

CLAIMS

1. A method of making a population of cells that comprise:

a first nucleic acid molecule that encodes a controllable chimeric antigen receptor (CCAR), or

a second nucleic acid molecule that encodes a chimeric antigen receptor (CAR) and a regulatory molecule,

the method comprising:

(i) contacting a population of cells with an agent that stimulates a CD3/TCR complex and/or an agent that stimulates a costimulatory molecule on the surface of the cells;

(ii) contacting the population of cells with a first nucleic acid molecule encoding a CCAR or a second nucleic acid molecule encoding a CAR and a regulatory molecule, thereby providing a population of cells comprising the first or second nucleic acid molecule, and

(iii) harvesting the population of cells for storage or administration, wherein the population of cells from step (iii) are not expanded, or expanded by no more than 40%, compared to the population of cells at the beginning of step (i).

2. The method of claim 1, wherein:

(a) step (ii) is performed together with step (i) or no later than 20 hours after the beginning of step (i), and step (iii) is performed no later than 30 hours after the beginning of step (i); or step (iii) is performed no later than 30 hours after the beginning of step (ii);

(b) the first or second nucleic acid molecule in step (ii) is on a viral vector;

(c) the population of cells comprises a population of T cells, optionally wherein the population of T cells is isolated from a frozen or fresh leukapheresis product; and/or

(d) the storage of step (iii) comprises reformulating the population of cells in cryopreservation media.

3. The method of claim 1 or 2, wherein:

(a) the agent that stimulates a CD3/TCR complex is an agent that stimulates CD3;

(b) the agent that stimulates a CD3/TCR complex or the agent that stimulates a costimulatory molecule is chosen from an antibody, a small molecule, or a ligand;

(c) the agent that stimulates a CD3/TCR complex or the agent that stimulates a costimulatory molecule does not comprise a bead;

(d) the agent that stimulates a costimulatory molecule is an agent that stimulates CD28, ICOS, CD27, HVEM, LIGHT, CD40, 4-1BB, OX40, DR3, GITR, CD30, TIM1, CD2, CD226, or any combination thereof; and/or

(e) the agent that stimulates a CD3/TCR complex is an anti-CD3 antibody.

4. The method of claim 3, wherein:

(a) the antibody is a single-domain antibody, a heavy chain variable domain antibody, a peptibody, a Fab fragment, or a scFv; and/or

(b) the ligand is a naturally-existing, recombinant, or chimeric ligand.

5. The method of any one of claims 1-4, wherein the agent that stimulates a CD3/TCR complex comprises an anti-CD3 antibody and the agent that stimulates a costimulatory molecule comprises an anti-CD28 antibody, optionally wherein:

(a) the agent that stimulates a CD3/TCR complex comprises an anti-CD3 antibody covalently attached to a colloidal polymeric nanomatrix and the agent that stimulates a costimulatory molecule comprises an anti-CD28 antibody covalently attached to a colloidal polymeric nanomatrix; or

(b) the agent that stimulates a CD3/TCR complex and the agent that stimulates a costimulatory molecule comprise T Cell TransAct™.

6. The method of any one of claims 1-5, wherein:

(I) (a) the percentage of naïve cells in the population of cells from step (iii) is the same as or differs by no more than 5 or 10% from the percentage of naïve cells in the population of cells at the beginning of step (i); or

(b) the percentage of naïve cells in the population of cells from step (iii) does not decrease, or decreases by no more than 5 or 10%, as compared to the percentage of naïve cells in the population of cells at the beginning of step (i); and/or

(II) (a) the percentage of central memory cells in the population of cells from step (iii) is the same as or differs by no more than 5 or 10% from the percentage of central memory cells in the population of cells at the beginning of step (i); or

(b) the percentage of central memory cells in the population of cells from step (iii) does not increase, or increases by no more than 5 or 10%, as compared to the percentage of central memory cells in the population of cells at the beginning of step (i);
optionally wherein said naïve cells are naïve T cells or CD45RA⁺ CD45RO⁻ CCR7⁺ T cells; or said central memory cells are central memory T cells, CD95⁺ central memory T cells, or CCR7⁺CD45RO⁺ cells.

7. The method of any one of claims 1-6, wherein:

(I) step (i) increases the percentage of cells that comprise the first or second nucleic acid molecule in the population of cells from step (iii), for example, the population of cells from step (iii) shows a higher percentage of cells that comprise the first or second nucleic acid molecule, compared with cells made by an otherwise similar method without step (i); and/or

(II) (a) the percentage of naïve cells in the population of cells from step (iii) is increased by, for example, at least 1.2-fold, as compared to the percentage of naïve cells in the population of cells at the beginning of step (i); or

(b) the percentage of naïve T cells that comprise the first or second nucleic acid molecule in the population of cells increases during the duration of step (ii), or between 18-24 hours after the beginning of step (ii); and/or

(III) (a) the population of cells from step (iii) shows a higher percentage of naïve cells compared with cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i);

(b) the percentage of naïve cells in the population of cells from step (iii) is higher than the percentage of naïve cells in cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i);

(c) the percentage of naïve T cells that comprise the first or second nucleic acid molecule in the population of cells from step (iii) is higher than the percentage of naïve T cells that comprise the first or second nucleic acid molecule in cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i);

(d) the population of cells from step (iii) shows a higher percentage of naïve cells compared with cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells in vitro for more than 3 days;

(e) the percentage of naïve cells in the population of cells from step (iii) is higher than the percentage of naïve cells in cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells in vitro for more than 3 days; or

(f) the percentage of naïve T cells that comprise the first or second nucleic acid molecule in the population of cells from step (iii) is higher than the percentage of naïve T cells that comprise the first or second nucleic acid molecule in cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells in vitro for more than 3 days;

(IV) (a) the percentage of central memory cells in the population of cells from step (iii) is reduced by at least 20%, as compared to the percentage of central memory cells in the population of cells at the beginning of step (i); or

(b) the percentage of central memory T cells that comprise the first or second nucleic acid molecule that comprise the first or second nucleic acid molecule, decreases during the duration of step (ii), or between 18-24 hours after the beginning of step (ii); and/or

(V) (a) the population of cells from step (iii) shows a lower percentage of central memory cells compared with cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i);

(b) the percentage of central memory cells in the population of cells from step (iii) is lower than the percentage of central memory cells in cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i);

(c) the percentage of central memory T cells that comprise the first or second nucleic acid molecule in the population of cells from step (iii) is lower than the percentage of central memory T cells that comprise the first or second nucleic acid molecule in cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i);

(d) the population of cells from step (iii) shows a lower percentage of central memory cells compared with cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells in vitro for more than 3 days;

(e) the percentage of central memory cells in the population of cells from step (iii) is lower than the percentage of central memory cells in cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells in vitro for more than 3 days; or

(f) the percentage of central memory T cells that comprise the first or second nucleic acid molecule in the population of cells from step (iii) is lower than the percentage of central memory T cells that comprise the first or second nucleic acid molecule, in cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells in vitro for more than 3 days; and/or

(VI) (a) the percentage of stem memory T cells, in the population of cells from step (iii) is increased, as compared to the percentage of stem memory T cells in the population of cells at the beginning of step (i);

(b) the percentage of stem memory T cells that comprise the first or second nucleic acid molecule in the population of cells from step (iii) is increased, as compared to the percentage of stem memory T cells that comprise the first or second nucleic acid molecule in the population of cells at the beginning of step (i);

(c) the percentage of stem memory T cells in the population of cells from step (iii) is higher than the percentage of stem memory T cell in cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i); or

(d) the percentage of stem memory T cells that comprise the first or second nucleic acid molecule in the population of cells from step (iii) is higher than the percentage of stem memory T cells that comprise the first or second nucleic acid molecule, in cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i);

(e) the percentage of stem memory T cells in the population of cells from step (iii) is higher than the percentage of stem memory T cells in cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells in vitro for more than 3 days; or

(f) the percentage of stem memory T cells that comprise the first or second nucleic acid molecule in the population of cells from step (iii) is higher than the percentage of stem memory T cells that comprise the first or second nucleic acid molecule in cells made by an otherwise

similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells in vitro for more than 3 days,

optionally wherein said naïve cells are naïve T cells or CD45RA⁺ CD45RO⁻ CCR7⁺ T cells;

said central memory cells are central memory T cells, CD95⁺ central memory T cells, or CCR7⁺CD45RO⁺ cells; and/or

said stem memory T cells are CD45RA⁺CD95⁺IL-2 receptor β +CCR7⁺CD62L⁺ T cell.

8. The method of any one of claims 1-7, wherein:

(a) the population of cells from step (iii), after being incubated with a cell expressing an antigen recognized by the CCAR or CAR, secretes IL-2 at a higher level than cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), or cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells in vitro for more than 3 days

(b) the population of cells from step (iii), after being administered in vivo, persists longer or expands at a higher level compared with cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), or compared with cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells in vitro for more than 3 days;

(c) the population of cells from step (iii), after being administered in vivo, shows a stronger anti-tumor activity than cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), or cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells in vitro for more than 3 days;

(d) the population of cells from step (iii) are not expanded, or expanded by no more than 5, 10, 15, 20, 25, 30, 35, or 40%, compared to the population of cells at the beginning of step (i);

(e) the population of cells from step (iii) are expanded by no more than 10%, compared to the population of cells at the beginning of step (i);

(f) the population of cells from step (iii) are not expanded, or expanded by no more than 5, 10, 15, 20, 25, 30, 35, or 40%, as assessed by the number of living cells, compared to the population of cells at the beginning of step (i);

(g) the population of cells from step (iii) are expanded by no more than 10% compared to the population of cells at the beginning of step (i);

(h) the number of living cells in the population of cells from step (iii) decreases from the number of living cells in the population of cells at the beginning of step (i);

(i) the population of cells from step (iii) are not expanded, or expanded by less than 2 hours, for example, less than 1 or 1.5 hours, compared to the population of cells at the beginning of step (i);

(j) the population of cells from step (iii) are not expanded; and/or

(k) the population of cells from step (iii) are not expanded, as assessed by the number of living cells, compared to the population of cells at the beginning of step (i).

9. The method of any one of claims 1-8, wherein:

(a) steps (i) and/or (ii) are performed in cell media comprising IL-2, IL-15, IL-7, IL-21, IL-6, a LSD1 inhibitor, a MALT1 inhibitor, or a combination thereof, optionally wherein said IL-15 is hetIL-15 or IL15/sIL15Ra and/or said IL-6 is IL-6/IL-6Ra;

(b) steps (i) and/or (ii) are performed in serum-free media comprising IL-2, IL-15, IL-7, IL-21, IL-6, a LSD1 inhibitor, a MALT1 inhibitor, or a combination thereof, optionally wherein said IL-15 is hetIL-15 or IL15/sIL15Ra and/or said IL-6 is IL-6/IL-6Ra;

(c) steps (i) and/or (ii) are performed in serum-free cell media comprising a serum replacement; and/or

(d) the serum replacement is CTS™ Immune Cell Serum Replacement (ICSR).

10. The method of any one of claims 1-9, further comprising prior to step (i):

(I) (iv) optionally receiving a fresh leukapheresis product or an alternative source of hematopoietic tissue such as a fresh whole blood product, a fresh bone marrow product, or a fresh tumor or organ biopsy or removal, or a fresh product from thymectomy from an entity, for example, a laboratory, hospital, or healthcare provider, and

(v) isolating the population of cells contacted in step (i) from a fresh leukapheresis product or an alternative source of hematopoietic tissue such as a fresh whole blood product, a fresh bone marrow product, a fresh tumor or organ biopsy or removal, or a fresh product from thymectomy, optionally wherein:

step (iii) is performed no later than 35 hours after the beginning of step (v), or the population of cells from step (iii) are not expanded, or expanded by no more than 40% compared to the population of cells at the end of step (v); or

(II) receiving cryopreserved T cells isolated from a leukapheresis product or an alternative source of hematopoietic tissue such as cryopreserved T cells isolated from whole blood, bone marrow, tumor or organ biopsy or removal, or thymectomy from an entity, for example, a laboratory, hospital, or healthcare provider; or

(III) (iv) optionally receiving a cryopreserved leukapheresis product or an alternative source of hematopoietic tissue such as a cryopreserved whole blood product, a cryopreserved bone marrow product, a cryopreserved tumor or organ biopsy or removal, or a cryopreserved product from thymectomy from an entity, for example, a laboratory, hospital, or healthcare provider, and

(v) isolating the population of cells contacted in step (i) from a cryopreserved leukapheresis product or an alternative source of hematopoietic tissue such as a cryopreserved whole blood product, a cryopreserved bone marrow product, a cryopreserved tumor or organ biopsy or removal, or a cryopreserved product from thymectomy, optionally wherein:

step (iii) is performed no later than 35 hours after the beginning of step (v), or the population of cells from step (iii) are not expanded, or expanded by no more than 5 40% compared to the population of cells at the end of step (v).

11. The method of any one of claims 1-10, further comprising step (vi):

culturing a portion of the population of cells from step (iii) for at least 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, or 7 days, for example, at least 2 days and no more than 7 days, and measuring CAR (e.g., CCAR) expression level in the portion (for example, measuring the percentage of viable, CAR-expressing cells (e.g., CCAR-expressing cells) in the portion), optionally wherein:

step (iii) comprises harvesting and freezing the population of cells and step (vi) comprises thawing a portion of the population of cells from step (iii), culturing the portion for at least 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, or 7 days, for example, at least 2 days and no more than 7 days, and measuring CAR (e.g., CCAR) expression level in the portion.

12. A method of making a population of cells (for example, T cells) that comprise:

a first nucleic acid molecule that encodes a controllable chimeric antigen receptor (CCAR), or

a second nucleic acid molecule that encodes a chimeric antigen receptor (CAR) and a regulatory molecule,

the method comprising:

(1) contacting a population of cells (for example, T cells, for example, T cells isolated from a frozen leukapheresis product) with a cytokine chosen from IL-2, IL-7, IL-15, IL-21, IL-6, or a combination thereof,

(2) contacting the population of cells (for example, T cells) with a first nucleic acid molecule (for example, a DNA or RNA molecule) encoding a CCAR or a second nucleic acid molecule (for example, a DNA or RNA molecule) encoding a CAR and a regulatory molecule, thereby providing a population of cells (for example, T cells) comprising the first or second nucleic acid molecule, and

(3) harvesting the population of cells (for example, T cells) for storage (for example, reformulating the population of cells in cryopreservation media) or administration, wherein:

(a) step (2) is performed together with step (1) or no later than 5 hours after the beginning of step (1), for example, no later than 1, 2, 3, 4, or 5 hours after the beginning of step (1), and

step (3) is performed no later than 26 hours after the beginning of step (1), for example, no later than 22, 23, or 24 hours after the beginning of step (1), for example, no later than 24 hours after the beginning of step (1), or

(b) the population of cells from step (3) are not expanded, or expanded by no more than 5, 10, 15, 20, 25, 30, 35, or 40%, for example, no more than 10%, for example, as assessed by the number of living cells, compared to the population of cells at the beginning of step (1),

optionally wherein the first or second nucleic acid molecule in step (2) is on a viral vector, optionally wherein the first or second nucleic acid molecule in step (ii) is an RNA molecule on a viral vector, optionally wherein step (ii) comprises transducing the population of cells (for example, T cells) with a viral vector comprising the first or second nucleic acid molecule,

wherein optionally, step (1) comprises contacting the population of cells (e.g., T cells) with IL-2, IL-7, IL-15, hetIL-15 (IL15/sIL-15Ra), IL-21, IL-6, or IL-6/sIL-6Ra; IL-7 and/or IL-15 (e.g., hetIL-15 (IL15/sIL-15Ra)); IL-7 and IL-21; IL-15 (e.g., hetIL-15 (IL15/sIL-15Ra)) and

IL-21; IL-7, IL-15 (e.g., hetIL-15 (IL15/sIL-15Ra)), and IL-21; IL-6 (e.g., IL-6/sIL-6Ra) and IL-15 (e.g., hetIL-15 (IL15/sIL-15Ra)); or IL-2 and IL-6 (e.g., IL-6/sIL-6Ra).

13. The method of claim 12, wherein:

(a) the population of cells from step (3) shows a higher percentage of naïve cells among cells that comprise the first or second nucleic acid molecule (for example, at least 10, 15, 20, 25, 30, 35, or 40% higher), compared with cells made by an otherwise similar method which further comprises contacting the population of cells with, for example, an anti-CD3 antibody;

(b) the population of cells from step (3) shows a higher percentage of naïve cells, for example, naïve T cells, for example, CD45RA⁺ CD45RO⁻ CCR7⁺ T cells (for example, at least 10, 20, 30, or 40% higher), compared with cells made by an otherwise similar method in which step (3) is performed more than 26 hours after the beginning of step (1), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (1);

(c) the population of cells from step (3) shows a higher percentage of naïve cells, for example, naïve T cells, for example, CD45RA⁺ CD45RO⁻ CCR7⁺ T cells (for example, at least 10, 20, 30, or 40% higher), compared with cells made by an otherwise similar method which further comprises, after step (2) and prior to step (3), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days;

(d) the population of cells from step (3), after being administered in vivo, persists longer or expands at a higher level (for example, as assessed using methods described in Example 1 with respect to FIG. 4C), compared with cells made by an otherwise similar method in which step (3) is performed more than 26 hours after the beginning of step (1), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (1);

(e) the population of cells from step (3), after being administered in vivo, persists longer or expands at a higher level (for example, as assessed using methods described in Example 1 with respect to FIG. 4C), compared with cells made by an otherwise similar method which further comprises, after step (2) and prior to step (3), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days;

(f) the population of cells from step (3) are not expanded, or expanded by no more than 5, 10, 15, 20, 25, 30, 35, or 40%, for example, no more than 10%, for example, as assessed by the

number of living cells, compared to the population of cells at the beginning of step (1), optionally wherein the number of living cells in the population of cells from step (3) decreases from the number of living cells in the population of cells at the beginning of step (1); (g) the population of cells from step (3) are not expanded, or expanded by less than 2 hours, for example, less than 1 or 1.5 hours, compared to the population of cells at the beginning of step (1);

(h) the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ T cells, in the population of cells from step (3):

is the same as or differs by no more than 5 or 10% from the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ cells, in the population of cells at the beginning of step (1), or

is increased, for example, increased by at least 10 or 20%, as compared to the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ cells, in the population of cells at the beginning of step (1);

(i) wherein steps (1) and/or (2) are performed in cell media comprising: no more than 5, 4, 3, 2, 1, or 0% serum, optionally wherein steps (1) and/or (2) are performed in cell media comprising about 2% serum, or a LSD1 inhibitor or a MALT1 inhibitor; and/or

(j) further comprising receiving a cryopreserved leukapheresis product (or an alternative source of hematopoietic tissue such as a cryopreserved whole blood product, a cryopreserved bone marrow product, or a cryopreserved tumor or organ biopsy or removal (for example, a cryopreserved product from thymectomy)) from an entity, for example, a laboratory, hospital, or healthcare provider.

14. The method of any one of claims 1-13, wherein:

(a) the population of cells at the beginning of step (i) or step (1) has been enriched for IL6R-expressing cells, optionally, cells that are positive for IL6R α and/or IL6R β ;

(b) the population of cells at the beginning of step (i) or step (1) comprises no less than 50% of IL6R-expressing cells, optionally, cells that are positive for IL6R α and/or IL6R β ;

(c) steps (i) and (ii) or steps (1) and (2) are performed in cell media comprising IL-15 or hetIL-15 (IL15/sIL-15Ra)), optionally wherein IL-15 increases the ability of the population of

cells to expand, for example, 10, 15, 20, or 25 days later and/or IL-15 increases the percentage of IL6R β -expressing cells in the population of cells.

15. The method of any one of claims 1-14, wherein the CCAR or CAR comprises an antigen binding domain, a transmembrane domain, and/or an intracellular signaling domain, optionally wherein:

- (I) the antigen binding domain binds to an antigen chosen from: CD19, CD20, CD22, BCMA, mesothelin, EGFRvIII, GD2, Tn antigen, sTn antigen, Tn-O-Glycopeptides, sTn-O-Glycopeptides, PSMA, CD97, TAG72, CD44v6, CEA, EPCAM, KIT, IL-13Ra2, leguman, GD3, CD171, IL-11Ra, PSCA, MAD-CT-1, MAD-CT-2, VEGFR2, LewisY, CD24, PDGFR-beta, SSEA-4, folate receptor alpha, ERBBs (for example, ERBB2), Her2/neu, MUC1, EGFR, NCAM, Ephrin B2, CAIX, LMP2, sLe, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, TEM7R, FAP, Legumain, HPV E6 or E7, ML-IAP, CLDN6, TSHR, GPRC5D, ALK, Polysialic acid, Fos-related antigen, neutrophil elastase, TRP-2, CYP1B1, sperm protein 17, beta human chorionic gonadotropin, AFP, thyroglobulin, PLAC1, globoH, RAGE1, MN-CA IX, human telomerase reverse transcriptase, intestinal carboxyl esterase, mut hsp 70-2, NA-17, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, NY-ESO-1, GPR20, Ly6k, OR51E2, TARP, GFR α 4, or a peptide of any of these antigens presented on MHC; and/or
- (II) the antigen binding domain comprises a VH and a VL, wherein the VH and VL are connected by a linker, optionally wherein the linker comprises the amino acid sequence of SEQ ID NO: 63 or 104; and/or
- (III) (a) the transmembrane domain comprises a transmembrane domain of a protein chosen from the alpha, beta or zeta chain of T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154,
 - (b) the transmembrane domain comprises a transmembrane domain of CD8,
 - (c) the transmembrane domain comprises the amino acid sequence of SEQ ID NO: 6, or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof, or
 - (d) the first or second nucleic acid molecule comprises a nucleic acid sequence encoding the transmembrane domain, wherein the nucleic acid sequence comprises the nucleic acid

sequence of SEQ ID NO: 17, or a nucleic acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof; and/or

(IV) the antigen binding domain is connected to the transmembrane domain by a hinge region, optionally wherein:

(a) the hinge region comprises the amino acid sequence of SEQ ID NO: 2, 3, or 4, or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof, or

(b) the first or second nucleic acid molecule comprises a nucleic acid sequence encoding the hinge region, wherein the nucleic acid sequence comprises the nucleic acid sequence of SEQ ID NO: 13, 14, or 15, or a nucleic acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof; and/or

(V) the intracellular signaling domain comprises a primary signaling domain, optionally wherein the primary signaling domain comprises a functional signaling domain derived from CD3 zeta, TCR zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, CD278 (ICOS), FcεRI, DAP10, DAP12, or CD66d, optionally wherein:

(a) the primary signaling domain comprises a functional signaling domain derived from CD3 zeta,

(b) the primary signaling domain comprises the amino acid sequence of SEQ ID NO: 9 or 10, or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof, or

(c) the first or second nucleic acid molecule comprises a nucleic acid sequence encoding the primary signaling domain, wherein the nucleic acid sequence comprises the nucleic acid sequence of SEQ ID NO: 20 or 21, or a nucleic acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof; and/or

(VI) the intracellular signaling domain comprises a costimulatory signaling domain, optionally wherein the costimulatory signaling domain comprises a functional signaling domain derived from a MHC class I molecule, a TNF receptor protein, an Immunoglobulin-like protein, a cytokine receptor, an integrin, a signaling lymphocytic activation molecule (SLAM protein), an activating NK cell receptor, BTLA, a Toll ligand receptor, OX40, CD2, CD7, CD27, CD28, CD30, CD40, CD80, ICAM-1, 4-1BB (CD137), B7-H3, ICOS (CD278), GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a,

ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, ITGB7, NKG2D, NKG2C, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, CD28-OX40, CD28-4-1BB, or a ligand that specifically binds with CD83, optionally wherein:

(a) the costimulatory signaling domain comprises a functional signaling domain derived from 4-1BB,

(b) the costimulatory signaling domain comprises the amino acid sequence of SEQ ID NO: 7, or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof, or

(c) the first or second nucleic acid molecule comprises a nucleic acid sequence encoding the costimulatory signaling domain, wherein the nucleic acid sequence comprises the nucleic acid sequence of SEQ ID NO: 18, or a nucleic acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof; and/or

(VII) the intracellular signaling domain comprises a functional signaling domain derived from 4-1BB and a functional signaling domain derived from CD3 zeta, optionally wherein the intracellular signaling domain comprises the amino acid sequence of SEQ ID NO: 7 (or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof) and the amino acid sequence of SEQ ID NO: 9 or 10 (or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof), optionally wherein the intracellular signaling domain comprises the amino acid sequence of SEQ ID NO: 7 and the amino acid sequence of SEQ ID NO: 9 or 10; and/or

(VIII) the CCAR or CAR further comprises a leader sequence comprising the amino acid sequence of SEQ ID NO: 1.

16. The method of claim 15, wherein the antigen binding domain comprises a complementarity determining region (CDR), heavy chain variable region (VH), light chain variable region (VL), or scFv sequence, wherein:

(a) the antigen binding domain binds to BCMA and comprises a CDR, VH, VL, scFv or CAR sequence disclosed in Tables 3-15, or a sequence having at least 80%, 85%, 90%, 95%, or 99% identity thereto;

(b) the antigen binding domain binds to CD19 and comprises a CDR, VH, VL, scFv or CAR sequence disclosed in Table 2, or a sequence having at least 80%, 85%, 90%, 95%, or 99% identity thereto;

(c) the antigen binding domain binds to CD20 and comprises a CDR, VH, VL, scFv or CAR sequence disclosed herein, or a sequence having at least 80%, 85%, 90%, 95%, or 99% identity thereto; or

(d) the antigen binding domain binds to CD22 and comprises a CDR, VH, VL, scFv or CAR sequence disclosed herein, or a sequence having at least 80%, 85%, 90%, 95%, or 99% identity thereto.

17. A population of cells that comprise the first or second nucleic acid molecule made by the method of any one of claims 1-16, optionally wherein the population of cells that comprise the first or the second nucleic acid molecule are autologous or allogeneic T cells or NK cells that comprise the first or second nucleic acid molecule.

18. A population of cells engineered to comprise:

a first nucleic acid molecule that encodes a CCAR, or

a second nucleic acid molecule that encodes a CAR and a regulatory molecule,

said population comprising:

(a) about the same percentage of naïve cells as compared to the percentage of naïve cells in the same population of cells prior to being engineered to comprise the first or second nucleic acid molecule;

(b) a change within about 5% to about 10% of naïve cells for example, as compared to the percentage of naïve cells in the same population of cells prior to being engineered to comprise the first or second nucleic acid molecule;

(c) an increased percentage of naïve cells for example, increased by at least 1.2-fold, as compared to the percentage of naïve cells in the same population of cells prior to being engineered to comprise the first or second nucleic acid molecule;

(d) about the same percentage of central memory cells as compared to the percentage of central memory cells in the same population of cells prior to being engineered to comprise the first or second nucleic acid molecule;

(e) a change within about 5% to about 10% of central memory cells as compared to the percentage of central memory cells in the same population of cells prior to being engineered to comprise the first or second nucleic acid molecule;

(f) a decreased percentage of central memory cells for example, decreased by at least 20%, as compared to the percentage of central memory cells in the same population of cells prior to being engineered to comprise the first or second nucleic acid molecule;

(g) about the same percentage of stem memory T cells as compared to the percentage of stem memory T cells in the same population of cells prior to being engineered to comprise the first or second nucleic acid molecule;

(h) a change within about 5% to about 10% of stem memory T cells as compared to the percentage of stem memory T cells in the same population of cells prior to being engineered to comprise the first or second nucleic acid molecule; or

(i) an increased percentage of stem memory T cells as compared to the percentage of stem memory T cells in the same population of cells prior to being engineered to comprise the first or second nucleic acid molecule.

19. A population of cells engineered to comprise:

a first nucleic acid molecule that encodes a CCAR, or

a second nucleic acid molecule that encodes a CAR and a regulatory molecule,

wherein:

(a) the median GeneSetScore (Up TEM vs. Down TSCM) of the population of cells is about the same as or differs by no more than (for example, increased by no more than) about 25, 50, 75, 100, or 125% from the median GeneSetScore (Up TEM vs. Down TSCM) of the same population of cells prior to being engineered to comprise the first or second nucleic acid molecule;

(b) the median GeneSetScore (Up Treg vs. Down Teff) of the population of cells is about the same as or differs by no more than (for example, increased by no more than) about 25, 50,

100, 150, or 200% from the median GeneSetScore (Up Treg vs. Down Teff) of the population of cells prior to being engineered to comprise the first or second nucleic acid molecule;

(c) the median GeneSetScore (Down stemness) of the population of cells is about the same as or differs by no more than (for example, increased by no more than) about 25, 50, 100, 150, 200, or 250% from the median GeneSetScore (Down stemness) of the population of cells prior to being engineered to comprise the first or second nucleic acid molecule;

(d) the median GeneSetScore (Up hypoxia) of the population of cells is about the same as or differs by no more than (for example, increased by no more than) about 125, 150, 175, or 200% from the median GeneSetScore (Up hypoxia) of the population of cells prior to being engineered to comprise the first or second nucleic acid molecule; or

(e) the median GeneSetScore (Up autophagy) of the population of cells is about the same as or differs by no more than (for example, increased by no more than) about 180, 190, 200, or 210% from the median GeneSetScore (Up autophagy) of the population of cells prior to being engineered to comprise the first or second nucleic acid molecule.

20. The method of any one of claims 1-16 or the population of cells of any one of claims 17-19, wherein the population of cells comprise the first nucleic acid molecule that encodes a CCAR.

21. The method of claim 20 or the population of cells of claim 20, wherein the CCAR is:

(a) a fusion polypeptide comprising a degradation polypeptide and a CAR polypeptide;
(b) a fusion polypeptide comprising a degradation domain and a CAR polypeptide, optionally wherein the degradation domain is separated from the CAR polypeptide by a heterologous protease cleavage site, optionally wherein the CCAR comprises, from the N-terminus to the C-terminus, the degradation domain, the heterologous protease cleavage site, and the CAR polypeptide; or

(c) a regulatable CAR (RCAR).

22. The method of claim 21 or the population of cells of claim 21, wherein:

(I) (i) the degradation polypeptide comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 310-315, 320-324, 337-339, 360-361, 367-369 and 374, or a sequence having at least 85, 87, 90, 95, 97, 98, 99, or 100% identity thereto, optionally

wherein the degradation polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 312;

(ii) the degradation polypeptide comprises a beta turn of IKZF1 or IKZF3, or a sequence having at least 85, 87, 90, 95, 97, 98, 99, or 100% identity thereto, optionally wherein the degradation polypeptide comprises a beta hairpin or a beta strand of IKZF1 or IKZF3, or a sequence having at least 85, 87, 90, 95, 97, 98, 99, or 100% identity thereto;

(iii) the degradation polypeptide comprises an alpha helix of IKZF1 or IKZF3, or a sequence having at least 85, 87, 90, 95, 97, 98, 99, or 100% identity thereto;

(iv) the degradation polypeptide comprises, from the N-terminus to the C-terminus, a first beta strand, a beta hairpin, a second beta strand, and a first alpha helix of IKZF1 or IKZF3, or a sequence having at least 85, 87, 90, 95, 97, 98, 99, or 100% identity thereto;

(v) the degradation polypeptide comprises, from the N-terminus to the C-terminus, a first beta strand, a beta hairpin, a second beta strand, a first alpha helix, and a second alpha helix of IKZF1 or IKZF3, or a sequence having at least 85, 87, 90, 95, 97, 98, 99, or 100% identity thereto, optionally wherein the beta hairpin and the second alpha helix are separated by no more than 60, 50, 40, or 30 amino acid residues;

(vi) the degradation polypeptide comprises about 10 to about 95 amino acid residues, about 15 to about 90 amino acid residues, about 20 to about 85 amino acid residues, about 25 to about 80 amino acid residues, about 30 to about 75 amino acid residues, about 35 to about 70 amino acid residues, about 40 to about 65 amino acid residues, about 45 to about 65 amino acid residues, about 50 to about 65 amino acid residues, or about 55 to about 65 amino acid residues of IKZF1 or IKZF3, or a sequence having at least 85, 87, 90, 95, 97, 98, 99, or 100% identity thereto;

(vii) the degradation polypeptide comprises at least 10 amino acids, at least 15 amino acids, at least 20 amino acids, at least 25 amino acids, at least 30 amino acids, at least 35 amino acids, at least 40 amino acids, at least 45 amino acids, at least 50 amino acids, at least 55 amino acids, at least 60 amino acids, at least 65 amino acids, at least 70 amino acids, at least 75 amino acids, at least 80 amino acids, at least 85 amino acids, at least 90 amino acids, at least 90 amino acids, or at least 95 amino acids of IKZF1 or IKZF3, or a sequence having at least 85, 87, 90, 95, 97, 98, 99, or 100% identity thereto;

(viii) the association of the fusion polypeptide with cereblon (CRBN) in the absence of COF1 or COF2, e.g., an immunomodulatory imide drug (IMiD), e.g., lenalidomide, pomalidomide, or thalidomide, is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20%, of the association of the fusion polypeptide with CRBN in the presence of COF1 or COF2, e.g., an IMiD, e.g., lenalidomide, pomalidomide, or thalidomide;

(ix) the ubiquitination of the fusion polypeptide in the absence of COF1 or COF2, e.g., an IMiD, e.g., lenalidomide, pomalidomide, or thalidomide, is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the fusion polypeptide in the presence of COF1 or COF2, e.g., an IMiD, e.g., lenalidomide, pomalidomide, or thalidomide;

(x) the degradation of the fusion polypeptide in the absence of COF1 or COF2, e.g., an IMiD, e.g., lenalidomide, pomalidomide, or thalidomide, is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70% of the degradation of the fusion polypeptide in the presence of COF1 or COF2, e.g., an IMiD, e.g., lenalidomide, pomalidomide, or thalidomide; and/or

(xi) the expression level of the fusion polypeptide in the presence of COF1 or COF2, e.g., an IMiD, e.g., lenalidomide, pomalidomide, or thalidomide, is decreased by, e.g., at least 40, 50, 60, 70, 80, 90, or 99%, as compared to the expression level of the fusion polypeptide in the absence of COF1 or COF2, e.g., an IMiD, e.g., lenalidomide, pomalidomide, or thalidomide; or

(II) (i) the degradation polypeptide comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 375-377, or a sequence having at least 85, 87, 90, 95, 97, 98, 99, or 100% identity thereto, optionally wherein the degradation polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 375;

(ii) the degradation polypeptide comprises a beta turn of IKZF2, or a sequence having at least 85, 87, 90, 95, 97, 98, 99, or 100% identity thereto, optionally wherein the degradation polypeptide comprises a beta hairpin or a beta strand of IKZF2, or a sequence having at least 85, 87, 90, 95, 97, 98, 99, or 100% identity thereto;

(iii) the degradation polypeptide comprises an alpha helix of IKZF2, or a sequence having at least 85, 87, 90, 95, 97, 98, 99, or 100% identity thereto;

(iv) the degradation polypeptide comprises, from the N-terminus to the C-terminus, a first beta strand, a beta hairpin, a second beta strand, and a first alpha helix of IKZF2, or a sequence having at least 85, 87, 90, 95, 97, 98, 99, or 100% identity thereto;

(v) the degradation polypeptide comprises, from the N-terminus to the C-terminus, a first beta strand, a beta hairpin, a second beta strand, a first alpha helix, and a second alpha helix of IKZF2, or a sequence having at least 85, 87, 90, 95, 97, 98, 99, or 100% identity thereto, optionally wherein the beta hairpin and the second alpha helix are separated by no more than 60, 50, 40, or 30 amino acid residues;

(vi) the degradation polypeptide comprises about 10 to about 95 amino acid residues, about 15 to about 90 amino acid residues, about 20 to about 85 amino acid residues, about 25 to about 80 amino acid residues, about 30 to about 75 amino acid residues, about 35 to about 70 amino acid residues, about 40 to about 65 amino acid residues, about 45 to about 65 amino acid residues, about 50 to about 65 amino acid residues, or about 55 to about 65 amino acid residues of IKZF2, or a sequence having at least 85, 87, 90, 95, 97, 98, 99, or 100% identity thereto;

(vii) the degradation polypeptide comprises at least 10 amino acids, at least 15 amino acids, at least 20 amino acids, at least 25 amino acids, at least 30 amino acids, at least 35 amino acids, at least 40 amino acids, at least 45 amino acids, at least 50 amino acids, at least 55 amino acids, at least 60 amino acids, at least 65 amino acids, at least 70 amino acids, at least 75 amino acids, at least 80 amino acids, at least 85 amino acids, at least 90 amino acids, at least 90 amino acids, or at least 95 amino acids of IKZF2, or a sequence having at least 85, 87, 90, 95, 97, 98, 99, or 100% identity thereto;

(viii) the association of the fusion polypeptide with cereblon (CRBN) in the absence of COF3, e.g., Compound I-112 disclosed in Table 29, is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20%, of the association of the fusion polypeptide with CRBN in the presence of COF3, e.g., Compound I-112 disclosed in Table 29;

(ix) the ubiquitination of the fusion polypeptide in the absence of COF3, e.g., Compound I-112 disclosed in Table 29, is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the fusion polypeptide in the presence of COF3, e.g., Compound I-112 disclosed in Table 29;

(x) the degradation of the fusion polypeptide in the absence of COF3, e.g., Compound I-112 disclosed in Table 29, is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70% of the degradation of the fusion polypeptide in the presence of COF3, e.g., Compound I-112 disclosed in Table 29; and/or

(viii) the expression level of the fusion polypeptide in the presence of COF3, e.g., Compound I-112 disclosed in Table 29, is decreased by, e.g., at least 40, 50, 60, 70, 80, 90, or 99%, as compared to the expression level of the fusion polypeptide in the absence of COF3, e.g., Compound I-112 disclosed in Table 29;

optionally wherein:

- (a) the degradation polypeptide is fused to the CAR polypeptide;
- (b) the degradation polypeptide and the CAR polypeptide are linked by a peptide bond;
- (c) the degradation polypeptide and the CAR polypeptide are linked by a bond other than a peptide bond;
- (d) the degradation polypeptide is linked directly to the CAR polypeptide;
- (e) the degradation polypeptide is linked indirectly to the CAR polypeptide;
- (f) the degradation polypeptide and the CAR polypeptide are operatively linked via a linker, e.g., a glycine-serine linker, e.g., a linker comprising the amino acid sequence of GGGGSGGGGTGGGGSG (SEQ ID NO: 335);
- (g) the degradation polypeptide is linked to the C-terminus or N-terminus of the CAR polypeptide; or
- (h) the degradation polypeptide is at the middle of the CAR polypeptide.

23. The method of claim 21 or the population of cells of claim 21, wherein:

- (I) (i) the degradation domain has a first state associated with a first level of expression of the fusion polypeptide and a second state associated with a second level of expression of the fusion polypeptide, wherein the second level is increased, e.g., by at least 2-, 3-, 4-, 5-, 10-, 20- or 30-fold over the first level in the presence of a stabilization compound, optionally wherein:
 - (a) in the absence of the stabilization compound, the fusion polypeptide is degraded by a cellular degradation pathway, e.g., at least 50%, 60%, 70%, 80%, 90% or greater of the fusion polypeptide is degraded;
 - (b) in the presence of the stabilization compound, the degradation domain assumes a conformation more resistant to cellular degradation relative to a conformation in the absence of the stabilization compound; and/or

- (c) in the presence of the stabilization compound, the conformation of the fusion polypeptide is more permissive to cleavage at the heterologous protease cleavage site relative to a conformation in the absence of the stabilization compound;
- (ii) the degradation domain is chosen from an estrogen receptor (ER) domain, an FKB protein (FKBP) domain, or a dihydrofolate reductase (DHFR) domain, optionally wherein:
- (a) the degradation domain is an estrogen receptor (ER) domain, e.g., the degradation domain comprising the amino acid sequence of SEQ ID NO: 342 or 344, or a sequence having at least 85, 87, 90, 95, 97, 98, 99, or 100% identity thereto, optionally wherein the stabilization compound is bazedoxifene or 4-hydroxy tamoxifen (4-OHT), or a pharmaceutically acceptable salt thereof;
- (b) the degradation domain is an FKB protein (FKBP) domain, e.g., the degradation domain comprising the amino acid sequence of SEQ ID NO: 346, or a sequence having at least 85, 87, 90, 95, 97, 98, 99, or 100% identity thereto, optionally wherein the stabilization compound is Shield-1, or a pharmaceutically acceptable salt thereof; or
- (c) the degradation domain is a dihydrofolate reductase (DHFR) domain, e.g., the degradation domain comprising the amino acid sequence of SEQ ID NO: 347, or a sequence having at least 85, 87, 90, 95, 97, 98, 99, or 100% identity thereto, optionally wherein the stabilization compound is trimethoprim, or a pharmaceutically acceptable salt thereof; and/or
- (II) (i) the heterologous protease cleavage site is cleaved by a mammalian intracellular protease, optionally wherein:
- (a) the heterologous protease cleavage site is cleaved by a protease selected from the group consisting of furin, PCSK1, PCSK5, PCSK6, PCSK7, cathepsin B, Granzyme B, Factor XA, Enterokinase, genenase, sortase, precision protease, thrombin, TEV protease, and elastase 1;
- (b) the heterologous protease cleavage site comprises a sequence having a cleavage motif selected from the group consisting of RX(K/R)R consensus motif (X can be any amino acid; SEQ ID NO: 348), RXXX[KR]R consensus motif (X can be any amino acid; SEQ ID NO: 349), RRX consensus motif (SEQ ID NO : 350), I-E-P-D-X consensus motif (SEQ ID NO: 351), Ile-Glu/Asp-Gly-Arg (SEQ ID NO : 352), Asp-Asp-

Asp-Asp-Lys (SEQ ID NO: 353), Pro-Gly-Ala-Ala-His-Tyr (SEQ ID NO: 354), LPXTG/A consensus motif (SEQ ID NO: 355), Leu-Glu-Val-Phe-Gln-Gly-Pro (SEQ ID NO: 356), Leu-Val-Pro-Arg-Gly-Ser (SEQ ID NO: 357), E-N-L-Y-F-Q-G (SEQ ID NO: 358), and [AGSV]-X (X can be any amino acid; SEQ ID NO: 359); or

(c) the heterologous protease cleavage site comprises a furin cleavage site selected from the group consisting of RTKR (SEQ ID NO: 378); GTGAEDPRPSRKRRSLGDVG (SEQ ID NO: 379); GTGAEDPRPSRKRR (SEQ ID NO: 381); LQWLEQQVAKRRTKR (SEQ ID NO: 383); GTGAEDPRPSRKRRSLGG (SEQ ID NO: 385); GTGAEDPRPSRKRRSLG (SEQ ID NO: 387); SLNLTESHNSRKRR (SEQ ID NO: 389); CKINGYPKRGRKRR (SEQ ID NO: 391); and SARNRQKR (SEQ ID NO: 336); or

(iii) the heterologous protease cleavage site is cleaved by a mammalian extracellular protease, optionally wherein:

(a) the heterologous protease cleavage site is cleaved by a protease selected from the group consisting of Factor XA, Enterokinase, genenase, sortase, precision protease, thrombin, TEV protease, and elastase 1; or

(b) the heterologous protease cleavage site comprises an amino acid sequence selected from the group consisting of Ile-Glu/Asp-Gly-Arg (SEQ ID NO : 352), Asp-Asp-Asp-Asp-Lys (SEQ ID NO: 353), Pro-Gly-Ala-Ala-His-Tyr (SEQ ID NO: 354), LPXTG/A consensus motif (SEQ ID NO: 355), Leu-Glu-Val-Phe-Gln-Gly-Pro (SEQ ID NO: 356), Leu-Val-Pro-Arg-Gly-Ser (SEQ ID NO: 357), E-N-L-Y-F-Q-G (SEQ ID NO: 358), and [AGSV]-X (X can be any amino acid; SEQ ID NO: 359).

24. The method of claim 21 or the population of cells of claim 21, wherein the RCAR comprises:

(I) (i) an intracellular signaling member comprising: an intracellular signaling domain, e.g., a primary intracellular signaling domain, and a first switch domain;

(ii) an antigen binding member comprising: an antigen binding domain and a second switch domain; and

(iii) a transmembrane domain, optionally wherein the transmembrane domain can be disposed on the intracellular signaling member and/or the antigen binding member; or

(II) (i) an intracellular signaling member comprising: an intracellular signaling domain, e.g., a primary intracellular signaling domain, and a first switch domain;

(ii) an inhibitory extracellular domain member comprising: an inhibitory extracellular domain (e.g., an inhibitory extracellular domain comprising an extracellular domain of B7-H1, B7-1, CD160, P1H, 2B4, PD1, TIM3, CEACAM, LAG3, TIGIT, CTLA-4, BTLA, LAIR1, or TGF-beta receptor, or a sequence having at least 85, 87, 90, 95, 97, 98, 99, or 100% identity thereto), and a second switch domain; and

(iii) a transmembrane domain, optionally wherein the transmembrane domain can be disposed on the intracellular signaling member and/or the inhibitory extracellular domain member; or

(III) (i) an intracellular signaling member comprising: an intracellular signaling domain, e.g., a primary intracellular signaling domain, and a first switch domain;

(ii) a costimulatory extracellular domain member comprising: a costimulatory extracellular domain (e.g., a costimulatory extracellular domain comprising an extracellular domain of ICOS, CD28, VEM, LIGHT, CD40L, 4-1BB, OX40, DR3, GITR, CD30, TIM1, SLAM, CD2, or CD226, or a sequence having at least 85, 87, 90, 95, 97, 98, 99, or 100% identity thereto), and a second switch domain; and

(iii) a transmembrane domain, optionally wherein the transmembrane domain can be disposed on the intracellular signaling member and/or the costimulatory extracellular domain member;

optionally wherein the first and second switch domains can form a dimerization switch, e.g., in the presence of a dimerization molecule, optionally wherein:

(a) the dimerization switch is an intracellular dimerization switch or an extracellular dimerization switch;

(b) the dimerization switch is a homodimerization switch or a heterodimerization switch;

(c) the dimerization switch comprises a FKBP-FRB based switch, e.g., a dimerization switch comprising a switch domain comprising a FRB binding fragment or analog of FKBP and a switch domain comprising a FKBP binding fragment or analog of FRB, optionally wherein the FKBP binding fragment or analog of FRB comprises one or more mutations disclosed herein (e.g., one or more mutations chosen from an E2032 mutation, a T2098 mutation, or an E2032

and a T2098 mutation), optionally wherein the dimerization molecule is an mTOR inhibitor, e.g., a rapamycin analogue, e.g., RAD001; and/or

(d) the antigen binding domain binds to a target antigen but does not promote an immune effector response of a T cell, until the dimerization molecule is present.

25. The method claim 24 or the population of cells of claim 24, wherein:

(i) the intracellular signaling member comprises a primary intracellular signaling domain, e.g., a primary intracellular signaling domain disclosed herein, e.g., a CD3zeta domain;

(ii) the intracellular signaling member comprises a costimulatory signaling domain, e.g., a costimulatory signaling domain disclosed herein, e.g., a 4-1BB domain or a CD28 domain;

(iii) the antigen binding member does not comprise a primary intracellular signaling domain, e.g., the antigen binding member comprises a costimulatory signaling domain and does not comprise a primary intracellular signaling domain;

(iv) the inhibitory extracellular domain member does not comprise a primary intracellular signaling domain, e.g., the inhibitory extracellular domain member comprises a costimulatory signaling domain and does not comprise a primary intracellular signaling domain; and/or

(v) the costimulatory extracellular domain member does not comprise a primary intracellular signaling domain, e.g., the costimulatory extracellular domain member comprises a costimulatory signaling domain and does not comprise a primary intracellular signaling domain.

26. The method of any one of claims 1-16 or the population of cells of any one of claims 17-19, wherein the population of cells comprise the second nucleic acid molecule that encodes a CAR and a regulatory molecule, wherein the second nucleic acid molecule comprises a nucleic acid sequence encoding the CAR and a nucleic acid sequence encoding the regulatory molecule, optionally wherein the nucleic acid sequence encoding the CAR and the nucleic acid sequence encoding the regulatory molecule are:

(i) disposed on a single nucleic acid molecule, e.g., wherein the nucleic acid sequence encoding the CAR and the nucleic acid sequence encoding the regulatory molecule are separated by a nucleic acid sequence encoding a self-cleavage site; or

(ii) disposed on separate nucleic acid molecules.

27. The method of claim 26 or the population of cells of claim 26, wherein the regulatory molecule comprises:

(a) a chimeric protein comprising: (i) a multimeric ligand binding region and (ii) a caspase 9 molecule; optionally, wherein

the caspase 9 molecule is a truncated caspase 9, optionally wherein the caspase 9 molecule lacks the caspase recruitment domain; and/or

the multimeric ligand binding region is selected from the group consisting of FKBP, cyclophilin receptor, steroid receptor, tetracycline receptor, heavy chain antibody subunit, light chain antibody subunit, single chain antibodies comprised of heavy and light chain variable regions in tandem separated by a flexible linker domain, and mutated sequences thereof, optionally wherein the multimeric ligand binding region is an FKBP12 region;

(b) a truncated epidermal growth factor receptor (EGFRt); optionally wherein the EGFRt has 1, 2, 3, 4, or all of the following properties:

(i) the EGFRt comprises one or both of an EGFR Domain III and an EGFR Domain IV;

(ii) the EGFRt does not comprise 1, 2, 3, or all of: an EGFR Domain I, an EGFR Domain II, an EGFR juxtamembrane domain, and an EGFR tyrosine kinase domain;

(iii) the EGFRt does not mediate signaling or trafficking;

(iv) the EGFRt does not bind an endogenous EGFR ligand, e.g., epidermal growth factor (EGF); and

(v) the EGFRt binds to an anti-EGFR-antibody molecule (e.g., cetuximab, matuzumab, necitumumab and panitumumab), an EGFR-specific siRNA, or a small molecule that targets EGFR.

28. A pharmaceutical composition comprising the population of cells of any one of claims 17-27 and a pharmaceutically acceptable carrier.

29. Use of the population of cells of any one of claims 17-27 or the pharmaceutical composition of claim 28 in the manufacture of a medicament for:

(a) increasing an immune response in a subject; or

(b) treating a cancer in a subject; optionally wherein:

(i) the cancer is a solid cancer, optionally wherein the solid cancer is chosen from: one or more of mesothelioma, malignant pleural mesothelioma, non-small cell lung cancer, small cell lung cancer, squamous cell lung cancer, large cell lung cancer, pancreatic cancer, pancreatic ductal adenocarcinoma, esophageal adenocarcinoma, breast cancer, glioblastoma, ovarian cancer, colorectal cancer, prostate cancer, cervical cancer, skin cancer, melanoma, renal cancer, liver cancer, brain cancer, thymoma, sarcoma, carcinoma, uterine cancer, kidney cancer, gastrointestinal cancer, urothelial cancer, pharynx cancer, head and neck cancer, rectal cancer, esophagus cancer, or bladder cancer, or a metastasis thereof; or

(ii) the cancer is a liquid cancer, optionally wherein the liquid cancer is chosen from: chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), multiple myeloma, acute lymphoid leukemia (ALL), Hodgkin lymphoma, B-cell acute lymphoid leukemia (BALL), T-cell acute lymphoid leukemia (TALL), small lymphocytic lymphoma (SLL), B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma (DLBCL), DLBCL associated with chronic inflammation, chronic myeloid leukemia, myeloproliferative neoplasms, follicular lymphoma, pediatric follicular lymphoma, hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma (extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue), Marginal zone lymphoma, myelodysplasia, myelodysplastic syndrome, non-Hodgkin lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, splenic marginal zone lymphoma, splenic lymphoma/leukemia, splenic diffuse red pulp small B-cell lymphoma, hairy cell leukemia-variant, lymphoplasmacytic lymphoma, a heavy chain disease, plasma cell myeloma, solitary plasmacytoma of bone, extraosseous plasmacytoma, nodal marginal zone lymphoma, pediatric nodal marginal zone lymphoma, primary cutaneous follicle center lymphoma, lymphomatoid granulomatosis, primary mediastinal (thymic) large B-cell lymphoma, intravascular large B-cell lymphoma, ALK+ large B-cell lymphoma, large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease,

primary effusion lymphoma, B-cell lymphoma, acute myeloid leukemia (AML), or unclassifiable lymphoma;

(iii) the cancer is selected from CLL, SLL, DLBCL, or relapsed and/or refractory DLBCL.

30. The use of claim 29, wherein:

(a) the use further comprising use of a second therapeutic agent to the subject; and/or

(b) the population of cells is formulated for administration at a dose determined based on the percentage of CAR-expressing cells (e.g., CCAR-expressing cells) measured in claim 10.

31. The use of claim 29 or 30, wherein the use further comprises, use of an effective amount of IMiD (e.g., thalidomide and derivatives thereof, e.g., lenalidomide, pomalidomide, and thalidomide) or Compound I-112 after use of the population of cells or the pharmaceutical composition, optionally wherein:

a) the subject has developed, is developing, or is anticipated to develop an adverse reaction after the use of the population of cells or the pharmaceutical composition,

b) the use of IMiD or Compound I-112 is in response to an occurrence of an adverse reaction in the subject, or in response to an anticipation of an occurrence of an adverse reaction in the subject, and/or

c) the use of IMiD or Compound I-112 reduces or prevents an adverse effect, optionally wherein the population of cells is the population of cells of any one of claims 20-22.

32. Use of the population of cells of any one of claims 20-22 in the manufacture of a medicament for treating a cancer in a subject, wherein the population of cells were made by a process comprising:

i) contacting the population of cells with IMiD (e.g., thalidomide and derivatives thereof, e.g., lenalidomide, pomalidomide, and thalidomide) or Compound I-112 *ex vivo*, optionally wherein:

in the presence of IMiD or Compound I-112, the expression level of the CCAR is decreased, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100

percent, relative to the expression level of the CCAR before the population of cells are contacted with IMiD or Compound I-112 *ex vivo*, and

ii) formulating the population of cells for administration to the subject, optionally wherein the use further comprises after step i) and prior to step ii):

reducing the amount of IMiD or Compound I-112 contacting the population of cells, e.g., inside and/or surrounding the population of cells.

33. The use of claim 32, wherein the use further comprises:

(I) after step ii):

iii) use of an effective amount of IMiD or Compound I-112, optionally wherein the use of IMiD or Compound I-112 decreases, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, the expression level of the CCAR relative to the expression level of the CCAR after step ii) and prior to step iii), optionally wherein:

a) the subject has developed, is developing, or is anticipated to develop an adverse reaction,

b) the use of IMiD or Compound I-112 is in response to an occurrence of an adverse reaction in the subject, or in response to an anticipation of an occurrence of an adverse reaction in the subject, and/or

c) the use of IMiD or Compound I-112 reduces or prevents an adverse effect;
and/or

(II) after step iii):

iv) discontinuing the use of IMiD or Compound I-112, optionally wherein discontinuing the use of IMiD or Compound I-112 increases, e.g., by at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, the expression level of the CCAR relative to the expression level of the CCAR after step iii) and prior to step iv) (e.g., wherein discontinuing the use of IMiD or Compound I-112 restores the expression level of the CCAR to the expression level after step ii) and prior to step iii)), optionally wherein:

a) the subject has relapsed, is relapsing, or is anticipated to relapse,

b) the discontinuation of the use of IMiD or Compound I-112 is in response to a tumor relapse in the subject, or in response to an anticipation of a relapse in the subject,
and/or

- c) the discontinuation of the use of IMiD or Compound I-112 treats or prevents a tumor relapse; and/or
- (III) after step iv):
 - v) repeating step iii) and/or step iv).

34. Use of the population of cells of any one of claims 20-22 in the manufacture of a medicament for treating cancer in a subject, wherein the population of cells were made by a process comprising:

- i) contacting the population of cells with IMiD (e.g., thalidomide and derivatives thereof, e.g., lenalidomide, pomalidomide, and thalidomide) or Compound I-112 *ex vivo* before use, optionally wherein:

- in the presence of IMiD or Compound I-112, the expression level of the CCAR is decreased, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, relative to the expression level of the CCAR before the population of cells are contacted with IMiD or Compound I-112 *ex vivo*, optionally wherein after the population of cells are contacted with IMiD or Compound I-112 *ex vivo* and before use of the population of cells to the subject, the amount of IMiD or Compound I-112 contacting the population of cells, e.g., inside and/or surrounding the population of cells, is reduced; and/or

- the population of cells are not contacted with IMiD or Compound I-112 *ex vivo* before use.

35. The use of claim 34, wherein the use further comprises:

- (I) after step i):
 - ii) use of IMiD or Compound I-112, optionally wherein the use of IMiD or Compound I-112 decreases, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, the expression level of the CCAR relative to the expression level of the CCAR after step i) and prior to step ii), optionally wherein:
 - a) the subject has developed, is developing, or is anticipated to develop an adverse reaction,

b) the use of IMiD or Compound I-112 is in response to an occurrence of an adverse reaction in the subject, or in response to an anticipation of an occurrence of an adverse reaction in the subject, and/or

c) the use of IMiD or Compound I-112 reduces or prevents an adverse effect; and/or

(II) after step ii):

iii) discontinuing the use of IMiD or Compound I-112, optionally wherein discontinuing the use of IMiD or Compound I-112 increases, e.g., by at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, the expression level of the CCAR relative to the expression level of the CCAR after step ii) and prior to step iii) (e.g., wherein discontinuing the use of IMiD or Compound I-112 restores the expression level of the CCAR to the expression level after step i) and prior to step ii)), optionally wherein:

a) the subject has relapsed, is relapsing, or is anticipated to relapse,

b) the discontinuation of the use of IMiD or Compound I-112 is in response to a tumor relapse in the subject, or in response to an anticipation of a relapse in the subject, and/or

c) the discontinuation of the use of IMiD or Compound I-112 treats or prevents a tumor relapse; and/or

(III) after step iii):

iv) repeating step ii) and/or step iii).

36. Use of IMiD (e.g., thalidomide and derivatives thereof, e.g., lenalidomide, pomalidomide, and thalidomide) or Compound I-112, in the manufacture of a medicament for treating a cancer in a subject, wherein the subject comprises the population of cells of any one of claims 20-22, optionally wherein the use of IMiD or Compound I-112 decreases, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, the expression level of the CCAR relative to the expression level of the CCAR before the use of IMiD or Compound I-112, optionally wherein:

a) the subject has developed, is developing, or is anticipated to develop an adverse reaction,

b) the use of IMiD or Compound I-112 is in response to an occurrence of an adverse reaction in the subject, or in response to an anticipation of an occurrence of an adverse reaction in the subject, and/or

c) the use of IMiD or Compound I-112 reduces or prevents an adverse effect.

37. The use of claim 36, wherein the use further comprises:

(I) discontinuing the use of IMiD or Compound I-112, optionally wherein discontinuing the use of IMiD or Compound I-112 increases, e.g., by at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, the expression level of the CCAR relative to the expression level of the CCAR after step i) and prior to step ii) (e.g., wherein discontinuing the use of IMiD or Compound I-112 restores the expression level of the CCAR to the expression level before the use of IMiD or Compound I-112), optionally wherein:

a) the subject has relapsed, is relapsing, or is anticipated to relapse,

b) the discontinuation of the use of IMiD or Compound I-112 is in response to a tumor relapse in the subject, or in response to an anticipation of a relapse in the subject, and/or

c) the discontinuation of the use of IMiD or Compound I-112 treats or prevents a tumor relapse; and/or

(II) after discontinuing the use of IMiD or Compound I-112, repeating the use of the IMiD or Compound I-112 and/or discontinuing the use of IMiD or Compound I-112.

38. Use of a stabilization compound and the population of cells of any one of claims 20, 21, or 23 in the manufacture of a medicament for treating a cancer in a subject, optionally wherein the expression level of the CCAR in the presence of the stabilization compound is e.g., at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, higher than the expression level of the CCAR in the absence of the stabilization compound; optionally wherein the population of cells are not contacted with the stabilization compound *ex vivo* before use.

39. The use of claim 38, wherein the use further comprises:

(i) discontinuing the use of the stabilization compound, optionally wherein discontinuing the use of the stabilization compound reduces, e.g., at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-,

40-, or 50-fold, the expression level of the CCAR relative to the expression of the CCAR after use and prior to discontinuing use, optionally wherein:

a) the subject responded to the treatment (e.g., the subject has a complete response to the treatment, the subject shows a shrinkage in tumor mass, the subject shows a decrease in tumor cells, or the treatment is effective in the subject), and/or

b) the discontinuation of the use of the stabilization compound is in response to a response of the subject to the treatment (e.g., the subject has a complete response to the treatment), the subject shows a shrinkage in tumor mass, the subject shows a decrease in tumor cells, or the treatment is effective in the subject);

(ii) discontinuing the use of the stabilization compound, optionally wherein discontinuing the use of the stabilization compound reduces, e.g., at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, the expression level of the CCAR relative to the expression of the CCAR after step i) and prior to step ii), optionally wherein:

a) the subject has developed, is developing, or is anticipated to develop an adverse reaction,

b) the discontinuation of the use of the stabilization compound is in response to an occurrence of an adverse reaction in the subject, or in response to an anticipation of an occurrence of an adverse reaction in the subject, and/or

c) the discontinuation of the use of the stabilization compound reduces or prevents an adverse effect.

40. The use of claim 39, wherein the use further comprises:

(A) after (i) or (ii):

(iii) use of an effective amount of a stabilization compound, optionally wherein the use of the stabilization compound increases, e.g., by at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, the expression level of the CCAR relative to the expression level of the CCAR after step ii) or iii) and prior to step iv), optionally wherein:

a) the subject has relapsed, is relapsing, or is anticipated to relapse,

b) the use of the stabilization compound is in response to a tumor relapse in the subject, or in response to an anticipation of a relapse in the subject, and/or

c) the use of the stabilization compound treats or prevents a tumor relapse; and/or

(B) after (iii): (iv) repeating (i), (ii), or (iii).

41. The use of any one of claims 38-40, further comprising prior to the use of the population of cells:

(v) contacting the population of cells with a stabilization compound *ex vivo*, optionally wherein the expression level of the CCAR in the presence of the stabilization compound is, e.g., at least about 1.5- fold, 2- fold, 3- fold, 4- fold, 5- fold, 10- fold, 20- fold, 30- fold, 40- fold, or 50-fold, higher than the expression level of the CCAR in the absence of the stabilization compound.