

SUMMARY

[0009] Aspects of the disclosure include antibodies that binds to CD20, comprising a heavy chain variable region comprising: (a) a CDR1 sequence comprising two or fewer substitutions in any one of the amino acid sequences of SEQ ID NOs: 1 or 4; and/or a CDR2 sequence comprising two or fewer substitutions in any one of the amino acid sequences of SEQ ID NOs: 2 or 5; and/or (c) a CDR3 sequence comprising two or fewer substitutions in any one of the amino acid sequences of SEQ ID NOs: 3 or 6.

[0010] In some examples, the CDR1, CDR2, and CDR3 sequences are present in a human framework. In some examples, an antibody further comprises a heavy chain constant region sequence in the absence of a CH1 sequence.

[0011] In some examples, an antibody comprises: (a) a CDR1 sequence selected from the group consisting of SEQ ID NOs: 1 and 4; and/or (b) a CDR2 sequence selected from the group consisting of SEQ ID NOs: 2 and 5; and/or (c) a CDR3 sequence selected from the group consisting of SEQ ID NOs: 3 and 6.

[0012] In some examples, an antibody comprises: (a) a CDR1 sequence selected from the group consisting of SEQ ID NOs: 1 and 4; and (b) a CDR2 sequence selected from the group consisting of SEQ ID NOs: 2 and 5; and (c) a CDR3 sequence selected from the group consisting of SEQ ID NOs: 3 and 6.

[0013] In some examples, an antibody comprises: (a) a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 2, and a CDR3 sequence of SEQ ID NO: 3; or (b) a CDR1 sequence of SEQ ID NO: 4, a CDR2 sequence of SEQ ID NO: 5, and a CDR3 sequence of SEQ ID NO: 6.

[0014] In some examples, an antibody comprises a heavy chain variable region having at least 95% sequence identity to any of the sequences of SEQ ID NOs: 7-8. In some examples, an antibody comprises a heavy chain variable region sequence selected from the group consisting of SEQ ID NOs: 7-8. In some examples, an antibody comprises a heavy chain variable region sequence of SEQ ID NO: 7. In some examples, an antibody comprises a heavy chain variable region sequence of SEQ ID NO: 8.

[0015] Aspects of the disclosure include antibodies that bind to CD20, comprising a heavy chain variable region comprising CDR1, CDR2 and CDR3 sequences in a human VH framework, wherein the CDR sequences are sequences having two or fewer substitutions in a CDR sequence selected from the group consisting of SEQ ID NOs: 1-6. In some examples, an antibody comprises a heavy chain variable region comprising CDR1, CDR2 and CDR3 sequences in a human VH framework, wherein the CDR sequences are selected from the group consisting of SEQ ID NOs: 1-6.

[0016] Aspects of the disclosure include antibodies that bind to CD20, comprising a heavy chain variable region comprising: (a) a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 2, and a CDR3 sequence of SEQ ID NO: 3, in a human VH framework; or (b) a CDR1 sequence of

SEQ ID NO: 4, a CDR2 sequence of SEQ ID NO: 5, and a CDR3 sequence of SEQ ID NO: 6, in a human VH framework. In one aspect, the present disclosure refers to an antibody that binds to CD20, comprising a heavy chain variable region comprising: (a) a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 2, and a CDR3 sequence of SEQ ID NO: 3; or (b) a CDR1 sequence of SEQ ID NO: 4, a CDR2 sequence of SEQ ID NO: 5, and a CDR3 sequence of SEQ ID NO: 6.

[0017] In some examples, an antibody is in a CAR-T format. In some examples, an antibody is multi-specific. In some examples, an antibody is bispecific. In some examples, an antibody binds to two different CD20 proteins. In some examples, an antibody binds to two different epitopes on the same CD20 protein. In some examples, an antibody binds to an effector cell. In some examples, an antibody binds to a T-cell antigen. In some examples, an antibody binds to CD3.

[0018] In some examples, an antibody comprises: (a) a heavy chain variable region comprising: (i) a CDR1 sequence of SEQ ID NO: 9, a CDR2 sequence of SEQ ID NO: 10, and a CDR3 sequence of SEQ ID NO: 11, in a human VH framework; or (ii) a CDR1 sequence of SEQ ID NO: 12, a CDR2 sequence of SEQ ID NO: 13, and a CDR3 sequence of SEQ ID NO: 14, in a human VH framework; and (b) a light chain variable region comprising a CDR1 sequence of SEQ ID NO: 15, a CDR2 sequence of SEQ ID NO: 16, and a CDR3 sequence of SEQ ID NO: 17, in a human VL framework.

[0019] In some examples, an antibody comprises: (a) a heavy chain variable region comprising: (i) a heavy chain variable region sequence having at least 95% sequence identity to SEQ ID NO: 18; or (ii) a heavy chain variable region sequence having at least 95% sequence identity to SEQ ID NO: 19; and (b) a light chain variable region sequence having at least 95% sequence identity to SEQ ID NO: 20.

[0020] In some examples, an antibody comprises: (a) a heavy chain variable region comprising: (i) a heavy chain variable region sequence comprising SEQ ID NO: 18; or (ii) a heavy chain variable region sequence comprising SEQ ID NO: 19; and (b) a light chain variable region sequence comprising SEQ ID NO: 20.

[0021] Aspects of the disclosure include bispecific three-chain antibody-like molecules (TCA) that bind to CD20 and CD3, comprising: (a) a first polypeptide consisting of SEQ ID NO: 32; (b) a second polypeptide selected from the group consisting of: SEQ ID NO: 33 and SEQ ID NO: 42; and (c) a third polypeptide selected from the group consisting of: SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, and SEQ ID NO: 39.

[0022] Aspects of the disclosure include CAR-T cells comprising a CAR comprising an extracellular antigen-binding domain that binds to CD20, comprising a heavy chain variable region comprising: (a) a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 2, and a CDR3 sequence of SEQ ID NO: 3; or (b) a CDR1 sequence of SEQ ID NO: 4, a CDR2 sequence of SEQ ID NO: 5, and a CDR3 sequence of SEQ ID NO: 6. In another aspect, the present disclosure refers to a CAR-T cell comprising a CAR comprising an extracellular antigen-binding domain that binds to CD20, comprising a heavy chain variable region comprising: (a) a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 2, and a CDR3 sequence of SEQ ID NO: 3; or (b) a CDR1 sequence of SEQ ID NO: 4, a CDR2 sequence

of SEQ ID NO: 5, and a CDR3 sequence of SEQ ID NO: 6. In some examples, the extracellular antigen-binding domain that binds to CD20 comprises a heavy chain variable region having at least 95% sequence identity to any of the sequences of SEQ ID NOs: 7-8. In some examples, the extracellular antigen-binding domain that binds to CD20 comprises a heavy chain variable region sequence selected from the group consisting of SEQ ID NOs: 7-8. In some examples, the extracellular antigen-binding domain that binds to CD20 comprises a heavy chain variable region sequence of SEQ ID NO: 7. In some examples, the

extracellular antigen-binding domain that binds to CD20 comprises a heavy chain variable region sequence of SEQ ID NO: 8.

[0023] Aspects of the disclosure include pharmaceutical compositions comprising an antibody as described herein, or a CAR-T cell as described herein. In yet another aspect, the present disclosure refers to a pharmaceutical composition comprising the CAR-T cell as described herein.

[0024] Aspects of the disclosure include methods for the treatment of a disorder characterized by expression of CD20, comprising administering to a subject with said disorder an antibody as described herein, a CAR-T cell as described herein, or a pharmaceutical composition as described herein. In some examples, the disorder is a hematological malignancy. In some examples, the hematological malignancy is non-Hodgkin lymphoma (NHL). In some examples, the hematological malignancy is chronic lymphocytic leukemia (CLL). In some examples, the hematological malignancy is follicular lymphoma (FL). In some examples, the hematological malignancy is diffuse large B-cell lymphoma (DLBCL). In some examples, the hematological malignancy is mantle cell lymphoma (MCL). In some examples, the hematological malignancy is acute lymphocytic leukemia (ALL). In some examples, the hematological malignancy is marginal zone lymphoma (MZL).

[0025] Aspects of the disclosure include polynucleotides encoding an antibody or a CAR of a CAR-T cell as described herein, vectors comprising such polynucleotides, and cells comprising such vectors.

[0026] Aspects of the disclosure include methods of producing an antibody as described herein, the methods comprising growing a cell as described herein under conditions permissive for expression of the antibody and isolating the antibody from the cell and/or a cell culture medium in which the cell is grown.

[0027] Aspects of the disclosure include methods of making an antibody as described herein, the methods comprising immunizing a UniRat animal with CD20 and identifying CD20-binding heavy chain sequences.

[0028] Aspects of the disclosure include methods of treatment, comprising administering to an individual in need an effective dose of an antibody, a CAR-T cell, or a pharmaceutical composition as described herein.

[0029] Aspects of the disclosure include use of an antibody a CAR-T cell as described herein in the preparation of a medicament for the treatment of a disease or disorder in an individual in need. In another aspect, the present disclosure refers to the use of a CAR-T cell as described herein in the manufacture of a medicament for treating a disorder characterized by expression of CD20.

[0030] Aspects of the disclosure include kits for treating a disease or disorder in an individual in need, comprising an antibody, a CAR-T cell, or a pharmaceutical composition as described herein, and instructions for use. In some examples, a kit further comprises at least one additional reagent. In some examples, the at least one additional reagent comprises a chemotherapeutic drug. In yet another aspect, the present disclosure refers to a kit for treating a disease or disorder in an individual in need, comprising the CAR-T cell as described herein and instructions for use.

[0031] These and further aspects will be further explained in the rest of the disclosure, including the Examples.

CLAIMS:

1. An antibody that binds to CD20, comprising a heavy chain variable region comprising:
 - (a) a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 2, and a CDR3 sequence of SEQ ID NO: 3; or
 - (b) a CDR1 sequence of SEQ ID NO: 4, a CDR2 sequence of SEQ ID NO: 5, and a CDR3 sequence of SEQ ID NO: 6.
2. A CAR-T cell comprising a CAR comprising an extracellular antigen-binding domain that binds to CD20, comprising a heavy chain variable region comprising:
 - (a) a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 2, and a CDR3 sequence of SEQ ID NO: 3; or
 - (b) a CDR1 sequence of SEQ ID NO: 4, a CDR2 sequence of SEQ ID NO: 5, and a CDR3 sequence of SEQ ID NO: 6.
3. The CAR-T cell of claim 2, wherein the CDR1, CDR2 and CDR3 sequences are present in a human VH framework.
4. The CAR-T cell of claim 2, wherein the heavy chain variable region comprises a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 2, and a CDR3 sequence of SEQ ID NO: 3.
5. The CAR-T cell of claim 2, wherein the heavy chain variable region comprises a CDR1 sequence of SEQ ID NO: 4, a CDR2 sequence of SEQ ID NO: 5, and a CDR3 sequence of SEQ ID NO: 6.
6. The CAR-T cell of claim 2, wherein the extracellular antigen-binding domain that binds to CD20 comprises a heavy chain variable region having at least 95% sequence identity to any one of the sequences of SEQ ID NOs: 7 and 8.
7. The CAR-T cell of claim 6, wherein the extracellular antigen-binding domain that binds to CD20 comprises a heavy chain variable region sequence selected from the group consisting of SEQ ID NOs: 7 and 8.

8. The CAR-T cell of claim 7, wherein the extracellular antigen-binding domain that binds to CD20 comprises a heavy chain variable region sequence of SEQ ID NO: 7.
9. The CAR-T cell of claim 7, wherein the extracellular antigen-binding domain that binds to CD20 comprises a heavy chain variable region sequence of SEQ ID NO: 8.
10. A pharmaceutical composition comprising the CAR-T cell of claim 2.
11. Use of a CAR-T cell of claim 2 in the manufacture of a medicament for treating a disorder characterized by expression of CD20.
12. The use of claim 11, wherein the disorder is a hematological malignancy.
13. The use of claim 12, wherein the hematological malignancy is non-Hodgkin lymphoma (NHL).
14. The use of claim 12, wherein the hematological malignancy is chronic lymphocytic leukemia (CLL).
15. The method of claim 12, wherein the hematological malignancy is follicular lymphoma (FL).
16. The use of claim 12, wherein the hematological malignancy is diffuse large B-cell lymphoma (DLBCL).
17. The use of claim 12, wherein the hematological malignancy is mantle cell lymphoma (MCL).
18. The use of claim 12, wherein the hematological malignancy is acute lymphocytic leukemia (ALL).
19. The use of claim 12, wherein the hematological malignancy is marginal zone lymphoma (MZL).
20. A kit for treating a disease or disorder in an individual in need, comprising the CAR-T cell of claim 2 and instructions for use.

21. The kit of claim 20, further comprising at least one additional reagent.
22. The kit of claim 21, wherein the at least one additional reagent comprises a chemotherapeutic drug.