

CLAIMS

What is claimed is:

~~1.~~ 1. An isolated nucleic acid encoding a chimeric antigen receptor (CAR), wherein the CAR comprises:

- (a) ~~—~~ a ROR-~~1~~ antigen binding domain;
- (b) ~~—~~ a spacer;
- (c) ~~—~~ a transmembrane domain; and
- (d) ~~—~~ a costimulatory (c) an intracellular signaling domain comprising 4 1BB or CD28, or both; and
- (e) ~~—~~ a CD3 zeta signaling domain.

~~2.~~ 2. The isolated nucleic acid of claim ~~1~~, 1, wherein the CAR comprises ~~at least one of:~~

- (a) ~~a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with at least one of the amino acid sequences as shown in sequence of any one of SEQ ID NOs: 3–14; or~~
- (b) ~~a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with at least one of amino acid sequences as shown in SEQ ID NOs: and 75–82.~~

3. The isolated nucleic acid of claim ~~1~~, wherein the ROR-1, wherein the ROR-1 antigen binding domain comprises a polypeptide having at least 90% identity with the amino acid sequence of any one of SEQ ID NOs: 15–74.

~~3.~~ 4. The isolated nucleic acid of claim 1, wherein the ROR-1 antigen binding domain comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 99% or 100% identify% identity with at least one of the amino acid sequences as shown in SEQ ID NOs: 15–74.

~~4.~~ The isolated nucleic acid of claim 1, wherein the ROR-1 antigen binding domain comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 99% or 100% identify

~~with at least one of amino acid sequences as shown in~~ sequence of any one of SEQ ID NOs: 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, and 73.

~~5.~~ 5. The isolated nucleic acid of claim 1, wherein the ROR-1 antigen binding domain comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 99% or 100% identity with at least one of the amino acid sequences as shown in sequence of any one of SEQ ID NOs: 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, and 74.

~~6.~~ The isolated nucleic acid of claim 1, wherein the intracellular signaling domain comprises a costimulatory signaling domain.

~~6.~~ 7. The isolated nucleic acid of claim 6, wherein the costimulatory signaling domain comprises 4-1BB.

~~8.~~ The isolated nucleic acid of claim 1, wherein the costimulatory signaling domain comprises a polypeptide having at least 90% identity with SEQ ID NO: 89.

~~7.~~ 9. The isolated nucleic acid of any claim 6, wherein the costimulatory signaling domain comprises CD28.

~~10.~~ The isolated nucleic acid of claim 1, wherein the costimulatory signaling domain comprises a polypeptide having at least 90% identity with SEQ ID NO: 90.

~~11.~~ The isolated nucleic acid of claim 1, wherein the intracellular signaling domain comprises a CD3 zeta signaling domain.

~~8.~~ 12. The isolated nucleic acid of claim 11, wherein the CD3 zeta signaling domain comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 93.

9. 13. The isolated ~~nucleic~~nucleic acid of claim ~~1,1~~, further ~~comprising~~encoding a polypeptide having at least 90%, ~~91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%~~ identity with the amino acid sequence of any one of SEQ ID NOs: 94–108.

10. 14. The isolated nucleic acid of ~~any of claims 1-9,~~claim 1, further ~~comprising~~encoding a cell tag.

11. 15. The isolated nucleic acid claim ~~10,14~~, wherein the cell tag is a truncated epidermal growth factor receptor.

12. 16. The isolated nucleic acid of claim ~~11,15~~, wherein the truncated epidermal growth factor receptor is ~~HER1t~~and comprises a polypeptide having at least 90%, ~~91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%~~ identity with the amino acid sequence of SEQ ID NO: 109.

13. 17. The isolated nucleic acid of claim ~~11,~~wherein the truncated epidermal growth factor receptor is ~~HER1t~~1 and comprises15, encoding a polypeptide having at least 90%, ~~91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%~~ identity with the amino acid sequence of SEQ ID NO: 110.

18. The isolated nucleic acid of claim ~~1,1~~, further ~~comprising an additional polynucleotide encoding gene switch polypeptides for ligand-inducible control~~a spacer.

19. The isolated nucleic acid of ~~heterologous gene expression~~claim 18, wherein the ~~gene switch polypeptides comprises~~spacer comprises a polypeptide having at least 90% identity with any one of SEQ ID NOs: 83–86.

20. The isolated nucleic acid of claim 1, wherein the transmembrane domain comprises a polypeptide having at least 90% identity with SEQ ID NO: 87 or 88.

~~14.~~ 21. The isolated nucleic acid of claim 1, further encoding: (a) a first gene switch polypeptide ~~that comprises~~comprising a DNA binding domain fused to a first nuclear receptor ligand binding domain; and (b) a second gene switch polypeptide ~~that comprises~~comprising a transactivation domain fused to a second nuclear receptor ligand binding domain; wherein the first gene switch polypeptide and the second gene switch polypeptide are connected by a linker.

22. The isolated nucleic acid of claim 1, further encoding a cytokine.

23. The isolated nucleic acid of claim 22, wherein the cytokine is IL-15 or IL-12.

24. The isolated nucleic acid of claim 1, further encoding a fusion protein comprising IL-15 and IL-15R α .

25. The isolated nucleic acid of claim 24, wherein fusion protein comprises an amino acid sequence having at least 90% identity with SEQ ID NO: 113.

26. The isolated nucleic acid of claim 1, further encoding a self-cleaving *Thosea asigna* virus (T2A) peptide.

~~15.~~ 27. A vector comprising a backbone and ~~a~~the nucleic acid ~~sequence encoding~~of any one of claims 1 to 26.

(1) —

28. The vector of claim 27, wherein the vector is a lentivirus vector, a retroviral vector, or a non-viral vector.

29. The vector of claim 27, wherein the backbone is a *Sleeping Beauty* transposon DNA plasmid.

30. An immune effector cell comprising the nucleic acid of any one of claims 1 to 26.

31. An immune effector cell comprising the vector of claim 27.

32. An immune effector cell comprising:

- (1) a cell tag;
- (2) —a cytokine; and
- (3)—a chimeric antigen receptor (CAR), wherein the CAR comprises:
 - (a)—a ROR-1 antigen binding domain;
 - (b)—a spacer;
 - (c)—a transmembrane domain; and
 - (d)—a costimulatory (c) an intracellular signaling domain comprising 4 1BB or CD28, or both; and
 - (e) a CD3 zeta signaling domain.

16. 33. The ~~vector~~immune effector cell of claim 15,32, wherein the ~~vector~~ is a lentivirus vector, a retroviral vector, or a non-viral vector, cytokine comprises IL-15.

34. The ~~vector~~immune effector cell of claim 15,33, wherein the IL-15 comprises a membrane bound IL-15.

17. 35. The immune effector cell of claim 32, wherein the cell tag ~~is~~comprises a truncated epidermal growth factor receptor.

18. 36. The ~~vector~~immune effector cell of claim 17, wherein the truncated epidermal growth factor receptor is a HER1t, HER1t-1 or a functional variant thereof.

19. The vector of claim 15, wherein the cytokine is IL-15.

20. The vector of claim 19, wherein the IL-15 is a membrane bound IL-15.

21. The vector of any of claims 15-20, further35, comprising a nucleotide sequence encoding a self-cleaving *Thosea asigna* virus (T2A) peptide.

22. The vector of any of claims 15-21, wherein the backbone is Sleeping Beauty transposon DNA plasmid.

23. ~~The vector of claim 15, wherein the CAR comprises at least one of:~~

~~(a) a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with at least one of amino acid sequences as shown in SEQ ID NOs: 3-14; or~~

~~(b) a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with at least one of the amino acid sequences as shown in sequence of SEQ ID NOs: 75-82; NO: 109 or 110.~~

24. ~~The vector of claim 15, wherein the ROR-1 antigen binding domain comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 99% or 100% identify with at least one of amino acid sequences as shown in SEQ ID NOs: 15-74.~~

25. ~~The vector of claim 15, wherein the ROR-1 antigen binding domain comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 99% or 100% identify with at least one of amino acid sequences as shown in SEQ ID NOs: 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, and 73.~~

26. ~~The vector of claim 15, wherein the ROR-1 antigen binding domain comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 99% or 100% identify with at least one of amino acid sequences as shown in SEQ ID NOs: 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, and 74.~~

27. ~~The vector of claim 16, wherein the non-viral vector is a Sleeping Beauty transposon.~~

28. ~~The vector of claim 15, further comprising an additional vector comprising a polynucleotide encoding gene-switch polypeptides for ligand-inducible control of heterologous gene expression, wherein the gene-switch polypeptides comprise: (a) a first gene-switch polypeptide that comprises a DNA binding domain fused to a first nuclear receptor ligand binding domain; and (b) a second gene-switch polypeptide that comprises a transactivation domain fused to a second nuclear receptor ligand binding domain; wherein the first gene-switch polypeptide and the second gene-switch polypeptide are connected by a linker.~~

29. ~~An immune effector cell comprising the isolated nucleic acid of any one of claims 1-14.~~

~~30. An immune effector cell comprising the vector of any one of claims 15-28.~~

~~31. An immune effector cell comprising (1) a cell tag; (2) a cytokine; and (3) a chimeric antigen receptor (CAR), wherein the CAR comprises (a) a ROR 1 antigen binding domain; (b) a spacer; (c) a transmembrane domain; (d) a costimulatory signaling domain comprising 4-1BB or CD28, or both; and (e) a CD3-zeta signaling domain.~~

~~32. The immune effector cell of claim 31, wherein the cytokine is IL-15.~~

~~33. The immune effector cell of claim 32, wherein the IL-15 is a membrane bound IL-15.~~

~~34. The immune effector cell of claim 31, wherein the cell tag is a truncated epidermal growth factor receptor.~~

~~35. The immune effector cell of claim 34, wherein the truncated epidermal growth factor receptor is a HER1t, HER1t-1 or a functional variant thereof.~~

~~The immune effector cell of any one of claims 31-35~~

~~36. 37. The immune effector cell of claim 30, wherein the cell is a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), or a regulatory T cell.~~

~~37. 38. The immune effector cell of claim 31,32, wherein the CAR comprises at least one of a polypeptide having at least 90% identity with the amino acid sequences as shown in sequence of any one of SEQ ID NOs: 3-14 or 75-82.~~

~~38. 39. The immune effector cell of claim 31,32, wherein the CAR comprises at least one of a polypeptide having at least 90% identity with the amino acid sequences as shown in sequence of any one of SEQ ID NOs: 15-74.~~

~~39. 40. The immune effector cell of claim 31,30, further comprising gene switch polypeptides for ligand-inducible control of heterologous gene expression, wherein the gene switch polypeptides comprise: (a) a first gene switch polypeptide that comprises comprising a DNA binding domain fused to a first nuclear receptor ligand binding domain; and (b) a second gene switch polypeptide that comprises comprising a transactivation domain fused to a second nuclear receptor~~

ligand binding domain; wherein the first gene switch polypeptide and the second gene switch polypeptide are connected by a linker.

~~A method for~~

~~40. 41. Use of the immune effector cell of claim 30 in the preparation of a medicament stimulating a T cell-mediated immune response to a target cell population or tissue in a human subject in need thereof, comprising administering to the human subject an effective amount of a cell genetically modified to express a CAR, wherein the CAR comprises, the immune effector cell comprising a truncated epidermal growth factor receptor and a CAR comprising:~~

- ~~(a)——a ROR-1 antigen binding domain;~~
- ~~(b)——a spacer;~~
- ~~(c)——a transmembrane domain;~~
- ~~(d)——a costimulatory signaling domain comprising 4-1BB or CD28, or both; and~~
- ~~(e)——a CD3 zeta signaling domain; and~~
- ~~(f)——a truncated epidermal growth factor receptor (HER1).~~

~~41. The method of claim 40, wherein the human has been diagnosed with at least one of lung cancer, breast cancer, pancreatic cancer, ovarian cancer, prostate cancer, adrenal cancer, melanoma, uterine cancer, testicular cancer, or bladder cancer.~~

~~The method of claim 40, wherein the human has been diagnosed with non-Hodgkin's~~

~~42. Use of the immune effector cell of claim 30 in the preparation of a medicament for treating a disease characterized by the overexpression of ROR-1.~~

~~42. 43. The use of claim 42, wherein the disease is non-Hodgkin's lymphoma, acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), hairy cell leukemia (HCL), multiple myeloma (MM), acute myeloid leukemia (AML), or chronic myeloid leukemia (CML).~~

~~A method of treating cancer in a subject in need thereof comprising administering to the subject one or more doses of an effective amount of engineered T cells~~

~~43. 44. The use of claim 42, wherein the engineered T cells comprise ROR-1 CAR and membrane bound IL-15.~~

~~44. The method of claim 43, wherein a first dose of an effective amount of engineered T cells is administered intraperitoneally or intravenously.~~

- ~~45. The method of claim 43, wherein the cancer is non-Hodgkin's lymphoma, acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), hairy cell leukemia (HCL), multiple myeloma (MM), acute myeloid leukemia (AML), or chronic myeloid leukemia (CML).~~
46. The method of claim 43, wherein the cancerdisease is lung cancer, breast cancer, pancreatic cancer, ovarian cancer, prostate cancer, adrenal cancer, melanoma, uterine cancer, testicular cancer, or bladder cancer.
- ~~47. The method of claim 43, wherein the ROR-1 CAR is encoded by any one of sequences as shown in SEQ ID NOs: 3-14, 75-82, or 15-74.~~
- ~~48. The method of claim 43, wherein the effective amount of engineered T-cells is at least 10^2 cells/kg, 10^4 cells/kg, or 10^5 cells/kg.~~
45. A method of detecting in a subject a disease that is associated with overexpression of ROR-1, the method comprising contacting cells obtained from the subject with the ROR-1 antigen binding domain encoded by the nucleic acid of any one of claims 1 to 26.
46. A composition comprising the vector of any one of claims 27 to 29.
47. A kit comprising the vector of any one of claims 27 to 29.
48. A composition comprising the immune effector cell of claim 30.
49. A system for expressing a CAR in an immune effector cell, the system comprising the vector of claim 27.
50. A method of expressing a CAR in an immune effector cell comprising contacting the immune effector cell with the system of claim 49.