

MULTISPECIFIC HEAVY CHAIN ANTIBODIES BINDING TO CD22 AND CD3

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority benefit of the filing date of US Provisional Patent Application Serial No. 62/861,708, filed on June 14, 2019, 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 July 10, 2020, is named TNO-0017-WO SL.txt and is 115,348 bytes in size.

FIELD OF THE INVENTION

[0002] The present invention concerns multispecific, human heavy chain antibodies (e.g., UniAbs™) binding to CD22 and CD3. 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 the expression of CD22.

BACKGROUND OF THE INVENTION

CD22

[0003] CD22, also known as SIGLEC-2 (UniProt P20273), is a cell-surface receptor that is expressed on mature B-cells. CD22 contains multiple Ig domains and is a member of the immunoglobulin superfamily. The extracellular domain of CD22 interacts with sialic acid moieties, including those present on the CD45 cell surface protein. CD22 is thought to function as an inhibitory receptor for B-cell receptor signaling. Along with CD20 and CD19, the restricted B-cell expression of CD22 makes it an attractive target for the therapeutic treatment of B-cell malignancies. Monoclonal antibodies specific to CD22 have been described in the literature (e.g., Jabbour, Elias, et al. "Monoclonal antibodies in acute lymphoblastic leukemia." *Blood* 125.26 (2015): 4010-4016) and have been used therapeutically as standard monoclonals (e.g., epratuzumab) as well as antibody-drug conjugates (inotuzumab ozogamicin). In addition, anti-CD22 chimeric antigen receptor T-cells have been used in the clinic to treat leukemia (Fry, Terry J., et al.

"CD22-targeted CAR T cells induce remission in B-ALL that is naive or resistant to CD19-targeted CAR immunotherapy." *Nature medicine* (2017)).

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 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

[0008a] In one aspect, the present disclosure refers to a multi-specific antibody comprising a first binding unit having binding affinity to CD22 and a second binding unit having binding affinity to CD3, wherein the first binding unit comprises a variable region comprising a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 12, and a CDR3 sequence of SEQ ID NO: 19.

[0008b] In another aspect, the present disclosure refers to a multi-specific antibody comprising:

- (i) a heavy chain variable region having binding affinity to CD3, comprising a CDR1 sequence of SEQ ID NO: 85, a CDR2 sequence of SEQ ID NO: 86, and a CDR3 sequence of SEQ ID NO: 87, in a human VH framework;
- (ii) a light chain variable region comprising a CDR1 sequence of SEQ ID NO: 88, a CDR2 sequence of SEQ ID NO: 89, and a CDR3 sequence of SEQ ID NO: 90, in a human VL framework; and
- (iii) an antigen-binding domain of an anti-CD22 heavy chain antibody, comprising a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 12, and a CDR3 sequence of SEQ ID NO: 19, in a human VH framework.

[0008c] In another aspect, the present disclosure refers to a pharmaceutical composition comprising a multi-specific antibody as described herein.

[0008d] In another aspect, the present disclosure refers to a use of a multi-specific antibody as described herein, in the preparation of a medicament for the treatment of a B-cell disorder characterized by expression of CD22.

[0008e] In yet another aspect, the present disclosure refers to a multi-specific antibody as described herein for use in therapy.

[0008f] In yet another aspect, the present disclosure refers to a polynucleotide encoding a multi-specific antibody as described herein.

[0008g] In yet another aspect, the present disclosure refers to a vector comprising the polynucleotide as described herein.

[0008h] In yet another aspect, the present disclosure refers to a cell comprising the vector as described herein.

[0009] Examples~~Aspects~~ of the disclosure~~invention~~ relate to heavy chain antibodies, including but not limited to UniAbs™, with binding affinity to CD22. Further examples~~aspects~~ of the disclosure~~invention~~ relate to methods of making such antibodies, compositions comprising such antibodies, and their use in the treatment of disorders that are characterized by the expression of CD22.

[0010] Examples~~Aspects~~ of the disclosure~~invention~~ include multi-specific binding compounds that bind to CD3, comprising: a heavy chain variable region comprising: (a) a CDR1 sequence having two or fewer substitutions in SEQ ID NO: 85; and/or (b) a CDR2 sequence having two or fewer substitutions in SEQ ID NO: 86; and/or (c) a CDR3 sequence having two or fewer substitutions in SEQ ID NO: 87; and a light chain variable region. In some examples~~embodiments~~, the heavy chain CDR1, CDR2 and CDR3 sequences are present in a human VH framework. In some examples~~embodiments~~, the heavy chain variable region comprises heavy chain CDR1, CDR2 and CDR3 sequences in a human VH framework, wherein each CDR sequence comprises a sequence with at least 85% identity to any one of SEQ ID NOs:85-87; and the binding compound also comprises a light chain variable region.

[0011] In some examples~~embodiments~~, the multi-specific binding compound comprises: a heavy chain variable region comprising: (a) a CDR1 sequence having two or fewer substitutions in SEQ ID NO: 85; and (b) a CDR2 sequence having two or fewer substitutions in SEQ ID NO: 86; and (c) a CDR3 sequence having two or fewer substitutions in SEQ ID NO: 87; and the binding compound also comprises a light chain variable region.

[0012] In some examples~~embodiments~~, the multi-specific binding compound comprises: a heavy chain variable region comprising a CDR1 sequence of SEQ ID NO: 85, a CDR2 sequence of SEQ ID NO: 86, and a CDR3 sequence of SEQ ID NO: 87; and the binding compound also comprises a light chain variable region.

[0013] In some examples~~embodiments~~, the light chain variable region comprises a CDR1, CDR2 and CDR3 sequence in a human VL framework, wherein each CDR sequence comprises a sequence with 3 or fewer amino acid substitutions relative to a CDR sequence or set of CDR sequences in SEQ ID NO: 92; or wherein the CDR sequences comprise a sequence with at least 85% identity to a CDR sequence

or set of CDR sequences in SEQ ID NO: 92. In some example embodiments, the light chain variable region comprises a CDR1 sequence of SEQ ID NO: 88, a CDR2 sequence of SEQ ID NO: 89, and a CDR3 sequence of SEQ ID NO: 90. In some example embodiments, the heavy chain variable region comprises an amino acid sequence having at least 95% identity to SEQ ID NO: 91. In some example embodiments, the heavy chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 91. In some example embodiments, the light chain variable region comprises an amino acid sequence having at least 95% identity to SEQ ID NO: 92. In some example embodiments, the light chain variable region comprises an amino acid sequence of SEQ ID NO: 92.

[0014] Examples ~~Aspects~~ of the disclosure ~~invention~~ include multi-specific binding compounds comprising a first binding unit having binding affinity to CD22 and a second binding unit having binding affinity to CD3, wherein the first binding unit comprises: (a) a CDR1 having two or fewer substitutions in any of the amino acid sequences of SEQ ID NOs: 1 to 10; and/or (b) a CDR2 having two or fewer substitutions in any of the amino acid sequences of SEQ ID NOs: 11 to 17; and/or (c) a CDR3 having two or fewer substitutions in any of the amino acid sequences of SEQ ID NOs: 18 to 23. In some example embodiments, the CDR1, CDR2, and CDR3 sequences of the first binding unit are present in a human framework. In some example embodiments, the first binding unit further comprises a heavy chain constant region sequence in the absence of a CH1 sequence.

[0015] In some example embodiments, the first binding unit comprises a heavy chain variable region comprising: (a) a CDR1 sequence selected from the group consisting of SEQ ID NOs: 1 to 10; and/or (b) a CDR2 sequence selected from the group consisting of SEQ ID NOs: 11 to 17; and/or (c) a CDR3 sequence selected from the group consisting of SEQ ID NOs: 18 to 23.

[0016] In some example embodiments, the multi-specific binding compound comprises: (a) a CDR1 sequence selected from the group consisting of SEQ ID NOs: 1 to 10; and (b) a CDR2 sequence selected from the group consisting of SEQ ID NOs: 11 to 17; and (c) a CDR3 sequence selected from the group consisting of SEQ ID NOs: 18 to 23.

[0017] In some example embodiments, the multi-specific binding compound comprises: (a) a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 11, and a CDR3 sequence of SEQ ID NO: 18; (b) a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 12, and a CDR3 sequence of SEQ ID NO: 19; or (c) a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 12, and a CDR3 sequence of SEQ ID NO: 20. In some example embodiments, the multi-specific binding compound comprises a heavy chain variable region having at least 95% sequence identity to any one of the sequences of SEQ ID NOs: 24 to 84. In some example embodiments, the multi-specific binding compound comprises a heavy chain variable region sequence selected from the group consisting of SEQ ID NOs: 24 to 84. In some example embodiments, the multi-specific binding compound comprises a heavy chain variable region sequence of SEQ ID NO: 24.

[0018] ExamplesAspects of the disclosureinvention include multi-specific binding compounds comprising a first binding unit having binding affinity to CD22 and a second binding unit having binding affinity to CD3, wherein the first binding unit comprises a heavy chain variable region comprising: (a) a CDR1 sequence of the formula: G X₁ S I X₂ X₃ X₄ X₅ X₆ Y (SEQ ID NO: 104), where X₁ is D or G; X₂ is S, T, I or N; X₃ is S or D; X₄ is G, S or N; X₅ is D, G or S; and X₆ is Y or H; and (b) a CDR2 sequence of the formula: X₇ X₈ Y X₉ G X₁₀ X₁₁ (SEQ ID NO: 105) where X₇ is I or V; X₈ is Y or H; X₉ is S or T; X₁₀ is A, V or S; and X₁₁ is T or A; and (c) a CDR3 sequence of the formula: X₁₂ R X₁₃ D S S X₁₄ W R S (SEQ ID NO: 106) where X₁₂ is T, A or K; X₁₃ is D or E; and X₁₄ is N or S.

[0019] ExamplesAspects of the disclosureinvention include multi-specific binding compounds comprising a first binding unit having binding affinity to CD22 and a second binding unit having binding affinity to CD3, wherein the first binding unit comprises a heavy chain variable region comprising CDR1, CDR2 and CDR3 sequences in a human VH framework, wherein the CDR sequences comprise a sequence having two or fewer substitutions in a CDR sequence selected from the group consisting of SEQ ID NOs: 1-23.

[0020] ExamplesAspects of the disclosureinvention include multi-specific binding compounds comprising a first binding unit having binding affinity to CD22 and a second binding unit having binding affinity to CD3, wherein the first binding unit 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: 1-23.

[0021] ExamplesAspects of the disclosureinvention include multi-specific binding compounds comprising a first binding unit having binding affinity to CD22 and a second binding unit having binding affinity to CD3, wherein the first binding unit comprises a heavy chain variable region comprising: (a) a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 11, and a CDR3 sequence of SEQ ID NO: 18; or (b) a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 12, and a CDR3 sequence of SEQ ID NO: 19; or (c) a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 12, and a CDR3 sequence of SEQ ID NO: 20, in a human VH framework.

[0022] In some examplesembodiments, the multi-specific binding compound is bispecific. In some examplesembodiments, the multi-specific binding compound is in a CAR-T format.

[0023] ExamplesAspects of the disclosureinvention include multi-specific binding compounds comprising: (i) a heavy chain variable region having binding affinity to CD3, comprising a CDR1 sequence of SEQ ID NO: 85, a CDR2 sequence of SEQ ID NO: 86, and CDR3 sequence of SEQ ID NO: 87, in a human VH framework; (ii) a light chain variable region comprising a CDR1 sequence of SEQ ID NO: 88, a CDR2 sequence of SEQ ID NO: 89, and CDR3 sequences of SEQ ID NO: 90, in a human VL framework; and (iii) an antigen-binding domain of an anti-CD22 heavy chain antibody, comprising a

CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 11, and a CDR3 sequence of SEQ ID NO: 18, in a human VH framework.

[0024] Examples/Aspects of the disclosure/invention include multi-specific binding compounds comprising: (i) a heavy chain variable region having binding affinity to CD3, comprising a CDR1 sequence of SEQ ID NO: 85, a CDR2 sequence of SEQ ID NO: 86, and CDR3 sequence of SEQ ID NO: 87, in a human VH framework; (ii) a light chain variable region comprising a CDR1 sequence of SEQ ID NO: 88, a CDR2 sequence of SEQ ID NO: 89, and CDR3 sequences of SEQ ID NO: 90, in a human VL framework; and (iii) an antigen-binding domain of an anti-CD22 heavy chain antibody, comprising a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 12, and a CDR3 sequence of SEQ ID NO: 19, in a human VH framework.

[0025] Examples/Aspects of the disclosure/invention include multi-specific binding compound comprising: (i) a heavy chain variable region having binding affinity to CD3, comprising a CDR1 sequence of SEQ ID NO: 85, a CDR2 sequence of SEQ ID NO: 86, and CDR3 sequence of SEQ ID NO: 87, in a human VH framework; (ii) a light chain variable region comprising a CDR1 sequence of SEQ ID NO: 88, a CDR2 sequence of SEQ ID NO: 89, and CDR3 sequences of SEQ ID NO: 90, in a human VL framework; and (iii) an antigen-binding domain of an anti-CD22 heavy chain antibody, comprising a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 12, and a CDR3 sequence of SEQ ID NO: 20, in a human VH framework.

[0026] In some example embodiments, the multispecific binding compound comprises a human IgG1 Fc region. In some example embodiments, the human IgG1 Fc region is a silenced human IgG1 Fc region. In some example embodiments, the multispecific binding compound comprises a human IgG4 Fc region. In some example embodiments, the human IgG4 Fc region is a silenced human IgG4 Fc region.

[0027] Examples/Aspects of the disclosure/invention include pharmaceutical compositions comprising a multi-specific binding compound as described herein.

[0028] Examples/Aspects of the disclosure/invention include methods for the treatment of a B-cell disorder characterized by expression of CD22, comprising administering to a subject with said disorder a multi-specific binding compound or a pharmaceutical composition as described herein.

[0029] Examples/Aspects of the disclosure/invention include use of a multi-specific binding compound in the preparation of a medicament for the treatment of a B-cell disorder characterized by expression of CD22.

[0030] In some example embodiments, the disorder is diffuse large B cell lymphoma (DLBCL). In some example embodiments, the disorder is non-Hodgkin's lymphoma (NHL). In some example embodiments, the disorder is systemic lupus erythematosus (SLE). In some example embodiments, the disorder is rheumatoid arthritis (RA). In some example embodiments, the disorder is multiple sclerosis (MS).

- [0031] ~~Examples/Aspects~~ of the ~~disclosureinvention~~ include polynucleotides encoding a multi-specific binding compound as described herein. ~~Examples/Aspects~~ of the ~~disclosureinvention~~ include vectors comprising the polynucleotides as described herein. ~~Examples/Aspects~~ of the ~~disclosureinvention~~ include cells comprising the vectors as described herein.
- [0032] ~~Examples/Aspects~~ of the ~~disclosureinvention~~ include methods of producing a multi-specific binding compound as described herein, comprising growing a cell as described herein under conditions permissive for expression of the binding compound, and isolating the binding compound from the cell.
- [0033] ~~Examples/Aspects~~ of the ~~disclosureinvention~~ include methods of making a multi-specific binding compound as described herein, comprising immunizing a UniRat animal with CD22 and identifying CD22-binding heavy chain sequences.
- [0034] ~~Examples/Aspects~~ of the ~~disclosureinvention~~ include methods of treatment, comprising administering to an individual an effective dose of a multi-specific binding compound as described herein, or a pharmaceutical composition as described herein.
- [0035] These and further ~~examplesaspects~~ will be further explained in the rest of the disclosure, including the Examples.

BRIEF DESCRIPTION OF THE DRAWINGS

- [0036] FIG. 1A is a graph depicting T cell mediated cytotoxicity of CD22 positive cells (Daudi) using resting human pan T cells.
- [0037] FIG. 1B is a graph depicting dose response curves of cytokine release by resting human pan T cells incubated with CD22 positive cells (Daudi) and treated with an anti-CD22xCD3_F2F multispecific binding compound and a positive control.
- [0038] FIG. 2A is a graph depicting T cell mediated cytotoxicity of CD22 positive cells (SUDHL10) using resting human pan T cells.
- [0039] FIG. 2B is a graph depicting dose response curves of cytokine release by resting human pan T cells incubated with CD22 positive cells (SUDHL10) and treated with an anti-CD22xCD3_F2F multispecific binding compound and a positive control.
- [0040] FIG. 3A shows a series of graphs depicting T cell mediated cytotoxicity of CD22 positive cells (RI-1) using resting human pan T cells.
- [0041] FIG. 3B shows a series of graphs depicting dose response curves of cytokine release by resting human pan T cells incubated with CD22 positive cells (RI-1) and treated with an anti-CD22xCD3_F2F multispecific binding compound and a positive control.
- [0042] FIG. 4 shows a series of graphs depicting T cell mediated cytotoxicity of CD22 positive cells using activated human pan T cells.
- [0043] FIG. 5 shows a series of graphs depicting cell binding of bispecific antibodies against CD22 and CD3.

[0087] 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.

[0088] 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 (SEQ ID NO: 107), such as 2, 3, 4, 5, 6, 7, 8, or 9. Non-limiting examples of such linkers include GGGGS (SEQ ID NO: 102) (n=1) and GGGSGGGGS (SEQ ID NO: 103) (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.

[0089] 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. A TCA protein makes use of a heavy chain-only antibody as hereinabove defined.

[0090] 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

variable region (VH) sequences of SEQ ID NOs: 24 to 84 set forth in Table 2. The antibodies described herein provide a number of benefits that contribute to utility as clinically therapeutic agent(s). The antibodies include members with a range of binding affinities, allowing the selection of a specific sequence with a desired binding affinity.

Table 1: Anti-CD22 heavy chain antibody unique CDR amino acid sequences.

SEQ_aa_CDR1	SEQ_aa_CDR2	SEQ_aaCDR3
GDSISSGDYY (SEQ ID NO: 1)	IYYSGVT (SEQ ID NO: 11)	TREDSSNWRS (SEQ ID NO: 18)
GDSISSGGYY (SEQ ID NO: 2)	IYYSGAT (SEQ ID NO: 12)	TRDDSSNWRS (SEQ ID NO: 19)
GGSISSGDYY (SEQ ID NO: 3)	~~IYYSGAT~~ -IYYSGST (SEQ ID NO: 13)	TREDSSSWRS (SEQ ID NO: 20)
GGSISSSSYY (SEQ ID NO: 4)	IYYTGST (SEQ ID NO: 14)	AREDSSSWRS (SEQ ID NO: 21)
GGSFSGYY (SEQ ID NO: 5)	VYYTGAT (SEQ ID NO: 15)	KRDDSSNWRS (SEQ ID NO: 22)
GDSISSSSYY (SEQ ID NO: 6)	IHYSGST (SEQ ID NO: 16)	ARDDSSNWRS (SEQ ID NO: 23)
GGSISSSSYY (SEQ ID NO: 7)	IYYSGSA (SEQ ID NO: 17)	
GGSISSSSHY (SEQ ID NO: 8)		
GGSISSSSYY (SEQ ID NO: 9)		
GGSISSSSHY (SEQ ID NO: 10)		

Table 2. Anti-CD22 heavy chain antibody variable domain amino acid sequences.

Clone ID #	SEQ_aa_FR1_FR4	SEQ ID NO:
335207	QLQLQESGPGLVKPSETLSLTCTVSGDSISSGDYYWGWRQPPGKGLEWIG HIYYSGVTYYNPSLKSRTISVDTSRNQFSLKLSSVTAADTAVYYCTREDSS NWRSRGQGLTVTVSS	24
335161	QLQLQESGPGLVKPSETLSLTCTVSGDSISSGDYYWGWRQPPGKGLEWIG HIYYSGATYYNPSLENRVITISVDTSKNQFSLKLSSVTAADTAVYYCTRDDS SNWRSRGQGLTVTVSS	25
335254	QLQLQESGPGLVKPSETLSLTCTVSGDSISSGDYYWGWRQPPGKGLEWIG HIYYSGVTYYNPSLKNRVITISVDTSKNQFSLKLSSVTAADTAVYYCTREDS SSWRSRGQGLTVTVSS	26
335260	QLQLQESGPGLVKPSETLSLTCTVSGDSISSGDYYWGWRQPPGKGLEWIG HIYYSGVTYYNPSLKNRVITISVDTSRNQFSLNLSSVTAADTAVYYCTREDS SSWRSRGQGLTVTVSS	27
335151	QLQLQESGPGLVKPSETLSLTCTVSGDSISSGDYYWGWRQPPGKGLEWIG HIYYSGATYYNPSLKNRVITISVDTSRNQFSLNLSSVTAADTAVYYCTRDDS SNWRSRGQGLTVTVSS	28
335170	QLQLQESGPGLVKPSETLSLTCTVSGDSISSGDYYWGWRQPPGKGLEWIG HIYYSGATYYNPSLKNRVITISVDTSRNQFSLKLSSVTAADTAVYYCTRDDS SNWRSRGQGLTVTVSS	29
335176	QLQLQESGPGLVKPSETLSLTCTVSGDSISSGDYYWGWRQPPGKGLEWIG HIYYSGATYYNPSLKNRVITISVDTSKNQFSLKLSSVTAADTAVYYCTRDDS SNWRSRGQGLTVTVSS	30
335181	QLQLQESGPGLVKPSETLSLTCTVSGDSISSGGYYWGWRQPPGKGLEWIG HIYYSGATYYNPSLKNRVITISVDTSKNQFSLKLSSVTAADTAVYYCTRDDS SNWRSRGQGLTVTVSS	31

CLAIMS:

1. ~~A multi-specific binding compound that binds to CD3, comprising:
a heavy chain variable region comprising:
(a) ~~a CDR1 sequence having two or fewer substitutions in SEQ ID NO: 85; and/or~~
(b) ~~a CDR2 sequence having two or fewer substitutions in SEQ ID NO: 86; and/or~~
(c) ~~a CDR3 sequence having two or fewer substitutions in SEQ ID NO: 87; and~~
a light chain variable region.~~
2. ~~The multi-specific binding compound of claim 1, wherein said heavy chain CDR1, CDR2 and CDR3 sequences are present in a human VH framework.~~
3. ~~The multi-specific binding compound of claim 1, comprising a heavy chain variable region comprising heavy chain CDR1, CDR2 and CDR3 sequences in a human VH framework, wherein each CDR sequence comprises a sequence with at least 85% identity to any one of SEQ ID NOs:85-87; and
a light chain variable region.~~
4. ~~The multi-specific binding compound of claim 1, comprising:
a heavy chain variable region comprising:
(a) ~~a CDR1 sequence having two or fewer substitutions in SEQ ID NO: 85; and~~
(b) ~~a CDR2 sequence having two or fewer substitutions in SEQ ID NO: 86; and~~
(c) ~~a CDR3 sequence having two or fewer substitutions in SEQ ID NO: 87; and~~
a light chain variable region.~~
5. ~~The multi-specific binding compound of claim 4, comprising:
a heavy chain variable region comprising a CDR1 sequence of SEQ ID NO: 85, a CDR2 sequence of SEQ ID NO: 86, and a CDR3 sequence of SEQ ID NO: 87; and
a light chain variable region.~~
6. ~~The multi-specific binding compound of any one of claims 1-5, wherein the light chain variable region comprises a CDR1, CDR2 and CDR3 sequence in a human VL framework, wherein each CDR sequence comprises a sequence with 3 or fewer amino acid substitutions relative to a CDR sequence or~~

~~set of CDR sequences in SEQ ID NO: 92; or wherein the CDR sequences comprise a sequence with at least 85% identity to a CDR sequence or set of CDR sequences in SEQ ID NO: 92.~~

~~7. — The multi-specific binding compound of any one of claims 1-6, wherein the light chain variable region comprises a CDR1 sequence of SEQ ID NO: 88, a CDR2 sequence of SEQ ID NO: 89, and a CDR3 sequence of SEQ ID NO: 90.~~

~~8. — The multi-specific binding compound of any one of claims 1-7, wherein the heavy chain variable region comprises an amino acid sequence having at least 95% identity to SEQ ID NO: 91.~~

~~9. — The multi-specific binding compound of any one of claims 1-7, wherein the heavy chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 91.~~

~~10. — The multi-specific binding compound of any one of claims 1-9, wherein the light chain variable region comprises an amino acid sequence having at least 95% identity to SEQ ID NO: 92.~~

~~11. — The multi-specific binding compound of any one of claims 1-9, wherein the light chain variable region comprises an amino acid sequence of SEQ ID NO: 92.~~

~~12. — A multi-specific binding compound comprising a first binding unit having binding affinity to CD22 and a second binding unit having binding affinity to CD3, wherein the first binding unit comprises:~~

~~(a) — a CDR1 having two or fewer substitutions in any of the amino acid sequences of SEQ ID NOs: 1 to 10; and/or~~

~~(b) — a CDR2 having two or fewer substitutions in any of the amino acid sequences of SEQ ID NOs: 11 to 17; and/or~~

~~(c) — a CDR3 having two or fewer substitutions in any of the amino acid sequences of SEQ ID NOs: 18 to 23.~~

~~13. — The multi-specific binding compound of claim 12, wherein said CDR1, CDR2, and CDR3 sequences of the first binding unit are present in a human framework.~~

~~14. — The multi-specific binding compound of any one of claims 12-13, wherein the first binding unit further comprises a heavy chain constant region sequence in the absence of a CH1 sequence.~~

~~15. The multi-specific binding compound of any one of claims 12 to 14, wherein the first binding unit comprises a heavy chain variable region comprising:~~

- ~~(a) a CDR1 sequence selected from the group consisting of SEQ ID NOs: 1 to 10; and/or~~
- ~~(b) a CDR2 sequence selected from the group consisting of SEQ ID NOs: 11 to 17; and/or~~
- ~~(c) a CDR3 sequence selected from the group consisting of SEQ ID NOs: 18 to 23.~~

~~16. The multi-specific binding compound of any one of claim 15, comprising:~~

- ~~(a) a CDR1 sequence selected from the group consisting of SEQ ID NOs: 1 to 10; and~~
- ~~(b) a CDR2 sequence selected from the group consisting of SEQ ID NOs: 11 to 17; and~~
- ~~(c) a CDR3 sequence selected from the group consisting of SEQ ID NOs: 18 to 23.~~

~~117. TheA multi-specific antibodybinding compound of any one of claims 12 to 16, comprising: a first binding unit having binding affinity to CD22 and a second binding unit having binding affinity to CD3, wherein the first binding unit comprises a variable region comprising~~

- ~~(a) a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 11, and a CDR3 sequence of SEQ ID NO: 18;~~
- ~~(b) a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 12, and a CDR3 sequence of SEQ ID NO: 19; or~~
- ~~(c) a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 12, and a CDR3 sequence of SEQ ID NO: 20.~~

~~218. The multi-specific antibodybinding compound of claim 112, wherein the first binding unit comprisescomprising a heavy chain variable region having at least 95% sequence identity to any one of the sequences of SEQ ID NOs: 2524 to 84.~~

~~19. The multi-specific binding compound of claim 18, comprising a heavy chain variable region sequence selected from the group consisting of SEQ ID NOs: 24 to 84.~~

~~320. The multi-specific antibodybinding compound of claim 192, comprising wherein the first binding unit comprises thea heavy chain variable region sequence of SEQ ID NO: 2425.~~

21. — A multi-specific binding compound comprising a first binding unit having binding affinity to CD22 and a second binding unit having binding affinity to CD3, wherein the first binding unit comprises a heavy chain variable region comprising:

— (a) — a CDR1 sequence of the formula:



where — X_1 is D or G;

— X_2 is S, T, I or N;

— X_3 is S or D;

— X_4 is G, S or N;

— X_5 is D, G or S; and

— X_6 is Y or H; and

— (b) — a CDR2 sequence of the formula:



where — X_7 is I or V;

— X_8 is Y or H;

— X_9 is S or T;

— X_{10} is A, V or S; and

— X_{11} is T or A; and

(c) — a CDR3 sequence of the formula:



where — X_{12} is T, A or K;

— X_{13} is D or E; and

— X_{14} is N or S.

22. — A multi-specific binding compound comprising a first binding unit having binding affinity to CD22 and a second binding unit having binding affinity to CD3, wherein the first binding unit comprises a heavy chain variable region comprising CDR1, CDR2 and CDR3 sequences in a human VH framework, wherein the CDR sequences comprise a sequence having two or fewer substitutions in a CDR sequence selected from the group consisting of SEQ ID NOs: 1-23.

23. — A multi-specific binding compound comprising a first binding unit having binding affinity to CD22 and a second binding unit having binding affinity to CD3, wherein the first binding unit 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: 1-23.~~

424. ~~The~~A multi-specific antibody~~binding compound~~ of claim 1, wherein the CDR1, CDR2 and CDR3 sequences are present in a human VH framework~~binding compound comprising a first binding unit having binding affinity to CD22 and a second binding unit having binding affinity to CD3, wherein the first binding unit comprises~~

~~a heavy chain variable region comprising:~~

(a) ~~— a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 11, and a CDR3 sequence of SEQ ID NO: 18; or~~

(b) ~~— a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 12, and a CDR3 sequence of SEQ ID NO: 19; or~~

(c) ~~— a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 12, and a CDR3 sequence of SEQ ID NO: 20,~~

~~in a human VH framework.~~

525. The multi-specific antibody~~binding compound~~ of any one of claims 1-424, which is bispecific.

26. ~~The multi-specific binding compound of any one of the preceding claims, which is in a CAR-T format.~~

~~27.~~ ~~A multi-specific binding compound comprising:~~

~~— (i) a heavy chain variable region having binding affinity to CD3, comprising a CDR1 sequence of SEQ ID NO: 85, a CDR2 sequence of SEQ ID NO: 86, and CDR3 sequence of SEQ ID NO: 87, in a human VH framework;~~

~~— (ii) a light chain variable region comprising a CDR1 sequence of SEQ ID NO: 88, a CDR2 sequence of SEQ ID NO: 89, and CDR3 sequences of SEQ ID NO: 90, in a human VL framework; and~~

~~— (iii) an antigen-binding domain of an anti-CD22 heavy chain antibody, comprising a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 11, and a CDR3 sequence of SEQ ID NO: 18, in a human VH framework.~~

628. A multi-specific antibody~~binding compound~~ comprising:

(i) a heavy chain variable region having binding affinity to CD3, comprising a CDR1 sequence of SEQ ID NO: 85, a CDR2 sequence of SEQ ID NO: 86, and a CDR3 sequence of SEQ ID NO: 87, in a human VH framework;

(ii) a light chain variable region comprising a CDR1 sequence of SEQ ID NO: 88, a CDR2 sequence of SEQ ID NO: 89, and a CDR3 sequences of SEQ ID NO: 90, in a human VL framework; and

(iii) an antigen-binding domain of an anti-CD22 heavy chain antibody, comprising a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 12, and a CDR3 sequence of SEQ ID NO: 19, in a human VH framework.

~~29. — A multi-specific binding compound comprising:~~

~~—— (i) a heavy chain variable region having binding affinity to CD3, comprising a CDR1 sequence of SEQ ID NO: 85, a CDR2 sequence of SEQ ID NO: 86, and CDR3 sequence of SEQ ID NO: 87, in a human VH framework;~~

~~—— (ii) a light chain variable region comprising a CDR1 sequence of SEQ ID NO: 88, a CDR2 sequence of SEQ ID NO: 89, and CDR3 sequences of SEQ ID NO: 90, in a human VL framework; and~~

~~—— (iii) an antigen-binding domain of an anti-CD22 heavy chain antibody, comprising a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 12, and a CDR3 sequence of SEQ ID NO: 20, in a human VH framework.~~

730. The multispecific antibodybinding compound of any one of claims 1-~~629~~, comprising a human IgG1 Fc region.

834. The multispecific antibodybinding compound of claim 730, wherein the human IgG1 Fc region is a silenced human IgG1 Fc region.

932. The multispecific antibodybinding compound of any one of claims 1-~~629~~, comprising a human IgG4 Fc region.

1033. The multispecific antibodybinding compound of claim 932, wherein the human IgG4 Fc region is a silenced human IgG4 Fc region.

1134. A pharmaceutical composition comprising a multi-specific antibodybinding compound of any one of claims 1 to 1033.

~~35. — A method for the treatment of a B-cell disorder characterized by expression of CD22, comprising administering to a subject with said disorder a multi-specific binding compound of any one of claims 1 to 33, or the pharmaceutical composition of claim 34.~~

1236. Use of a multi-specific ~~antibody~~binding compound of any one of claims 1 to 1033, in the preparation of a medicament for the treatment of a B-cell disorder characterized by expression of CD22.

1337. A multi-specific ~~binding compound~~antibody of any one of claims 1 to 3310 for use in ~~the treatment of a B-cell disorder characterized by expression of CD22~~therapy.

1438. The ~~method, use or multi-specific binding compound of any one of claims 1235-37~~, wherein the disorder is selected from the group consisting of: diffuse large B cell lymphoma (DLBCL), non-Hodgkin's lymphoma (NHL), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and multiple sclerosis (MS).

1539. The ~~method, use or multi-specific binding compound of any one of claims 1235-37~~, wherein the disorder is B-cell chronic lymphocytic leukemia~~wherein the disorder is non-Hodgkin's lymphoma (NHL).~~

1640. The ~~method, use or multi-specific binding compound of any one of claims 1235-37~~, wherein the disorder is follicular lymphoma~~wherein the disorder is systemic lupus erythematosus (SLE).~~

~~41. — The method, use or multi-specific binding compound of any one of claims 35-37, wherein the disorder is rheumatoid arthritis (RA).~~

~~42. — The method, use or multi-specific binding compound of any one of claims 35-37, wherein the disorder is multiple sclerosis (MS).~~

4317. A polynucleotide encoding a multi-specific ~~antibody~~binding compound of any one of claims 1 to 1033.

1844. A vector comprising the polynucleotide of claim 1743.

1945. A cell comprising the vector of claim 1844.

~~46. — A method of producing a multi-specific binding compound of any of claims 1 to 33, comprising growing a cell according to claim 45 under conditions permissive for expression of the binding compound, and isolating the binding compound from the cell.~~

~~47. — A method of making a multi-specific binding compound of any one of claims 1 to 33, comprising immunizing a UniRat animal with CD22 and identifying CD22-binding heavy chain sequences.~~

~~48. — A method of treatment, comprising administering to an individual an effective dose of the multi-specific binding compound of any one of claims 1 to 33, or the pharmaceutical composition of claim 34.~~

20. The multispecific binding antibody of claim 6, wherein the light chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 92.

21. The multispecific binding antibody of claim 20, wherein the human VH framework of the heavy chain variable region having binding affinity to CD3 comprises an amino acid sequence set forth in SEQ ID NO: 98.

22. The multispecific binding antibody of claim 20, wherein the human VH framework of the heavy chain variable region having binding affinity to CD3 comprises an amino acid sequence set forth in SEQ ID NO: 99.

23. The multispecific binding antibody of claim 20, wherein the human VH framework of the heavy chain variable region having binding affinity to CD3 comprises an amino acid sequence set forth in SEQ ID NO: 100.

24. The multispecific binding antibody of claim 20, wherein the human VH framework of the heavy chain variable region having binding affinity to CD3 comprises an amino acid sequence set forth in SEQ ID NO: 101.