

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 costimulatory 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 costimulatory domain; and/or a second primary signaling domain,

wherein the first CAR and the second CAR each comprise an HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, as recited in SEQ ID NO: 12 or 16;

wherein the CAR molecule comprises the amino acid sequence of SEQ ID NO: 12 or 16, or an amino acid sequence having at least 95%, 96%, 97%, 98%, or 99% identity thereto;

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 costimulatory signaling domain and the second costimulatory 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 costimulatory signaling domain and is comprised in the nucleic acid molecule is different from a nucleotide sequence that encodes the second costimulatory 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 costimulatory 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 costimulatory 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 costimulatory 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 costimulatory signaling domain; and a second primary signaling domain.

3. 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 costimulatory 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 complementarity determining region 1 (HC CDR1), a heavy chain complementarity determining region 2 (HC CDR2), and a heavy chain complementarity determining region 3 (HC CDR3), and a light chain variable region (VL) comprising a light

chain complementarity determining region 1 (LC CDR1), a light chain complementarity determining region 2 (LC CDR2), and a light chain 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 costimulatory 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 SEQ ID NOs: 35, 37, 39, 40, 41, and 42, respectively; wherein the CAR molecule comprises the amino acid sequence of SEQ ID NO: 12 or 16, or an amino acid sequence having at least 95%, 96%, 97%, 98%, or 99% identity thereto.

4. 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 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 85%, 90%, 95%, or 99% sequence identity thereto.

5. 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.

6. The nucleic acid molecule of any of the preceding claims, wherein the first costimulatory signaling domain and the second costimulatory 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.

7. 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.

8. 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.

9. A T cell or an NK 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 costimulatory 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 costimulatory domain; and/or a second primary signaling domain,

wherein the first CAR and the second CAR each comprise an HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, as recited in SEQ ID NO: 12 or 16;

wherein the CAR molecule comprises the amino acid sequence of SEQ ID NO: 12 or 16, or an amino acid sequence having at least 95%, 96%, 97%, 98%, or 99% identity thereto;

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 costimulatory signaling domain and the second costimulatory 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 costimulatory signaling domain and is comprised in the nucleic acid molecule is different from a nucleotide sequence that encodes the second costimulatory 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.

10. A T cell or an NK 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 costimulatory 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 costimulatory 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 SEQ ID NOs: 35, 37, 39, 40, 41, and 42, respectively;

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

11. A T cell or NK 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 costimulatory 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 costimulatory domain; and/or a second primary signaling domain,

wherein the first CAR and the second CAR each comprise an HCDR1, HCDR2,

HCDR3, LCDR1, LCDR2, and LCDR3, as recited in SEQ ID NO: 12 or 16;

wherein the CAR molecule comprises the amino acid sequence of SEQ ID NO: 12 or 16, or an amino acid sequence having at least 95%, 96%, 97%, 98%, or 99% identity thereto; 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 costimulatory signaling domain and the second costimulatory 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 costimulatory signaling domain and is comprised in a nucleic acid molecule is different from a nucleotide sequence that encodes the second costimulatory 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.

12. A T cell or NK 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 costimulatory 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 costimulatory 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 SEQ ID NOs: 35, 37, 39, 40, 41, and 42, respectively; wherein the CAR molecule comprises the amino acid sequence of SEQ ID NO: 12 or 16, or an amino acid sequence having at least 95%, 96%, 97%, 98%, or 99% identity thereto.

13. The T cell or NK cell of any of claims 9-12, 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 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 85%, 90%, 95%, or 99% sequence identity thereto.

14. The T cell or NK cell of any of claims 9-13, 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.

15. A pharmaceutical composition comprising the nucleic acid molecule of any one of claims 1-8, wherein the pharmaceutical composition comprises an excipient, a carrier, a diluent and/or a stabilizer.

16. Use of of a cell comprising the nucleic acid molecule of any one of claims 1 to 8 in the manufacture of a medicament for providing anti-tumor immunity, wherein an effective amount of the medicament is to be administered to a subject in need thereof.

17. Use of a T cell or NK cell comprising the nucleic acid molecule of any one of claims 1 to 8, in the manufacture of a medicament for the treatment of a disease associated with CD19 and/or CD22, wherein an effective amount of the medicament is to be administered to the subject in need thereof.

18. The use of claim 16 or 17, wherein the T cell is a CD4⁺ T cell or a CD8⁺ T cell.

19. The use of claim 17, wherein the disease is a cancer.

20. The use of claim 19, wherein the cancer is a hematological cancer.

21. The use of claim 20, wherein the hematological cancer is selected from the group consisting of acute myeloid leukemia (AML), B-cell acute lymphoblast leukemia (BALL), small lymphocytic lymphoma (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, and a combination thereof.

22. The use of claim 20, wherein the hematological cancer is pediatric BALL or adult BALL.

23. An effective amount of a T cell or NK cell comprising the nucleic acid molecule of any one of claims 1 to 8 for use in the treatment of a disease associated with CD19 and/or CD22.