

CLAIMS

What is claimed is:

1. A polynucleotide that encodes an anti-CD70 chimeric antigen receptor (CAR) comprising a signal peptide, an anti-CD70 antigen binding domain, transmembrane domain, and at least one intracellular domain, wherein the anti-CD70 antigen binding domain does not comprise an antibody, and optionally wherein a transmembrane domain of the CAR is not a CD27 transmembrane domain.

2. The polynucleotide of claim 1, wherein the anti-CD70 antigen binding domain is comprised in an extracellular domain of CD27, optionally, wherein the sequence that encodes the antigen binding domain is codon optimized.

~~3. The polynucleotide of claim 1 or 2, wherein the CAR comprises an antigen binding domain of CD27 that is codon optimized.~~

34. The polynucleotide of claim 1 or 2, ~~or 3~~, wherein the antigen binding domain comprises, consists of, or consists essentially of SEQ ID NO:2.

~~5. The polynucleotide of any one of claims 1, 2, or 3, wherein the antigen binding domain and transmembrane domain comprise, consist of, or consist essentially of SEQ ID NO:1.~~

~~6. The polynucleotide of any one of claims 1-5, wherein the sequence that encodes the transmembrane domain is codon optimized.~~

47. The ~~polynucleotidemethod~~ of any one of claims 1-34, wherein the transmembrane domain is 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, CD 16, CD22, CD33, CD37, CD64, CD80, CD86, CD 134, CD137, CD154, ICOS/CD278, GITR/CD357, NKG2D, DAP10, or DAP12, optionally, wherein the sequence that encodes the transmembrane domain is codon optimized.

58. The ~~polynucleotidemethod~~ of any one of claims 1-47, wherein the ~~CD28~~ transmembrane domain comprises, consists of, or consists essentially of SEQ ID NO:3 or SEQ ID NO: 7.

~~96.~~ The polynucleotide of any one of claims 1-~~58~~, wherein the CAR comprises two, three, or more intracellular domains, optionally.

~~10.~~ ~~The polynucleotide of any one of claims 1-8, wherein the CAR comprises three or more intracellular domains.~~

~~11.~~ ~~The polynucleotide of any one of claims 1-10, wherein the intracellular domain comprises CD3zeta;~~

~~12.~~ ~~The polynucleotide of any one of claims 1-8, wherein the intracellular domain is an intracellular costimulatory domain.~~

~~13.~~ ~~The polynucleotide of claim 9, wherein the intracellular domain comprises an ITAM-containing signaling domain; and/or the intracellular domain is an intracellular costimulatory domain.~~

~~14.~~ ~~The polynucleotide of claim 9, wherein the intracellular costimulatory domain is selected from the group consisting of CD27, CD28, 4-1BB, DAP12, NKG2D, OX-40 (CD134), DAP10, CD40L, 2B4, DNAM, CS1, CD48, NKp30, NKp44, NKp46, NKp80, and a combination thereof.~~

~~745.~~ The polynucleotide of any one of claims 1-~~614~~, wherein the intracellular domain comprises one or more of SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:12 is a costimulatory domain.

~~846.~~ The polynucleotide of any one of claims 1-~~745~~, wherein the signal peptide is from CD27 or granulocyte-macrophage colony-stimulating factor receptor (GMSCF-R), optionally, -

~~17.~~ ~~The polynucleotide of claim 16, wherein the sequence of the CD27 signal peptide comprises, consists of, or consists essentially of SEQ ID NO:6.~~

~~948.~~ The polynucleotide of any one of claims 1-8, wherein the signal peptide is from CD27, the anti-CD70 antigen binding domain is from CD27, the transmembrane domain is from CD27, and an intracellular domain is from CD27.

~~19.~~ ~~The polynucleotide of any one of claims 1-18, wherein the signal peptide comprises, consists of, or consists essentially of SEQ ID NO:6.~~

1020. The polynucleotide of any one of claims 1-~~919~~, wherein the CAR comprises, consists of, or consists essentially of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:5.

~~21. The polynucleotide of any one of claims 1-20, wherein the transmembrane domain comprises, consists of, or consists essentially of SEQ ID NO:3 or SEQ ID NO:7.~~

~~22. The polynucleotide of any one of claims 1-21, wherein the intracellular domain comprises one or more of SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:12.~~

~~23. The polynucleotide of any one of claims 1-22, wherein the CAR comprises, consists of, or consists essentially of SEQ ID NO:5.~~

~~24. The polynucleotide of any one of claims 1-23, wherein the CAR comprises, consists of, or consists essentially of SEQ ID NO:1.~~

1125. The polynucleotide of any one of claims ~~12-1024~~, wherein the CAR comprises one or more of the following:

- (a) CD27 signal peptide (SP), CD27 extracellular domain (EC), CD27 transmembrane domain (TMD), DAP12 intracellular domain (ICD), and CD3zeta;
- (b) GMSCF-R SP, codon optimized CD27 EC, codon optimized CD27 TMD, DAP12 ICD;
- (c) CD27 SP, CD27 EC, CD28 TMD, DAP12 ICD, and CD3zeta;
- (d) GMSCF-R SP, codon optimized CD27 EC, CD28 TMD, DAP12 ICD, and CD3zeta;
- (e) CD27 SP, CD27 EC, CD27 TMD, Natural killer group 2 member D (NKG2D) ICD, and CD3zeta;
- (f) GMSCF-R SP, codon optimized CD27 EC, codon optimized CD27 TMD, NKG2D ICD, and CD3zeta;
- (g) CD27 SP, CD27 EC, CD28 TMD, Natural killer group 2 member D (NKG2D) ICD, and CD3zeta;

- (h) GMSCF-R SP, codon optimized CD27 EC, CD28 TMD, NKG2D ICD, and CD3zeta;
- (i) CD27 SP, CD27 EC, CD27 TMD, 4-1BB ICD, and CD3zeta;
- (j) GMSCF-R SP, codon optimized CD27 EC, codon optimized CD27 TMD, 4-1BB ICD, and CD3zeta;
- (k) CD27 SP, CD27 EC, CD28 TMD, 4-1BB ICD, and CD3zeta;
- (l) GMSCF-R SP, codon optimized CD27 EC, CD28 TMD, 4-1BB ICD, and CD3zeta;
- (m) CD27 SP, CD27 EC, CD27 TMD, DAP10 ICD, and CD3zeta;
- (n) GMSCF-R SP, codon optimized CD27 EC, codon optimized CD27 TMD, DAP10 ICD, and CD3zeta;
- (o) CD27 SP, CD27 EC, CD28 TMD, DAP10 ICD, and CD3zeta;
- (p) GMSCF-R SP, codon optimized CD27 EC, CD28 TMD, DAP10 ICD, and CD3zeta;
- (q) CD27 SP, CD27 full length (FL), CD27 TMD, CD27 ICD, and CD3zeta;
- (r) GMSCF-R SP, codon optimized CD27 FL, codon optimized CD27 TMD, codon optimized CD27 ICD, and CD3zeta;
- (s) CD27 SP, CD27 FL, CD27 TMD, CD27 ICD, CD28 ICD, and CD3zeta;
- (t) GMSCF-R SP, codon optimized CD27 FL, codon optimized CD27 TMD, codon optimized CD27 ICD, CD28 ICD, and CD3zeta;
- (u) CD27 SP, CD27 FL, CD27 TMD, CD27 ICD, 4-1BB ICD, and CD3zeta;
- (v) GMSCF-R SP, codon optimized CD27 FL, codon optimized CD27 TMD, codon optimized CD27 ICD, 4-1BB ICD, and CD3zeta;
- (w) CD27 SP, CD27 FL, CD27 TMD, CD27 ICD, DAP10 ICD, and CD3zeta;

- (x) GMSCF-R SP, CD27 FL, CD27 TMD, CD27 ICD, DAP10 ICD, and CD3zeta;
- (y) CD27 SP, CD27 FL, CD27 TMD, CD27 ICD, DAP12 ICD, and CD3zeta;
- (z) GMSCF-R SP, codon optimized CD27 FL, codon optimized CD27 TMD, codon optimized CD27 ICD, DAP12 ICD, and CD3zeta;
- (aa) CD27 SP, CD27 FL, CD27 TMD, CD27 ICD, NKG2D ICD, and CD3zeta;
- (bb) GMSCF-R SP, codon optimized CD27 FL, codon optimized CD27 TMD, codon optimized CD27 ICD, NKG2D ICD, and CD3zeta;
- (cc) CD27 SP, CD27 EC, CD27 TMD, and CD3zeta;
- (dd) GMSCF-R SP, codon optimized CD27 EC, codon optimized CD27 TMD, and CD3zeta;
- (ee) CD27 SP, CD27 EC, CD28 TMD, and CD3zeta;
- (ff) GMSCF-R SP, codon optimized CD27 EC, codon optimized CD28 TMD, and CD3zeta;
- (gg) CD27 SP, CD27 EC, CD27 TMD, CD28 ICD, and CD3zeta;
- (hh) GMSCF-R SP, codon optimized CD27 EC, codon optimized CD27 TMD, CD28 ICD, and CD3zeta;
- (ii) CD27 SP, CD27 EC, CD28 TMD, CD28 ICD, and CD3zeta; or
- (jj) GMSCF-R SP, codon optimized CD27 EC, CD28 TMD, and CD3zeta.

1226. An immune cell comprising the polynucleotide of any one of claims 1-1125.

~~27. The cell of claim 26, wherein the cell is an immune cell.~~

~~28. The immune cell of claim 27, wherein the immune cell is a Natural Killer (NK) cell, T cell, gamma delta T cells, invariant NKT (iNKT) cell, B cell, macrophage, MSCs, or dendritic cell.~~

~~29. The immune cell of claim 27 or 28, wherein the cell is a NK cell.~~

~~30. — The cell of claim 28 or 29, wherein the NK cell is derived from cord blood, peripheral blood, induced pluripotent stem cells, bone marrow, from a cell line, or a mixture thereof.~~

~~31. — The immune cell of claim 30, wherein the NK cell line is NK 92 cell line or another NK cell line derived from a tumor or from a healthy NK cell, or is a progenitor cell.~~

~~32. — The immune cell of any one of claims 29-31, wherein the NK cell is derived from a cord blood mononuclear cell.~~

~~33. — The immune cell of any one of claims 28-32, wherein the NK cell is a CD56+ NK cell.~~

~~34. — The immune cell of any one of claims 28-33, wherein the NK cells express one or more exogenously provided cytokines.~~

~~35. — The immune cell of claim 34, wherein the cytokine is IL-15, IL-2, IL-12, IL-18, IL-21, IL-7, or a combination thereof.~~

1336. A population of the immune cells of ~~any one of claims 1227-35~~, said cells present in a suitable medium.

~~37. — The population of claim 36, wherein the immune cells are NK cells.~~

1438. Use of the polynucleotide of any one of claims 1-11 in the manufacture of a medicament for A method of killing CD70-positive cells in an individual, wherein comprising the step of administering to the individual a therapeutically effective amount of cells harboring the polynucleotide of any one of claims 1-~~1125~~ is to be administered to the individual.

~~39. — The method of claim 38, wherein the cells are NK cells, T cells, gamma delta T cells, invariant NKT (iNKT) cells, B cells, macrophages, gamma delta T cells, or dendritic cells.~~

~~40. — The method of claim 39, wherein the NK cells are derived from cord blood, peripheral blood, induced pluripotent stem cells, bone marrow, or from a cell line.~~

~~41. — The method of any one of claims 39-40, wherein the NK cells are derived from cord blood mononuclear cells.~~

~~42. The method of any one of claims 38-41, wherein the CD70-positive cells are not cancer cells.~~

~~43. The method of claim 42, wherein the CD70-positive cells are T-regulatory cells.~~

~~44. The method of any one of claims 28-41, wherein the individual has a CD70-expressing cancer.~~

~~45. The method of any one of claims 28-41, wherein the individual has acute myeloid leukemia, lymphoma, lung cancer, renal cancer, bladder cancer, melanoma, glioblastoma, breast cancer, head and neck cancer, mesothelioma, multiple myeloma, pancreatic cancer or a combination thereof.~~

~~46. The method of any one of claims 28-45, wherein the cells are allogeneic with respect to the individual.~~

~~47. The method of any one of claims 28-45, wherein the cells are autologous with respect to the individual.~~

~~48. The method of any one of claims 28-47, wherein the individual is a human.~~

~~49. The method of any one of claims 28-48, wherein the cells are administered to the individual once or more than once.~~

~~50. The method of claim 49, wherein the duration of time between administrations of the cells to the individual is 1-24 hours, 1-7 days, 1-4 weeks, 1-12 months, or one or more years.~~

1551. The usemethod of any one of claims ~~1428-50~~, further comprising ~~the step of providing to the individual~~ an effective amount of an additional therapy to be provided to the individual; and/or

~~52. The method of claim 51, wherein the additional therapy comprises surgery, radiation, gene therapy, immunotherapy, or hormone therapy.~~

~~53. The method of claim 51 or 52, wherein the additional therapy comprises one or more antibodies.~~

~~54. The method of any one of claims 28-53, wherein the cells are 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.~~

~~55. The method of any one of claims 28-54, further comprising the step of identifying CD70-positive cells in the individual.~~

16. The polynucleotide of any one of claims 1-11 for use in therapy.