CLAIMS

What is claimed is:

1. A polynucleotide that encodes an anti-TROP-2 chimeric antigen receptor (CAR), the CAR comprising (i) an anti-TROP-2 antigen binding region of a TROP-2 specific antibody, (ii) a transmembrane domain, and (iii) an intracellular region.

2. The polynucleotide of claim 1, wherein:

(a) (i) (A) the TROP-2 specific antibody is an RS7 antibody, optionally wherein the RS7 antibody is:

(I) humanized RS7 (hRS7); or

(II) murine RS7 (mRS7); and/or

(B) the anti-TROP-2 antigen binding region comprises a sequence having at least 85% sequence identity with SEQ ID NO:9; and/or

(C) the anti-TROP-2 antigen binding region comprises a sequence having at least 85% sequence identity with SEQ ID NO:14; and/or

(D) the anti-TROP-2 antigen binding region comprises a first sequence having at least 85% sequence identity with SEQ ID NO:9 and a second sequence having at least 85% sequence identity with SEQ ID NO:14; and/or

(E) the anti-TROP-2 antigen binding region comprises SEQ ID NO:9; and/or

(F) the anti-TROP-2 antigen binding region comprises SEQ ID NO:14; and/or

(G) the anti-TROP-2 antigen binding region comprises SEQ ID NO:9 and SEQ ID NO:14; or

(ii) (A) the TROP-2 specific antibody is a 2G10 antibody, optionally wherein the 2G10 antibody is:

(I) murine 2G10 (m2G10); or

(II) human 2G10 (h2G10); and/or

(B) wherein the anti-TROP-2 antigen binding region comprises:

(I) SEQ ID NO:44; or

(II) SEQ ID NO:46; and/or

(b) the anti-TROP-2 antigen binding region is a codon optimized anti-TROP-2 antigen binding region; and/or

(c) the transmembrane domain is a transmembrane domain from CD28, the alpha chain of the T- cell receptor, beta chain of the T- cell receptor, zeta chain of the T-cell receptor, CD3 zeta, CD3 epsilon, CD3 gamma, CD3 delta, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD27, CD33, CD37, CD64, CD80, CD86, CD 134, CD137, CD154, ICOS/CD278, GITR/CD357, NKG2D, DAP10, or DAP12, optionally wherein the transmembrane domain:

(i) is a CD28 transmembrane domain, optionally wherein the transmembrane domain comprises SEQ ID NO:23; or

(ii) is a CD27 transmembrane domain, optionally wherein the transmembrane domain comprises SEQ ID NO:22; or

(iii) is a CD8 transmembrane domain, optionally wherein the transmembrane domain comprises SEQ ID NO:24; and/or

(d) the intracellular domain is an intracellular domain from CD28, CD3 zeta, CD27, 4-1BB, DAP12, NKG2D, OX-40 (CD134), DAP10, CD40L, 2B4, DNAM, CS1, CD48, NKp30, NKp44, NKp46, or NKp80, optionally wherein the intracellular domain is:

(i) a CD28 intracellular domain; or

(ii) a CD3 zeta intracellular domain, further optionally wherein the intracellular domain comprises SEQ ID NO:29; and/or

(e) the CAR comprises two or more intracellular domains, optionally wherein the two or more intracellular domains comprise:

(i) a CD3 zeta intracellular domain and an additional intracellular domain selected from a CD28, DAP10, DAP12, 4-1BB, NKG2D, and 2B4 intracellular domain; and/or

(ii) a CD3 zeta intracellular domain and:

(A) a CD28 intracellular domain; or

(B) a DAP10 intracellular domain; and/or

(f) the polynucleotide further comprises a signal peptide, optionally wherein the signal peptide is a signal peptide from CD8, CD27, granulocyte-macrophage colony-stimulating factor receptor (GMSCF-R), Ig heavy chain (IgH), CD3, or CD4, further optionally wherein

(i) the signal peptide:

(A) is a GMSCF-R signal peptide; and/or

(B) comprises SEQ ID NO:21; or

(ii) the signal peptide is a IgH signal peptide, optionally wherein the signal peptide comprises SEQ ID NO:20; and/or

(g) the CAR comprises a CD28 transmembrane domain, an IgH signal peptide, an anti-TROP-2 antigen binding region of an RS7 antibody, and a CD3 zeta intracellular domain; and/or

(h) the polynucleotide further encodes an additional polypeptide of interest, optionally, wherein:

(i) the sequence encoding the additional polypeptide of interest and the sequence encoding the CAR are separated on the polynucleotide by a 2A element, optionally wherein the 2A element is an E2A element, further optionally wherein the E2A element comprises SEQ ID NO:38; and/or

(ii) the additional polypeptide of interest is a therapeutic protein or a protein that enhances cell activity, expansion, and/or persistence; and/or

(iii) the additional polypeptide of interest is a suicide gene product, a cytokine, or a human or viral protein that enhances proliferation, expansion and/or metabolic fitness; and/or

(iv) the additional polypeptide of interest is a cytokine, optionally wherein the cytokine is IL-15, IL-2, IL-12, IL-18, IL-21, IL-23, or IL-7, optionally wherein the cytokine is IL-15, further optionally wherein the cytokine comprises SEQ ID NO:37; and/or

(v) wherein the additional polypeptide of interest is a suicide gene product, optionally wherein:

(A) the suicide gene product is Caspase 9; and/or

(B) the polynucleotide encodes for a sequence having at least 95% identity with SEQ ID NO:42, optionally wherein the polynucleotide encodes for SEQ ID NO:42; and/or

(i) the polynucleotide encodes for a sequence having at least 95% sequence identity:

(i) with SEQ ID NO:2, optionally wherein the polynucleotide encodes for SEQ ID NO:2, further optionally wherein the polynucleotide comprises SEQ ID NO:1; or

(ii) with SEQ ID NO:4, optionally wherein the polynucleotide encodes for SEQ ID NO:4, further optionally wherein the polynucleotide comprises SEQ ID NO:3; or

(iii) with SEQ ID NO:6, optionally wherein the polynucleotide encodes for SEQ ID NO:6, further optionally wherein the polynucleotide comprises SEQ ID NO:5; or

(iv) with SEQ ID NO:8, optionally wherein the polynucleotide encodes for SEQ ID NO:8; further optionally wherein the polynucleotide comprises SEQ ID NO:7; or

(v) with SEQ ID NO:51, optionally wherein the polynucleotide encodes for SEQ ID NO:51, further optionally wherein the polynucleotide comprises SEQ ID NO:50; or

(vi) with SEQ ID NO:53, optionally wherein the polynucleotide encodes for SEQ ID NO:53, further optionally wherein the polynucleotide comprises SEQ ID NO:52; or

(vii) with SEQ ID NO:55, optionally wherein the polynucleotide encodes for SEQ ID NO:55, further optionally wherein the polynucleotide comprises SEQ ID NO:54; or

(viii) with SEQ ID NO:57, optionally the polynucleotide encodes for SEQ ID NO:57; further optionally wherein the polynucleotide comprises SEQ ID NO:56; or

(ix) with SEQ ID NO:59; optionally wherein the polynucleotide encodes for SEQ ID NO:59, further optionally wherein the polynucleotide comprises SEQ ID NO:58; or

(x) with SEQ ID NO:61, optionally wherein the polynucleotide encodes for SEQ ID NO:61, further optionally, wherein the polynucleotide comprises SEQ ID NO:60.

3. A vector comprising the polynucleotide of claims 1 or 2, optionally wherein the vector is:

(a) a viral vector, optionally wherein the viral vector is an adenoviral vector, adeno-associated viral vector, lentiviral vector, or retroviral vector; or

(b) a non-viral vector, optionally wherein the non-viral vector is a plasmid.

4. An immune cell comprising the polynucleotide of claims 1 and 2(a)-(i)(i) or the vector of claim 3, optionally wherein:

(a) the immune cell is a natural killer (NK) cell, T cell, gamma delta T cell, alpha beta T cell, invariant NKT (iNKT) cell, B cell, macrophage, mesenchymal stromal cell, or dendritic cell, optionally wherein the immune cell is a NK cell, further optionally wherein:

(i) the NK cell is derived from cord blood, peripheral blood, induced pluripotent stem cells, hematopoietic stem cells, bone marrow, or from a cell line, optionally wherein the NK cell is derived from:

(A) a cell line, wherein the NK cell line is NK-92; or

(B) a cord blood mononuclear cell; and/or

(ii) the NK cell is a $CD56^+$ NK cell; and/or

(iii) the NK cell expresses a recombinant cytokine, optionally wherein the cytokine is IL-15, IL-2, IL-12, IL-18, IL-21, IL-7, or IL-23, further optionally wherein the cytokine is IL-15; and/or

(b) expression of one or more endogenous genes in the immune cell has been modified, optionally, wherein the one or more genes comprise NKG2A, SIGLEC-7, LAG3, TIM3, CISH, FOXO1, TGFBR2, TIGIT, CD96, ADORA2, NR3C1, PD1, PDL-1, PDL-2, CD47, SIRPA, SHIP1, ADAM17, RPS6, 4EBP1, CD25, CD40, IL21R, ICAM1, CD95, CD80, CD86, IL10R, CD5, CD7, CTLA-4, TDAG8, CD38, or a combination thereof.

5. A population of immune cells comprising the immune cell of claim 4.

6. Use of cells harboring the polynucleotide of claims 1 and 2(a)-(i)(i) in the manufacture of a medicament for killing TROP-2-positive cells in an individual, wherein said cells are to be administered to the individual.

7. The use according to claim 6, wherein:

(a) the cells harboring the polynucleotide are immune cells, optionally wherein:

(i) the immune cells are NK cells, T cells, gamma delta T cells, alpha beta T cells, invariant NKT (iNKT) cells, B cells, macrophages, dendritic cells, or a mixture thereof, optionally wherein the immune cells comprise NK cells, wherein the NK cells are derived from cord blood, peripheral blood,

induced pluripotent stem cells, hematopoietic stem cells, bone marrow, from a cell line, or a mixture thereof, further optionally wherein the NK cells are derived from cord blood mononuclear cells; and/or

(ii) the immune cells are:

(A) allogeneic with respect to the individual; or

(B) autologous with respect to the individual; and/or

(b) the individual is human; and/or

(c) the individual has cancer, optionally wherein the individual has breast cancer, acute myeloid leukemia, multiple myeloma, or glioblastoma; and/or (d) wherein the cells harboring the polynucleotide are to be administered to the individual once or more than once, optionally wherein the duration of time between administrations of the cells harboring the polynucleotide to the individual is 1-24 hours, 1-7 days, 1-4 weeks, 1-12 months, or one or more years; and/or

(e) an additional therapy is to be further provided to the individual, optionally wherein the additional therapy comprises surgery, radiation, gene therapy, immunotherapy, or hormone therapy; and/or

(f) the cells harboring the polynucleotide are to be administered to the individual by injection, intravenously, intraarterially, intraperitoneally, intratracheally, intratumorally, intramuscularly, endoscopically, intralesionally, intracranially, percutaneously, subcutaneously, regionally, by perfusion, in a tumor microenvironment, or a combination thereof; and/or

(g) identifying TROP-2-positive cancer in the individual; and/or

(h) producing the cells harboring the polynucleotide.

8. A polynucleotide that encodes:

(a) an anti-TROP-2 chimeric antigen receptor (CAR), the CAR comprising (i) an anti-TROP-2 antigen binding region of an RS7 antibody, (ii) a transmembrane domain, and (iii) an intracellular region;

(b) IL-15; and

(c) Caspase 9;

optionally wherein the RS7 antibody is a:

(i) humanized RS7 antibody; or

(ii) murine RS7 antibody.

9. A polynucleotide that encodes:

(a) an anti-TROP-2 chimeric antigen receptor (CAR), the CAR comprising (i) an anti-TROP-2 antigen binding region of a 2G10 antibody, (ii) a transmembrane domain, and (iii) an intracellular region;

(b) IL-15; and

(c) Caspase 9;

optionally wherein the 2G10 antibody is:

(i) a murine 2G10 antibody; or

(ii) a humanized 2G10 antibody.

10. An NK cell comprising the polynucleotide of claims 8 or 9.

11. A natural killer cell comprising:

(a) a first polynucleotide encoding an anti-TROP-2 chimeric antigen receptor;

(b) a second polynucleotide encoding IL-15; and

(c) a third polynucleotide encoding Caspase 9,

optionally wherein the first polynucleotide, the second polynucleotide, and the third polynucleotide are:

(i) present on the same nucleic acid molecule; or

(ii) present on two different nucleic acid molecules; or

(iii) present on three different nucleic acid molecules.

12. A polynucleotide that encodes for SEQ ID NO:2, optionally wherein the polynucleotide comprises a sequence having at least 90% identity with SEQ ID NO:1, further optionally wherein the polynucleotide comprises SEQ ID NO:1.

13. A polynucleotide that encodes for SEQ ID NO:4, optionally wherein the polynucleotide comprises a sequence having at least 90% identity with SEQ ID NO:3, further optionally wherein the polynucleotide comprises SEQ ID NO:3.

14. A polynucleotide that encodes for SEQ ID NO:6, optionally wherein the polynucleotide comprises a sequence having at least 90% identity with SEQ ID NO:5, further optionally wherein the polynucleotide comprises SEQ ID NO:5.

15. A polynucleotide that encodes for SEQ ID NO:8, optionally wherein the polynucleotide comprises a sequence having at least 90% identity with SEQ ID NO:7, further optionally wherein the polynucleotide comprises SEQ ID NO:7.

16. Cells harboring the polynucleotide of claims 1 and 2(a)-(i)(i) for use in therapy.