

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 heavy chain antibody that binds to CD38, comprising a variable region sequence that 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.

[0013b] In another aspect, the present disclosure refers to a bispecific three-chain antibody like molecule that binds to a first CD38 epitope and a second, non-overlapping CD38 epitope, the bispecific three-chain antibody like molecule 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 heavy chain antibody or bispecific three-chain antibody like molecule as described herein in the manufacture of a medicament for the treatment of a disorder characterized by CD38 ectoenzymatic activity, wherein the heavy chain or bispecific three-chain antibody like molecule 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

CLAIMS:

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

12. The bispecific three-chain antibody like molecule of claim 11, 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.

13. The bispecific three-chain antibody like molecule of claim 11 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.

14. Use of the heavy chain antibody or bispecific three-chain antibody like molecule of any one of claims 1 to 13 in the manufacture of a medicament for the treatment of a disorder characterized by CD38 ectoenzymatic activity, wherein the heavy chain antibody or bispecific three-chain antibody like molecule of any one of claims 1 to 13 is to be administered to a subject with said disorder.

15. The use of claim 14, wherein the disorder is characterized by a hydrolase enzymatic activity of CD38.

16. The use of claim 14, wherein the disorder is colitis.

17. The use of claim 14, wherein the disorder is multiple sclerosis (MS), systemic sclerosis or fibrosis.

18. The use of claim 14, wherein the subject is to be further administered a second heavy chain antibody that binds to CD38.
19. The use of claim 18, wherein the second heavy chain antibody that binds to CD38 is isatuximab or daratumumab.
20. The antibody of any one of claims 1 to 13 for use as a therapy.