



(51) International Patent Classification:

C07K 16/24 (2006.01) C12N 5/00 (2006.01)

(21) International Application Number:

PCT/US2021/025906

(22) International Filing Date:

06 April 2021 (06.04.2021)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

63/005,684 06 April 2020 (06.04.2020) US

(71) Applicant: CELLEDIT LLC [US/US]; 100 Barber Avenue, Worcester, MA 01606 (US).

(72) Inventor: HU, Biliang; c/o Celledit LLC, 100 Barber Avenue, Worcester, MA 01606 (US).

(74) Agent: CHEN, Yahua; c/o McDermott Will & Emery LLP, 500 North Capitol Street, NW, Washington, DC 20001 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: GENETICALLY MODIFIED IMMUNE CELLS EXPRESSING A CHIMERIC ANTIGEN RECEPTOR AND HAVING REDUCED PROINFLAMMATORY CYTOKINE SIGNALING

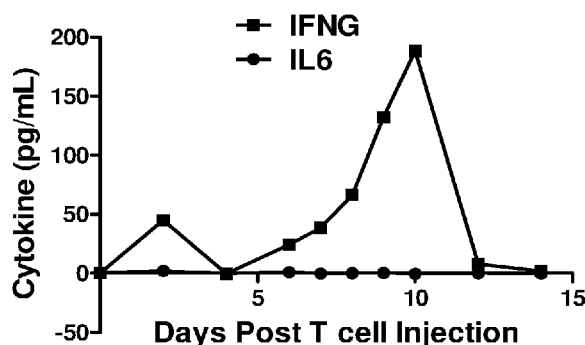


FIG. 1C

(57) Abstract: A population of immune cells comprising modified immune cells co-expressing a chimeric antigen receptor comprising, inter alia, an IB-2K β cytoplasmic signaling domain. Also provided herein are genetically engineered immune cells having reduced production of interferon gamma (IFN γ). Such genetically engineered immune cells may have a disrupted endogenous IFN γ gene, a disrupted endogenous IFN γ receptor (IFN γ R) gene, or both. Alternatively, the immune cells may express an IFN γ antagonist.

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

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.

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.

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 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). 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.

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.

5 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.

10 In some embodiments, 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
NCRNTGPWLKKVLKCNTDPDSKFFSQLSSEHGGDVQKWLSSPFPSSSFSPGGLAPEISP
LEVLERDKVTQLLPLNTDAYLSLQELQGQDPHTLV (SEQ ID NO: 2). Alternatively or in
15 addition, the CD3 ζ signaling domain comprises the amino acid sequence set forth
inRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQ
EGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPP
R (SEQ ID NO: 3).

20 In some embodiments, 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
25 Immunoglobulin-Like Receptor (KIR), or any combination thereof.

In some embodiments, 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.

30 In some embodiments, 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

5 RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQE
GLYNELQKDKMAEAYSEIGMKGERRRGKGHDLGLYQGLSTATKDTYDAYRHQALPPR
(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, CD
10 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 VEGFR2. In some embodiments, the extracellular antigen binding domain is a single-chain antibody fragment (scFv). In some examples, the extracellular antigen binding domain in the
15 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
20 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.

25 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 embodiments, the anti-IL6 or anti-IL6R antibody may comprise the same heavy chain complementarity
30 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.

25 In some instances, at least two of the first plurality of immune cells, the second plurality of 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-
30 1 antagonist.

The immune cells may be 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. 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 addition, provided herein is a method for producing a population of modified
10 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, the first nucleic acid
15 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 embodiments, 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 other
20 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 embodiments, 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.

Accordingly, the present disclosure also provides a population of immune cells
30 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 embodiments, 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 embodiments, the genetically engineered cells express the IFN γ antagonist. For example, the genetically engineered cells secrete 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 embodiments, 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), (c) a
25 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 bind 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 antibodies
30 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 (CD3 ζ) signaling domain, an interleukin 2 receptor beta subunit (IL-2R β) 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-2R β) 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 IFN γ 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 IFN γ signaling, expressing an IL-6 antagonist, and/or expressing an IL-1 antagonist, and a pharmaceutically acceptable carrier.

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 embodiments, 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 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.

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 embodiments of the invention are set forth in the description below. Other features or advantages of the present invention will be apparent from the following drawings and detailed description of several embodiments, 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.

FIG. 5 shows the peak frequencies of anti-CD19 CAR-T cells in acute lymphocytic leukemia (ALL) and lymphoma patients and anti-BCMA CAR-T cells in multiple myeloma (MM) patient.

FIG. 6A shows the efficiency of various anti-IFN γ scFv antibodies on inhibiting IFN γ signaling: 1, Amg-LH; 2, Fon-LH; 3, Ema-LH; 4, Amg-HL; 5, Fon-HL; and 6, Ema-HL (LH meaning the orientation of light chain variable region to heavy chain variable region and HL meaning orientation of heavy chain variable region to light chain variable region). Amg = AMG 811 (U.S. Pat. Appl. No: US20130142809); Fon = fontulizumab; and Ema = emapalumab

FIG. 6B shows the efficiency of different signal peptides on Amg derived scFv for inhibiting IFN γ signaling. 1, a signal peptide from albumin (SEQ ID NO: 47); 2, a signal peptide from CD8 (SEQ ID NO: 44); 3, a signal peptide from a growth hormone (SEQ ID NO: 29); 4, a modified signal peptide from albumin (SEQ ID NO: 48); 5, a modified signal peptide from IL-2 (SEQ ID NO: 49); 6, a signal peptide from an antibody light chain (SEQ ID NO: 45); and 7, a signal peptide from GL (*Gaussia* luciferase) (SEQ ID NO: 46).

FIG. 7 shows the changes of IFN γ in the peripheral blood of a patient suffering from acute lymphocytic leukemia (ALL) and was treated with anti-CD19 CAR-T cells with 41BB-IL2R β -CD3 ζ signaling, CRISPR edited IFN γ knockout (KO) and co-expressing IL6 and IL1 blockers.

FIG. 8A shows the changes of IFN γ in the peripheral blood of a patient diagnosed with refractory and relapsed multiple myeloma (MM) and was treated with anti-BCMA CAR-T cells with 41BB-IL2R β -CD3 ζ signaling, CRISPR edited IFN γ KO and co-expressing IL6 and IL1 blockers.

FIG. 8B shows the changes of IgG levels in the peripheral blood of the same patient over time after treatment.

FIG. 9 shows the changes of IFN γ in peripheral blood of a patient diagnosed with refractory and relapsed lymphoma and was treated with anti-CD19 CAR-T cells with 41BB-IL2R β -CD3 ζ signaling, and co-expressing IFN γ blocking scFv derived from emapalumab and IL6 blocking scFv derived from sirukumab.

FIG. 10A shows the changes of IFN γ in peripheral blood of patient #1 diagnosed with refractory and relapsed MM and were treated with anti-BCMA CAR-T cells with 41BB-IL2R β -CD3 ζ signaling, and co-expressing IFN γ blocking scFv derived from emapalumab and IL6 blocking scFv derived from sirukumab.

FIG. 10B shows the changes of IFN γ and the changes of IgG levels in the peripheral blood of patient #2 same patient with refractory and relapsed MM and were treated with anti-BCMA CAR-T cells with 41BB-IL2R β -CD3 ζ signaling, and co-expressing IFN γ blocking scFv derived from emapalumab and IL6 blocking scFv derived from sirukumab.

5

DETAILED DESCRIPTION OF THE INVENTION

Adoptive cell transfer immunotherapy relies on immune cell activation and cytokine secretion to eliminate disease cells. However, CAR-T do not always expand well in patients. The present disclosure aims to overcome this limitation, in part, via the development of immune cells having reduced inflammatory properties. The present disclosure is based, at least in part, on the development of CARs that include an IL2R β signaling domain. This CAR construct is expected to achieve superior therapeutic effects via inducing more effective proliferation of T cells upon activation by tumor target cells.

Accordingly, provided herein are CARs comprising an IL2R β signaling domain, modified immune cells expressing such CARs, and therapeutic applications thereof (including CAR-T therapy). Without being bound by theory, the modified immune cells expressing (1) the CAR comprising an IL2R β signaling domain, (2) the antagonistic antibody specific to IL-6 or IL-6R, and/or (3) the IL-1 antagonist or antagonistic antibody specific to IL-1 α or IL-1 β significantly reduced the cytokine release syndrome (CRS) in patients treated with these cells. No additional anti-IL-6 medication was needed to prevent or suppress CRS in these patients. The presence of the IL2R β signaling domain in the CAR promoted sustain persistence of the CAR-T cells *in vivo* in the treated patients, compared to CAR-T cells having CARs that did not have the IL2R β signaling domain.

IFN γ is one of the most essential cytokines involved in T cell cytotoxicity and IFN γ secretion is therefore a primary parameter reflecting the potency of a CAR-T therapy. During CAR-T therapy, IFN γ is one of the most elevated cytokines during CRS. Due to IFN γ 's important role in CART killing tumor target cells, blocking IFN γ signaling would have been expected to significantly impair CAR-T therapeutic efficacy. Surprising, it is reported in the present disclosure that CAR-T cells having reduced production of IFN γ , *e.g.*, by knocking out the endogenous IFN γ gene or expressing an anti-IFN γ scFv (*e.g.*, a secreted scFv) could achieve robust clinical responses, despite the low level of IFN γ observed in patients. These results suggest that reducing the IFN γ level would be an approach for achieving robust and safe immune cell therapy (*e.g.*, CAR-T therapy).

Accordingly, provided herein are genetically engineered immune cells that have (1) a disrupted genomic IFN γ gene or IFN γ R gene so that the expression of the endogenous IFN γ or IFN γ R is reduced; (2) expresses an IFN γ antagonist, or a combination of both. The cell may further comprise a CAR that specifically targets and binds a tumor associated antigen. The genetically engineered immune cell described herein may also inhibit IL-6 or IL-1 or both IL-6 and IL-1 signaling *in vivo* via the expression of IL-6 and IL-1 antagonists.

As used herein, the term “endogenous” refers to naturally originating from within an organism.

For purpose of the present disclosure, it will be explicitly understood that the term “antagonist” encompass all the identified terms, titles, and functional states and characteristics whereby the target protein itself, a biological activity of the target protein, or the consequences of the biological activity, are substantially nullified, decreased, or neutralized in any meaningful degree, *e.g.*, by at least 20%, 50%, 70%, 85%, 90%, or above.

I. Chimeric Antigen Receptor Comprising IL2R β signaling domain

One aspect of the present disclosure provides chimeric antigen receptors comprising, *inter alia*, an IL2R β signaling domain. A chimeric antigen receptor (CAR) disclosed herein is an artificial (non-naturally occurring) receptor having a binding specificity to a target antigen of interest (*e.g.*, a tumor cell antigen) and capable of triggering immune responses in immune cells expression such upon binding to the target antigen. A CAR often comprises an extracellular antigen binding domain fused to at least an intracellular signaling domain. Cartellieri et al., *J Biomed Biotechnol* 2010:956304, 2010. The CAR disclosed herein comprise an IL2R β signaling domain, which may be in combination with other intracellular signaling domains such as one or more co-stimulatory signaling domain and/or a cytoplasmic signaling domain comprising an immunoreceptor tyrosine-based activation motif (ITAM), such as a CD3 ζ signaling domain (also referred to as CD3z). The CAR may also have a transmembrane domain, a hinge domain, and/or a STAT3 binding site. The transmembrane domain is located between extracellular antigen binding domain and the intracellular signaling domain. The hinge domain may be located between the extracellular antigen binding domain and the transmembrane domain, between the transmembrane domain and the intracellular signaling domain, and also within the intracellular signaling domain when the intracellular signaling domain comprises a combination of one or more co-stimulatory signaling domain and/or a cytoplasmic signaling domain.

In one embodiment, provided herein is a CAR having an intracellular domain comprising a IL2R β signaling domain, an ITAM-containing cytoplasmic signaling domain, such as a CD3 ζ signaling domain, and an additional co-stimulatory domain such as that from 4-1BB. Without being bound by theory, the presence of the IL2R β signaling domain significantly improved persistence *in vivo* of the CAR-T cells expressing the CAR. The IL2R β signaling domain also induced sustainable B cell aplasia *in vivo* in treated patients.

(A) *IL2R β signaling domain*

IL2R β is the β chain of the interleukin-2 receptor (IL-2R). An IL-2R β signaling domain refers to the fragment in an IL2R β polypeptide (*e.g.*, of a suitable species such as human) that is capable of triggering the signaling pathway mediated by the IL-2/IL-2R interaction. IL-2 polypeptides and the signaling domains therein are known in the art. For example, an exemplary human IL2R β polypeptide is provided in GENBANK accession number NP_000869.1 (the contents of which are incorporated herein by reference). IL2R β polypeptides from other species can be obtained from publically available gene databases such as GENBANK.

In some embodiments, the IL2R β signaling domain used in the CAR constructs disclosed herein comprise an amino acid sequence at least 80% (*e.g.*, at least 85%, 90%, 95%, 98% or above) identical to the amino acid sequence of NCRNTGPWLKKVLKCNTDPDSKFFSQLSSEHGGDVQKWLSSPFPSSSFSPGGLAPEISP LEVLERDKVTQLLPLNTDAYLSLQELQGQDPHTLV (SEQ. ID. NO: 2).

The “percent identity” of two amino acid sequences is determined using the algorithm of Karlin and Altschul Proc. Natl. Acad. Sci. USA 87:2264-68, 1990, modified as in Karlin and Altschul Proc. Natl. Acad. Sci. USA 90:5873-77, 1993. Such an algorithm is incorporated into the NBLAST and XBLAST programs (version 2.0) of Altschul, et al. J. Mol. Biol. 215:403-10, 1990. BLAST protein searches can be performed with the XBLAST program, score=50, wordlength=3 to obtain amino acid sequences homologous to the protein molecules of interest. Where gaps exist between two sequences, Gapped BLAST can be utilized as described in Altschul et al., Nucleic Acids Res. 25(17):3389-3402, 1997. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used.

Alternatively or in addition, the IL2R β signaling domain used in the CAR constructs may contain one or more mutations (*e.g.*, amino acid residue substitutions) relative to a wild-type counterpart, for example, SEQ. ID. NO: 2. In some examples, the IL2R β signaling domain

may contain up to 15 (*e.g.*, up to 12, 10, 8, 6, 5, 4, 3, 2, or 1) amino acid residue substitutions relative to the wild-type counterpart (*e.g.*, SEQ. ID. NO: 2). In some examples, the one or more amino acid residue substitutions are conservative amino acid residue substitutions.

As used herein, a “conservative amino acid substitution” refers to an amino acid substitution that does not alter the relative charge or size characteristics of the protein in which the amino acid substitution is made. Variants can be prepared according to methods for altering polypeptide sequence known to one of ordinary skill in the art such as are found in references which compile such methods, *e.g.* Molecular Cloning: A Laboratory Manual, J. Sambrook, et al., eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989, or Current Protocols in Molecular Biology, F.M. Ausubel, et al., eds., John Wiley & Sons, Inc., New York. Conservative substitutions of amino acids include substitutions made amongst amino acids within the following groups: (a) A → G, S; (b) R → K, H; (c) N → Q, H; (d) D → E, N; (e) C → S, A; (f) Q → N; (g) E → D, Q; (h) G → A; (i) H → N, Q; (j) I → L, V; (k) L → I, V; (l) K → R, H; (m) M → L, I, Y; (n) F → Y, M, L; (o) P → A; (p) S → T; (q) T → S; (r) W → Y, F; (s) Y → W, F; and (t) V → I, L.

In specific examples, the IL2R β signaling domain used in the CAR constructs disclosed herein comprises (*e.g.*, consisting of) the amino acid sequence of SEQ ID NO: 2.

(B) Extracellular antigen binding domain

The extracellular antigen binding domain used in the CAR constructs disclosed herein is specific to an antigen of interest (*e.g.*, a pathologic antigen such as a cancer antigen). In some instances, it can be a single-chain antibody fragment (scFv), which typically comprises a heavy chain variable domain (V_H) and a light chain variable domain (V_L) connected by a peptide linker. Peptide linkers used in scFv constructs are well known in the art. In some embodiments, the extracellular antigen binding domain used herein targets a tumor antigen, such as CD19 or BCMA.

In some examples, the extracellular antigen binding domain (*e.g.*, a scFv) binds CD19. Such an anti-CD19 scFv may comprise the amino acid sequence of SEQ. ID. NO: 6. Alternatively, the anti-CD19 scFv may be a variant derived from SEQ. ID. NO: 6, for example, having the same heavy chain and light chain complementary determining regions (CDRs) as those in SEQ. ID. NO: 6. Alternatively, the variant may have the same V_H and V_L as in SEQ. ID. NO: 6 and a different peptide linker. In other instances, the variant may have a different V_H→V_L orientation as in SEQ. ID. NO: 6.

In some examples, the extracellular antigen binding domain (*e.g.*, a scFv) binds B-cell maturation antigen (BCMA). Such an anti-BCMA scFv may comprise the amino acid sequence

of SEQ. ID. NO: 7. Alternatively, the anti-BCMA scFv may be a variant derived from SEQ. ID. NO: 7, for example, having the same heavy chain and light chain complementary determining regions (CDRs) as those in SEQ. ID. NO: 7. Alternatively, the variant may have the same V_H and V_L as in SEQ. ID. NO: 7 and a different peptide linker. In other instances, the variant may have a different V_H→V_L orientation as in SEQ. ID. NO: 7.

Two antibodies having the same CDR or same V_H/V_L means that the two antibodies have the same amino acid sequence of that CDR as determined by the same method.

(C) Other CAR components

In addition to the IL2Rβ signaling domain and the extracellular antigen binding domain disclosed above, any of the CAR constructs disclosed herein may further comprise one or more co-stimulatory domains, a cytoplasmic signaling domain comprising an ITAM such as CD3ζ signaling domain, or a combination thereof. In some instances, the CAR may further comprise a STAT3 binding site, which may be located C-terminal to the CD3ζ signaling domain.

In some examples, the CAR disclosed herein may comprise a co-stimulatory domain from co-stimulatory receptor 4-1BB (aka CD137), for example, from human 4-1BB. Non-limiting sources for co-stimulatory domains include OX40, CD70, CD27, CD28, CD5, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), DAP10, and DAP12. Hence, the CAR may have a co-stimulatory domain derived from 4-1BB, OX40, CD70, CD27, CD28, CD5, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), DAP10, and DAP12 or any combination thereof. One example includes the 4-1BB co-stimulatory signaling domain comprises (*e.g.*, consists of) the amino acid sequence: KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCEL (SEQ. ID. NO: 1). Alternatively or in addition, the CAR may comprise a CD3ζ signaling domain, which may comprise (*e.g.*, consist of) the amino acid sequence: RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDRRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ. ID. NO: 3).

In some examples, the CAR construct disclosed herein contains a STAT3 binding motif linked to the CD3ζ signaling domain (to its C-terminal). The STAT3 binding motif may have the amino acid sequence YX₁X₂Q, where X₁ and X₂ are each independently an amino acid. In particular, the YX₁X₂Q motif may be YRHQ (SEQ. ID. NO: 4). In some examples, the fragment in the CAR construct containing the CD3ζ signaling domain and the STAT3 binding motif may comprise (*e.g.*, consist of) the amino acid sequence:

RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPGEMGGKPRRKNPQE
 GLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAYRHQALPPR
 (SEQ. ID. NO: 5).

In addition, the CAR construct disclosed herein may further comprise a transmembrane
 5 domain, a hinge domain, or both, which may be located between the extracellular antigen
 binding domain and the intracellular signaling domains. Any transmembrane domains and/or
 hinge domains commonly used in CAR constructs can be used here. In some embodiments, the
 transmembrane domain may be obtained from a suitable cell-surface receptor, such as the
 transmembrane domain of a cell surface receptor of the alpha, beta or zeta chain of the T-cell
 10 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). In some examples, the hinge domain may be of CD28,
 CD8, or an IgG, such as IgG1 or IgG4.

In specific examples, the CAR constructs disclosed herein may comprise an
 15 extracellular antigen binding domain, a 4-1BB co-stimulatory domain, an IL-2R β cytoplasmic
 signaling domain, and a CD3 ζ signaling domain. The foregoing domains may be arranged,
 respectively, from the N-terminus to the C-terminus of the CAR. The CAR may further
 comprise a transmembrane domain, which may be located C-terminal to the extracellular
 antigen binding domain and N-terminal to the 4-1BB co-stimulatory domain. In other
 20 examples, the CAR may also comprise a hinge domain, which may be linked to the C-terminus
 of the extracellular antigen binding domain and the N-terminus of the transmembrane domain.
 In some examples, the CAR may comprise a signal peptide at the N-terminus of the CAR.

Provided below are amino acid sequences of CAR components such as antigen binding
 scFvs, IL2R β signaling domain, hinge domains, transmembrane domains, signal peptides, and
 25 CD3 ζ signal domain for constructing exemplary CAR constructs. Additionally, exemplary
 CAR constructs are disclosed below. Contemplated CAR constructs can be made using any
 methods known in the art, *e.g.*, molecular cloning methods.

Anti-CD19 scFv:

30 DIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVP
 SRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGKLEITGGGGSGGGGS
 GGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIW
 GSETTYYN SALKSRLTIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDY
 WGQGTSVTVSS (SEQ. ID. NO: 6)

DIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVP
 SRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGKLEITG**STSGSGKPGS**
GEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVI
 WGSETTYNSALKSRLTIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMD
 5 YWGQGTSTVTVSS (SEQ. ID. NO: 39)

DVVMTQSPSSIPVTLGESVSISCRSSKSLQNVNGNTYLYWFQQRPGQSPQLLIYRMSNL
 NSGVPDRFSGSGSGTDFTLRISGVEPEDVGVYYCMQHLEYPLTFGAGTKLEIK**GGGGS**
GGGGSGGGGSQVQLVQSGPELIKPGGSVKMSCKASGYTFTSYVMHWVRQKPGQGL
 10 EWIGYINPYNDGTYNEKFKGRATLTSDKSSSTAYMELSSLRSEDSAVYYCARGTYY
 YGSRVFDYWGQGTSTVTVSS (SEQ. ID. NO: 40, an anti-CD19 scFv as disclosed in WO
 2020/135335, the content is incorporated herein by reference in its entirety)

DVVMTQSPSSIPVTLGESVSISCRSSKSLQNVNGNTYLYWFQQRPGQSPQLLIYRMSNL
 15 NSGVPDRFSGSGSGTDFTLRISGVEPEDVGVYYCMQHLEYPTFGAGTKLEIK**GGGGS**
GGGGSGGGGSQVQLVQSGPELIKPGGSVKMSCKASGYTFTSYVMHWVRQKPGQGL
 EWIGYINPYNDGTYNEKFKGRATLTSDKSSSTAYMELSSLRSEDSAVYYCARGTYY
 YGSRVFDYWGQGTSTVTVSS (SEQ. ID. NO: 41, an anti-CD19 scFv as disclosed in WO
 2020/135335, the content is incorporated herein by reference in its entirety)

20

Anti-BCMA scFv:

DIVLTQSPPSLAMSLGKRATISCRASESVTILGSHLIHWYQQKPGQPPTLLIQLASNVQT
 GVPARFSGSGSRDFTLTIDPVEEDDVAVYYCLQSRTPRTFGGKLEIK**GSTSGSGK**
PGSGEGSTKGQIQLVQSGPELKKPGETVKISCKASGYTFTDYSINWVKRAPGKGLKW
 25 MGWINTETREPAYAYDFRGRFAFSLETSASTAYLQINNLYEDTATYFCALDYSYAM
 DYWGQGTSTVTVSS (SEQ. ID. NO: 7)

CD8 hinge domain:

TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ. ID. NO: 10)
 30

CD8 Transmembrane domain:

IYIWAPLAGTCGVLLLSLVITLYC (SEQ. ID. NO: 11)

CD8 hinge domain and CD8 transmembrane:

TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVL
LLSLVITLYC (SEQ. ID. NO: 42)

5 4-1BB co-stimulatory domain:

KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCEL (SEQ. ID. NO: 1)

IL-2R β domain (truncated form of IL-2R β cytoplasmic domain:

NCRNTGPWLKKVLKCNTPDPSKFFSQLSSEHGGDVQKWLSSPFPSSSFSPGGLAPEISP
10 LEVLERDKVTQLLPLNTDAYLSLQELQGQDPTHLV (SEQ. ID. NO: 2)

CD3 ζ signal domain:

RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQE
GLYNELQKDKMAEAYSEIGMKGERRRRGKGGHDGLYQGLSTATKDTYDAYRHQALPPR
15 (SEQ. ID. NO: 5)

CD8 signal peptide:

MALPVTALLPLALLHAARP (SEQ. ID. NO: 44)

20 Anti-CD19 CAR:

DIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVP
SRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGKLEITGGGGSGGGGS
GGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVW
GSETTYNSALKSRLTIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDY
25 WQQTGTSVTVSSTTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI
WAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEE
GGCELNCRNTGPWLKKVLKCNTPDPSKFFSQLSSEHGGDVQKWLSSPFPSSSFSPGGL
APEISPLEVLERDKVTQLLPLNTDAYLSLQELQGQDPTHLVRVKFSRSADAPAYKQQQ
NQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE
30 IGMKGERRRRGKGGHDGLYQGLSTATKDTYDAYRHQALPPR (SEQ. ID. NO: 12)

Anti-CD19 CAR:

DIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVP
SRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGKLEITGSTSGSGKPGS

GEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVI
 WGSETTYNSALKSRLTIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMD
 YWGQGTSVTVSSTTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI
 WAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEE
 5 GGCELNCRNTGPWLKKVLKCNTDPDSKFFSQLSSEHGGDVQKWLSSPFPSSSFSPGGL
 APEISPLEVLERDKVTQLLPLNTDAYLSLQELQGQDPTHLVRVKFSRSADAPAYKQGG
 NQLYNELNLGRREEYDVLDKRRGRDPGEMGGKPRRKNPQEGLYNELQKDKMAEAYSE
 IGMKGERRRGKGHDGLYQGLSTATKDTYDAYRHQALPPR (SEQ. ID. NO: 43)

10 Anti-BCMA CAR:

DIVLTQSPPSLAMSLGKRATISCRASESVTILGSHLIHWYQQKPGQPPTLLIQLASNVQT
 GVPARFSGSGSRTDFTLTIDPVEEDDVAVYYCLQSRTIPRTFGGGKLEIK**GSTSGSGK**
PGSGEGSTKGQIQLVQSGPELKKPGETVKISCKASGYTFTDYSINWVKRAPGKGLKW
 MGWINTETREPAYAYDFRGRFAFSLETSASTAYLQINNLYEDTATYFCALDYSYAM
 15 DYWGQGTSVTVSSTTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDI
 YIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEE
 EGGCELNCRNTGPWLKKVLKCNTDPDSKFFSQLSSEHGGDVQKWLSSPFPSSSFSPGG
 LAPEISPLEVLERDKVTQLLPLNTDAYLSLQELQGQDPTHLVRVKFSRSADAPAYKQG
 QNQLYNELNLGRREEYDVLDKRRGRDPGEMGGKPRRKNPQEGLYNELQKDKMAEAY
 20 SEIGMKGERRRGKGHDGLYQGLSTATKDTYDAYRHQALPPR (SEQ. ID. NO: 13)

II. Genetically Modified Immune Cells

In another aspect, the present disclosure provides genetically modified immune cells
 expressing any of the CAR constructs disclosed herein, optionally in combination with other
 25 genetic edits. Such modified immune cells comprise the CAR construct, which is specific to an
 antigen of interest (*e.g.*, a cancer antigen), thereby eliminating the target disease cells *via, e.g.*,
 the effector activity of the immune cells. In another aspect, the present disclosure provides
 genetically modified immune cells expressing an antigen specific TCR. The TCR is specific to
 an antigen of interest (*e.g.*, a cancer antigen), thereby eliminating the target disease cells *via*,
 30 *e.g.*, the effector activity of the immune cells. The modified immune cells disclosed herein may
 also comprise one or more IL-6 antagonistic antibodies disclosed herein. In some instances, the
 modified immune cells may further comprise one or more IL-1 antagonists, *e.g.*, IL-1RA or
 others known in the art or disclosed herein. In some embodiments, the modified immune cells
 may further comprise one or more IFN γ antagonists, *e.g.*, an antagonistic IFN γ antibody or

others known in the art or disclosed herein. These CARs, TCRs, IL-6 antagonistic antibodies, IFN γ antagonists, or IL-1 antagonists may be knock-in modifications in the modified cells. In some embodiments, the modified immune cells may further comprise one or more knock-out modifications of endogenous genes (*e.g.*, GM-CSF, TCR, IFN γ , or B2M). Preferably, the

5 knock-out of the endogenous IFN γ gene.

(i) *Antagonistic IL-6 Antibodies*

IL-6 signals through a complex comprising the membrane glycoprotein gp130 and the IL-6 receptor (IL-6R) (see, *e.g.*, Hibi et al., *Cell*, 63(6):1149-57, 1990). IL-6 binding to IL-6R on target cells promotes gp130 homo-dimerization and subsequent signal transduction. As used

10 herein, IL-6R includes both membrane bound and soluble forms of IL-6R (sIL-6R). When bound to IL-6, soluble IL-6R (sIL-6R) acts as an agonist and can also promote gp130 dimerization and signaling. Trans-signaling can occur whereby sIL-6R secretion by a particular cell type induces cells that only express gp130 to respond to IL-6 (see, *e.g.*, Taga et al., *Annu Rev Immunol.*, 15:797-819, 1997; and Rose-John et al., *Biochem J.*, 300 (Pt 2):281-90, 1994).

15 In one example, sIL-6R comprises the extracellular domain of human IL-6R (see *e.g.*, Peters et al., *J Exp Med.*, 183(4):1399-406, 1996).

In some embodiments, the modified immune cells disclosed herein express an IL-6 antagonist, which may be an antibody that binds to IL-6 or to an IL-6 receptor (IL-6R). Such antibodies (antagonistic antibodies) can interfere with binding of IL-6/IL-6R on immune cells,

20 thereby suppressing cell signaling mediated by IL-6.

A typical antibody molecule comprises a heavy chain variable region (V_H) and a light chain variable region (V_L), which are usually involved in antigen binding. The V_H and V_L regions can be further subdivided into regions of hypervariability, also known as “complementarity determining regions” (“CDR”), interspersed with regions that are more

25 conserved, which are known as “framework regions” (“FR”). Each V_H and V_L is typically composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The extent of the framework region and CDRs can be precisely identified using methodology known in the art, for example, by the Kabat definition, the Chothia definition, the AbM definition, and/or the contact

30 definition, all of which are well known in the art. See, *e.g.*, Kabat, E.A., et al. (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, Chothia et al., (1989) *Nature* 342:877; Chothia, C. et al. (1987) *J. Mol. Biol.* 196:901-917, Al-lazikani et al (1997) *J. Molec. Biol.* 273:927-948; and Almagro, J. *Mol. Recognit.* 17:132-143 (2004). See also the Human Genome

Mapping Project Resources at the Medical Research Council in the United Kingdom and the antibody rules described at the Bioinformatics and Computational Biology group website at University College London.

An antibody (interchangeably used in plural form) as used herein is an immunoglobulin molecule capable of specific binding to a target protein, *e.g.*, IL-6 or IL-6R, through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule. As used herein, the term “antibody” encompasses not only intact (*e.g.*, full-length) antibodies and heavy chain antibodies (*e.g.*, an Alpaca heavy chain IgG antibody), but also antigen-binding fragments thereof (such as Fab, Fab', F(ab')₂, Fv), single chain (scFv), single-domain antibody (sdAb; VHH), also known as a nanobody, mutants thereof, fusion proteins comprising an antibody portion, humanized antibodies, chimeric antibodies, diabodies, linear antibodies, single chain antibodies, multispecific antibodies (*e.g.*, bispecific antibodies) and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity, including glycosylation variants of antibodies, amino acid sequence variants of antibodies, and covalently modified antibodies. An antibody includes an antibody of any class, such as IgD, IgE, IgG, IgA, or IgM (or sub-class thereof), and the antibody need not be of any particular class. Depending on the antibody amino acid sequence of the constant domain of its heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

In some embodiments, the antibodies described herein that “bind” a target protein or a receptor thereof may specifically bind to the target protein or receptor. An antibody that “specifically binds” (used interchangeably herein) to a target or an epitope is a term well understood in the art, and methods to determine such specific binding are also well known in the art. A molecule is said to exhibit “specific binding” if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular target antigen than it does with alternative targets. An antibody “specifically binds” to a target cytokine if it binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances. For example, an antibody that specifically (or preferentially) binds to an IL-6 or an IL-6R epitope is an antibody that binds this IL-6 epitope or IL-6R

epitope with greater affinity, avidity, more readily, and/or with greater duration than it binds to other IL-6 epitopes, non-IL-6 epitopes, other IL-6R epitopes or non-IL-6R epitopes. It is also understood by reading this definition that, for example, an antibody that specifically binds to a first target antigen may or may not specifically or preferentially bind to a second target antigen.

5 As such, “specific binding” or “preferential binding” does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means preferential binding.

In some embodiments, an antagonistic antibody of a target protein as described herein has a suitable binding affinity for the target protein (*e.g.*, human IL-6 or human IL-6R) or
10 antigenic epitopes thereof. As used herein, “binding affinity” refers to the apparent association constant or K_A . The K_A is the reciprocal of the dissociation constant (K_D). The antagonistic antibody described herein may have a binding affinity (K_D) of at least 10^{-5} , 10^{-6} , 10^{-7} , 10^{-8} , 10^{-9} , 10^{-10} M, or lower for the target antigen or antigenic epitope. An increased binding affinity corresponds to a decreased K_D . Higher affinity binding of an antibody for a first antigen
15 relative to a second antigen can be indicated by a higher K_A (or a smaller numerical value K_D) for binding the first antigen than the K_A (or numerical value K_D) for binding the second antigen. In such cases, the antibody has specificity for the first antigen (*e.g.*, a first protein in a first conformation or mimic thereof) relative to the second antigen (*e.g.*, the same first protein in a second conformation or mimic thereof; or a second protein). In some embodiments, the
20 antagonistic antibodies described herein have a higher binding affinity (a higher K_A or smaller K_D) to the target protein in mature form as compared to the binding affinity to the target protein in precursor form or another protein, *e.g.*, an inflammatory protein in the same family as the target protein. Differences in binding affinity (*e.g.*, for specificity or other comparisons) can be at least 1.5, 2, 3, 4, 5, 10, 15, 20, 37.5, 50, 70, 80, 91, 100, 500, 1000, 10,000 or 10^5
25 fold.

Binding affinity (or binding specificity) can be determined by a variety of methods including equilibrium dialysis, equilibrium binding, gel filtration, ELISA, surface plasmon resonance, or spectroscopy (*e.g.*, using a fluorescence assay). Exemplary conditions for evaluating binding affinity are in HBS-P buffer (10 mM HEPES pH7.4, 150 mM NaCl,
30 0.005% (v/v) Surfactant P20). These techniques can be used to measure the concentration of bound binding protein as a function of target protein concentration. The concentration of bound binding protein ([Bound]) is generally related to the concentration of free target protein ([Free]) by the following equation:

$$[\text{Bound}] = [\text{Free}] / (K_d + [\text{Free}])$$

It is not always necessary to make an exact determination of KA, though, since sometimes it is sufficient to obtain a quantitative measurement of affinity, *e.g.*, determined using a method such as ELISA or FACS analysis, is proportional to KA, and thus can be used for comparisons, such as determining whether a higher affinity is, *e.g.*, 2-fold higher, to obtain
5 a qualitative measurement of affinity, or to obtain an inference of affinity, *e.g.*, by activity in a functional assay, *e.g.*, an *in vitro* or *in vivo* assay.

In some embodiments, the IL-6 antagonistic antibody as described herein can bind and inhibit the IL-6 signaling by at least 50% (*e.g.*, 60%, 70%, 80%, 90%, 95% or greater). The inhibitory activity of an IL-6 antagonistic antibody described herein can be determined by
10 routine methods known in the art.

The antibodies described herein can be murine, rat, human, or any other origin (including chimeric or humanized antibodies). Such antibodies are non-naturally occurring, *e.g.*, would not be produced in an animal without human act (*e.g.*, immunizing such an animal with a desired antigen or fragment thereof).

15 Any of the antibodies described herein can be either monoclonal or polyclonal. A “monoclonal antibody” refers to a homogenous antibody population and a “polyclonal antibody” refers to a heterogeneous antibody population. These two terms do not limit the source of an antibody or the manner in which it is made.

In one example, the antibody used in the methods described herein is a humanized
20 antibody. Humanized antibodies refer to forms of non-human (*e.g.*, murine) antibodies that are specific chimeric immunoglobulins, immunoglobulin chains, or antigen-binding fragments thereof that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from
25 a CDR of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, the humanized antibody may comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences, but are included to further refine and optimize
30 antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region or domain

(Fc), typically that of a human immunoglobulin. Antibodies may have Fc regions modified as described in WO 99/58572. Other forms of humanized antibodies have one or more CDRs (one, two, three, four, five, and/or six), which are altered with respect to the original antibody, which are also termed one or more CDRs “derived from” one or more CDRs from the original
 5 antibody. Humanized antibodies may also involve affinity maturation.

The heavy chain variable domains (V_H) and light chain variable domains (V_L) of exemplary anti-IL-6 antibodies and anti-IL-6R antibodies are provided below (Reference Antibodies 1-6) with the CDRs shown in boldface (determined following the antibody rules described by the Bioinformatics and Computational Biology group website at University
 10 College London).

Antibody 1 (binding to IL-6R):

V_H:

EVQLVESGGGLVQGRSLRLSCAAS**RFTEDDYAMH**WVRQAPGKGLEWVSG**ISWNS**
 15 **GRIGYADSV**KGRFTISRDN AENSLFLQMNGLR AEDTALYYCAK**GRDSFDI**WGQGTMT
 VTVSS (SEQ. ID. NO: 14)

V_L:

DIQMTQSPSSVSASVGDRVITTC**RASQGISSWLA**WYQQKPGKAPKLLIY**GASSLES**GV
 20 PSRFGSGSGTDFTLTISSLQPEDFASYYC**QQANSFPYT**FGQGGTKLEIK (SEQ. ID.
 NO: 15)

Antibody 2 (binding to IL-6):

V_H:

EVQLVESGGGLVQPGGSLRLSCAAS**SGFTFSPFAMS**WVRQAPGKGLEWVAK**ISPGGS**
 25 **WTYYSDTV**TGRFTISRDN AKNSLYLQMNSLR AEDTAVYYCAR**QLWGYIALDI**WGQ
 GTTVTVSS (SEQ. ID. NO: 16)

V_L:

EIVLTQSPATLSLSPGERATLSC**SASISVSYMY**WYQQKPGQAPRLLIY**DMSNLAS**GIPA
 30 RFGSGSGSGTDFTLTISLLEPEDFAVYYC**MOWSGYPYT**FGGGGTKVEIK (SEQ. ID.
 NO: 17)

Antibody 3 (binding to IL-6):

V_H:

EVQLVESGGKLLKPGGSLKLSCAASSGFTFSSFAMSWFRQSPEKRLEWVAEISSGGSY
TYYPDTVTGRFTISRDNKNTLYLEMSSLRSEDAMYYCARGGLWGYALDYWGQG

5 TSVTVSS (SEQ. ID. NO: 18)

V_L:

QIVLIQSPAIMASAPGEKVTMTCSSASSVSMYWYQQKPGSSPRLIYDTSNLASGV
 RFGSGSGTSYSLTISRMEAEDAATYYCQQWSGYPYTFGGGTKLEIK (SEQ. ID.

10 NO: 19)

Antibody 4 (binding to IL-6R):

V_H:

QVQLQESGPGLVRPSTLSLTCTVSGYSITSDHAWSWVRQPPGRGLEWIGYISYSGIT
 15 TYNPSLKSRVTMLRDTSKNQFSLRLSSVTAADTAVYYCARSLARTTAMDYWGQGSL

VTVSS (SEQ. ID. NO: 20)

V_L:

DIQMTQSPSSLSASVGDRVITTCRASQDISSYLNWYQQKPGKAPKLLIYYTSRLHSGV

20 PSRFGSGSGTDFTFTISSLPEDIATYYCQQGNLTPYTFGQGTKVEIK (SEQ. ID.

NO: 21)

Antibody 5 (binding to IL-6):

V_H:

25 EVQLVESGGGLVQPGGSLRLSCAASGFSLSNYYVTWVRQAPGKGLEWVGHIIYGSDET
AYATSAIGRFTISRDNKNTLYLQMNSLRAEDTAVYYCARDDSSDWDKFNLWGQG

TLVTVSS (SEQ. ID. NO: 22)

V_L:

30 AIQMTQSPSSLSASVGDRVITTCQASQSINNELSWYQQKPGKAPKLLIYRASTLASGV
 PSRFGSGSGTDFTLTISLQPDFAATYYCQOGYSLRNIDNAFGGGTKVEIK (SEQ. ID.

NO: 23)

Antibody 6 (binding to gp130):

V_H:

EVQLVESGGGLVQPGGSLRLSCAASGFNFNDYFMNWVRQAPGKGLEWVAQMRNK
NYQYGTYYAESLEGRFTISRDDSKNSLYLQMNSLKTEDTAVYYCAREESYYGFTSYW

5 GQGTLLTVSS (SEQ. ID. NO: 24)

V_L:

DIQMTQSPSSLSASVGDRVTITCQASQDIGISLSWYQQKPGKAPKLLIYNANNLADGV
 PSRFGSGSGTDFTLTISSLQPEDFATYYCLOHNSAPYTFGQGTKLEIK (SEQ. ID.

10 NO: 25)

In some embodiments, the IL-6 antagonistic antibodies described herein bind to the same epitope in an IL-6 antigen (*e.g.*, human IL-6) or in an IL-6R (*e.g.*, human IL-6R) as one of the reference antibodies provided herein (*e.g.*, Antibody 1 or Antibody 2) or compete against the reference antibody from binding to the IL-6 or IL-6R antigen. Reference antibodies provided herein include Antibodies 1-6, the structural features and binding activity of each of which are provided herein. An antibody that binds the same epitope as a reference antibody described herein may bind to exactly the same epitope or a substantially overlapping epitope (*e.g.*, containing less than 3 non-overlapping amino acid residue, less than 2 non-overlapping amino acid residues, or only 1 non-overlapping amino acid residue) as the reference antibody. Whether two antibodies compete against each other from binding to the cognate antigen can be determined by a competition assay, which is well known in the art. Such antibodies can be identified as known to those skilled in the art, *e.g.*, those having substantially similar structural features (*e.g.*, complementary determining regions), and/or those identified by assays known in the art. For example, competition assays can be performed using one of the reference antibodies to determine whether a candidate antibody binds to the same epitope as the reference antibody or competes against its binding to the IL-6 or IL-6R antigen.

In some instances, the IL-6 antagonistic antibodies disclosed herein may comprise the same heavy chain CDRs and/or the same light chain CDRs as a reference antibody as disclosed herein (*e.g.*, Antibody 1 or Antibody 2). The heavy chain and/or light chain CDRs are the regions/residues that are responsible for antigen binding; such regions/residues can be identified from amino acid sequences of the heavy chain/light chain sequences of the reference antibody (shown above) by methods known in the art. See, *e.g.*, antibody rules described at the Bioinformatics and Computational Biology group website at University College London;

Almagro, J. Mol. Recognit. 17:132-143 (2004); Chothia et al., J. Mol. Biol. 227:799-817 (1987), as well as others known in the art or disclosed herein. Determination of CDR regions in an antibody is well within the skill of the art, for example, the methods disclosed herein, *e.g.*, the Kabat method (Kabat et al. Sequences of Proteins of Immunological Interest, (5th ed., 5 1991, National Institutes of Health, Bethesda Md.)) or the Chothia method (Chothia et al., 1989, Nature 342:877; Al-lazikani et al (1997) J. Molec. Biol. 273:927-948)). As used herein, a CDR may refer to the CDR defined by any method known in the art. Two antibodies having the same CDR means that the two antibodies have the same amino acid sequence of that CDR as determined by the same method.

10 Also within the scope of the present disclosure are functional variants of any of the exemplary anti-IL-6 or anti-IL-6R antibodies as disclosed herein (*e.g.*, Antibody 1 or Antibody 2). A functional variant may contain one or more amino acid residue variations in the V_H and/or V_L, or in one or more of the HC CDRs and/or one or more of the LC CDRs as relative to the reference antibody, while retaining substantially similar binding and biological activities 15 (*e.g.*, substantially similar binding affinity, binding specificity, inhibitory activity, or a combination thereof) as the reference antibody.

In some examples, the IL-6 antagonistic antibody disclosed herein comprises a HC CDR1, a HC CDR2, and a HC CDR3, which collectively contains no more than 10 amino acid variations (*e.g.*, no more than 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) as compared with 20 the HC CDR1, HC CDR2, and HC CDR3 of a reference antibody such as Antibody 1 or Antibody 2. "Collectively" means that the total number of amino acid variations in all of the three HC CDRs is within the defined range. Alternatively or in addition, the anti-IL-6 or anti-IL-6R antibody may comprise a LC CDR1, a LC CDR2, and a LC CDR3, which collectively contains no more than 10 amino acid variations (*e.g.*, no more than 9, 8, 7, 6, 5, 4, 3, 2 or 1 25 amino acid variation) as compared with the LC CDR1, LC CDR2, and LC CDR3 of the reference antibody.

In some examples, the IL-6 antagonistic antibody disclosed herein may comprise a HC CDR1, a HC CDR2, and a HC CDR3, at least one of which contains no more than 5 amino acid variations (*e.g.*, no more than 4, 3, 2, or 1 amino acid variation) as the counterpart HC 30 CDR of a reference antibody such as Antibody 1 or Antibody 2. In specific examples, the antibody comprises a HC CDR3, which contains no more than 5 amino acid variations (*e.g.*, no more than 4, 3, 2, or 1 amino acid variation) as the HC CDR3 of a reference antibody such as Antibody 1 or Antibody 2. Alternatively or in addition, an IL-6 antagonistic antibody may comprise a LC CDR1, a LC CDR2, and a LC CDR3, at least one of which contains no more

than 5 amino acid variations (*e.g.*, no more than 4, 3, 2, or 1 amino acid variation) as the counterpart LC CDR of the reference antibody. In specific examples, the antibody comprises a LC CDR3, which contains no more than 5 amino acid variations (*e.g.*, no more than 4, 3, 2, or 1 amino acid variation) as the LC CDR3 of the reference antibody.

5 In some instances, the amino acid residue variations can be conservative amino acid residue substitutions. See disclosures herein.

 In some embodiments, the IL-6 antagonistic antibody disclosed herein may comprise heavy chain CDRs that collectively are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) identical to the heavy chain CDRs of a reference antibody such as Antibody 1 or Antibody 2.

10 Alternatively or in addition, the antibody may comprise light chain CDRs that collectively are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) identical to the light chain CDRs of the reference antibody. In some embodiments, the IL-6 antagonistic antibody may comprise a heavy chain variable region that is at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) identical to the heavy chain variable region of a reference antibody such as Antibody 1 or Antibody 2 and/or a light chain variable region that is at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) identical to the light chain variable region of the reference antibody.

 The present disclosure also provides germlined variants of any of the reference IL-6 antagonistic antibodies disclosed herein. A germlined variant contains one or more mutations in the framework regions as relative to its parent antibody towards the corresponding germline sequence. To make a germlined variant, the heavy or light chain variable region sequence of the parent antibody or a portion thereof (*e.g.*, a framework sequence) can be used as a query against an antibody germline sequence database (*e.g.*, the antibody rules described at the Bioinformatics and Computational Biology group website at University College London; thevbase2 website, or the IMGT®, the international ImMunoGeneTics information system® website) to identify the corresponding germline sequence used by the parent antibody and amino acid residue variations in one or more of the framework regions between the germline sequence and the parent antibody. One or more amino acid substitutions can then be introduced into the parent antibody based on the germline sequence to produce a germlined variant.

30 In some examples, the antagonistic antibodies described herein are human antibodies or humanized antibodies. Alternatively or in addition, the antagonistic antibodies are scFv. Exemplary scFv antibodies are provided below.

IL-6/IL-6R scFv 1:

DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQKPGKAPKLLIYGASSLESQV

SRFSGSGSGTDFTLTISSLQPEDFASYCQQANSFPYTFGQGTKLEIKGGGGSGGGGSG
 GGGSEVQLVESGGGLVQPGRSLRLSCAASRFTFDDYAMHWVRQAPGKGLEWVSGIS
 WNSGRIGYADSVKGRFTISRDNNAENSLFLQMNGSLRAEDTALYYCAKGRDSFDIWGQG
 TMVTVSS (SEQ. ID. NO: 8)

5

IL-6/IL-6R scFv 2:

EIVLTQSPATLSLSPGERATLSCSASISVSYMYWYQQKPGQAPRLLIYDMSNLASGIPA
 RFSGSGSGTDFTLTISSLEPEDFAVYYCMQWSGYPTYTFGGGTKVEIKGGGGSGGGGSG
 GGGSEVQLVESGGGLVQPGGSLRLSCAASGFTFSPFAMSWVRQAPGKGLEWVAKISP
 10 GGSWTYYSDTVTGRFTISRDNNAKNSLYLQMNSLRAEDTAVYYCARQLWGYYALDIW
 QGGTTVTVSS (SEQ. ID. NO: 9)

IL-6/IL-6R scFv 3:

QIVLIQSPAIMASAPGEKVTMTCSASSSVSYMYWYQQKPGSSPRLLIYDTSNLASGVVPV
 15 RFSGSGSGTSYSLTISRMEAEDAATYYCQQWSGYPTYTFGGGTKLEIKGGGGSGGGGSG
 GGGSEVQLVESGGKLLKPGGSLKLSCAASGFTFSSFAMSWFRQSPEKRLEWVAEISSG
 GSYTYYPDVTGRFTISRDNNAKNTLYLEMSSLRSEDAMYYCARGLWGYYALDYWG
 QGTSVTVSS (SEQ. ID. NO: 26)

20 IL-6/IL-6R scFv 4:

QVQLQESGPGLVRRPSQTLSTCTVSGYSITSDHAWSWVRQPPGRGLEWIGYISYSGITT
 YNPSLKSRTVMTLRDTSKNQFSLRLSSVTAADTAVYYCARSLARTTAMDYWGQGS
 TVSSGGGGSGGRASGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQDISSYLN
 WYQQKPGKAPKLLIYYTSLRHSGLVPSRFSGSGSGTDFTLTISSLQPEDTATYYCQQGNT
 25 LPYTFGQGTKVEIK (SEQ. ID. NO: 27)

(ii) *IL-1 Antagonist*

Interleukin-1 is a cytokine known in the art and includes two isoforms, IL-1 α and IL-1 β . IL-1 plays important roles in up- and down-regulation of acute inflammation, as well as
 30 other biological pathways.

In some embodiments, the IL-1 antagonist expressed in the modified immune cells disclosed herein can be an interleukin-1 receptor antagonist (IL-1RA). IL-1RA is a naturally-occurring polypeptide, which can be secreted by various types of cells, such as immune cells,

epithelial cells, and adipocytes. It binds to cell surface IL-1R receptor and thereby preventing the cell signaling triggered by IL-1/IL-1R interaction. A human IL-1RA is encoded by the *IL1RN* gene. Below is an exemplary amino acid sequence of a human IL-1RA:

5 ***MALETICRPSGRKSSKMQAFRIWDVNQKTFYLRNNQLVAGYLQGPNVNLEEKIDVVPI***
EPHALFLGIHGGKMCLSCVKSGDETRLQLEAVNITDLSNRKQDKRFAFIRSDSGPTTS
FESAACPGWFLCTAMEADQPVSLTNMPDEGVMVTKFYFQEDE (SEQ. ID. NO: 28)

10 The N-terminal fragment in boldface and italicized refers to the signal peptide in the native IL-1RA. The IL-1RA for use in the instant application may comprise the amino acid sequence corresponding to the mature polypeptide of the human IL-1RA noted above (excluding the signal peptide)

15 ***RPSGRKSSKMQAFRIWDVNQKTFYLRNNQLVAGYLQGPNVNLEEKIDVVPIEPHALFL***
GIHGGKMCLSCVKSGDETRLQLEAVNITDLSNRKQDKRFAFIRSDSGPTTSFESAACP
GWFLCTAMEADQPVSLTNMPDEGVMVTKFYFQEDE (SEQ. ID. NO: 51).

20 In some instances, this signal peptide can be replaced with a different signal sequence, for example, ***MATGSRTSLLLAFLGCLLPWLQEGSA*** (SEQ. ID. NO: 29). The resultant IL-1RA would have the whole sequence:

25 ***MATGSRTSLLLAFLGCLLPWLQEGSARPSGRKSSKMQAFRIWDVNQKTFYLRNNQLV***
AGYLQGPNVNLEEKIDVVPIEPHALFLGIHGGKMCLSCVKSGDETRLQLEAVNITDLS
NRKQDKRFAFIRSDSGPTTSFESAACPGWFLCTAMEADQPVSLTNMPDEGVMVTKFY
FQEDE (SEQ. ID. NO: 30)

30 Other IL-1 antagonists include, but are not limited to, anti-IL-1 α or anti-IL-1 β antibodies (see Fredericks ZL, et al., 2004, *Protein Eng Des Sel.* 17(1):95-106); U.S. Patent No. 7,531,166 and 8,383,778, the contents are incorporated herein by reference in their entirety.

(iii) Interferon Gamma Modification

In some instances, the present disclosure provides genetically modified immune cells that have reduced production of IFN γ . Such genetically modified immune cells would produce

no or less IFN γ relative to their wild-type counterpart cells that are not modified. The amount of IFN γ in culture or *in vivo* in a patient may be determined by any method known in the art, *e.g.*, by an ELISA assay of the cell culture media or the blood IFN γ level of a patient treated with such modified cells. By less IFN γ means at least 10% lower compared to their wild-type counterpart cells that are not modified to reduce IFN γ expression. In other embodiments, less IFN γ means at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% lower compared to their wild-type counterpart cells that are not modified to reduce IFN γ expression. In one embodiment, the genetically modified immune cells that have reduced expression of IFN γ may have the endogenous IFN γ gene knocked out, *e.g.*, by genetic editing. In some embodiments, the genetically modified immune cells having reduced IFN γ expression may comprise a CAR comprising an extracellular antigen binding domain; a co-stimulatory domain; a cytoplasmic signaling domain, or a combination thereof; and optionally a transmembrane domain. The genetically modified immune cells may further comprise an IL-6 antagonist. The CAR of genetically modified immune cells that have reduced production of IFN γ may comprise an IL-2R β cytoplasmic signaling domain. Additionally or alternatively, the genetically modified immune cells that have reduced production of IFN γ may comprise an IL-1 antagonist.

In other instances, the present disclosure provides genetically modified immune cells that have reduced expression of IFN γ R. Preferably, the IFN γ R1. Such genetically modified immune cells would express no, little or less IFN γ R as relative to their wild-type counterpart. The amount of IFN γ R may be determined by any method known in the art, *e.g.*, by an ELISA assay. By less IFN γ R means at least 10% lower compared to their wild-type counterpart cells that are not modified to reduce IFN γ R expression. In one embodiment, the genetically modified immune cells that have reduced expression of IFN γ R may have the endogenous IFN γ R gene knocked out, *e.g.*, by genetic editing. In some embodiments, the genetically modified immune cells having reduced IFN γ R expression may comprise a CAR comprising an extracellular antigen binding domain; a co-stimulatory domain; a cytoplasmic signaling domain, or a combination thereof; and optionally a transmembrane domain. The genetically modified immune cells may further comprise an IL-6 antagonist. The CAR may comprise an IL-2R β cytoplasmic signaling domain.

In one aspect, also provided herein are genetically modified immune cells that can bring about interferon gamma blockade *in vivo*. The interferon gamma blockade *in vivo* is effectuated via genomic gene editing of the IFN γ or IFN γ R gene or by expressing and secreting

an IFN γ antagonist. In some embodiments, the genetically modified immune cells can bring about interferon gamma blockade *in vivo* may comprise a CAR comprising an extracellular antigen binding domain; a co-stimulatory domain; a cytoplasmic signaling domain, or a combination thereof; and optionally a transmembrane domain. The genetically modified
5 immune cells may further comprise an IL-6 antagonist. The CAR may comprise an IL-2R β cytoplasmic signaling domain.

(a) Blockade of IFN γ Signaling via IFN γ or IFN γ R Gene Knock-Out

Genomic gene editing of the IFN γ or IFN γ R (*e.g.*, the R1 subunit) gene aims to knockout either the IFN γ or the IFN γ R genes or both of them. By doing so, it is envisioned that
10 any CRS initiated by IFN γ signaling *in vivo* would be limited when there is less IFN γ ligand or IFN γ R available.

Accordingly, provided herein is a genetically modified immune cell comprising a disrupted endogenous IFN γ or the IFN γ R genes or both of them. In some embodiments, the genetically modified immune cells comprising a disrupted endogenous IFN γ or the IFN γ R
15 genes or both of them may comprise a CAR comprising an extracellular antigen binding domain; a co-stimulatory domain; a cytoplasmic signaling domain, or a combination thereof; and optionally a transmembrane domain. The genetically modified immune cells may further comprise an IL-6 antagonist. Alternatively or in addition, the genetically modified immune cells may further comprise an IFN γ antagonist. The CAR in these modified cells may comprise
20 an IL-2R β cytoplasmic signaling domain.

Any methods known in the art for down-regulating the expression of an endogenous gene in a host cell can be used to reduce the expression level of IFN γ or IFN γ R as described herein. The genomic information for the human IFN γ and IFN γ R1 are found in GENBANK Gene ID: 3458 and Gene ID: 3459 respectively. Any gene editing method may involve use of
25 an endonuclease that is capable of cleaving the target region in the endogenous allele. Non-homologous end joining in the absence of a template nucleic acid may repair double-strand breaks in the genome and introduce mutations (*e.g.*, insertions, deletions and/or frameshifts) into a target site.

In some examples, a knocking-out event can be coupled with a knocking-in event –
30 an exogenous nucleic acid coding for a desired molecule (*e.g.*, the IL-1RA described herein) can be inserted into a genomic locus of IFN γ or IFN γ R gene via gene editing, thereby disrupting the gene expression as a result of the insertion.

In some instances, any of the knock-out modification may be achieved using

antisense oligonucleotides (*e.g.*, interfering RNAs such as shRNA or siRNA) or ribozymes *via* methods known in the art. An antisense oligonucleotide specific to a target cytokine/protein refers to an oligonucleotide that is complementary or partially complementary to a target region of an endogenous gene of the cytokine or an mRNA encoding such. Such antisense oligonucleotides can be delivered into target cells via conventional methods. Alternatively, expression vectors such as lentiviral vectors or equivalent thereof can be used to express such an antisense oligonucleotides.

Alternatively, knocking-out the endogenous IFN γ or IFN γ R gene can be achieved using the gene editing methods such as the CRISPR technology, for example, using a CRISPR/Cas9 system. To disrupt the IFN γ gene, the single guide RNAs (sgRNA) that target the protospacer adjacent motif (PAM) sequence in the human IFN γ gene may be used with the CRISPR/Cas9 system. The sgRNAs molecules contains both the custom-designed short crRNA sequence fused to the scaffold tracrRNA sequence. The DNA sequences used for *in vitro* transcription of IFN γ sgRNA are provided herein. The bold sequences are the targeted PAM sequences in the first exon of the human IFN γ gene.

sgRNA 1

GAAATATACAAGTTATATCTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG
CTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT (SEQ. ID.

NO: 31)

sgRNA 2

GTTTCAGCTCTGCATCGTTTTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG
CTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT (SEQ. ID.

NO: 32)

sgRNA 3

GTTTCAGCTCTGCATCGTTTTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG
CTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT (SEQ. ID.

NO: 33)

sgRNA 4

**GCATCGTTTTGGGTCTCTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCT
AGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT** (SEQ. ID. NO: 34)

sgRNA 5

5 **GTCTCTTGGCTGTTACTGCCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG
CTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT** (SEQ. ID.
NO: 35)

sgRNA 6

10 **GTTCTTTTACATATGGGTCCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG
CTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT** (SEQ. ID.
NO: 36)

sgRNA 7

15 **GTTCTGCTTCTTTTACATATGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
TAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT** (SEQ. ID.
NO: 37)

sgRNA 8

20 **GTTTCTGCTTCTTTTACATAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
TAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT** (SEQ. ID.
NO: 38)

To disrupt the IFN γ R gene, commercially available IFN γ R1 Human Gene Knockout
25 Kit (CRISPR) Cat# KN202761 from OriGENE may be used. Methods of using such kits are
known in the art.

(b) Blockade of IFN γ Signaling via IFN γ antagonist

In some embodiments of the genetically engineered immune cell described herein, the
cell comprises the IFN γ antagonist. In other embodiments, provided herein is a genetically
30 modified immune cell comprising an IFN γ antagonist. In some embodiments, the genetically
modified immune cells comprising an IFN γ antagonist may comprise a CAR comprising an
extracellular antigen binding domain; a co-stimulatory domain; a cytoplasmic signaling
domain, or a combination thereof; and optionally a transmembrane domain. The genetically

modified immune cells may further comprise an IL-6 antagonist. Alternatively or in addition, the genetically modified immune cells may have a disrupted endogenous IFN γ or the IFN γ R genes or both of them. The CAR in these modified cells may comprise an IL-2R β cytoplasmic signaling domain.

5 The IFN γ antagonist blocks the formation of the ternary IFN γ /IFN γ R1/IFN γ R2. IFN γ R1 is required for ligand binding and signaling. The IFN γ antagonist can be an antagonistic anti-IFN γ antibody or antigen-binding fragment thereof; a secreted IFN γ receptor or a ligand-binding fragment of the receptor; and an antagonistic anti-IFN γ R antibody or antigen-binding fragment thereof, whereby the IFN γ antagonist blocks IFN γ /IFN γ R interaction and downstream
10 signaling. In one embodiment, the IFN γ antagonist is secreted. The antagonistic anti-IFN γ antibody or antigen-binding fragment thereof binds the IFN γ ligand that is released *in vivo* and thus the IFN γ ligand is not available to interact with its native receptor, IFN γ R1, expressed on cell surfaces. The secreted IFN γ receptor or a ligand-binding fragment functions as decoy receptor and captures the IFN γ ligand that is released *in vivo* and thus the IFN γ ligand is also
15 not available to interact with its native receptor, IFN γ R1 that is expressed on cell surfaces. In one embodiment, the secreted IFN γ R or a ligand-binding fragment is the extracellular portion of a native human IFN γ receptor. The antagonistic anti-IFN γ R antibody or antigen-binding fragment thereof binds to the IFN γ receptor expressed on cells and prevents the interaction of the IFN γ ligand with the receptor and the consequential ligand-induced assembly of the
20 complete receptor complex that contains two IFN γ R1 and two IFN γ R2 subunits. The complete receptor complex is necessary for the IFN γ signaling pathway.

In one embodiment, the antagonistic anti-IFN γ antibody or antigen-binding fragment thereof is a scFv of the antibody such as an anti-IFN γ scFv. ScFv consist of a variable heavy (V_H) and a variable light (V_L) antibody chains linked with a peptide linker. Non-limiting
25 examples of V_{HS} and V_{LS} from anti-IFN γ antibodies for constructing an anti-IFN γ scFv are as follows:

V_L:

DIQMTQSPSTLSASVGDRVTITCKASENVDITYVSWYQQKPGKAPKLLIYGASNRYTGV
30 PSRFGSGSGTDFTLTISLQPDDEFATYYCGQSYNYPFTFGQGTKVEVKR (SEQ. ID.
NO: 52)

V_H:

QVQLVQSGAELKKPGSSVKVSCKASGYIFTSSWINWVKQAPGQGLEWIGRIDPSDGEV
HYNQDFKDKATLTVDKSTNTAYMELSSLRSEDNAVYYCARGFLPWFADWGQGTLVT
VSS (SEQ. ID. NO: 53)

5

V_L:

NFMLTQPHSVSESPGKTVTISCTRSSGSIASNYVQWYQQRPGSSPTTVIYEDNQRPSGV
PDRFSGSIDSSNSASLTISGLKTEDEADYYCQSYDGSNRWMFGGGTKLTVL (SEQ. ID.
NO: 55)

10

V_H:

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGST
YYADSVKGRFTISRDN SKNTLYLQMNSLRAEDNAVYYCAKDGSSGWYVPHWFDPWG
QGTLVTVSS (SEQ. ID. NO: 56)

15

V_L:

EIVLTQSPGTLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIP
DRFSGSGSGTDFTLTISRLEPEDFAVYYCQRSGGSSFTFGPGTKVDIK (SEQ. ID. NO: 58)

20

V_H:

EVQLVQSGAEVKKPGESLKISCKGSGYNFTSYWIGWVRQMPGKGLELMGIIYPGDSDT
RYSPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCGSGSYFYFDLWGRGTLTVS
S (SEQ. ID. NO: 59)

25

Non-limiting examples of an anti-IFN γ scFv are as follows, with the flexible glycine-serine
peptide linker shown in bold:

DIQMTQSPSTLSASVGDRVITITCKASENVDITYVSWYQQKPGKAPKLLIYGASNRYTG
VPSRFSGSGSGTDFTLTISLQPDDEFATYYCGQSYNYPFTFGQGTKVEVKR**GGGGSGGG**
30 **GSGGGGS**QVQLVQSGAELKKPGSSVKVSCKASGYIFTSSWINWVKQAPGQGLEWIGR
IDPSDGEVHYNQDFKDKATLTVDKSTNTAYMELSSLRSEDNAVYYCARGFLPWFADW
QGTLVTVSS(SEQ. ID. NO: 54)

NFMLTQPHSVSESPGKTVTISCTRSSGSIASNYVQWYQQRPGSSPTTVIYEDNQRP
 PDRFSGSIDSSSNSASLTISGLKTEDEADYYCQSYDGSNRWMFGGGTKLTVLGGGGSG
 GGGSGGGGSEVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEW
 VSAISGSGGSTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKDGSSGW
 5 YVPHWFDPWGQGTLVTVSS (SEQ. ID. NO: 57)

EIVLTQSPGTLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIP
 DRFSGSGSGTDFTLTISRLEPEDFAVYYCQRSGGSSFTFGPGTKVDIKGGGGSGGGGS
 GGGGSEVQLVQSGAEVKKPGESLKISCKGSGYNFTSYWIGWVRQMPGKGLELMGIY
 10 PGDS DTRYSPSFQGGQVTISADKSISTAYLQWSSLKASDTAMYYCGSGSYFYFDLWGRG
 TLVTVSS (SEQ. ID. NO: 60)

In some embodiments, 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
 15 comprising the amino acid sequence of SEQ ID NO: 52; or comprises 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 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 further
 20 embodiments, the anti-IFN γ scFv comprises the amino acid sequence of SEQ. ID. NO: 54; 57,
 or 60.

Other antagonistic anti-IFN γ antibodies or antigen-binding fragments thereof can be
 found in U.S. Patent No: 9,682,142, the content of which is incorporated by reference in its
 entirety.

25 Soluble IFN γ R fragment are known in the art, for example, the extracellular portion of
 a native human IFN γ receptor is described in U.S. Patent No: 5,578,707 and 7,449,176. The
 high-affinity IFN γ receptor complex is made up of two type I membrane proteins, IFN γ R1
 (IFN γ R alpha) and IFN γ R2 (IFN γ R beta). Both proteins are members of the type II cytokine
 receptor family and share approximately 52% overall sequence identity. IFN γ R1 is the ligand-
 30 binding subunit that is necessary and sufficient for IFN γ binding and receptor internalization.
 IFN γ R2 is required for IFN γ signaling but does not bind IFN γ by itself. Human IFN γ R1 cDNA
 encodes a 499 amino acid (aa) residue protein with a 17 aa signal peptide, a 228 aa
 extracellular domain, a 23 aa transmembrane domain, and a 221 aa intracellular domain.

Soluble IFN γ R fragments that antagonizes the IFN γ signaling may comprises the 228 aa extracellular domain.

Antagonistic anti-IFN γ R antibodies or antigen-binding fragments thereof described in U.S. Patent No: 4,897,264 and 7,449,176. The contents of these patents are incorporated herein
5 by reference in their entirety.

In some embodiments of the IFN γ antagonists described herein, the IFN γ antagonist may further comprising a signal peptide located at the N-terminus of the IFN γ antagonist, optionally the signal peptide is selected from albumin, CD8, a growth hormone, IL-2, an antibody light chain; and *Gaussia* luciferase, or modified version thereof. For examples:
10 CD8 signal peptide, MALPVTALLLPLALLLHAARP (SEQ. ID. NO: 44); antibody light chain signal peptide, MKYLLPTAAAGLLLLAAQPAMA (SEQ. ID. NO: 45); *Gaussia* luciferase signal peptide, MGVKVLFALICIAVAEA (SEQ ID NO: 46); human albumin signal peptide, MKWVTFISLLFLFSSAYS (SEQ. ID. NO: 47); modified human albumin signal peptide, MKWVTFISLLFLFSSSSRA (SEQ. ID. NO: 48); modified IL2 signal peptide,
15 MRRMQLLLLIALSLALVTNS (SEQ. ID. NO: 49); growth hormone signal peptide, MATGSRTSLLAFGLLCLPWLQEGSA (SEQ. ID. NO: 29); and native IL-IRA signal peptide, MALETIC (SEQ. ID. NO: 50).

The modified immune cells disclosed herein may further comprise knock-out of one or more inflammatory proteins (*e.g.*, inflammatory cytokines or soluble receptors thereof,
20 inflammatory growth factors, or cytotoxic molecules), knock-in of one or more antagonists of the inflammatory proteins or immune suppressive cytokines, or a combination thereof.

Exemplary inflammatory cytokines or a soluble receptor thereof include interleukin 1 alpha (IL1 α), interleukin 1 beta (IL1 β), interleukin 2 (IL-2), interleukin 5 (IL-5), interleukin 6 (IL-6), interleukin 7 (IL-7), interleukin 8 (IL-8), interleukin 9 (IL-9), interleukin (IL-12),
25 interleukin 15 (IL-15), interleukin 17 (IL-17), interleukin 18 (IL-18), interleukin 21 (IL-21), interleukin 23 (IL-23), sIL-1RI, sIL-2R α , soluble IL-6 receptor (sIL-6R), interferon α (IFN α), interferon β (IFN β), Macrophage inflammatory proteins (*e.g.*, MIP α and MIP β), Macrophage colony-stimulating factor 1 (CSF1), leukemia inhibitory factor (LIF), granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), C-
30 X-C motif chemokine ligand 10 (CXCL10), chemokine (C-C motif) ligand 5 (CCL5), eotaxin, tumor necrosis factor (TNF), monocyte chemoattractant protein 1 (MCP1), monokine induced by gamma interferon (MIG), receptor for advanced glycation end-products (RAGE), c-reactive protein (CRP), angiopoietin-2, and von Willebrand factor (VWF).

Examples of target inflammatory proteins include, but are not limited to, inflammatory cytokines or soluble receptors thereof (*e.g.*, IL2, IL1 α , IL1 β , IL-5, IL-6, IL-7, IL-8, IL-9, IL-12, IL-15, IL-17, IL-18, IL-21, IL-23, sIL-1RI, sIL-2R α , sIL-6R, IFN α , IFN β , IFN γ , MIP α , MIP β , CSF1, LIF, G-CSF, GM-CSF, CXCL10, CCL5, eotaxin, TNF, MCP1, MIG, RAGE, CRP, angiopoietin-2, and VWF), inflammatory growth factors (*e.g.*, TGF α , VEGF, EGF, HGF, and FGF) and cytotoxic molecules (*e.g.*, perforin, granzyme, and ferritin).

(iv) *Populations of modified immune cells*

Also provided herein are one or more populations of modified immune cells comprising the CAR, modified immune cells comprising the IL-6 antagonistic antibody (*e.g.*, scFv1 or scFv2), modified immune cells comprising the IL-1 antagonist, modified immune cells comprising the disrupted IFN γ gene, the modified immune cells comprising the IFN γ antagonist or a combination thereof as described herein. One or more of the IL-6 antagonist, the IFN γ antagonist and the IL-1 antagonist may be a knock-in modification of immune cells.

In one embodiment, at least one population of immune cells comprises the CAR. In another embodiment, at least one population of immune cells comprises an antigen specific TCR. Methods of making such TCRs are described in U.S. Pat. No. 10,117,918 and in U.S. Pat. Publication No. US20190169261, the contents are incorporated herein by reference in their entirety. In some embodiments, the genetically modified immune cells may comprise knock-in modifications of the IL-6 antagonist (*e.g.*, an anti-IL-6 antagonistic antibody such as scFv1 or scFv2), the IFN γ antagonist (*e.g.*, an anti-IFN γ antagonistic antibody such as AmG811) and/or an IL-1 antagonist such as IL-1RA. Immune cells described herein may not express one or more of TCR, CD52, IFN γ , B2M, and GM-CSF. The lack of expression in the immune cells may be due to disruption of the respective endogenous gene or genes (*e.g.*, a knock-out). CD52, which is an important marker for producing UCART. Exemplary of combinations of modifications in the immune cells include B2M knockout and an IL-1 antagonist; GM-CSF knockout and an IL-1 antagonist; CD52 knockout and an IL-1 antagonist; TCR knockout and an IL-1 antagonist; GM-CSF knockout and an IL-6 antagonist; B2M knockout and an IL-6 antagonist; CD52 knockout and an IL-6 antagonist; and TCR knockout and an IL-6 antagonist.

The modified immune cells disclosed herein comprise knock-in modifications to express the CAR, the antagonistic IL-6 antibody, the IL-1 antagonist, the IFN γ antagonist or a combination thereof. Knock-in modifications may comprise delivering to host cells (*e.g.*, immune cells as described herein) one or more exogenous nucleic acids coding for the CAR, the IL-6 antagonist antibodies, the IL-1 antagonist or the IFN γ antagonist as disclosed herein,

or a combination thereof. The exogenous nucleic acids are in operative linkage to suitable promoters such that the encoded proteins (*e.g.*, cytokine antagonists and/or immune suppressive cytokines) can be expressed in the host cells. In some instances, the exogenous nucleic acids coding for the CAR, the IL-6 antagonistic antibodies, the IFN γ antagonist and the IL-1 antagonist, or a combination thereof, may integrate into the genome of the host cells. In other instances, the exogenous nucleic acids may remain extrachromosomal (not integrated into the genome).

The modified immune cells comprising one or more knock-in modifications may comprise one or more exogenous nucleic acids (*e.g.*, exogenous expression cassettes) for expressing immune suppressive cytokines and/or antagonists of one or more target inflammatory proteins as described herein. For purpose of the present disclosure, it will be explicitly understood that the term “antagonist” encompass all the previously identified terms, titles, and functional states and characteristics whereby the target protein itself, a biological activity of the target protein, or the consequences of the biological activity, are substantially nullified, decreased, or neutralized in any meaningful degree, *e.g.*, by at least 20%, 50%, 70%, 85%, 90%, or above.

A population of modified immune cells may comprise one or more populations of the immune cells comprising the CAR, the IL-6 antagonist, and the IL-1 antagonist. The one or more populations may be overlapping. In one example, at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, or 80% of the immune cells, or a range between two of the foregoing amounts, express the CAR or an antigen specific TCR, the IL-6 antagonist, and the IL-1 antagonist. For example, at least 10% of the immune cells may express the IL-6 antagonist, and the IL-1 antagonist. In another embodiment, about 50-70% of the immune cells may express the CAR, the IL-6 antagonist, and the IL-1 antagonist. In another example, at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, or 80% of the immune cells, or a range between two of the foregoing amounts, express the CAR or an antigen specific TCR, the IL-6 antagonist, and the IFN γ antagonist. For example, at least 10% of the immune cells may express the IL-6 antagonist, and the IFN γ antagonist. In another embodiment, about 50-70% of the immune cells may express the CAR or an antigen specific TCR, the IL-6 antagonist, and the IFN γ antagonist. Also contemplated is a population of modified immune cells that comprises the CAR or an antigen specific TCR and can bring about interferon blockade *in vivo*. For example, the modified immune cells have the IFN γ antagonist or having the disrupted IFN γ gene, or the combination of both. In some embodiments, provided herein is a population of modified

immune cells that comprises one or more populations of the immune cells comprising the CAR or an antigen specific TCR and the IFN γ antagonist or the disrupted IFN γ gene, or the combination of both.

In some embodiments, the present disclosure provides a population of genetically engineered immune cells (*e.g.*, T cells), in which about 5-50% of the immune cells express a CAR or an antigen specific TCR (*e.g.*, any CAR constructs disclosed herein) and the IL-1 antagonist, and at least 50% (*e.g.*, 70%) of the immune cells have a disrupted endogenous IFN γ and/or GM-CSF gene. Alternatively or in addition, about 5-50% of the immune cells may express an anti-IL6 antagonistic antibody and/or an anti-IFN γ antagonistic antibody, such as those disclosed herein. In further additions, these populations of engineered immune cells may include about 5-50% cells of the immune cells may express an IL-1 antagonist or about 5-50% cells of the immune cells may express an GM-CSF antagonist.

The immune cell population as described herein can be further modified to express an exogenous cytokine, a chimeric synNotch receptor, a chimeric immunoreceptor, a chimeric costimulatory receptor, a chimeric killer-cell immunoglobulin-like receptor (KIR), and/or an exogenous T cell receptor. This can be done either before, after, or concurrently with the knock-in and/or knock-out modifications. Such receptors may be cloned and integrated into any suitable expression vector using routine recombinant technology. Considerations for design of chimeric antigen receptors are also known in the art. See, *e.g.*, Sadelain et al., Cancer Discov., 3(4):388-98, 2013.

In some embodiments, an immune cell can be derived from, for example without limitation, a stem cell. The stem cells can be adult stem cells, non-human embryonic stem cells, more particularly non-human stem cells, cord blood stem cells, progenitor cells, bone marrow stem cells, induced pluripotent stem cells, totipotent stem cells or hematopoietic stem cells. Representative human cells are CD34+ cells. The immune cells disclosed herein may be T-cells, 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 combinations thereof. The T-cells may be selected from the group consisting of inflammatory T-lymphocytes, cytotoxic T-lymphocytes, regulatory T-lymphocytes or helper T-lymphocytes. In some embodiments, the T-cells can be derived from the group consisting of CD4+ T-lymphocytes and CD8+ T-lymphocytes.

Specific knock-in and knock-out genetic modifications for CAR-T cells, including IL-6 antagonists and IL-1 antagonists, can be found in WO2019/178259 and PCT/US2020/012329,

the relevant disclosures of each of which are incorporated by reference for the purpose and subject matter disclosed herein.

Specifically provided herein is a genetically engineered immune cell comprising: (a) a disrupted endogenous IFN γ gene or IFN γ R gene; or (b) an IFN γ antagonist, or a combination of both, whereby the cell inhibits interferon gamma signaling *in vivo*. The engineered cell, in addition to IFN γ signaling blockade, may further comprise a CAR or an antigen specific TCR. The CAR may comprise an extracellular antigen binding domain, a co-stimulatory signaling, a cytoplasmic domain that may be a cytoplasmic signaling domain, or a combination thereof. The CAR may comprise an IL-2R β cytoplasmic signaling domain with a co-stimulatory signaling. For example, the CAR may comprise a 4-1BB co-stimulatory domain; an IL-2R β cytoplasmic signaling domain, a CD3 ζ signaling domain, and optionally a transmembrane domain, a hinge domain, and/or a STAT3 binding site. For example, the genetically engineered immune cell may comprise the CAR, an IFN γ antagonist, and an IL-6 antagonist. The genetically engineered immune cell may further comprise an IL-1 antagonist. Alternatively, the genetically engineered immune cell may comprise the CAR, a disrupted endogenous IFN γ gene or IFN γ R gene, an IFN γ antagonist, and an IL-6 antagonist. The genetically engineered immune cell may further comprise an IL-1 antagonist. In another embodiment, the genetically engineered immune cell may comprise the CAR, a disrupted endogenous IFN γ gene or IFN γ R gene, and an IL-6 antagonist. The genetically engineered immune cell may further comprise an IL-1 antagonist.

III. Methods of Preparing Modified Immune Cells

Any of the knock-in and knock-out modifications may be introduced into suitable immune cells by routine methods and/or approaches described herein. Typically, such methods would involve delivery of genetic material into the suitable immune cells to either down-regulate expression of a target endogenous inflammatory protein, express a cytokine antagonist of interest or express an immune suppressive cytokine of interest.

(A) Knocking In Modification

To generate a knock-in of one or more CARs, IL-6 antagonists, IFN γ antagonists and IL-1 antagonists described herein, a coding sequence of the one or more the CARs, IL-6 antagonists, IFN γ antagonists, and IL-1 antagonists may be cloned into a suitable expression vector (*e.g.*, including but not limited to lentiviral vectors, retroviral vectors, adenoviral vectors, adeno-associated vectors, PiggyBac transposon vector and Sleeping Beauty transposon

vector) and introduced into host immune cells using conventional recombinant technology. Sambrook et al., Molecular Cloning, A Laboratory Manual, 3rd Ed., Cold Spring Harbor Laboratory Press. As a result, modified immune cells of the present disclosure may comprise one or more exogenous nucleic acids encoding at least one CAR, IL-6 antagonist, the IFN γ antagonist or IL-1 antagonist. In some instances, the coding sequence of such molecules is integrated into the genome of the cell. In some instances, the coding sequence of such molecules is not integrated into the genome of the cell.

An exogenous nucleic acid comprising a coding sequence of interest may further comprise a suitable promoter, which can be in operable linkage to the coding sequence. A promoter, as used herein, refers to a nucleotide sequence (site) on a nucleic acid to which RNA polymerase can bind to initiate the transcription of the coding DNA (*e.g.*, for a cytokine antagonist) into mRNA, which will then be translated into the corresponding protein (*e.g.*, expression of a gene). A promoter is considered to be “operably linked” to a coding sequence when it is in a correct functional location and orientation relative to the coding sequence to control (“drive”) transcriptional initiation and expression of that coding sequence (to produce the corresponding protein molecules). In some instances, the promoter described herein can be constitutive, which initiates transcription independent other regulatory factors. In some instances, the promoter described herein can be inducible, which is dependent on regulatory factors for transcription. Exemplary promoters include, but are not limited to ubiquitin, RSV, CMV, EF1 α and PGK1. In one example, one or more nucleic acids encoding one or more antagonists of one or more inflammatory cytokines as those described herein, operably linked to one or more suitable promoters can be introduced into immune cells *via* conventional methods to drive expression of one or more antagonists.

Additionally, the exogenous nucleic acids described herein may further contain, for example, some or all of the following: a selectable marker gene, such as the neomycin gene for selection of stable or transient transfectants in mammalian cells; enhancer/promoter sequences from the immediate early gene of human CMV for high levels of transcription; transcription termination and RNA processing signals from SV40 for mRNA stability; SV40 polyoma origins of replication and ColE1 for proper episomal replication; versatile multiple cloning sites; and T7 and SP6 RNA promoters for *in vitro* transcription of sense and antisense RNA. Suitable methods for producing vectors containing transgenes are well known and available in the art. Sambrook et al., Molecular Cloning, A Laboratory Manual, 3rd Ed., Cold Spring Harbor Laboratory Press.

In some instances, one or more CARs, IL-6 antagonists, the IFN γ antagonists or IL-1 antagonists can be constructed in one expression cassette in a multi-cistronic manner such that the various molecules are expressed as separate polypeptides. In some examples, an internal ribosome entry site can be inserted between two coding sequences to achieve this goal.

5 Alternatively, a nucleotide sequence coding for a self-cleaving peptide (*e.g.*, T2A or P2A) can be inserted between two coding sequences. Exemplary designs of such multi-cistronic expression cassettes are provided in Examples below.

(B) *Knocking Out Modification*

Any methods known in the art for down-regulating the expression of an endogenous
10 gene in a host cell can be used to reduce the production level of a target endogenous cytokine/protein as described herein. A gene editing method may involve use of an endonuclease that is capable of cleaving the target region in the endogenous allele. Non-homologous end joining in the absence of a template nucleic acid may repair double-strand breaks in the genome and introduce mutations (*e.g.*, insertions, deletions and/or frameshifts)
15 into a target site. Gene editing methods are generally classified based on the type of endonuclease that is involved in generating double stranded breaks in the target nucleic acid. Examples include, but are not limited to, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/endonuclease systems, transcription activator-like effector-based nuclease (TALEN), zinc finger nucleases (ZFN), endonucleases (*e.g.*, ARC homing endonucleases),
20 meganucleases (*e.g.*, mega-TALs), or a combination thereof.

Various gene editing systems using meganucleases, including modified meganucleases, have been described in the art; see, *e.g.*, the reviews by Steentoft et al., Glycobiology 24(8):663-80, 2014; Belfort and Bonocora, Methods Mol Biol. 1123:1-26, 2014; Hafez and Hausner, Genome 55(8):553-69, 2012; and references cited therein. In some
25 examples, a knocking-out event can be coupled with a knocking-in event – an exogenous nucleic acid coding for a desired molecule such as those described herein can be inserted into a locus of a target endogenous gene of interest via gene editing.

In some instances, knocking-out an endogenous gene can be achieved using the CRISPR technology. Exemplary target endogenous genes include one or more of a TCR,
30 CD52, IFN- γ , B2M, and GM-CSF.

Alternatively, any of the knock-out modification may be achieved using antisense oligonucleotides (*e.g.*, interfering RNAs such as shRNA or siRNA) or ribozymes *via* methods known in the art. An antisense oligonucleotide specific to a target cytokine/protein refers to an

oligonucleotide that is complementary or partially complementary to a target region of an endogenous gene of the cytokine or an mRNA encoding such. Such antisense oligonucleotides can be delivered into target cells via conventional methods. Alternatively, expression vectors such as lentiviral vectors or equivalent thereof can be used to express such an antisense
5 oligonucleotides.

(C) Preparation of Immune Cell Population Comprising Modified Immune Cells

A population of immune cells comprising any of the modified immune cells described herein, or a combination thereof, may be prepared by introducing into a population of host immune cells one or more of the knock-in modifications, one or more of the knock-out
10 modifications, or a combination thereof. The knock-in and knock-out modifications can be introduced into the host cells in any order.

In some instances, one or more modifications are introduced into the host cells in a sequential manner without isolation and/or enrichment of modified cells after a preceding modification event and prior to the next modification event. In that case, the resultant immune
15 cell population may be heterogeneous, comprising cells harboring different modifications or different combination of modifications. Such an immune cell population may also comprise unmodified immune cells. The level of each modification event occurring in the immune cell population can be controlled by the amount of genetic materials that induce such modification as relative to the total number of the host immune cells. See also above discussions.

In other instances, modified immune cells may be isolated and enriched after a first
20 modification event before performing a second modification event. This approach would result in the production of a substantially homogenous immune cell population harboring all of the knock-in and/or knock-out modifications introduced into the cells.

In some examples, the knock-in modification(s) and the knock-out modification(s) are
25 introduced into host immune cells separately. For example, a knock-out modification is performed *via* gene editing to knock out an endogenous gene for a target cytokine and a knock-in modification is performed by delivering into the host immune cells a separate exogenous expression cassette for producing one or more cytokine antagonists. In some instances, the knock-in and knock-out event can be occurred simultaneously, for example, the knock-in
30 cassette can be inserted into the locus of a target gene to be knocked-out.

IV. Therapeutic Applications

Any of the immune cell populations comprising the modified immune cells as described herein may be used in an adoptive immune cell therapy (*e.g.*, CAR-T) for treating a

target disease, such as leukemia or lymphoma. Due to the knock-in and knock-out modifications introduced into the immune cells, particularly the knock-in of the CAR, the knock-in of the IL-6 antagonistic antibody, the IFN γ antagonist, the IL-1 antagonist, or a combination thereof, the therapeutic uses of such would be expected to improve proliferation
5 of the therapeutic cells and/or reduce cytokine toxicity in the patient being treated, while achieving the same or better therapeutic effects.

To practice the therapeutic methods described herein, an effective amount of the immune cell population, comprising any of the modified immune cells as described herein, may be administered to a subject who needs treatment via a suitable route (*e.g.*, intravenous
10 infusion). One or more of the immune cell populations may be mixed with a pharmaceutically acceptable carrier to form a pharmaceutical composition prior to administration, which is also within the scope of the present disclosure. The immune cells may be autologous to the subject, *e.g.*, the immune cells are obtained from the subject in need of the treatment, modified to reduce expression of one or more target cytokines/proteins, for example, those described
15 herein, to express one or more cytokine antagonists described herein, to express a CAR construct and/or exogenous TCR, or a combination thereof. The resultant modified immune cells can then be administered to the same subject. Administration of autologous cells to a subject may result in reduced rejection of the immune cells as compared to administration of non-autologous cells. Alternatively, the immune cells can be allogeneic cells, *e.g.*, the cells are
20 obtained from a first subject, modified as described herein and administered to a second subject that is different from the first subject but of the same species. For example, allogeneic immune cells may be derived from a human donor and administered to a human recipient who is different from the donor.

The subject to be treated may be a mammal (*e.g.*, human, mouse, pig, cow, rat, dog,
25 guinea pig, rabbit, hamster, cat, goat, sheep or monkey). The subject may be suffering from cancer, have an infectious disease or an immune disorder. Exemplary cancers include but are not limited to hematologic malignancies (*e.g.*, B-cell acute lymphoblastic leukemia, chronic lymphocytic leukemia and multiple myeloma). Exemplary infectious diseases include but are not limited to human immunodeficiency virus (HIV) infection, Epstein-Barr virus (EBV) infection,
30 human papillomavirus (HPV) infection, dengue virus infection, malaria, sepsis and *Escherichia coli* infection. Exemplary immune disorders include but are not limited to, autoimmune diseases, such as rheumatoid arthritis, type I diabetes, systemic lupus erythematosus, inflammatory bowel disease, multiple sclerosis, Guillain-Barre syndrome, chronic

inflammatory demyelinating polyneuropathy, psoriasis, Graves' disease, Hashimoto's thyroiditis, myasthenia gravis, and vasculitis.

In some examples, the subject to be treated in the methods disclosed herein may be a human cancer patient. In some examples, the cancer may be lymphoblastic leukemia, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, mantle cell lymphoma, large B-cell lymphoma, or non-Hodgkin's lymphoma. In particular, for treating such cancers, the immune cells may express a CAR that binds CD19. In some examples, the cancer may be multiple myeloma, relapsed multiple myeloma, or refractory multiple myeloma. In particular, for treating such cancers, the immune cells may express a CAR that binds BCMA. Alternatively, the human patient may have breast cancer, gastric cancer, neuroblastoma, or osteosarcoma.

In some embodiments, the CAR-T cells described herein are useful for treating B-cell related cancers. Non-limiting B-cell related cancers include multiple myeloma, malignant plasma cell neoplasm, Hodgkin's lymphoma, nodular lymphocyte predominant Hodgkin's lymphoma, Kahler's disease and Myelomatosis, plasma cell leukemia, plasmacytoma, B-cell prolymphocytic leukemia, hairy cell leukemia, B-cell non-Hodgkin's lymphoma (NHL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), chronic myeloid leukemia (CML), follicular lymphoma, Burkitt's lymphoma, marginal zone lymphoma, mantle cell lymphoma, large cell lymphoma, precursor B-lymphoblastic lymphoma, myeloid leukemia, Waldenstrom's macroglobulinemia, diffuse large B cell lymphoma, follicular lymphoma, marginal zone lymphoma, mucosa-associated lymphatic tissue lymphoma, small cell lymphocytic lymphoma, mantle cell lymphoma, Burkitt lymphoma, primary mediastinal (thymic) large B-cell lymphoma, lymphoplasmacytic lymphoma, Waldenström macroglobulinemia, nodal marginal zone B cell lymphoma, splenic marginal zone lymphoma, intravascular large B-cell lymphoma, primary effusion lymphoma, lymphomatoid granulomatosis, T cell/histiocyte-rich large B-cell lymphoma, primary central nervous system lymphoma, primary cutaneous diffuse large B-cell lymphoma (leg type), EBV positive diffuse large B-cell lymphoma of the elderly, diffuse large B-cell lymphoma associated with inflammation, intravascular large B-cell lymphoma, ALK-positive large B-cell lymphoma, plasmablastic lymphoma, large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease, B-cell lymphoma unclassified with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma, B-cell lymphoma unclassified with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma, and other B-cell related lymphoma.

The term “an effective amount” as used herein refers to the amount of each active agent required to confer therapeutic effect on the subject, either alone or in combination with one or more active agents. Effective amounts vary, as recognized by those skilled in the art, depending on the particular condition being treated, the severity of the condition, individual patient parameters including age, physical condition, size, gender and weight, the duration of treatment, route of administration, excipient usage, co-usage (if any) with other active agents and like factors within the knowledge and expertise of the health practitioner. The quantity to be administered depends on the subject to be treated, including, for example, the capacity of the individual's immune system to produce a cell-mediated immune response. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner. However, suitable dosage ranges are readily determinable by one skilled in the art.

The term “treating” as used herein refers to the application or administration of a composition including one or more active agents to a subject, who has a target disease, a symptom of the target disease, or a predisposition toward the target disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the disease, the symptoms of the disease, or the predisposition toward the disease.

An effective amount of the immune cells may be administered to a human patient in need of the treatment via a suitable route, *e.g.*, intravenous infusion. In some instances, about 1×10^6 to about 1×10^8 CAR+ T cells may be given to a human patient (*e.g.*, a leukemia patient, a lymphoma patient, or a multiple myeloma patient). In some examples, a human patient may receive multiple doses of the immune cells. For example, the patient may receive two doses of the immune cells on two consecutive days. In some instances, the first dose is the same as the second dose. In other instances, the first dose is lower than the second dose, or *vice versa*.

In any of the treatment methods disclosed herein, which involves the use of the immune cells, the subject may be administered IL-2 concurrently with the cell therapy. More specifically, an effective amount of IL-2 may be given to the subject via a suitable route before, during, or after the cell therapy. In some embodiments, IL-2 is given to the subject after administration of the immune cells.

Alternatively or in addition, the subject being treated by the cell therapy disclosed herein may be free from treatment involving an IL-6 antagonist (aside from an IL-6 antagonist produced by the immune cells used in the cell therapy) after immune cell infusion.

The immune cell populations comprising the modified immune cells as described herein may be utilized in conjunction with other types of therapy for cancer, such as chemotherapy, surgery, radiation, gene therapy, and so forth. Such therapies can be

administered simultaneously or sequentially (in any order) with the immunotherapy described herein. When co-administered with an additional therapeutic agent, suitable therapeutically effective dosages for each agent may be lowered due to the additive action or synergy.

The subject being treated may also receive immunosuppressive steroids such as
5 methylprednisolone and dexamethasone in conjunction with infusion of the immune cells disclosed herein.

In some examples, the subject is subject to a suitable anti-cancer therapy (*e.g.*, those disclosed herein) to reduce tumor burden prior to the CAR-T therapy disclosed herein. For example, the subject (*e.g.*, a human cancer patient) may be subject to a chemotherapy (*e.g.*,
10 comprising a single chemotherapeutic agent or a combination of two or more chemotherapeutic agents) at a dose that substantially reduces tumor burden. In some instances, the chemotherapy may reduce the total white blood cell count in the subject to lower than $10^8/L$, *e.g.*, lower than $10^7/L$. Tumor burden of a patient after the initial anti-cancer therapy, and/or after the CAR-T cell therapy disclosed herein may be monitored via routine methods. If a patient showed a high
15 growth rate of cancer cells after the initial anti-cancer therapy and/or after the CAR-T therapy, the patient may be subject to a new round of chemotherapy to reduce tumor burden followed by any of the CAR-T therapy as disclosed herein.

Non-limiting examples of other anti-cancer therapeutic agents useful for combination with the modified immune cells described herein include, but are not limited to, immune
20 checkpoint inhibitors (*e.g.*, PDL1, PD1, and CTLA4 inhibitors), anti-angiogenic agents (*e.g.*, TNP-470, platelet factor 4, thrombospondin-1, tissue inhibitors of metalloproteases, prolactin, angiostatin, endostatin, bFGF soluble receptor, transforming growth factor beta, interferon alpha, interferon gamma, soluble KDR and FLT-1 receptors, and placental proliferin-related protein); a VEGF antagonist (*e.g.*, anti-VEGF antibodies, VEGF variants, soluble VEGF
25 receptor fragments); chemotherapeutic compounds. Exemplary chemotherapeutic compounds include pyrimidine analogs (*e.g.*, 5-fluorouracil, floxuridine, capecitabine, gemcitabine and cytarabine); purine analogs (*e.g.*, fludarabine); folate antagonists (*e.g.*, mercaptopurine and thioguanine); antiproliferative or antimitotic agents, for example, vinca alkaloids; microtubule disruptors such as taxane (*e.g.*, paclitaxel, docetaxel), vincristin, vinblastin, nocodazole,
30 epothilones and navelbine, and epididodophyllotoxins; DNA damaging agents (*e.g.*, actinomycin, amsacrine, anthracyclines, bleomycin, busulfan, camptothecin, carboplatin, chlorambucil, cisplatin, cyclophosphamide, cytoxan, dactinomycin, daunorubicin, doxorubicin, epirubicin, hexamethylnelamineoxaliplatin, iphosphamide, melphalan, merchlorheptamine,

mitomycin, mitoxantrone, nitrosourea, plicamycin, procarbazine, taxol, taxotere, teniposide, triethylenethiophosphoramidate and etoposide).

In some embodiments, radiation or radiation and chemotherapy is used in combination with the cell populations comprising modified immune cells described herein.

5 Additional useful agents and therapies can be found in Physician's Desk Reference, 59th edition, (2005), Thomson P D R, Montvale N.J.; Gennaro et al., Eds. Remington's The Science and Practice of Pharmacy 20th edition, (2000), Lippincott Williams and Wilkins, Baltimore Md.; Braunwald et al., Eds. Harrison's Principles of Internal Medicine, 15th edition, (2001), McGraw Hill, NY; Berkow et al., Eds. The Merck Manual of Diagnosis and
10 Therapy, (1992), Merck Research Laboratories, Rahway N.J.

V. Kits for Therapeutic Uses or Making Modified Immune Cells

The present disclosure also provides kits for use of any of the target diseases described herein involving one or more of the immune cell population described herein and kits for use in
15 making the modified immune cells as described herein.

A kit for therapeutic use as described herein may include one or more containers comprising an immune cell population, which may be formulated to form a pharmaceutical composition. The immune cell population comprises any of the modified immune cells described herein or a combination thereof. The population of immune cells, such as T
20 lymphocytes, NK cells, and others described herein may further express a CAR construct and/or an exogenous TCR, and or an antigen specific TCR, as described herein.

In some embodiments, the kit can additionally comprise instructions for use of the immune cell population in any of the methods described herein. The included instructions may comprise a description of administration of the immune cell population or a pharmaceutical
25 composition comprising such to a subject to achieve the intended activity in a subject. The kit may further comprise a description of selecting a subject suitable for treatment based on identifying whether the subject is in need of the treatment. In some embodiments, the instructions comprise a description of administering the immune cell population or the pharmaceutical composition comprising such to a subject who is in need of the treatment.

30 The instructions relating to the use of the immune cell population or the pharmaceutical composition comprising such as described herein generally include information as to dosage, dosing schedule, and route of administration for the intended treatment. The containers may be unit doses, bulk packages (*e.g.*, multi-dose packages) or sub-unit doses. Instructions supplied in the kits of the disclosure are typically written instructions on a label or package insert. The

label or package insert indicates that the pharmaceutical compositions are used for treating, delaying the onset, and/or alleviating a disease or disorder in a subject.

The kits provided herein are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging, and the like. Also contemplated are packages for use in combination with a specific device, such as an inhaler, nasal administration device, or an infusion device. A kit may have a sterile access port (for example, the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The container may also have a sterile access port. At least one active agent in the pharmaceutical composition is a population of immune cells (*e.g.*, T lymphocytes or NK cells) that comprise any of the modified immune cells or a combination thereof.

Kits optionally may provide additional components such as buffers and interpretive information. Normally, the kit comprises a container and a label or package insert(s) on or associated with the container. In some embodiment, the disclosure provides articles of manufacture comprising contents of the kits described above.

Also provided here are kits for use in making the modified immune cells as described herein. Such a kit may include one or more containers each containing reagents for use in introducing the knock-in and/or knock-out modifications into immune cells. For example, the kit may contain one or more components of a gene editing system for making one or more knock-out modifications as those described herein. Alternatively or in addition, the kit may comprise one or more exogenous nucleic acids for expressing cytokine antagonists as also described herein and reagents for delivering the exogenous nucleic acids into host immune cells. Such a kit may further include instructions for making the desired modifications to host immune cells.

VI. General Techniques

The practice of the present disclosure will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as *Molecular Cloning: A Laboratory Manual*, second edition (Sambrook, et al., 1989) Cold Spring Harbor Press; *Oligonucleotide Synthesis* (M. J. Gait, ed. 1984); *Methods in Molecular Biology*, Humana Press; *Cell Biology: A Laboratory Notebook* (J. E. Cellis, ed., 1989) Academic Press; *Animal Cell Culture* (R. I. Freshney, ed. 1987); *Introduction to Cell and Tissue Culture* (J. P. Mather and P. E. Roberts, 1998) Plenum Press; *Cell and Tissue Culture: Laboratory Procedures* (A.

Doyle, J. B. Griffiths, and D. G. Newell, eds. 1993-8) J. Wiley and Sons; Methods in Enzymology (Academic Press, Inc.); Handbook of Experimental Immunology (D. M. Weir and C. C. Blackwell, eds.): Gene Transfer Vectors for Mammalian Cells (J. M. Miller and M. P. Calos, eds., 1987); Current Protocols in Molecular Biology (F. M. Ausubel, et al. eds. 1987);

5 PCR: The Polymerase Chain Reaction, (Mullis, et al., eds. 1994); Current Protocols in Immunology (J. E. Coligan et al., eds., 1991); Short Protocols in Molecular Biology (Wiley and Sons, 1999); Immunobiology (C. A. Janeway and P. Travers, 1997); Antibodies (P. Finch, 1997); Antibodies: a practice approach (D. Catty., ed., IRL Press, 1988-1989); Monoclonal antibodies: a practical approach (P. Shepherd and C. Dean, eds., Oxford University Press,

10 2000); Using antibodies: a laboratory manual (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999); The Antibodies (M. Zanetti and J. D. Capra, eds. Harwood Academic Publishers, 1995); *DNA Cloning: A practical Approach*, Volumes I and II (D.N. Glover ed. 1985); *Nucleic Acid Hybridization* (B.D. Hames&S.J. Higgins eds.(1985; *Transcription and Translation* (B.D. Hames&S.J. Higgins, eds. (1984; *Animal Cell Culture* (R.I. Freshney, ed.

15 (1986; *Immobilized Cells and Enzymes* (IRL Press, (1986); B. Perbal, *A practical Guide To Molecular Cloning* (1984); F.M. Ausubelet al. (eds.); *Chimeric Antigen Receptor (CAR) Immunotherapy* (D. W. Lee and N. N. Shah, eds., Elsevier, 2019, ISBN:9780323661812); *Basics of Chimeric Antigen Receptor (CAR) Immunotherapy* (M. Y. Balkhi, Academic Press, Elsevier Science, 2019, ISBN:9780128197479); *Chimeric Antigen*

20 *Receptor T Cells Development and Production* (V. Picanço-Castro, K. C. R. Malmegrim, K.Swiech, eds., Springer US, 2020, ISBN:9781071601488); *Cell and Gene Therapies* (C. Bollard, S. A. Abutalib, M.-A. Peraleseds., Springer International, 2018; ISBN:9783319543680) and *Developing Costimulatory Molecules for Immunotherapy of Diseases* (M. A. Mir, Elsevier Science, 2015, ISBN:9780128026755).

25 The present disclosure is not limited in its application to the details of construction and the arrangements of component set forth in the description herein or illustrated in the drawings. The present disclosure is capable of other embodiments and of being practice or of being carried out in various ways. Also, the phraseology and terminology used herein is for the purpose of description and should not be regarded as limiting. The use of “including,”

30 “comprising,” or “having,” “containing,” “involving,” and variations thereof herein, is meant to encompass the items listed thereafter and equivalents thereof as well as additional items. As also used in this specification and the appended claims, the singular forms “a,” “an,” and “the” include plural references unless the context clearly dictates otherwise.

Without further elaboration, it is believed that one skilled in the art can, based on the above description, utilize the present invention to its fullest extent. The following specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. All publications cited herein are

5 incorporated by reference for the purposes or subject matter referenced herein.

EXAMPLES

Example 1: Treating Cancer Using CAR-T Therapy

This example demonstrates that CAR-T cells expressing a CAR construct described herein is effective for treating cancer in a patient with a heavy tumor burden, while also

10 resulting in relatively low IFN- γ production during CAR-T therapy. A human patient diagnosed with mantle cell lymphoma was treated with anti-CD19/IL-6/IL-1 CAR-T cells as follows. Structural features of the anti-CD19 CAR, IL-6 antagonist, and IL-1 antagonist are as provided herein. The human patient was treated with chemotherapy to lower tumor burden, followed by fludarabine/cyclophosphamide pretreatment to deplete endogenous lymphocytes

15 to place the patient in condition for CAR-T cell transplantation. Afterwards, the patient received 0.2×10^8 (D0) anti-CD19/IL-6/IL-1 CAR-T cells as disclosed herein (with wild type GM-CSF and TCR genes). The patient was injected with recombinant IL-2 during the therapy. After treatment, an enormous number of lymphocytes ($13.02 \times 10^9/L$) at D0 in peripheral blood decreased to normal levels ($0.44 \times 10^9/L$) at D19 (**FIG. 1A**) and complete response was

20 achieved. During treatment, the patient only experienced mild fever (**FIG. 1B**) and only grade 1 cytokine release syndrome (CRS) without hypotension, hypoxia or neurotoxicity. During treatment, the patient did not receive tocilizumab, an antagonistic monoclonal antibody that binds to the receptor for IL-6. Analysis of cytokine levels revealed a very low level of IL-6 in the patient after the T-cell infusion (**FIG. 1C**), and low levels of peak IFN γ (**FIG. 1C**). **FIGS.**

25 **1D** and **1E** show levels of CRP and Ferritin after the T-cell infusion. In sum, these results revealed a relatively low level of IFN γ production while CAR-T cells eradicated an enormous number of tumor cells, suggesting that IFN γ might be dispensable during CAR-T therapy, and that knock out of IFN γ may be an appealing way to minimize cytokine toxicity associated with CAR-T therapy.

Example 2: IFN- γ Knock-Out

This example describes IFN- γ knock-outs. T-cells from a normal donor were stimulated and activated by anti-CD3/anti-CD28 dynabeads (Thermo). Three days later, T-cells were electroporated with a ribonucleoprotein (RNP) complex of Cas9 protein (thermo) and single

guide RNA (sgRNA) candidates targeting the protospacer adjacent motif (PAM) sequence in the first exon of the human IFN- γ gene. A sgRNA targeting B2M was included as a control.

The DNA sequences used for *in vitro* transcription of IFN- γ sgRNA were as follows:

DNA Sequence for *in vitro* transcription of IFN γ sgRNA

5

sgRNA 1

GAAATATACAAGTTATATCTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG
CTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT (SEQ ID
NO: 31)

10

sgRNA 2

GTTTCAGCTCTGCATCGTTTTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG
CTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT (SEQ ID
NO: 32)

15

sgRNA 3

GTTTCAGCTCTGCATCGTTTTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG
CTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT (SEQ ID
NO: 33)

20

sgRNA 4

GCATCGTTTTGGGTTCTCTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCT
AGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT (SEQ ID NO: 34)

25

sgRNA 5

GTCTCTTGGCTGTTACTGCCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG
CTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT (SEQ ID
NO: 35)

30

sgRNA 6

GTTCTTTTACATATGGGTCCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG
CTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT (SEQ ID
NO: 36)

sgRNA 7

GTTCTGCTTCTTTTACATATGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
TAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT (SEQ ID NO: 37)

5 sgRNA 8

GTTTCTGCTTCTTTTACATAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
TAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT (SEQ ID NO: 38)

Two days after electroporation, T-cells were analyzed by intracellular staining of IFN- γ , and the results indicated that sgRNA 4 was most effective in reducing IFN- γ production (FIG. 2).

Example 3: Improved persistence of CAR-T *in vivo*

This example describes improved persistence of CAR-T with intracellular IL-2R β signaling in patients after CAR-T cell transplantation. Patients diagnosed for refractory or relapsed Multiple Myeloma (MM) were subject to treatment with anti-BCMA CAR-T cells. Two types of anti-BCMA CAR-T cells were used. Patient #1 was infused with anti-BCMA CAR-T cells that had the intracellular signaling domains of 41BB, IL-2R β and CD3 ζ . Patient #2 was infused with anti-BCMA CAR-T cells that had the intracellular signaling domains of 41BB and CD3 ζ without the IL-2R β co-stimulatory signaling domains. The human patients were treated with fludarabine/cyclophosphamide pretreatment to deplete endogenous lymphocytes so as to place the patient in condition for CAR-T cell transplantation. The result showed that the frequency of CAR+ T cells was maintained around 40% at Day 31 post infusion in patient #1, as compared to around 37% at Day16 post infusion in patient #2, which indicates that addition of IL2R β signaling would improve long term persistence of CAR+ T cells in patients. See FIG. 3.

Example 4: Sustained clinical response to cell-based therapy *in vivo*

This example describes sustained clinical response by anti-CD19 CAR-T cells with intracellular signaling comprising domains from 41BB, IL-2R β , CD3 ζ in patients. Patients diagnosed with refractory and relapsed acute lymphocytic leukemia (ALL) were treated with anti-CD19 CAR-T cells with 41BB, IL-2R β and CD3 ζ signaling. After treatment, the three patients achieved complete response as determined by very low numbers or undetectable of CD19+ B-cells in circulation after the CAR-T treatment compared to prior to treatment. More importantly, IL-2R β signaling powered CART cells showed long term persistence of CAR+ T cells and induced sustainable B cell aplasia in the treated patients. See FIG. 4.

Example 5: Expansion of CAR-T cells with intracellular signaling of 41BB, IL-2R β , and CD3 ζ in patients.

This example describes *in vivo* expansion of CAR-T cells having the intracellular signaling domains comprising of 41BB, IL-2R β , CD3 ζ . Patient diagnosed for refractory or relapsed ALL (Acute Lymphoblastic Leukemia), lymphoma or MM (Multiple Myeloma) were subject to treatment with anti-CD19 or anti-BCMA CAR-T cells with intracellular signaling of 41BB, IL-2R β , CD3 ζ . The human patient was treated with fludarabine/cyclophosphamide pretreatment to deplete endogenous lymphocytes so as to place the patient in condition for CAR-T cell transplantation. Afterwards, the patient received the respective CAR-T cells as disclosed herein (with 41BB, IL-2R β , CD3 ζ signaling domains). **FIG. 5** showed the median peak frequency of anti-BCMA CAR-T cells in T cell population was about 60%, and the median peak frequency of anti-CD19 CAR-T cells in T cell population was about 10%. This result indicated that the combination of 41BB, IL-2R β , and CD3 ζ signaling induces significant expansion of both anti-CD19 and anti-BCMA CAR-T cells in patients.

Example 6: Effects of interferon gamma (IFN γ) antagonistic antibodies expressed in 293T cells in inhibiting IFN γ Signaling in the cells.

HEK293T cells were transfected with a 3rd generation self-inactivating (SIN) lentiviral transfer vectors encoding single-chain variable fragment (scFv) antibody derived from reference antibodies Amg (AMG811), Fon (fontulizumab) and Ema (emapalumab) disclosed herein, which target IFN γ , by LIPOFECTAMINE 2000 (Thermo Scientific). A growth hormone (GH) leading sequence (single peptide sequence for the expression of proteins destined to be secreted move through the secretory pathway) is located before the anti-IFN γ scFv construct. The supernatants of transfected cells, containing the secreted scFv antibodies expressed by the transfected HEK293T cells, were collected, diluted, and added to HEK-Blue IFN γ reporter cells (INVIVOGEN) in the presence of 2 ng/ml human IFN γ . HEK-Blue IFN γ reporter cells were used because they are capable of producing Secreted Embryonic Alkaline Phosphatase (SEAP) upon human IFN γ stimulation. After overnight incubation, the supernatant of HEK-Blue IFN γ cells was collected and incubated with Quant-Blue substrate solution. SEAP production was quantified by measuring optical absorbance of converted substrate Quant Blue (INVIVOGEN) at 650nm wave length through a spectrophotometer.

The amino acid sequences of the V_L and V_H of Amg, Fon, and Ema used in constructing the various anti-IFN γ scFv-CARs are as follows (SEQ ID Nos: 52-60):

V_L of fontulizumab (SEQ. ID. NO: 52):

DIQMTQSPSTLSASVGDRVTITCKASENVDITYVSWYQQKPGKAPKLLIYGASNRYTGV
PSRFSGSGSGTDFTLTISLQPDFFATYYCGQSYNYPFTFGQGTKVEVKR

5 V_H of fontulizumab (SEQ. ID. NO: 53):

QVQLVQSGAELKKPGSSVKVSCKASGYIFTSSWINWVKQAPGQGLEWIGRIDPSDGEV
HYNQDFKDKATLTVDKSTNTAYMELSSLRSEDNAVYYCARGFLPWFADWGQGLVT
VSS

10 An anti-IFN γ scFv from fontulizumab (SEQ. ID. NO: 54)

DIQMTQSPSTLSASVGDRVTITCKASENVDITYVSWYQQKPGKAPKLLIYGASNRYTGV
PSRFSGSGSGTDFTLTISLQPDFFATYYCGQSYNYPFTFGQGTKVEVKR**GGGGSGGG**
GSGGGGSQVQLVQSGAELKKPGSSVKVSCKASGYIFTSSWINWVKQAPGQGLEWIGR
IDPSDGEVHYNQDFKDKATLTVDKSTNTAYMELSSLRSEDNAVYYCARGFLPWFADW
15 GQGLVTVSS

V_L of emapalumab (SEQ. ID. NO: 55):

NFMLTQPHSVSESPGKTVTISCTRSSGSIASNYVQWYQQRPGSSPTTVIYEDNQRPSGV
PDRFSGSIDSSSNSASLTISGLKTEDEADYYCQSYDGSNRWMFGGGTKLTVL

20

V_H of emapalumab (SEQ. ID. NO: 56):

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGST
YYADSVKGRFTISRDN SKNTLYLQMNSLRAEDNAVYYCAKDGSSGWYVPHWFDPWG
QGLTVTVSS

25

An anti-IFN γ scFv from emapalumab (SEQ. ID. NO: 57):

NFMLTQPHSVSESPGKTVTISCTRSSGSIASNYVQWYQQRPGSSPTTVIYEDNQRPSGV
PDRFSGSIDSSSNSASLTISGLKTEDEADYYCQSYDGSNRWMFGGGTKLTVL**GGGGSG**
GGGSGGGGSEVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEW
30 VSAISGSGGSTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDNAVYYCAKDGSSGW
YVPHWFDPWGQGLTVTVSS

Amino acid sequence of anti-human IFN- γ AMG811 are disclosed in U.S. Pat. Appl. No: 20130142809 and U.S. Patent 7,335,743, the relevant portions of which are incorporated herein by reference.

5 V_L of AMG811 (SEQ. ID. NO: 58):

EIVLTQSPGTLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSR
ATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQRSGGSSFTFGPGTKVDIK

V_H of AMG811 (SEQ. ID. NO: 59):

10 EVQLVQSGAEVKKPGESLKISCKGSGYNFTSYWIGWVRQMPGKGLELMGIIYP
GDSDTRYSPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCGSGSYFYFDLWGRGT
LTVTVSS

Anti-IFN γ scFv from AMG811 (SEQ. ID. NO: 60)

15 EIVLTQSPGTLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIP
DRFSGSGSGTDFTLTISRLEPEDFAVYYCQRSGGSSFTFGPGTKVDIKGGGGSGGGGS
GGGGSEVQLVQSGAEVKKPGESLKISCKGSGYNFTSYWIGWVRQMPGKGLELMGIIY
PGDSDTRYSPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCGSGSYFYFDLWGRG
TLVTVSS

20

As shown in **FIG. 6A**, scFv antibodies 1 and 4 are able to inhibit IFN γ signaling in the reporter cells. Amongst the 6 scFv antibodies tested, the scFv antibodies derived from antibody Amg811 (Amg) exhibited higher efficiency in inhibiting IFN γ signaling as compared to the scFv antibodies derived from reference antibodies fontulizumab (Fon) and emapalumab (Ema).
25 For example, scFv antibodies derived from reference Amg showed close to 80% inhibition of IFN γ signaling at dilution 0.5, whereas those derived from reference antibodies Fon and Ema showed very low inhibition efficiency at the same dilution. This result indicated that scFvs from antibody Amg are more effective in blocking IFN γ signaling in the auto-secretion style of lentivector system.

30

These *in vitro* data **FIG. 6A** showed that CAR vector encoding anti-IFN γ scFv from emapalumab was not as effective as the anti-IFN γ scFv from Amg811 in inhibiting IFN γ signaling. However, the clinical data of patients treated with CART co-expressing anti-IFN γ scFv from emapalumab showed that very low level of IFN γ was observed during CART

therapy. See further examples below, **FIGS. 9 and 10**. One possible reason might be that CART synthesized anti-IFN γ scFv from emapalumab was not secreted efficiently and trapped inside the cells.

Several signal peptide sequences from various proteins were tested to explore their effects on the expression and secretion of the IFN γ scFv from cells, and thus the effective amount of the secreted anti-IFN γ scFv available for inhibiting IFN γ signaling *in vivo*. The influence of signal peptide on inhibition of IFN γ signaling by Amg-LH scFv are shown in **Fig. 6B**: 1, a signal peptide from albumin (SEQ. ID. NO: 47); 2, a signal peptide from CD8 (SEQ. ID. NO: 44); 3, a signal peptide from a growth hormone (SEQ. ID. NO: 29); 4, a modified signal peptide from albumin (SEQ. ID. NO: 48); 5, a modified signal peptide from IL2 (SEQ. ID. NO: 49); 6, a signal peptide from an antibody light chain (SEQ. ID. NO: 45); and 7, a signal peptide from GL (*Gaussia* luciferase) (SEQ. ID. NO: 46). The results revealed that the signal peptide from CD8 was most effective in producing secreted anti-IFN γ scFv available for inhibition of IFN γ signaling.

Example 7: Inhibition of IFN γ signaling prevents severe cytokine release syndrome (CRS) in patients treated with CAR-T Therapy for cancer.

Several approaches to reduce the IFN γ signaling *in vivo* in patients undergoing CAR-T cells therapy were next tested. Surprisingly, with relatively lower IFN γ signaling achieved via IFN γ gene knockout or expressing IFN γ antagonist, the CAR-T cells were still effective, effecting cytotoxic activity against target cells.

A. Reduced expression of endogenous IFN γ in the CAR-T cells used to treat patients.

Acute lymphocytic leukemia (ALL) patient treated with IFN γ knockout (KO) CAR-T

cells

A patient diagnosed with refractory and relapsed ALL was treated with anti-CD19 CAR-T cells with 41BB- IL-2R β -CD3 ζ signaling, CRISPR edited IFN γ KO and co-expressing both IL-6 antagonist and IL-1 antagonist. After treatment, this patient achieved complete response and has low levels of peak IFN γ (**FIG. 7**), showing that anti-CD19 CAR-T cells with IFN γ KO are capable of inducing complete response in clinical efficacy. In the patient, B cell aplasia was observed at day 14 after CART infusion, and no tumor cells were detected in bone marrow examination result, suggesting complete response was achieved after treatment. During the treatment, only grade 2 CRS was observed.

Multiple myeloma (MM) patient treated with IFN γ KO CAR-T cells

A patient diagnosed with refractory and relapsed MM was treated with anti-BCMA CAR-T cells with 41BB- IL-2R β -CD3 ζ signaling, CRISPR edited IFN γ KO and co-expressing both IL-6 antagonist and IL-1 antagonist. After treatment, this patient achieved complete
 5 response, and has moderate levels of peak IFN γ (**FIG. 8A**), indicating that CAR-T cells with anti-BCMA IFN γ KO are capable of inducing complete response in clinical efficacy. During the treatment, only grade 1 CRS (fever, hypoxia and hypotension) was observed. Compared to other patients, this patient had a relatively higher IFN γ peak, likely because of very high tumor burden. However, the CRS symptoms of fever, hypoxia and hypotension were mild. In **FIG.**
 10 **8B**, the IgG level of the patient decreased to very low level after treatment. Further immunofixation electrophoresis during follow up indicated negative result of monoclonal protein (M protein), suggesting complete response.

B. Co-expressing soluble antagonist anti-IFN γ scFv in the CAR-T cells used to treat patients.

Lymphoma patient treated with antagonistic anti-IFN γ scFv expressing CAR-T cells

A patient diagnosed with refractory and relapsed lymphoma was treated with anti-CD19 CAR-T cells with 41BB- IL-2R β -CD3 ζ signaling, and co-expressing IFN γ blocking scFv derived from emapalumab and IL6 blocking scFv derived from sirukumab. After
 treatment, this patient achieved complete response, and the very low level of peak IFN γ was
 20 detected (**FIG. 9**), showing that anti-CD19 CAR-T cells with co-expression of Ema scFv are capable of inducing complete response in clinical efficacy. During the treatment, only grade 0 CRS was observed. PET-CT scanning result during follow up indicated that the tumor spot disappeared at day106 after treatment.

MM patient treated with antagonistic anti- IFN γ scFv expressing CAR-T cells

Two patients diagnosed with refractory and relapsed MM were treated with anti-BCMA
 25 CAR-T cells with 41BB- IL-2R β -CD3 ζ signaling, and co-expressing IFN γ blocking scFv derived from emapalumab and IL6 blocking scFv derived from sirukumab. After treatment, the patients achieved very good partial response, and the very low levels of peak IFN γ were detected (**FIGS. 10A and 10B**) indicating that anti-BCMA CAR-T cells with co-expression of
 30 Ema scFv are capable of inducing very good partial response in clinical efficacy. During the treatment, only grade 1 CRS was observed. For patient #1, immunofixation electrophoresis during follow up indicated only residual level of monoclonal protein (M protein) at day 102 after treatment. For patient #2, IgG level decreased to normal level at day 39 after treatment.

Table 1. Summary of the CRS experienced by patients treated with the various CAR-T cells in CAR-T cell therapy

CAR T type	Cancer type	IFN γ blockade	IL-6 blockade	IL-1 blockade	CRS Grade
Mono-specific scFv; anti-CD19	mantle cell lymphoma	None	Yes, IL-6 blocking scFv (scFv from sirukumab)	Yes, IL-1RA	1
Mono-specific scFv; anti-CD19	Acute lymphocytic leukemia (ALL)	Yes, CRISPR Knock Out	Yes, IL-6 blocking scFv (scFv from sirukumab)	Yes, IL-1RA	2
Mono-specific scFv; anti-BCMA	Multiple myeloma (MM)	Yes, CRISPR Knock Out	Yes, IL-6 blocking scFv (scFv from sirukumab)	Yes, IL-1RA	1
Mono-specific scFv; anti-CD19	Lymphoma	Yes, IFN γ blocking scFv (scFv from emapalumab)	Yes, IL-6 blocking scFv (scFv from sirukumab)	None	0
Mono-specific scFv; anti-BCMA	MM	Yes, IFN γ blocking scFv (scFv from emapalumab)	Yes, IL-6 blocking scFv (scFv from sirukumab)	None	1

OTHER EMBODIMENTS

5 All of the features disclosed in this specification may be combined in any combination. Each feature disclosed in this specification may be replaced by an alternative feature serving the same, equivalent, or similar purpose. Thus, unless expressly stated otherwise, each feature disclosed is only an example of a generic series of equivalent or similar features.

From the above description, one skilled in the art can easily ascertain the essential
10 characteristics of the present invention, and without departing from the spirit and scope thereof,

can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, other embodiments are also within the claims.

EQUIVALENTS

5 While several inventive embodiments have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other means and/or structures for performing the function and/or obtaining the results and/or one or more of the advantages described herein, and each of such variations and/or modifications is deemed to be within the scope of the inventive embodiments described herein. More generally, those skilled in the art
10 will readily appreciate that all parameters, dimensions, materials, and configurations described herein are meant to be exemplary and that the actual parameters, dimensions, materials, and/or configurations will depend upon the specific application or applications for which the inventive teachings is/are used. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific inventive embodiments
15 described herein. It is, therefore, to be understood that the foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and equivalents thereto, inventive embodiments may be practiced otherwise than as specifically described and claimed. Inventive embodiments of the present disclosure are directed to each individual feature, system, article, material, kit, and/or method described herein. In addition,
20 any combination of two or more such features, systems, articles, materials, kits, and/or methods, if such features, systems, articles, materials, kits, and/or methods are not mutually inconsistent, is included within the inventive scope of the present disclosure.

 All definitions, as defined and used herein, should be understood to control over dictionary definitions, definitions in documents incorporated by reference, and/or ordinary
25 meanings of the defined terms.

 All references, patents and patent applications disclosed herein are incorporated by reference with respect to the subject matter for which each is cited, which in some cases may encompass the entirety of the document.

 The indefinite articles “a” and “an,” as used herein in the specification and in the
30 claims, unless clearly indicated to the contrary, should be understood to mean “at least one.”

 The phrase “and/or,” as used herein in the specification and in the claims, should be understood to mean “either or both” of the elements so conjoined, *e.g.*, elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with “and/or” should be construed in the same fashion, *e.g.*, “one or more” of the

elements so conjoined. Other elements may optionally be present other than the elements specifically identified by the “and/or” clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to “A and/or B”, when used in conjunction with open-ended language such as “comprising” can refer, in one
5 embodiment, to A only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

As used herein in the specification and in the claims, “or” should be understood to have the same meaning as “and/or” as defined above. For example, when separating items in a list,
10 “or” or “and/or” shall be interpreted as being inclusive, *e.g.*, the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as “only one of” or “exactly one of,” or, when used in the claims, “consisting of,” will refer to the inclusion of exactly one element of a number or list of elements. In general, the term “or” as used herein shall only be
15 interpreted as indicating exclusive alternatives (*e.g.* “one or the other but not both”) when preceded by terms of exclusivity, such as “either,” “one of,” “only one of,” or “exactly one of.” “Consisting essentially of,” when used in the claims, shall have its ordinary meaning as used in the field of patent law.

As used herein in the specification and in the claims, the phrase “at least one,” in
20 reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified
25 within the list of elements to which the phrase “at least one” refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, “at least one of A and B” (or, equivalently, “at least one of A or B,” or, equivalently “at least one of A and/or B”) can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment,
30 to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

What Is Claimed Is:

1. A chimeric antigen receptor (CAR) comprising:
 - (a) an extracellular antigen binding domain;
 - 5 (b) a 4-1BB co-stimulatory domain;
 - (c) an IL-2R β cytoplasmic signaling domain; and
 - (d) a CD3 ζ signaling domain.

- 10 2. The CAR of claim 1, wherein the 4-1BB co-stimulatory signaling domain comprises the amino acid sequence set forth in
 KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL (SEQ ID NO: 1).

- 15 3. The CAR of claim 1 or 2, wherein the IL-2R β cytoplasmic signaling domain comprises the amino acid sequence set forth in
 NCRNTGPWLKKVLKCNTPDPSKFFSQLSSEHGGDVQKWLSSPFPSSSFSPGGLAPE
 ISPLEVLERDKVTQLLPLNTDAYLSLQELQGQDPHTLV (SEQ ID NO: 2).

- 20 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
 NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALH
 MQALPPR (SEQ ID NO: 3).

- 25 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.

- 30 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.

5 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

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).

15

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

20

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 VEGFR1.

30

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.

5 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.

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

15 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 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.

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.

30 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.

5 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.

10 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.

15 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.

20 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.

25 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 .

30 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.

5

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

10

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

15

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.

20

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.

25

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.

30

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.

5 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.

10 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.

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

20 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.

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

- 30 (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. The population of immune cells of claim 50, wherein the genetically engineered immune cells comprise the disrupted endogenous IFN γ or IFN γ R gene.

5 52. The population of immune cells of claim 51, 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. The population of immune cells of any one of claims 50- 52, wherein the genetically engineered cells express the IFN γ antagonist.

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

15 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).

20 56. The population of immune cells of claim 55, wherein the IFN γ antagonist is an anti-IFN γ scFv.

25 57. 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.

30 58. The population of immune cells of claim 57, 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.

5 60. The population of immune cells of any one of claims 50-59, further comprises a chimeric antigen receptor (CAR).

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

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

15 62. The population of immune cells of claim 61, 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 VEGFR11.

20

64. The population of immune cells of claim 63, wherein the tumor associated antigen is CD19 and the extracellular antigen binding domain comprises a scFv that binds CD19.

25

65. The population of immune cells of claim 64, wherein the scFv that binds CD19 comprises the amino acid sequence of SEQ. ID. NO: 6, 39, 40, or 41.

30 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.

5 68. 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.

10 69. The population of immune cells of any one of claims 61-68, wherein the cytoplasmic signaling domain is a CD3zeta (CD3 ζ) signaling domain, an interleukin 2 receptor beta subunit (IL-2R β) cytoplasmic signaling domain, or a combination thereof.

15 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.

20 71. 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).

25 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.

30 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.

75. The population of immune cells of claim 74, 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).

5

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.

10

77. The population of immune cells of any one of claims 50-76, 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.

15

78. The population of immune cells of any one of claims 50-77, 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

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.

25

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

82. A pharmaceutical composition comprising the population of immune cells of any one of claims 50-81 and a pharmaceutically acceptable carrier.

30

83. 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 of any one of claims 50-81 or the pharmaceutical composition of claim 82.

5

84. The method of claim 83, 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 VEGFR1L.

10

85. The method of claim 84, wherein the cancer is a solid tumor cancer.

15

86. The method of claim 85, 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.

20

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.

25

89. The method of claim 88, 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.

30

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.

5 91. The method of any one of claims 83-90, wherein prior to the cell therapy, the subject received a lymphodepleting treatment to condition the subject for the cell therapy.

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

10

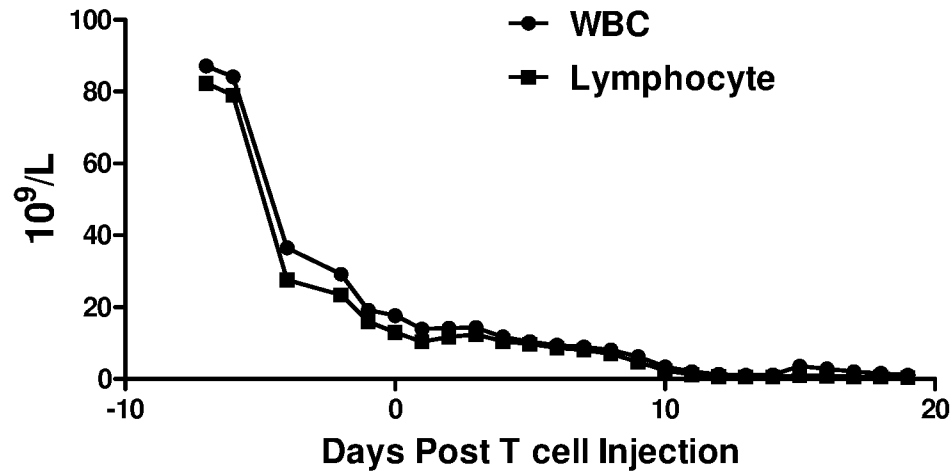


FIG. 1A

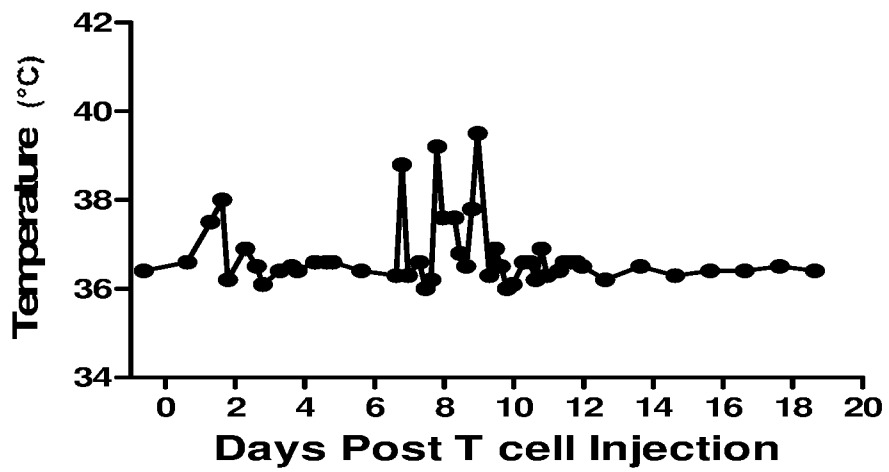


FIG. 1B

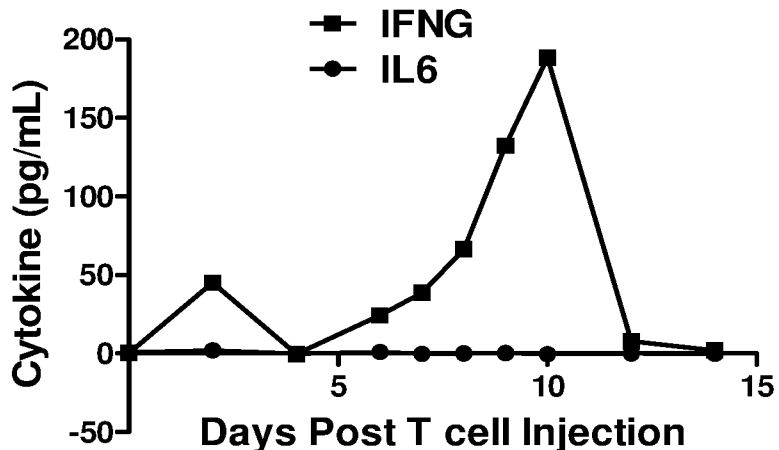


FIG. 1C

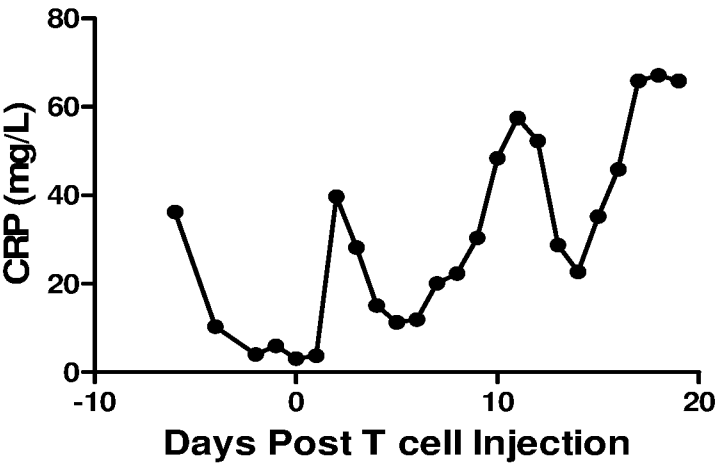


FIG. 1D

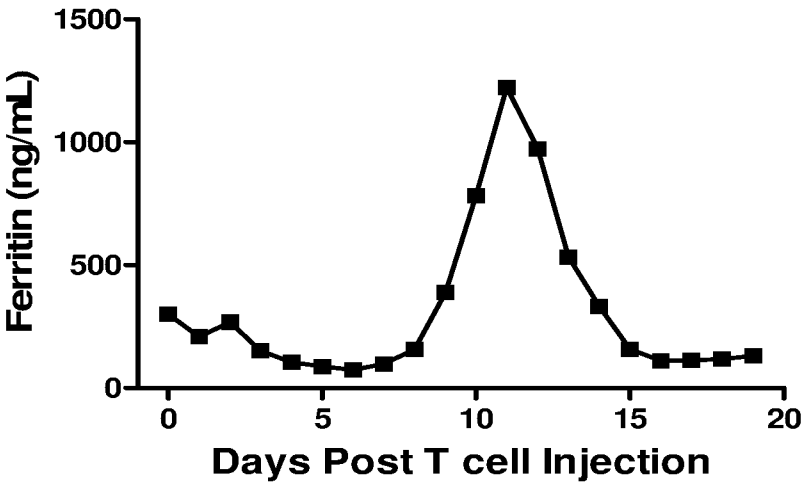


FIG. 1E

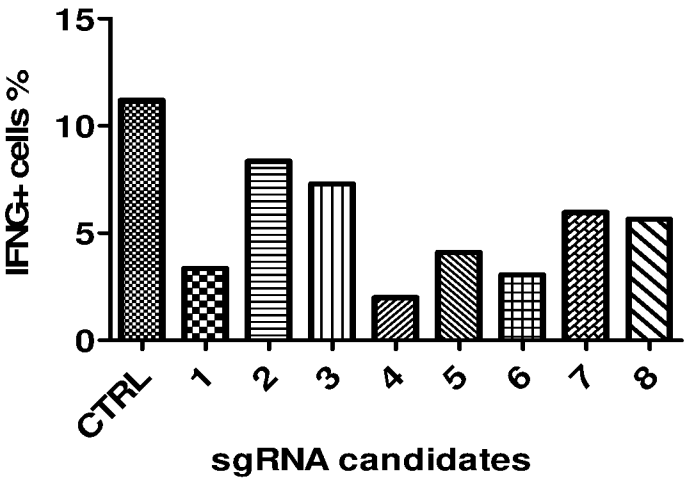


FIG. 2

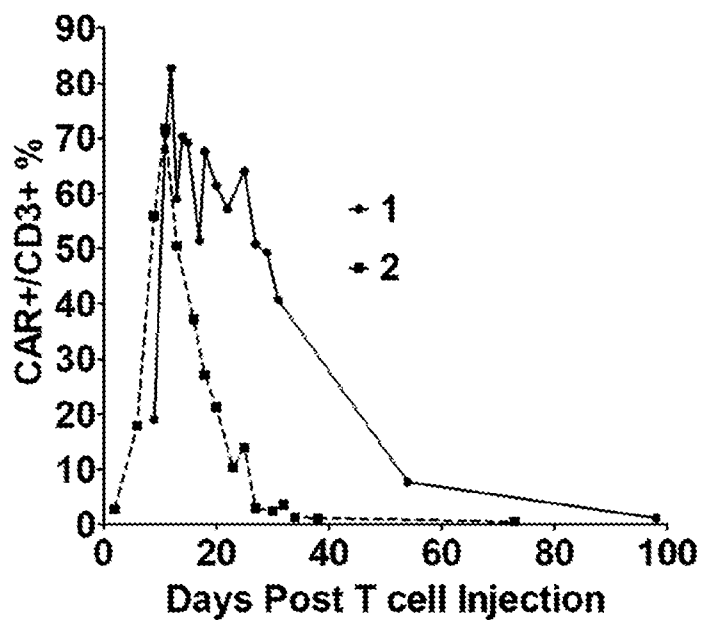


FIG. 3

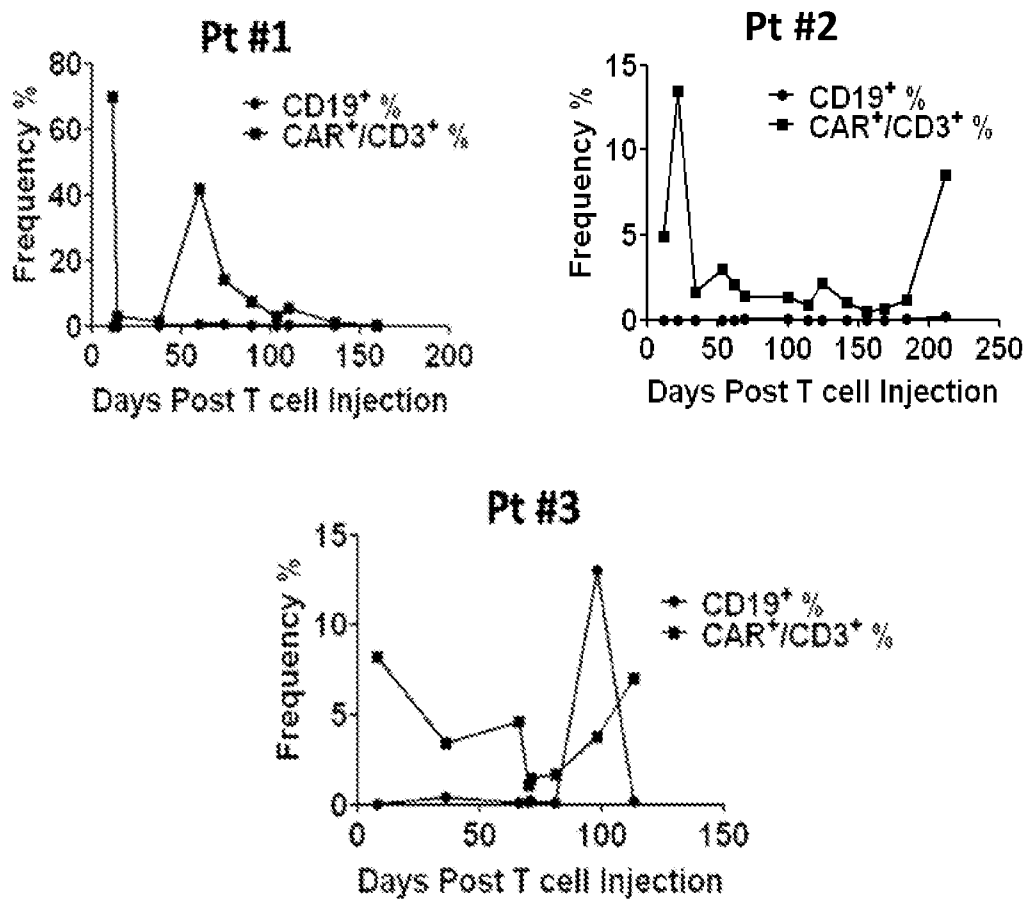


FIG. 4

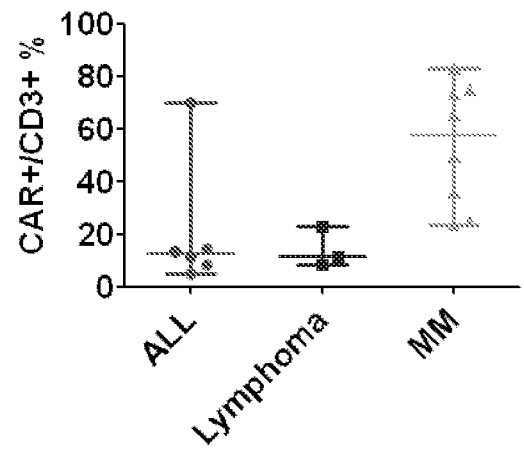


FIG. 5

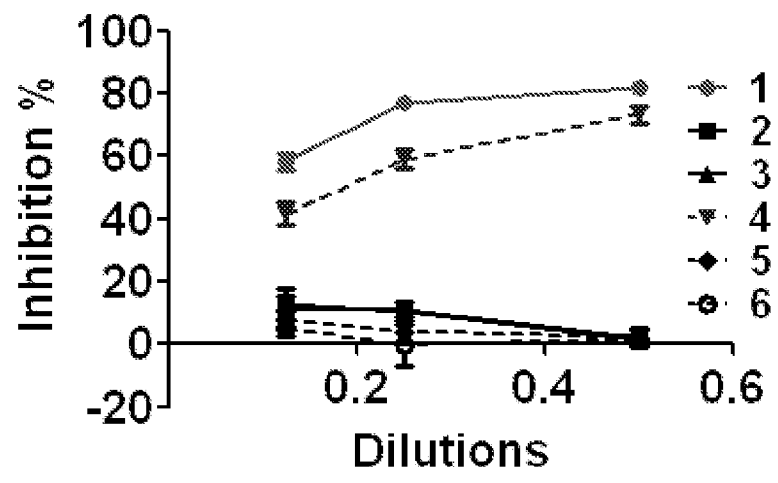


FIG. 6A

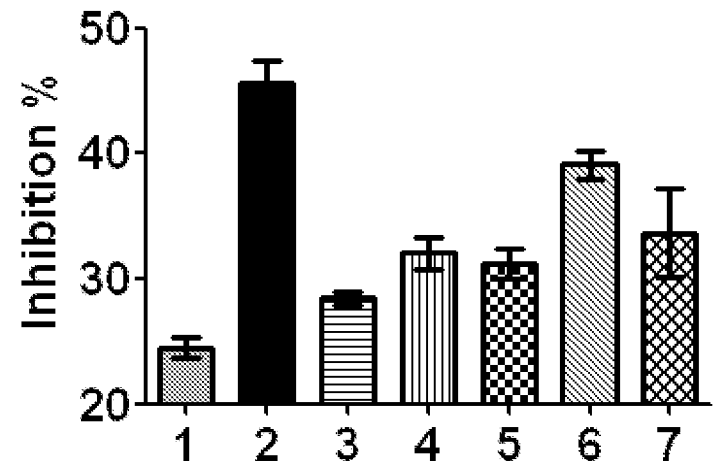


FIG. 6B

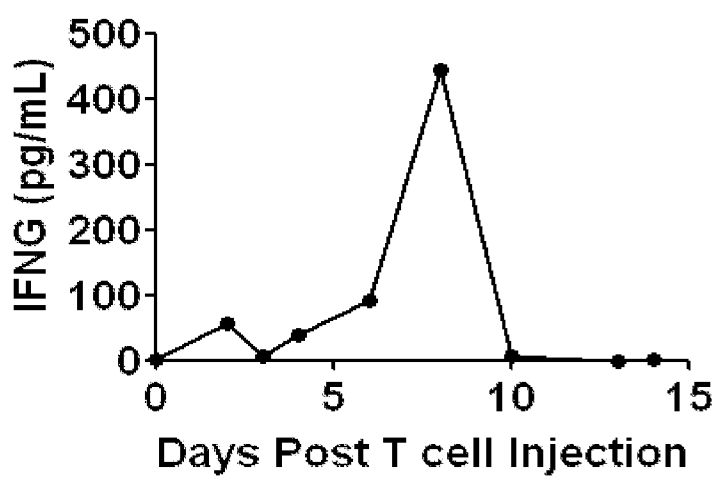


FIG. 7

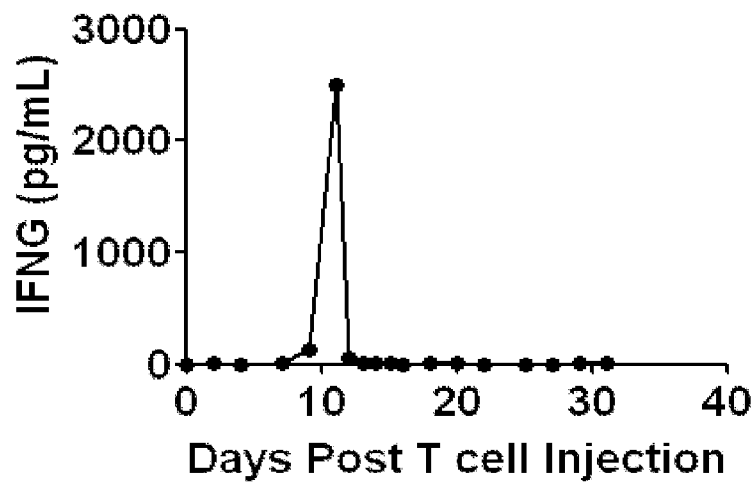


FIG. 8A

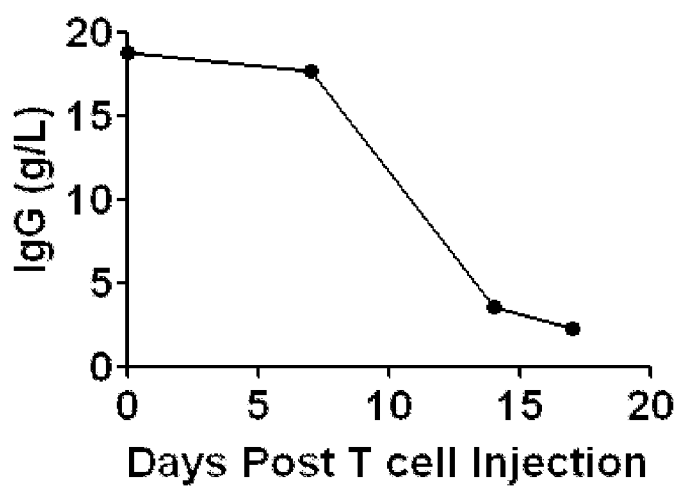


FIG. 8B

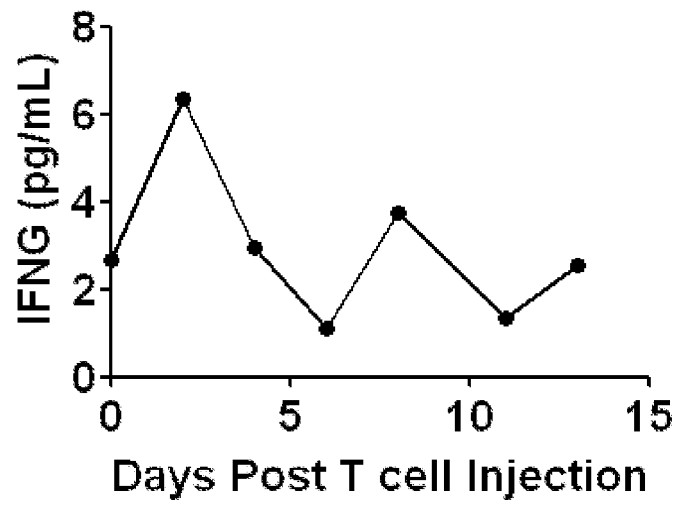


FIG. 9

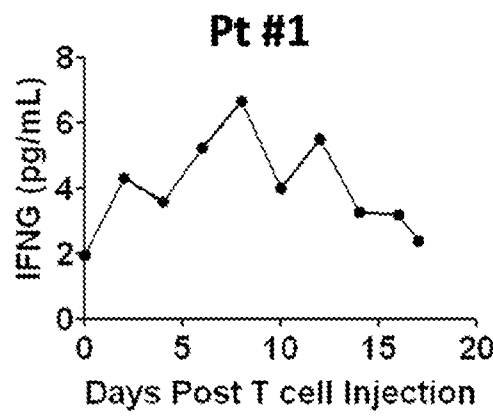


FIG. 10A

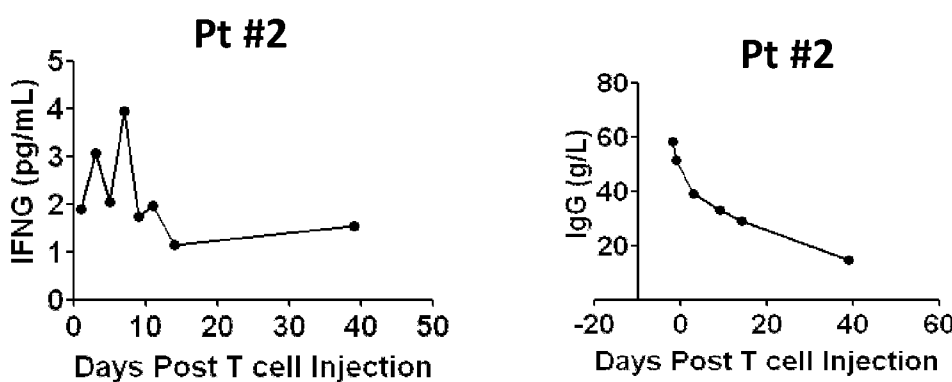


FIG. 10B

SEQUENCE LISTING

<110> Celledit LLC

<120> GENETICALLY MODIFIED IMMUNE CELLS EXPRESSING A CHIMERIC ANTIGEN RECEPTOR AND HAVING REDUCED PROINFLAMMATORY CYTOKINE SIGNALING

<130> 112126-0025-70003W000

<140> Not Yet Assigned

<141> Concurrently Herewith

<150> US 63/005,684

<151> 2020-04-06

<160> 60

<170> PatentIn version 3.5

<210> 1

<211> 42

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic

<400> 1

Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met
1 5 10 15

Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe
20 25 30

Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu
35 40

<210> 2

<211> 94

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic

<400> 2

Asn Cys Arg Asn Thr Gly Pro Trp Leu Lys Lys Val Leu Lys Cys Asn
1 5 10 15

Thr Pro Asp Pro Ser Lys Phe Phe Ser Gln Leu Ser Ser Glu His Gly
20 25 30

Gly Asp Val Gln Lys Trp Leu Ser Ser Pro Phe Pro Ser Ser Ser Phe
35 40 45

Ser Pro Gly Gly Leu Ala Pro Glu Ile Ser Pro Leu Glu Val Leu Glu
50 55 60

Arg Asp Lys Val Thr Gln Leu Leu Pro Leu Asn Thr Asp Ala Tyr Leu

65 70 75 80

Ser Leu Gln Glu Leu Gln Gly Gln Asp Pro Thr His Leu Val
85 90

<210> 3
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 3

Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Lys Gln Gly
1 5 10 15

Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr
20 25 30

Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys
35 40 45

Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys
50 55 60

Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg
65 70 75 80

Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala
85 90 95

Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
100 105 110

<210> 4
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 4

Tyr Arg His Gln
1

<210> 5
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 5

Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Lys Gln Gly
1 5 10 15

Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr
20 25 30

Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys
35 40 45

Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys
50 55 60

Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg
65 70 75 80

Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala
85 90 95

Thr Lys Asp Thr Tyr Asp Ala Tyr Arg His Gln Ala Leu Pro Pro Arg
100 105 110

<210> 6
<211> 242
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 6

Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu Gly
1 5 10 15

Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile Ser Lys Tyr
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Ile
35 40 45

Tyr His Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu Gln
65 70 75 80

Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Tyr
85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr Gly Gly Gly Gly Ser
100 105 110

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Val Lys Leu Gln Glu
115 120 125

Ser Gly Pro Gly Leu Val Ala Pro Ser Gln Ser Leu Ser Val Thr Cys
130 135 140

Thr Val Ser Gly Val Ser Leu Pro Asp Tyr Gly Val Ser Trp Ile Arg
145 150 155 160

Gln Pro Pro Arg Lys Gly Leu Glu Trp Leu Gly Val Ile Trp Gly Ser
165 170 175

Glu Thr Thr Tyr Tyr Asn Ser Ala Leu Lys Ser Arg Leu Thr Ile Ile
180 185 190

Lys Asp Asn Ser Lys Ser Gln Val Phe Leu Lys Met Asn Ser Leu Gln
195 200 205

Thr Asp Asp Thr Ala Ile Tyr Tyr Cys Ala Lys His Tyr Tyr Tyr Gly
210 215 220

Gly Ser Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val
225 230 235 240

Ser Ser

<210> 7
<211> 246
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 7

Asp Ile Val Leu Thr Gln Ser Pro Pro Ser Leu Ala Met Ser Leu Gly
1 5 10 15

Lys Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Thr Ile Leu
20 25 30

Gly Ser His Leu Ile His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
35 40 45

Thr Leu Leu Ile Gln Leu Ala Ser Asn Val Gln Thr Gly Val Pro Ala
50 55 60

Arg Phe Ser Gly Ser Gly Ser Arg Thr Asp Phe Thr Leu Thr Ile Asp
65 70 75 80

Pro Val Glu Glu Asp Asp Val Ala Val Tyr Tyr Cys Leu Gln Ser Arg
85 90 95

Thr Ile Pro Arg Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Gly
100 105 110

Ser Thr Ser Gly Ser Gly Lys Pro Gly Ser Gly Glu Gly Ser Thr Lys
115 120 125

Gly Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly
130 135 140

Glu Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp
145 150 155 160

Tyr Ser Ile Asn Trp Val Lys Arg Ala Pro Gly Lys Gly Leu Lys Trp
165 170 175

Met Gly Trp Ile Asn Thr Glu Thr Arg Glu Pro Ala Tyr Ala Tyr Asp
180 185 190

Phe Arg Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala
195 200 205

Tyr Leu Gln Ile Asn Asn Leu Lys Tyr Glu Asp Thr Ala Thr Tyr Phe
210 215 220

Cys Ala Leu Asp Tyr Ser Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr
225 230 235 240

Ser Val Thr Val Ser Ser
245

<210> 8
<211> 238
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 8

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Trp
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Gly Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Ser Tyr Tyr Cys Gln Gln Ala Asn Ser Phe Pro Tyr

85

90

95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Ser
 100 105 110

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Val Glu
 115 120 125

Ser Gly Gly Gly Leu Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys
 130 135 140

Ala Ala Ser Arg Phe Thr Phe Asp Asp Tyr Ala Met His Trp Val Arg
 145 150 155 160

Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Gly Ile Ser Trp Asn
 165 170 175

Ser Gly Arg Ile Gly Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile
 180 185 190

Ser Arg Asp Asn Ala Glu Asn Ser Leu Phe Leu Gln Met Asn Gly Leu
 195 200 205

Arg Ala Glu Asp Thr Ala Leu Tyr Tyr Cys Ala Lys Gly Arg Asp Ser
 210 215 220

Phe Asp Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser Ser
 225 230 235

<210> 9
 <211> 240
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic

<400> 9

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Ser Ala Ser Ile Ser Val Ser Tyr Met
 20 25 30

Tyr Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr
 35 40 45

Asp Met Ser Asn Leu Ala Ser Gly Ile Pro Ala Arg Phe Ser Gly Ser
 50 55 60

Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu
 65 70 75 80

Asp Phe Ala Val Tyr Tyr Cys Met Gln Trp Ser Gly Tyr Pro Tyr Thr
85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Gly Gly Gly Gly Ser Gly
100 105 110

Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Val Glu Ser
115 120 125

Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala
130 135 140

Ala Ser Gly Phe Thr Phe Ser Pro Phe Ala Met Ser Trp Val Arg Gln
145 150 155 160

Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Lys Ile Ser Pro Gly Gly
165 170 175

Ser Trp Thr Tyr Tyr Ser Asp Thr Val Thr Gly Arg Phe Thr Ile Ser
180 185 190

Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg
195 200 205

Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Gln Leu Trp Gly Tyr
210 215 220

Tyr Ala Leu Asp Ile Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
225 230 235 240

<210> 10
<211> 45
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 10

Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala
1 5 10 15

Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly
20 25 30

Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp
35 40 45

<210> 11
<211> 24
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 11

Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu Leu Leu
1 5 10 15

Ser Leu Val Ile Thr Leu Tyr Cys
20

<210> 12

<211> 559

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic

<400> 12

Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu Gly
1 5 10 15

Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile Ser Lys Tyr
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Ile
35 40 45

Tyr His Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu Gln
65 70 75 80

Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Tyr
85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr Gly Gly Gly Gly Ser
100 105 110

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Val Lys Leu Gln Glu
115 120 125

Ser Gly Pro Gly Leu Val Ala Pro Ser Gln Ser Leu Ser Val Thr Cys
130 135 140

Thr Val Ser Gly Val Ser Leu Pro Asp Tyr Gly Val Ser Trp Ile Arg
145 150 155 160

Gln Pro Pro Arg Lys Gly Leu Glu Trp Leu Gly Val Ile Trp Gly Ser
165 170 175

Glu Thr Thr Tyr Tyr Asn Ser Ala Leu Lys Ser Arg Leu Thr Ile Ile
180 185 190

Lys Asp Asn Ser Lys Ser Gln Val Phe Leu Lys Met Asn Ser Leu Gln
195 200 205

Thr Asp Asp Thr Ala Ile Tyr Tyr Cys Ala Lys His Tyr Tyr Tyr Gly
210 215 220

Gly Ser Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val
225 230 235 240

Ser Ser Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr
245 250 255

Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala
260 265 270

Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile
275 280 285

Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu Leu Leu Ser
290 295 300

Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg Lys Lys Leu Leu Tyr
305 310 315 320

Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu
325 330 335

Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu
340 345 350

Leu Asn Cys Arg Asn Thr Gly Pro Trp Leu Lys Lys Val Leu Lys Cys
355 360 365

Asn Thr Pro Asp Pro Ser Lys Phe Phe Ser Gln Leu Ser Ser Glu His
370 375 380

Gly Gly Asp Val Gln Lys Trp Leu Ser Ser Pro Phe Pro Ser Ser Ser
385 390 395 400

Phe Ser Pro Gly Gly Leu Ala Pro Glu Ile Ser Pro Leu Glu Val Leu
405 410 415

Glu Arg Asp Lys Val Thr Gln Leu Leu Pro Leu Asn Thr Asp Ala Tyr
420 425 430

Leu Ser Leu Gln Glu Leu Gln Gly Gln Asp Pro Thr His Leu Val Arg
435 440 445

Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Lys Gln Gly Gln
450 455 460

Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp

465		470		475		480									
Val	Leu	Asp	Lys	Arg	Arg	Gly	Arg	Asp	Pro	Glu	Met	Gly	Gly	Lys	Pro
				485					490					495	
Arg	Arg	Lys	Asn	Pro	Gln	Glu	Gly	Leu	Tyr	Asn	Glu	Leu	Gln	Lys	Asp
			500					505					510		
Lys	Met	Ala	Glu	Ala	Tyr	Ser	Glu	Ile	Gly	Met	Lys	Gly	Glu	Arg	Arg
		515					520					525			
Arg	Gly	Lys	Gly	His	Asp	Gly	Leu	Tyr	Gln	Gly	Leu	Ser	Thr	Ala	Thr
	530					535					540				
Lys	Asp	Thr	Tyr	Asp	Ala	Tyr	Arg	His	Gln	Ala	Leu	Pro	Pro	Arg	
545					550					555					
<210> 13															
<211> 563															
<212> PRT															
<213> Artificial Sequence															
<220>															
<223> Synthetic															
<400> 13															
Asp	Ile	Val	Leu	Thr	Gln	Ser	Pro	Pro	Ser	Leu	Ala	Met	Ser	Leu	Gly
1				5					10					15	
Lys	Arg	Ala	Thr	Ile	Ser	Cys	Arg	Ala	Ser	Glu	Ser	Val	Thr	Ile	Leu
			20					25					30		
Gly	Ser	His	Leu	Ile	His	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln	Pro	Pro
		35					40					45			
Thr	Leu	Leu	Ile	Gln	Leu	Ala	Ser	Asn	Val	Gln	Thr	Gly	Val	Pro	Ala
	50					55					60				
Arg	Phe	Ser	Gly	Ser	Gly	Ser	Arg	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Asp
65					70					75					80
Pro	Val	Glu	Glu	Asp	Asp	Val	Ala	Val	Tyr	Tyr	Cys	Leu	Gln	Ser	Arg
				85					90					95	
Thr	Ile	Pro	Arg	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Gly
			100					105					110		
Ser	Thr	Ser	Gly	Ser	Gly	Lys	Pro	Gly	Ser	Gly	Glu	Gly	Ser	Thr	Lys
			115					120				125			
Gly	Gln	Ile	Gln	Leu	Val	Gln	Ser	Gly	Pro	Glu	Leu	Lys	Lys	Pro	Gly
	130					135					140				

Glu Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp
145 150 155 160

Tyr Ser Ile Asn Trp Val Lys Arg Ala Pro Gly Lys Gly Leu Lys Trp
165 170 175

Met Gly Trp Ile Asn Thr Glu Thr Arg Glu Pro Ala Tyr Ala Tyr Asp
180 185 190

Phe Arg Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala
195 200 205

Tyr Leu Gln Ile Asn Asn Leu Lys Tyr Glu Asp Thr Ala Thr Tyr Phe
210 215 220

Cys Ala Leu Asp Tyr Ser Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr
225 230 235 240

Ser Val Thr Val Ser Ser Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr
245 250 255

Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala
260 265 270

Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp Phe
275 280 285

Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val
290 295 300

Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg Lys
305 310 315 320

Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr
325 330 335

Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu
340 345 350

Gly Gly Cys Glu Leu Asn Cys Arg Asn Thr Gly Pro Trp Leu Lys Lys
355 360 365

Val Leu Lys Cys Asn Thr Pro Asp Pro Ser Lys Phe Phe Ser Gln Leu
370 375 380

Ser Ser Glu His Gly Gly Asp Val Gln Lys Trp Leu Ser Ser Pro Phe
385 390 395 400

Pro Ser Ser Ser Phe Ser Pro Gly Gly Leu Ala Pro Glu Ile Ser Pro
405 410 415

Leu Glu Val Leu Glu Arg Asp Lys Val Thr Gln Leu Leu Pro Leu Asn

420

425

430

Thr Asp Ala Tyr Leu Ser Leu Gln Glu Leu Gln Gly Gln Asp Pro Thr
435 440 445

His Leu Val Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr
450 455 460

Lys Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg
465 470 475 480

Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met
485 490 495

Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu
500 505 510

Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys
515 520 525

Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu
530 535 540

Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Tyr Arg His Gln Ala Leu
545 550 555 560

Pro Pro Arg

<210> 14
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 14

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Arg Phe Thr Phe Asp Asp Tyr
20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Gly Ile Ser Trp Asn Ser Gly Arg Ile Gly Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Glu Asn Ser Leu Phe
65 70 75 80

Leu Gln Met Asn Gly Leu Arg Ala Glu Asp Thr Ala Leu Tyr Tyr Cys
85 90 95

Ala Lys Gly Arg Asp Ser Phe Asp Ile Trp Gly Gln Gly Thr Met Val
100 105 110

Thr Val Ser Ser
115

<210> 15
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 15

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Trp
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Gly Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Ser Tyr Tyr Cys Gln Gln Ala Asn Ser Phe Pro Tyr
85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 16
<211> 119
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 16

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Pro Phe
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val

35

40

45

Ala Lys Ile Ser Pro Gly Gly Ser Trp Thr Tyr Tyr Ser Asp Thr Val
 50 55 60

Thr Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg Gln Leu Trp Gly Tyr Tyr Ala Leu Asp Ile Trp Gly Gln Gly
 100 105 110

Thr Thr Val Thr Val Ser Ser
 115

<210> 17
 <211> 106
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic

<400> 17

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Ser Ala Ser Ile Ser Val Ser Tyr Met
 20 25 30

Tyr Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr
 35 40 45

Asp Met Ser Asn Leu Ala Ser Gly Ile Pro Ala Arg Phe Ser Gly Ser
 50 55 60

Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu
 65 70 75 80

Asp Phe Ala Val Tyr Tyr Cys Met Gln Trp Ser Gly Tyr Pro Tyr Thr
 85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
 100 105

<210> 18
 <211> 119
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic

<400> 18

Glu Val Gln Leu Val Glu Ser Gly Gly Lys Leu Leu Lys Pro Gly Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe
20 25 30

Ala Met Ser Trp Phe Arg Gln Ser Pro Glu Lys Arg Leu Glu Trp Val
35 40 45

Ala Glu Ile Ser Ser Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Thr Val
50 55 60

Thr Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Glu Met Ser Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Arg Gly Leu Trp Gly Tyr Tyr Ala Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Ser Val Thr Val Ser Ser
115

<210> 19

<211> 106

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic

<400> 19

Gln Ile Val Leu Ile Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly
1 5 10 15

Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
20 25 30

Tyr Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Arg Leu Leu Ile Tyr
35 40 45

Asp Thr Ser Asn Leu Ala Ser Gly Val Pro Val Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Met Glu Ala Glu
65 70 75 80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Gly Tyr Pro Tyr Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys

100

105

<210> 20
 <211> 119
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic

<400> 20

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
 1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Thr Ser Asp
 20 25 30

His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp
 35 40 45

Ile Gly Tyr Ile Ser Tyr Ser Gly Ile Thr Thr Tyr Asn Pro Ser Leu
 50 55 60

Lys Ser Arg Val Thr Met Leu Arg Asp Thr Ser Lys Asn Gln Phe Ser
 65 70 75 80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg Ser Leu Ala Arg Thr Thr Ala Met Asp Tyr Trp Gly Gln Gly
 100 105 110

Ser Leu Val Thr Val Ser Ser
 115

<210> 21
 <211> 107
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic

<400> 21

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile Ser Ser Tyr
 20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
 35 40 45

Tyr Tyr Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Gly Asn Thr Leu Pro Tyr
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 22
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 22

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Ser Asn Tyr
20 25 30

Tyr Val Thr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Gly Ile Ile Tyr Gly Ser Asp Glu Thr Ala Tyr Ala Thr Ser Ala Ile
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Asp Asp Ser Ser Asp Trp Asp Ala Lys Phe Asn Leu Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 23
<211> 110
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 23

Ala Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Ser Ile Asn Asn Glu
20 25 30

Leu Ser Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Arg Ala Ser Thr Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Gly Tyr Ser Leu Arg Asn
85 90 95

Ile Asp Asn Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 24
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 24

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Phe Asn Asp Tyr
20 25 30

Phe Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Gln Met Arg Asn Lys Asn Tyr Gln Tyr Gly Thr Tyr Tyr Ala Glu
50 55 60

Ser Leu Glu Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Ser
65 70 75 80

Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
85 90 95

Tyr Cys Ala Arg Glu Ser Tyr Tyr Gly Phe Thr Ser Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 25
<211> 107

<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 25

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Gly Ile Ser
20 25 30

Leu Ser Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Asn Ala Asn Asn Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln His Asn Ser Ala Pro Tyr
85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 26
<211> 240
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 26

Gln Ile Val Leu Ile Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly
1 5 10 15

Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
20 25 30

Tyr Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Arg Leu Leu Ile Tyr
35 40 45

Asp Thr Ser Asn Leu Ala Ser Gly Val Pro Val Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Met Glu Ala Glu
65 70 75 80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Gly Tyr Pro Tyr Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Ser Gly
100 105 110

Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Val Glu Ser
115 120 125

Gly Gly Lys Leu Leu Lys Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala
130 135 140

Ala Ser Gly Phe Thr Phe Ser Ser Phe Ala Met Ser Trp Phe Arg Gln
145 150 155 160

Ser Pro Glu Lys Arg Leu Glu Trp Val Ala Glu Ile Ser Ser Gly Gly
165 170 175

Ser Tyr Thr Tyr Tyr Pro Asp Thr Val Thr Gly Arg Phe Thr Ile Ser
180 185 190

Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Glu Met Ser Ser Leu Arg
195 200 205

Ser Glu Asp Thr Ala Met Tyr Tyr Cys Ala Arg Gly Leu Trp Gly Tyr
210 215 220

Tyr Ala Leu Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser
225 230 235 240

<210> 27
<211> 246
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 27

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Thr Ser Asp
20 25 30

His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp
35 40 45

Ile Gly Tyr Ile Ser Tyr Ser Gly Ile Thr Thr Tyr Asn Pro Ser Leu
50 55 60

Lys Ser Arg Val Thr Met Leu Arg Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Leu Ala Arg Thr Thr Ala Met Asp Tyr Trp Gly Gln Gly
100 105 110

Ser Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Arg Ala
115 120 125

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr
130 135 140

Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile
145 150 155 160

Thr Cys Arg Ala Ser Gln Asp Ile Ser Ser Tyr Leu Asn Trp Tyr Gln
165 170 175

Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Tyr Thr Ser Arg
180 185 190

Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr
195 200 205

Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr
210 215 220

Tyr Tyr Cys Gln Gln Gly Asn Thr Leu Pro Tyr Thr Phe Gly Gln Gly
225 230 235 240

Thr Lys Val Glu Ile Lys
245

<210> 28
<211> 159
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 28

Met Ala Leu Glu Thr Ile Cys Arg Pro Ser Gly Arg Lys Ser Ser Lys
1 5 10 15

Met Gln Ala Phe Arg Ile Trp Asp Val Asn Gln Lys Thr Phe Tyr Leu
20 25 30

Arg Asn Asn Gln Leu Val Ala Gly Tyr Leu Gln Gly Pro Asn Val Asn
35 40 45

Leu Glu Glu Lys Ile Asp Val Val Pro Ile Glu Pro His Ala Leu Phe
50 55 60

Leu Gly Ile His Gly Gly Lys Met Cys Leu Ser Cys Val Lys Ser Gly

65		70		75		80									
Asp	Glu	Thr	Arg	Leu	Gln	Leu	Glu	Ala	Val	Asn	Ile	Thr	Asp	Leu	Ser
			85						90					95	
Glu	Asn	Arg	Lys	Gln	Asp	Lys	Arg	Phe	Ala	Phe	Ile	Arg	Ser	Asp	Ser
			100					105					110		
Gly	Pro	Thr	Thr	Ser	Phe	Glu	Ser	Ala	Ala	Cys	Pro	Gly	Trp	Phe	Leu
		115					120					125			
Cys	Thr	Ala	Met	Glu	Ala	Asp	Gln	Pro	Val	Ser	Leu	Thr	Asn	Met	Pro
	130					135					140				
Asp	Glu	Gly	Val	Met	Val	Thr	Lys	Phe	Tyr	Phe	Gln	Glu	Asp	Glu	
145					150					155					

<210> 29
 <211> 26
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> Synthetic

 <400> 29

Met	Ala	Thr	Gly	Ser	Arg	Thr	Ser	Leu	Leu	Leu	Ala	Phe	Gly	Leu	Leu
1				5					10					15	

Cys Leu Pro Trp Leu Gln Glu Gly Ser Ala
 20 25

<210> 30
 <211> 178
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> Synthetic

 <400> 30

Met	Ala	Thr	Gly	Ser	Arg	Thr	Ser	Leu	Leu	Leu	Ala	Phe	Gly	Leu	Leu
1				5					10					15	

Cys Leu Pro Trp Leu Gln Glu Gly Ser Ala Arg Pro Ser Gly Arg Lys
 20 25 30

Ser Ser Lys Met Gln Ala Phe Arg Ile Trp Asp Val Asn Gln Lys Thr
 35 40 45

Phe Tyr Leu Arg Asn Asn Gln Leu Val Ala Gly Tyr Leu Gln Gly Pro
 50 55 60

Asn Val Asn Leu Glu Glu Lys Ile Asp Val Val Pro Ile Glu Pro His
 65 70 75 80

Ala Leu Phe Leu Gly Ile His Gly Gly Lys Met Cys Leu Ser Cys Val
85 90 95

Lys Ser Gly Asp Glu Thr Arg Leu Gln Leu Glu Ala Val Asn Ile Thr
100 105 110

Asp Leu Ser Glu Asn Arg Lys Gln Asp Lys Arg Phe Ala Phe Ile Arg
115 120 125

Ser Asp Ser Gly Pro Thr Thr Ser Phe Glu Ser Ala Ala Cys Pro Gly
130 135 140

Trp Phe Leu Cys Thr Ala Met Glu Ala Asp Gln Pro Val Ser Leu Thr
145 150 155 160

Asn Met Pro Asp Glu Gly Val Met Val Thr Lys Phe Tyr Phe Gln Glu
165 170 175

Asp Glu

<210> 31
<211> 100
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 31
gaaatataca agttatatct gtttttagagc tagaaatagc aagttaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgctttt 100

<210> 32
<211> 100
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 32
gtttcagctc tgcacgcttt gtttttagagc tagaaatagc aagttaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgctttt 100

<210> 33
<211> 100
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 33
gttcagctct gcatcgcttt gtttttagagc tagaaatagc aagttaaaat aaggctagtc 60

cgttatcaac ttgaaaaagt ggcaccgagt cggtgctttt 100

<210> 34
<211> 99
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 34
gcatcgtttt gggttctctg ttttagagct agaaatagca agttaaata aggctagtcc 60
gttatcaact tgaaaaagtg gcaccgagtc ggtgctttt 99

<210> 35
<211> 100
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 35
gtctcttggc tgttactgcc gtttagagc tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgctttt 100

<210> 36
<211> 100
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 36
gttcttttac atatgggtcc gtttagagc tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgctttt 100

<210> 37
<211> 100
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 37
gttctgcttc tttacatat gtttagagc tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgctttt 100

<210> 38
<211> 100
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 38
gtttctgctt cttttacata gtttagagc tagaaatagc aagttaaat aaggctagtc 60

cgttatcaac ttgaaaaagt ggcaccgagt cgggtgctttt

100

<210> 39

<400> 39
000

<210> 40

<400> 40
000

<210> 41

<400> 41
000

<210> 42

<400> 42
000

<210> 43

<400> 43
000

<210> 44

<400> 44
000

<210> 45

<400> 45
000

<210> 46

<400> 46
000

<210> 47

<400> 47
000

<210> 48

<400> 48
000

<210> 49

<400> 49
000

<210> 50

<400> 50
000

<210> 51

<400> 51
000

<210> 52
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 52

Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Glu Asn Val Asp Thr Tyr
20 25 30

Val Ser Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Gly Ala Ser Asn Arg Tyr Thr Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Asp Asp Phe Ala Thr Tyr Tyr Cys Gly Gln Ser Tyr Asn Tyr Pro Phe
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Val Lys Arg
100 105

<210> 53
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 53

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Leu Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ile Phe Thr Ser Ser
20 25 30

Trp Ile Asn Trp Val Lys Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Arg Ile Asp Pro Ser Asp Gly Glu Val His Tyr Asn Gln Asp Phe
50 55 60

Lys Asp Lys Ala Thr Leu Thr Val Asp Lys Ser Thr Asn Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys

85

90

95

Ala Arg Gly Phe Leu Pro Trp Phe Ala Asp Trp Gly Gln Gly Thr Leu
 100 105 110

Val Thr Val Ser Ser
 115

<210> 54
 <211> 240
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic

<400> 54

Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Glu Asn Val Asp Thr Tyr
 20 25 30

Val Ser Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
 35 40 45

Tyr Gly Ala Ser Asn Arg Tyr Thr Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
 65 70 75 80

Asp Asp Phe Ala Thr Tyr Tyr Cys Gly Gln Ser Tyr Asn Tyr Pro Phe
 85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Val Lys Arg Gly Gly Gly Gly
 100 105 110

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val
 115 120 125

Gln Ser Gly Ala Glu Leu Lys Lys Pro Gly Ser Ser Val Lys Val Ser
 130 135 140

Cys Lys Ala Ser Gly Tyr Ile Phe Thr Ser Ser Trp Ile Asn Trp Val
 145 150 155 160

Lys Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile Gly Arg Ile Asp Pro
 165 170 175

Ser Asp Gly Glu Val His Tyr Asn Gln Asp Phe Lys Asp Lys Ala Thr
 180 185 190

Leu Thr Val Asp Lys Ser Thr Asn Thr Ala Tyr Met Glu Leu Ser Ser
195 200 205

Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Gly Phe Leu
210 215 220

Pro Trp Phe Ala Asp Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
225 230 235 240

<210> 55
<211> 111
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 55

Asn Phe Met Leu Thr Gln Pro His Ser Val Ser Glu Ser Pro Gly Lys
1 5 10 15

Thr Val Thr Ile Ser Cys Thr Arg Ser Ser Gly Ser Ile Ala Ser Asn
20 25 30

Tyr Val Gln Trp Tyr Gln Gln Arg Pro Gly Ser Ser Pro Thr Thr Val
35 40 45

Ile Tyr Glu Asp Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Ile Asp Ser Ser Ser Asn Ser Ala Ser Leu Thr Ile Ser Gly
65 70 75 80

Leu Lys Thr Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Gly
85 90 95

Ser Asn Arg Trp Met Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105 110

<210> 56
<211> 123
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 56

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val

35

40

45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Lys Asp Gly Ser Ser Gly Trp Tyr Val Pro His Trp Phe Asp Pro
 100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
 115 120

<210> 57
 <211> 249
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic

<400> 57

Asn Phe Met Leu Thr Gln Pro His Ser Val Ser Glu Ser Pro Gly Lys
 1 5 10 15

Thr Val Thr Ile Ser Cys Thr Arg Ser Ser Gly Ser Ile Ala Ser Asn
 20 25 30

Tyr Val Gln Trp Tyr Gln Gln Arg Pro Gly Ser Ser Pro Thr Thr Val
 35 40 45

Ile Tyr Glu Asp Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60

Gly Ser Ile Asp Ser Ser Ser Asn Ser Ala Ser Leu Thr Ile Ser Gly
 65 70 75 80

Leu Lys Thr Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Gly
 85 90 95

Ser Asn Arg Trp Met Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
 100 105 110

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Val
 115 120 125

Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu
 130 135 140

Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr Ala Met
145 150 155 160

Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Ala
165 170 175

Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val Lys Gly
180 185 190

Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln
195 200 205

Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Lys
210 215 220

Asp Gly Ser Ser Gly Trp Tyr Val Pro His Trp Phe Asp Pro Trp Gly
225 230 235 240

Gln Gly Thr Leu Val Thr Val Ser Ser
245

<210> 58
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 58

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Arg Ser Gly Gly Ser Ser
85 90 95

Phe Thr Phe Gly Pro Gly Thr Lys Val Asp Ile Lys
100 105

<210> 59
<211> 117
<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic

<400> 59

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
1 5 10 15

Ser Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Asn Phe Thr Ser Tyr
20 25 30

Trp Ile Gly Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Leu Met
35 40 45

Gly Ile Ile Tyr Pro Gly Asp Ser Asp Thr Arg Tyr Ser Pro Ser Phe
50 55 60

Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr
65 70 75 80

Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Gly Ser Gly Ser Tyr Phe Tyr Phe Asp Leu Trp Gly Arg Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 60

<211> 240

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic

<400> 60

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Arg Ser Gly Gly Ser Ser
85 90 95

Phe Thr Phe Gly Pro Gly Thr Lys Val Asp Ile Lys Gly Gly Gly Gly
100 105 110

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Val
115 120 125

Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu Ser Leu Lys Ile Ser
130 135 140

Cys Lys Gly Ser Gly Tyr Asn Phe Thr Ser Tyr Trp Ile Gly Trp Val
145 150 155 160

Arg Gln Met Pro Gly Lys Gly Leu Glu Leu Met Gly Ile Ile Tyr Pro
165 170 175

Gly Asp Ser Asp Thr Arg Tyr Ser Pro Ser Phe Gln Gly Gln Val Thr
180 185 190

Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr Leu Gln Trp Ser Ser
195 200 205

Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys Gly Ser Gly Ser Tyr
210 215 220

Phe Tyr Phe Asp Leu Trp Gly Arg Gly Thr Leu Val Thr Val Ser Ser
225 230 235 240