decreasing patient wait time, and increasing accessibility of CAR T therapies to more patients. But allogeneic CAR T cells may be recognized by the host as foreign and rejected by the patient immune system, thereby limiting expansion and persistence of the infused allogeneic CAR T cells. In addition, although CD19 is an effective target for, e.g., non-Hodgkin's lymphoma, a propensity of pathogenic target cells such as tumor cells to down-modulate CD19 post CAR T cell treatment has been observed in preclinical and clinical studies (see Spiegel *et al*., 2021, Nature Medicine 27:1419-1431).

[0006] Thus, there is a need for improved anti-CD19 CAR T cells with greater potency, increased persistence, and/or enhanced functionality.

SUMMARY

[0007] Described herein are engineered immune cells, e.g., engineered T cells, comprising and/or expressing an anti-CD19, or CD19-specific, chimeric antigen receptor (CAR) and a CD70-binding protein and/or a chimeric cytokine receptor (CCR). Further described herein are engineered immune cells comprising one or more polynucleotides that encode one or more recombinant proteins. In some examples, the one or more polynucleotides comprise one or more, at least two, or two or more coding sequences encoding the CD19-specific CAR, the CD70-binding protein, the CCR, and/or other recombinant proteins. In one aspect, there is described a method of making an engineered immune cell comprising introducing to the immune cell an engineered endonuclease that cleaves at a target sequence within a human TRAC gene and the polynucleotide as described herein or the vector as described herein, and culturing the immune cell under suitable conditions.

[0008] In some examples, the engineered immune cells comprise or express a CD19-specific CAR and a CD70-binding protein, or a CD70-specific CAR. In some examples, the CD19-specific CAR comprises the amino acid sequence of SEQ ID NO: 2, 3, 4 or 5, with or without the signal peptide, and the CD70-specific CAR comprises the amino acid sequence of SEQ ID NO: 16, 17, 18, or 19, with or without the signal peptide.

[0009] In some examples, the engineered immune cells comprise a polynucleotide that comprises a coding sequence encoding a CD19-specific CAR and a coding sequence encoding a CD70-specific CAR. In some examples the coding sequence encoding the CD19-specific CAR is linked to the coding sequence encoding the CD70-specific CAR by a nucleotide sequence encoding a P2A peptide.

[0010] Also described herein are polynucleotides comprising a first coding sequence that encodes a CD19-specific CAR and a second coding sequence that encodes a CD170-specific CAR. In some examples the coding sequence encoding the CD19-specific CAR is linked to the coding sequence encoding the CD70-specific CAR by a nucleotide sequence

encoding a P2A peptide. In some examples, the CD19-specific CAR comprises the amino acid sequence of SEQ ID NO: 2, 3, 4 or 5, with or without the signal peptide, and the CD70-specific CAR comprises the amino acid sequence of SEQ ID NO: 16, 17, 18, or 19, with or without the signal peptide.

[0011] In some examples, the polynucleotide comprises a truncated PGK promoter, a first coding sequence and a second coding sequence, wherein the truncated PGK promoter comprises at least 50 nucleotides deletion from the 5' end of a PGK promoter, or a wildtype or long PGK promoter having the nucleotide sequence of SEQ ID NO: 34, wherein the truncated PGK promoter is operably linked to the first coding sequence and the second coding sequence, and wherein either the first or the second coding sequence encodes an anti-CD19 chimeric antigen receptor (CAR). In one aspect, there is described a polynucleotide comprising, from 5' to 3', a truncated PGK promoter, a first coding sequence and a second coding sequence, wherein the truncated PGK promoter comprises at least 50 nucleotides deletion from the 5' end of the long PGK promoter having the nucleotide sequence of SEQ ID NO: 34, wherein the truncated PGK promoter is operably linked to the first coding sequence and the second coding sequence, and wherein either the first or the second coding sequence encodes an anti-CD19 chimeric antigen receptor (CAR). In some examples, the first and second coding sequences are linked by a P2A peptide.

[0012] In some examples, the anti-CD19 CAR comprises the amino acid sequence of SEQ ID NO: 4. In some examples, the anti-CD19 CAR further comprises a CD8 hinge and transmembrane domains. In some examples, the CD8 hinge domain comprises the amino acid sequence of SEQ ID NO: 9. In some examples, the CD8 transmembrane domain comprises the amino acid sequence of SEQ ID NO: 10 or 99. In some examples, the anti-CD19 CAR further comprises a CD3z signaling domain and/or a 4-1BB costimulatory domain. In some examples, the CD3z signaling domain comprises the amino acid sequence of SEQ ID NO: 11. In some examples, the 4-1BB costimulatory domain comprises the amino acid sequence of SEQ ID NO: 12. In some examples, the anti-CD19 CAR comprises the amino acid sequence of SEQ ID NO: 5 or 7, with or without a signal peptide. In some examples, the anti-CD19 CAR comprises the amino acid sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to SEQ ID NO: 5 or 7, with or without a signal peptide. In some examples, the coding sequence that encodes the anti-CD19 CAR comprises a nucleotide

sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to SEQ ID NO: 6 or 8.

[0013] In some examples, the truncated PGK promoter comprises at least 100 nucleotides, at least 150 nucleotides, at least 200 nucleotides, at least 250 nucleotides, at least 300 nucleotides, or at least 350 nucleotides deletion from the 5' end of the long PGK promoter having the nucleotide sequence of SEQ ID NO: 34. In some examples, the truncated PGK promoter comprises a nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the nucleotide sequence of SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, or SEQ ID NO: 38. In some examples, the truncated PGK promoter comprises a nucleotide sequence that comprises (a) about 300, about 310, about 320, about 330, about 340, about 350, about 360, about 370, about 380, or about 390 nucleotides deletion from the 5' end of SEQ ID NO: 34 and having at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the nucleotide sequence of SEQ ID NO: 35; (b) about 210, about 220, about 230 about 240, about 250, about 260, about 270, about 280, or about 290 nucleotides deletion from the 5' end of SEQ ID NO: 34 and having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the nucleotide sequence of SEQ ID NO: 36; (c) about 100, about 110, about 120, about 130, about 140, abut 150, about 160, about 170, about 180, or about 190 nucleotides deletion from the 5' end of SEQ ID NO: 34 and having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the nucleotide sequence of SEQ ID NO: 37; or (d) about 50, about 60, about 70, about 80, about 90 or about 100 nucleotides deletion from the 5' end of SEQ ID NO: 34 and having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the nucleotide sequence of SEQ ID NO: 38. In some examples, the truncated PGK promoter consists essentially of or consists of a nucleotide sequence of SEQ ID NO: 35, 36, 37 or 38. In some examples, the truncated PGK promoter consists essentially of or consists of a nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 98% or at least 99% sequence identity of SEQ ID NO: 35, 36, 37 or 38.

[0014] In some examples, the first coding sequence encodes the anti-CD19 CAR and the second coding sequence encodes a CD70-binding protein. In some examples, the first coding sequence encodes the CD70-binding protein and the second coding sequence

encodes the anti-CD19 CAR. In certain examples, the CD-70 binding protein is or comprises an anti-CD70 CAR.

[0015] In some examples, the first coding sequence encodes the anti-CD19 CAR and the second coding sequence encodes a chimeric cytokine receptor (CCR). In some examples, the first coding sequence encodes the CCR and the second coding sequence encodes the anti-CD19 CAR.

[0016] In some examples, the CD70-binding protein is a recombinant CD70-binding protein. In some embodiment, the CD70-binding protein comprises an anti-CD70 antibody or an antigen-binding fragment thereof, and a transmembrane domain. In some examples, the anti-CD70 antibody comprises the amino acid sequence of SEQ ID NOs: 16 and/or 17. In some examples, the anti-CD70 antibody comprises the amino acid sequence of SEQ ID NO: 18. In some examples, the transmembrane domain comprises the amino acid sequence of SEQ ID NO: 10. In some examples, the CD70-binding protein further comprises a hinge domain. In certain examples, the hinge domain and the transmembrane domain comprise a CD8 hinge and a CD8 transmembrane domain. In some examples, the CD8 hinge domain comprises the amino acid sequence of SEQ ID NO: 9. In some examples, the CD8 transmembrane domain comprises the amino acid sequence of SEQ ID NO: 10 or 99. In other examples, the hinge domain and the transmembrane domain comprise a CD28 hinge and a CD28 transmembrane domain. In some examples, the CD70-binding protein further comprises a CD3 signaling domain. In some examples, the CD70-binding protein does not comprise a costimulatory domain. In some examples, the CD70-binding protein does not comprise a 4-1BB costimulatory domain. In some examples, the CD70-binding protein comprises a CD3 signaling domain comprising the amino acid sequence of SEQ ID NO: 11. In some examples, the CD70-binding protein comprises the amino acid sequence of SEQ ID NO: 19 or 21, or comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95% or at least about 99% sequence identity to SEQ ID NO: 19 or 21, with or without a signal peptide. In some examples, the coding sequence that encodes the CD70-binding protein comprises a nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to SEQ ID NO: 20 or 22.

[0017] In some examples, the CCR comprises a thrombopoietin receptor/ myeloproliferative leukemia protein receptor (TPOR/MPLR) transmembrane and JAK binding domain and an intracellular recruiting domain. In some examples, the TPOR/MPLR transmembrane and JAK binding domain comprises the amino acid sequence of SEQ ID NO: 25 or 51. In some examples, the intracellular recruiting domain comprises the amino acid sequence of SEQ ID NO: 26. In some examples, the CCR comprises the amino acid sequence of SEQ ID NO: 27 or 29, or an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 99% sequence identity to SEQ ID NO: 27 or 29, with or without the signal peptide. In some examples, the coding sequence that encodes a CCR comprises the nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 28 or 30.

[0018] In some examples, the polynucleotides further comprise a 5' homology arm and a 3' homology arm. In some examples, the 5' and 3' homology arms are each at least about 100, at least about 150, at least about 200, at least about 250, or at least about 300 nucleotides in length or more. In some examples, the 5' homology arm and the 3' homology arm each comprises a nucleotide sequence homologous to a nucleotide sequence of a T cell receptor alpha constant region (TRAC) gene.

[0019] In related examples, described herein are vectors comprising the polynucleotides, and engineered immune cells (e.g., engineered T cells or CAR T cells) comprising the vectors or the polynucleotides. In one aspect, described herein is a vector comprising the polynucleotide sequence as described herein. In some examples, the vector is an adeno-associated virus (AAV) vector or a lentiviral vector.

[0020] In some examples, the immune cells comprise the polynucleotides integrated into the TRAC gene. In some examples, the integration of the polynucleotide into the human TRAC gene is mediated by an endonuclease. In some examples, the endonuclease is a CRISPR endonuclease. In some examples, the endonuclease is not TALEN. In some examples, the integration of the polynucleotide into the human TRAC gene is mediated by a CRISPR endonuclease and the integration prevents or reduces the expression or activities of the TRAC gene. In some examples, the CRISPR endonuclease is Cas 9 or Cas12, e.g., Cas12i. In some examples, the CRISPR endonuclease makes a double strand break to create an integration site in a target sequence within the TRAC gene and the polynucleotide is integrated into the integration site by

AAV-mediated site-specific integration, optionally wherein the integration site is in the first, second or third exon of the TRAC gene, and further optionally wherein the target sequence comprises the nucleic acid sequence of GACCCTGCC or SEQ ID NO: 92.

[0021] In some examples, the immune cells further comprise an additional polynucleotide that comprises a promoter operably linked to one or more coding sequences, wherein the one or more coding sequences expresses either a CCR or a CD70-binding protein, as described herein. In some examples, the one or more coding sequences of the additional polynucleotide further express a PD-1 dominant negative receptor. In some examples, the additional polynucleotide further comprises a 5' homology arm and a 3' homology arm. In some examples, the 5' and 3' homology arms each are at least 100 nucleotides in length. In some examples, the 5' and 3' homology arms each comprises a nucleotide sequence homologous to a nucleotide sequence of a CD52 gene. In some examples, the promoter comprises an EFS promoter or a truncated PGK promoter described herein. In some examples, the additional polynucleotide is integrated into the CD52 gene. In some examples, the integration of the additional polynucleotide into the human CD52 gene is mediated by a CRISPR endonuclease. In some examples, the CRISPR endonuclease is Cas 9 or Cas12, e.g., Cas12i, and the integration prevents or reduces the expression or activities of the CD52 gene. In some examples, the CRISPR endonuclease makes a double strand break to create an integration site in a target sequence within the CD52 gene and the polynucleotide is integrated into the integration site by AAV-mediated site-specific integration.

[0022] In some aspect, the disclosure describes an immune cell, or a population of immune cells, comprising a polynucleotide integrated into a human TRAC gene, wherein the polynucleotide comprises a nucleic acid sequence comprising a 5' region of the human TRAC gene, the nucleic acid sequence encoding a CD19-specific CAR and a CD70-binding protein or a CD70-specific CAR, and a 3' region of the human TRAC gene, wherein the integrated polynucleotide prevents or reduces the expression or activities of the human TRAC gene. In some examples, the anti-CD19 CAR comprises the amino acid of SEQ ID NO: 5 or 7, and the CD70-binding protein (or the CD70-specific CAR) comprises the amino acid of SEQ ID NO: 19 or 21. In some examples, the polynucleotide encodes the CD19-specific CAR and the CD70-specific CAR linked by a P2A sequence. In some examples, the integration of the polynucleotide at the human TRAC gene is mediated a CRISPR endonuclease, for example, Cas 9 or Cas12 or Cas12i. In certain examples, the

CRISPR endonuclease makes a double strand break to create an integration site in a target sequence within the TRAC gene and the polynucleotide is integrated into the integration site by AAV-mediated site-specific integration. In some examples, the integration site is in the first, second or third exon of the TRAC gene. In some examples, the integration stie is in the first exon of the TRAC gene. In some examples, the target sequence comprises the nucleic acid sequence of GACCCTGCC or SEQ ID NO: 92.

[0023] In one aspect, the disclosure describes an immune cell comprising the polynucleotide as described herein. In another example, the disclosure describes an immune cell comprising a polynucleotide encoding an anti-CD19 CAR and a CD70-binding protein, wherein the anti-CD19 CAR comprises the amino acid sequence of SEQ ID NO: 5 or 7, and the CD70-binding protein comprises the amino acid sequence of SEQ ID NO: 19 or 21. In certain examples, the polynucleotide encodes a polypeptide of CD19-specific CAR and CD70-specific CAR connected by a P2A peptide that comprises an amino acid sequence that is at least 85%, 88%, 90%, 95%, 98%, or 99% or 100% identical to the polypeptide sequence of SEQ ID NO: 97. In some examples, the polynucleotide comprises a nucleic acid sequence that is at least 85%, 88%, 90%, 95%, 98%, or 99% or 100% identical to the polynucleotide sequence of SEQ ID NO: 96. In certain examples, the polynucleotide is integrated into a human TRAC gene by an endonuclease. In some examples, the endonuclease is a CRISPR endonuclease. In some examples, the endonuclease is not a TALEN endonuclease. In certain examples, the polynucleotide is integrated into a human TRAC gene by a CRISPR endonuclease, wherein the CRISPR endonuclease makes a double strand break to create an integration site in a target sequence within the TRAC gene and the polynucleotide is integrated into the integration site by AAV-mediated site-specific integration. In some examples, the integrated polynucleotide prevents or reduces the expression or activities of the human TRAC gene. In some examples, the integration site is in the first, second or third exon of the TRAC gene. In some examples, the integration stie is in the first exon of the TRAC gene. In some examples, the target sequence comprises the nucleic acid sequence of GACCCTGCC or SEQ ID NO: 92.

[0024] In some examples, the polynucleotide encoding the CD19 CAR and the CD70-binding protein or anti-CD70 CAR is operably linked to a truncated PGK promoter disclosed herein.

[0025] In some examples, the immune cell is a T cell, an NK cell, an NK-T cell, a tumor infiltrating lymphocyte (TIL), or a T cell of NK cell derived from an iPSC.

[0026] In related examples, described are immune cells comprising a recombinant polynucleotide sequence integrated at a human TRAC gene, wherein the recombinant polynucleotide sequence comprises a 5' region of the human TRAC gene, the polynucleotide as described herein, and a 3' region of the human TRAC gene, wherein the integrated recombinant polynucleotide sequence prevents expression of the human TRAC gene. In some examples, the immune cells further comprise an additional recombinant polynucleotide sequence integrated at a human CD52 gene, wherein the additional recombinant polynucleotide sequence comprises a 5' region of the human CD52 gene, one or more coding sequences, and a 3' region of the human CD52 gene.

[0027] In yet related examples, populations of immune cells and pharmaceutical compositions of immune cells or populations of immune cells are described. In one aspect, there is described a population of immune cells comprising a plurality of the immune cells as described herein. In another aspect, there is described a pharmaceutical composition comprising the population of the immune cell as described herein, and a pharmaceutically acceptable excipient. In a further example, methods of treating a disease condition in a subject are provided comprising administering to the subject an immune cell, a population of immune cells and a pharmaceutical composition comprising the immune cell, an effective amount of the immune cells, an effective amount of the population of immune cells, or an effective amount of the pharmaceutical compositions, as described throughout. In one aspect, there is described a use of the pharmaceutical composition as described herein in the manufacture of a medicament for treating a disease condition in a subject. In some examples, the subject is a human.

[0028] In some examples, the disease condition is cancer, including without limitation, non-Hodgkin's lymphoma, large B cell lymphoma (LBCL), follicular lymphoma (FL), T cell lymphoma (TCL), B cell acute lymphoblastic leukemia (BALL), T cell acute lymphoblastic leukemia (TALL), primary central nervous system lymphoma (PCNSL), Mantle cell lymphoma (MCL), chronic lymphocytic leukemia (CLL), and peripheral T cell lymphoma (PTCL). In some examples, the immune cell is autologous with respect to the subject. In some examples, the immune cell is allogeneic with respect to the subject.

[0029] In some examples, the disease condition is an autoimmune disease or disorder, including without limitation, lupus, systemic lupus erythematosus (SLE), lupus nephritis, rheumatoid arthritis, systemic sclerosis, scleroderma, multiple sclerosis (MS), relapse remitting multiple sclerosis (RRMS), secondary progressive multiple sclerosis (SPMS), primary progressive multiple sclerosis (PPMS), neuromyelitis optica spectrum disorder (NMOSD), myasthenia gravis, Sjogren's syndrome, stiff person syndrome, graft v. host

disease (GvHD), autoimmune hemolytic anemia (AIHA), immune thrombocytopenia (ITP), and myositis. In some examples, the immune cells are capable of reducing or depleting B cells, pathogenic B cells, autoreactive B cells, pathogenic T cells, autoreactive T cells, CD70+ T cells, or alloreactive T cells. In some examples, the immune cell is autologous with respect to the subject. In other examples, the immune cell is allogeneic with respect to the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

[0030] **FIGs. 1A-1C** show results of in vitro long-term killing assays of the anti-CD19 CAR T cells with or without co-expressing a chimeric cytokine receptor (CCR).

[0031] **FIGs. 2A-2B** show results of in vivo cytotoxicity assay of the anti-CD19 CAR T cells with or without co-expressing the CCR (CD19CAR/CCR).

[0032] **FIG. 3A** depicts data showing reduced in vivo anti-tumor activity of the anti-CD19 CAR T cells co-expressing a CCR (CD19 CAR/CCR T cells) generated by site-specific integration as compared to CD19 CAR/CCR T cells generated by LVV transduction. **FIG. 3B** shows higher in vivo number of, *i.e.*, higher persistence of, CAR+ T cells generated by LVV transduction as compared to CAR T cells generated by site-specific integration.

[0033] **FIGs. 4A-4D** show results of experiments investigating the effects of different promoters driving the expression of the CCR and the CAR in CAR T cells generated by site-specific integration. **FIG. 4A** shows the median fluorescence intensity (MFI) of the CD19 CAR/CCR T cells at the end of CAR T cell production. **FIG. 4B** shows the results of CAR T cell killing of target Raji cells in a flow-based serial restimulation assay. **FIG. 4C** shows the CAR MFI, and **FIG. 4D** shows the CAR+ T cell counts, during the flow-based serial restimulation assay.

[0034] **FIGs. 5A-5C** compare the efficacy of CAR/CCR T cells generated by site-specific integration driven by either the EFS promoter or the PGK SSI-1 promoter, with the CD19 CAR/CCR T cells generated by LVV transduction, in a long-term killing assay (**FIG. 5A**), or in a single-stimulation flow-based potency assay (**FIGs. 5B-5C**) against the target Raji cells.

[0035] **FIGs. 6A-6B** compare the in vivo efficacy of CD19 CAR/CCR T cells generated by site-specific integration driven by the EFS promoter, the CypA300 promoter, or the PGK

What is claimed is:

1. A polynucleotide comprising a truncated PGK promoter, a first coding sequence and a second coding sequence, wherein the truncated PGK promoter comprises at least 50 nucleotides deletion from the 5' end of a long PGK promoter having the nucleotide sequence of SEQ ID NO: 34, wherein the truncated PGK promoter is operably linked to the first coding sequence and the second coding sequence, and wherein either the first or the second coding sequence encodes an anti-CD19 chimeric antigen receptor (CAR).

2. The polynucleotide of claim 1, wherein the coding sequence that encodes the anti-CD19 CAR comprises a nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to SEQ ID NO: 6 or 8.

3. The polynucleotide of any one of the preceding claims, wherein the truncated PGK promoter comprises a nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the nucleotide sequence of SEQ ID NO: 35, SEQ ID NO: 36, or SEQ ID NO: 37.

4. The polynucleotide of claim 3, wherein the truncated PGK promoter comprises a nucleotide sequence:

(a) comprising about 300, about 310, about 320, about 330, about 340, about 350, about 360, about 370, about 380, or about 390 nucleotides deletion from the 5' end of SEQ ID NO: 34 and having at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the nucleotide sequence of SEQ ID NO: 35;

(b) comprising about 210, about 220, about 230 about 240, about 250, about 260, about 270, about 280, or about 290 nucleotides deletion from the 5' end of SEQ ID NO: 34 and having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the nucleotide sequence of SEQ ID NO: 36;

(c) comprising about 100, about 110, about 120, about 130, about 140, abut 150, about 160, about 170, about 180, or about 190 nucleotides deletion from the 5' end of SEQ ID NO: 34 and having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the nucleotide sequence of SEQ ID NO: 37; or

(d) comprising about 50, about 60, about 70, about 80, about 90 or about 100 nucleotides deletion from the 5' end of SEQ ID NO: 34 and having at least

85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the nucleotide sequence of SEQ ID NO: 38.

5. The polynucleotide of any one of the preceding claims, wherein the first coding sequence encodes the anti-CD19 CAR and the second coding sequence encodes a CD70-binding protein, or wherein the second coding sequence encodes the anti-CD19 CAR and the first coding sequence encodes a CD70 binding protein.

6. The polynucleotide of claim 5, wherein the CD70-binding protein comprises the amino acid sequence of SEQ ID NO: 19 or 21.

7. The polynucleotide of claim 5, wherein the coding sequence that encodes the CD70-binding protein comprises a nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 20 or 22.

8. The polynucleotide of any one of the preceding claims, further comprising a 5' homology arm and a 3' homology arm, wherein the 5' and 3' homology arms are each at least 100 nucleotides in length.

9. The polynucleotide of claim 8, wherein the 5' homology arm and the 3' homology arm each comprises a nucleotide sequence homologous to a nucleotide sequence of a T cell receptor alpha constant region (TRAC) gene.

10. A vector comprising the polynucleotide sequence of any one of the preceding claims.

11. The vector of claim 10, wherein the vector is an adeno-associated virus (AAV) vector or a lentiviral vector.

12. An immune cell comprising the polynucleotide of any one of the preceding claims.

13. The immune cell of claim 12, further comprising an additional polynucleotide that comprises a promoter operably linked to one or more coding sequences, optionally wherein the one or more coding sequence encodes a chimeric cytokine receptor (CCR).

14. A population of immune cells comprising a plurality of the immune cells of claim 12 or 13.

15. A pharmaceutical composition comprising the population of the immune cell of claim 14, and a pharmaceutically acceptable excipient.

16. Use of the pharmaceutical composition of claim 15 in the manufacture of a medicament for treating a disease condition in a subject.

17. The use of claim 16, wherein the disease condition is a non-Hodgkin's lymphoma or an autoimmune disease.

18. The use of claim 17, wherein the disease condition is large B cell lymphoma (LBCL), follicular lymphoma (FL), T cell lymphoma (TCL), B cell acute lymphoblastic leukemia (BALL), T cell acute lymphoblastic leukemia (TALL), primary central nervous system lymphoma (PCNSL), Mantle cell lymphoma (MCL), chronic lymphocytic leukemia (CLL), peripheral T cell lymphoma (PTCL).

19. The use of claim 17, wherein the autoimmune disease or disorder is lupus, systemic lupus erythematosus (SLE), lupus nephritis, rheumatoid arthritis, systemic sclerosis, scleroderma, multiple sclerosis (MS), relapse remitting multiple sclerosis (RRMS), secondary progressive multiple sclerosis (SPMS), primary progressive multiple sclerosis (PPMS), neuromyelitis optica spectrum disorder (NMOSD), myasthenia gravis, Sjogren's syndrome, and myositis.

20. A method of making an engineered immune cell comprising introducing to the immune cell an engineered endonuclease that cleaves at a target sequence within a human TRAC gene and the polynucleotide of any one of claims 1-9 or the vector of claim 10 or 11, and culturing the immune cell under suitable conditions.