

ANTI-CD33 CHIMERIC ANTIGEN RECEPTORS AND THEIR USES

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This patent application claims the benefit of U.S. Provisional Patent Application No. 62/643,015, filed March 14, 2018, which is incorporated by reference herein in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

[0002] This invention was made with Government support under project number ZIA BC 011565 by the National Institutes of Health, National Cancer Institute. The Government has certain rights in the invention.

INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0003] Incorporated by reference in its entirety herein is a computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: one 69,707 Byte ASCII (Text) file named “741580_ST25.txt,” dated March 14, 2019.

BACKGROUND OF THE INVENTION

[0004] Acute myelogenous leukemia is a highly aggressive acute leukemia, representing the second most common leukemia occurring in children and adolescents and young adults (AYAs). Despite current treatment regimens, which include intensive cycles of multi-agent chemotherapy, and frequently consolidation with allogeneic donor stem cell transplantation to achieve cure, only 60% of children and AYAs with AML will be achieve long-term remission. New therapeutic strategies are needed to increase remission rates, decrease relapse and to improve overall survival.

BRIEF SUMMARY

[0005] Examples of the disclosure provide chimeric antigen receptors (CARs) comprising an antigen binding domain specific for CD33, a transmembrane domain, and an intracellular T cell

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 (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.

[0005c] 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 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.

[0005d] 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 pharmaceutical composition as described herein, thereby forming a complex, and (b) detecting the complex, wherein detection of the complex is indicative of the presence of cancer.

[0005e] 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.

[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.

[0012] Figures 5A-5C present graphs of an IncuCyte killing assay. CD33 CAR transduced T cells were incubated with target leukemia cells as indicated in the figure. The differences of the live leukemia cells relative to the original plated cells were plotted.

[0013] Figures 5D and 5E present graphs of an IncuCyte killing assay. CD123 CAR transduced T cells were incubated with target leukemia cells as indicated in the figure. The differences of the live leukemia cell were normalized to the tumor only control. 5D: Killing of MOLM14 cell. 5E: Killing of THP1 cells.

[0014] Figures 6A-6E present bioluminescent images that were used to track the leukemia progression with different treatments *in vivo*, as shown. The anti-CD19 CAR is non-specific to the CD33 antigen.

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 (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 hP67.6wherein 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.

8. The CAR according to any one of claims 1-7, 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.

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

10. A chimeric antigen receptor (CAR) comprising (a) the amino acid sequence of 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.

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

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

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

14. A recombinant expression vector comprising the nucleic acid according to claim 12 or 13.

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

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

17. A pharmaceutical composition comprising 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, the host cell according to claim 15, or the population of cells according to claim 16, and a pharmaceutically acceptable carrier.

18. 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, the host cell according to claim 15, the population of cells according to claim 16, or the pharmaceutical composition 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. Use of 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, the host cell according to claim 15, the population of cells according to claim 16, or the pharmaceutical composition according to claim 17 in the manufacture of a medicament for the treatment or prevention of cancer in a mammal.

20. 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, the host cell according to claim 15, the population of cells according to claim 16, or the pharmaceutical composition according to claim 17 for use in the treatment or prevention of cancer in a mammal.

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