

human antibody agents, multi-specific binding agents (e.g., bi-specific antibodies) and chimeric antigen receptors effectively mediate killing of cancer cells characterized by CD19 expression (e.g., lymphomas and/or leukemias).

[004a] In one aspect, provided herein is an antibody or antibody fragment that specifically binds human cluster of differentiation 19 (CD19), the antibody or antibody fragment comprising:

heavy chain-complementarity determining region 1 (HC-CDR1) of SEQ ID NO: 151, heavy chain-complementarity determining region 2 (HC-CDR2) of SEQ ID NO: 152, and heavy chain-complementarity determining region 3 (HC-CDR3) of SEQ ID NO: 153, and

light chain-complementarity determining region 1 (LC-CDR 1) of SEQ ID NO: 259,

light chain-complementarity determining region 2 (LC-CDR2) of SEQ ID NO: 260, and

light chain-complementarity determining region 3 (LC-CDR3) of SEQ ID NO: 261,

wherein the antibody or antibody fragment may have one of the following substitutions (i) to (vi):

(i) substitution of M residue for the L residue at the 21st from the N-terminus of the amino acid sequence of SEQ ID NO: 153 of HC-CDR3;

(ii) substitution of M residue for the L residue at the 21st from the N-terminus of the amino acid sequence of SEQ ID NO: 153 of HC-CDR3, and

substitution of E residue for the D residue at the 8th from the N-terminus of the amino acid sequence of SEQ ID NO:261 of LC-CDR3;

(iii) substitution of M residue for the L residue at the 21st from the N-terminus of the amino acid sequence of SEQ ID NO: 153 of HC-CDR3, and

substitution of G residue for the D residue at the 8th from the N-terminus of the amino acid sequence of SEQ ID NO:261 of LC-CDR3;

(iv) substitution of M residue for the L residue at the 21st from the N-terminus of the amino acid sequence of SEQ ID NO: 153 of HC-CDR3, and

substitution of T residue for the S residue at in the 6th from the N-terminus of the amino acid sequence of SEQ ID NO:261 of LC-CDR3;

(v) substitution of M residue for the L residue at the 21st from the N-terminus of the amino acid sequence of SEQ ID NO: 153 of HC-CDR3, and

substitution of N residue for the Y residue at the 12th from the N-terminus of the amino acid sequence of SEQ ID NO: 153 of HC-CDR3; and

(vi) substitution of M residue for the L residue at the 21st from the N-terminus of the amino acid sequence of SEQ ID NO: 153 of HC-CDR3,

substitution of F residue for the Y residue at the 7th from the N-terminus of the amino acid sequence of SEQ ID NO: 151 of HC-CDR1, and

substitution of V residue for the A residue at the 1st from the N-terminus of the amino acid sequence of SEQ ID NO: 153 of HC-CDR3.

[004b] In one aspect, provided herein is a bi-specific antibody comprising a first antigen-binding site and a second antigen-binding site, wherein the first antigen-binding site is from an antibody or antibody fragment as described herein, and the bi-specific antibody specifically binds human cluster of differentiation 19 (CD19) by the first antigen-binding site.

[004c] In one aspect, provided herein is a chimeric antigen receptor comprising an antigen-binding site of an antibody or antibody fragment as described herein and specifically binding human cluster of differentiation 19 (CD19).

[004d] In one aspect, provided herein is a nucleic acid molecule encoding, in whole, the antibody or antibody fragment as described herein, the bi-specific antibody of as described herein, or the chimeric antigen receptor as described herein.

[004e] In one aspect, provided herein is a vector comprising the nucleic acid molecule as described herein.

[004f] In one aspect, provided herein is cell comprising the nucleic acid molecule as described herein or the vector as described herein or expressing the antibody or antibody fragment as described herein, the bi-specific antibody as described herein, or the chimeric antigen receptor as described herein.

[004g] In one aspect, provided herein is an effector cell expressing the antibody or antibody fragment as described herein, the bi-specific antibody as described herein, or the chimeric antigen receptor as described herein.

[004h] In one aspect, provided herein is a pharmaceutical composition comprising the antibody or antibody fragment as described herein, the bi-specific antibody as described herein, the chimeric antigen receptor as described herein, the nucleic acid molecule as described herein, the vector as described herein, or the effector cell as described herein, and a pharmaceutically acceptable carrier or diluent.

[004i] In one aspect, provided herein is a use of a composition comprising the antibody or antibody fragment as described herein, the bi-specific antibody as described herein, the chimeric antigen receptor as described herein, the nucleic acid molecule as described herein, the vector as described herein, the effector cell as described herein, or the pharmaceutical composition as described herein in the manufacture of a medicament for treating a medical condition characterized by CD19 expression in a subject.

[004j] In one aspect, provided herein is a use of a composition comprising the antibody or antibody fragment as described herein, the bi-specific antibody as described herein, the chimeric antigen receptor as described herein, the nucleic acid molecule of as described herein, the vector as described herein, the effector cell as described herein, or the pharmaceutical composition as described herein in the manufacture of a medicament for treating cancer.

[004k] In one aspect, provided herein is a use of a medical composition comprising the antibody or antibody fragment as described herein, the bi-specific antibody as described herein, the chimeric antigen receptor as described herein, the nucleic acid molecule as described herein, the vector as described herein, the effector cell as described herein, or the pharmaceutical composition as described herein in the manufacture of a medicament for inhibiting tumor growth or killing tumor cells.

[004l] In one aspect, provided herein is the antibody or antibody fragment as described herein, the bi-specific antibody as described herein, the chimeric antigen receptor as described herein, the nucleic acid molecule as described herein, the vector as described herein, the effector cell as described herein, or the pharmaceutical composition as described herein for use in therapy.

[005] Although embodiments employing antibody agents that contain human sequences (i.e., human heavy and light chain variable region sequences including human CDR sequences) are extensively discussed herein, the present invention also provides non-human antibody agents. In some embodiments, non-human antibody agents comprise human CDR sequences from an antibody agent as described herein and non-human framework sequences. Non-human framework sequences include, in some embodiments, any sequence that can be used for generating synthetic heavy and/or light chain variable regions using one or more human CDR sequences as described herein, including, e.g., mammals, e.g., mouse, rat, rabbit, pig, bovine (e.g., cow, bull, buffalo), deer, sheep, goat, chicken, cat, dog, ferret, primate (e.g., marmoset, rhesus monkey), etc. In some embodiments, a provided antibody agent includes an antibody agent generated by grafting one or more human CDR sequences as described herein onto a non-human framework sequence (e.g., a mouse or chicken framework sequence). In many embodiments, provided antibody agents are human antibody agents (e.g., a human monoclonal

antibody or fragment thereof, human antigen-binding protein or polypeptide, human multi-specific binding agent [e.g., a human bi-specific antibody], a human polypeptide having one or more structural components of a human immunoglobulin polypeptide).

[006] In some embodiments, the present invention provides an antibody agent that specifically binds human CD19, wherein the antibody agent comprises a heavy chain CDR1 (HC-CDR1), heavy chain CDR2 (HC-CDR2) and a heavy chain CDR3 (HC-CDR3), and a light chain CDR1 (LC-CDR1), light chain CDR2 (LC-CDR2) and a light chain CDR3 (LC-CDR3),

wherein the HC-CDR1 comprises G-X₁-X₂-F-X₃-S-X₄-X₅ (SEQ ID NO:291), and wherein X₁ is F, G, Y or V, X₂ is S or T, X₃ is S or T, X₄ is N or Y, and X₅ is A, W or Y;

wherein the HC-CDR2 comprises I-X₆-P-X₇-X₈-X₉-X₁₀-T (SEQ ID NO:292), and wherein X₆ is S, D or Y, X₇ is E, S, G or I, X₈ is D, F or V, X₉ is G or S, and X₁₀ is K, E, Y, D or T;

wherein the LC-CDR1 comprises S-S-N-X₁₁-G-X₁₂-X₁₃-X₁₄ (SEQ ID NO:293) or N-I-G-S-X₁₅-S (SEQ ID NO:294), and wherein X₁₁ is I or V, X₁₂ is N, S or T, X₁₃ is N, H or K, X₁₄ is Y, A or T, and X₁₅ is K or E; and/or

wherein the LC-CDR2 comprises X₁₆-X₁₇-X₁₈ (SEQ ID NO:295), and wherein X₁₆ is D, E, S, R or Y, X₁₇ is N or D, and X₁₈ is N, Y, S or D.

[007] In some embodiments, the present invention provides a human antibody agent that specifically binds human CD19, wherein the human antibody agent comprises one or more

CLAIMS

1. An antibody or antibody fragment that specifically binds human cluster of differentiation 19 (CD19), the antibody or antibody fragment comprising:

heavy chain-complementarity determining region 1 (HC-CDR1) of SEQ ID NO: 151, heavy chain-complementarity determining region 2 (HC-CDR2) of SEQ ID NO: 152, and heavy chain-complementarity determining region 3 (HC-CDR3) of SEQ ID NO: 153, and light chain-complementarity determining region 1 (LC-CDR 1) of SEQ ID NO: 259, light chain-complementarity determining region 2 (LC-CDR2) of SEQ ID NO: 260, and light chain-complementarity determining region 3 (LC-CDR3) of SEQ ID NO: 261,

wherein the antibody or antibody fragment may have one of the following substitutions (i) to (vi):

(i) substitution of M residue for the L residue at the 21st from the N-terminus of the amino acid sequence of SEQ ID NO: 153 of HC-CDR3;

(ii) substitution of M residue for the L residue at the 21st from the N-terminus of the amino acid sequence of SEQ ID NO: 153 of HC-CDR3, and

substitution of E residue for the D residue at the 8th from the N-terminus of the amino acid sequence of SEQ ID NO:261 of LC-CDR3;

(iii) substitution of M residue for the L residue at the 21st from the N-terminus of the amino acid sequence of SEQ ID NO: 153 of HC-CDR3, and

substitution of G residue for the D residue at the 8th from the N-terminus of the amino acid sequence of SEQ ID NO:261 of LC-CDR3;

(iv) substitution of M residue for the L residue at the 21st from the N-terminus of the amino acid sequence of SEQ ID NO: 153 of HC-CDR3, and

substitution of T residue for the S residue at in the 6th from the N-terminus of the amino acid sequence of SEQ ID NO:261 of LC-CDR3;

- (v) substitution of M residue for the L residue at the 21st from the N-terminus of the amino acid sequence of SEQ ID NO: 153 of HC-CDR3, and
- substitution of N residue for the Y residue at the 12th from the N-terminus of the amino acid sequence of SEQ ID NO: 153 of HC-CDR3; and
- (vi) substitution of M residue for the L residue at the 21st from the N-terminus of the amino acid sequence of SEQ ID NO: 153 of HC-CDR3,
- substitution of F residue for the Y residue at the 7th from the N-terminus of the amino acid sequence of SEQ ID NO: 151 of HC-CDR1, and
- substitution of V residue for the A residue at the 1st from the N-terminus of the amino acid sequence of SEQ ID NO: 153 of HC-CDR3.
2. The antibody or antibody fragment of claim 1, wherein the antibody or antibody fragment may comprise the substitution (i), the substitution (ii), the substitution (iii) or the substitution (vi).
3. The antibody or antibody fragment of claim 1, wherein the antibody or antibody fragment does not have the substitutions (i) to (vi).
4. The antibody or antibody fragment of claim 1, wherein the antibody or antibody fragment comprises the sequence of SEQ ID NO: 42 as an amino acid sequence comprising the HC-CDR1, HC-CDR2, HC-CDR3, LC-CDR1, LC-CDR2 and LC-CDR3.
5. The antibody or antibody fragment of any one of claims 1 to 4, wherein the antibody or antibody fragment is or comprises an immunoglobulin, Fab, F(ab')₂, Fd, Fv, single chain Fv (scFv), or a dAb.
6. The antibody or antibody fragment of any one of claims 1 to 5, wherein the antibody or antibody fragment is conjugated to a therapeutic agent, a detection agent, a cytotoxic agent or moiety, or a radio-isotope.
7. The antibody or antibody fragment of any one of claims 1 to 6, wherein the antibody or antibody fragment is a human antibody or antibody fragment.

8. A bi-specific antibody comprising a first antigen-binding site and a second antigen-binding site, wherein the first antigen-binding site is from an antibody or antibody fragment of any one of claims 1 to 7, and the bi-specific antibody specifically binds human cluster of differentiation 19 (CD19) by the first antigen-binding site.
9. The bi-specific antibody of claim 8, wherein the second antigen-binding site binds an immune cell selected from the group consisting of a T cell, NK cell, B cell, dendritic cell, monocyte, macrophage, neutrophil, mesenchymal stem cell and neural stem cell.
10. A chimeric antigen receptor comprising an antigen-binding site of an antibody or antibody fragment of any one of claims 1 to 7 and specifically binding human cluster of differentiation 19 (CD19).
11. The chimeric antigen receptor of claim 10, further comprising a transmembrane domain and an intracellular signaling domain, wherein the intracellular signaling domain comprises a CD3 ζ intracellular signaling sequence and a CD28 intracellular signaling sequence.
12. A nucleic acid molecule encoding, in whole, the antibody or antibody fragment of any one of claims 1-7, the bi-specific antibody of claim 8 or 9, or the chimeric antigen receptor of claim 10 or 11.
13. A vector comprising the nucleic acid molecule of claim 12.
14. A cell comprising the nucleic acid molecule of claim 12 or the vector of claim 13 or expressing the antibody or antibody fragment of any one of claims 1-7, the bi-specific antibody of claim 8 or 9, or the chimeric antigen receptor of claim 10 or 11.
15. An effector cell expressing the antibody or antibody fragment of any one of claims 1-7, the bi-specific antibody of claim 8 or 9, or the chimeric antigen receptor of claim 10 or 11.
16. The effector cell of claim 15, wherein the effector cell is a T cell.
17. A pharmaceutical composition comprising the antibody or antibody fragment of any one of claims 1-7, the bi-specific antibody of claim 8 or 9, the chimeric antigen receptor of claim 10

or 11, the nucleic acid molecule of claim 12, the vector of claim 13, or the effector cell of claim 15 or 16, and a pharmaceutically acceptable carrier or diluent.

18. Use of a composition comprising the antibody or antibody fragment of any one of claims 1-7, the bi-specific antibody of claim 8 or 9, the chimeric antigen receptor of claim 10 or 11, the nucleic acid molecule of claim 12, the vector of claim 13, the effector cell of claim 15 or 16, or the pharmaceutical composition of claim 17 in the manufacture of a medicament for treating a medical condition characterized by CD19 expression in a subject.

19. The use of claim 18, wherein the medical condition characterized by CD19 expression is B cell lymphoma, acute lymphoblastic leukemia, chronic lymphocytic leukemia, Burkitt lymphoma, non-Hodgkin's lymphoma, or acute myeloid leukemia.

20. Use of a composition comprising the antibody or antibody fragment of any one of claims 1-7, the bi-specific antibody of claim 8 or 9, the chimeric antigen receptor of claim 10 or 11, the nucleic acid molecule of claim 12, the vector of claim 13, the effector cell of claim 15 or 16, or the pharmaceutical composition of claim 17 in the manufacture of a medicament for treating cancer.

21. Use of a medical composition comprising the antibody or antibody fragment of any one of claims 1-7, the bi-specific antibody of claim 8 or 9, the chimeric antigen receptor of claim 10 or 11, the nucleic acid molecule of claim 12, the vector of claim 13, the effector cell of claim 15 or 16, or the pharmaceutical composition of claim 17 in the manufacture of a medicament for inhibiting tumor growth or killing tumor cells.

22. The antibody or antibody fragment of any one of claims 1-7, the bi-specific antibody of claim 8 or 9, the chimeric antigen receptor of claim 10 or 11, the nucleic acid molecule of claim 12, the vector of claim 13, the effector cell of claim 15 or 16, or the pharmaceutical composition of claim 17 for use in therapy.