

CHIMERIC ANTIGEN RECEPTORS AND THEIR USES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This patent application claims the benefit of U.S. Provisional Patent Application No. 62/506,268, filed May 15, 2017, 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 Z01 BC011565 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 180,939 Byte ASCII (Text) file named “739267_ST25.TXT” dated May 15, 2018.

BACKGROUND OF THE INVENTION

[0004] Cancer is a public health concern. Despite advances in treatments such as chemotherapy, the prognosis for many cancers, including hematological malignancies, may be poor. Accordingly, there exists an unmet need for additional treatments for cancer, particularly hematological malignancies.

BRIEF SUMMARY

[0004a] In one aspect, the present disclosure provides for a chimeric antigen receptor (CAR) amino acid construct comprising: (a) a cleavable domain; (b) a first CAR comprising a first antigen binding domain, a first transmembrane domain, and a first intracellular T cell signaling domain; and (c) a second CAR comprising a second antigen binding domain, a second transmembrane domain, and a second intracellular T cell signaling domain; wherein the first and second CARs are linked through the cleavable domain, and (i) wherein the first antigen binding domain comprises the heavy chain variable region CDR sequences of SEQ ID NOS: 4, 6, and 8 and the light chain variable region CDR sequences of SEQ ID NOS: 12, 14, and 16, and wherein

when the first CAR is cleaved from the construct, the first antigen binding domain has antigenic specificity for CD22; or (ii) wherein the first antigen binding domain comprises the light chain variable region CDR sequences of SEQ ID NOS: 24, 26, and 28 and the heavy chain variable region CDR sequences of SEQ ID NOS: 32, 34, and 36, and wherein when the first CAR is cleaved from the construct, the first antigen binding domain has antigenic specificity for CD19.

[0004b] In another aspect, the present disclosure provides for a chimeric antigen receptor (CAR) amino acid construct comprising: (a) two or more cleavable domains; (b) a first CAR comprising a first antigen binding domain, a first transmembrane domain, and a first intracellular T cell signaling domain; and (c) a second CAR comprising a second antigen binding domain, a second transmembrane domain, and a second intracellular T cell signaling domain; wherein the first and second CARs are linked through the two or more cleavable domains.

[0004c] In another aspect, the present disclosure provides for a nucleic acid comprising a nucleotide sequence encoding the CAR amino acid construct as described herein.

[0004d] In another aspect, the present disclosure provides for an isolated host cell comprising a recombinant expression vector comprising the nucleic acid as described herein.

[0004e] In another aspect, the present disclosure provides for a pharmaceutical composition comprising the CAR construct as described herein, the nucleic acid as described herein, the host cell as described herein or a population of cells thereof, and a pharmaceutically acceptable carrier.

[0004f] In another aspect, the present disclosure provides for the use of the host cell as described herein or a population of cells thereof in the manufacture of a medicament for the treatment of cancer in a mammal.

[0004g] In another aspect, the present disclosure provides for a method of making a chimeric antigen receptor (CAR) amino acid construct, the method comprising designing two or more cleavable domains between (a) a first CAR comprising a first antigen binding domain, a first transmembrane domain, and a first intracellular T cell signaling domain; and (b) a second CAR comprising a second antigen binding domain, a second transmembrane domain, and a second intracellular T cell signaling domain; wherein the first and second CARs are linked through the two or more cleavable domains; and cloning into a plasmid a sequence comprising from N-terminus to C-terminus the first CAR, the two or more cleavable domains, and the second CAR.

[0005] An example of the disclosure provides a chimeric antigen receptor (CAR) amino acid construct comprising (a) a cleavable domain; (b) a first CAR comprising a first antigen binding domain, a first transmembrane domain, and a first intracellular T cell signaling domain; and (c) a second CAR comprising a second antigen binding domain, a second transmembrane domain, and a second intracellular T cell signaling domain; wherein the first and second CARs are linked through the cleavable domain, wherein the first antigen binding domain comprises an antigen binding domain of the m971 antibody, wherein when the first CAR is cleaved from the construct, the first antigen binding domain has antigenic specificity for CD22.

[0006] Another example of the disclosure provides a chimeric antigen receptor (CAR) amino acid construct comprising (a) a cleavable domain; (b) a first CAR comprising a first antigen

binding domain, a first transmembrane domain, and a first intracellular T cell signaling domain; and (c) a second CAR comprising a second antigen binding domain, a second transmembrane domain, and a second intracellular T cell signaling domain; wherein the first and second CARs are linked through the cleavable domain, wherein the first antigen binding domain comprises an antigen binding domain of the FMC63 antibody, wherein when the first CAR is cleaved from the construct, the first antigen binding domain has antigenic specificity for CD19.

[0007] Another example of the disclosure provides chimeric antigen receptor (CAR) amino acid construct comprising (a) two or more cleavable domains; (b) a first CAR comprising a first antigen binding domain, a first transmembrane domain, and a first intracellular T cell signaling domain; and (c) a second CAR comprising a second antigen binding domain, a second transmembrane domain, and a second intracellular T cell signaling domain; wherein the first and second CARs are linked through the two or more cleavable domains.

[0008] Another example of the disclosure provides a method of making a chimeric antigen receptor (CAR) amino acid construct, the method comprising designing two or more cleavable domains between (a) a first CAR comprising a first antigen binding domain, a first transmembrane domain, and a first intracellular T cell signaling domain; and (b) a second CAR comprising a second antigen binding domain, a second transmembrane domain, and a second intracellular T cell signaling domain; wherein the first and second CARs are linked through the two or more cleavable domains; and cloning into a plasmid a sequence comprising from N-terminus to C-terminus the first CAR, the two or more cleavable domains, and the second CAR.

[0009] Another example of the disclosure provides CAR amino acid constructs comprising the amino acid sequences as described herein.

[0010] Further examples of the disclosure provide related nucleic acids, recombinant expression vectors, host cells, populations of cells, and pharmaceutical compositions relating to the CAR amino acid constructs of the disclosure.

[0011] 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

[0012] Figure 1 presents schematic diagrams of exemplary CAR constructs, in accordance with embodiments of the invention.

[0013] Figures 2A-2C present fluorescence-activated cell sorting dot plots comparing surface expression on human T cells of (2A) anti-CD19 CAR (the anti-CD19 CAR denoted as “CD19 CAR”) and CD3 when the T cells are transduced with a vector encoding the single anti-CD19 CAR or the V1 CAR construct (also denoted as bicistronic-V1 or bicis-V1), (2B) anti-CD22 CAR (the anti-CD22 CAR denoted as “CD22 CAR”) and CD3 when the T cells are transduced with a vector encoding the single anti-CD22 CAR or the V1 CAR construct, and (2C) anti-CD19 CAR and anti-CD22 CAR when the cells are transduced with a vector encoding the V1 CAR construct, in accordance with embodiments of the invention.

[0014] Figure 3 presents fluorescence-activated cell sorting dot plots comparing surface expression of anti-CD19 CAR and anti-CD22 CAR on human T cells when transduced with a vector encoding the single anti-CD19 CAR, the single anti-CD22 CAR, the LoopCAR6, or co-transduced with separate vectors encoding the single anti-CD19 CAR and the single anti-CD22 CAR.

[0015] Figure 4 presents fluorescence-activated cell sorting dot plots comparing surface expression of anti-CD19 CAR and anti-CD22 CAR on human T cells when transduced with a vector encoding the V1 CAR construct, the V5 CAR construct (also denoted as bicistronic-V5 or bicis-V5), or the LoopCAR6, in accordance with embodiments of the invention.

[0016] Figures 5A, 5B, 6A, 6B, 7A, 7B, and 8 are bar graphs showing *in vitro* activity based on cytokine production, in accordance with embodiments of the invention. Figure 5A shows IL2 levels, and Figure 5B shows IFN γ levels, measured when K562 cells expressing CD19, CD22, both, or none are contacted with T cells transduced with a vector encoding the V1 CAR construct, the single anti-CD19 CAR (CAR19), or the single anti-CD22 CAR (CAR22), in accordance with embodiments of the invention. Figure 6A shows IL2 levels, and Figure 6B shows IFN γ levels, measured when K562 cells expressing CD19, CD22, both, or none are

Claims

1. A chimeric antigen receptor (CAR) amino acid construct comprising:
 - (a) a cleavable domain;
 - (b) a first CAR comprising
 - a first antigen binding domain,
 - a first transmembrane domain, and
 - a first intracellular T cell signaling domain; and
 - (c) a second CAR comprising
 - a second antigen binding domain,
 - a second transmembrane domain, and
 - a second intracellular T cell signaling domain;

wherein the first and second CARs are linked through the cleavable domain, and

 - (i) wherein the first antigen binding domain comprises the heavy chain variable region CDR sequences of SEQ ID NOS: 4, 6, and 8 and the light chain variable region CDR sequences of SEQ ID NOS: 12, 14, and 16, and

wherein when the first CAR is cleaved from the construct, the first antigen binding domain has antigenic specificity for CD22; or

 - (ii) wherein the first antigen binding domain comprises the light chain variable region CDR sequences of SEQ ID NOS: 24, 26, and 28 and the heavy chain variable region CDR sequences of SEQ ID NOS: 32, 34, and 36, and

wherein when the first CAR is cleaved from the construct, the first antigen binding domain has antigenic specificity for CD19.

2. The CAR construct according to claim 1, wherein the first antigen binding domain comprises the heavy chain variable region CDR sequences of SEQ ID NOS: 4, 6, and 8 and the light chain variable region CDR sequences of SEQ ID NOS: 12, 14, and 16.

3. The CAR construct according to claim 1, wherein the first antigen binding domain comprises the amino acid sequences of the light chain variable region CDR sequences of

SEQ ID NOS: 24, 26, and 28 and the heavy chain variable region CDR sequences of SEQ ID NOS: 32, 34, and 36.

4. The CAR construct according to claim 2, wherein the second antigen binding domain comprises the light chain variable region CDR sequences of SEQ ID NOS: 24, 26, and 28 and the heavy chain variable region CDR sequences of SEQ ID NOS: 32, 34, and 36.

5. The CAR construct according to any one of claims 1-4, wherein the cleavable domain is 2A or furin.

6. The CAR construct according to any one of claims 1-4, wherein the cleavable domain is 2A and furin.

7. The CAR construct according to any one of claim 1-6, wherein the CAR construct comprises exactly two CARs being the first and second CARs, respectively.

8. The CAR construct according to claim 1, wherein the CAR comprises the amino acid sequence of SEQ ID NO: 48, 49, 50, 51, or 52 or an amino acid sequence having 90% or greater sequence identity with any one of SEQ ID NOS: 63-70.

9. The CAR according to claim 1, wherein the CAR comprises the amino acid sequence of SEQ ID NO: 49.

10. A chimeric antigen receptor (CAR) amino acid construct comprising:

- (a) two or more cleavable domains;
- (b) a first CAR comprising
 - a first antigen binding domain,
 - a first transmembrane domain, and
 - a first intracellular T cell signaling domain; and
- (c) a second CAR comprising
 - a second antigen binding domain,

a second transmembrane domain, and
a second intracellular T cell signaling domain;
wherein the first and second CARs are linked through the two or more cleavable domains.

11. The CAR construct of claim 10, wherein there are exactly two cleavable domains.
12. A nucleic acid comprising a nucleotide sequence encoding the CAR amino acid construct of any one of claims 1-11.
13. An isolated host cell comprising a recombinant expression vector comprising the nucleic acid of claim 12.
14. A pharmaceutical composition comprising the CAR construct of any one of claims 1-11, the nucleic acid of claim 12, the host cell of claim 13 or a population of cells thereof, and a pharmaceutically acceptable carrier.
15. Use of the host cell of claim 13 or a population of cells thereof in the manufacture of a medicament for the treatment of cancer in a mammal.
16. The CAR construct for the use of claim 15, wherein the cancer is a hematological malignancy.
17. A method of making a chimeric antigen receptor (CAR) amino acid construct, the method comprising designing two or more cleavable domains between
 - (a) a first CAR comprising
 - a first antigen binding domain,
 - a first transmembrane domain, and
 - a first intracellular T cell signaling domain; and
 - (b) a second CAR comprising
 - a second antigen binding domain,
 - a second transmembrane domain, and

a second intracellular T cell signaling domain;
wherein the first and second CARs are linked through the two or more cleavable domains;
and
cloning into a plasmid a sequence comprising from N-terminus to C-terminus the first CAR, the two or more cleavable domains, and the second CAR.