

MULTISPECIFIC HEAVY CHAIN ANTIBODIES WITH MODIFIED HEAVY CHAIN CONSTANT REGIONS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority benefit of the filing date of the filing date of US Provisional Patent Application Serial No. 63/017,589, filed on April 29, 2020, as well as US Provisional Patent Application Serial No. 63/108,796, filed on November 2, 2020, the disclosures of which applications are incorporated by reference herein in their entireties.

SEQUENCE LISTING

[0001a] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on July 13, 2021, is named 60792_00035WO01_(TNO-0018-WO)_SL.txt and is 199,159 bytes in size.

FIELD OF THE INVENTION

[0002] The present invention concerns multispecific, human heavy chain antibodies (e.g., UniAbs™) that have modified heavy chain constant regions that impart advantageous properties. The invention further concerns methods of making such antibodies, compositions, including pharmaceutical compositions, comprising such antibodies, and their use to treat disorders that are characterized by expression of one or more of the binding targets described herein.

BACKGROUND OF THE INVENTION

Modified Fc Regions

[0003] Advances in protein engineering have led to the successful manufacture and clinical utilization of multispecific antibodies having binding affinity to two or more targets. However, due to their heterodimeric nature, appropriate measures must be utilized to facilitate proper pairing of the desired combination of binding sequences, and thus the polypeptide subunits, in a multispecific antibody. Wang et al., *mAbs* 10:8, 1226-1235 (2018).

[0004] One approach to circumvent the problem of mispaired polypeptide subunits is known as “knobs-into-holes” (KiH), and it aims at forcing the pairing of two different antibody heavy chains by introducing

mutations in the CH2 and/or CH3 domains to modify the contact interface. On one chain, bulky amino acids are replaced by amino acids with short side chains to create a “hole”. Conversely, amino acids with large side chains are introduced into the other heavy chain to create a “knob”. By co-expressing these two heavy chains, a higher yield of heterodimer formation (“knob-hole”) versus homodimer formation (“hole-hole” or “knob-knob”) is observed due to the more favorable stability of the knob-hole pair (Ridgway, J.B., et al, *Protein Eng.* 9 (1996) 617-621; and WO 96/027011).

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 OF THE INVENTION

[00015a] In one aspect, the present disclosure refers to an isolated multispecific antibody comprising:
a first binding moiety that has binding specificity for CD3, comprising:
a first heavy chain polypeptide subunit 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 first light chain polypeptide subunit comprising 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;
a second binding moiety that has binding specificity for CD19, comprising:
a second heavy chain polypeptide subunit comprising a heavy chain variable domain;
wherein the first heavy chain polypeptide subunit further comprises a mutated human IgG4 constant region comprising mutations S228P, F234A, L235A, and T366W; and the second heavy chain polypeptide subunit comprises a mutated human IgG4 constant region comprising mutations S228P, F234A, L235A, T366S, L368A, and Y407V.

[00015b] In another aspect, the present disclosure refers to a pharmaceutical composition comprising a multispecific antibody as described herein.

[00015c] In another aspect, the present disclosure refers to a polynucleotide encoding a multispecific antibody as described herein.

[00015d] In another aspect, the present disclosure refers to a vector comprising a polynucleotide as described herein.

[00015e] In another aspect, the present disclosure refers to a cell comprising a vector as described herein.

[00015f] In another aspect, the present disclosure refers to a method 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.

[00015g] In yet another aspect, the present disclosure refers to the multispecific antibody as described herein, or the pharmaceutical composition as described herein, for use in therapy in an individual in need.

[00015h] In yet another aspect, the present disclosure refers to a use of the multispecific antibody as described herein, or the pharmaceutical composition comprising the multispecific antibody as described herein in the manufacture of medicament for treating a disease or condition or disorder characterized by expression of CD19, wherein the multispecific antibody or the pharmaceutical composition comprising the multispecific antibody is to be administered to an individual in need in an effective dose.

[00015i] In yet another aspect, the present disclosure refers to a kit for treating a disease or disorder in an individual in need, comprising a multispecific antibody as described herein, or the pharmaceutical composition as described herein, and instructions for use.

[00015j] In yet another aspect, the present disclosure refers to a bispecific three-chain antibody like molecule that binds to human CD3 and human CD19, 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 CD19, comprising the amino acid sequence of SEQ ID NO: 67.

[00015k] In yet another aspect, the present disclosure refers to a bispecific three-chain antibody like molecule that binds to human CD3 and human CD19, 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 CD19, comprising the amino acid sequence of SEQ ID NO: 78.

[00015l] In yet another aspect, the present disclosure refers to 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 CD19,

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 monovalent second heavy chain comprising the amino acid sequence of SEQ ID NO: 67.

[00015m] In yet another aspect, the present disclosure refers to 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 CD19,

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 monovalent second heavy chain comprising The amino acid sequence of SEQ ID NO: 78.

[00016] Aspects-Examples of the ~~invention-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 ~~embodiments~~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 ~~embodiments~~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 ~~embodiments~~examples, multispecific antibodies in accordance with embodiments of the ~~disclosure~~invention 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 [embodimentsexamples](#), 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 [embodimentsexamples](#), 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 [embodimentsexamples](#), 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 [embodimentsexamples](#), multispecific antibodies in accordance with [embodimentsexamples](#) of the [inventiondisclosure](#) further comprise a second binding moiety having binding specificity to a protein other than CD3. In some [embodimentsexamples](#), the second binding moiety comprises a single heavy chain variable region, in a monovalent or bivalent configuration. In some [embodimentsexamples](#), 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 [embodimentsexamples](#), the light chain polypeptide subunit of the first binding moiety comprises a light chain constant domain. In some [embodimentsexamples](#), the protein other than CD3 is a tumor-associated antigen (TAA) or a tumor-specific antigen (TSA). In some [embodimentsexamples](#), the TAA is B-cell maturation antigen (BCMA). In some [embodimentsexamples](#), the TAA is CD19. In some [embodimentsexamples](#), the TAA is prostate specific membrane antigen (PSMA).

[00020] [AspectsExamples](#) of the [inventiondisclosure](#) 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] [AspectsExamples](#) of the [inventiondisclosure](#) 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] [AspectsExamples](#) of the [inventiondisclosure](#) 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] [AspectsExamples](#) of the [inventiondisclosure](#) 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] [AspectsExamples](#) of the [inventiondisclosure](#) 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 [embodimentsexamples](#), the disease is an

autoimmune disease. In some [embodimentsexamples](#), the disease is a cancer. In some [embodimentsexamples](#), the cancer is a myeloma. In some [embodimentsexamples](#), the myeloma is multiple myeloma.

[00025] [AspectsExamples](#) of the [inventiondisclosure](#) 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 [embodimentsexamples](#), the disease is a cancer. In some [embodimentsexamples](#), the cancer is prostate cancer.

[00026] [AspectsExamples](#) of the [inventiondisclosure](#) 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 [embodimentsexamples](#), the disorder is diffuse large B-cell lymphoma (DLBCL). In some [embodimentsexamples](#), the disorder is acute lymphoblastic leukemia (ALL). In some [embodimentsexamples](#), the disorder is non-Hodgkin's lymphoma (NHL). In some [embodimentsexamples](#), the disorder is systemic lupus erythematosus (SLE). In some [embodimentsexamples](#), the disorder is rheumatoid arthritis (RA). In some [embodimentsexamples](#), the disorder is multiple sclerosis (MS).

[00027] [AspectsExamples](#) of the [inventiondisclosure](#) 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 [embodimentsexamples](#), a kit further comprises at least one additional reagent. In some [embodimentsexamples](#), the at least one additional reagent comprises a chemotherapeutic drug.

[00028] [AspectsExamples](#) of the [inventiondisclosure](#) 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 [embodimentsexamples](#), the third polypeptide subunit comprises a heavy chain-only variable region in a bivalent configuration that has binding specificity to BCMA.

[00029] [AspectsExamples](#) of the [inventiondisclosure](#) 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] [AspectsExamples](#) of the [inventiondisclosure](#) 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] AspectsExamples of the inventiondisclosure 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] AspectsExamples of the inventiondisclosure 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] AspectsExamples of the inventiondisclosure 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] AspectsExamples of the inventiondisclosure 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 embodimentsexamples, the disease is an autoimmune disease. In some embodimentsexamples, the disease is a cancer. In some embodimentsexamples, the cancer is a myeloma. In some embodimentsexamples, the myeloma is multiple myeloma.
- [00035] AspectsExamples of the inventiondisclosure 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 embodimentsexamples, a kit further comprises at least one additional reagent. In some embodimentsexamples, the at least one additional reagent comprises a chemotherapeutic drug.
- [00036] These and further aspectsexamples 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.

second antigen-binding domain on a polypeptide are connected by a polypeptide linker. One non-limiting example of such a polypeptide linker is a GS linker, having an amino acid sequence of four glycine residues, followed by one serine residue, and wherein the sequence is repeated n times, where n is an integer ranging from 1 to about 10, such as 2, 3, 4, 5, 6, 7, 8, or 9 (SEQ ID NO: 93). Non-limiting examples of such linkers include GGGGS (SEQ ID NO: 70) (n=1) and GGGSGGGGS (SEQ ID NO: 71) (n=2). Other suitable linkers can also be used, and are described, for example, in Chen et al., Adv Drug Deliv Rev. 2013 October 15; 65(10): 1357-69, the disclosure of which is incorporated herein by reference in its entirety.

[000103] The term “three-chain antibody like molecule” or “TCA” is used herein to refer to antibody-like molecules comprising, consisting essentially of, or consisting of three polypeptide subunits, two of which comprise, consist essentially of, or consist of one heavy and one light chain of a monoclonal antibody, or functional antigen-binding fragments of such antibody chains, comprising an antigen-binding region and at least one CH domain. This heavy chain/light chain pair has binding specificity for a first antigen. The third polypeptide subunit comprises, consists essentially of, or consists of a heavy-chain only antibody comprising an Fc portion comprising CH2 and/or CH3 and/or CH4 domains, in the absence of a CH1 domain, and one or more antigen binding domains (e.g., two antigen binding domains) that binds an epitope of a second antigen or a different epitope of the first antigen, where such binding domain is derived from or has sequence identity with the variable region of an antibody heavy or light chain. Parts of such variable region may be encoded by V_H and/or V_L gene segments, D and J_H gene segments, or J_L gene segments. The variable region may be encoded by rearranged V_HDJ_H , V_LDJ_H , V_HJ_L , or V_LJ_L gene segments.

[000104] A TCA binding compound makes use of a “heavy chain only antibody” or “heavy chain antibody” or “heavy chain polypeptide” which, as used herein, mean a single chain antibody comprising heavy chain constant regions CH2 and/or CH3 and/or CH4 but no CH1 domain. In one embodiment, the heavy chain antibody is composed of an antigen-binding domain, at least part of a hinge region and CH2 and CH3 domains. In another embodiment, the heavy chain antibody is composed of an antigen-binding domain, at least part of a hinge region and a CH2 domain. In a further embodiment, the heavy chain antibody is composed of an antigen-binding domain, at least part of a hinge region and a CH3 domain. Heavy chain antibodies in which the CH2 and/or CH3 domain is truncated are also included herein. In a further embodiment, the heavy chain is composed of an antigen binding domain, and at least one CH (CH1, CH2, CH3, or CH4) domain but no hinge region. The heavy chain only antibody can be in the form of a dimer, in which two heavy chains are disulfide bonded other otherwise covalently or non-covalently attached to each other, and can optionally include an asymmetric interface between one or more of the CH domains to facilitate proper pairing between polypeptide chains. The heavy-chain antibody may belong to the IgG subclass, but antibodies belonging to other subclasses, such as IgM, IgA, IgD and IgE subclass, are also included herein. In a particular embodiment, the heavy chain antibody is of the IgG1, IgG2, IgG3, or IgG4