-Hodgkin's lymphoma or Hodgkin's lymphoma.

In some examples, the genetically engineered immune cells (e.g., T cells) express a CAR that binds CD19 (e.g., those disclosed herein). The subject is a human patient having lymphoblastic leukemia, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, mantle cell lymphoma, large B-cell lymphoma, or non-Hodgkin's lymphoma. In other examples, the genetically engineered immune cells (e.g., T cells) express a CAR that binds

BCMA (*e.g.*, those disclosed herein). The subject is a human patient having multiple myeloma, relapsed multiple myeloma, or refractory multiple myeloma.

Any of the methods disclosed herein may comprise, prior to the cell therapy, performing a lymphodepleting treatment to the subject to condition the subject for the cell therapy. In some instances, the lymphodepleting treatment comprises administering to the
5 subject one or more of fludarabine and cyclophosphamide.

Alternatively or in addition, the human patient received a therapy against the cancer to reduce tumor burden prior to the cell therapy. Exemplary prior therapy includes a chemotherapy, an immunotherapy, a radiotherapy, or a surgery.

10 In some instances, the subject may have an infectious disease, or an immune disorder.

Also within the scope of the present disclosure are any of the genetically engineered immune cells as disclosed herein for use in treating any of the target diseases also disclosed

herein (e.g., cancer), as well as use of such genetically engineered immune cells for manufacturing a medicament for treatment of the target disease.

Also within the scope of the present disclosure are immune cell populations as described herein for use in treating the target disease as also described herein, and uses of such immune cell population in manufacturing a medicament for use in treatment of a target disease.

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

BRIEF DESCRIPTION OF THE DRAWINGS

FIGs. 1A-1E include diagrams showing anti-tumor efficiency achieved by CAR-T cells expressing an exemplary CAR constructs disclosed herein.

FIG. 1A is a chart showing the numbers of white blood cells (WBC) and lymphocytes in a human patient at different time points as indicated after the T cell infusion.

FIG. 1B is a chart showing the chart showing body temperature (°C) of a human patient at different time points as indicated after T-cell infusion.

FIG. 1C is a chart showing the levels of IFN γ and IL-6 in the human patient at different time points as indicated after T-cell infusion.

FIG. 1D is a chart showing the levels of CRP (C reactive protein) in a human patient at different time points as indicated after T-cell infusion.

FIG. 1E is a chart showing the levels of Ferritin in a human patient at different time points as indicated after T-cell infusion.

FIG. 2 is a chart summarizing the levels of IFN γ in T-cells where the endogenous IFN- γ was gene edited with different sgRNA candidates targeting IFN γ exon 1 and using the CRISPR system. The levels of IFN γ were determined by intracellular staining of the T-cells.

FIG. 3 shows the frequencies of CAR $^{+}$ T cells post infusion in treated multiple myeloma (MM) patients. Patient #1 (circle) was infused with anti-BCMA CAR-T cells that had the intracellular signaling domains of 41BB, IL-2R β receptor and CD3 ζ . Patient #2 (square) was infused with anti-BCMA CAR-T cells that had the intracellular signaling domains of 41BB and CD3 ζ without the IL-2R β receptor co-stimulatory signaling domains.

FIG. 4 shows the changes of CD19 $^{+}$ cells and CAR $^{+}$ /CD3 $^{+}$ cells in the peripheral blood of three patients, Pt#1, Pt#2, and Pt#3 infused with anti-CD19 CAR-T cells with 41BB, IL-2R β and CD3 ζ signaling.

What Is Claimed Is:

1. ~~A chimeric antigen receptor (CAR) comprising:~~

- ~~(a) — an extracellular antigen binding domain;~~
- ~~(b) — a 4-1BB co-stimulatory domain;~~
- ~~(c) — an IL-2R β cytoplasmic signaling domain; and~~
- ~~(d) — a CD3 ζ signaling domain.~~

2. ~~The CAR of claim 1, wherein the 4-1BB co-stimulatory signaling domain comprises the amino acid sequence set forth in~~

~~KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFEEEEGGCEL (SEQ ID NO: 1).~~

3. ~~The CAR of claim 1 or 2, wherein the IL-2R β cytoplasmic signaling domain comprises the amino acid sequence set forth in~~

~~NCRNTGPWLKKVLKCNTDPDSKFFSQLSSEHGGDVQKWLSSFPSSSFSPGGLAPE
ISPLEVLERDKVTQLLPLNTDAYLSLQELQGQDPHTLV (SEQ ID NO: 2).~~

4. ~~The CAR of any one of claims 1-3, wherein the CD3 ζ signaling domain comprises the amino acid sequence set forth~~

~~in RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK
NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH
MQALPPR (SEQ ID NO: 3).~~

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

~~domain, which is C-terminal to the extracellular antigen binding domain and N-terminal to the 4-1BB co-stimulatory domain.~~

6. ~~The CAR of claim 5, wherein the transmembrane domain is derived from a cell surface receptor selected from the group consisting of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, CD271, TNFRSF19 and Killer Cell Immunoglobulin-Like Receptor (KIR), or any combination thereof.~~

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

~~(a) providing a population of immune cells; and~~

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

~~50.1.~~ A population of immune cells comprising a first plurality of genetically engineered immune cells, wherein the genetically engineered immune cells

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

51.2. The population of immune cells of claim 501, wherein the genetically engineered immune cells comprise the disrupted endogenous IFN γ or IFN γ R gene; and wherein the disrupted endogenous IFN γ or IFN γ R gene is produced by gene editing.

5 52.3. The population of immune cells of claim 512, ~~wherein the disrupted endogenous IFN γ or IFN γ R gene is produced by gene editing, optionally~~ wherein the gene editing is mediated by a CRISPR/Cas gene editing system.

10 53.4. The population of immune cells of any one of claims 501- 523, wherein the genetically engineered cells express or secrete the IFN γ antagonist; wherein the IFN γ antagonist is selected from the group consisting of an anti-IFN γ antibody; a secreted IFN γ receptor; and an anti-IFN γ R antibody.

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

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

~~56. The population of immune cells of claim 554, wherein the IFN γ antagonist is an anti-IFN γ scFv; and~~

25 ~~57.5. The population of immune cells of claim 56,~~ wherein the anti-IFN γ scFv comprises (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 53 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 52; (b) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 56 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 55; or (c) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 59 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 58.

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~~58.6.~~ The population of immune cells of claim ~~575~~, wherein the anti-IFN γ scFv comprises the amino acid sequence of SEQ. ID. NO: 54; 57, or 60.

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

~~60.7.~~ The population of immune cells of any one of claims ~~501-596~~, further comprises a chimeric antigen receptor (CAR);

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

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

~~62.8.~~ The population of immune cells of claim ~~617~~, wherein the extracellular antigen binding domain comprises a single chain variable fragment (scFv).

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

~~64.9.~~ The population of immune cells of claim ~~638~~, wherein ~~the tumor associated antigen is CD19 and the~~ extracellular antigen binding domain comprises ~~a the~~ scFv that binds CD19 or B cell maturation antigen (BCMA).

~~65.10.~~ The population of immune cells of claim ~~649~~, wherein the scFv that binds CD19 comprises the amino acid sequence of SEQ. ID. NO: 6, 39, 40, or 41; or wherein the scFv that binds BCMA comprises the amino acid sequence of SEQ. ID. NO: 7.

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

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

~~68-11. The population of immune cells of any one of claims 61-67, wherein the co-stimulatory domain is a co-stimulatory domain from 4-1BB (CD137), OX40, CD70, CD27, CD28, CD5, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), DAP10, and DAP12, or any combination thereof;~~

~~69. The population of immune cells of any one of claims 61-68, wherein the cytoplasmic signaling domain is a CD3zeta (CD3ζ) signaling domain, an interleukin 2 receptor beta subunit (IL-2Rβ) cytoplasmic signaling domain, or a combination thereof; and/or;~~

~~70. The population of immune cells of any one of claims 61-69, wherein the transmembrane domain is from a cell surface receptor selected from the group consisting of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, CD271, TNFRSF19 and Killer Cell Immunoglobulin-Like Receptor (KIR), or any combination thereof.~~

~~71.12. The population of immune cells of any one of claims 61-70, wherein the CAR further comprises a hinge or a spacer or a combination of both to connect the functional domains of (a)-(d);~~

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

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

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

5 ~~75.13. ____~~ The population of immune cells of claim ~~74~~12, wherein the STAT3 binding motif comprises the amino sequence set forth in YX₁X₂Q, wherein X₁ and X₂ are each independently an amino acid, optionally, wherein the STAT3 binding motif comprises the amino acid sequence set forth in YRHQ (SEQ ID NO: 4).

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

15 ~~77.14. ____~~ The population of immune cells of any one of claims ~~501-76~~13, wherein the immune cells comprise T cells, Natural Killer (NK) cells, tumor infiltrating lymphocytes, dendritic cells, macrophages, neutrophils, eosinophils, basophils, mast cells, myeloid-derived suppressor cells, mesenchymal stem cells or precursors thereof, or a combination thereof; optionally wherein the immune cells are human immune cells.

20 ~~78.15. ____~~ The population of immune cells of any one of claims ~~501-77~~14, further comprising a second plurality of immune cells that express an antibody specific to interleukin-6 (IL-6) or IL-6 receptor (IL-6R); and/or

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

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

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

~~82.16.~~ A pharmaceutical composition comprising the population of immune cells of any one of claims ~~501-8115~~ and a pharmaceutically acceptable carrier.

~~83.17.~~ A method for reducing or eliminating undesired cells in a subject, the method comprising administering to a subject in need thereof a therapeutically effective amount Use of the population of immune cells of any one of claims ~~501-8115~~ or the pharmaceutical composition of claim ~~8216 in the manufacture of a medicament for reducing or eliminating undesired cells in a subject.~~

~~84.18.~~ The ~~method-use~~ of claim ~~8317~~, wherein the subject is a human cancer patient and the genetically engineered immune cell expresses the CAR, which is specific to a tumor associated antigen, optionally wherein the tumor associated antigen is selected from the group consisting of 5T4, CD2, CD5, CD3, CD7, CD19, CD20, CD22, CD30, CD33, CD38, CD70, CD123, CD133, CD171, CEA, CS1, Claudin 18.2, BCMA, BAFF-R, PSMA, PSCA, desmoglein (Dsg3), HER-2, FAP, FSHR, NKG2D, GD2, EGFRVIII, mesothelin, ROR1, MAGE, MUC1, MUC16, GPC3, Lewis Y, and VEGFR11.

~~85.19.~~ The ~~method-use~~ of claim ~~8418~~, wherein the cancer is a solid tumor cancer or a hematological cancer.

~~86.20.~~ The ~~method-use~~ of claim ~~8519~~, wherein the cancer is selected from the group consisting of breast cancer, lung cancer, pancreatic cancer, liver cancer, glioblastoma (GBM), prostate cancer, ovarian cancer, mesothelioma, colon cancer, and stomach cancer; or-

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

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

~~89.21.~~ 89.21. The ~~method-use~~ of claim ~~8820~~, wherein the CAR binds CD19 and the subject is a human patient having lymphoblastic leukemia, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, mantle cell lymphoma, large B-cell lymphoma, or non-Hodgkin's lymphoma; ~~or-~~

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

~~91.22.~~ 91.22. The ~~method-use~~ of any one of claims ~~8317-9021~~, wherein prior to the cell therapy, the subject received a lymphodepleting treatment to condition the subject for the cell therapy.

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

23. A chimeric antigen receptor (CAR) comprising:

- (a) an extracellular antigen binding domain;
- (b) a 4-1BBco-stimulatory domain;
- (c) an IL-2R β cytoplasmic signaling domain; and
- (d) aCD3 ζ signaling domain.

24. A population of immune cells, comprising a first plurality of immune cells that express the CAR of claim 23.

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

- (a) providing a population of immune cells; and
- (b) introducing into the immune cells a first nucleic acid coding for the CAR of claim 23.

26. Use of the population of immune cells of claim 24 in the manufacture of a cell therapy-based medicament for treating a cancer, an infectious disease, or an immune disorder.

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27. The population of immune cells of claim 24 for use in therapy.