

CLAIMS

1. A CD19 binding molecule that specifically binds to human CD19, wherein the CD19 binding molecule is a trispecific binding molecule (TBM) comprising:

- (a) an antigen-binding module 1 (ABM1) which is an antibody or antigen-binding domain thereof that binds specifically to CD19; and
- (b) an antigen-binding module 2 (ABM2), which is an antibody or antigen-binding domain thereof and binds specifically to a component of a human T-cell receptor (TCR) complex and
- (c) an antigen-binding module 3 (ABM3), in which ABM3 binds specifically to human CD2,

wherein the CD19 binding molecule comprises CD19 binding sequences selected from the group consisting of

a) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:14, SEQ ID NO:15, and SEQ ID NO:16 respectively;

b) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:17, SEQ ID NO:18, and SEQ ID NO:19 respectively;

c) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:20, SEQ ID NO:21, and SEQ ID NO:22 respectively; and

d) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:23, SEQ ID NO:24, and SEQ ID NO:25 respectively.

2. The CD19 binding molecule of claim 1, which comprises CD19 binding sequences which comprise a VH having the amino acid sequence of SEQ ID NO:13 and/or which comprises a VL having the amino acid sequence of SEQ ID NO:26.

3. The CD19 binding molecule of any one of claims 1 or 2, wherein ABM1 is a Fab.

4. The CD19 binding molecule of any one of claims 1 to 3, wherein ABM2 is an scFv.

5. The CD19 binding molecule of any one of claims 1 to 4, wherein the component of the TCR complex is CD3.

6. The CD19 binding molecule of claim 5, wherein ABM2 is an anti-CD3 antibody or an antigen-binding domain thereof and comprises the CDR sequences of any one of the CDR sequence sets selected from the group consisting of the following

1.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:192, SEQ ID NO:212, and SEQ ID NO:236 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:193, SEQ ID NO:213, and SEQ ID NO:237 respectively;

2.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:194, SEQ ID NO:214, and SEQ ID NO:238 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:195, SEQ ID NO:215, and SEQ ID NO:239 respectively;

3.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:196, SEQ ID NO:216, and SEQ ID NO:240 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:197, SEQ ID NO:217, and SEQ ID NO:241 respectively;

4.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:192, SEQ ID NO:212, and SEQ ID NO:236 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:198, SEQ ID NO:218, and SEQ ID NO:242 respectively;

5.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:192, SEQ ID NO:219, and SEQ ID NO:236 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:193, SEQ ID NO:213, and SEQ ID NO:237 respectively;

6.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:199, SEQ ID NO:220, and SEQ ID NO:243 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:200, SEQ ID NO:221, and SEQ ID NO:244 respectively;

7.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:194, SEQ ID NO:222, and SEQ ID NO:245 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:195, SEQ ID NO:215, and SEQ ID NO:239 respectively;

8.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:201, SEQ ID NO:223, and SEQ ID NO:246 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:202, SEQ ID NO:224, and SEQ ID NO:247 respectively;

9.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:203, SEQ ID NO:214, and SEQ ID NO:248 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:202, SEQ ID NO:224, and SEQ ID NO:247 respectively;

10.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:194, SEQ ID NO:223, and SEQ ID NO:238 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:204, SEQ ID NO:215, and SEQ ID NO:239 respectively;

11.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:194, SEQ ID NO:223, and SEQ ID NO:249 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:204, SEQ ID NO:215, and SEQ ID NO:250 respectively;

12.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:192, SEQ ID NO:219, and SEQ ID NO:251 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:193, SEQ ID NO:213, and SEQ ID NO:237 respectively;

13.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:205, SEQ ID NO:225, and SEQ ID NO:252 respectively, and CDR-L1, CDR-L2, and CDR-L3 having

the amino acid sequences of SEQ ID NO:206, SEQ ID NO:226, and SEQ ID NO:253 respectively;

14.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:194, SEQ ID NO:227, and SEQ ID NO:249 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:204, SEQ ID NO:215, and SEQ ID NO:250 respectively;

15.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:194, SEQ ID NO:223, and SEQ ID NO:254 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:204, SEQ ID NO:215, and SEQ ID NO:250 respectively;

16.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:194, SEQ ID NO:223, and SEQ ID NO:255 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:204, SEQ ID NO:215, and SEQ ID NO:250 respectively;

17.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:194, SEQ ID NO:223, and SEQ ID NO:256 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:204, SEQ ID NO:215, and SEQ ID NO:250 respectively;

18.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:207, SEQ ID NO:223, and SEQ ID NO:249 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:204, SEQ ID NO:215, and SEQ ID NO:250 respectively;

19.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:208, SEQ ID NO:223, and SEQ ID NO:238 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:204, SEQ ID NO:215, and SEQ ID NO:239 respectively;

20.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:207, SEQ ID NO:223, and SEQ ID NO:238 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:204, SEQ ID NO:215, and SEQ ID NO:239 respectively;

21.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:194, SEQ ID NO:228, and SEQ ID NO:238 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:204, SEQ ID NO:215, and SEQ ID NO:239 respectively;

22.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:194, SEQ ID NO:229, and SEQ ID NO:238 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:204, SEQ ID NO:215, and SEQ ID NO:239 respectively;

23.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:194, SEQ ID NO:230, and SEQ ID NO:238 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:204, SEQ ID NO:215, and SEQ ID NO:239 respectively;

24.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:194, SEQ ID NO:223, and SEQ ID NO:249 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:204, SEQ ID NO:215, and SEQ ID NO:239 respectively;

25.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:194, SEQ ID NO:223, and SEQ ID NO:257 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:204, SEQ ID NO:215, and SEQ ID NO:239 respectively;

26.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:194, SEQ ID NO:223, and SEQ ID NO:258 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:204, SEQ ID NO:215, and SEQ ID NO:239 respectively;

27.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:194, SEQ ID NO:223, and SEQ ID NO:238 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:209, SEQ ID NO:215, and SEQ ID NO:239 respectively;

28.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:194, SEQ ID NO:223, and SEQ ID NO:238 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:210, SEQ ID NO:215, and SEQ ID NO:239 respectively;

29.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:194, SEQ ID NO:223, and SEQ ID NO:238 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:204, SEQ ID NO:231, and SEQ ID NO:239 respectively;

30.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:194, SEQ ID NO:223, and SEQ ID NO:238 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:204, SEQ ID NO:232, and SEQ ID NO:239 respectively;

31.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:194, SEQ ID NO:223, and SEQ ID NO:238 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:204, SEQ ID NO:233, and SEQ ID NO:239 respectively;

32.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:194, SEQ ID NO:223, and SEQ ID NO:238 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:204, SEQ ID NO:234, and SEQ ID NO:239 respectively;

33.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:194, SEQ ID NO:223, and SEQ ID NO:238 respectively, and CDR-L1, CDR-L2, and CDR-L3 having

the amino acid sequences of SEQ ID NO:204, SEQ ID NO:215, and SEQ ID NO:259 respectively;

34.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:194, SEQ ID NO:223, and SEQ ID NO:238 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:204, SEQ ID NO:215, and SEQ ID NO:250 respectively;

35.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:194, SEQ ID NO:223, and SEQ ID NO:238 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:195, SEQ ID NO:215, and SEQ ID NO:239 respectively;

36.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:194, SEQ ID NO:223, and SEQ ID NO:238 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:211, SEQ ID NO:215, and SEQ ID NO:239 respectively;

37.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:194, SEQ ID NO:227, and SEQ ID NO:249 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:204, SEQ ID NO:215, and SEQ ID NO:239 respectively;

38.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:194, SEQ ID NO:223, and SEQ ID NO:256 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:204, SEQ ID NO:215, and SEQ ID NO:239 respectively;

39.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:207, SEQ ID NO:227, and SEQ ID NO:256 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:204, SEQ ID NO:215, and SEQ ID NO:239 respectively;

40.) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:194, SEQ ID NO:235, and SEQ ID NO:238 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:204, SEQ ID NO:215, and SEQ ID NO:239 respectively; and

41) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:194, SEQ ID NO:214, and SEQ ID NO:260 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:209, SEQ ID NO:215, and SEQ ID NO:239 respectively.

7. The CD19 binding molecule of any one of claims 1 to 6, wherein ABM3 is a CD58 moiety.

8. The CD19-binding molecule of claim 7, wherein the CD58 moiety comprises sequences selected from the group consisting of

the amino acid sequence of SEQ ID NO:322,

the amino acid sequence of SEQ ID NO:323,

the amino acid sequence of SEQ ID NO:324,

the amino acid sequence of SEQ ID NO:325,

the amino acid sequence of SEQ ID NO:326,

the amino acid sequence of SEQ ID NO:327,

the amino acid sequence of SEQ ID NO:328,

the amino acid sequence of SEQ ID NO:329,

the amino acid sequence of SEQ ID NO:330,

the amino acid sequence of SEQ ID NO:331 and

the amino acid sequence of SEQ ID NO:332.

9. The CD19-binding molecule of claim 8, wherein the CD58 moiety comprises the amino acid sequence of SEQ ID NO:327.
10. A pharmaceutical composition comprising (a) the CD19 binding molecule of any one of claims 1 to 9 and (b) an excipient.
11. The CD19 binding molecule of any one of claims 1 to 9, or the pharmaceutical composition of claim 10 for use in therapy.
12. The CD19 binding molecule of any one of claims 1 to 9, or the pharmaceutical composition of claim 10 for use in a method of treating a subject with a CD19-associated disease or disorder, wherein the disease or disorder is a B cell malignancy that expresses cell surface CD19, wherein an effective amount of the CD19 binding molecule, or the pharmaceutical composition is to be administered to the subject.
13. A nucleic acid or plurality of nucleic acids encoding the CD19-binding molecule of any one of claims 1 to 9.
14. A cell engineered to express the CD19 binding molecule of any one of claims 1 to 9.
15. A method of producing a CD19 binding molecule, comprising:
- (a) culturing the cell of claim 14 in conditions under which the CD19 binding molecule is expressed; and
 - (b) recovering the CD19-binding molecule from the cell culture.
16. A CD19 binding molecule which is a trispecific binding molecule (TBM) comprising:
- (a) an antigen-binding module 1 (ABM1) that binds specifically to CD19 and comprises CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6 respectively, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:17, SEQ ID NO:18, and SEQ ID NO:19 respectively;

(b) an antigen-binding module 2 (ABM2) that binds specifically to a component of a human T-cell receptor (TCR) complex;

(c) an antigen-binding module 3 (ABM3) which is a CD58 moiety that binds specifically to human CD2

and

d) an Fc domain.

17. The CD19 binding molecule of claim 16, in which ABM1 is a Fab.

18. The CD19 binding molecule of claim 17, in which the Fab comprises a VH having the amino acid sequence of SEQ ID NO:13 and a VL having the amino acid sequence of SEQ ID NO:26.

19. The CD19 binding molecule of any one of claims 16 to 18, wherein the component of the TCR complex is CD3.

20. The CD19 binding molecule of claim 19, wherein ABM2 is an anti-CD3 antibody or an antigen-binding domain thereof and wherein ABM2 comprises the heavy and light chain variable sequences of SEQ ID Nos: 172 and 173 respectively.

21. The CD19 binding molecule of claim 20, wherein the anti-CD3 antibody or antigen-binding domain thereof is in the form of a scFv.

22. The CD19 binding molecule of any one of claims 16 to 21, wherein ABM3 comprises the amino acid sequence of SEQ ID NO:327.

23. The CD19 binding molecule of any one of claims 16 to 22, which comprises a first variant Fc region and a second variant Fc region that together form an Fc heterodimer.

24. The CD19 binding molecule of any one of claims 16 to 23, wherein the Fc domain is a human IgG1 Fc domain which comprises:

(a) a first CH3 domain comprising the modification T366W; and

(b) a second CH3 domain that heterodimerizes with the first CH3 domain and comprises the modifications T366S, L368A and Y407V.

25. The CD19 binding molecule of any one of claims 16 to 24, wherein the Fc domain comprises a human IgG1 sequence of SEQ ID NO:1109 with a mutation at 1, 2, 3, 4, 5 or 6 positions of positions 233, 234, 235, 236, 237, 239, 265, 266, 267, 268, 269, 297, 299, 322, 327, 328, 329, 330, 331 and 332 (EU numbering).

26. The CD19 binding molecule of any one of claims 16 to 25, wherein the Fc domain comprises a human IgG1 Fc domain modified by substituting the aspartate residue at position 265 with an alanine residue, the asparagine residue at position 297 with an alanine residue and the proline residue at position 329 with an alanine residue (D265A/N297A/P329A).

27. A CD19 binding molecule comprising:

(a) first half antibody heavy chain whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:758 and a Fc sequence;

(b) a first half antibody light chain whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:759;

(c) a second half antibody whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:760 and a Fc sequence.

28. A CD19 binding molecule comprising:

a) first half antibody heavy chain whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:758;

b) a first half antibody light chain whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:759; and

c) a second half antibody whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:760.

29. A CD19 binding molecule comprising:

(a) a first polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1077;

(b) a second polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:759; and

(c) a third polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1078 or SEQ ID NO:1086.

30. A CD19 binding molecule comprising:

(a) a first polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1077;

(b) a second polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:759; and

(c) a third polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1086.

31. A pharmaceutical composition comprising (a) the CD19 binding molecule of any one of claims 16 to 30 and (b) a pharmaceutically acceptable excipient.