

HEAVY CHAIN ANTIBODIES BINDING TO CD38

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority benefit of the filing date of US Provisional Patent Application No. 62/751,520, filed on October 26, 2018, the disclosure of which application is herein incorporated by reference in its entirety.

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 January 8, 2020, is named TNO-0011-WO_SL.txt and is 49,692 bytes in size.

FIELD OF THE INVENTION

[0002] The present invention concerns binding compounds, such as human heavy-chain antibodies (e.g., UniAbs™) binding to CD38. Aspects of the invention relate to anti-CD38 heavy chain antibodies, combinations, including synergistic combinations, of anti-CD38 heavy chain antibodies targeting non-overlapping epitopes on CD38, multi-specific anti-CD38 heavy chain antibodies with binding specificity to more than one non-overlapping epitope on CD38, as well as methods of making such binding compounds, compositions, including pharmaceutical compositions, comprising such binding compounds, and their various uses.

BACKGROUND OF THE INVENTION

CD38 Ectoenzyme

[0003] The CD38 ectoenzyme is a membrane protein that has its catalytic site on the outside of the membrane in the extracellular compartment. This cell surface protein facilitates many functions and is found on a wide variety of cells, such as immune cells, endothelial cells, and neuronal tissue cells.

[0004] CD38, also known as ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase 1, is a single-pass type II transmembrane protein with ectoenzymatic activities. Using NAD(P) as a substrate, it catalyzes the formation of several products: cyclic ADP-ribose (cADPR); ADP-ribose (ADPR); nicotinic acid adenine dinucleotide phosphate (NAADP); nicotinic acid (NA); ADP-ribose-2'-phosphate (ADPRP) (see, e.g. H. C. Lee, *Mol. Med.*, 2006, 12: 317-323). CD38 can also use Nicotinamide Mononucleotide (NMN) as a substrate and convert it to nicotinamide and R5P (Liu et al., "Covalent and noncovalent intermediates of an NAD utilizing enzyme, human CD38." *Chem Biol* 15(10): 1068-78.

[0005] CD38 is expressed predominantly on immune cells, including plasma cells, activated effector T cells, antigen-presenting cells, smooth muscle cells in the lung, Multiple Myeloma (MM) cells, B cell lymphoma, B cell leukemia cells, T cell lymphoma cells, breast cancer cells, myeloid derived suppressor cells, B regulatory cells, and T regulatory cells. CD38 on immune cells interacts with CD31/PECAM-1 expressed by endothelial cells and other cell lineages. This interaction promotes leukocyte proliferation, migration, T cell activation, and monocyte-derived DC maturation.

J Biotechnol 74:277–302; Revets et al., 2005; *Expert Opin Biol Ther* 5:111–124). Cartilaginous fish, such as sharks, have also evolved a distinctive type of immunoglobulin, designated as IgNAR, which lacks the light polypeptide chains and is composed entirely by heavy chains. IgNAR molecules can be manipulated by molecular engineering to produce the variable domain of a single heavy chain polypeptide (vNARs) (Nuttall et al. *Eur. J. Biochem.* 270, 3543-3554 (2003); Nuttall et al. *Function and Bioinformatics* 55, 187-197 (2004); Dooley et al., *Molecular Immunology* 40, 25-33 (2003)).

[0011] The ability of heavy chain-only antibodies devoid of light chain to bind antigen was established in the 1960s (Jaton *et al.* (1968) *Biochemistry*, 7, 4185-4195). Heavy chain immunoglobulin physically separated from light chain retained 80% of antigen-binding activity relative to the tetrameric antibody. Sitia et al. (1990) *Cell*, 60, 781-790 demonstrated that removal of the CH1 domain from a rearranged mouse μ gene results in the production of a heavy chain-only antibody, devoid of light chain, in mammalian cell culture. The antibodies produced retained VH binding specificity and effector functions.

[0012] Heavy chain antibodies with a high specificity and affinity can be generated against a variety of antigens through immunization (van der Linden, R. H., et al. *Biochim. Biophys. Acta.* 1431, 37-46 (1999)) and the VHH portion can be readily cloned and expressed in yeast (Frenken, L. G. J., et al. *J. Biotechnol.* 78, 11-21 (2000)). Their levels of expression, solubility and stability are significantly higher than those of classical F(ab) or Fv fragments (Ghahroudi, M. A. et al. *FEBS Lett.* 414, 521-526 (1997)).

[0013] Mice in which the λ (lambda) light (L) chain locus and/or the λ and κ (kappa) L chain loci have been functionally silenced, and antibodies produced by such mice, are described in U.S. Patent Nos. 7,541,513 and 8,367,888. Recombinant production of heavy chain-only antibodies in mice and rats has been reported, for example, in WO2006008548; U.S. Application Publication No. 20100122358; Nguyen et al., 2003, *Immunology*; 109(1), 93-101; Brüggemann *et al.*, *Crit. Rev. Immunol.*; 2006, 26(5):377-90; and Zou *et al.*, 2007, *J Exp Med*; 204(13): 3271–3283. The production of knockout rats via embryo microinjections of zinc-finger nucleases is described in Geurts et al., 2009, *Science*, 325(5939):433. Soluble heavy chain-only antibodies and transgenic rodents comprising a heterologous heavy chain locus producing such antibodies are described in U. S. Patent Nos. 8,883,150 and 9,365,655. CAR-T structures comprising single-domain antibodies as a binding (targeting) domain are described, for example, in Iri-Sofla *et al.*, 2011, *Experimental Cell Research* 317:2630-2641 and Jamnani *et al.*, 2014, *Biochim Biophys Acta*, 1840:378-386.0

SUMMARY

[0013a] In one aspect, the present disclosure refers to an antibody that binds to CD38, comprising a variable region sequence comprising:

- (a) a CDR1 sequence comprising any one of SEQ ID NOs: 1-5; and

- (b) a CDR2 sequence comprising any one of SEQ ID NOs: 6-12; and
- (c) a CDR3 sequence comprising any one of SEQ ID NOs: 13-17.

[0013b] In another aspect, the present disclosure refers to a bispecific antibody that binds to a first CD38 epitope and a second, non-overlapping CD38 epitope, the bispecific antibody comprising:

- (a) a first polypeptide subunit comprising a heavy chain variable region comprising a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 6, and a CDR3 sequence of SEQ ID NO: 13 in a human heavy chain framework;

- (b) a second polypeptide subunit comprising a light chain variable region comprising a CDR1 sequence of SEQ ID NO: 49, a CDR2 sequence of SEQ ID NO: 50, and a CDR3 sequence of SEQ ID NO: 51, in a human light chain framework;

wherein the first polypeptide subunit and the second polypeptide subunit together have binding affinity to the first CD38 epitope; and

- (c) a third polypeptide subunit comprising an antigen-binding domain of a heavy-chain only antibody comprising a CDR1 sequence of SEQ ID NO: 3, a CDR2 sequence of SEQ ID NO: 9, and a CDR3 sequence of SEQ ID NO: 16 in a human heavy chain framework, in a monovalent or bivalent configuration;

wherein the third polypeptide subunit has binding affinity to the second, non-overlapping CD38 epitope.

[0013c] In yet another aspect, the present disclosure refers to a use of the antibody as described herein in the manufacture of a medicament for the treatment of a disorder characterized by expression of CD38, wherein the antibody as described herein is to be administered to a subject with said disorder.

[0013d] In yet another aspect, the present disclosure refers to the antibody as described herein for use as a therapy.

[0014] Examples of the disclosure include bispecific binding compounds comprising a first polypeptide having binding affinity to a first epitope on an ectoenzyme, and a second polypeptide having binding affinity to a second, non-overlapping epitope on the ectoenzyme. In some examples, the first

polypeptide comprises an antigen-binding domain of a heavy-chain antibody having binding affinity to the first epitope. In some examples, the second polypeptide comprises an antigen-binding domain of a heavy-chain antibody having binding affinity to the second epitope. In some examples, the first and second polypeptides each comprise at least a portion of a hinge region. In some examples, the first and second polypeptides each comprise at least one CH domain. In some examples, the CH domain comprises a CH2 and/or a CH3 and/or a CH4 domain. In some examples, the CH domain comprises a CH2 domain and a CH3 domain. In some examples, the CH domain comprises a CH2 domain, a CH3 domain, and a CH4 domain. In some examples, the CH domain comprises a human IgG1 Fc region. In some examples, the human IgG1 Fc region is a silenced human IgG1 Fc region. In some examples, the CH domain comprises a human IgG4 Fc region. In some examples, the human IgG4 Fc region is a silenced human IgG4 Fc region. In some examples, the CH domain does not comprise a CH1 domain. In some examples, an asymmetric interface is present between the CH2 and/or the CH3 and/or the CH4 domains of the first and second polypeptides.

[0015] In some examples, the first polypeptide comprises a first antigen-binding domain of a heavy-chain antibody having binding affinity to the first epitope, and a second antigen-binding domain of a heavy-chain antibody having binding affinity to the second epitope. In some examples, the second polypeptide comprises a first antigen-binding domain of a heavy-chain antibody having binding affinity to the first epitope, and a second antigen-binding domain of a heavy-chain antibody having binding affinity to the second epitope. In some examples, the first and second antigen-binding domains are connected by a polypeptide linker. In some examples, the polypeptide linker consists of the sequence of SEQ ID NO: 45.

[0016] In some examples, a bispecific binding compound comprises a first and a second heavy chain polypeptide, each comprising an antigen-binding domain of a heavy-chain antibody having binding affinity to the first epitope, and a first and a second light chain polypeptide, each comprising an antigen-binding domain of a heavy-chain antibody having binding affinity to the second epitope. In some examples, the first and second light chain polypeptides each comprise a CL domain.

[0017] In some examples, the ectoenzyme is CD38.

[0018] Examples of the disclosure include heavy-chain antibodies that bind to CD38 and that comprise an antigen-binding domain comprising: (i) a CDR1 sequence having two or fewer substitutions in any of the amino acid sequences of SEQ ID NOs: 1-5; and/or (ii) a CDR2 sequence having two or fewer substitutions in any of the amino acid sequences of SEQ ID NOs: 6-12; and/or (iii) a CDR3 sequence having two or fewer substitutions in any of the amino acid sequences of SEQ ID NOs: 13-17. In some examples, the CDR1, CDR2, and CDR3 sequences are present in a human framework. In some examples, a heavy-chain antibody further comprises a heavy chain constant region sequence in the absence of a CH1 sequence.

[0019] In some examples, a heavy-chain antibody comprises a variable region sequence having at least 95% sequence identity to any of the sequences of SEQ ID NOs: 18-28. In some examples, a heavy-chain antibody comprises a variable region sequence selected from the group consisting of SEQ ID NOs: 18-28. In some examples, a heavy-chain antibody is monospecific. In some examples, a heavy-chain antibody is multi-specific. In some examples, a heavy-chain antibody is bispecific. In some examples, a heavy-chain antibody has binding affinity to two different epitopes on the same CD38 protein. In some examples, the two different epitopes are non-overlapping epitopes. In some examples, a heavy-chain antibody has binding affinity to an effector cell. In some examples, a heavy-chain antibody has binding affinity to a T-cell antigen. In some examples, a heavy-chain antibody has binding affinity to CD3. In some examples, a heavy-chain antibody is in a CAR-T format.

[0020] Examples of the disclosure include pharmaceutical compositions comprising a binding compound or a heavy-chain antibody described herein.

[0021] Examples of the disclosure include therapeutic combinations comprising a binding compound or a heavy-chain antibody described herein and a second antibody that binds to CD38. In some examples, the second antibody that binds to CD38 is isatuximab or daratumumab.

[0022] Examples of the disclosure include methods for the treatment of a disorder characterized by expression of CD38, the methods comprising administering to a subject with said disorder a binding compound or a heavy-chain antibody, a pharmaceutical composition, and/or a therapeutic combination as described herein. In some examples, the disorder is characterized by a hydrolase enzymatic activity of CD38. In some examples, the disorder is colitis. In some examples, the disorder is multiple myeloma (MM). In some examples, the disorder is an autoimmune disorder. In some examples, the disorder is rheumatoid arthritis (RA). In some examples, the disorder is pemphigus vulgaris (PV). In some examples, the disorder is systemic lupus erythematosus (SLE). In some examples, the disorder is multiple sclerosis (MS), systemic sclerosis or fibrosis. In some examples, the disorder is an ischemic injury. In some examples, the ischemic injury is an ischemic brain injury, an ischemic cardiac injury, an ischemic gastro-intestinal injury, or an ischemic kidney injury. In some examples, a method further comprises administering to the subject a second antibody that binds to CD38. In some examples, the second antibody that binds to CD38 is isatuximab or daratumumab.

[0023] These and further examples will be further explained in the rest of the disclosure, including the Examples.

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: 53). Non-limiting examples of such linkers include GGGGS (SEQ ID NO: 29) (n=1) and GGGSGGGGS (SEQ ID NO: 45) (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.

[0081] The term “bispecific 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. In some embodiments, a TCA comprises a light chain polypeptide subunit comprising a CDR1 sequence of SEQ ID NO: 49, a CDR2 sequence of SEQ ID NO: 50, and a CDR3 sequence of SEQ ID NO: 51, in a human light chain framework. In some embodiments, the human light chain framework is a human kappa (Vkappa) or a human lambda (Vlambda) framework. In some embodiments, a TCA comprises a light chain polypeptide subunit comprising a light chain variable region (VL) comprising a sequence having at least about 80%, 85%, 90%, 95%, or 99% identity to the sequence of SEQ ID NO: 52. In some embodiments, a TCA comprises a light chain polypeptide subunit that comprises the sequence of SEQ ID NO: 52. In some embodiments, a TCA comprises a light chain polypeptide subunit that comprises a light chain constant region (CL). In some embodiments, the light chain constant region is a human kappa light chain constant region or a human lambda light chain constant region. In some embodiments, a TCA comprises a light chain polypeptide subunit comprising a full length light chain comprising a sequence having at least about 80%, 85%, 90%, 95%, or 99% identity to the sequence of SEQ ID NO: 48. In some embodiments, a TCA comprises a light chain polypeptide subunit that comprises the sequence of SEQ ID NO: 48. 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 an antigen binding domain 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.

polypeptides to form the binding compound. Asymmetric interfaces in accordance with embodiments of the invention are further described herein.

[0146] In some embodiments, a binding compound includes a first and a second polypeptide, i.e., a first and a second polypeptide subunit, wherein each polypeptide comprises two antigen binding domains. In some embodiments, each of the first and second polypeptides further includes a hinge region, or at least a portion of a hinge region, which can facilitate formation of at least one disulfide bond between the first and second polypeptides. In some embodiments, each of the first and second polypeptides further includes at least one heavy chain constant region (CH) domain, such as a CH2 domain, and/or a CH3 domain, and/or a CH4 domain. In certain embodiments, the CH domain lacks a CH1 domain. The antigen-binding domain of each of the first and second polypeptides can incorporate any of the CDR sequences and/or variable region sequences described herein in order to impart antigen-binding capability on the binding compound. As such, in certain embodiments, each polypeptide in the binding compound can include two antigen-binding domains, having binding specificity to the same epitope, or to different epitopes (e.g., a first and a second, non-overlapping epitope on a CD38 protein).

[0147] A non-limiting example of a binding compound in accordance with embodiments of the invention is depicted in FIG. 11, Panel B. In the depicted embodiment, the binding compound is a bispecific, tetravalent binding compound that comprises a first polypeptide comprising two antigen-binding domains, one with binding specificity to a first epitope and one with binding specificity to a second, non-overlapping epitope, at least a portion of a hinge region, a CH domain comprising a CH2 and a CH3 domain (and lacking a CH1 domain), and a second polypeptide comprising two antigen-binding domains, one with binding specificity to the first epitope and one with binding specificity to the second, non-overlapping epitope, at least a portion of a hinge region, a CH domain comprising a CH2 and a CH3 domain (and lacking a CH1 domain). The depicted embodiment includes at least one disulfide bond in the hinge region that connects the first and second polypeptides to form the binding compound.

[0148] In some embodiments, the first and second antigen-binding domains on each polypeptide are connected by a polypeptide linker. One non-limiting example of a polypeptide linker that can connect the first and second antigen-binding domains is a GS linker, such as the G4S linker (SEQ ID NO: 29) having the amino acid sequence GGGGS (SEQ ID NO: 29). 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.

[0149] In some embodiments, a binding compound includes a first and a second heavy chain polypeptide, i.e., first and second heavy chain polypeptide subunits, as well as a first and a second light chain polypeptide, i.e., first and second light chain polypeptide subunits. In some embodiments, each of the heavy chain polypeptides comprises an antigen-binding domain of a heavy-chain antibody. In

CLAIMS:

1. An antibody that binds to CD38, comprising a variable region sequence comprising:
 - (a) a CDR1 sequence comprising any one of SEQ ID NOs: 1-5; and
 - (b) a CDR2 sequence comprising any one of SEQ ID NOs: 6-12; and
 - (c) a CDR3 sequence comprising any one of SEQ ID NOs: 13-17.
2. The antibody of claim 1, wherein the variable region sequence comprises:
a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 6, and a CDR3 sequence of SEQ ID NO: 13; or
a CDR1 sequence of SEQ ID NO: 3, a CDR2 sequence of SEQ ID NO: 9, and a CDR3 sequence of SEQ ID NO: 16; or
a CDR1 sequence of SEQ ID NO: 4, a CDR2 sequence of SEQ ID NO: 11, and a CDR3 sequence of SEQ ID NO: 17.
3. The antibody of claim 1, wherein said CDR1, CDR2, and CDR3 sequences are present in a human VH framework.
4. The antibody of claim 1, comprising a variable region sequence selected from the group consisting of SEQ ID NOs: 18-28.
5. The antibody of claim 1, wherein the variable region sequence comprises SEQ ID NO: 18 or SEQ ID NO: 23.
6. The antibody of claim 1, further comprising a heavy chain constant region sequence that does not comprise a CH1 domain.
7. The antibody of claim 1, which is monospecific.
8. The antibody of claim 1, which is multi-specific.
9. The antibody of claim 8, which is bispecific.
10. The antibody of claim 9, which has binding affinity to two different epitopes on the same CD38 protein.
11. The antibody of claim 10, wherein the two different epitopes are non-overlapping epitopes.

12. A bispecific antibody that binds to a first CD38 epitope and a second, non-overlapping CD38 epitope, the bispecific antibody comprising:

(a) a first polypeptide subunit comprising a heavy chain variable region comprising a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 6, and a CDR3 sequence of SEQ ID NO: 13 in a human heavy chain framework;

(b) a second polypeptide subunit comprising a light chain variable region comprising a CDR1 sequence of SEQ ID NO: 49, a CDR2 sequence of SEQ ID NO: 50, and a CDR3 sequence of SEQ ID NO: 51, in a human light chain framework;

wherein the first polypeptide subunit and the second polypeptide subunit together have binding affinity to the first CD38 epitope; and

(c) a third polypeptide subunit comprising an antigen-binding domain of a heavy-chain only antibody comprising a CDR1 sequence of SEQ ID NO: 3, a CDR2 sequence of SEQ ID NO: 9, and a CDR3 sequence of SEQ ID NO: 16 in a human heavy chain framework, in a monovalent or bivalent configuration;

wherein the third polypeptide subunit has binding affinity to the second, non-overlapping CD38 epitope.

13. The bispecific antibody of claim 12, comprising an Fc region selected from the group consisting of: a human IgG1 Fc region, a human IgG4 Fc region, a silenced human IgG1 Fc region, and a silenced human IgG4 Fc region.

14. The bispecific antibody of claim 12 comprising:

(a) a first heavy chain polypeptide comprising the sequence of SEQ ID NO: 46;

(b) a first light chain polypeptide comprising the sequence of SEQ ID NO: 48; and

(c) a second heavy chain polypeptide comprising the sequence of SEQ ID NO: 47.

15. Use of the antibody of any one of claims 1 to 14 in the manufacture of a medicament for the treatment of a disorder characterized by expression of CD38, wherein the antibody of any one of claims 1 to 14 is to be administered to a subject with said disorder.

16. The use of claim 15, wherein the disorder is characterized by a hydrolase enzymatic activity of CD38.

17. The use of claim 15, wherein the disorder is colitis.

18. The use of claim 15, wherein the disorder is multiple sclerosis (MS), systemic sclerosis or fibrosis.
19. The use of claim 15, wherein the subject is to be further administered a second antibody that binds to CD38.
20. The use of claim 19, wherein the second antibody that binds to CD38 is isatuximab or daratumumab.
21. The antibody of any one of claims 1 to 14 for use as a therapy.