What is claimed is:

1. A method of making a population of cells that express a chimeric antigen receptor (CAR), 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 nucleic acid molecule encoding the CAR, thereby providing a population of cells comprising the 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 step (ii) is performed together with step (i) or no later than 20 hours after the beginning of step (i), and

(a) step (iii) is performed no later than 30 hours after the beginning of step (i); or

(b) step (iii) is performed no later than 30 hours after the beginning of step (ii).

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

(a) the nucleic acid molecule in step (ii) is on a viral vector;

(b) 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

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

4. The method of any one of claims 1-3, 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.

5. The method of claim 4, 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.

6. The method of claim 4 or 5, 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^{TM}.

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

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

(I) step (i) increases the percentage of CAR-expressing cells in the population of cells from step (iii), for example, the population of cells from step (iii) shows a higher percentage of CAR-expressing cells, compared with cells made by an otherwise similar method without step (i);

(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 CAR-expressing naïve T cells 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 CAR-expressing naïve T cells in the population of cells from step (iii) is higher than the percentage of CAR-expressing naïve T 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);

(d) the population of cells from step (iii) 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 (ii) and prior to step (iii), 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;

(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 CAR-expressing naïve T cells in the population of cells from step (iii) is higher than the percentage of CAR-expressing naïve 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; and/or

(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 CAR-expressing central memory T cells 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, for example, central memory T 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 CAR-expressing central memory T cells in the population of cells from step (iii) is lower than the percentage of CAR-expressing central memory T 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);

(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 CAR-expressing central memory T cells in the population of cells from step (iii) is lower than the percentage of CAR-expressing central 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; 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 CAR-expressing stem memory T cells in the population of cells from step (iii) is increased, as compared to the percentage of CAR-expressing stem memory T cells 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 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);

(d) the percentage of CAR-expressing stem memory T cells in the population of cells from step (iii) is higher than the percentage of CAR-expressing stem memory T 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);

(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 CAR-expressing stem memory T cells in the population of cells from step (iii) is higher than the percentage of CAR-expressing 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;

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.

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

(a) the population of cells from step (iii), after being incubated with a cell expressing an antigen recognized by the 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 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);

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

(g) 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).

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

(a) steps (i) and/or (ii) are performed in cell media comprising IL-2, IL-15, IL-7, IL-21, IL-6, 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) steps (i) and/or (ii) are performed in serum-free cell media comprising is CTS™ Immune Cell Serum Replacement (ICSR).

11. The method of any one of claims 1-10, 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, 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, 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 40%, compared to the population of cells at the end of step (v).

12. The method of any one of claims 1-11, 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 expression level in the portion (for example, measuring the percentage of viable, CAR-expressing cells in the portion), optionally wherein:

step (iii) comprises harvesting and freezing the population of cells (for example, T 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 expression level in the portion (for example, measuring the percentage of viable, CAR-expressing cells in the portion).

13. A method of making a population of cells (for example, T cells) that express a chimeric antigen receptor (CAR), 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 nucleic acid molecule (for example, a DNA or RNA molecule) encoding the CAR, thereby providing a population of cells (for example, T cells) comprising the 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 nucleic acid molecule in step (2) is on a viral vector, optionally wherein the 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 a nucleic acid molecule encoding the CAR,

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).

14. The method of claim 13, wherein:

(a) the population of cells from step (3) shows a higher percentage of naïve cells among CAR-expressing cells (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) 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.

15. The method of any one of claims 1-14, 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 IL6Rα and/or IL6Rβ;

(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 IL6Rα and/or IL6Rβ)

(c) steps (i) and (ii) or steps (1) and (2) are performed in cell media comprising IL-15 and/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 IL6Rβ-expressing cells in the population of cells.

16. The method of any one of claims 1-15, wherein the CAR comprises an antigen binding domain, a transmembrane domain, and 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, GFRα4, 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 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 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 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, CDS, 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 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 CAR further comprises a leader sequence comprising the amino acid sequence of SEQ ID NO: 1.

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

18. The method of any one of claims 1-17, wherein the CAR comprises:

(I) an antigen binding domain, a transmembrane domain, and an intracellular signaling domain, wherein the antigen binding domain binds to CD19 and comprises a heavy chain

complementarity determining region 1 (HC CDR1), an HC CDR2, an HC CDR3, a light chain complementarity determining region 1 (LC CDR 1), an LC CDR2, and an LC CDR3, wherein:

(a) the HC CDR1 comprises the amino acid sequence of SEQ ID NO: 295;

(b) the HC CDR2 comprising the amino acid sequence of SEQ ID NO: 296;

(c) the HC CDR3 comprising the amino acid sequence of SEQ ID NO: 297;

(d) the LC CDR1 comprising the amino acid sequence of SEQ ID NO: 298;

(e) the LC CDR2 comprising the amino acid sequence of SEQ ID NO: 299; and

(f) the LC CDR3 comprising the amino acid sequence of SEQ ID NO: 300;

optionally wherein the CAR comprises the amino acid sequence of SEQ ID NO: 301, or a sequence having at least 80%, 85%, 90%, 95%, or 99% identity thereto; and/or the nucleic acid molecule encoding the CAR comprises the nucleotide sequence of SEQ ID NO: 302, or a sequence having at least 80%, 85%, 90%, 95%, or 99% identity thereto; or

(II) an antigen binding domain, a transmembrane domain, and an intracellular signaling domain, wherein the antigen binding domain binds to BCMA and comprises a HC CDR1, an HC CDR2, an HC CDR3, a LC CDR 1, an LC CDR2, and an LC CDR3, wherein:

(i) the HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2 and LC CDR3 sequences comprise the amino acid sequences of SEQ ID NO: 86, 87, 88, 95, 96, and 97, respectively;

(ii) the HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2 and LC CDR3 sequences comprise the amino acid sequences of SEQ ID NO: 47, 89, 88, 98, 99, and 100, respectively;

(iii) the HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2 and LC CDR3 sequences comprise the amino acid sequences of SEQ ID NO: 90, 91, 92, 101, 99, and 97, respectively;

optionally wherein, the CAR comprises the amino acid sequence of SEQ ID NO: 107, or a sequence having at least 80%, 85%, 90%, 95%, or 99% identity thereto; and/or the nucleic acid molecule encoding the CAR comprises the nucleotide sequence of SEQ ID NO: 108, or a sequence having at least 80%, 85%, 90%, 95%, or 99% identity thereto.

19. A population of CAR-expressing cells made by the method of any one of claims 1-18, optionally wherein the CAR-expressing cells are autologous or allogeneic CAR-expressing T cells or NK cells.

20. A population of cells engineered to express a CAR ("a population of CAR-expressing cells"), 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 express the CAR;

(b) a change within about 5% to about 10% of naïve cells, for example, as compared to the percentage of naïve cells, for example, naïve T cells in the same population of cells prior to being engineered to express the CAR;

(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 express the CAR;

(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 express the CAR;

(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 express the CAR;

(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 express the CAR;

(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 express the CAR;

(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 express the CAR; 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 express the CAR;

optionally wherein said naïve cells are naïve T cells or CD45RO- CCR7+ T cells, said central memory cells are central memory T cells or CCR7+CD45RO+ T cells, or said stem memory T cells are CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells.

21. A population of cells engineered to express a CAR ("a population of CAR-expressing cells"), 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 express the CAR;

(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 express the CAR;

(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 express the CAR;

(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 express the CAR; 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 express the CAR.

22. The population of CAR-expressing cell of any one of claims 19-21, wherein:

(I) the CAR comprises an antigen binding domain, a transmembrane domain, and an intracellular signaling domain, wherein the antigen binding domain binds to CD19 and comprises a heavy chain complementarity determining region 1 (HC CDR1), an HC CDR2, an

HC CDR3, a light chain complementarity determining region 1 (LC CDR 1), an LC CDR2, and an LC CDR3, wherein:

(a) the HC CDR1 comprises the amino acid sequence of SEQ ID NO: 295;

(b) the HC CDR2 comprising the amino acid sequence of SEQ ID NO: 296;

(c) the HC CDR3 comprising the amino acid sequence of SEQ ID NO: 297;

(d) the LC CDR1 comprising the amino acid sequence of SEQ ID NO: 298;

(e) the LC CDR2 comprising the amino acid sequence of SEQ ID NO: 299; and

(f) the LC CDR3 comprising the amino acid sequence of SEQ ID NO: 300;

optionally wherein the CAR comprises the amino acid sequence of SEQ ID NO: 301, or a sequence having at least 80%, 85%, 90%, 95%, or 99% identity thereto; and/or the nucleic acid molecule encoding the CAR comprises the nucleotide sequence of SEQ ID NO: 302, or a sequence having at least 80%, 85%, 90%, 95%, or 99% identity thereto; or

(II) the CAR comprises an antigen binding domain, a transmembrane domain, and an intracellular signaling domain, wherein the antigen binding domain binds to BCMA and comprises a HC CDR1, an HC CDR2, an HC CDR3, a LC CDR 1, an LC CDR2, and an LC CDR3, wherein:

(i) the HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2 and LC CDR3 sequences comprise the amino acid sequences of SEQ ID NO: 86, 87, 88, 95, 96, and 97, respectively;

(ii) the HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2 and LC CDR3 sequences comprise the amino acid sequences of SEQ ID NO: 47, 89, 88, 98, 99, and 100, respectively;

(iii) the HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2 and LC CDR3 sequences comprise the amino acid sequences of SEQ ID NO: 90, 91, 92, 101, 99, and 97, respectively;

optionally wherein, the CAR comprises the amino acid sequence of SEQ ID NO: 107, or a sequence having at least 80%, 85%, 90%, 95%, or 99% identity thereto; and/or the nucleic acid molecule encoding the CAR comprises the nucleotide sequence of SEQ ID NO: 108, or a sequence having at least 80%, 85%, 90%, 95%, or 99% identity thereto.

23. A pharmaceutical composition comprising the population of CAR-expressing cells of any one of claims 19-22 and a pharmaceutically acceptable carrier.

24. Use of the population of CAR-expressing cells of any one of claims 19-22 or the pharmaceutical composition of claim 23, in the manufacture of a medicament for:

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

(b) treating the 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;

(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 plasmocytoma of bone, extraosseous plasmocytoma, 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; or

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

25. The use of claim 24, further comprising administering a second therapeutic agent to the subject, optionally wherein:

(a) the second therapeutic agent is IL-15, for example, hetIL-15 (IL15/sIL-15Ra));

(b) the second therapeutic agent is ibrutinib;

(c) the second therapeutic agent is ibrutinib, wherein ibrutinib is administered once daily at a dose of about 600mg, about 550mg, about 500mg, about 480mg, about 460mg, about 440mg, about 420mg, about 400mg, about 350mg, about 300mg, about 280mg, about 250mg, about 200mg, about 190mg, about 180mg, about 170mg, about 160mg, about 150mg, about 140mg, about 130mg, about 120mg or 100mg;

(d) the second therapeutic agent is ibrutinib, wherein ibrutinib is administered once daily at a dose of 420mg, 280mg, or 140mg; or

(e) the second therapeutic agent is ibrutinib, wherein ibrutinib is administered prior to, concurrently with, or after the administration of the population of CAR-expressing cells.

26. The use of claim 24 or 25, wherein the population of CAR-expressing cells is administered at a dose determined based on the percentage of CAR-expressing cells measured in claim 8.

27. The use of any one of claims 24-26, wherein:

(a) the population of CAR-expressing cells (for example, CD19 CAR-expressing cells) is administered at a dose of about 0.5×10^6 to 50×10^6 viable CAR-expressing cells, for example, about 5×10^6 viable CAR-expressing cells, optionally wherein the population of CAR-expressing cells (for example, CD19 CAR-expressing cells) is administered at a dose of 5×10^6 viable CAR-expressing cells;

(b) the population of CAR-expressing cells (for example, CD19 CAR-expressing cells) is administered at a dose of about 2.5 x 10^6 to 2.5 x 10^8 viable CAR-expressing cells, for example, about 2.5 x 10^7 viable CAR-expressing cells, optionally wherein the population of CAR-expressing cells (for example, CD19 CAR-expressing cells) is administered at a dose of 2.5 x 10^7 viable CAR-expressing cells;

(c) the population of CAR-expressing cells (for example, CD19 CAR-expressing cells) is administered at a dose of about 1.25 x 10^7 to 1.25 x 10^9 viable CAR-expressing cells, for example, about 1.25 x 10^8 viable CAR-expressing cells, optionally wherein the population of CAR-expressing cells (for example, CD19 CAR-expressing cells) is administered at a dose of 1.25 x 10^8 viable CAR-expressing cells;

(d) the population of CAR-expressing cells (for example, BCMA CAR-expressing cells) is administered at a dose of about 2.5 x 10^6 to 2.5 x 10^8 viable CAR-expressing cells, for example, about 1 x 10^7 or 5 x 10^7 viable CAR-expressing cells; or

(e) the CAR is a CD19 CAR and the population of CD19 CAR-expressing cells is administered at a dose of about 1 x 10^7 to about 2 x 10^7 cells.

28. The use of any one of claims 24-27, wherein the subject is monitored for a sign of Cytokine Release Syndrome, for example, for at least 2, 2.5, 3, 3.5, or 4 days, or for about 3 days.