

[0006] The interaction of the T cell receptor (TCR) with its peptide-MHC ligand determines the activity of a T cell. The binding characteristics of this interaction has been studied in great detail and shown to control T cell function. The strength and nature of the TCR-peptide/MHC interaction determines whether T cells exert effector functions or are inactivated and deleted. Antibodies against CD3 activate T cells by changing the conformation of the CD3 ϵ chain and depending on the epitope may have either agonistic or antagonistic effects on T cells (Yoon et al., 1994 Immunity 1:563-569). In light of the significant side-effects of many T cell agonists it may be preferred to maintain potent anti-tumor effects while reducing the release of pro-inflammatory cytokines. However, partial agonistic anti-CD3 antibodies may alter the CD3 ϵ chain sub-optimally resulting in ineffective signaling, and most anti-CD3 antibodies are full agonists for both pathways. It is unclear whether these effector functions can be separated. Many existing anti-CD3 antibodies (for example SP-34, UCHT1, OKT3) have affinities in the range of 1-50 nM KD, however this may not be optimal for therapeutic use.

[0007] CD3 specific antibodies, and bispecific antibodies derived therefrom are provided by the invention.

Publications

[0008] CD3 antibodies are disclosed, for example, in U.S. Pat. Nos. 5,585,097; 5,929,212; 5,968,509; 6,706,265; 6,750,325; 7,381,803; 7,728,114. Bispecific antibodies with CD3 binding specificity are disclosed, for example, in U.S. Pat. Nos. 7,262,276; 7,635,472; 7,862,813; and 8,236,308, each herein specifically incorporated by reference.

SUMMARY

[0009] Compositions and methods of use thereof are provided for a family of closely related antibodies that bind to and activate signaling through CD3, e.g. activation of CD3⁺ T cells. The antibody family comprises a set of CDR sequences as defined herein. The family of antibodies provides a number of benefits that contribute to utility as clinically therapeutic agent(s). The antibodies within the family include members with a range of binding affinities, allowing the selection of a specific sequence with a desired affinity. The ability to fine tune affinity is of particular importance to manage the level of CD3 activation in an individual being treated, and thereby reduce toxicity.

[0009a] In one aspect, disclosed herein is a bispecific antibody comprising: (a) a first binding moiety specific for human CD3, wherein the first binding moiety comprises: (i) a first polypeptide subunit comprising a heavy chain variable (VH) domain comprising a CDR1 having an amino acid sequence of GFTFDDYA (SEQ ID NO:29), a CDR2 having an amino acid sequence of ISWNSGSI (SEQ ID NO:24), and a CDR3 having an amino acid sequence of AKDSRGYGDYRLGGAY (SEQ ID NO:41) in a human framework; and (ii) a second polypeptide

subunit comprising a light chain variable (VL) domain comprising a CDR1 having an amino acid sequence of QSVSSN (SEQ ID NO:35), a CDR2 having an amino acid sequence of GAS (SEQ ID NO:38), and a CDR3 having an amino acid sequence of QQYNNWPWT (SEQ ID NO:45); and (b) a second binding moiety specific for human BCMA, wherein the second binding moiety comprises: (i) a third polypeptide subunit comprising a VH domain comprising a CDR1 having an amino acid sequence of GFTVSSYG (SEQ ID NO:36), a CDR2 having an amino acid sequence of IRGSDGST (SEQ ID NO:39), and a CDR3 having an amino acid sequence of AKQGENDGPFDH (SEQ ID NO:46).

[0009b] In another aspect, disclosed herein is a pharmaceutical composition comprising the bispecific antibody of the disclosure, and a pharmaceutically acceptable carrier.

[0009c] In another aspect, disclosed herein is one or more polynucleotides encoding the bispecific antibody of the disclosure.

[0009d] In another aspect, disclosed herein is a cell comprising the one or more vectors of the disclosure.

[0009e] In another aspect, disclosed herein is a method of producing a bispecific antibody, comprising growing the cell of the disclosure under conditions permissive for expression of the bispecific antibody, and isolating the bispecific antibody from the cell.

[0009f] In another aspect, disclosed herein is a kit comprising the bispecific antibody of the disclosure, and instructions for use.

[0009g] 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.

[0009h] In another aspect, disclosed herein is the bispecific antibody of the disclosure for use in therapy.

[0010] In some examples, anti-CD3 antibodies have an affinity (KD) for CD3 ranging from around about 10^{-6} to around about 10^{-11} . Anti-CD3 antibodies that have affinities (KD) of 50 nM or greater, 100 nM or greater, 500 nM or greater, or 1 μ M or greater can be desirable to more closely mimic the TCR/MHC interaction and minimize toxic cytokine release while maintaining effective tumor cell lysis. In some examples, anti-CD3 antibodies are characterized or selected for reduced propensity to induce cytokine release, upon binding to a competent T cell,

e.g. for release of IL-2 and IFN γ . Antibodies may be selected for therapeutic use that optimize killing of tumor cells and reduced release of cytokines, e.g. an antibody that, within the family of antibody sequences described herein, induces a cytokine release that is less than about half the maximum observed for a family member in a comparative assay, and may be less, e.g. less and about 25% the maximum observed for a family member in a comparative assay. In some examples, bispecific or multispecific antibodies are provided, which comprise at least a heavy chain variable region from the antibody family and may comprise a heavy and light chain variable region provided herein. Bispecific antibodies comprise at least the heavy chain variable region of an antibody specific for a protein other than CD3, and may comprise a heavy and light chain variable region. In some such examples, the second antibody specifically binds to a tumor associated antigen, a targeting antigen, *e.g.* integrins, *etc.*, a pathogen antigen, a checkpoint protein, and the like. Various formats of bispecific antibodies are within the ambit of the disclosure, including without limitation single chain polypeptides, two chain polypeptides, three chain polypeptides, four chain polypeptides, and multiples thereof.

[0011] Each of the CD3 specific antibodies comprises a VH domain, comprising CDR1, CDR2 and CDR3 sequences in a human VH framework. The family 2 CDR sequences may be situated, as an example, in the region of around amino acid residues 26-33; 51-58; and 97-112 for CDR1, CDR2 and CDR3, respectively, of the provided exemplary variable region sequences set forth in SEQ ID NO: 1-18. It will be understood by one of skill in the art that the CDR sequences may be in different position if a different framework sequence is selected, although generally the order of the sequences will remain the same.

[0012] The CDR sequences for a family 2 antibody may have the following sequence formulas. An X indicates a variable amino acid, which may be specific amino acids as indicated below.

CDR1

G₁ F₂ T₃ F₄ X₅ X₆ Y₇ A₈

where:

X₅ may be any amino acid; in some examples X₅ is D, A or H; in some examples X₅ is D.

X₆ may be any amino acid; in some examples X₆ is D or N; in some examples D₆ is D.

In some examples a CDR1 sequence of a family 2 anti-CD3 antibody comprises the sequence set forth in any of SEQ ID NO:1-18, residues 26-33.

CDR2

I₁' S₂' W₃' N₄' S₅' G₆' S₇' I₈'

In some examples a CDR2 sequence of a family 2 anti-CD3 antibody comprises the sequence set forth in any of SEQ ID NO: 1-18, residues 51-58.

CDR3

$A_1'' K_2'' D_3'' S_4'' R_5'' G_6'' Y_7'' G_8'' X_9'' Y_{10}'' X_{11}'' X_{12}'' G_{13}'' G_{12}'' A_{15}'' Y_{16}''$

where:

X_9'' may be any amino acid, in some examples X_9'' is D or S; in some examples X_9'' is D;

X_{11}'' may be any amino acid, in some examples X_{11}'' is R or S;

X_{12}'' may be any amino acid, in some examples X_{12}'' is L or R.

[0013] In some examples a CD3 sequence of a family 2 anti-CD3 antibody has the formula $A K D S R G Y G D Y X_{11}'' X_{12}'' G G A Y$ where X_{11}'' and X_{12}'' are as defined above. In some examples a CDR3 sequence of a family 2 anti-CD3 antibody comprises the sequence set forth in any of SEQ ID NO: 1-18, residues 97-112. In some examples the CD3-binding VH domain of a family 2 antibody is paired with a light chain variable region domain. In some such examples the light chain is a fixed light chain.

[0014] In some examples the light chain comprises a VL domain with CDR1, CDR2 and CDR3 sequences in a human VL framework. The CDR sequences may be those of SEQ ID NO:19. In some examples, the CDR1 sequence comprises amino acid residues 27-32; 50-52; 89-97 for CDR1, CDR2, CDR3, respectively.

[0015] In some examples the CDR sequences of an antibody of the disclosure are a sequence with at least 85% identity, at least 90% identity, at least 95% identity, at least 99% identity relative to a CDR sequence or set of CDR sequences in SEQ ID NO: 1-18. In some examples a CDR sequence of the disclosure comprises one, two, three or more amino acid substitutions relative to a CDR sequence or set of CDR sequences in any one of SEQ ID NO: 1-18. In some examples said amino acid substitution(s) are one or more of position 5 or 10 of CDR1, position 2, 6 or 7 of CDR2, position 1, 8, 9 or 10 of CDR3, relative to the family 2 formulas provided above.

[0016] In some examples, a bispecific antibody of the disclosure comprises a CD3-binding variable region described herein, paired with a light chain. In some examples the light chain comprises the variable region sequence set forth in SEQ ID NO:19, or a variable region comprising the set of CDR sequences in SEQ ID NO:19 and framework sequences. Various Fc sequences find use, including without limitation human IgG1, IgG2a, IgG2b, IgG3, IgG4, etc. In some examples, the second arm of the bispecific antibody comprises a variable region that specifically binds to a tumor-associated antigen. In some examples, the second arm of the bispecific antibody comprises a variable region that specifically binds to BCMA. In some examples the anti-BCMA arm is a single chain variable region, for example as shown in

Figure 2B. In some examples the anti-BCMA arm comprises the variable region sequence set forth in SEQ ID NO:20; or the tandem variable region sequence set forth in SEQ ID NO:21. The Fc sequence of the anti-BCMA arm may be, without limitation, human IgG1, IgG2a, IgG2b, IgG3, IgG4, etc. The CDR sequences may be those contained in SEQ ID NO:20. In some examples, the CDR sequence comprises amino acid residues 26-33; 51-58; 97-108 for CDR1, CDR2, CDR3, respectively.

[0017] In other examples, pharmaceutical compositions are provided, comprising at least a CD3-binding VH domain of the disclosure, e.g. a monospecific, bispecific, etc. antibody or antibody-like protein comprising at least a CD3-binding VH domain of the disclosure; and a pharmaceutically acceptable excipient. The composition may be lyophilized, suspended in solution, etc. and may be provided in a unit dose formulation.

[0018] In some examples, a method is provided for treatment of cancer, the method comprising administering to an individual in need thereof an effective dose of a mono-specific, bi-specific, *etc.* antibody of the disclosure. Where the antibody is bispecific, a second antigen-binding site may specifically bind a tumor antigen, a checkpoint protein, etc. In various examples, the cancer is selected from the group consisting of ovarian cancer, breast cancer, gastrointestinal, brain cancer, head and neck cancer, prostate cancer, colon cancer, lung cancer, leukemia, lymphoma, sarcoma, carcinoma, neural cell tumors, squamous cell carcinomas, germ cell tumors, metastases, undifferentiated tumors, seminomas, melanomas, myelomas, neuroblastomas, mixed cell tumors, and neoplasias caused by infectious agents.

[0019] In some examples, a method is provided for treatment of infectious disease, the method comprising administering to an individual in need thereof an effective dose of a mono-specific, bi-specific, etc. antibody of the disclosure. Where the antibody is bispecific, a second antigen-binding site may specifically bind a pathogen antigen, e.g. bacteria, viruses or parasites.

[0020] In other examples, a method is provided for the production of a bispecific antibody of the present disclosure comprising expressing the antibody sequences, e.g. one or more light chain encoding sequences, one or more heavy chain encoding sequences, in a single host cell. In various examples, the host cell may be a prokaryotic or an eukaryotic cell, such as a mammalian cell.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] The invention is best understood from the following detailed description when read in conjunction with the accompanying drawings. The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee. It is emphasized that, according to common practice, the various features of the drawings are not

WHAT IS CLAIMED IS:

1. A bispecific antibody comprising:
 - (a) a first binding moiety specific for human CD3, wherein the first binding moiety comprises:
 - (i) a first polypeptide subunit comprising a heavy chain variable (VH) domain comprising a CDR1 having an amino acid sequence of GFTFDDYA (SEQ ID NO:29), a CDR2 having an amino acid sequence of ISWNSGSI (SEQ ID NO:24), and a CDR3 having an amino acid sequence of AKDSRGYGDYRLGGAY (SEQ ID NO:41) in a human framework; and
 - (ii) a second polypeptide subunit comprising a light chain variable (VL) domain comprising a CDR1 having an amino acid sequence of QSVSSN (SEQ ID NO:35), a CDR2 having an amino acid sequence of GAS (SEQ ID NO:38), and a CDR3 having an amino acid sequence of QQYNNWPWT (SEQ ID NO:45); and
 - (b) a second binding moiety specific for human BCMA, wherein the second binding moiety comprises:
 - (i) a third polypeptide subunit comprising a VH domain comprising a CDR1 having an amino acid sequence of GFTVSSYG (SEQ ID NO:36), a CDR2 having an amino acid sequence of IRGSDGST (SEQ ID NO:39), and a CDR3 having an amino acid sequence of AKQGENDGPFDH (SEQ ID NO:46).
2. The bispecific antibody of claim 1, wherein the CDR1, CDR2 and CDR3 sequences in the VL domain of the second polypeptide subunit are present in a human VL framework.
3. The bispecific antibody of claim 1, wherein the first polypeptide subunit comprises a VH domain having an amino acid sequence of SEQ ID NO:1, or wherein the second polypeptide subunit comprises a VL domain having an amino acid sequence SEQ ID NO:19.
4. The bispecific antibody of claim 1, wherein the first polypeptide subunit comprises a VH domain having an amino acid sequence of SEQ ID NO: 1 and the second polypeptide subunit comprises a VL domain having an amino acid sequence of SEQ ID NO: 19.
5. The bispecific antibody of claim 1 or claim 3, wherein the third polypeptide subunit comprises a VH domain having an amino acid sequence of SEQ ID NO:21.

6. The bispecific antibody of claim 1 or claim 3, wherein the third polypeptide subunit has an amino acid sequence of SEQ ID NO:20.

7. The bispecific antibody according to claims 4 and 5 comprising:

(a) a first binding moiety specific for human CD3, wherein the first binding moiety comprises a first polypeptide subunit comprising a VH domain having an amino acid sequence of SEQ ID NO: 1 and a second polypeptide subunit comprising a VL domain having an amino acid sequence of SEQ ID NO: 19; and

(b) a second binding moiety specific for human BCMA, wherein the second binding moiety comprises a third polypeptide subunit comprising a VH domain having an amino acid sequence of SEQ ID NO:21.

8. The bispecific antibody of claim 7, wherein the third polypeptide subunit comprises a first VH domain having an amino acid sequence of SEQ ID NO:21 and a second VH domain having an amino acid sequence of SEQ ID NO:21, wherein the first VH domain and the second VH domain are arranged in a tandem configuration.

9. The bispecific antibody of claim 8, wherein the first VH domain and the second VH domain of the third polypeptide subunit are arranged in a tandem configuration and joined by a linker.

10. The bispecific antibody according to claims 4 and 6 comprising:

(a) a first binding moiety specific for human CD3, wherein the first binding moiety comprises a first polypeptide subunit comprising a VH domain having an amino acid sequence of SEQ ID NO: 1 and a second polypeptide subunit comprising a VL domain having an amino acid sequence of SEQ ID NO: 19; and

(b) a second binding moiety specific for human BCMA, wherein the second binding moiety comprises a third polypeptide subunit having an amino acid sequence of SEQ ID NO:20.

11. A pharmaceutical composition comprising the bispecific antibody of any one of claims 1 to 10, and a pharmaceutically acceptable carrier.

12. One or more polynucleotides encoding the bispecific antibody of any one of claims 1 to 10.

13. One or more vectors comprising the one or more polynucleotides of claim 12.

14. A cell comprising the one or more vectors of claim 13.
15. A method of producing a bispecific antibody, comprising growing the cell of claim 14 under conditions permissive for expression of the bispecific antibody, and isolating the bispecific antibody from the cell.
16. A kit comprising the bispecific antibody of any one of claims 1 to 10, and instructions for use.
17. Use of the bispecific antibody of any one of claims 1 to 10 in the manufacture of a medicament for treating a BCMA- expressing cancer in a human subject in need thereof.
18. The bispecific antibody of any one of claims 1 to 10 for use in therapy.