

What is claimed is:

1. A nucleic acid molecule encoding a chimeric antigen receptor (CAR) molecule, wherein said CAR molecule comprises:

(a) a first CAR comprising a first antigen binding domain which binds to CD22 and a first transmembrane domain; a first co-stimulatory signaling domain; and/or a first primary signaling domain; and

(b) a second CAR comprising a second antigen binding domain which binds to CD19 and a second transmembrane domain; a second co-stimulatory domain; and/or a second primary signaling domain,

wherein:

(i) the first transmembrane domain and the second transmembrane domain comprise the amino acid sequence of SEQ ID NO: 65 or an amino acid sequence with at least 90% identity thereto, and wherein a nucleotide sequence that encodes the first transmembrane domain and is comprised in the nucleic acid molecule is different from a nucleotide sequence that encodes the second transmembrane domain and is comprised in the nucleic acid molecule;

(ii) the first co-stimulatory signaling domain and the second co-stimulatory signaling domain comprise the amino acid sequence of SEQ ID NO: 70 or an amino acid sequence with at least 90% identity thereto, and wherein a nucleotide sequence that encodes the first co-stimulatory signaling domain and is comprised in the nucleic acid molecule is different from a nucleotide sequence that encodes the second co-stimulatory signaling domain and is comprised in the nucleic acid molecule; and/or

(iii) the first primary signaling domain and the second primary signaling domain comprise the amino acid sequence of SEQ ID NO: 75 or an amino acid sequence with at least 90% identity thereto, and wherein a nucleotide sequence that encodes the primary signaling domain and is comprised in the nucleic acid molecule is different from a nucleotide sequence that encodes the second primary signaling domain and is comprised in the nucleic acid molecule.

2. The nucleic acid molecule of claim 1, wherein the first CAR comprises:

a first antigen binding domain which binds to CD22, a first transmembrane domain, and a first co-stimulatory signaling domain;

a first antigen binding domain which binds to CD22, a first transmembrane domain; and a first primary signaling domain; or

a first antigen binding domain which binds to CD22, a first transmembrane domain, a first co-stimulatory signaling domain, and a first primary signaling domain,

and wherein the second CAR comprises:

a second antigen binding domain which binds to CD19; a second transmembrane domain; and a second co-stimulatory signaling domain;

a second antigen binding domain which binds to CD19; a second transmembrane domain; and a second primary signaling domain; or

a second antigen binding domain which binds to CD19; a second transmembrane domain; a second co-stimulatory signaling domain; and a second primary signaling domain.

~~3. The nucleic acid molecule of claim 1 or 2, wherein the second CAR comprises:~~

~~—— a second antigen binding domain which binds to CD19; a second transmembrane domain; and a second co-stimulatory signaling domain;~~

~~—— a second antigen binding domain which binds to CD19; a second transmembrane domain; and a second primary signaling domain; or~~

~~—— a second antigen binding domain which binds to CD19; a second transmembrane domain; a second co-stimulatory signaling domain; and a second primary signaling domain.~~

~~43. A nucleic acid molecule encoding~~

(a) a first CAR comprising a first antigen binding domain which binds to CD22; a first transmembrane domain; a first co-stimulatory signaling domain; and/or a first primary signaling domain; and wherein the CD22 binding domain comprises a heavy chain variable region (VH) comprising a heavy chain ~~complementary-complementarity~~ determining region 1 (HC CDR1), a heavy chain ~~complementary-complementarity~~ determining region 2 (HC CDR2), and a heavy chain ~~complementary-complementarity~~ determining region 3 (HC CDR3), and a light chain variable region (VL) comprising a light chain ~~complementary-complementarity~~ determining region 1 (LC CDR1), a light chain ~~complementary-complementarity~~ determining region 2 (LC CDR2), and a light chain ~~complementary-complementarity~~ determining region 3 (LC CDR3), wherein the HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2, and LC CDR3 comprise the amino acid sequences of:

(i) SEQ ID NOs: 20, 21, 22, 28, 29, and 30, respectively;

(ii) SEQ ID NOs: 23, 24, 22, 31, 32, and 33, respectively; or

(iii) SEQ ID NOs: 25, 26, 27, 34, 32, and 30, respectively; and

(b) a second CAR comprising a second antigen binding domain which binds to CD19; a second transmembrane domain; a second co-stimulatory domain; and/or a second primary signaling domain, wherein the CD19 binding domain comprises a VH comprising a HC CDR1, a HC CDR2, and a HC CDR3, and a VL comprising a LC CDR1, a LC CDR2, and a LC CDR3,

wherein the HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2, and LC CDR3 comprise the amino acid sequences of:

- (i) SEQ ID NOs: 35, 36, 39, 40, 41, and 42, respectively;
- (ii) SEQ ID NOs: 35, 37, 39, 40, 41, and 42, respectively; or
- (iii) SEQ ID NOs: 35, 38, 39, 40, 41, and 42, respectively.

54. The nucleic acid molecule of any of the preceding claims, wherein

(i) the CD22 binding domain of the first CAR comprises the amino acid sequences of SEQ ID NO: 50 or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereto; and/or

(ii) the CD19 binding domain of the second CAR comprises the amino acid sequences of SEQ ID NO: 44 or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereto.

65. The nucleic acid molecule of any of the preceding claims, wherein the first transmembrane domain and the second transmembrane domain comprise an amino acid sequence with at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the amino acid sequence of SEQ ID NO: 65.

~~7. The nucleic acid molecule of any of the preceding claims, wherein the nucleotide sequence encoding the first transmembrane domain is at least 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, or 100% different from the nucleotide sequence encoding the second transmembrane domain.~~

86. The nucleic acid molecule of any of the preceding claims, wherein the first co-stimulatory signaling domain and the second co-stimulatory signaling domain comprise an amino acid sequence with at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the amino acid sequence of SEQ ID NOs: 70.

~~9. The nucleic acid molecule of any of the preceding claims, wherein the nucleotide sequence encoding the first co-stimulatory signaling domain is at least 1%, 5%, 10%, 20%, 30%, 40%,~~

~~50%, 60%, 70%, 80%, 85%, 90%, 95%, or 100% different from the nucleotide sequence encoding the second co-stimulatory signaling domain.~~

~~107.~~ The nucleic acid molecule of any of the preceding claims, wherein the first primary signaling domain and the second primary signaling domain comprise an amino acid sequence with at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the amino acid sequence of SEQ ID NOs: 75.

~~11.~~ The nucleic acid molecule of any of the preceding claims, wherein the nucleotide sequence encoding the first primary signaling domain is at least 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, or 100% different from the nucleotide sequence encoding the second primary signaling domain.

~~128.~~ The nucleic acid molecule of any of the preceding claims, wherein

(i) the first CAR comprises the amino acid sequence of SEQ ID NO: 13 or 18, or an amino acid having at least 95%, 96%, 97%, 98%, or 99% identity thereto; and/or

(ii) the second CAR comprises the amino acid sequence of SEQ ID NO: 14 or 17, or an amino acid having at least 95%, 96%, 97%, 98%, or 99% identity thereto.

~~139.~~ The nucleic acid molecule of any of the preceding claims, wherein the CAR molecule comprises the amino acid sequence of SEQ ID NO: 12 or 16, or an amino acid having at least 95%, 96%, 97%, 98%, or 99% identity thereto.

~~1410.~~ A cell (e.g., an immune effector cell) comprising a nucleic acid molecule encoding a chimeric antigen receptor (CAR) molecule, wherein said CAR molecule comprises:

(a) a first CAR comprising a first antigen binding domain which binds to CD22; a first transmembrane domain; a first co-stimulatory signaling domain; and/or a first primary signaling domain; and

(b) a second CAR comprising a second antigen binding domain which binds to CD19; a second transmembrane domain; a second co-stimulatory domain; and/or a second primary signaling domain,

wherein:

(i) the first transmembrane domain and the second transmembrane domain comprise the amino acid sequence of SEQ ID NO: 65 or an amino acid sequence with at least 90% identity thereto, and wherein a nucleotide sequence that encodes the first transmembrane domain and is comprised in the nucleic acid molecule is different from a nucleotide sequence that encodes the second transmembrane domain and is comprised in the nucleic acid molecule;

(ii) the first co-stimulatory signaling domain and the second co-stimulatory signaling domain comprise the amino acid sequence of SEQ ID NO: 70 or an amino acid sequence with at least 90% identity thereto, and wherein a nucleotide sequence that encodes the first co-stimulatory signaling domain and is comprised in the nucleic acid molecule is different from a nucleotide sequence that encodes the second co-stimulatory signaling domain and is comprised in the nucleic acid molecule; and/or

(iii) the first primary signaling domain and the second primary signaling domain comprise the amino acid sequence of SEQ ID NOs: 75 or an amino acid sequence with at least 90% identity thereto, and wherein a nucleotide sequence that encodes the primary signaling domain and is comprised in the nucleic acid molecule is different from a nucleotide sequence that encodes the second primary signaling domain and is comprised in the nucleic acid molecule.

4511. A cell (e.g., an immune effector cell) comprising a nucleic acid molecule encoding a chimeric antigen receptor (CAR) molecule, wherein said CAR molecule comprises:

(a) a first CAR comprising a first antigen binding domain which binds to CD22; a first transmembrane domain; a first co-stimulatory signaling domain; and/or a first primary signaling domain; and wherein the CD22 binding domain comprises a VH comprising a HC CDR1, a HC CDR2, and a HC CDR3, and a VL comprising a LC CDR1, a LC CDR2, and a LC CDR3, wherein the HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2, and LC CDR3 comprise the amino acid sequences of:

(i) SEQ ID NOs: 20, 21, 22, 28, 29, and 30, respectively;

(ii) SEQ ID NOs: 23, 24, 22, 31, 32, and 33, respectively; or

(iii) SEQ ID NOs: 25, 26, 27, 34, 32, and 30, respectively; and

(b) a second CAR comprising a second antigen binding domain which binds to CD19; a second transmembrane domain; a second co-stimulatory domain; and/or a second primary signaling

domain, wherein the CD19 binding domain comprises a VH comprising a HC CDR1, a HC CDR2, and a HC CDR3, and a VL comprising a LC CDR1, a LC CDR2, and a LC CDR3, wherein the HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2, and LC CDR3 comprise the amino acid sequences of:

- (i) SEQ ID NOs: 35, 36, 39, 40, 41, and 42, respectively;
- (ii) SEQ ID NOs: 35, 37, 39, 40, 41, and 42, respectively; or
- (iii) SEQ ID NOs: 35, 38, 39, 40, 41, and 42, respectively.

~~16~~12. The cell of any of the preceding claims, wherein

(i) the CD22 binding domain of the first CAR comprises the amino acid sequences of SEQ ID NO: 50 or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereto; and/or

(ii) the CD19 binding domain of the second CAR comprises the amino acid sequences of SEQ ID NO: 44 or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereto.

~~17~~13. The cell of any of the preceding claims, wherein:

~~(i) the first CAR comprises the amino acid sequence of SEQ ID NO: 13 or 18, or an amino acid having at least 95%, 96%, 97%, 98%, or 99% identity thereto; and/or~~

~~(ii) the second CAR comprises the amino acid sequence of SEQ ID NO: 14 or 17, or an amino acid having at least 95%, 96%, 97%, 98%, or 99% identity thereto. The cell of any of the preceding claims, wherein~~

(i) the CD22 binding domain of the first CAR comprises the amino acid sequences of SEQ ID NO: 50 or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereto; and/or

(ii) the CD19 binding domain of the second CAR comprises the amino acid sequences of SEQ ID NO: 44 or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereto.

~~18~~14. The cell of any of the preceding claims, wherein:

(i) the first CAR comprises the amino acid sequence of SEQ ID NO: 13 or 18, or an amino acid having at least 95%, 96%, 97%, 98%, or 99% identity thereto; and/or

(ii) the second CAR comprises the amino acid sequence of SEQ ID NO: 14 or 17, or an amino acid having at least 95%, 96%, 97%, 98%, or 99% identity thereto.

1915. A cell (e.g., an immune effector cell) comprising a chimeric antigen receptor (CAR) molecule, wherein said CAR molecule comprises:

(a) a first CAR comprising a first antigen binding domain which binds to CD22 and a first transmembrane domain; a first co-stimulatory signaling domain; and/or a first primary signaling domain; and

(b) a second CAR comprising a second antigen binding domain which binds to CD19 and a second transmembrane domain; a second co-stimulatory domain; and/or a second primary signaling domain,

wherein:

(i) the first transmembrane domain and the second transmembrane domain comprise the amino acid sequence of SEQ ID NO: 65 or an amino acid sequence with at least 90% identity thereto, and wherein a nucleotide sequence that encodes a first transmembrane domain and is comprised in a nucleic acid molecule is different from a nucleotide sequence that encodes the second transmembrane domain and is comprised in the nucleic acid molecule;

(ii) the first co-stimulatory signaling domain and the second co-stimulatory signaling domain comprise the amino acid sequence of SEQ ID NO: 70 or an amino acid sequence with at least 90% identity thereto, and wherein a nucleotide sequence that encodes the first co-stimulatory signaling domain and is comprised in a nucleic acid molecule is different from a nucleotide sequence that encodes the second co-stimulatory signaling domain and is comprised in the nucleic acid molecule; and/or

(iii) the first primary signaling domain and the second primary signaling domain comprise the amino acid sequence of SEQ ID NO: 75 or an amino acid sequence with at least 90% identity thereto, and wherein a nucleotide sequence that encodes the primary signaling domain and is comprised in a nucleic acid molecule is different from a nucleotide sequence that encodes the second primary signaling domain and is comprised in the nucleic acid molecule.

~~20~~16. A cell comprising (e.g., an immune effector cell) comprising a CAR molecule, wherein the CAR molecule comprises

(a) a first CAR comprising a first antigen binding domain which binds to CD22; a first transmembrane domain; a first co-stimulatory signaling domain; and/or a first primary signaling domain; and wherein the CD22 binding domain comprises a VH comprising a HC CDR1, a HC CDR2, and a HC CDR3, and a VL comprising a LC CDR1, a LC CDR2, and a LC CDR3, wherein the HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2, and LC CDR3 comprise the amino acid sequences of:

(i) SEQ ID NOs: 20, 21, 22, 28, 29, and 30, respectively;

(ii) SEQ ID NOs: 23, 24, 22, 31, 32, and 33, respectively; or

(iii) SEQ ID NOs: 25, 26, 27, 34, 32, and 30, respectively; and

(b) a second CAR comprising a second antigen binding domain which binds to CD19; a second transmembrane domain; a second co-stimulatory domain; and/or a second primary signaling domain, wherein the CD19 binding domain comprises a VH comprising a HC CDR1, a HC CDR2, and a HC CDR3, and a VL comprising a LC CDR1, a LC CDR2, and a LC CDR3, wherein the HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2, and LC CDR3 comprise the amino acid sequences of:

(i) SEQ ID NOs: 35, 36, 39, 40, 41, and 42, respectively;

(ii) SEQ ID NOs: 35, 37, 39, 40, 41, and 42, respectively; or

(iii) SEQ ID NOs: 35, 38, 39, 40, 41, and 42, respectively.

~~24~~17. The cell of any of the preceding claims, wherein

(i) the CD22 binding domain of the first CAR comprises the amino acid sequences of SEQ ID NO: 50 or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereto; and/or

(ii) the CD19 binding domain of the second CAR comprises the amino acid sequences of SEQ ID NO: 44 or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereto.

~~22~~18. The cell of any of the preceding claims, wherein

(i) the first CAR comprises the amino acid sequence of SEQ ID NO: 13 or 18, or an amino acid having at least 95%, 96%, 97%, 98%, or 99% identity thereto; and/or

(ii) the second CAR comprises the amino acid sequence of SEQ ID NO: 14 or 17, or an amino acid having at least 95%, 96%, 97%, 98%, or 99% identity thereto.

~~2319.~~ A pharmaceutical composition comprising the nucleic acid ~~encoding the CAR molecule of any one of claims 1 to 13, optionally~~ molecule of any one of claims 1-9 wherein the pharmaceutical composition comprises an excipient, a carrier, a diluent and/or a stabilizer.

~~2420.~~ A method of providing anti-tumor immunity, ~~comprising administering to a subject in need thereof, wherein~~ an effective amount of a cell, e.g., a population of immune effector cells, comprising, e.g., ~~expressing~~, the nucleic acid ~~encoding a CAR molecule of any one of claims 1 to 13~~ 9 is to be administered to a subject in need thereof.

~~2521.~~ A method of treating a subject having a disease associated with an antigen (e.g., CD19 and/or CD22), ~~comprising administering to the subject in need thereof, an effective amount~~ Use of a cell, e.g., a population of immune effector cells, comprising, e.g., ~~expressing~~, the nucleic acid molecule encoding a CAR molecule of any one of claims 1 to 139, in the manufacture of a medicament for the treatment of a disease associated with an antigen (e.g., CD19 and/or CD22), wherein an effective amount of the medicament is to be administered to the subject in need thereof.

~~2622.~~ The method of claims ~~24-20~~ or the use of claim 2521, wherein the cell is a T cell (e.g., a CD4+ T cell or a CD8+ T cell) or an NK cell.

~~27.~~ The method use of claim 25, wherein the disease associated with CD19 and/or CD22 is selected from a proliferative disease, e.g., a cancer or malignancy, a precancerous condition, e.g., a myelodysplasia, a myelodysplastic syndrome, or a preleukemia, or a non-cancer related indication associated with expression of CD19 and/or CD22.

~~2823.~~ The method use of claim ~~2721~~, wherein the disease is a cancer, e.g., a hematological cancer.

~~2924.~~ The method use of claim ~~2823~~, wherein the hematological cancer is ~~chosen~~ selected from the group consisting of acute myeloid leukemia (AML), B-cell acute lymphoblast leukemia (BALL), small lymphocytic leukemia (SLL), acute lymphoblast leukemia (ALL), chronic myelogenous leukemia (CML), chronic

lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma (DLBCL), follicular lymphoma, hairy cell leukemia, small cell-lymphoma, large cell-follicular lymphoma, a malignant lymphoproliferative condition, MALT lymphoma, Marginal zone lymphoma, multiple myeloma, myelodysplasia, or myelodysplastic syndrome, myeloproliferative neoplasm, non-Hodgkin's lymphoma (NHL), Hodgkin's lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, preleukemia, ~~or~~ and a combination thereof.

~~3025.~~ The ~~method~~ use of claim ~~2823~~, wherein the hematological cancer is pediatric BALL or adult BALL.

~~31.~~ The ~~method of claim 28, wherein the hematological cancer is adult BALL.~~

~~32.~~ The ~~method of claim 28, wherein the hematological cancer is NHL.~~

~~3326.~~ A method of making a population of cells of any one of claims ~~14 to 2210-18~~, the method comprising:

(i) contacting (for example, binding) a population of cells (for example, T cells, for example, T cells isolated from a frozen or fresh leukapheresis product) 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 (for example, T cells) with the nucleic acid molecule of any one of claims 1-~~139~~, thereby providing a population of cells (for example, T cells) comprising the nucleic acid molecule, and

(iii) 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 (ii) is performed together with step (i) or no later than 20 hours after the beginning of step (i), for example, no later than 12, 13, 14, 15, 16, 17, or 18 hours after the beginning of step (i), for example, no later than 18 hours after the beginning of step (i), and

step (iii) is performed no later than 30 (for example, 26) hours after the beginning of step (i), for example, no later than 22, 23, 24, 25, 26, 27, 28, 29, or 30 hours after the beginning of step (i), for example, no later than 24 hours after the beginning of step (i),

(b) step (ii) is performed together with step (i) or no later than 20 hours after the beginning of step (i), for example, no later than 12, 13, 14, 15, 16, 17, or 18 hours after the beginning of step (i), for example, no later than 18 hours after the beginning of step (i), and

step (iii) is performed no later than 30 hours after the beginning of step (ii), for example, no later than 22, 23, 24, 25, 26, 27, 28, 29, or 30 hours after the beginning of step (ii),
or

(c) 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%, 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 (i), optionally wherein the nucleic acid molecule in step (ii) 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.

27. An effective amount of a cell, e.g., a population of immune effector cells, comprising the nucleic acid molecule of any one of claims 1 to 9 for use in the treatment of a disease associated with an antigen (e.g., CD19 and/or CD22).