

CLAIMS

1. A memory-like natural killer (ML NK) cell comprising A ~~a~~ chimeric antigen receptor (CAR) construct comprising:

- (i) a targeting antibody fragment against a disease-associated antigen;
- (ii) a transmembrane domain; and
- (iii) at least one intracellular signaling domain,

wherein the CAR construct is capable of being expressed and capable of ~~er~~ functioning in a memory-like natural killer (ML NK) cell.

2. The ML NK cell comprising the CAR construct of claim 1, wherein the disease-associated antigen is selected from the group consisting of CD19, CD33, CD123, CD20, BCMA, Mesothelin, EGFR, CD3, CD4 BAFF-R, EGFR, HER2, gp120, or gp41.

3. The ML NK cell comprising the CAR construct of claim 1, wherein the transmembrane domain is selected from the group consisting of NKG2D, FcγRIIIa, NKp44, NKp30, NKp46, actKIR, NKG2C, CD8α, and IL15Rb.

4. The ML NK cell comprising the CAR construct of claim 1, wherein the at least one intracellular signaling domain is selected from the group consisting of CD137/41BB, DNAM-1, NKp80, 2B4, NTBA, CRACC, CD2, CD27, one or more integrins, IL-15R, IL-18R, IL-12R, IL-21R, IRE1a, and combinations thereof.

5. The ML NK cell comprising the CAR construct of claim 1, wherein the at least one intracellular signaling domain is a transmembrane adapter.

6. The ML NK cell comprising the CAR construct of claim 1, further comprising a transmembrane adapter or hinge.

7. The ML NK cell comprising the CAR construct of any one of claims 5 to 6, wherein the transmembrane adapter is selected from the group consisting of FcεR1γ, CD3ζ, DAP12, DAP10, and combinations thereof.

8. The ML NK cell comprising the CAR construct of claim 4, wherein the one or more integrins are selected from the group consisting of ITGB1, ITGB2, ITGB3, and combinations thereof.

9. The ML NK cell comprising the CAR construct of claim 1, wherein the targeting antibody fragment against a disease-associated antigen comprises a scFv selected from the group consisting of:

- (i) anti-CD19 scFv comprising an amino acid sequence of SEQ ID NO: 1;
- (ii) anti-CD33 scFv comprising an amino acid sequence of SEQ ID NO: 2; and
- (iii) anti-CD123 scFv comprising an amino acid sequence of SEQ ID NO: 3.

10. The ML NK cell comprising the CAR construct of claim 3, wherein the transmembrane domain is selected from the group consisting of:

NKG2D comprising an amino acid sequence of SEQ ID NO: 5;
FcγRIIIa comprising an amino acid sequence of SEQ ID NO: 7;
NKp44 comprising an amino acid sequence of SEQ ID NO: 9;
NKp30 comprising an amino acid sequence of SEQ ID NO: 11;
NKp46 comprising an amino acid sequence of SEQ ID NO: 13;
actKIR comprising an amino acid sequence of SEQ ID NO: 15;
NKG2C comprising an amino acid sequence of SEQ ID NO: 17;
CD8α comprising an amino acid sequence of SEQ ID NO: 19; and
IL15Rb comprising an amino acid sequence of SEQ ID NO: 21.

11. The ML NK cell comprising the CAR construct of claim 6, wherein the hinge is selected from the group consisting of:

NKG2D comprising an amino acid sequence of SEQ ID NO: 4;
FcγRIIIa comprising an amino acid sequence of SEQ ID NO: 6;
NKp44 comprising an amino acid sequence of SEQ ID NO: 8;

NKp30 comprising an amino acid sequence of SEQ ID NO: 10;
NKp46 comprising an amino acid sequence of SEQ ID NO: 12;
actKIR comprising an amino acid sequence of SEQ ID NO: 14;
NKG2C comprising an amino acid sequence of SEQ ID NO: 16;
CD8 α comprising an amino acid sequence of SEQ ID NO: 18; and
IL15R β comprising an amino acid sequence of SEQ ID NO: 20.

12. The ML NK cell comprising the CAR construct of claim 1, wherein the at least one intracellular signaling domain is selected from the group consisting of:
CD137/41BB comprising an amino acid sequence of SEQ ID NO: 22;
DNAM-1 comprising an amino acid sequence of SEQ ID NO: 23;
NKp80 comprising an amino acid sequence of SEQ ID NO: 24;
2B4 comprising an amino acid sequence of SEQ ID NO: 25;
NTBA comprising an amino acid sequence of SEQ ID NO: 26;
CRACC comprising an amino acid sequence of SEQ ID NO: 27;
CD2 comprising an amino acid sequence of SEQ ID NO: 28);
CD27 comprising an amino acid sequence of SEQ ID NO: 29);
integrins, ITGB1 comprising an amino acid sequence of SEQ ID NO: 30,
ITGB2 comprising an amino acid sequence of SEQ ID NO: 31, or ITGB3 comprising an amino acid sequence of SEQ ID NO: 32;
IL15RB comprising an amino acid sequence of SEQ ID NO: 33;
IL18R comprising an amino acid sequence of SEQ ID NO: 34;
IL12R, IL12RB1 comprising an amino acid sequence of SEQ ID NO: 35 and
IL12RB2 comprising an amino acid sequence of SEQ ID NO: 36;
IL21R comprising an amino acid sequence of SEQ ID NO: 37;
IRE1a comprising an amino acid sequence of SEQ ID NO: 38; and
combinations thereof.

~~13. A memory-like natural killer (ML NK) cell comprising the CAR construct of claim 1.~~

~~14. A method of generating chimeric antigen receptor memory-like natural killer (CARML NK) cells comprising:~~

~~providing NK cells, activating cytokines comprising IL-12/15/18, and IL-15;~~

~~contacting the NK cells and activating cytokines for an amount of time sufficient to form cytokine-activated memory-like (ML) NK cells;~~

~~transducing a chimeric antigen receptor (CAR) via a viral vector into the cytokine-activated ML NK cells in the presence of IL-15 for an amount of time sufficient to virally transduce CAR into the cytokine-activated ML NK cells, resulting in CAR-transduced ML NK cells; and~~

~~incubating the CAR-transduced ML NK cells in the presence of IL-15 for an amount of time sufficient to form CAR-expressing ML NK (CARML NK cells).~~

~~15. The method of claim 14, wherein the NK cells were isolated from peripheral blood mononuclear cells (PBMCs).~~

~~16. The method of claim 14, wherein the amount of time sufficient to form cytokine-activated NK cells is between about 8 and about 24 hours, about 12 hours, or about 16 hours.~~

~~17. The method of claim 14, wherein the amount of time sufficient to virally transduce CAR into the ML NK cells is between about 12 hours and about 24 hours.~~

~~18. The method of claim 14, wherein the amount of time sufficient to form ML NK cells expressing CAR (CARML NK cells) is at least between about 3 days and about 8 days or about 7 days.~~

~~19. The method of claim 14, wherein the viral vector comprises a chimeric antigen receptor (CAR) is a CAR lentivirus.~~

~~20. The method of claim 14, wherein the viral vector is a lentiviral vector selected from the group consisting of pMND-G, pMND-Lg, pMDN-REV, and combinations thereof.~~

~~21. The method of claim 14, wherein transducing a chimeric antigen receptor (CAR) via a viral vector into the cytokine-activated ML NK cells is performed in the absence of polybrene.~~

~~22. A chimeric antigen receptor memory-like natural killer (CARML NK) cell made according to the method of any one of claims 14 to 21 comprising the CAR construct of any one of claims 1 to 13.~~

2313. A memory-like natural killer (ML NK) cell comprising a chimeric antigen receptor (CAR) construct comprising:

(i) an anti-CD19 scFv comprising SEQ ID NO: 1, an anti-CD33 scFv comprising SEQ ID NO: 2, or an anti-CD123 scFv comprising SEQ ID NO: 3;

(ii) a CD8a transmembrane domain comprising SEQ ID NO: 19, a NKp30 transmembrane domain comprising SEQ ID NO: 11, or a NKG2D transmembrane domain comprising SEQ ID NO: 5; and

(iii) a CD137 intracellular signaling domain comprising SEQ ID NO: 22, a IL-15R intracellular signaling domain comprising SEQ ID NO: 33, or a 2B4 intracellular signaling domain comprising SEQ ID NO: 25;

wherein the CAR construct is capable of being expressed or functioning in a memory-like natural killer (ML NK) cell.

14. The ML NK cell comprising the CAR construct of claim 1, comprising: a transmembrane domain selected from the group consisting of NKG2D, FcγRIIIa, NKp44, NKp30, NKp46, actKIR, NKG2C, CD8α, and IL15Rb;

at least one intracellular signaling domain selected from the group consisting of:

CD137/41BB, DNAM-1, NKp80, 2B4, NTBA, CRACC, CD2, CD27, one or more integrins.

IL-15R, IL-18R, IL-12R, IL-21R, IRE1a,

FcεR1γ, CD3ζ, DAP12, DAP10,

and combinations thereof.

15. The ML NK cell comprising the CAR construct of claim 14, wherein the one or more integrins are selected from the group consisting of ITGB1, ITGB2, ITGB3, and combinations thereof.

16. The ML NK cell comprising the CAR construct of claim 1, comprising a transmembrane domain and adapter combination chosen from:

the transmembrane domain NKG2D and the adapter DAP10;

the transmembrane domain FCγRIIIa and the adapter CD3ζ;

the transmembrane domain FCγRIIIa and the adapter FcεR1γ;

the transmembrane domain NKp30 and the adapter CD3ζ;

the transmembrane domain NKp30 and the adapter FcεR1γ;

the transmembrane domain NKp44 and the adapter DAP12;

the transmembrane domain NKp46 and the adapter CD3ζ;

the transmembrane domain NKp46 and the adapter FcεR1γ;

the transmembrane domain actKIR and the adapter DAP12; and

the transmembrane domain NKG2C and the adapter DAP12.

17. The ML NK cell comprising the CAR construct of claim 1, comprising a combination chosen from:

a targeting antibody fragment against a disease-associated antigen comprising an anti-CD19 scFv, a transmembrane domain comprising CD8a, and an intracellular signaling domain comprising CD137 and CD3ζ;

a targeting antibody fragment against a disease-associated antigen comprising an anti-CD19 scFv, a transmembrane domain comprising NKG2D, a transmembrane adaptor comprising DAP10, and an intracellular signaling domain comprising DNAM1, CD137, and CD3ζ;

a targeting antibody fragment against a disease-associated antigen comprising an anti-CD19 scFv, a transmembrane domain comprising NKG2D, a transmembrane adaptor comprising DAP10, and an intracellular signaling domain comprising IL-15R, 2B4, and DAP12;

a targeting antibody fragment against a disease-associated antigen comprising an anti-CD33 scFv, a transmembrane domain comprising CD8a, and an intracellular signaling domain comprising CD137 and CD3ζ;

a targeting antibody fragment against a disease-associated antigen comprising an anti-CD123 scFv, a transmembrane domain comprising CD8a, and an intracellular signaling domain comprising CD137 and CD3ζ;

a targeting antibody fragment against a disease-associated antigen comprising an anti-CD19 scFv, a transmembrane domain comprising CD8a, and an intracellular signaling domain comprising IL-15R;

a targeting antibody fragment against a disease-associated antigen comprising an anti-CD19 scFv, a CD8a hinge, a transmembrane domain comprising NKp30, and an intracellular signaling domain comprising 2B4 and CD3ζ; and

a targeting antibody fragment against a disease-associated antigen comprising an anti-CD19 scFv, a CD8a hinge, a transmembrane domain comprising NKp30, a transmembrane adaptor comprising DAP10, and an intracellular signaling domain comprising 2B4 and CD3ζ.

18. A chimeric antigen receptor (CAR) construct comprising:

(i) an anti-CD19 scFv comprising SEQ ID NO: 1, an anti-CD33 scFv comprising SEQ ID NO: 2, or an anti-CD123 scFv comprising SEQ ID NO: 3;

(ii) a CD8a transmembrane domain comprising SEQ ID NO: 19, a NKp30 transmembrane domain comprising SEQ ID NO: 11, or a NKG2D transmembrane domain comprising SEQ ID NO: 5; and

(iii) a CD137 intracellular signaling domain comprising SEQ ID NO: 22, a IL-15R intracellular signaling domain comprising SEQ ID NO: 33, or a 2B4 intracellular signaling domain comprising SEQ ID NO: 25;

wherein the CAR construct is capable of being expressed or functioning in a memory-like natural killer (ML NK) cell.