

Claims

1. A bispecific antibody or an antigen-binding portion thereof, comprising a first binding domain that binds to CD20 on the surface of a target cell and a second binding domain that binds to CD3 on the surface of a T cell, wherein the first binding domain comprises a first light chain and a first heavy chain, and the first binding domain comprises a light chain and heavy chain complementarity-determining regions (CDR) combination, wherein the first light chain CDR1 comprising the sequence shown in SEQ ID NO: 61, the first light chain CDR2 comprising the sequence shown in SEQ ID NO: 62, and the first light chain CDR3 comprising the sequence shown in SEQ ID NO: 63, and the first heavy chain CDR1 comprising the sequence shown in SEQ ID NO: 76, the first heavy chain CDR2 comprising the sequence shown in SEQ ID NO: 77 and the first heavy chain CDR3 comprising the sequence shown in SEQ ID NO: 85; and
- the second binding domain comprises a second light chain and a second heavy chain, and the second binding domain comprises a light chain and heavy chain CDR combination, wherein the second light chain CDR1 comprising the sequence shown in SEQ ID NO: 7, the second light chain CDR2 comprising the sequence shown in SEQ ID NO: 8, and the second light chain CDR3 sequences comprising the sequence shown in SEQ ID NO: 21, and the second heavy chain

CDR1 comprising the sequence shown in SEQ ID NO: 26, the second heavy chain CDR2 comprising the sequence shown in SEQ ID NO: 27 and the second heavy chain CDR3 sequences comprising the sequence shown in SEQ ID NO: 28.

- 5 2. The bispecific antibody or the antigen-binding portion thereof according to claim 1, wherein the first binding domain comprises a first light chain and a first heavy chain selected from:

(1) the first binding domain comprises a first light chain of amino acid sequence SEQ ID NO: 100, and a first heavy chain of amino acid sequence SEQ ID NO:

10 98;

(2) the first binding domain comprises a first light chain of amino acid sequence SEQ ID NO: 102, and a first heavy chain of amino acid sequence SEQ ID NO: 104;

(3) the first binding domain comprises a first light chain of amino acid sequence
15 SEQ ID NO: 110, and a first heavy chain of amino acid sequence SEQ ID NO: 112;

(4) the first binding domain comprises a first light chain of amino acid sequence SEQ ID NO: 118, and a first heavy chain of amino acid sequence SEQ ID NO: 120.

- 20 3. The bispecific antibody or the antigen-binding portion thereof according to

claim 1 or 2, wherein the second binding domain comprises a second light chain and a second heavy chain selected from:

(1) the second binding domain comprises a second light chain of amino acid sequence SEQ ID NO: 92, and a second heavy chain of amino acid sequence

5 SEQ ID NO: 90;

(2) the second binding domain comprises a second light chain of amino acid sequence SEQ ID NO: 106, and a second heavy chain of amino acid sequence SEQ ID NO: 108;

(3) the second binding domain comprises a second light chain of amino acid
10 sequence SEQ ID NO: 114, and a second heavy chain of amino acid sequence SEQ ID NO: 116.

4. The bispecific antibody or the antigen-binding portion thereof according to claim 1, wherein bispecific antibody comprises:

(1) the bispecific antibody comprises a first light chain of amino acid sequence
15 SEQ ID NO: 100, a first heavy chain of amino acid sequence SEQ ID NO: 98, a second light chain of amino acid sequence SEQ ID NO: 90, and a second heavy chain of amino acid sequence SEQ ID NO: 92;

(2) the bispecific antibody comprises a first light chain of amino acid sequence SEQ ID NO: 102, a first heavy chain of amino acid sequence SEQ ID NO: 104,
20 a second light chain of amino acid sequence SEQ ID NO: 106, and a second

heavy chain of amino acid sequence SEQ ID NO: 108;

(3) the bispecific antibody comprises a first light chain of amino acid sequence SEQ ID NO: 110, a first heavy chain of amino acid sequence SEQ ID NO: 112, a second light chain of amino acid sequence SEQ ID NO: 114, and a second
5 heavy chain of amino acid sequence SEQ ID NO: 116;

(4) the bispecific antibody comprises a first light chain of amino acid sequence SEQ ID NO: 118, a first heavy chain of amino acid sequence SEQ ID NO: 120, a second light chain of amino acid sequence SEQ ID NO: 114, and a second heavy chain of amino acid sequence SEQ ID NO: 116;

10 (5) the bispecific antibody comprises a first light chain of amino acid sequence SEQ ID NO: 110, a first heavy chain of amino acid sequence SEQ ID NO: 112, a second light chain of amino acid sequence SEQ ID NO: 106, and a second heavy chain of amino acid sequence SEQ ID NO: 108;

(6) the bispecific antibody comprises a first light chain of amino acid sequence
15 SEQ ID NO: 102, a first heavy chain of amino acid sequence SEQ ID NO: 104, a second light chain of amino acid sequence SEQ ID NO: 114, and a second heavy chain of amino acid sequence SEQ ID NO: 116.

5. A nucleic acid encoding the bispecific antibody or the antigen-binding portion thereof according to any one of claims 1 to 4.

20 6. The nucleic acid according to claim 5, wherein the nucleic acid comprises:

(1) the nucleic acid sequence as shown in SEQ ID NO: 101 encoding the first light chain, the nucleic acid sequence as shown in SEQ ID NO: 99 encoding the first heavy chain, the nucleic acid sequence as shown in SEQ ID NO: 91 encoding the second light chain, and the nucleic acid sequence as shown in SEQ ID NO: 93 encoding the second heavy chain;

(2) the nucleic acid sequence as shown in SEQ ID NO: 103 encoding the first light chain, the nucleic acid sequence as shown in SEQ ID NO: 105 encoding the first heavy chain, the nucleic acid sequence as shown in SEQ ID NO: 107 encoding the second light chain, and the nucleic acid sequence as shown in SEQ ID NO: 109 encoding the second heavy chain;

(3) the nucleic acid sequence as shown in SEQ ID NO: 111 encoding the first light chain, the nucleic acid sequence as shown in SEQ ID NO: 113 encoding the first heavy chain, the nucleic acid sequence as shown in SEQ ID NO: 115 encoding the second light chain, and the nucleic acid sequence as shown in SEQ ID NO: 117 encoding the second heavy chain;

(4) the nucleic acid sequence as shown in SEQ ID NO: 119 encoding the first light chain, the nucleic acid sequence as shown in SEQ ID NO: 121 encoding the first heavy chain, the nucleic acid sequence as shown in SEQ ID NO: 115 encoding the second light chain, and the nucleic acid sequence as shown in SEQ ID NO: 117 encoding the second heavy chain;

- (5) the nucleic acid sequence as shown in SEQ ID NO: 111 encoding the first light chain, the nucleic acid sequence as shown in SEQ ID NO: 113 encoding the first heavy chain, the nucleic acid sequence as shown in SEQ ID NO: 107 encoding the second light chain, and the nucleic acid sequence as shown in SEQ ID NO: 109 encoding the second heavy chain; or
- (6) the nucleic acid sequence as shown in SEQ ID NO: 103 encoding the first light chain, the nucleic acid sequence as shown in SEQ ID NO: 105 encoding the first heavy chain, the nucleic acid sequence as shown in SEQ ID NO: 115 encoding the second light chain, and the nucleic acid sequence as shown in SEQ ID NO: 117 encoding the second heavy chain.
7. A vector comprising the nucleic acid according to claim 5 or 6.
8. A cell comprising the nucleic acid according to claim 5 or 6 or the vector according to claim 7.
9. A composition, comprising the bispecific antibody or the antigen-binding portion thereof according to any one of claims 1 to 4, the nucleic acid according to claim 5 or 6, the vector according to claim 7 and/or the cell according to claim 8.
10. An antibody-drug conjugate, comprising the bispecific antibody or the antigen-binding portion thereof according to any one of claims 1 to 4 that is covalently attached to therapeutic moiety;

preferably, the therapeutic moiety being selected from a cytotoxic moiety, a chemotherapeutic agent, a cytokine, an immunosuppressant, an immunostimulator, a lytic peptide, and a radioisotope;

preferably, the cytotoxic moiety being selected from paclitaxel; cytochalasin B;

5 gramicidin D; ethidium bromide; emetine; mitomycin; etoposide; teniposide; vincristine; vinblastine; colchicine; doxorubicin; daunorubicin; dihydroxy anthrenedione; a tubulin inhibitor such as maytansine or an analog or derivative thereof; an antimitotic agent such as monomethyl auristatin E or F or an analog or derivative thereof; dolastatin 10 or 15 or an analog thereof; irinotecan or an

10 analog thereof; mitoxantrone; mithramycin; actinomycin D; 1-dehydrotestosterone; a glucocorticoid; procaine; tetracaine; lidocaine; propranolol; puromycin; calicheamicin or an analog or derivative thereof; an anti-metabolite such as methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, fludarabine, 5-fluorouracil, decarbazine, hydroxyurea, asparaginase,

15 gemcitabine, or cladribine; an alkylating agent such as dichloromethyldiethylamine, a thiopurine, chlorambucil, melphalan, carmustine (BSNU), lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, dacarbazine (DTIC), procarbazine, mitomycin C; a platinum derivative such as cisplatin or carboplatin; duocarmycin A, duocarmycin SA,

20 rachelmycin (CC-1065) or an analog or derivative thereof; an antibiotic such as

actinomycin, bleomycin, daunorubicin, doxorubicin, idarubicin, mithramycin, mitomycin, mitoxantrone, perimycin, anthramycin (AMC); pyrrolo[2,1-c][1,4]-benzodiazepine (PDB); diphtheria toxin and related molecules such as diphtheria A chain and an active fragment and a hybrid molecule thereof, ricin such as ricin A or deglycosylated ricin A chain toxin, cholera toxin, a Shiga-like toxin such as SLT I, SLT II, SLT IIV, LT toxin, C3 toxin, a Shiga toxin, pertussis toxin, tetanus toxin, a soybean Bowman-Birk protease inhibitor, *Pseudomonas* exotoxin, alorin, saporin, modeccin, gelatin, abrin A chain, modeccin A chain, α -sarcin, *Aleurites fordii* protein, dianthin protein, a *Phytolacca americana* protein such as PAPI, PAPII and PAP-S, a *Momordica charantia* inhibitor, curcin, crotin, a *Sapaonaria officinalis* inhibitor, gelonin, mitomycin, restrictocin, phenomycin, and enomycin toxin; ribonuclease (RNase); DNase I, Staphylococcal endotoxin A; pokeweed antiviral protein; diphtheria toxin and *Pseudomonas* endotoxin;

preferably, the cytokine being selected from IL-2, IL-4, IL-6, IL-7, IL-10, IL-12, IL-13, IL-15, IL-18, IL-23, IL-24, IL-27, IL-28a, IL-28b, IL-29, KGF, IFN α , IFN β , IFN γ , GM-CSF, CD40L, Flt3 ligand, a stem cell factor, anxistim, and TNF α ;

preferably, the radioisotope being selected from ^3H , ^{14}C , ^{15}N , ^{35}S , ^{67}Cu , ^{90}Y , ^{99}Tc , ^{125}I , ^{131}I , ^{186}Re , ^{188}Re , ^{211}At , ^{212}Bi , ^{212}Pb , ^{213}Bi , ^{225}Ac , and ^{227}Th .

11. A kit, comprising the bispecific antibody or the antigen-binding portion thereof according to any one of claims 1 to 4, the nucleic acid according to claim 5 or 6, the vector according to claim 7, the cell according to claim 8, the composition according to claim 9 and/or the antibody-drug conjugate according to claim 10.

12. Use of the bispecific antibody or the antigen-binding portion thereof according to any one of claims 1 to 4, a composition comprising the bispecific antibody or the antigen-binding portion thereof according to any one of claims 1 to 4 and/or the antibody-drug conjugate according to claim 10 in the preparation of a drug or kit for diagnosing or treating a CD20-associated disease, wherein the CD20-associated disease is selected from non-Hodgkin lymphoma (NHL), acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), mantle cell lymphoma (MCL), marginal zone lymphoma (MZL), multiple myeloma (MM), and Hodgkin lymphoma (HL).