

[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.

[0010] In some embodimentsexamples, 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 examplesembodiments, 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 example embodiments, 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 example embodiments, 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 example embodiments X₅ is D, A or H; in some example embodiments X₅ is D.

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

In some examples ~~embodiments~~ 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 exampleembodiments 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

A₁¹ K₂² D₃³ S₄⁴ R₅⁵ G₆⁶ Y₇⁷ G₈⁸ X₉⁹ Y₁₀¹⁰ X₁₁¹¹ X₁₂¹² G₁₃¹³ G₁₂¹² A₁₅¹⁵ Y₁₆¹⁶

where:

X₉⁹ may be any amino acid, in some exampleembodiments X₉⁹ is D or S; in some exampleembodiments X₉⁹ is D;

X₁₁¹¹ may be any amino acid, in some exampleembodiments X₁₁¹¹ is R or S;

X₁₂¹² may be any amino acid, in some exampleembodiments X₁₂¹² is L or R.

[0013] In some exampleembodiments a CD3 sequence of a family 2 anti-CD3 antibody has the formula A K D S R G Y G D Y X₁₁¹¹ X₁₂¹² G G A Y where X₁₁¹¹ and X₁₂¹² are as defined above. In some exampleembodiments 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 exampleembodiments the CD3-binding VH domain of a family 2 antibody is paired with a light chain variable region domain. In some such exampleembodiments the light chain is a fixed light chain.

[0014] In some exampleembodiments 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 exampleembodiments, the CDR1 sequence comprises amino acid residues 27-32; 50-52; 89-97 for CDR1, CDR2, CDR3, respectively.

[0015] In some exampleembodiments 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 exampleembodiments 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 exampleembodiments 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 exampleembodiments, a bispecific antibody of the disclosure comprises a CD3-binding variable region described herein, paired with a light chain. In some exampleembodiments 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 exampleembodiments, the second arm of the bispecific antibody comprises a variable region that specifically binds to a tumor-associated antigen. In some exampleembodiments, the second arm of the bispecific antibody comprises a variable region that specifically binds to BCMA. In some exampleembodiments the anti-BCMA arm is a single chain variable region, for example as shown in

Figure 2B. In some example embodiments 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 example embodiments, the CDR sequence comprises amino acid residues 26-33; 51-58; 97-108 for CDR1, CDR2, CDR3, respectively.

[0016a] In one aspect, there is provided a multispecific antibody comprising:

(i) a first binding moiety that has binding specificity for human CD3δ/CD3, comprising:

a heavy chain variable domain comprising (A) a CDR1 sequence comprising GFTFDDYA ~~or GFTFHNYA~~, a CDR2 sequence comprising ISWNSGSI, and a CDR3 sequence comprising AKDSRGYGDYRLGGAY ~~or AKDSRGYGDYSLGGAY, or~~

(B) a CDR1 sequence comprising GFTFHNYA, a CDR2 sequence comprising ISWNSGSI, and a CDR3 sequence comprising AKDSRGYGDYSLGGAY; and

a light chain variable domain comprising a CDR1 sequence comprising QSVSSN, a CDR2 sequence comprising GAS, and a CDR3 sequence comprising QQYNNWPWT; and (ii) a second binding moiety that has binding specificity for a tumor-associated antigen (TAA) or a tumor-specific antigen (TSA).

~~[0016b] In another aspect, there is provided a bispecific three-chain antibody like molecule comprising:~~

~~a first polypeptide subunit comprising:~~

~~a light chain variable domain (VL) comprising the sequence of SEQ ID NO: 19; and~~

~~a light chain constant domain (CL);~~

~~a second polypeptide subunit comprising:~~

~~a heavy chain variable domain (VH) comprising:~~

~~(i) the sequence of SEQ ID NO: 1; or~~

~~(ii) the sequence of SEQ ID NO: 13; and~~

~~a heavy chain constant domain (CH) comprising a CH1 domain, a hinge region, a CH2 domain, a CH3 domain;~~

~~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 single heavy chain variable region, in a single or tandem configuration, that has binding specificity for a protein other than CD3; and~~

~~a heavy chain constant domain (CH) comprising a hinge region, a CH2 domain, and a CH3 domain, in the absence of a CH1 domain.~~

[0016c] In another aspect, there is provided a bispecific antibody comprising:

~~— (a) a first binding moiety that has binding specificity for CD3, comprising:~~

~~(i) a heavy chain variable domain comprising a CDR1 sequence comprising GFTFDDYA, a CDR2 sequence comprising ISWNSGSI, and a CDR3 sequence comprising AKDSRGYGDYRLGGAY; and~~

~~(ii) — a light chain variable domain comprising a CDR1 sequence comprising QSVSSN, a CDR2 sequence comprising GAS, and a CDR3 sequence comprising QQYNNWPWT; and~~

~~(b) — a second binding moiety comprising a tandem configuration of two single heavy chain variable regions that have binding specificity for BCMA, wherein the two single heavy chain variable regions are connected by a linker sequence, and wherein each single heavy chain variable region comprises:~~

~~(i) — a CDR1 sequence comprising GFTVSSYG, a CDR2 sequence comprising IRGSDGST, and a CDR3 sequence comprising AKQGENDGPFDH.~~

~~[0016d] In another aspect, there is provided a bispecific three-chain antibody like molecule comprising:~~

~~a first polypeptide subunit comprising:~~

~~a light chain variable domain (VL) comprising the sequence of SEQ ID NO: 19;~~

~~and~~

~~a light chain constant domain (CL);~~

~~a second polypeptide subunit comprising:~~

~~a heavy chain variable domain (VH) comprising the sequence of SEQ ID NO: 1;~~

~~and~~

~~a heavy chain constant domain (CH) comprising a CH1 domain, a hinge region, a CH2 domain, and a CH3 domain;~~

~~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 tandem configuration of two single heavy chain variable regions, comprising the sequence of SEQ ID NO: 20; and~~

~~a heavy chain constant domain (CH) comprising a hinge region, a CH2 domain, and a CH3 domain, in the absence of a CH1 domain;~~

~~wherein the tandem configuration of two single heavy chain variable regions forms a second binding moiety that has binding specificity for BCMA.~~

[0017] In other example embodiments, 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. In another aspect, there is provided a pharmaceutical composition comprising a multispecific antibody disclosed herein. In another example aspect, there is provided a pharmaceutical composition comprising the bispecific three-chain antibody like molecule disclosed herein. In another example aspect, there is provided a

pharmaceutical composition comprising a bispecific antibody disclosed herein. The composition may be lyophilized, suspended in solution, etc. and may be provided in a unit dose formulation.

[0018] In some example embodiments, 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 example embodiments, 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 example embodiments, 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.

[0019a] In another aspect, there is provided a multispecific antibody disclosed herein, or the pharmaceutical composition disclosed herein, for use in therapy in an individual in need. ~~In another aspect, there is provided a bispecific three-chain antibody like molecule disclosed herein, or the pharmaceutical composition disclosed herein, for use in therapy in an individual in need. In another aspect, there is provided a bispecific antibody disclosed herein, or the pharmaceutical composition disclosed herein, for use in therapy in an individual in need. In another aspect, there is provided a bispecific three-chain antibody like molecule disclosed herein, or the pharmaceutical composition disclosed herein, for use in therapy in an individual in need.~~

[0019b] In another aspect, there is provided use of an effective dose of the multispecific antibody disclosed herein, or the pharmaceutical composition disclosed herein, in the preparation of a medicament for treating a disease or condition in an individual in need, wherein the disease is an autoimmune disease or a cancer. ~~In another aspect, there is provided use of an effective dose of the bispecific antibody disclosed herein, or the pharmaceutical composition disclosed herein, in the preparation of a medicament for treating a disease or condition in an individual in need, wherein the disease is an autoimmune disease or a cancer. In another aspect, there is provided use of an effective dose of the bispecific antibody disclosed herein, or the pharmaceutical composition disclosed herein, in the preparation of a medicament for treating a cancer in an individual with cancer. In another aspect, there is provided use of a bispecific three-chain antibody like molecule disclosed herein, or the pharmaceutical~~

~~composition disclosed herein, in the preparation of a medicament for the treatment of a disease or disorder in an individual in need.~~

[0020] In other example embodiments, 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 another aspect, there is provided a method of producing a multispecific antibody disclosed herein, comprising growing a cell disclosed herein under conditions permissive for expression of the multispecific antibody, and isolating the multispecific antibody from the cell. In another example aspect, there is provided a method of producing a bispecific antibody disclosed herein, comprising growing a cell disclosed herein under conditions permissive for expression of the bispecific antibody, and isolating the bispecific antibody from the cell. In various example embodiments, the host cell may be a prokaryotic or an eukaryotic cell, such as a mammalian cell.

[0020a] In another aspect, there is provided a polynucleotide encoding a multispecific antibody disclosed herein. In another aspect, there is provided a polynucleotide encoding a bispecific antibody disclosed herein.

[0020b] In another aspect, there is provided a vector comprising a polynucleotide disclosed herein.

[0020c] In another aspect, there is provided a cell comprising a vector disclosed herein.

[0020d] In another aspect, there is provided a kit for treating a disease or disorder in an individual in need, comprising a multispecific antibody disclosed herein, or the pharmaceutical composition disclosed herein, and instructions for use, wherein the disease or disorder is an autoimmune disease or a cancer. ~~In another aspect, there is provided a kit for treating a disease or disorder in an individual in need, comprising a bispecific three-chain antibody like molecule disclosed herein, or the pharmaceutical composition disclosed herein, and instructions for use.~~

BRIEF DESCRIPTION OF THE DRAWINGS

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 multispecific antibody comprising:

(i) a first binding moiety that has binding specificity for ~~CD3~~-human CD3 $\delta\epsilon$, comprising:

a heavy chain variable domain comprising

(A) a CDR1 sequence comprising GFTFDDYA-~~or~~ GFTFHNYA, a CDR2 sequence comprising ISWNSGSI, and a CDR3 sequence comprising AKDSRGYGDYRLGGAY-~~or~~ AKDSRGYGDYSLGGAY, or

(B) a CDR1 sequence comprising GFTFHNYA, a CDR2 sequence comprising ISWNSGSI, and a CDR3 sequence comprising AKDSRGYGDYSLGGAY; and

a light chain variable domain comprising a CDR1 sequence comprising QSVSSN, a CDR2 sequence comprising GAS, and a CDR3 sequence comprising QQYNNWPWT; and

(ii) a second binding moiety that has binding specificity for a tumor-associated antigen (TAA) or a tumor-specific antigen (TSA).

2. The multispecific antibody of claim 1, wherein the first binding moiety comprises a heavy chain variable domain comprising a CDR1 sequence comprising GFTFDDYA, a CDR2 sequence comprising ISWNSGSI, and a CDR3 sequence comprising AKDSRGYGDYRLGGAY.

3. The multispecific antibody of claim 1, wherein the first binding moiety comprises a heavy chain variable domain comprising a CDR1 sequence comprising GFTFHNYA, a CDR2 sequence comprising ISWNSGSI, and a CDR3 sequence comprising AKDSRGYGDYSLGGAY.

4. The multispecific antibody of any one of claims 1-3, wherein:

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.

5. The multispecific antibody of claim 4, wherein:

the heavy chain variable domain of the first binding moiety comprises a sequence having at least 95% identity to SEQ ID NO: 1; and

the light chain variable domain of the first binding moiety comprises a sequence having at least 95% identity to SEQ ID NO: 19.

6. The multispecific antibody of claim 4, wherein:
the heavy chain variable domain of the first binding moiety comprises a sequence having at least 95% identity to SEQ ID NO: 13; and
the light chain variable domain of the first binding moiety comprises a sequence having at least 95% identity to SEQ ID NO: 19.

7. The multispecific antibody of claim 5, wherein:
the heavy chain variable domain of the first binding moiety comprises the sequence of SEQ ID NO: 1; and
the light chain variable domain of the first binding moiety comprises the sequence of SEQ ID NO: 19.

8. The multispecific antibody of claim 6, wherein:
the heavy chain variable domain of the first binding moiety comprises the sequence of SEQ ID NO: 13; and
the light chain variable domain of the first binding moiety comprises the sequence of SEQ ID NO: 19.

~~9. The multispecific antibody of any one of claims 1-8, further comprising a second binding moiety having binding specificity to a protein other than CD3.~~

~~910.~~ The multispecific antibody of claim ~~91~~, wherein the second binding moiety comprises a single heavy chain variable region, in a single or tandem configuration.

~~1044.~~ The multispecific antibody of claim ~~91~~, wherein 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.

~~1211.~~ The multispecific antibody of claim ~~104~~, wherein the light chain polypeptide subunit of the first binding moiety comprises a light chain constant domain.

~~1312.~~ The multispecific antibody of claim ~~104~~, wherein the heavy chain polypeptide subunit of the first binding moiety comprises at least one heavy chain constant domain.

134. The multispecific antibody of claim 132, wherein the heavy chain polypeptide subunit of the first binding moiety comprises a CH1 domain, a CH2 domain, and a CH3 domain.

145. The multispecific antibody of claim 143, wherein the heavy chain polypeptide subunit of the first binding moiety comprises a hinge region positioned between the CH1 domain and the CH2 domain.

165. The multispecific antibody of claim 140, wherein the heavy chain polypeptide subunit of the second binding moiety comprises at least one constant domain.

167. The multispecific antibody of claim 156, wherein the heavy chain polypeptide subunit of the second binding moiety comprises a CH2 domain and a CH3 domain, but no CH1 domain.

178. The multispecific antibody of claim 156 or 167, wherein the heavy chain polypeptide subunit of the second binding moiety comprises a hinge region positioned between the second binding moiety and the CH2 domain.

189. The multispecific antibody of any one of claims 134-178, wherein the CH2 and CH3 domains of the heavy chain polypeptide subunit of the first binding moiety and the CH2 and CH3 domains of the heavy chain polypeptide subunit of the second binding moiety together form an Fc region.

1920. The multispecific antibody of claim 189, wherein the Fc region comprises a native-sequence human IgG1 Fc region.

204. The multispecific antibody of claim 189, wherein the Fc region comprises a variant human IgG1 Fc region.

212. The multispecific antibody of claim 204, wherein the variant human IgG1 Fc region has been engineered to reduce one or more effector functions.

223. The multispecific antibody of claim 189, wherein the Fc region comprises a native-sequence human IgG4 Fc region.

234. The multispecific antibody of claim 189, wherein the Fc region comprises a variant human IgG4 Fc region.

245. The multispecific antibody of claim 234, wherein the variant human IgG4 Fc region has been engineered to reduce one or more effector functions.

256. The multispecific antibody according to any one of claims 189-245, wherein the Fc region has been engineered to reduce homodimer formation.

~~27. The multispecific antibody of any one of claims 9-26, wherein the second binding moiety has binding specificity for a protein other than CD3.~~

~~28. The multispecific antibody of claim 27, wherein the protein other than CD3 is a tumor-associated antigen (TAA) or a tumor-specific antigen (TSA).~~

~~29. The multispecific antibody of claim 28, wherein the TAA is B-cell maturation antigen (BCMA).~~

2630. A pharmaceutical composition comprising a multispecific antibody according to any one of claims 1-259.

2731. A polynucleotide encoding a multispecific antibody according to any one of claims 1-259.

2832. A vector comprising a polynucleotide according to claim 2731.

2933. A cell comprising a vector according to claim 2832.

3034. A method of producing a multispecific antibody according to any one of claims 1-2529, comprising growing a cell according to claim 2933 under conditions permissive for expression of the multispecific antibody, and isolating the multispecific antibody from the cell.

3135. The multispecific antibody of any one of claims 1-2529, or the pharmaceutical composition of claim 2630, for use in therapy in an individual in need.

3236. Use of an effective dose of the multispecific antibody according to any one of claims 1-2529, or the pharmaceutical composition of claim 2630, in the preparation of a medicament for treating a disease of condition in an individual in need, wherein the disease is an autoimmune disease or a cancer.

3337. A kit for treating a disease or disorder in an individual in need, comprising a multispecific antibody of any one of claims 1-2529, or the pharmaceutical composition of claim 2630, and instructions for use, wherein the disease or disorder is an autoimmune disease or a cancer.

3438. The kit of claim 337, further comprising at least one additional reagent.

3539. The kit of claim 338, wherein the at least one additional reagent comprises a chemotherapeutic drug.

~~40. — A bispecific three-chain antibody like molecule comprising:~~
~~a first polypeptide subunit comprising:~~
~~a light chain variable domain (VL) comprising the sequence of SEQ ID NO: 19; and~~
~~a light chain constant domain (CL);~~
~~a second polypeptide subunit comprising:~~
~~a heavy chain variable domain (VH) comprising:~~
~~(i) — the sequence of SEQ ID NO: 1; or~~
~~(ii) — the sequence of SEQ ID NO: 13; and~~
~~a heavy chain constant domain (CH) comprising a CH1 domain, a hinge region, a CH2 domain, a CH3 domain;~~
~~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 single heavy chain variable region, in a single or tandem configuration, that has binding specificity for a protein other than CD3; and~~
~~a heavy chain constant domain (CH) comprising a hinge region, a CH2 domain, and a CH3 domain, in the absence of a CH1 domain.~~

~~41. — The bispecific three-chain antibody like molecule of claim 40, wherein the heavy chain constant domain of the second polypeptide subunit and the heavy chain constant domain of the third polypeptide subunit have been engineered to reduce one or more effector functions.~~

~~42. — The bispecific three chain antibody like molecule of claim 40 or 41, wherein the heavy chain constant domain of the second polypeptide subunit and the heavy chain constant domain of the third polypeptide subunit have been engineered to reduce homodimer formation.~~

~~43. — A pharmaceutical composition comprising the bispecific three chain antibody like molecule of any one of claims 40-42.~~

~~44. — The bispecific three chain antibody like molecule of any one of claims 40-42, or the pharmaceutical composition of claim 43, for use in therapy in an individual in need.~~

~~45. — Use of an effective dose of the bispecific antibody according to any one of claims 40-42, or the pharmaceutical composition of claim 43, in the preparation of a medicament for treating a disease or condition in an individual in need, wherein the disease is an autoimmune disease or a cancer.~~

~~46. — A kit for treating a disease or disorder in an individual in need, comprising a bispecific three chain antibody like molecule of any one of claims 40-42, or the pharmaceutical composition of claim 43, and instructions for use.~~

~~47. — The kit of claim 46, further comprising at least one additional reagent.~~

~~48. — The kit of claim 47, wherein the at least one additional reagent comprises a chemotherapeutic drug.~~

~~49. — A bispecific antibody comprising:~~

~~— (a) — a first binding moiety that has binding specificity for CD3, comprising:~~

~~(i) — a heavy chain variable domain comprising a CDR1 sequence comprising GFTFDDYA, a CDR2 sequence comprising ISWNSGSI, and a CDR3 sequence comprising AKDSRCYGDYRLGGAY; and~~

~~(ii) — a light chain variable domain comprising a CDR1 sequence comprising QSVSSN, a CDR2 sequence comprising GAS, and a CDR3 sequence comprising QQYNNWPWT; and~~

~~(b) — a second binding moiety comprising a tandem configuration of two single heavy chain variable regions that have binding specificity for BCMA, wherein the two single heavy chain variable~~

~~regions are connected by a linker sequence, and wherein each single heavy chain variable region comprises:~~

~~(i) a CDR1 sequence comprising GFTVSSYG, a CDR2 sequence comprising IRGSDGST, and a CDR3 sequence comprising AKQGENDGPFDH.~~

~~50. The bispecific antibody of claim 49, wherein:~~

~~the CDR1, CDR2 and CDR3 sequences in the heavy chain variable domain of the first binding moiety are present in a human VH framework;~~

~~the CDR1, CDR2 and CDR3 sequences in the light chain variable domain of the first binding moiety are present in a human Vkappa framework; and~~

~~the CDR1, CDR2 and CDR3 sequences in each single heavy chain variable region are present in a human VH framework.~~

~~51. The bispecific antibody of claim 50, wherein:~~

~~the heavy chain variable domain of the first binding moiety comprises a sequence having at least 95% identity to SEQ ID NO: 1;~~

~~the light chain variable domain of the first binding moiety comprises a sequence having at least 95% identity to SEQ ID NO: 19; and~~

~~each single heavy chain variable region sequence in the second binding moiety comprises a sequence having at least 95% identity to SEQ ID NO: 21.~~

~~52. The bispecific antibody of claim 51, wherein:~~

~~the heavy chain variable domain of the first binding moiety comprises the sequence of SEQ ID NO: 1;~~

~~the light chain variable domain of the first binding moiety comprises the sequence of SEQ ID NO: 19; and~~

~~each single heavy chain variable region sequence in the second binding moiety comprises the sequence of SEQ ID NO: 21.~~

~~53. The bispecific antibody of any one of claims 49-52, wherein 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.~~

~~_____~~

~~54. The bispecific antibody of claim 53, wherein the light chain polypeptide subunit of the first binding moiety comprises a light chain constant domain.~~

~~55. The bispecific antibody of claim 53, wherein the heavy chain polypeptide subunit of the first binding moiety comprises at least one heavy chain constant domain.~~

~~56. The bispecific antibody of claim 55, wherein the heavy chain polypeptide subunit of the first binding moiety comprises a CH1 domain, a CH2 domain, and a CH3 domain.~~

~~57. The bispecific antibody of claim 56, wherein the heavy chain polypeptide subunit of the first binding moiety comprises a hinge region positioned between the CH1 domain and the CH2 domain.~~

~~58. The bispecific antibody of claim 53, wherein the heavy chain polypeptide subunit of the second binding moiety comprises at least one constant domain.~~

~~59. The bispecific antibody of claim 58, wherein the heavy chain polypeptide subunit of the second binding moiety comprises a CH2 domain and a CH3 domain, but no CH1 domain.~~

~~60. The bispecific antibody of claim 58 or 59, wherein the heavy chain polypeptide subunit of the second binding moiety comprises a hinge region positioned between the tandem configuration of single heavy chain variable domains and the CH2 domain.~~

~~61. The bispecific antibody of any one of claim 59 or 60, wherein the CH2 and CH3 domains of the heavy chain polypeptide subunit of the first binding moiety and the CH2 and CH3 domains of the heavy chain polypeptide subunit of the second binding moiety together form an Fc region.~~

~~62. The bispecific antibody of claim 61, wherein the Fc region comprises a native sequence human IgG1 Fc region.~~

~~63. The bispecific antibody of claim 62, wherein the Fc region comprises a variant human IgG1 Fc region.~~

~~64. The bispecific antibody of claim 63, wherein the variant human IgG1 Fc region has been engineered to reduce one or more effector functions.~~

~~65. — The bispecific antibody of claim 61, wherein the Fc region comprises a native sequence human IgG4 Fc region.~~

~~66. — The bispecific antibody of claim 61, wherein the Fc region comprises a variant human IgG4 Fc region.~~

~~67. — The bispecific antibody of claim 66, wherein the variant human IgG4 Fc region has been engineered to reduce one or more effector functions.~~

~~68. — The bispecific antibody according to any one of claims 61-67, wherein the Fc region has been engineered to reduce homodimer formation.~~

~~69. — A pharmaceutical composition comprising a bispecific antibody according to any one of claims 49-68.~~

~~70. — A polynucleotide encoding a bispecific antibody according to any one of claims 49-68.~~

~~71. — A vector comprising a polynucleotide according to claim 70.~~

~~72. — A cell comprising a vector according to claim 71.~~

~~73. — A method of producing a bispecific antibody according to any one of claims 49-68, comprising growing a cell according to claim 72 under conditions permissive for expression of the bispecific antibody, and isolating the bispecific antibody from the cell.~~

~~74. — The bispecific antibody of any one of claims 49-68, or the pharmaceutical composition of claim 69, for use in therapy in an individual in need.~~

~~75. — Use of an effective dose of the bispecific antibody according to any one of claims 49-68, or the pharmaceutical composition of claim 69, in the preparation of a medicament for treating a cancer in an individual with cancer.~~

~~76. — The use according to claim 75, wherein the cancer is a myeloma.~~

- ~~77. — A bispecific three chain antibody like molecule comprising:~~
~~a first polypeptide subunit comprising:~~
~~a light chain variable domain (VL) comprising the sequence of SEQ ID NO: 19; and~~
~~a light chain constant domain (CL);~~
~~a second polypeptide subunit comprising:~~
~~a heavy chain variable domain (VH) comprising the sequence of SEQ ID NO: 1; and~~
~~a heavy chain constant domain (CH) comprising a CH1 domain, a hinge region, a CH2 domain, and a CH3 domain;~~
~~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 tandem configuration of two single heavy chain variable regions, comprising the sequence of SEQ ID NO: 20; and~~
~~a heavy chain constant domain (CH) comprising a hinge region, a CH2 domain, and a CH3 domain, in the absence of a CH1 domain;~~
~~wherein the tandem configuration of two single heavy chain variable regions forms a second binding moiety that has binding specificity for BCMA.~~
- ~~78. — The bispecific three chain antibody like molecule of claim 77, wherein the heavy chain constant domain of the second polypeptide subunit and the heavy chain constant domain of the third polypeptide subunit have been engineered to reduce one or more effector functions.~~
- ~~79. — The bispecific three chain antibody like molecule of claim 77 or 78, wherein the heavy chain constant domain of the second polypeptide subunit and the heavy chain constant domain of the third polypeptide subunit have been engineered to reduce homodimer formation.~~
- ~~80. — A pharmaceutical composition comprising the bispecific three chain antibody like molecule of any one of claims 77-79.~~
- ~~81. — Use of a bispecific three chain antibody like molecule of any one of claims 77-79, or the pharmaceutical composition of claim 80, in the preparation of a medicament for the treatment of a disease or disorder in an individual in need.~~

~~82. The bispecific three chain antibody like molecule of any one of claims 77-79, or the pharmaceutical composition of claim 80, for use in therapy in an individual in need.~~

~~83. A kit for treating a disease or disorder in an individual in need, comprising a bispecific three-chain antibody like molecule of any one of claims 77-79, or the pharmaceutical composition of claim 80, and instructions for use.~~

~~84. The kit of claim 83, further comprising at least one additional reagent.~~

~~85. The kit of claim 84, wherein the at least one additional reagent comprises a chemotherapeutic drug.~~

36. The multispecific antibody of claim 1, wherein the multispecific antibody is a bispecific antibody.