to functionally express an anti-CD70 antigen binding protein e.g. a CD70-binding protein such as an anti-CD70 CAR in addition to the first antigen binding protein e.g. CAR that the cell functionally expresses.

[0008] This approach potentially provides a safer alternative to deep lymphodepletion regimens since CD70-negative immune cells are not expected to be targeted by the CD70 CAR, thus avoiding long-term immunosuppression. Additionally, CD70 is also expressed by malignant cells in a variety of tumors, including renal cell carcinoma, lymphoma and acute myeloid leukemia. Engineering T cells to express CARs against CD70 and a second tumor-specific target, such as CD19 or CD20, either as separate CARs or as a single CAR with dual specificity, may therefore enhance the antitumor efficacy and persistence of CAR T cells and delay rejection by the host immune system. In one aspect, the present disclosure refers to the use of engineered immune cells that comprise or functionally express a CD70 binding protein that comprises a CD70 binding domain and a transmembrane domain in the manufacture of a medicament for treating or preventing a host v. graft rejection or reaction in a patient in need thereof, wherein the CD70 binding protein inhibits proliferation and/or activity of CD70 positive cells in the patient.

[0009] Thus, in one aspect, the present disclosure provides a method of inhibiting proliferation and/or activity of CD70 positive cells in vitro or in a patient, comprising the step of contacting the CD70 positive cells with engineered immune cells that comprise or functionally express a CD70-binding protein comprising an extracellular ligand-binding domain that binds to CD70 (or a CD70 binding domain) and a transmembrane domain. In a related aspect, the present disclosure provides a method of lymphodepletion in a patient in need thereof, comprising the step of administering engineered immune cells to the patient, wherein the engineered immune cells comprise or functionally express a CD70-binding protein comprising an extracellular ligand-binding domain that binds to CD70 (or a CD70 binding domain) and a transmembrane domain, and wherein the engineered immune cells inhibit proliferation and/or activity of CD70 positive cells in the patient. In another aspect, the present disclosure refers to the use of engineered immune cells in the manufacture of a medicament for lymphodepletion in a patient in need thereof, wherein the engineered immune cells comprise or functionally express a CD70 binding protein that comprises a CD70 binding domain and a transmembrane domain, and wherein the engineered immune cells inhibit proliferation and/or activity of CD70 positive cells in the patient. In some examples, the CD70 positive cells are T cells, B cells or NK cells. In some examples, the CD70 positive cells are normal or non-cancerous

lymphocytes. In some examples, the CD70 positive cells are activated lymphocytes. In some examples, the CD70 positive cells are activated T cells.

[0010] In another aspect, the present disclosure provides an engineered immune cell that functionally expresses a protein comprising a first antigen binding domain and a protein comprising a second antigen binding domain, wherein the first antigen binding domain specifically binds a target of interest and the second antigen binding domain specifically binds CD70 (e.g., a CD70-binding protein). In yet another aspect, the present disclosure refers to an engineered immune cell that functionally expresses a protein comprising a first antigen binding domain and a protein comprising a second antigen binding domain, wherein the first antigen binding domain specifically binds a target of interest and the second antigen binding domain specifically binds CD70, wherein optionally the second antigen binding domain comprises an anti-CD70 scFv, an anti-CD70 VHH, an anti-CD70 VH or a receptor for CD70, wherein optionally the receptor for CD70 is CD27 or a CD70-binding fragment thereof. In various examples, the protein comprising the first antigen binding domain is a first protein and the protein comprising the second antigen binding domain is a second protein separate and distinct from the first protein. In various examples, the protein comprising the first antigen binding domain is a first CAR and the protein

comprising the second antigen binding domain is a second CAR, i.e. a CD70 CAR. In various examples, one antigen binding protein e.g. one CAR comprises both the first antigen binding domain and the second antigen binding domain, e.g. the CAR is a bispecific CAR that recognizes both a target of interest and CD70. In other examples, the protein comprising the first antigen binding domain recognizing a target of interest and the protein comprising the second antigen binding domain, i.e., the CD70-binding protein, are different proteins.

[0011] In some examples, the target of interest of the first antigen binding domain, e.g. the target of interest of the first CAR, is any molecule of interest, including, for example, without limitation, CD70, BCMA, EGFRvIII, Flt-3, WT-1 , CD20, CD22, CD23, CD30, CD38, CD33, CD133, WT1, TSPAN10, MHC-PRAME, HER2, MSLN, PSMA, PSCA, GPC3, Liv1, ADAM10, CHRNA2, LeY, NKG2D, CS1, CD44v6, ROR1, CD19, Claudin-18.2 (also referred to as Claudin-18A2 or Claudin18 isoform 2), DLL3 (also referred to as Delta-like protein 3, Drosophila Delta homolog 3, Delta3), Muc17, Muc3, Muc16, FAP alpha (Fibroblast Activation Protein alpha), Ly6G6D (also referred to as Lymphocyte antigen 6 complex locus protein G6d, c6orf23, G6D, MEGT1, NG25), or RNF43 (also referred to as E3 ubiquitin-protein ligase RNF43, RING finger protein 43), specifically including the human form of any of the listed exemplary targets. The target of interest can be, for example, any molecule, e.g. any protein, that is expressed on the surface of a cell and is characteristic in some respect of a condition or disease, such as any form of cancer, the inhibition, reduction or elimination of which would be therapeutically beneficial or otherwise desirable.

[0012] In some examples, the protein comprising the second antigen binding domain that specifically binds CD70 (e.g., the CD70-binding protein) is or comprises a CD70 CAR. In some examples, the protein comprising the second antigen binding domain comprises an anti-CD70 scFv, an anti-CD70 VH or a receptor for CD70, or comprises any two or more of these. In some examples, the receptor for CD70 is CD27 or a fragment of CD27 that retains binding specificity for CD70, e.g. a CD70-binding fragment of CD27. In some examples, CD27 has or comprises the amino acid sequence of human CD27 as disclosed in UniProtKB entry P26842. In some examples, the protein comprising the second antigen binding domain is a CAR that comprises a CD3 intracellular signaling domain. In some examples, the protein comprising the second antigen binding domain is a CAR that comprises one or more than one costimulatory domain. In some examples, the protein comprising the second antigen binding domain is a CAR that comprises a CD3 intracellular signaling domain without a costimulatory domain (a first-generation CAR). In some examples, the protein

comprising the second antigen binding domain is a CAR that comprises a CD3 intracellular signaling domain and a costimulatory domain (a second-generation CAR). In some examples, the protein comprising the second antigen binding domain is a CAR that does not comprise a costimulatory domain. In some examples, the protein comprising the second antigen binding domain, e.g., the CD70-binding protein, does not comprise an intracellular signaling domain. In some examples, the protein comprising the second antigen binding domain is a CAR that optionally comprises one or more than one costimulatory domain. In various examples of the protein comprising the second antigen binding domain, the protein further comprises an intracellular signaling domain such as a CD3ζ intracellular signaling domain and/or one or more costimulatory domains. In some examples, the protein comprising the second antigen binding domain comprises full-length CD27 or a CD70-binding fragment of CD27 and further comprises a CD3ζ intracellular signaling domain.

[0013] In various examples, the engineered immune cell functionally expresses one or more of CD70, TRAC and CD52 at a reduced level. In some examples, the reduced level of expression, stated relative to the expression level in a corresponding but non-engineered immune cell, is 0%, for example when both chromosomal copies of a gene are knocked out, or 50% (i.e. 50% of the level in a non-engineered control immune cell), for example when one of the two chromosomal copies of a gene is knocked out and there is no compensatory increase in expression of the other chromosomal copy of that gene. In some examples, the cell expresses one or more of CD70, TRAC and CD52 at a level not greater than 90%, not greater than 75%, not greater than 50%, not greater than 25%, or not greater than 10% of the expression level in a non-engineered immune cell. In some examples, the level of expression of one or more of CD70, TRAC and CD52 in the engineered immune cell is any value between 0% and 90% of the level in a control cell not correspondingly engineered with respect to CD70, TRAC and/or CD52. In some examples, the expression level in the engineered cell is, for example, between 10% and 90%, between 25% and 90%, between 25% and 75%, between 10% and 50%, between 25% and 50%, between 50% and 90%, or between 50% and 75% of the level in a control cell. In some examples, a reduced level of expression other than 0% or 50% is obtained when, for example, only one chromosomal copy of a gene is knocked out and a compensatory mechanism causes an increase in the level of expression of the remaining chromosomal copy, or reduction in expression is achieved by a method other than gene knockout, such as known knockdown methods e.g. those that employ any of various RNA-based techniques (e.g. antisense RNA, miRNA, shRNA, siRNA; *see, e.g.*, Lam *et al.*, Mol.

Ther.-Nucleic Acids 4:e252 (2015), doi:10.1038/mtna.2015.23; Sridharan and Gogtay, Brit. J. Clin. Pharmacol. 82: 659-72 (2016)). In some examples, the engineered immune cell disclosed herein exhibits a reduced level of expression of an MHC class I protein or complex at the cell surface relative to a suitable control. In various examples, the cell is a T cell e.g. a human T cell. In some examples, the cell comprises a mutation in one or more of the CD70, CD52 and TRAC locus or gene and/or a disruption in one or more of the CD70, CD52 and TRAC locus or gene that causes a reduction in functional expression of the disrupted locus or gene. In an example, the mutation or disruption is introduced into one or more of the CD70, CD52 and TRAC genes or loci by any gene mutation or gene editing technique, including but not limited to known homologous recombination techniques and techniques that employ any one or more of meganucleases, TALEN, zinc fingers, shRNA, Cas-CLOVER, and a CRISPR/Cas system. In some examples, the cell is a non-human cell, e.g. a primate cell or a non-primate mammalian cell. In some examples, the cell is a human cell. In some examples, the mutation or disruption is produced by knocking in a nucleic acid e.g. a nucleic acid that encodes one or more proteins or gene products to be expressed in the cell. In some examples, the nucleic acid encodes either or both of the first CAR and the second CAR.

[0014] Provided herein is an engineered immune cell that functionally expresses a protein comprising a first antigen binding domain and a protein comprising a second antigen binding domain, wherein the first antigen binding domain specifically binds a target of interest and the second antigen binding domain specifically binds CD70 (or a CD70-binding protein), and the uses thereof.

[0015] In various examples, the protein comprising the first antigen binding domain is a first CAR and the protein comprising the second antigen binding domain is a second CAR. In some examples, the second antigen binding domain is a CAR e.g. second CAR that comprises one or more than one costimulatory domains. In some examples, the second antigen binding domain is a CAR e.g. second CAR that does not comprise a costimulatory domain. In some examples, the second antigen binding domain is a CAR e.g. second CAR that optionally comprises one or more than one costimulatory domains.

[0016] In various examples, one protein comprises both the first antigen binding domain and the second antigen binding domain. In certain examples, the one protein is a bispecific CAR.

[0017] In certain examples, the target of interest is a protein selected from the group consisting of CD70, BCMA, EGFRvIII, Flt-3, WT-1 , CD20, CD22, CD23, CD30, CD38, CD33, CD133, WT1, TSPAN10, MHC-PRAME, HER2, MSLN, PSMA, PSCA, GPC3, Liv1, ADAM10, CHRNA2, LeY, NKG2D, CS1, CD44v6, ROR1 , CD19, Claudin-18.2 (Claudin-18A2, or Claudin18 isoform 2), DLL3 (Delta-like protein 3, Drosophila Delta homolog 3, Delta3), Muc17, Muc3, Muc16, FAP alpha (Fibroblast Activation Protein alpha), Ly6G6D (Lymphocyte antigen 6 complex locus protein G6d, c6orf23, G6D, MEGT1, NG25), or RNF43 (E3 ubiquitin-protein ligase RNF43, RING finger protein 43). In certain examples, the target of interest is the human form of a protein selected from the group consisting of CD70, BCMA, EGFRvIII, Flt-3, WT-1 , CD20, CD22, CD23, CD30, CD38, CD33, CD133, WT1, TSPAN10, MHC-PRAME, HER2, MSLN, PSMA, PSCA, GPC3, Liv1, ADAM10, CHRNA2, LeY, NKG2D, CS1, CD44v6, ROR1 , CD19, Claudin-18.2 (Claudin-18A2, or Claudin18 isoform 2), DLL3 (Delta-like protein 3, Drosophila Delta homolog 3, Delta3), Muc17, Muc3, Muc16, FAP alpha (Fibroblast Activation Protein alpha), Ly6G6D (Lymphocyte antigen 6 complex locus protein G6d, c6orf23, G6D, MEGT1, NG25), or RNF43 (E3 ubiquitin-protein ligase RNF43, RING finger protein 43).

[0018] In various examples, the engineered immune cell disclosed herein further comprises one or more genomic modifications of one or more of an endogenous TRAC gene, an endogenous CD52 gene, and an endogenous CD70 gene. In certain examples, the cell comprises a knockout at one or both alleles of TRAC, a knockout at one or both alleles of CD52, or a knockout at one or both alleles of CD70, or a knockout at one or both alleles of one or more of TRAC, CD52 and CD70. In various examples, the engineered immune cell expresses one or more of TRAC, CD52 and CD70 at a level not greater than 90%, not greater than 75%, not greater than 50%, not greater than 25%, or not greater than 10% of the expression level in a non-engineered immune cell.

[0019] In various examples, the engineered immune cell disclosed herein is an engineered T cell. In various examples, the engineered immune cell disclosed herein is an engineered NK cell.

[0020] In various examples, the engineered immune cell disclosed herein comprises a first nucleic acid encoding the protein comprising a first antigen binding domain and a second nucleic acid encoding the protein comprising a second antigen binding domain. In certain examples, a first vector comprises the first nucleic acid and a second vector comprises the second nucleic acid. In some examples, one or both vectors are lentiviral vectors. In some

examples, one or both vectors are adeno-associated virus (AAV) vectors. In certain examples, the first nucleic acid and/or the second nucleic acid is located within a disrupted TRAC, CD52 or CD70 locus. In certain examples, one vector comprises both the first nucleic acid and the second nucleic acid. In some examples, the vector is a lentiviral vector or an AAV vector.

[0021] In certain examples, the engineered immune cell comprises one nucleic acid that encodes both the protein comprising a first antigen binding domain and the protein comprising a second antigen binding domain. In some examples, a vector comprises the one nucleic acid. In some examples, the vector is a lentiviral vector or an AAV vector. In certain examples, the one nucleic acid is located within a disrupted TRAC, CD52 or CD70 locus.

[0022] In various examples, the engineered immune cell comprises or further comprises one or more genomic modifications, e.g. a modification of an endogenous genetic locus, for example, of one or both alleles of one or more of the following: an endogenous CD70 gene, an endogenous TCRa gene and an endogenous CD52 gene. In various examples, the one or more genomic modifications cause a reduction or absence of functional expression of the gene that contains the modification. In various examples, the modification comprises a disruption of the locus, e.g. a knockout, knockdown or knock-in. In various examples, the disruption comprises the insertion of a first nucleic acid encoding the protein comprising a first antigen binding domain and/or a second nucleic acid encoding the protein comprising a second antigen binding domain. In various examples, the modification causes a reduction or absence of functional expression of the gene by knockdown, e.g., antisense RNA, siRNA, miRNA, shRNA-mediated knockdown.

[0023] The engineered immune cell provided herein can be derived from or prepared from cells from any of various sources. The engineered immune cell can be prepared or derived from one or more cells e.g. stem cells or immune cells from a person other than the person to whom the engineered immune cells will be administered, e.g. a donor (e.g. a healthy volunteer) other than the recipient, or can be prepared or derived from cells e.g. stem cells or immune cells from the person to whom the engineered immune cells will be administered (the recipient), or can be derived from one or more induced pluripotent stem cells (iPSCs). In various examples, the engineered immune cell disclosed herein is or is derived from an immune cell obtained from a healthy volunteer, is obtained from a patient, or is derived from an iPSC.

[0024] Also provided herein is a population of engineered immune cells as disclosed herein. In various examples, the population comprises between 10^3 and 10^{10} engineered immune cells provided herein, e.g. 10^3, 10^4, 10^5, 10^6, 10^7, 10^8, 10^9, or 10^{10} engineered immune cells, or any range between any two of those values. In another aspect, the present disclosure refers to a population of cells comprising between 10^3 and 10^{10} engineered immune cells. In certain examples, no more than 75%, no more than 50%, or no more than 25% of the engineered immune cells of the population functionally express any one of TRAC, CD52 and CD70 or any two or more of TRAC, CD52 and CD70. In another aspect, the present disclosure refers to a population of engineered immune cells as described herein wherein no more than 75% of the engineered immune cells functionally express one or more of TRAC, CD52 and CD70. In certain examples, no more than 75%, no more than 50%, or no more than 25% of the engineered immune cells of the population functionally express TRAC. In certain examples, no more than 75%, no more than 50%, or no more than 25% of the engineered immune cells of the population functionally express CD52. In certain examples, no more than 75%, no more than 50%, or no more than 25% of the engineered immune cells of the population functionally express TRAC, and no more than 75%, no more than 50%, or no more than 25% of the engineered immune cells of the population functionally express CD52.

[0025] Also provided herein is a population of cells, wherein the population of cells comprises at least 10% engineered immune cells disclosed herein, at least 20% engineered immune cells disclosed herein, at least 30% engineered immune cells disclosed herein, at least 40% engineered immune cells disclosed herein, at least 50% engineered immune cells disclosed herein, at least 75% engineered immune cells disclosed herein, or at least 90% engineered immune cells disclosed herein. In certain examples, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 75%, or at least 90% of the engineered cells comprise one or more genomic modifications of one or more of an endogenous TCRa (or TRAC) gene, an endogenous CD52 gene and an endogenous CD70 gene. In various examples, the population of cells comprises between 10^3 and 10^{10} cells.

[0026] In various examples, the populations of cells and of engineered immune cells disclosed herein are derived from one or more immune cells obtained from a healthy volunteer, from one or more immune cells obtained from a patient, or from one or more induced pluripotent stem cells (iPSCs).

[0027] Also provided herein is a pharmaceutical composition comprising one or more of the engineered immune cells disclosed herein or comprising a population of cells or of engineered immune cells disclosed herein, and further comprising at least one pharmaceutically acceptable carrier or excipient. In another aspect, the present disclosure refers to a pharmaceutical composition comprising one or more of the engineered immune cells as described herein or the population of engineered immune cells as described herein, and further comprising at least one pharmaceutically acceptable carrier or excipient.

[0028] Further provided herein is a method of making the engineered immune cell disclosed herein that comprises one or more genomic modifications of one or more of an endogenous TCRa gene, an endogenous CD52 gene and an endogenous CD70 gene, the method comprising the use of one or more gene editing technologies selected from the group consisting of TALENs, zinc fingers, Cas-CLOVER, and a CRISPR/Cas system and/or the use of any known gene knockdown methods e.g. those that employ any of various RNA-based techniques (e.g. shRNA, antisense RNA, miRNA, siRNA; *see, e.g.*, Lam *et al.*, Mol. Ther.-Nucleic Acids 4:e252 (2015), doi:10.1038/mtna.2015.23; Sridharan and Gogtay, Brit. J. Clin. Pharmacol. 82: 659-72 (2016)) to reduce functional expression of one or more than one of TRAC, CD52 and CD70 in an engineered immune cell disclosed herein, and/or functional expression from any other locus. In yet another aspect, the present disclosure refers to a method of making the engineered immune cell as described herein comprising the use of one or more gene editing technologies selected from the group consisting of TALENs, zinc fingers, Cas-CLOVER, and a CRISPR/Cas system to reduce functional expression of one or more of TRAC, CD52 and CD70 in an immune cell.

[0029] In another aspect, the present disclosure refers to a method of making the engineered immune cell as described herein comprising introducing into an immune cell a first nucleic acid encoding the protein comprising a first antigen binding domain and a second nucleic acid encoding the protein comprising a second antigen binding domain. In certain examples, one vector comprises both the first nucleic acid and the second nucleic acid. In various examples, the vector is a lentiviral vector. In various examples, a first vector comprises the first nucleic acid and a second vector comprises the second nucleic acid. In certain examples, either or both of the first vector and the second vector are lentiviral vectors. In various examples, the protein comprising a first antigen binding domain is a CAR and/or the protein comprising a second antigen binding domain is a CD70 CAR. In various examples, the protein comprising a first antigen binding domain and the protein comprising a second antigen binding domain (i.e. CD70 binding domain) are physically separate and distinct proteins. In certain examples, the separate and distinct proteins are expressed from a bicistronic expression cassette, separated by, for example, a P2A or T2A peptide. In some examples, the protein comprising the first antigen binding domain is N-terminal to the protein comprising the CD70 binding domain. In some examples, the protein comprising the CD70 binding domain is N-terminal to the protein comprising the first antigen binding domain. In various examples, the protein comprising a first antigen binding domain and the protein comprising a second antigen binding domain (i.e. CD70 binding domain) are the same protein, e.g. a bispecific CAR.

[0030] Also provided herein is a method of treating a condition or disease in a patient comprising administering to the patient one or more of any one or more of the engineered immune cells disclosed herein, or a population of cells or of engineered immune cells as

disclosed herein, or a composition as disclosed herein. In another aspect, the present disclosure refers to the use of engineered immune cells that comprise or functionally express a CD70 binding protein that comprises a CD70 binding domain and a transmembrane domain, and optionally a separate therapeutic agent in the manufacture of a medicament for treating a disease condition in a patient in need thereof, wherein the engineered immune cells inhibit proliferation and/or activity of CD70 positive cells in the patient. In another aspect, the present disclosure refers to the use of one or more of the engineered immune cells as described herein, the population of engineered immune cells as described herein, or the pharmaceutical composition as described herein in the manufacture of a medicament for treating a condition or disease in a patient. In various examples, the condition or disease is a solid tumor or a hematological tumor. In various examples, the condition or disease is a viral disease, a bacterial disease, a cancer, an inflammatory disease, an immune disease, or an aging-associated disease. In various examples, the condition or disease is selected from the group consisting of gastric cancer, sarcoma, lymphoma (including Non-Hodgkin's lymphoma), leukemia, head and neck cancer, thymic cancer, epithelial cancer, salivary cancer, liver cancer, stomach cancer, thyroid cancer, lung cancer, ovarian cancer, breast cancer, prostate cancer, esophageal cancer, pancreatic cancer, glioma, leukemia, multiple myeloma, renal cell carcinoma, bladder cancer, cervical cancer, choriocarcinoma, colon cancer, oral cancer, skin cancer, and melanoma. In various examples, the patient is a previously treated adult subject with locally advanced or metastatic melanoma, squamous cell head and neck cancer (SCHNC), ovarian carcinoma, sarcoma, or relapsed or refractory classic Hodgkin's Lymphoma (cHL).

[0031] In various examples, the method comprises administering about 10^3 or 10^4 to about 10^9 engineered immune cells disclosed herein per kg body weight, or about 10^5 to about 10^6 cells of engineered immune cells disclosed herein per kg body weight, or between $0.1x10^6$ and $5x10^6$ engineered immune cells disclosed herein per kg body weight.

[0032] In various examples, the cells are administered as a single dose. In various examples, the cells are administered as more than one dose over a period of time.

BRIEF DESCRIPTION OF THE DRAWINGS

[0033] FIG. 1 illustrates the functions of an allogeneic CAR T cell that comprises both a first CAR that can recognize and bind to a tumor antigen and a second CAR, termed "CD70 Dagger" in the drawing, that can recognize and bind to CD70. The first, anti-tumor antigen CAR, causes the recognized tumor cell to lyse. The second, anti-CD70 CAR, a.k.a. CD70 Dagger, causes the lysis of immune cells (e.g. NK and/or T cells) of the patient (recipient of the allogeneic CAR T cell) that respond to and/or attack the allogeneic CAR T cell. The allogeneic CAR T cell thereby persists longer by preventing or limiting the attack by the patient's immune cells.

[0034] FIGs. 2A-2D. Graft donor CD70 CAR T cells are protected from killing by recipient alloreactive T cells. PBMCs from eight recipient donors were co-cultured with irradiated graft donor T cells for 7 days to allow for priming and expansion of alloreactive recipient T cells (RTCs). **FIG. 2A.** Primed alloreactive RTCs were then isolated and co-cultured either with

WHAT IS CLAIMED IS

1. Use of engineered immune cells in the manufacture of a medicament for lymphodepletion in a patient in need thereof, wherein the engineered immune cells comprise or functionally express a CD70 binding protein that comprises a CD70 binding domain and a transmembrane domain, and wherein the engineered immune cells inhibit proliferation and/or activity of CD70 positive cells in the patient.

2. The use of claim 1, wherein the CD70 binding domain comprises a CD70 antibody, or a receptor for CD70 or a CD70 binding fragment thereof, optionally the anti-CD70 antibody is a scFv.

3. The use of any one of the preceding claims, wherein the CD70 binding protein further comprises a hinge domain, optionally the hinge domain comprises a CD8 hinge.

4. The use of any one of the preceding claims, wherein the CD70 binding protein further comprises one or more intracellular signaling domains selected from the group consisting of a CD3ζ signaling domain, a CD3δ signaling domain, a CD3γ signaling domain, a CD3ε signaling domain, a CD28 signaling domain, a CD2 signaling domain, an OX40 signaling domain, and a 4-1BB signaling domain, or a variant thereof.

5. The use of any one of the preceding claims, wherein the CD70 binding protein comprises a CD3ζ or a CD3γ signaling domain and does not comprise a costimulatory domain.

6. The use of any one of claims 1-4, wherein the CD70 binding protein comprises a 4-1BB signaling domain, a CD2 signaling domain, a CD28 signaling domain, or a variant thereof, and does not comprise a CD3ζ signaling domain.

7. The use of any one of claims 1-4, wherein the CD70 binding protein comprises a 4-1BB signaling domain and a CD3ζ signaling domain.

8. The use of any one of claims 1-4, wherein the one or more intracellular domain comprises the amino acid sequence of one or more of SEQ ID NOs: 265, 271- 278, 281-295, 311-337, 580-591, or 616- 617.

9. The use of any one of claims 1-3 wherein the CD70 binding protein does not comprise an intracellular signaling domain.

10. The use of any one of the preceding claims, wherein the CD70 positive cells are normal or non-cancerous or alloreactive lymphocytes in the patient.

11. The use of claim 10, wherein the CD70 positive cells are T cells, B cells, or NK cells, optionally wherein the CD70 positive cells are activated T cells.

12. The use of any one of the preceding claims, wherein the engineered immune cells are peripheral blood mononuclear cells (PBMC), T cells, NK cells, or a mixture thereof, or derived or developed from iPSCs.

13. The use of any one of the preceding claims, wherein the engineered immune cells are autologous or allogeneic to the patient.

14. The use of any one of the preceding claims, wherein the engineered immune cells further comprise one or more genomic modifications of one or more of an endogenous TCRa gene, an endogenous CD52 gene, and an endogenous CD70 gene, wherein the engineered immune cells exhibit reduced level of TCRa, CD52 and/or CD70 protein expression and/or activity as compared to a control immune cell without the one or more genomic modifications.

15. The use of any one of the preceding claims, wherein the patient has or is expected to have a host v. graft rejection.

16. The use of any one of the preceding claims, wherein the patient is in need for a transplant, optionally wherein the transplant is a bone marrow transplant, stem cell transplant, or tissue transplant.

17. The use of any one of claims 1-16, wherein the engineered immune cells further comprise or functionally express an additional antigen binding domain specific for a target of interest, optionally the additional antigen binding domain comprises an antibody that binds to the target of interest.

18. The use of claim 17, wherein one protein comprises the additional antigen binding domain and the CD70 binding protein, and wherein the additional antigen binding

domain comprises an antibody that binds to the target of interest, and optionally the additional antigen binding domain comprises a scFv.

19. The use of claim 18, wherein the one protein is a bispecific CAR, and wherein the engineered immune cells are CAR T cells.

20. The use of claim 17, wherein the additional antigen binding protein is expressed as a separate protein from the CD70 binding protein.

21. The use of claim 20, wherein the separate protein further comprises a transmembrane domain, and an intracellular signaling domain.

22. The use of claim 21, wherein the separate protein comprises an intracellular signaling domain selected from the group consisting of a CD3ζ signaling domain, a CD28 signaling domain and a 4-1BB signaling domain.

23. The use of any one of claims 20-22, wherein the separate protein is a CAR specific for the target of interest, and wherein the engineered immune cells are CAR T cells.

24. The use of claim 19 or 23, wherein the patient is to be further administered a chemotherapy agent before, simultaneously, or following administration of the CAR T cells, and optionally the chemotherapy agent is cyclophosphamide.

25. The use of any one of claims 19, 23 and 24, wherein the patient is not to be administered fludarabine before, simultaneously, or following administration of the CAR T cells.

26. The use of any one of claims 17-25, wherein the target of interest is a target associated with a disease condition.

27. The use of claim 26, wherein the disease condition is cancer or an autoimmune disorder.

28. The use of claim 26 or 27, wherein the target of interest is BCMA, EGFRvIII, Flt-3, WT-1 , CD20, CD22, CD23, CD30, CD38, CD33, CD133, WT1, TSPAN10, MHC-PRAME, HER2, MSLN, PSMA, PSCA, GPC3, Liv1, ADAM10, CHRNA2, LeY, NKG2D, CS1, CD44v6, ROR1 , CD19, Claudin-18.2 (Claudin-18A2, or Claudin18 isoform 2), DLL3 (Delta-like protein 3, Drosophila Delta homolog 3, Delta3), Muc17, Muc3, Muc16, FAP alpha (Fibroblast Activation Protein alpha), Ly6G6D (Lymphocyte antigen 6 complex locus

protein G6d, c6orf23, G6D, MEGT1, NG25), or RNF43 (E3 ubiquitin-protein ligase RNF43, RING finger protein 43).

29. The use of any one of the preceding claims, wherein the engineered immune cells are genetically modified at the CD70 locus to reduce the level of CD70 expression.

30. The use of any one of claims 1-29, wherein the engineered immune cells are not genetically modified at the CD70 locus to reduce the level of CD70 expression.

31. Use of engineered immune cells that comprise or functionally express a CD70 binding protein that comprises a CD70 binding domain and a transmembrane domain, and optionally a separate therapeutic agent in the manufacture of a medicament for treating a disease condition in a patient in need thereof, wherein the engineered immune cells inhibit proliferation and/or activity of CD70 positive cells in the patient.

32. The use of claim 31, wherein the CD70 binding domain comprises a CD70 antibody, or a receptor for CD70 or a CD70 binding fragment thereof, optionally wherein the anti-CD70 antibody is a scFv.

33. The use of any one of claims 31-32, wherein the CD70 positive cells are normal or non-cancerous or alloreactive lymphocytes.

34. The use of claim 33, wherein the normal lymphocytes are T cells, B cell, or NK cells.

35. The use of any one of claims 31-34, wherein the engineered immune cells are PBMCs, T cells, NK cells, or a mixture thereof, or derived or developed from iPSCs.

36. The use of any one of claims 31-35, wherein the engineered immune cells further comprise or functionally express an additional antigen binding domain specific for a target of interest, optionally the antigen binding domain comprises an antibody that binds to the target of interest.

37. The use of claim 36, wherein the additional antigen binding domain comprises an antibody that binds to the target of interest, and optionally the antibody that binds to the target of interest is a scFv.

38. The use of claim 36 or 37, wherein one protein comprises the additional antigen binding domain and the CD70 binding protein.

39. The use of claim 38, wherein the one protein is a bispecific CAR, and the engineered immune cells are CAR T cells.

40. The use of claim 36 or 37, wherein the additional antigen binding domain is expressed as a separate protein from the CD70 binding protein.

41. The use of claim 40, wherein the separate protein is a CAR specific for the target of interest, and the engineered immune cells are CAR T cells.

42. The use of claim 39 or 41, wherein the patient is to be administered a chemotherapy agent before, simultaneously, or following administration of the CAR T cells, and optionally the chemotherapy agent is cyclophosphamide.

43. The use of any one of claims 39, 41 and 42, wherein the patient is not to be administered fludarabine before, simultaneously, or following administration of the CAR T cells.

44. The use of claim 41, wherein the CAR specific for the target of interest further comprises a transmembrane domain, and an intracellular signaling domain.

45. The use of claim 44, wherein the intracellular signaling domain comprises a CD3z signaling domain, a CD28 signaling domain or a 4-1BB signaling domain.

46. The use of any one of claims 36-45, wherein the target of interest is a target associated with a disease condition.

47. The use of any one of claims 36-46, wherein the target of interest is BCMA, EGFRvIII, Flt-3, WT-1 , CD20, CD22, CD23, CD30, CD38, CD33, CD133, WT1, TSPAN10, MHC-PRAME, HER2, MSLN, PSMA, PSCA, GPC3, Liv1, ADAM10, CHRNA2, LeY, NKG2D, CS1, CD44v6, ROR1 , CD19, Claudin-18.2 (Claudin-18A2, or Claudin18 isoform 2), DLL3 (Delta-like protein 3, Drosophila Delta homolog 3, Delta3), Muc17, Muc3, Muc16, FAP alpha (Fibroblast Activation Protein alpha), Ly6G6D (Lymphocyte antigen 6 complex locus protein G6d, c6orf23, G6D, MEGT1, NG25), or RNF43 (E3 ubiquitin-protein ligase RNF43, RING finger protein 43).

48. The use of claim 46, wherein the disease condition is a cancer or an autoimmune disorder.

49. The use of claim 48, wherein the disease condition is cancer and the cancer is selected from the group consisting of gastric cancer, sarcoma, lymphoma (including Non-Hodgkin's lymphoma), leukemia, head and neck cancer, thymic cancer, epithelial cancer, salivary cancer, liver cancer, stomach cancer, thyroid cancer, lung cancer, ovarian cancer, breast cancer, prostate cancer, esophageal cancer, pancreatic cancer, glioma, leukemia, multiple myeloma, renal cell carcinoma, bladder cancer, cervical cancer, choriocarcinoma, colon cancer, oral cancer, skin cancer, and melanoma. In some embodiments, the subject is a previously treated adult subject with locally advanced or metastatic melanoma, squamous cell head and neck cancer (SCHNC), ovarian carcinoma, sarcoma, or relapsed or refractory classic Hodgkin's Lymphoma (cHL).

50. Use of engineered immune cells that comprise or functionally express a CD70 binding protein that comprises a CD70 binding domain and a transmembrane domain in the manufacture of a medicament for treating or preventing a host v. graft rejection or reaction in a patient in need thereof, wherein the CD70 binding protein inhibits proliferation and/or activity of CD70 positive cells in the patient.

51. An engineered immune cell that functionally expresses a protein comprising a first antigen binding domain and a protein comprising a second antigen binding domain, wherein the first antigen binding domain specifically binds a target of interest and the second antigen binding domain specifically binds CD70, wherein optionally the second antigen binding domain comprises an anti-CD70 scFv, an anti-CD70 VHH, an anti-CD70 VH or a receptor for CD70, wherein optionally the receptor for CD70 is CD27 or a CD70-binding fragment thereof.

52. The engineered immune cell of claim 51, wherein the protein comprising the first antigen binding domain is a first CAR and the protein comprising the second antigen binding domain is a second CAR.

53. The engineered immune cell of claim 51, wherein one protein comprises both the first antigen binding domain and the second antigen binding domain, and wherein the one protein is a bispecific CAR.

54. The engineered immune cell of any one of claims 51-53, wherein the target of interest is a protein selected from the group consisting of BCMA, EGFRvIII, Flt-3, WT-1 , CD20, CD22, CD23, CD30, CD38, CD33, CD133, WT1, TSPAN10, MHC-PRAME, HER2, MSLN, PSMA, PSCA, GPC3, Liv1, ADAM10, CHRNA2, LeY, NKG2D, CS1, CD44v6, ROR1, CD19, Claudin-18.2 (Claudin-18A2, or Claudin18 isoform 2), DLL3 (Delta-like protein 3, Drosophila Delta homolog 3, Delta3), Muc17, Muc3, Muc16, FAP alpha (Fibroblast Activation Protein alpha), Ly6G6D (Lymphocyte antigen 6 complex locus protein G6d, c6orf23, G6D, MEGT1, NG25), or RNF43 (E3 ubiquitin-protein ligase RNF43, RING finger protein 43).

55. The engineered immune cell of any one of claims 51-54, wherein the engineered immune cell further comprises one or more genomic modifications of one or more of an endogenous TCRa gene, an endogenous CD52 gene, and an endogenous CD70 gene, optionally wherein the one or more genomic modifications comprise a knockout.

56. The engineered immune cell of any one of claims 51-55, wherein the engineered immune cell is an engineered T cell.

57. The engineered immune cell of any one of claims 51-56, wherein the engineered immune cell comprises a first nucleic acid encoding the protein comprising a first antigen binding domain and a second nucleic acid encoding the protein comprising a second antigen binding domain or wherein a single nucleic acid comprises both the first nucleic acid and the second nucleic acid.

58. The engineered immune cell of claim 57, wherein a first vector comprises the first nucleic acid and a second vector comprises the second nucleic acid, and optionally wherein one or both vectors are lentiviral vectors or adeno-associated virus vectors, or wherein one vector comprises both the first nucleic acid and the second nucleic acid, and optionally wherein the vector is a lentiviral vector or an adeno-associated virus vector.

59. The engineered immune cell of claim 57, wherein the first nucleic acid and/or the second nucleic acid is located within a disrupted TRAC locus, a disrupted CD52 locus or a disrupted CD70 locus or wherein the single nucleic acid is located within a disrupted TRAC locus, a disrupted CD52 locus or a disrupted CD70 locus.

60. The engineered immune cell of any one of claims 51-59, wherein the immune cell is or is derived from an immune cell obtained from a healthy volunteer, is obtained from a patient, or is derived from an iPSC.

61. A population of engineered immune cells of any one of claims 55-60 wherein no more than 75% of the engineered immune cells functionally express one or more of TRAC, CD52 and CD70.

62. A population of cells comprising between 10^3 and 10^{10} engineered immune cells of one or more of claims 51-60.

63. A pharmaceutical composition comprising one or more of the engineered immune cells of any one of claims 51-60 or the population of engineered immune cells of any one of claims 61-62, and further comprising at least one pharmaceutically acceptable carrier or excipient.

64. A method of making the engineered immune cell of any one of claims 55-60 comprising the use of one or more gene editing technologies selected from the group consisting of TALENs, zinc fingers, Cas-CLOVER, and a CRISPR/Cas system to reduce functional expression of one or more of TRAC, CD52 and CD70 in an immune cell.

65. A method of making the engineered immune cell of any one of claims 51-60 comprising introducing into an immune cell a first nucleic acid encoding the protein comprising a first antigen binding domain and a second nucleic acid encoding the protein comprising a second antigen binding domain.

66. The method of claim 65, wherein one vector comprises both the first nucleic acid and the second nucleic acid, optionally wherein the vector is a lentiviral vector or adeno-associated viral vector.

67. The method of claim 65, wherein a first vector comprises the first nucleic acid and a second vector comprises the second nucleic acid, optionally wherein either or both of the first vector and the second vector are lentiviral vectors or either or both of the first vector and the second vector are adeno-associated viral vectors.

68. The method of 67, wherein the first nucleic acid and the second nucleic acid are introduced into the engineered immune cell by site-specific integration and optionally either or both of the first vector and the second vector are adeno-associated viral vectors.

69. Use of one or more of the engineered immune cells of any one of claims 51-60, the population of engineered immune cells of any one of claims 61-62, or the pharmaceutical composition of claim 63 in the manufacture of a medicament for treating a condition or disease in a patient.

70. The use of claim 69, wherein the condition or disease is a viral disease, a bacterial disease, a cancer, an inflammatory disease, an immune disease, an autoimmune disease, or an aging-associated disease.

71. The use of claim 69 or 70, wherein the condition or disease is selected from the group consisting of gastric cancer, sarcoma, lymphoma (including Non-Hodgkin's lymphoma), leukemia, head and neck cancer, thymic cancer, epithelial cancer, salivary cancer, liver cancer, stomach cancer, thyroid cancer, lung cancer, ovarian cancer, breast cancer, prostate cancer, esophageal cancer, pancreatic cancer, glioma, leukemia, multiple myeloma, renal cell carcinoma, bladder cancer, cervical cancer, choriocarcinoma, colon cancer, oral cancer, skin cancer, and melanoma.

72. The use of claim 69, wherein the patient is a previously treated adult subject with locally advanced or metastatic melanoma, squamous cell head and neck cancer (SCHNC), ovarian carcinoma, sarcoma, or relapsed or refractory classic Hodgkin's Lymphoma (cHL).

73. The use of any one of claims 69-72, wherein the engineered immune cells inhibit proliferation and/or activity of CD70 positive lymphocytes of the patient.

74. The use of claim 73, wherein the CD70 positive lymphocytes of the patient are normal or non-cancerous or alloreactive lymphocytes of the patient.