

signaling domain. Another example of the disclosure provides CAR constructs comprising the amino acid sequences as described herein.

[0005a] In one aspect, the present disclosure refers to a chimeric antigen receptor (CAR) comprising an antigen binding domain having antigenic specificity for CD33, a transmembrane domain, and an intracellular T cell signaling domain, wherein the antigen binding domain comprises from N-terminus to C-terminus the amino acid sequences of SEQ ID NOS: 15 and 16 (a) ~~SEQ ID NOS: 15, 4, and 16~~ or (b) ~~SEQ ID NOS: 13, 4, and 14~~.

~~**[0005b]** In another aspect, the present disclosure refers to a chimeric antigen receptor (CAR) comprising an antigen binding domain having antigenic specificity for CD33, a transmembrane domain, and an intracellular T cell signaling domain, wherein (a) the antigen binding domain comprises the light chain variable CDR1, CDR2, and CDR3 regions of hP67.6; and/or (b) the antigen binding domain comprises the heavy chain variable CDR1, CDR2, and CDR3 regions of hP67.6 wherein the CDR regions are the amino acid sequences of SEQ ID NOS: 47-52.~~

~~**[0005e0005b]**~~ In another aspect, the present disclosure refers to a chimeric antigen receptor (CAR) comprising (a) the amino acid sequence of any one of SEQ ID NO: 22 or 23~~SEQ ID NOS: 18-23~~ or (b) the amino acid sequence of SEQ ID NO: 23 wherein the amino acid Q within the amino acid sequence of SEQ ID NO: 37 within the amino acid sequence of SEQ ID NO: 23 is substituted with K. In yet another aspect, the present disclosure refers to a nucleic acid comprising a nucleotide sequence encoding the CAR as described herein. In yet another aspect, the present disclosure refers to a recombinant expression vector comprising the nucleic acid as described herein. In yet another aspect, the present disclosure refers to an isolated host cell comprising the recombinant expression vector as described herein. In yet another aspect, the present disclosure refers to a population of cells comprising at least one host cell as described herein.

~~**[0005d0005c]**~~ In a further aspect, the present disclosure refers to a pharmaceutical composition comprising the CAR as described herein, the nucleic acid as described herein, the recombinant expression vector as described herein, the host cell as described herein, or the population of cells as described herein, and a pharmaceutically acceptable carrier. In a further aspect, the present disclosure refers to an *in vitro* method of detecting the presence of cancer, comprising:

(a) contacting a sample comprising one or more cells with the CAR as described herein, ~~the nucleic acid as described herein, the recombinant expression vector as described herein,~~ the host cell as described herein, the population of cells as described herein, or ~~the a~~ pharmaceutical composition ~~thereof as described herein,~~ thereby forming a complex, and (b) detecting the complex, wherein detection of the complex is indicative of the presence of cancer.

[0005e0005d] In a further aspect, the present disclosure refers to the use of the CAR as described herein, the nucleic acid as described herein, the recombinant expression vector as described herein, the host cell as described herein, the population of cells as described herein, or the pharmaceutical composition as described herein in the manufacture of a medicament for the treatment or prevention of cancer in a mammal. In a further aspect, the present disclosure refers to the CAR as described herein, the nucleic acid as described herein, the recombinant expression vector as described herein, the host cell as described herein, the population of cells as described herein, or the pharmaceutical composition as described herein for use in the treatment or prevention of cancer in a mammal.

[0006] Further examples of the disclosure provide related nucleic acids, recombinant expression vectors, host cells, populations of cells, and pharmaceutical compositions relating to the CAR constructs of the disclosure.

[0007] Additional examples of the disclosure provide methods of detecting the presence of cancer in a mammal and methods of treating or preventing cancer in a mammal.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] Figures 1A and 1B present diagrams of certain CAR embodiments of the invention.

Mylo: Mylotarg, humanized antibody hP67.6 targeting human CD33. M195: humanized monoclonal murine IgG2a antibody (M195) targeting human CD33 from a mouse immunized with live human leukemic myeloblasts. Hu195: humanized antibody targeting human CD33.

[0009] Figures 2A-2F present graphs showing transduction efficiency of CARs.

[0010] Figures 3A and 3B present graphs showing flow cytometric analysis of CD33 and CD123 target antigen expression (indicated as fluorescence intensity) on Leukemia cells.

[0011] Figures 4A-4F present graphs showing cytokine production by CD33 and CD123 CAR T cells following *in vitro* stimulation. CD33 or CD123 CAR transduced T cells were incubated with target leukemia cells as indicated in the figure. The interferon gamma or IL-2 level in the supernatant were detected by ELISA.

Claims

1. A chimeric antigen receptor (CAR) comprising an antigen binding domain having antigenic specificity for CD33, a transmembrane domain, and an intracellular T cell signaling domain, wherein the antigen binding domain comprises from N-terminus to C-terminus the amino acid sequences of SEQ ID NOS: 15 and 16 ~~(a) SEQ ID NOS: 15, 4, and 16 or (b) SEQ ID NOS: 13, 4, and 14.~~

~~2. A chimeric antigen receptor (CAR) comprising an antigen binding domain having antigenic specificity for CD33, a transmembrane domain, and an intracellular T cell signaling domain, wherein~~

~~(a) the antigen binding domain comprises the light chain variable CDR1, CDR2, and CDR3 regions of hP67.6; and/or~~

~~(b) the antigen binding domain comprises the heavy chain variable CDR1, CDR2, and CDR3 regions of hP67.6~~

~~wherein the CDR regions are the amino acid sequences of SEQ ID NOS: 47-52.~~

~~3. The CAR according to claim 2, wherein the antigen binding domain comprises the amino acid sequence of SEQ ID NO: 3.~~

~~4. The CAR according to claim 2 or 3, wherein the antigen binding domain comprises the amino acid sequence of SEQ ID NO: 5.~~

~~5. The CAR according to any one of claims 2-4, wherein the antigen binding domain comprises the amino acid sequence of SEQ ID NO: 4.~~

~~6. The CAR according to any one of claims 2-5, wherein the antigen binding domain comprises the amino acid sequences of SEQ ID NOS: 3, 4, and 5.~~

~~7. The CAR according to any one of claims 2-6, wherein the CAR further comprises a spacer.~~

2. The CAR according to claim 1, wherein the antigen binding domain comprises from N-terminus to C-terminus the amino acid sequences of SEQ ID NOS: 15, 4, and 16.

38. The CAR according to ~~any one of claims 1-7~~ claim 1 or 2, wherein the CAR comprises (i) the amino acid sequence of SEQ ID NO: 7 and the amino acid sequence of SEQ ID NO: 6, or (ii) the amino acid sequence of SEQ ID NO: 11 and the amino acid sequence of SEQ ID NO: 10.

49. The CAR according to any one of ~~claims 1-8~~ claims 1-3, wherein the CAR comprises the amino acid sequence of SEQ ID NO: 8, the amino acid sequence of SEQ ID NO: 9, or both.

5. The CAR according to claim 1 or 2, wherein the CAR comprises the amino acid sequences of SEQ ID NOS: 6, 7, 11, and 9.

610. A chimeric antigen receptor (CAR) comprising (a) the amino acid sequence of SEQ ID NO: 22 or 23 ~~any one of SEQ ID NOS: 18-23~~ or (b) the amino acid sequence of SEQ ID NO: 23 wherein the amino acid Q within the amino acid sequence of SEQ ID NO: 37 within the amino acid sequence of SEQ ID NO: 23 is substituted with K.

117. The CAR according to ~~claim 10~~ claim 6, wherein the CAR comprises the amino acid sequence of SEQ ID NO: 23.

128. A nucleic acid comprising a nucleotide sequence encoding the CAR according to any one of ~~claims 1-11~~ claims 1-7.

139. The nucleic acid according to ~~claim 12~~ claim 8, wherein the nucleotide sequence is codon-optimized.

1410. A recombinant expression vector comprising the nucleic acid according to ~~claim 12 or 13~~ claim 8 or 9.

~~15~~11. An isolated host cell comprising the recombinant expression vector according to ~~claim 14~~ claim 10.

~~16~~12. A population of cells comprising at least one host cell according to ~~claim 15~~ claim 11.

~~17~~13. A pharmaceutical composition comprising the CAR according to any one of ~~claims 1-11~~ claims 1-7, the nucleic acid according to ~~claim 12 or 13~~ claim 8 or 9, the recombinant expression vector according to ~~claim 14~~ claim 10, the host cell according to ~~claim 15~~ claim 11, or the population of cells according to ~~claim 16~~ claim 12, and a pharmaceutically acceptable carrier.

~~18~~14. An *in vitro* method of detecting the presence of cancer, comprising:

(a) contacting a sample comprising one or more cells with the CAR according to any one of ~~claims 1-11, the nucleic acid according to claim 12 or 13, the recombinant expression vector according to claim 14~~ claims 1-7, the host cell according to ~~claim 15~~ claim 11, the population of cells according to ~~claim 16~~ claim 12, or ~~the a~~ pharmaceutical composition ~~thereof according to claim 17~~, thereby forming a complex, and

(b) detecting the complex, wherein detection of the complex is indicative of the presence of cancer.

~~19~~15. Use of the CAR according to any one of ~~claims 1-11~~ claims 1-7, the nucleic acid according to ~~claim 12 or 13~~ claim 8 or 9, the recombinant expression vector according to ~~claim 14~~ claim 10, the host cell according to ~~claim 15~~ claim 11, the population of cells according to ~~claim 16~~ claim 12, or the pharmaceutical composition according to ~~claim 17~~ claim 13 in the manufacture of a medicament for the treatment or prevention of cancer in a mammal.

~~20~~16. The CAR according to any one of ~~claims 1-11~~ claims 1-7, the nucleic acid according to ~~claim 12 or 13~~ claim 8 or 9, the recombinant expression vector according to ~~claim 14~~ claim 10, the host cell according to ~~claim 15~~ claim 11, the population of cells according to

~~claim 16~~ claim 12, or the pharmaceutical composition according to ~~claim 17~~ claim 13 for use in the treatment or prevention of cancer in a mammal.

~~2417~~. The method according to ~~claim 18~~ claim 14, the use according to ~~claim 19~~ claim 15, or the CAR, ~~nucleic acid, recombinant expression vector~~, host cell, population of cells, or pharmaceutical composition for use according to ~~claim 20~~ claim 16, wherein the cancer is acute myeloid leukemia.

18. The nucleic acid or recombinant expression vector for use according to claim 16, wherein the cancer is acute myeloid leukemia.