BISPECIFIC CHIMERIC ANTIGEN RECEPTORS BINDING TO CD19 AND CD22

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of the filing date of U.S. Provisional Application No. 63/140,752, filed January 22, 2021, the entire contents of which are incorporated by reference herein.

BACKGROUND OF THE INVENTION

Chimeric antigen receptor (CAR-T) T cells are genetically engineered T cells expressing an artificial T cell receptor for use in immunotherapy. The artificial T cell receptor (known as chimeric antigen receptor) can specifically bind disease cell antigens, such as cancer antigens. Upon binding to the disease cell, the CAR-T cells would be activated and eliminate the disease cell.

While CAR-T cell therapy have demonstrated efficacy in treatment of a few blood cancer, efficacy of the treatment may be affected by various factors, for example, tumor antigen escape, for example, the expression level of the tumor antigen may reduce to a level that CAR-T cells cannot engage and mediate cytotoxic activity. In some instances, tumor cells may escape killing by expressing an alternative form of the target antigen that lacks the binding epitope to the CAR. In other instances, tumor cells may escape killing by switching to a genetically related but phenotypically different disease (so called lineage switch).

Accordingly, it is of great interest to develop improved CAR-T approaches to address such challenges.

SUMMARY ~~OF THE INVENTION~~

The present disclosure is based, at least in part, on the development of anti-CD19/CD22 bispecific chimeric antigen receptors (CARs) having superior antigen binding affinity and specificity and superior anti-tumor effects as observed in an animal model. Accordingly, provided herein are anti-CD19/CD22 bispecific CARs, nucleic acid encoding such, host cells such as immune cells expressing the bispecific CAR, and therapeutic applications thereof.

In one aspect ~~some aspects~~, the present disclosure features a bi-specific chimeric antigen receptor (CAR) specific to CD19 and CD22, comprising a first antigen binding moiety specific to CD19, a second antigen binding moiety to CD22, a co-stimulatory signaling domain, and a cytoplasmic signaling domain. The first antigen binding moiety may comprise the same heavy

chain complementary determining regions (CDRs) and/or the same light chain CDRs as reference antibody EPC-001-1, which binds CD19. The second antigen binding moiety may comprise the same heavy chain CDRs and/or the same light chain CDRs as reference antibody EPC-001-2, EPC-001-3, or EPC-001-4, each of which binds CD22.

In some ~~embodiment~~examples, the first antigen binding moiety may comprise the same heavy chain variable region (V_H) and the same light chain variable region (V_L) as the reference antibody EPC-001-1. Alternatively, or in addition, the second antigen binding moiety comprises the same heavy chain variable region (V_H) and the same light chain variable region (V_L) as the reference antibody EPC-001-2, EPC-001-2, or EPC-001-3.

In some ~~embodiment~~examples, the first antigen binding moiety, the second antibody binding moiety, or both can be single-chain variable fragments (scFvs). For example, the first antigen binding moiety is a scFv comprising the amino acid sequence of SEQ ID NO: 9. Alternatively, or in addition, the second antigen binding moiety is a scFv comprising the amino acid sequence of SEQ ID NO: 18, 27, or 36.

In any of the CAR constructs disclosed herein, the co-stimulatory signaling domain can be from a co-stimulatory molecule selected from the CD28, 4-1BB, OX40, ICOS, CD27, CD40, or CD40L. In some ~~embodiment~~examples, the cytoplasmic signaling domain is from CD3ζ. Any of the CAR disclosed herein may further comprising a hinge domain and a transmembrane domain. In some instances, the hinge and transmembrane domains may be located the antigen binding moieties and the co-stimulatory signaling domain.

In some examples, the bi-specific CAR comprises a fusion polypeptide comprising, from N-terminus to C-terminus, (i) the first antigen binding moiety, (ii) the second antigen binding moiety, (iii) the co-stimulatory signaling domain, and (iv) the cytoplasmic signaling domain. In other examples, the bi-specific CAR comprises a fusion polypeptide comprising, from N-terminus to C-terminus, (i) the second antigen binding moiety, (ii) the first antigen binding moiety, (iii) the co-stimulatory signaling domain, and (iv) the cytoplasmic signaling domain.

In some instances, the bi-specific CAR may further comprise a peptide linker connecting the first antigen binding moiety and the second antigen binding moiety. Exemplary peptide linkers include, but are not limited to, GGGGS (SEQ ID NO:38), GGGGSGGGGS (SEQ ID NO:39), GGGGSGGGGSGGGGS (SEQ ID NO:40), or GSTSGSGKPGSGEGSTKG (SEQ ID NO:41).

In some ~~embodiment~~examples, the bi-specific CAR disclosed here may comprise the amino acid sequence of any one of SEQ ID NOs: 48-53. In some examples, the bi-specific CAR

disclosed herein may comprise the amino acid sequence of any one of SEQ ID NOs.: 55-60 and 63-66.

In another aspect, provided herein is a nucleic acid or a set of nucleic acid, which collectively encode any of the bi-specific CARs disclosed herein. For example, the nucleic acid may comprise a nucleotide sequence encoding a CAR comprising an amino acid sequence of any one of SEQ ID NOs: 48-53. In specific examples, the nucleic acid may comprise a nucleotide sequence encoding a CAR comprising an amino acid sequence of any one of SEQ ID NOs: 55-60 and 63-67.

In some instances, the nucleic acid or the set of nucleic acids may further comprises (i) a nucleotide sequence encoding a truncated epithelium growth factor receptor (EGFR) domain, which may comprise an extracellular domain and a transmembrane domain of an EGFR receptor, and (ii) a nucleotide sequence encoding a self-cleaving peptide, which is located between the nucleotide sequence encoding the bi-specific CAR and the nucleotide sequence encoding the truncated EGFR domain. In some examples, the truncated EGFR domain comprises the amino acid sequence of SEQ ID NO:68.

Any of the nucleic acid or set of nucleic acid can be an expression vector(s). In some examples, the expression vector(s) may be a viral vector(s).

In yet another aspect, the present disclosure features a genetically engineered immune cell, which expresses any of the bi-specific CARs disclosed herein. Such a genetically engineered immune cell may comprise any of the nucleic acids encoding the bi-specific CAR as disclosed herein. In some examples, the genetically engineered immune cell is a T cell. In some examples, the genetically engineered immune cell is an NK cell. In other examples, the genetically engineered immune cell may be a macrophage.

Also within the scope of the present disclosure are anti-CD19 CAR and anti-CD22 CAR, nucleic acids encoding such, and genetically engineered immune cells (*e.g.*, T cells) expressing such.

In some ~~embodiment~~examples, the anti-CD19 CAR may comprise an extracellular antigen binding domain that binds CD19, a co-stimulatory signaling domain, and a cytoplasmic signaling domain. The extracellular antigen binding domain may be an anti-CD19 single chain variable fragment (scFv) comprising the same heavy chain complementary determining regions (CDRs) and the same light chain CDRs as anti-CD19 antibody EPC-001-1. In some examples, the anti-CD19 scFv comprises the same heavy chain variable domain and the same light chain variable domain as anti-CD19 antibody EPC-001-1. In one example, the anti-CD19 scFv comprises the amino acid sequence of SEQ ID NO: 9. In one specific example, the anti-CD19

CAR may comprise the amino acid sequence of SEQ ID NO: 62.

In some ~~embodiment~~examples, the anti-CD22 chimeric antigen receptor (CAR) may comprise an extracellular antigen binding domain that binds CD22, a co-stimulatory signaling domain, and a cytoplasmic signaling domain. In some examples, the extracellular antigen binding domain can be an anti-CD22 single chain variable fragment (scFv) comprising the same heavy chain complementary determining regions (CDRs) and/or the same light chain CDRs as anti-CD22 antibody EPC-001-2. For example, the anti-CD22 scFv may comprises the same heavy chain variable domain and/or the same light chain variable domain as anti-CD22 antibody EPC-001-2. In some examples, the extracellular antigen binding domain can be an anti-CD22 single chain variable fragment (scFv) comprising the same heavy chain complementary determining regions (CDRs) and/or the same light chain CDRs as anti-CD22 antibody EPC-001-3. For example, the anti-CD22 scFv may comprises the same heavy chain variable domain and/or the same light chain variable domain as anti-CD22 antibody EPC-001-3. In some examples, the extracellular antigen binding domain can be an anti-CD22 single chain variable fragment (scFv) comprising the same heavy chain complementary determining regions (CDRs) and/or the same light chain CDRs as anti-CD22 antibody or EPC-001-4. For example, the anti-CD22 scFv may comprises the same heavy chain variable domain and/or the same light chain variable domain as anti-CD22 antibody EPC-001-4. In some examples, the anti-CD22 scFv comprises the amino acid sequence of SEQ ID NO: 18, 27, or 36. In specific examples, the anti-CD22 CAR may comprise the amino acid sequence of SEQ ID NO: 61.

In a further aspect, the present disclosure provides a use of a genetically engineered immune cell in the manufacture of a medicament for treating cancer in a subject, wherein the genetically engineered immune cell is as described herein; wherein the subject is a human patient having $CD19^+$ and/or $CD22^+$ cancer cells. In a further aspect, the present disclosure provides a use of the genetically engineered immune cell as described herein for use in therapy. In addition, the present disclosure features a method for eliminating undesired cells in a subject, the method comprising administering to a subject in need thereof an effective amount of the genetically engineered immune cell disclosed herein, which expresses an anti-CD19 CAR, an anti-CD22 CAR, or an anti-CD19/CD22 CAR as those disclosed herein, or a pharmaceutical composition comprising such. In some instances, the undesired cells are cancer cells.

In some ~~embodiment~~examples, the subject is a human cancer patient. For example, the subject can be a human cancer patient comprise $CD19^+$ and/or $CD22^+$ cancer cells. In some instances, the human cancer patient may have a hematopoietic malignancy, for example, a T cell malignancy

or a B cell malignancy.

The details of one or more ~~embodiment~~examples of the ~~invention~~disclosure are set forth in the description below. Other features or advantages of the present ~~invention~~disclosure will be apparent from the following drawings and detailed description of several ~~embodiment~~examples, and also from the appended claims.

WHAT IS CLAIMED IS:

1. A bi-specific chimeric antigen receptor (CAR) specific to CD19 and CD22, comprising a first antigen binding moiety specific to CD19, a second antigen binding moiety to CD22, a co-stimulatory signaling domain, and a cytoplasmic signaling domain;

wherein the first antigen binding moiety comprises the same heavy chain complementary determining regions (CDRs) and the same light chain CDRs as reference antibody EPC-001-1, which binds CD19; and

wherein the second antigen binding moiety comprises the same heavy chain CDRs and the same light chain CDRs as reference antibody EPC-001-2, EPC-001-3, or EPC-001-4, each of which binds CD22.

2. The bi-specific CAR of claim 1, wherein the first antigen binding moiety comprises the same heavy chain variable region (V_H) and the same light chain variable region (V_L) as the reference antibody EPC-001-1; and/or ~~.~~

~~3. The bi-specific CAR of claim 1 or claim 2,~~ wherein the second antigen binding moiety comprises the same heavy chain variable region (V_H) and the same light chain variable region (V_L) as the reference antibody EPC-001-2, EPC-001-3, or EPC-001-4.

~~4~~3. The bi-specific CAR of ~~any one of claims 1-3~~claim 1 or claim 2, wherein the first antigen binding moiety, the second antibody binding moiety, or both are single-chain variable fragments (scFvs).

~~5~~4. The bi-specific CAR of claim ~~4~~3, wherein the first antigen binding moiety is a scFv comprising the amino acid sequence of SEQ ID NO: 9; and/or~~.~~

~~6. The bi-specific CAR of claim 4 or claim 5,~~ wherein the second antigen binding moiety is a scFv comprising the amino acid sequence of SEQ ID NO: 18, 27, or 36.

~~7~~5. The bi-specific CAR of any one of claims 1-~~4~~6, wherein the co-stimulatory signaling domain is from a co-stimulatory molecule selected from the CD28, 4-1BB, OX40, ICOS, CD27, CD40, or CD40L; and/or~~.~~

~~8. The bi-specific CAR of any one of claims 1-7,~~ wherein the cytoplasmic signaling domain is from CD3ζ.

~~9~~6. The bi-specific CAR of any one of claims 1-~~5~~8, wherein the bi-specific CAR comprises:

(a) a fusion polypeptide comprising, from N-terminus to C-terminus, (i) the first antigen binding moiety, (ii) the second antigen binding moiety, (iii) the co-stimulatory signaling domain, and (iv) the cytoplasmic signaling domain; or

(b) a fusion polypeptide comprising, from N-terminus to C-terminus, (i) the second antigen binding moiety, (ii) the first antigen binding moiety, (iii) the co-stimulatory signaling domain, and (iv) the cytoplasmic signaling domain.

~~10~~7. The bi-specific CAR of claim ~~6~~9, further comprising a hinge domain and a transmembrane domain, which are located between (ii) and (iii).

~~11~~8. The bi-specific CAR of claim ~~9 or claim 10~~6 or claim 7, further comprising a peptide linker connecting the first antigen binding moiety and the second antigen binding moiety.

~~12~~9. The bi-specific CAR of claim ~~11~~8, wherein the peptide linker comprises the amino acid sequence of GGGGS (SEQ ID NO:38), GGGGSGGGGS (SEQ ID NO:39), GGGGSGGGGSGGGGS (SEQ ID NO:40), or GSTSGSGKPGSGEGSTKG (SEQ ID NO:41).

10~~3~~. The bi-specific CAR of claim 9~~12~~, which comprises the amino acid sequence of any one of SEQ ID NOs: 48-53.

11~~4~~. The bi-specific CAR of claim 10~~3~~, which comprises the amino acid sequence of any one of SEQ ID NOs.: 55-60 and 63-67.

125. A nucleic acid or a set of nucleic acid, which collectively encode the bi-specific CAR of any one of claims 1-11 ~~14~~.

136. The nucleic acid or set of nucleic acid of claim ~~15~~12, which comprises a nucleotide sequence encoding the bi-specific CAR ~~of any one of claims 9-15~~of any one of claims 6-11.

147. The nucleic acid or set of nucleic acid of claim 13~~6~~, which further comprises a nucleotide sequence encoding a truncated epithelium growth factor receptor (EGFR) domain comprising an extracellular domain and a transmembrane domain of an EGFR receptor, and a nucleotide sequence encoding a self-cleaving peptide, which is located between the nucleotide sequence encoding the bi-specific CAR and the nucleotide sequence encoding the truncated EGFR domain.

158. The nucleic acid or set of nucleic acid of claim 14~~7~~, wherein the truncated EGFR domain comprises the amino acid sequence of SEQ ID NO:68.

169. The nucleic acid or set of nucleic acid of ~~any one of claims 15-18~~any one of claims 12-15, wherein the nucleic acid(s) is an expression vector(s), which optionally is a viral vector(s).

17~~20~~. A genetically engineered immune cell, which expresses the bi-specific CAR of any one of claims 1-11~~14~~.

18~~21~~. The genetically engineered immune cell of claim ~~20~~17, which comprises the nucleic acid of any one of claims 125-179 encoding the bi-specific CAR.

19~~22~~. The genetically engineered immune cell of claim ~~20 or claim 21~~17 or claim 18, which is a T cell, an NK cell, or a macrophage, optionally wherein the immune cell is a T cell.

~~23. An anti-CD19 chimeric antigen receptor (CAR), comprising an extracellular antigen binding domain that binds CD19, a co-stimulatory signaling domain, and a cytoplasmic signaling domain; wherein the extracellular antigen binding domain is an anti-CD19 single chain variable fragment (scFv) comprising the same heavy chain complementary determining regions (CDRs) and the same light chain CDRs as anti-CD19 antibody EPC-001-1.~~

~~24. The anti-CD19 CAR of claim 23, wherein the anti-CD19 scFv comprises the same heavy chain variable domain and the same light chain variable domain as anti-CD19 antibody EPC-001-1.~~

~~25. The anti-CD19 CAR of claim 24, wherein the anti-CD19 scFv comprises the amino acid sequence of SEQ ID NO: 9.~~

~~26. The anti-CD19 CAR of claim 25, which comprises the amino acid sequence of SEQ ID NO: 62.~~

~~27. An anti-CD22 chimeric antigen receptor (CAR), comprising an extracellular antigen binding domain that binds CD22, a co-stimulatory signaling domain, and a cytoplasmic signaling domain; wherein the extracellular antigen binding domain is an anti-CD22 single chain variable fragment (scFv) comprising the same heavy chain complementary determining regions (CDRs) and the same light chain CDRs as anti-CD22 antibody EPC-001-2, EPC-001-3, or EPC-001-4.~~

~~28. The anti-CD22 CAR of claim 27, wherein the anti-CD22 scFv comprises the same heavy chain variable domain and the same light chain variable domain as anti-CD22 antibody EPC-001-2, EPC-001-3, or EPC-001-4.~~

~~29. The anti-CD22 CAR of claim 28, wherein the anti-CD22 scFv comprises the amino acid sequence of SEQ ID NO: 18, 27, or 36.~~

~~30. The anti-CD22 CAR of claim 29, which comprises the amino acid sequence of SEQ ID NO: 61.~~

~~31. A nucleic acid, which encodes the anti-CD19 CAR of any one of claims 23-26 or encodes the anti-CD22 CAR of any one of claims 27-30.~~

~~32. The nucleic acid of claim 31, which is an expression vector, optionally wherein the expression vector is a viral vector.~~

~~33. A genetically engineered immune cell, which expresses the anti-CD19 CAR of any one of claims 23-26 or the anti-CD22 CAR of any one of claims 27-30.~~

~~34. The genetically engineered immune cell of claim 33, which is a T cell.~~

~~35~~20. ~~A method for eliminating undesired cells in a subject, the method comprising administering to a subject in need thereof an effective amount of the~~Use of a genetically engineered immune cell in the manufacture of a medicament for treating cancer in a subject, wherein the genetically engineered immune cell is set forth in any one of claims 17-19; wherein the subject is a human patient having $CD19^+$ and/or $CD22^+$ cancer cells. ~~of any one of claims 18-20 and 33-34, or a pharmaceutical composition comprising such.~~

~~36. The method of claim 35, wherein the undesired cells are cancer cells.~~

~~37. The method of claim 35 or claim 36, wherein the subject is a human cancer patient.~~

~~38. The method of claim 37, wherein the human cancer patient comprise $CD19^+$ and/or $CD22^+$ cancer cells.~~

21~~39~~. The use of claim 20, ~~method of claim 37 or claim 38,~~ wherein the human cancer patient has a hematopoietic malignancy, which optionally is a T cell malignancy or a B cell malignancy.

22. The genetically engineered immune cell is set forth in any one of claims 17-19 for use in therapy.