

CLAIMS

What is claimed is:

1. An isolated nucleic acid encoding a chimeric antigen receptor (CAR), wherein the CAR comprises a polypeptide having the amino acid sequence of SEQ ID NO: 31.

(a) a MUC16 antigen binding domain;

(b) a stalk domain;

(c) a transmembrane domain;

(d) a costimulatory signaling domain comprising 4-1BB or CD28, or both; and

(e) a CD3 zeta signaling domain.

2. The isolated nucleic acid of claim 1, wherein the MUC16 antigen binding domain 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: 1, 3, 5, 7, 9, 12, and 14;

(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: 2, 4, 6, 8, 10, 11, 13, and 15; and

(c) 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: 27-57.

3. The isolated nucleic acid of claims 1 or 2, wherein the MUC16 antigen binding domain is 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: 27-57.

4. The isolated nucleic acid of claims 1 or 2, wherein the stalk domain is 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: 16.

5. The isolated nucleic acid of any of claims 1-4, wherein the costimulatory signaling domain comprises 4-1BB.

6. The isolated nucleic acid of claim 5, wherein the costimulatory signaling domain of 4-1BB 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: 22.

7. The isolated nucleic acid of any of claims 1-5, wherein the costimulatory signaling domain comprises CD28.

~~8. The isolated nucleic acid of claim 7, wherein the costimulatory signaling domain of CD28 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: 23.~~

~~9. The isolated nucleic acid of any of claims 1-8, 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: 26.~~

~~2.40. The isolated nucleic acid of claim 1, any one of claims 1-9, further encoding comprising a truncated epidermal growth factor receptor.~~

~~3.44. The isolated nucleic acid of claim 2.40, 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: 65 or SEQ ID NO: 66.~~

~~12. The isolated nucleic acid of claim 10, wherein the truncated epidermal growth factor receptor is HER1t-1 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: 66.~~

~~13. The isolated nucleic acid of claim 1, wherein the CAR comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with an amino acid sequence shown in SEQ ID NOs: 27-57.~~

~~4.44. A vector comprising a backbone and a nucleic acid sequence encoding:~~

- ~~(1) a truncated epidermal growth factor receptor comprising at least one of HER1t, HER1t-1, or a functional variant thereof;~~
- ~~(2) a cytokine; and~~
- ~~(3) a chimeric antigen receptor (CAR) comprising a polypeptide having the amino acid sequence of SEQ ID NO: 31., wherein the CAR comprises~~
 - ~~(a) a MUC16 antigen binding domain;~~
 - ~~(b) a stalk domain;~~
 - ~~(c) a transmembrane domain;~~
 - ~~(d) a costimulatory signaling domain comprising 4-1BB or CD28, or both; and~~
 - ~~(e) a CD3 zeta signaling domain.~~

~~5.45. The vector of claim 4.44, wherein the cytokine is IL-15 or IL-12.~~

~~6.16.~~ The vector of claim ~~14 or 15~~, wherein the vector is a lentivirus vector, a retroviral vector, or a non-viral vector.

~~17.~~ The vector of claim ~~14~~, wherein the truncated epidermal growth factor receptor 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: ~~65 or SEQ ID NO: 66~~.

~~7.18.~~ The vector of claim ~~515~~, wherein the IL-15 is membrane bound IL-15.

~~8.19.~~ The vector of claim ~~718~~, wherein the membrane bound IL-15 comprises a nucleotide sequence that encodes a polypeptide having the amino acid sequence of SEQ ID NO: ~~69~~, encoding for SEQ ID NO: ~~161~~.

~~9.20.~~ The vector of claim ~~4~~, any one of claims ~~14–19~~, further comprising a nucleotide sequence encoding a self-cleaving Thosea asigna virus (T2A) peptide.

~~21.~~ The vector of any of claims ~~14–20~~, wherein the backbone is Sleeping Beauty transposon DNA plasmid or pFUGW.

~~22.~~ The vector of any of claims ~~14–21~~, further comprising a promoter.

~~23.~~ The vector of claim ~~22~~, wherein the promoter is hEF1a1.

~~24.~~ The vector of any of claims ~~14–23~~, wherein the MUC16 antigen binding domain 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 NO: ~~1, 3, 5, 7, 9, 12, and 14~~;

(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: ~~2, 4, 6, 8, 10, 11, 13, and 15~~; and;

(c) 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: ~~27–57~~.

~~25.~~ The vector of any of claims ~~14–24~~, wherein the MUC16 antigen binding domain is 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 NO: ~~27–57~~.

- ~~26. The vector of any of claims 14-25, wherein the stalk 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: 16.~~
- ~~27. The vector of any of claims 14-26, wherein the costimulatory signaling domain comprises 4-1BB.~~
- ~~28. The vector of claim 27, wherein the costimulatory signaling domain of 4-1BB comprises a nucleic acid sequence 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: 22.~~
- ~~29. The vector of any of claims 14-26, wherein the costimulatory signaling domain comprises CD28.~~
- ~~30. The vector of claim 29, wherein the costimulatory signaling domain of CD28 comprises a nucleic acid sequence 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: 23.~~
- ~~31. The vector of any of claims 14-30, wherein the CD3 zeta signaling domain comprises a nucleic acid sequence 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: 26.~~
- ~~32. The vector of any one of claims 14-31, wherein the vector comprises a plasmid.~~
- ~~33. The vector of claim 14-31, wherein each the vector comprises an expression plasmid.~~
- ~~34. The vector of claim 16, wherein the non-viral vector is a Sleeping Beauty transposon.~~
- ~~35. An immune effector cell comprising the nucleotide of any of claims 1-13.~~
- ~~36. An immune effector cell comprising (1) a cell tag (2) IL-15 and (3) a chimeric antigen receptor (CAR), wherein the CAR comprises (a) a MUC16 antigen binding domain; (b) a stalk domain; (c) a transmembrane domain; (d) a costimulatory signaling domain comprising 4-1BB or CD28, or both; and (e) a CD3 zeta signaling domain.~~
- ~~37. The immune effector cell of claim 36, wherein the IL-15 is membrane bound IL-15.~~

~~38. The immune effector cell of claim 36, wherein the membrane bound IL-15 comprises the polypeptide sequence of SEQ ID NO: 161.~~

~~39. The immune effector cell of claim 36, wherein the cell tag comprises HER1t, and the HER1t comprises the polypeptide sequence of SEQ ID NO: 65.~~

~~40. The immune effector cell of claim 36, wherein the cell tag comprises HER1t-1, and the HER1t-1 comprises the polypeptide sequence of SEQ ID NO: 66.~~

~~10.41. An immune effector cell comprising the vector of claim 4, any one of claims 14-34.~~

~~11.42. The immune effector cell of claim 10, claims 35-41, wherein the cell is a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), or a regulatory T cell.~~

~~43. The immune effector cell of claim 36, wherein the CAR comprises at least one of amino acid sequences SEQ ID Nos: 27-57.~~

~~44. A method for 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~~
~~(a) a MUC16 antigen-binding domain;~~
~~(b) a stalk domain;~~
~~(c) a transmembrane domain;~~
~~(d) a costimulatory signaling domain comprising 4-1BB or CD28, or both;~~
~~(e) a CD3 zeta signaling domain; and~~
~~(f) a truncated epidermal growth factor receptor (HER1t).~~

~~45. The method of claim 44, wherein the human has been diagnosed with at least one of ovarian and breast cancer.~~

~~46. The method of claim 45, wherein the ovarian or breast cancer is relapsed or refractory ovarian or breast cancer.~~

~~47. An isolated nucleic acid encoding a chimeric antigen receptor (CAR), wherein the CAR comprises:~~

- ~~(a) a MUC16 antigen-binding domain with at least one of amino acid sequences of as shown in SEQ ID NO: 1-15 or 27-57;~~
- ~~(b) a stalk domain with the amino acid sequence of SEQ ID NO: 16;~~

~~(c) a costimulatory signaling domain comprising CD28 with the amino acid sequence of SEQ ID NO: 23;~~

~~(d) a HER1 tag which comprises at least one of HER1t with the amino acid sequence of SEQ ID NO: 65 and HER1t 1 with the amino acid sequence of SEQ ID NO: 66; and~~

~~(e) a CD3 zeta signaling domain with the amino acid sequence of SEQ ID NO: 26.~~

~~48. An isolated nucleic acid encoding a chimeric antigen receptor (CAR), wherein the CAR comprises:~~

~~(a) a MUC16 antigen binding domain with at least one of amino acid sequences as shown in SEQ ID NO: 1-15 or 27-57;~~

~~(b) a stalk domain with the amino acid sequence of SEQ ID NO: 16;~~

~~(c) a costimulatory signaling domain comprising 4-1BB with the amino acid sequence of SEQ ID NO: 23;~~

~~(d) a HER1 tag which comprises at least one of HER1t with the amino acid sequence of SEQ ID NO: 65 and HER1t 1 with the amino acid sequence of SEQ ID NO: 66; and~~

~~(e) a CD3 zeta signaling domain with the amino acid sequence of SEQ ID NO: 26.~~

~~49. A vector comprising any one or more of the polynucleotides in any of claims 47 and 48.~~

~~50. The vector of claim 49, wherein the vector is a lentivirus vector, a retroviral vector, or a non-viral vector.~~

~~51. The vector of claim 50, wherein the non-viral vector is a Sleeping Beauty transposon.~~

~~52. The vector of claim 51, wherein the vector is a plurality of vectors.~~

~~53. A system for expressing a CAR in an immune effector cell, the system comprising one or more vectors encoding an isolated nucleic acid as provided in any one of claims 1-13 and 47-48.~~

~~54. The system of claim 53, wherein the immune effector cell is a T cell or NK cell.~~

~~55. The system of any one of claims 53-54, further comprising a nucleic acid encoding at least one additional gene.~~

~~56. The system of claim 55, wherein the additional gene comprises a cytokine.~~

~~57. The system of claim 56, wherein the cytokine comprises at least one of IL-2, IL-15, IL-12, IL-21, and a fusion of IL-15 and IL-15Ra.~~

~~58. The system of claim 56, wherein the cytokine is in secreted form.~~

~~59. The system of claim 56, wherein the cytokine is in membrane bound form.~~

- ~~60. The system of any one of claims 53-59, wherein the system comprises one vector.~~
- ~~61. The system of any one of claims 53-60, wherein the one or more vectors is a lentivirus vector, a retroviral vector, or a non-viral vector.~~
- ~~62. The system of claim 61, wherein the non-viral vector is a Sleeping Beauty transposon.~~
- ~~63. The system of claim 62, further comprising a Sleeping Beauty transposase.~~
- ~~64. The system of claim 63, wherein the Sleeping Beauty transposase is SB11, SB100X or SB110.~~
- ~~65. The system of any one of claims 53-64, wherein the immune effector cell is a mammalian cell.~~
- ~~66. A method of expressing a CAR in an immune effector cell comprising contacting the immune effector cell with a system of any one of claims 53-65.~~
- ~~67. A method of stimulating the proliferation and/or survival of engineered T-cells comprising:~~
- ~~(a) obtaining a sample of cells from a subject, the sample comprising T-cells or T-cell progenitors;~~
 - ~~(b) transfecting the cells with one or more vectors encoding an isolated nucleic acid as provided in any one of claims 1-13 and 44-45 and a vector encoding a transposase, to provide a population of engineered MUC16 CAR-expressing T-cells;~~
 - ~~(c) and optionally, culturing the population of MUC16 CAR T-cells *ex vivo* for 2 days or less.~~
- ~~68. The method of claim 67, further comprising transfecting the cells with a vector encoding a cytokine.~~
- ~~69. The method of claim 68, wherein the cytokine is a fusion protein comprising IL-15 and IL-15Ra.~~
- ~~70. The method of any one of claims 67-69, wherein the one or more vectors is a lentivirus vector, a retroviral vector, or a non-viral vector.~~
- ~~71. The method of claim 70, wherein the non-viral vector is a Sleeping Beauty transposon.~~

72. — The method of claim 71, further comprising a Sleeping Beauty transposase.
73. — The method of claim 72, wherein the Sleeping Beauty transposase is SB11, SB100X or SB110.
74. — 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, wherein the engineered T cells comprise MUC16 CAR and membrane bound IL-15.
75. — The method of claim 74, wherein a first dose of an effective amount of engineered T cells is administered intraperitoneally.
76. — The method of claim 75, wherein a second dose of an effective amount of engineered T cells is administered intravenously.
77. — The method of claim 74, wherein the cancer is ovarian cancer.
78. — The method of claim 74, wherein the cancer is breast cancer.
79. — The method of claim 74, wherein the MUC16 CAR is encoded by any one of sequences as shown in SEQ ID NOs: 95-107, 119-149, or 194-195.
80. — The method of claim 74, wherein the membrane bound IL-15 is encoded by SEQ ID NO: 161.
81. — The method of claim 74, wherein an effective amount of engineered T cells is at least 102 cells/kg.
82. — The method of claim 74, wherein an effective amount of engineered T cells is at least 104 cells/kg.
83. — The method of claim 74, wherein an effective amount of engineered T cells is at least 105 cells/kg.