

CHIMERIC ANTIGEN RECEPTORS TARGETING CD-19

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This patent application claims the benefit of U.S. Provisional Patent Application No. 62/006,313, filed June 2, 2014, which is incorporated by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH AND
DEVELOPMENT

[0001a] This invention was made with Government support under project number Z01 BC001415 by the National Institutes of Health, National Cancer Institute. The Government has certain rights in the invention.

INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0002] Incorporated by reference in its entirety herein is a computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: One 58,356 Byte ASCII (Text) file named "720755_ST25.TXT," created on June 1, 2015.

BACKGROUND OF THE INVENTION

[0003] B-cell malignancies, such as lymphoma and leukemia, occur when the regulation of B-cell differentiation and activation is disrupted. Malignancies of mature B-cells include follicular lymphoma, mantle-cell lymphoma, Burkitt lymphoma, multiple myeloma, diffuse large B-cell lymphoma, Hodgkin lymphoma, lymphoplasmacytic lymphoma, marginal-zone lymphoma, and chronic lymphocytic leukemia (Shaffer et al., *Nature Reviews Immunology*, 2: 920-933 (2002)). Standard therapies such as chemotherapy, therapeutic monoclonal antibodies (e.g., Rituximab (RITUXANTM)), and allogeneic stem cell transplantation (alloHSCT) do not cure B-cell malignancies (see, e.g., Dreger et al., *Leukemia*, 21(1): 12-17 (2007); Gribben, J.G., *Blood*, 109(11): 4617-4626 (2007); and Armitage, J.O., *Blood*, 110(1): 29-36 (2007)). In particular, monoclonal antibodies are not curative as single agents, and alloHSCT is associated

with high levels of mortality and morbidity (see, e.g., Dreger et al., *supra*, Armitage et al., *supra*, and McLaughlin et al., *Journal of Clinical Oncology*, 16(8): 2825-2833 (1998)).

[0004] T-cells can be genetically modified to express chimeric antigen receptors (CARs), which are fusion proteins comprised of an antigen recognition moiety and T-cell activation domains (see, e.g., Kershaw et al., *supra*, Eshhar et al., *Proc. Natl. Acad. Sci. USA*, 90(2): 720-724 (1993), and Sadelain et al., *Curr. Opin. Immunol.*, 21(2): 215-223 (2009)). For B-cell

lineage malignancies, adoptive T-cell approaches that utilize CARs which target CD19 have been developed (see, e.g., Jensen et al., *Biology of Blood and Marrow Transplantation*, 16: 1245-1256 (2010); Kochenderfer et al., *Blood*, 116(20): 4099-4102 (2010); Porter et al., *The New England Journal of Medicine*, 365(8): 725-733 (2011); Savoldo et al., *Journal of Clinical Investigation*, 121(5): 1822-1826 (2011), Cooper et al., *Blood*, 101(4): 1637-1644 (2003); Brentjens et al., *Nature Medicine*, 9(3): 279-286 (2003); Kalos et al., *Science Translational Medicine*, 3(95): 95ra73 (2011); Cheadle et al., *Journal of Immunology*, 184(4): 1885-1896 (2010); Brentjens et al., *Clinical Cancer Research*, 13(18 Pt 1): 5426-5435 (2007); Kochenderfer et al., *Blood*, 116(19): 3875-3886 (2010); Brentjens et al., *Blood*, 118(18): 4817-4828 (2011); and Kochenderfer et al., *Blood*, December 8, 2011 (epublication ahead of print (2012)). The B-cell antigen CD19 has been chosen as target for CARs because its expression is limited to normal and malignant B-cells (see, e.g., Nadler et al., *Journal of Immunology*, 131(1): 244-250 (1983)).

[0005] One disadvantage associated with the anti-CD19 CAR therapies reported to date is that they can induce significant toxicity associated with elevated levels of serum cytokines. The generation of human anti-mouse immune responses also is a potential risk associated with current anti-CD19 CARs, which contain murine sequences (see, e.g., Jensen et al., *supra*; Lamers et al., *Blood*, 117(1): 72-82 (2011); and Maus et al., *Cancer Immunol Res*, 2: 112-120 (2014)).

[0006] Thus, there remains a need for compositions that can be used in methods to treat B-cell malignancies which have reduced toxicity and immunogenicity in humans. This invention provides such compositions and methods.

BRIEF SUMMARY OF THE INVENTION

[0006a] In one aspect, the present invention refers to a chimeric antigen receptor (CAR) directed against CD19, which comprises an amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, or SEQ ID NO: 13. In another aspect, the present invention refers to chimeric antigen receptor (CAR) comprising the amino acid sequence of each of the following that are present in SEQ ID NO: 4 or SEQ ID NO: 9: (i) the CD8 α extracellular spacer, (ii) the transmembrane domain from a human CD8 α molecule, and (iii) the intracellular T-cell signaling domains from (a) both of a human CD28

molecule and a human CD27 molecule, and (b) a human CD3 ζ molecule. In another aspect, the present invention refers to chimeric antigen receptor (CAR) comprising the amino acid sequence of each of the following that are present in SEQ ID NO: 10 or SEQ ID NO: 11: (i) the CD8 α extracellular spacer, (ii) the transmembrane domain from a human CD8 α molecule, and (iii) the intracellular T-cell signaling domains from (a) one or both of a human CD28 molecule and a human CD27 molecule, and (b) the gamma chain of Fc ϵ RI. In another aspect, the present invention refers to chimeric antigen receptor (CAR) comprising the amino acid sequence of each of the following that are present in SEQ ID NO: 12 or SEQ ID NO: 13: (i) the CD8 α extracellular spacer, (ii) the transmembrane domain from a human CD8 α molecule, and (iii) the intracellular T-cell signaling domains from a human CD28 molecule and the gamma chain of Fc ϵ RI. In yet another aspect, the present invention refers to a nucleic acid encoding the CAR of the present invention. In another aspect, there is provided a vector comprising the nucleic acid of the present invention. In another aspect, there is provided a T-cell or NK cell comprising the vector of the present invention. In another aspect, the present invention refers to a T-cell or NK cell of the present invention for use in preventing or treating a B-cell malignancy in a subject. In yet another aspect, the present invention provides a method of destroying malignant B-cells *in vitro*, which method comprises contacting one or more of the T-cells or NK cells of the present invention with a population of malignant B-cells cells that express CD19, whereby the CAR binds to CD19 on the malignant B-cells and the malignant B-cells are destroyed. In another aspect of the invention, there is provided a method of making a cell expressing a chimeric antigen receptor (CAR), the method comprising introducing a vector encoding the CAR of any one of the present invention into a T-cell or NK cell.

[0007] The disclosure describes an isolated or purified chimeric antigen receptor (CAR) directed against CD19, which comprises an amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, or SEQ ID NO: 13.

[0008] In addition, also disclosed are isolated or purified nucleic acid sequences encoding the foregoing CARs, vectors comprising such nucleic acid sequences, isolated T-cells comprising such vectors, and methods of destroying malignant B-cells by contacting such isolated T-cells with a population of malignant CD19-expressing B-cells cells *in vivo* or *ex vivo*.

[0009] Also described herein is an isolated or purified CAR comprising the following elements that are present in SEQ ID NO: 4 or SEQ ID NO: 9: (i) the extracellular spacer, (i) the transmembrane domain derived from a human CD8 α molecule, and (iii) the intracellular T-cell signaling domains derived from a human CD28 molecule, a human CD27 molecule, and a human CD3 ζ molecule.

[0010] Also described herein is an isolated or purified CAR comprising the following elements that are present in SEQ ID NO: 10 or SEQ ID NO: 11: (i) the extracellular spacer, (i) the transmembrane domain derived from a human CD8 α molecule, and (iii) the intracellular T-cell signaling domains derived from a human CD28 molecule, a human CD27 molecule, and the gamma chain of Fc ϵ RI.

[0010A] Also described herein is an isolated or purified nucleic acid encoding the CAR as described herein.

[0010B] Also described herein is a vector comprising the isolated or purified nucleic acid as described herein.

[0010C] Also described herein is an isolated T-cell or NK cell comprising the vector as described herein.

[0010D] Also described herein is an isolated T-cell or NK cell as described herein for use in preventing or treating a B-cell malignancy in a subject.

[0010E] Also described herein is a method of destroying malignant B-cells in vitro, which method comprises contacting one or more of the isolated T-cells or NK cells as described herein with a population of malignant B-cells cells that express CD19, whereby the CAR binds to CD19 on the malignant B-cells and the malignant B-cells are destroyed.

[0010F] Also described herein is a method of making a cell expressing a chimeric antigen receptor (CAR), the method comprising introducing a vector encoding the CAR as described herein into a T-cell or NK cell.

CLAIMS:

1. A chimeric antigen receptor (CAR) directed against CD19, which comprises an amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, or SEQ ID NO: 13.

2. The CAR of claim 1, wherein the CAR comprises an amino acid sequence of SEQ ID NO: 10.

3. A chimeric antigen receptor (CAR) comprising the amino acid sequence of each of the following that are present in SEQ ID NO: 4 or SEQ ID NO: 9: (i) the CD8 α extracellular spacer, (ii) the transmembrane domain from a human CD8 α molecule, and (iii) the intracellular T-cell signaling domains from (a) both of a human CD28 molecule and a human CD27 molecule, and (b) a human CD3 ζ molecule.

4. A chimeric antigen receptor (CAR) comprising the amino acid sequence of each of the following that are present in SEQ ID NO: 10 or SEQ ID NO: 11: (i) the CD8 α extracellular spacer, (ii) the transmembrane domain from a human CD8 α molecule, and (iii) the intracellular T-cell signaling domains from (a) one or both of a human CD28 molecule and a human CD27 molecule, and (b) the gamma chain of Fc ϵ RI.

5. A chimeric antigen receptor (CAR) comprising the amino acid sequence of each of the following that are present in SEQ ID NO: 12 or SEQ ID NO: 13: (i) the CD8 α extracellular spacer, (ii) the transmembrane domain from a human CD8 α molecule, and (iii) the intracellular T-cell signaling domains from a human CD28 molecule and the gamma chain of Fc ϵ RI.

6. A nucleic acid encoding the CAR of any one of claims 1-5.

7. A vector comprising the nucleic acid of claim 6.

8. A T-cell or NK cell comprising the vector of claim 7.

9. A T-cell or NK cell of claim 8 for use in preventing or treating a B-cell malignancy in a subject.
10. A method of destroying malignant B-cells *in vitro*, which method comprises contacting one or more of the T-cells or NK cells of claim 8 with a population of malignant B-cells cells that express CD19, whereby the CAR binds to CD19 on the malignant B-cells and the malignant B-cells are destroyed.
11. The method of claim 10, wherein the malignant B-cells are lymphoma cells.
12. The method of claim 10, wherein the malignant B-cells are leukemia cells.
13. A method of making a cell expressing a chimeric antigen receptor (CAR), the method comprising introducing a vector encoding the CAR of any one of claims 1-5 into a T-cell or NK cell.