

HEAVY CHAIN ANTIBODIES BINDING TO CD19

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority benefit to U.S Provisional Patent Application No. 62/701,281, filed on July 20, 2018, the disclosure of which is incorporated by reference herein 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 September 4, 2019, is named TNO-0013-WO_SL.txt and is 26,773 bytes in size.

FIELD OF THE INVENTION

[0002] The present invention concerns human heavy chain antibodies (e.g., UniAbsTM) binding to CD19. The invention further concerns methods of making such antibodies, compositions, including pharmaceutical compositions, comprising such antibodies, and their use to treat B-cell disorders that are characterized by the expression of CD19.

BACKGROUND OF THE INVENTION

CD19

[0003] 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).

Heavy Chain Antibodies

[0004] In a conventional IgG antibody, the association of the heavy chain and light chain is due in part to a hydrophobic interaction between the light chain constant region and the CH1 constant domain of the heavy chain. There are additional residues in the heavy chain framework 2 (FR2) and framework 4 (FR4) regions that also contribute to this hydrophobic interaction between the heavy and light chains.

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.

SUMMARY OF THE INVENTION

[0009] Aspects of the invention relate to heavy chain antibodies, including but not limited to UniAbs™, with binding affinity to CD19. Further aspects of the invention relate to methods of making such antibodies, compositions comprising such antibodies, and their use in the treatment of B-cell disorders that are characterized by the expression of CD19.

[0009a] In one aspect, provided herein is a heavy chain-only antibody binding to CD19 comprising a heavy chain variable region comprising a CDR1 sequence of SEQ ID NO: 4, a CDR2 sequence of SEQ ID NO: 10, and a CDR3 sequence of SEQ ID NO: 13.

[0010] In some embodiments, a heavy chain-only antibody binding to CD19 comprises a heavy chain variable region comprising: (a) a CDR1 having two or fewer substitutions in any of the amino acid sequences of SEQ ID NOs: 1 to 6; and/or (b) a CDR2 having two or fewer substitutions in any of the amino acid sequences of SEQ ID NOs: 7 to 12; and/or (c) a CDR3 having two or fewer substitutions in the amino acid sequence of SEQ ID NO: 13. In some embodiments, the CDR1, CDR2, and CDR3 sequences are present in a human framework. In some embodiments, a heavy chain-only antibody further comprises a heavy chain constant region sequence in the absence of a CH1 sequence.

[0011] In some embodiments, a heavy chain-only antibody comprises: (a) a CDR1 sequence selected from the group consisting of SEQ ID NOs: 1 to 6; and/or (b) a CDR2 sequence selected from the group consisting of SEQ ID NOs: 7 to 12; and/or (c) a CDR3 sequence of SEQ ID NO: 13. In some embodiments, a heavy chain-only antibody comprises: (a) a CDR1 sequence selected from the group consisting of SEQ ID NOs: 1 to 6; and (b) a CDR2 sequence selected from the group consisting of SEQ ID NOs: 7 to 12; and (c) a CDR3 sequence of SEQ ID NO: 13.

[0012] In some embodiments, a heavy chain-only antibody comprises: (a) a CDR1 sequence of SEQ ID NO: 4, a CDR2 sequence of SEQ ID NO: 10, and a CDR3 sequence of SEQ ID NO: 13. In some embodiments, a heavy chain-only antibody comprises a heavy chain variable region having at least 95% sequence identity to any of the sequences of SEQ ID NOs: 14 to 21. In some embodiments, a heavy chain-only antibody comprises a heavy chain variable region sequence selected from the group consisting of SEQ ID NOs: 14 to 21. In some embodiments, a heavy chain-only antibody comprises a heavy chain variable region sequence of SEQ ID NO: 17.

[0013] In some embodiments, a heavy chain-only antibody binding to CD19 comprises a heavy chain variable region comprising (a) a CDR1 sequence of the formula:

G F X₁ F S X₂ X₃ W (SEQ ID NO: 22)

where X₁ is T or S; X₂ is S or N; X₃ is Y or F; and (b) a CDR2 sequence of the formula:

X₄ X₅ X₆ X₇ G S X₈ X₉ (SEQ ID NO: 23)

where X₄ is I or M; X₅ is N, S or K; X₆ is Q or K; X₇ is D or A; X₈ is D or E; X₉ is K or E; and (c) a CDR3 sequence of ASGVYSFDY (SEQ ID NO: 13).

[0014] In some embodiments, a heavy chain-only antibody binding to CD19 comprises a heavy chain variable region comprising CDR1, CDR2 and CDR3 sequences in a human VH framework, wherein the CDR sequences are sequences having two or fewer substitutions in a CDR sequence selected from the group consisting of SEQ ID NOs: 1-13.

[0015] In some embodiments, a heavy chain-only antibody comprises a heavy chain variable region comprising CDR1, CDR2 and CDR3 sequences in a human VH framework, wherein the CDR sequences are selected from the group consisting of SEQ ID NOs: 1-13.

[0016] In one aspect, provided herein is a heavy chain-only antibody binding to CD19 comprises a heavy chain variable region comprising: (a) a CDR1 sequence of SEQ ID NO: 4, a CDR2 sequence of SEQ ID NO: 10, and a CDR3 sequence of SEQ ID NO: 13, in a human VH framework.

[0017] In some embodiments, a heavy chain-only antibody is multi-specific. In some embodiments, a heavy chain-only antibody is bispecific. In some embodiments, a heavy chain-only antibody has binding affinity to two different CD19 proteins. In some embodiments, a heavy chain-only antibody has binding affinity to two different epitopes on the same CD19 protein. In some embodiments, a heavy chain-only antibody has binding affinity to an effector cell. In some embodiments, a heavy chain-only antibody has binding affinity to a T-cell antigen. In some embodiments, a heavy chain-only antibody has binding affinity to CD3. In some embodiments, a heavy chain-only antibody is in a CAR-T format.

[0018] Aspects of the invention relate to pharmaceutical compositions comprising a heavy chain-only antibody described herein.

[0019] Aspects of the invention relate to methods for the treatment of a B-cell disorder characterized by expression of CD19 comprising administering to a subject with said disorder an antibody or a pharmaceutical composition described herein. In some embodiments, the disorder is diffuse large B cell lymphoma (DLBCL). In some embodiments, the disorder is acute lymphoblastic leukemia (ALL). In some embodiments, the disorder is non-Hodgkin's lymphoma (NHL). In some embodiments, the disorder is systemic lupus erythematosus (SLE). In some embodiments, the disorder is rheumatoid arthritis (RA). In some embodiments, the disorder is multiple sclerosis (MS).

[0019a] In one aspect, provided herein is a use of an antibody as described herein, or a pharmaceutical composition as described herein, in the manufacture of a medicament for the treatment of a B-cell disorder characterized by expression of CD19.

- [0020] Aspects of the invention relate to polynucleotides encoding an antibody described herein, vectors comprising such polynucleotides, and cells comprising such vectors.
- [0021] Aspects of the invention relate to methods of producing an antibody described herein, comprising growing a cell described herein under conditions permissive for expression of the antibody, and isolating the antibody from the cell and/or a cell culture medium in which the cell is grown.
- [0022] Aspects of the invention relate to methods of making an antibody described herein, comprising immunizing a UniRat animal with CD19 and identifying CD19-binding heavy chain sequences.
- [0023] Aspects of the invention relate to methods of treatment, comprising administering to an individual in need an effective dose of an antibody or pharmaceutical composition as described herein.
- [0024] Aspects of the invention relate to use of an antibody or pharmaceutical composition as described herein in the preparation of a medicament for the treatment of a disease or disorder in an individual in need.
- [0025] Aspects of the invention relate to a kit for treating a disease or disorder in an individual in need, comprising an antibody or pharmaceutical composition as described herein, and instructions for use. In some embodiments, a kit further comprises at least one additional reagent. In some embodiments, the at least one additional reagent comprises a chemotherapeutic drug.
- [0025a] In one aspect, provided herein is a kit for treating a B-cell disorder characterized by expression of CD19 in an individual in need, the kit comprising the antibody as described herein, or the pharmaceutical composition as described herein, and instructions for use.
- [0025b] In one aspect, provided herein is an antibody as described herein or a pharmaceutical composition as described herein for use in therapy.
- [0026] These and further aspects will be further explained in the rest of the disclosure, including the Examples.

BRIEF DESCRIPTION OF THE DRAWINGS

- [0027] FIG. 1 shows anti-CD19 heavy chain antibody unique CDR amino acid sequences.
- [0028] FIG. 2 shows anti-CD19 heavy chain antibody variable domain amino acid sequences.
- [0029] FIG. 3 shows anti-CD19 heavy chain antibody CDR1, CDR2 and CDR3 amino acid sequences.
- [0030] FIG. 4 shows anti-CD19 heavy chain antibody biological activities.
- [0031] FIG. 5A is a graph depicting percent specific lysis as a function of antibody concentration.
- [0032] FIG. 5B is a schematic illustration of a bispecific anti-CD19 x anti-CD3 in accordance with one embodiment of the invention.
- [0033] FIG. 6 is a graph showing protein binding response as a function of time.
- [0034] FIG. 7 is a graph showing tumor burden as a function of time (days post implantation) for a tumor model experiment.
- [0035] FIG. 8 is a graph showing target cell lysis (cytotoxicity) as a function of antibody concentration.

- [0064]** The term “Fc-region-comprising antibody” refers to an antibody that comprises an Fc region. The C-terminal lysine (residue 447 according to the EU numbering system) of the Fc region may be removed, for example, during purification of the antibody or by recombinant engineering of the nucleic acid encoding the antibody. Accordingly, an antibody having an Fc region according to this invention can comprise an antibody with or without K447.
- [0065]** Aspects of the invention include binding compounds having multi-specific configurations, which include, without limitation, bispecific, trispecific, etc. A large variety of methods and protein configurations are known and used in bispecific monoclonal antibodies (BsMAB), tri-specific antibodies, etc.
- [0066]** Various methods for the production of multivalent artificial antibodies have been developed by recombinantly fusing variable domains of two or more antibodies. In some embodiments, a first and a 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: 30). Non-limiting examples of such linkers include GGGGS (SEQ ID NO: 24) (n=1) and GGGSGGGGS (SEQ ID NO: 25) (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.
- [0067]** 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., a single heavy chain variable region (a “single configuration”), or two antigen binding domains (e.g., two single heavy chain variable regions) in a “tandem configuration”, where the two antigen binding domains are linked together by a linker sequence, as described above) 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. A TCA protein makes use of a heavy chain-only antibody as hereinabove defined.

CLAIMS:

1. A heavy chain-only antibody binding to CD19 comprising a heavy chain variable region comprising a CDR1 sequence of SEQ ID NO: 4, a CDR2 sequence of SEQ ID NO: 10, and a CDR3 sequence of SEQ ID NO: 13.
2. The heavy chain-only antibody of claim 1, wherein said CDR1, CDR2, and CDR3 sequences are present in a human framework.
3. The heavy chain-only antibody of claim 1, further comprising a heavy chain constant region sequence in the absence of a CH1 sequence.
4. The heavy chain-only antibody of claim 1, comprising a heavy chain variable region having at least 95% sequence identity to SEQ ID NO: 17.
5. The heavy chain-only antibody of claim 1, comprising a heavy chain variable region comprising SEQ ID NO: 17.
6. A heavy chain-only antibody binding to CD19, comprising a heavy chain variable region comprising a CDR1 sequence of SEQ ID NO: 4, a CDR2 sequence of SEQ ID NO: 10, and a CDR3 sequence of SEQ ID NO: 13, in a human VH framework.
7. The heavy chain-only antibody of claim 1, which is multi-specific.
8. The heavy chain-only antibody of claim 7, which is bispecific.
9. The heavy chain-only antibody of claim 7, having binding affinity to an effector cell.
10. The heavy chain-only antibody of claim 7, having binding affinity to a T-cell antigen.
11. The heavy chain-only antibody of claim 10, having binding affinity to CD3.
12. The heavy chain-only antibody of claim 1, which is in a CAR-T format.

13. A pharmaceutical composition comprising the heavy chain-only antibody of claim 1.
14. Use of an antibody of any one of claims 1-12, or a pharmaceutical composition of claim 13, in the manufacture of a medicament for the treatment of a B-cell disorder characterized by expression of CD19.
15. The use of claim 14, wherein the disorder is diffuse large B-cell lymphoma (DLBCL).
16. The use of claim 14, wherein the disorder is acute lymphoblastic leukemia (ALL).
17. The use of claim 14, wherein the disorder is non-Hodgkin's lymphoma (NHL).
18. The use of claim 14, wherein the disorder is systemic lupus erythematosus (SLE).
19. The use of claim 14, wherein the disorder is rheumatoid arthritis (RA).
20. The use of claim 14, wherein the disorder is multiple sclerosis (MS).
21. A kit for treating a B-cell disorder characterized by expression of CD19 in an individual in need, the kit comprising the antibody of claim 1, or the pharmaceutical composition of claim 13, and instructions for use.
22. The kit of claim 21, further comprising at least one additional reagent.
23. The kit of claim 22, wherein the at least one additional reagent comprises a chemotherapeutic drug.
24. An antibody of any one of claims 1-12, or a pharmaceutical composition of claim 13 for use in therapy.