28345023). In addition, radionuclide conjugates specific to PSMA are being investigated for imaging and treatment of prostate cancer (e.g., Hofman et al., 2018 PMID: 29752180).

CD19

[00015] CD19, also known as B-Lymphocyte Surface Antigen B4 (UniProt P15391), is a cell surface receptor that is expressed on all human B-cells, but is not found on plasma cells. CD19 is a transmembrane protein that recruits cytoplasmic signaling proteins to the membrane and works within the CD19/CD21 complex to decrease the threshold for B-cell receptor signaling pathways. CD19 has a relatively large, 240 amino acid, cytoplasmic tail. The extracellular Ig-like domains are divided by a potential disulfide linked non-Ig-like domain and N-linked carbohydrate addition sites. The cytoplasmic tail contains at least nine tyrosine residues near the C-terminus, some of which have been shown to be phosphorylated. Along with CD20 and CD22, the restricted expression of CD19 to the B-cell lineage makes it an attractive target for the therapeutic treatment of B-cell malignancies. Many monoclonal antibodies and antibody drug conjugates specific to CD19 have been described (e.g., Naddafi et al. 2015, PMC4644525). In addition, anti-CD19 chimeric antigen receptor T-cells have been approved to treat leukemia (e.g., Sadelain et al. 2017, PMID: 29245005).

SUMMARY

[00015a] In one aspect, disclosed herein is a bispecific monoclonal antibody that binds to human CD3 and human BCMA, comprising: a first polypeptide subunit comprising the amino acid sequence of SEQ ID NO: 49; a second polypeptide subunit comprising the amino acid sequence of SEQ ID NO: 56; wherein the first and second polypeptide subunits together form a first binding moiety that binds to human CD3; and a third polypeptide subunit that binds to human BCMA, comprising the amino acid sequence of SEQ ID NO:58.

[00015b] In another aspect, disclosed herein is a bispecific monoclonal antibody that binds to human CD3 and human BCMA, comprising: (i) a first polypeptide subunit comprising the amino acid sequence of SEQ ID NO:49; (ii) a second polypeptide subunit comprising the amino acid sequence of SEQ ID NO:75, wherein the first and second polypeptide subunits together form a first binding moiety that binds to human CD3; and (iii) a third polypeptide subunit that binds to human BCMA, comprising the amino acid sequence of SEQ ID NO:76.

[00015c] In another aspect, disclosed herein is a human, monoclonal IgG4 bispecific antibody comprising: (i) a first binding arm that binds to human CD3; and (ii) a second binding arm that binds to human BCMA, wherein the first binding arm comprises a first heavy chain comprising the amino acid sequence of SEQ ID NO:56 and a light chain comprising the amino acid sequence of SEQ ID NO:49; and wherein the second

binding arm comprises a bivalent second heavy chain comprising the amino acid sequence of SEQ ID NO:58.

[00015d] In another aspect, disclosed herein is a human, monoclonal IgG4 bispecific antibody comprising: (i) a first binding arm that binds to human CD3; and (ii) a second binding arm that binds to human BCMA, wherein the first binding arm comprises a first heavy chain comprising the amino acid sequence of SEQ ID NO:75 and a light chain comprising the amino acid sequence of SEQ ID NO:49; and wherein the second binding arm comprises a bivalent second heavy chain comprising the amino acid sequence of SEQ ID NO:76.

[00015e] In another aspect, disclosed herein is a pharmaceutical composition comprising the bispecific antibody of the disclosure, and a pharmaceutically acceptable carrier.

[00015f] In another aspect, disclosed herein is one or more polynucleotides encoding a bispecific antibody of the disclosure.

[00015g] In another aspect, disclosed herein is one or more vectors comprising the one or more polynucleotides of the disclosure.

[00015h] In another aspect, disclosed herein is a cell comprising the one or more vectors of the disclosure.

[00015i] In another aspect, disclosed herein is a method of producing a bispecific antibody, comprising growing the cell according to the disclosure under conditions permissive for expression of the bispecific antibody, and isolating the bispecific antibody from the cell.

[00015j] In another aspect, disclosed herein is a kit comprising the bispecific antibody of the disclosure and instructions for use.

[00015k] In another aspect, disclosed herein is a use of the bispecific antibody of the disclosure in the manufacture of a medicament for treating a BCMA-expressing cancer in a human subject in need thereof.

[00016] Example of the disclosure include isolated multispecific antibodies comprising: a first heavy chain polypeptide subunit comprising a mutated human IgG4 constant region comprising mutations S228P, F234A, L235A, and T366W; and a second heavy chain polypeptide subunit comprising a mutated human IgG4 constant region comprising mutations S228P, F234A, L235A, T366S, L368A, and Y407V. In some examples, the mutated human IgG4 constant region of the first heavy chain polypeptide subunit or the mutated human IgG4 constant region of second heavy chain polypeptide subunit lacks a CH1 domain. In some examples, the mutated human IgG4 constant region of the first heavy chain polypeptide subunit comprises a sequence of SEQ ID NO: 73 or 55, and the mutated human IgG4 constant region of the second heavy chain polypeptide subunit comprises a sequence of SEQ ID NO: 72 or 54.

[00017] In some examples, multispecific antibodies in accordance with examples of the disclosure further comprise a first binding moiety that has binding specificity for CD3, comprising: a heavy chain variable domain comprising a CDR1 sequence comprising a sequence of SEQ ID NO: 36, a CDR2 sequence

comprising a sequence of SEQ ID NO: 37, and a CDR3 sequence comprising a sequence of SEQ ID NO: 38; and a light chain variable domain comprising a CDR1 sequence comprising a sequence of SEQ ID NO:

39, a CDR2 sequence comprising a sequence of SEQ ID NO: 40, and a CDR3 sequence comprising a sequence of SEQ ID NO: 41.

[00018] In some examples, the CDR1, CDR2 and CDR3 sequences in the heavy chain variable domain of the first binding moiety are present in a human VH framework; and the CDR1, CDR2 and CDR3 sequences in the light chain variable domain of the first binding moiety are present in a human Vkappa framework. In some examples, the heavy chain variable domain of the first binding moiety comprises a sequence having at least 95% identity to SEQ ID NO: 42; and the light chain variable domain of the first binding moiety comprises a sequence having at least 95% identity to SEQ ID NO: 43. In some examples, the heavy chain variable domain of the first binding moiety comprises the sequence of SEQ ID NO: 42; and the light chain variable domain of the first binding moiety comprises the sequence of SEQ ID NO: 43.

[00019] In some examples, multispecific antibodies in accordance with examples of the disclosure further comprise a second binding moiety having binding specificity to a protein other than CD3. In some examples, the second binding moiety comprises a single heavy chain variable region, in a monovalent or bivalent configuration. In some examples, the first binding moiety comprises a light chain polypeptide subunit and a heavy chain polypeptide subunit, and wherein the second binding moiety comprises a heavy chain polypeptide subunit. In some examples, the light chain polypeptide subunit of the first binding moiety comprises a light chain constant domain. In some examples, the protein other than CD3 is a tumor-associated antigen (TAA) or a tumor-specific antigen (TSA). In some examples, the TAA is B-cell maturation antigen (BCMA). In some examples, the TAA is CD19. In some examples, the TAA is prostate specific membrane antigen (PSMA).

[00020] Examples of the disclosure include pharmaceutical compositions comprising a multispecific antibody as described herein, polynucleotides encoding a multispecific antibody as described herein, vectors comprising such polynucleotides, and cells comprising such vectors.

[00021] Examples of the disclosure include methods of producing a multispecific antibody as described herein, comprising growing a cell as described herein under conditions permissive for expression of the multispecific antibody, and isolating the multispecific antibody from the cell.

[00022] Examples of the disclosure include methods of treatment comprising administering to an individual in need an effective dose of a multispecific antibody, or the pharmaceutical composition, described herein.

[00023] Examples of the disclosure include use of a multispecific antibody described herein in the preparation of a medicament for the treatment of a disease or disorder in an individual in need.

[00024] Examples of the disclosure include methods for treating a disease or condition characterized by expression of BCMA, comprising administering to an individual in need an effective dose of a multispecific antibody, or a pharmaceutical composition, described herein. In some examples, the disease is an

autoimmune disease. In some examples, the disease is a cancer. In some examples, the cancer is a myeloma. In some examples, the myeloma is multiple myeloma.

[00025] Examples of the disclosure include methods for treating a disease or condition characterized by expression of PSMA, comprising administering to an individual in need an effective dose of a multispecific antibody, or a pharmaceutical composition, described herein. In some examples, the disease is a cancer. In some examples, the cancer is prostate cancer.

[00026] Examples of the disclosure include methods for treating a disease or condition characterized by expression of CD19, comprising administering to an individual in need an effective dose of a multispecific antibody, or a pharmaceutical composition, described herein. In some examples, the disorder is diffuse large B-cell lymphoma (DLBCL). In some examples, the disorder is acute lymphoblastic leukemia (ALL). In some examples, the disorder is non-Hodgkin's lymphoma (NHL). In some examples, the disorder is systemic lupus erythematosus (SLE). In some examples, the disorder is rheumatoid arthritis (RA). In some examples, the disorder is multiple sclerosis (MS).

[00027] Examples of the disclosure include kits for treating a disease or disorder in an individual in need, comprising a multispecific antibody, or a pharmaceutical composition, 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.

[00028] Examples of the disclosure include bispecific three-chain antibody like molecules comprising: a first polypeptide subunit comprising: a light chain variable domain (VL) comprising a sequence of SEQ ID NO: 43; and a light chain constant domain (CL); a second polypeptide subunit comprising: a heavy chain variable domain (VH) comprising a sequence of SEQ ID NO: 42; and a heavy chain constant domain (CH) comprising a sequence of SEQ ID NO: 72 or 73; wherein the light chain variable domain and the heavy chain variable domain together form a first binding moiety that has binding specificity for CD3; and a third polypeptide subunit comprising: a heavy chain-only variable region, in a monovalent or bivalent configuration, that has binding specificity for a protein other than CD3; and a heavy chain constant domain (CH) comprising a sequence of SEQ ID NO: 54 or 55. In some examples, the third polypeptide subunit comprises a heavy chain-only variable region in a bivalent configuration that has binding specificity to BCMA.

[00029] Examples of the disclosure include bispecific three-chain antibody like molecules comprising: a first polypeptide subunit comprising a sequence of SEQ ID NO: 49; a second polypeptide subunit comprising a sequence of SEQ ID NO: 56; and a third polypeptide subunit comprising a sequence of SEQ ID NO: 58.

[00030] Examples of the disclosure include pharmaceutical compositions comprising a bispecific three-chain antibody like molecule described herein, polynucleotides encoding a bispecific three-chain antibody like molecule described herein, vectors comprising such polynucleotides, and cells comprising such vectors.

[00031] Examples of the disclosure include methods of producing a bispecific three-chain antibody like molecule described herein, comprising growing a cell described herein under conditions permissive for expression of the bispecific three-chain antibody like molecule, and isolating the bispecific three-chain antibody like molecule from the cell.

[00032] Examples of the disclosure include methods of treatment, comprising administering to an individual in need an effective dose of a bispecific three-chain antibody like molecule, or a pharmaceutical composition, described herein.

[00033] Examples of the disclosure include use of a bispecific three-chain antibody like molecule described herein in the preparation of a medicament for the treatment of a disease or disorder in an individual in need.

[00034] Examples of the disclosure include methods for treating a disease or condition characterized by expression of BCMA, comprising administering to an individual in need an effective dose of a bispecific three-chain antibody like molecule, or a pharmaceutical composition, described herein. In some examples, the disease is an autoimmune disease. In some examples, the disease is a cancer. In some examples, the cancer is a myeloma. In some examples, the myeloma is multiple myeloma.

[00035] Examples of the disclosure include kits for treating a disease or disorder in an individual in need, comprising a bispecific three-chain antibody like molecule, or a pharmaceutical composition, 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.

[00036] These and further examples will be further explained in the rest of the disclosure, including the Examples.

BRIEF DESCRIPTION OF THE DRAWINGS

[00037] FIG. 1, panels A-C, provide illustrations of various multispecific antibodies in accordance with embodiments of the invention.

[00038] FIG. 2, panels A-B, show images of non-reducing SDS-PAGE analyses of various antibody species purified via protein A chromatography.

[00039] FIG. 3, panel A, shows an image of non-reducing SDS-PAGE analysis of various antibody species purified via protein A chromatography. Panel B shows an image of a reducing SDS-PAGE analysis of the same antibody species shown in panel A.

[00040] FIG. 4, panels A-D are graphs depicting Fc gamma receptor – antibody interactions for various IgG1 antibody species in accordance with embodiments of the invention.

[00041] FIG. 5, panels A-E are graphs depicting Fc gamma receptor – antibody interactions for various IgG4 antibody species in accordance with embodiments of the invention.

CLAIMS:

1. A bispecific monoclonal antibody that binds to human CD3 and human BCMA, comprising:
   (i) a first polypeptide subunit comprising the amino acid sequence of SEQ ID NO:49;
   (ii) a second polypeptide subunit comprising the amino acid sequence of SEQ ID NO:56, wherein the first and second polypeptide subunits together form a first binding moiety that binds to human CD3; and
   (iii) a third polypeptide subunit that binds to human BCMA, comprising the amino acid sequence of SEQ ID NO:58.

2. The bispecific monoclonal antibody of claim 1, wherein the first polypeptide subunit comprises a light chain.

3. The bispecific monoclonal antibody of claim 1, wherein the second polypeptide subunit comprises a heavy chain.

4. The bispecific monoclonal antibody of claim 1, wherein the third polypeptide subunit comprises a bivalent heavy chain.

5. The bispecific monoclonal antibody of claim 4, wherein the bivalent heavy chain comprises a heavy chain-only variable region in a bivalent configuration.

6. The bispecific monoclonal antibody of claim 4, wherein the bivalent heavy chain lacks a CH1 domain.

7. A bispecific monoclonal antibody that binds to human CD3 and human BCMA, comprising:
   (i) a first polypeptide subunit comprising the amino acid sequence of SEQ ID NO:49;
   (ii) a second polypeptide subunit comprising the amino acid sequence of SEQ ID NO:75, wherein the first and second polypeptide subunits together form a first binding moiety that binds to human CD3; and
   (iii) a third polypeptide subunit that binds to human BCMA, comprising the amino acid sequence of SEQ ID NO:76.

8. The bispecific monoclonal antibody of claim 7, wherein the first polypeptide subunit comprises a light chain.

9. The bispecific monoclonal antibody of claim 7, wherein the second polypeptide subunit comprises a heavy chain.

10. The bispecific monoclonal antibody of claim 7, wherein the third polypeptide subunit comprises a bivalent heavy chain.

11. The bispecific monoclonal antibody of claim 10, wherein the bivalent heavy chain comprises a heavy chain-only variable region in a bivalent configuration.

12. The bispecific monoclonal antibody of claim 10, wherein the bivalent heavy chain lacks a CH1 domain.

13. A human, monoclonal IgG4 bispecific antibody comprising:

(i) a first binding arm that binds to human CD3; and

(ii) a second binding arm that binds to human BCMA,

wherein the first binding arm comprises a first heavy chain comprising the amino acid sequence of SEQ ID NO:56 and a light chain comprising the amino acid sequence of SEQ ID NO:49; and wherein the second binding arm comprises a bivalent second heavy chain comprising the amino acid sequence of SEQ ID NO:58.

14. The human, monoclonal IgG4 bispecific antibody of claim 13, wherein the second binding arm does not comprise a light chain.

15. The human, monoclonal IgG4 bispecific antibody of claim 13, wherein the bivalent second heavy chain comprises a heavy chain-only variable region in a bivalent configuration.

16. The human, monoclonal IgG4 bispecific antibody of claim 13, wherein the bivalent second heavy chain lacks a CH1 domain.

17. A human, monoclonal IgG4 bispecific antibody comprising:

(i) a first binding arm that binds to human CD3; and

(ii) a second binding arm that binds to human BCMA,

wherein the first binding arm comprises a first heavy chain comprising the amino acid sequence of SEQ ID NO:75 and a light chain comprising the amino acid sequence of SEQ ID NO:49; and wherein the second binding arm comprises a bivalent second heavy chain comprising the amino acid sequence of SEQ ID NO:76.

18. The human, monoclonal IgG4 bispecific antibody of claim 17, wherein the second binding arm does not comprise a light chain.

19. The human, monoclonal IgG4 bispecific antibody of claim 17, wherein the bivalent second heavy chain comprises a heavy chain-only variable region in a bivalent configuration.

20. The human, monoclonal IgG4 bispecific antibody of claim 17, wherein the bivalent second heavy chain lacks a CH1 domain.

21. A pharmaceutical composition comprising the bispecific antibody of any one of claims 1 to 20, and a pharmaceutically acceptable carrier.

22. One or more polynucleotides encoding the bispecific antibody of any one of claims 1 to 20.

23. One or more vectors comprising the one or more polynucleotides of claim 22.

24. A cell comprising the one or more vectors of claim 23.

25. A method of producing a bispecific antibody, comprising growing the cell of claim 24 under conditions permissive for expression of the bispecific antibody, and isolating the bispecific antibody from the cell.

26. A kit comprising the bispecific antibody of any one of claims 1 to 20, and instructions for use.

27. Use of the bispecific antibody of any one of claims 1 to 20 in the manufacture of a medicament for treating a BCMA-expressing cancer in a human subject in need thereof.