CD19 BINDING MOLECULES AND USES THEREOF

1. CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority benefit of U.S. provisional application nos. 63/110,501, filed November 6, 2020, 63/114,371, filed November 16, 2020, 63/147,501, filed February 9, 2021 and 63/110, 490 filed November 6, 2020, the contents of each of which are incorporated herein in their entireties by reference thereto.

2. SEQUENCE LISTING

[0002] 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 November 3, 2021, is named NOV-014WO_SL.txt and is 768,034 bytes in size.

3. FIELD OF INVENTION

[0003] The disclosure generally relates to CD19 binding molecules that specifically bind to CD19, including monospecific, bispecific and trispecific binding molecules, and their use for treating diseases and disorders associated with expression of CD19.

4. BACKGROUND

[0004] B cells express a wide array of cell surface molecules during their differentiation and proliferation. CD19 is a pan-B cell membrane glycoprotein that is expressed from early stages of pre-B cell development through terminal differentiation, regulating B lymphocyte development and function. Expression of CD19 was identified on most cancers of lymphoid origin, on the vast majority of Non-Hodgkin lymphoma (NHL) and on leukemias, including Chronic Lymphocytic Leukemia (CLL), Acute Lymphoblastic Leukemia (ALL) and Waldenstrom's Macroglobulinemia (WM).

[0005] Blinatumomab, a CD19-CD3 bispecific T cell engager, is approved for the treatment of the treatment of ALL. However, treatment with blinatumomab lacks a durable response and is characterized by a high relapse rate. Von Stackelberg *et al.*, 2016, Journal of Clinical Oncology 34(36):4381-4389. Moreover, blinatumomab has a short half-life, which requires continuous exposure for the drug to exert sufficient efficacy and manageable toxicity. Porter *et al.*, 2013, Clin Pharmacol. 5(Suppl 1): 5–11.

[0006] Despite major improvements in cancer therapy, B cell malignancies, such as the B cell subtypes of non-Hodgkin's lymphomas, and chronic lymphocytic leukemia, are major

contributors of cancer-related deaths. Accordingly, there is still a need for further therapeutic agents for the treatment of B cell malignancies.

5. SUMMARY

[0007] The disclosure provides CD19 binding molecules that specifically bind to human CD19, *e.g.*, antibodies, antigen-binding fragments thereof, and multispecific molecules that specifically bind to human CD19. The CD19 binding molecules of the dislcosure typically comprise a Fc domain comprising a first variant human IgG1 Fc region and a second variant human IgG1 Fc region having L234A, L235A, and G237A ("LALAGA") substitutions, L234A, L235A, S267K, and P329A ("LALASKPA") substitutions, D265A, P329A, and S267K ("DAPASK") substitutions, G237A, D265A, and P329A ("GADAPA") substitutions, G237A, D265A, P329A, and S267K ("GADAPASK") substitutions, L234A, L235A, and P329G ("LALAPG") substitutions, or L234A, L235A, and P329A ("LALAPA") substitutions.

[0008] In one aspect, the disclosure provides monospecific CD19 binding molecules (*e.g.*, antibodies and antigen-binding fragments thereof) comprising a CD19 antigen-binding domain or antigen-binding module ("ABM"). Exemplary CD19 binding molecules, which can be monospecific, are described in Section 7.2 and specific embodiments 1 to 15, *infra*.

[0009] In another aspect, the disclosure provides multispecific binding molecules ("MBMs") comprising the CD19 ABMs of the disclosure.

[0010] In certain embodiments, the MBMs are bispecific binding molecules ("BBMs"). The BBMs of the disclosure comprise a first ABM that specifically binds to human CD19 ("ABM1" or "CD19 ABM") and a second ABM that specifically binds to a second antigen ("ABM2"), *e.g.*, human CD3 or other component of a T cell receptor (TCR) complex (sometimes referred to herein as a "TCR ABM"). The terms ABM1, ABM2, CD19 ABM, and TCR ABM are used merely for convenience and are not intended to convey any particular configuration of a BBM. In some embodiments, a TCR ABM binds to CD3 (referred to herein a "CD3 ABM" or the like). Accordingly, disclosures relating to ABM2 and TCR ABMs are also applicable to CD3 ABMs. Such multispecific molecules can be used to direct CD3+ effector T cells to CD19+ sites, thereby allowing the CD3+ effector T cells to attack and lyse the CD19+ cells and tumors. Features of exemplary MBMs are described in Sections 7.5 to 7.6 and specific embodiments 16 to 783, *infra*.

[0011] The present disclosure also extends the principles of redirected targeted T-cell lysis (RTCC) by providing trispecific binding molecules ("TBMs") that engage CD19, CD3 or other component of a TCR complex on T-cells, and either CD2 or a human tumor-associated antigen ("TAA"), for example a B cell antigen other than CD19. The TBMs of the disclosure comprise at least three antigen-binding modules ("ABMs") that can bind (i) CD19 (ABM1), (ii) a component

of a TCR complex (ABM2), and (iii) either CD2 or a TAA (ABM3). TBMs that bind to (1) human CD19, (2) CD3 or other component of a TCR complex, and (3) CD2 are referred to herein as "Type 1 TBMs" for convenience. TBMs that bind to (1) human CD19, (2) CD3 or other component of a TCR complex, and (3) a TAA are referred to herein as "Type 2 TBMs" for convenience.

[0012] Without being bound by theory, the inventors believe that combining CD2- and TCR complex-engagement in a Type 1 TBM can stimulate both a primary signaling pathway that promotes T-cell mediated lysis of tumor cells (by clustering TCRs, for example) and a second co-stimulatory pathway to induce T-cell proliferation and potentially overcome anergy. Also without being bound by theory, it is believed that engaging a TAA in addition to CD19 and a component of a TCR complex a Type 2 TBM will improve the clinical outcomes of RTCC therapy of cancer, *e.g.*, B cell malignancies, by targeting a greater number of cancerous B cells than using bispecific engagers that target only a CD19 and a TCR complex component.

[0013] Accordingly, in one aspect, the present disclosure provides Type 1 TBMs that bind to (1) human CD19, (2) CD3 or other component of a TCR complex, and (3) CD2.

[0014] In another aspect, the present disclosure provides Type 2 TBMs that bind to (1) human CD19, (2) CD3 or other component of a TCR complex, and (3) a TAA.

[0015] Unless expressly indicated otherwise or unless the context dictates otherwise, a reference to TBMs in the present disclosure applies to both Type 1 and Type 2 TBMs.

[0016] In some embodiments, each antigen-binding module of a MBM of the disclosure is capable of binding its respective target at the same time as each of the one or more additional antigen-binding modules is bound to its respective target. ABM1 is immunoglobulin based, while ABM2 and, when present, ABM3 can be immunoglobulin- or non-immunoglobulin-based. Therefore the MBMs can include immunoglobulin-based ABMs or any combination of immunoglobulin- and non-immunoglobulin-based ABMs. Immunoglobulin-based ABMs that can be used in the MBMs are described in Section 7.3.1 and specific embodiments 17 to 21, 24 to 29, *infra*. Non-immunoglobulin-based ABMs that can be used in the MBMs are described in Section 7.3.2 and specific embodiments 22 to 23, *infra*. Further features of exemplary ABMs that bind to human CD19 are described in Section 7.2 and specific embodiments 17 to 21, *infra*. Further features of exemplary ABMs that bind to a component of a TCR complex are described in Section 7.7 and specific embodiments 30 to 223, *infra*. Further features of exemplary ABMs that bind to CD2 are described in Section 7.8 and specific embodiments 325 to 374, *infra*. Further features of exemplary ABMs that bind to TAAs are described in Section 7.9 and specific embodiments 375 to 493, *infra*.

[0017] The ABMs of a MBM (or portions thereof) can be connected to each other, for example, by short peptide linkers or by an Fc domain. Methods and components for connecting ABMs to form a MBM are described in Section 7.4 and specific embodiments 497 to 770, *infra*.

[0018] BBMs have at least two ABMs (*e.g.*, a BBM is at least bivalent) and TBMs have at least three ABMs (*e.g.*, a TBM is at least trivalent), but they can have greater valencies. For example, a BBM can have three, four or more ABMs (*i.e.*, is trivalent, tetravalent, or has a valency that is greater than tetravalent). Exemplary bivalent, trivalent, and tetravalent BBM configurations are shown in FIG. 1 and described in Section 7.5 and specific embodiments 226 to 286, *infra*.

[0019] A TBM can have four ABMs (*i.e.*, is tetravalent), five ABMs (*i.e.*, is pentavalent), or six ABMs (*i.e.*, is hexavalent), provided that the TBM has at least one ABM that can bind CD19, at least one ABM that can bind a component of a TCR complex, and at least one ABM that can bind either CD2 or a TAA. Exemplary trivalent, tetravalent, pentavalent, and hexavalent TBM configurations are shown in FIG. 2 and described in Section 7.6 and specific embodiments 289 to 323, *infra*.

[0020] The disclosure further provides nucleic acids encoding the CD19 binding molecules (either in a single nucleic acid or a plurality of nucleic acids) and recombinant host cells and cell lines engineered to express the nucleic acids and CD19 binding molecules of the disclosure. Exemplary nucleic acids, host cells, and cell lines are described in Section 7.10 and specific embodiments 885 to 892, *infra*.

[0021] The present disclosure further provides drug conjugates comprising the CD19 binding molecules of the disclosure. Such conjugates are referred to herein as "antibody-drug conjugates" or "ADCs" for convenience, notwithstanding that some of the ABMs can be non-immunoglobulin domains. Examples of ADCs are described in Section 7.12 and specific embodiments 784 to 822, *infra*. Pharmaceutical compositions comprising the CD19 binding molecules are also provided. Examples of pharmaceutical compositions are described in Section 7.15 and specific embodiment 823, *infra*.

[0022] Further provided herein are methods of using the CD19 binding molecules, the ADCs, and the pharmaceutical compositions of the disclosure, for example for treating proliferative conditions (*e.g.*, cancers), on which CD19 is expressed, for treating autoimmune disorders, and for treating other diseases and conditions associated with expression of CD19. In some embodiments, the cancer is B cell malignancy (*e.g.*, a NHL such as DLBCL or MCL). In some embodiments, the CD19 binding molecules, the ADCs, and the pharmaceutical compositions of the disclosure are administered to a subject who has a NHL, for example DLBCL or MCL, and (i) has failed at least one prior line (and optionally up to five prior lines) of standard of care

therapy, *e.g.*, an anti-CD20 therapy such as rituximab and/or (ii) is intolerant to or ineligible for one or more other approved therapies, *e.g.,* autologous stem cell transplant (ASCT) and/or (iii) is a non-responder to a chimeric antigen receptor (CAR) T cell therapy. The NHL can be relapsed and/or refractory. Further exemplary methods are described in Section 7.16 and specific embodiments 824 to 881, *infra*.

[0023] The disclosure further provides methods of using the CD19 binding molecules, the ADCs, and the pharmaceutical compositions in combination with other agents and therapies. Exemplary agents, therapies, and methods of combination therapy are described in Section 7.17 and specific embodiment 881, *infra*.

[0024] Provided herein is a CD19 binding molecule that specifically binds to human CD19 and comprises (a) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:14, SEQ ID NO:15, and SEQ ID NO:16; and (b) a first variant human IgG1 Fc region and a second variant human IgG1 Fc region forming a Fc domain, wherein the first and second variant Fc regions comprise L234A, L235A, and G237A ("LALAGA") substitutions, L234A, L235A, S267K, and P329A ("LALASKPA") substitutions, D265A, P329A, and S267K ("DAPASK") substitutions, G237A, D265A, and P329A ("GADAPA") substitutions, G237A, D265A, P329A, and S267K ("GADAPASK") substitutions, L234A, L235A, and P329G ("LALAPG") substitutions, or L234A, L235A, and P329A ("LALAPA") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

[0025] Provided herein is a CD19 binding molecule that specifically binds to human CD19 and comprises (a) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:17, SEQ ID NO:18, and SEQ ID NO:19; and (b) a first variant human IgG1 Fc region and a second variant human IgG1 Fc region forming a Fc domain, wherein the first and second variant Fc regions comprise L234A, L235A, and G237A ("LALAGA") substitutions, L234A, L235A, S267K, and P329A ("LALASKPA") substitutions, D265A, P329A, and S267K ("DAPASK") substitutions, G237A, D265A, and P329A ("GADAPA") substitutions, G237A, D265A, P329A, and S267K ("GADAPASK") substitutions, L234A, L235A, and P329G ("LALAPG") substitutions, or L234A, L235A, and P329A ("LALAPA") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

[0026] Provided herein is a CD19 binding molecule that specifically binds to human CD19 and comprises (a) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID

NO:7, SEQ ID NO:8, and SEQ ID NO:9, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:20, SEQ ID NO:21, and SEQ ID NO:22; and (b) a first variant human IgG1 Fc region and a second variant human IgG1 Fc region forming a Fc domain, wherein the first and second variant Fc regions comprise L234A, L235A, and G237A ("LALAGA") substitutions, L234A, L235A, S267K, and P329A ("LALASKPA") substitutions, D265A, P329A, and S267K ("DAPASK") substitutions, G237A, D265A, and P329A ("GADAPA") substitutions, G237A, D265A, P329A, and S267K ("GADAPASK") substitutions, L234A, L235A, and P329G ("LALAPG") substitutions, or L234A, L235A, and P329A ("LALAPA") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

[0027] Provided herein is a CD19 binding molecule that specifically binds to human CD19 and comprises (a) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:23, SEQ ID NO:24, and SEQ ID NO:25; and a first human IgG1 variant Fc region and a second human IgG1 variant Fc region forming a Fc domain, wherein the first and second variant Fc regions comprise L234A, L235A, and G237A ("LALAGA") substitutions, L234A, L235A, S267K, and P329A ("LALASKPA") substitutions, D265A, P329A, and S267K ("DAPASK") substitutions, G237A, D265A, and P329A ("GADAPA") substitutions, G237A, D265A, P329A, and S267K ("GADAPASK") substitutions, L234A, L235A, and P329G ("LALAPG") substitutions, or L234A, L235A, and P329A ("LALAPA") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

[0028] In some embodiments, the CD19 binding molecule can comprise a VH having the amino acid sequence of SEQ ID NO:13. The CD19 binding molecule can also comprise a VL having the amino acid sequence of SEQ ID NO:26.

[0029] In some embodiments, the CD19 binding molecule is a multispecific binding molecule (MBM) comprising (a) an antigen-binding module 1 (ABM1) that binds specifically to CD19; and (b) an antigen-binding module 2 (ABM2) that binds specifically to a different target molecule. In some embodiments, the target molecule is a component of a human T-cell receptor (TCR) complex.

[0030] In some embodiments, the CD19 binding molecule has an ABM1 that is an antibody, an antibody fragment, an scFv, a dsFv, a Fv, a Fab, an scFab, a (Fab')2, a single domain antibody (SDAB), a VH or VL domain, or a camelid VHH domain. For example, in some embodiments, the ABM1 is a Fab. In other embodiments, the ABM1 is an anti-CD19 antibody or an antigen-binding domain thereof.

[0031] In some embodiments, the CD19 binding molecule has an ABM2 that is an immunoglobulin scaffold based ABM. For example, the ABM2 can be an antibody, an antibody fragment, an scFv, a dsFv, a Fv, a Fab, an scFab, a (Fab')2, a single domain antibody (SDAB), a VH or VL domain, or a camelid VHH domain. As a specific example, the ABM2 is an scFv. In some embodiments, the ABM2 can bind specifically to a component of a human T-cell receptor (TCR) complex. For example, the component of the TCR complex can be CD3. In some embodiments, the ABM2 is an anti-CD3 antibody or an antigen-binding domain thereof. Should the CD19 binding molecule bind to CD3, the ABM2 can comprise the CDR sequences of any one of CD3-1 to CD3-130. More specifically, the ABM2 can comprise the CDR sequences of CD3-21.

[0032] In some embodiments, the CDR sequences of the CD3 binding portion of the binding molecule as described herein can be defined by Kabat numbering, as set forth in Table 12B. The CDR sequences of the CD3 binding portion of the binding molecule as described herein can also be defined by Chothia numbering, as set forth in Table 12C. The CDR sequences of the CD3 binding portion of the binding molecule as described herein can also be defined by a combination of Kabat and Chothia numbering, as set forth in Table 12D.

[0033] In some embodiments, the CD19 binding molecule can have an ABM2 that comprises the heavy and light chain variable sequences of CD3-21, as set forth in Table 12A.

[0034] More specifically, the CD19 binding molecule can be a trispecific binding molecule (TBM) comprising an antigen-binding module 3 (ABM3) that binds specifically to a target molecule other than CD19.

[0035] In some embodiments, the CD19 binding molecule can have an ABM2 that binds specifically to a component of a human T-cell receptor (TCR) complex and ABM3 binds specifically to human CD2. In some embodiments, the CD19 binding molecule is trivalent. The CD19 binding molecule can have any one of the configurations depicted in FIGS. 2A-2P. For example, the CD19 binding molecule has the configuration depicted in FIG. 2I. In some embodiments, the CD19 binding molecule has the configuration referred to as T2 in Section 7.6.1.

[0036] In a specific embodiment, the CD19 binding molecule has an ABM3 that binds specifically to human CD2. In some embodiments, the ABM3 is a non-immunoglobulin scaffold based ABM. Where the ABM3 binds to human CD2, the ABM3 can comprise a receptor binding domain of a CD2 ligand. For example, the ABM3 can be a CD58 moiety. In some embodiments, the CD58 moiety comprises the amino acid sequence of CD58-6 as set forth in Table 15.

[0037] The CD19 binding molecule can have modifications in its Fc region. For example, the first variant Fc region and the second variant Fc region can comprise L234A, L235A, and G237A ("LALAGA") substitutions, where the amino acid residues are numbered according to the EU numbering system. In another embodiment, the first variant Fc region and the second variant Fc region can comprise L234A, L235A, S267K, and P329A ("LALASKPA") substitutions, where the amino acid residues are numbered according to the EU numbering system. In still another embodiment, the first variant Fc region and the second variant Fc region can comprise D265A, P329A, and S267K ("DAPASK") substitutions, where the amino acid residues are numbered according to the EU numbering system. In other embodiments, the first variant Fc region and the second variant Fc region can comprise G237A, D265A, and P329A ("GADAPA") substitutions, where the amino acid residues are numbered according to the EU numbering system. In another embodiment, the first variant Fc region and the second variant Fc region can comprise G237A, D265A, P329A, and S267K ("GADAPASK") substitutions, where the amino acid residues are numbered according to the EU numbering system. In another embodiment, the first variant Fc region and the second variant Fc region can comprise L234A, L235A, and P329G ("LALAPG") substitutions, where the amino acid residues are numbered according to the EU numbering system. In another embodiment, the first variant Fc region and the second variant Fc region can comprise L234A, L235A, and P329A ("LALAPA") substitutions, where the amino acid residues are numbered according to the EU numbering system.

[0038] The CD19 binding molecule's Fc regions can have a first variant Fc region and the second variant Fc region, which together form an Fc heterodimer. For example, the Fc heterodimer can comprise knob-in-hole ("KIH") modifications. In some embodiments, the first and second variant Fc regions can comprise the amino acid substitutions T366W : T366S/L368A/Y407V.

[0039] In a specific example, the CD19 binding molecule is trispecific binding molecule (TBM) comprising (a) an antigen-binding module 1 (ABM1) that binds specifically to CD19 and comprises CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:17, SEQ ID NO:18, and SEQ ID NO:19; (b) an antigen-binding module 2 (ABM2) that binds specifically to a component of a human T-cell receptor (TCR) complex; (c) an antigen-binding module 3 (ABM3) that binds specifically to human CD2; and (d) a first variant human IgG1 Fc region and a second variant human IgG1 Fc region forming a Fc domain, where the first and second variant Fc regions comprise L234A, L235A, and G237A ("LALAGA") substitutions, L234A, L235A, S267K, and P329A ("LALASKPA") substitutions, D265A, P329A, and S267K ("DAPASK") substitutions, G237A, D265A, and P329A

("GADAPA") substitutions, G237A, D265A, P329A, and S267K ("GADAPASK") substitutions, L234A, L235A, and P329G ("LALAPG") substitutions, or L234A, L235A, and P329A ("LALAPA") substitutions, where the amino acid residues are numbered according to the EU numbering system. In this specific example, the CD19 binding molecule is trivalent. Additionally, the ABM1 is a Fab. In this specific example, the Fab comprises a VH having the amino acid sequence of SEQ ID NO:13 and a VL having the amino acid sequence of SEQ ID NO:26. Further, the CD19 binding molecule can bind to a component of the TCR complex that is CD3. In this example, the ABM2 is an anti-CD3 antibody or an antigen-binding domain thereof. The sequences of the ABM2 comprises the CDR sequences of CD3-21. For example, the ABM2 comprises the heavy and light chain variable sequences of CD3-21, as set forth in Table 12A. The anti-CD3 antibody or antigen-binding domain thereof is in the form of a scFv in this example. Further, the ABM2 comprises the amino acid sequence of the scFv designated as CD3-21 in Table 12A. Regarding the ABM3 arm in this example, ABM3 is a CD58 moiety. More specifically, the ABM3 comprises the amino acid sequence of CD58-6 as set forth in Table 15.

[0040] In another specific example, the CD19 binding molecule is a trispecific binding molecule (TBM) comprising (a) an antigen-binding module 1 (ABM1) that binds specifically to CD19 and which is a Fab comprising CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:17, SEQ ID NO:18, and SEQ ID NO:19; (b) an antigen-binding module 2 (ABM2) that binds specifically to CD3 and which comprises the amino acid sequence of the scFv designated as CD3-21 in Table 12A; (c) an antigen-binding module 3 (ABM3) that binds specifically to human CD2 and which comprises the amino acid sequence of CD58-6 as set forth in Table 15; and (d) a first variant human IgG1 Fc region and a second variant human IgG1 Fc region forming a Fc domain, where the first and second variant Fc regions comprise L234A, L235A, and G237A ("LALAGA") substitutions, L234A, L235A, S267K, and P329A ("LALASKPA") substitutions, D265A, P329A, and S267K ("DAPASK") substitutions, G237A, D265A, and P329A ("GADAPA") substitutions, G237A, D265A, P329A, and S267K ("GADAPASK") substitutions, L234A, L235A, and P329G ("LALAPG") substitutions, or L234A, L235A, and P329A ("LALAPA") substitutions, where the amino acid residues are numbered according to the EU numbering system. In this example, the ABM1 comprises a VH having the amino acid sequence of SEQ ID NO:13 and a VL having the amino acid sequence of SEQ ID NO:26. Further, the CD19 binding molecule has the configuration depicted in FIG. 2I and referred to as T2 in Section 7.6.1.

[0041] The CD19 binding molecules described throughout can be made into a pharmaceutical composition. For example, the pharmaceutical composition comprises (a) any one of the CD19

binding molecules or conjugates described throughout the specification and (b) an excipient. Further, the CD19 binding molecules or conjugates described throughout the specification can be used in a combination therapy. For example, described is a combination comprising the CD19 binding molecule described throughout and at least one additional therapeutic agent. The one or more additional therapeutic agents can comprise an immunomodulatory imide drug (IMiD). For example, the IMiD can be lenalidomide, thalidomide, pomalidomide, or iberdomide. In a specific example, the IMiD is lenalidomide.

[0042] The CD19 binding molecule (or composition or combination) described throughout can be used as a medicament. The CD19 binding molecule (or composition or combination) described throughout can also be used to treat a subject in need thereof. For example, the CD19 binding molecule (or composition or combination) can be used in a method of treating a subject with a CD19-associated disease or disorder, comprising administering to the subject an effective amount of the CD19 binding molecule described throughout. The conjugate described throughout can also be used to treat the subject in need thereof. In some embodiments, the pharmaceutical composition described throughout can be used in a method of treating a subject with a CD19-associated disease or disorder, comprising administering to the subject an effective amount of the pharmaceutical composition (or combination) described throughout. The CD19-associated disease or disorder can be diffuse large B-cell lymphoma (DLBCL). More specifically, the DLBCL can be relapsed or refractory DLBCL. The CD19-associated disease or disorder can be Acute Lymphoblastic Leukemia (ALL). More specifically, the ALL can be relapsed or refractory ALL.

[0043] The CD19 binding molecules described throughout can be encoded by a nucleic acid or a plurality of nucleic acids.

[0044] A cell can be engineered to express the CD19 binding molecules described throughout. These cells can be used in a method of producing the CD19 binding molecule. For example, described is a method of producing a CD19 binding molecule (as described throughout) comprising (a) culturing the cell engineered to express the CD19 binding molecules described throughout in conditions under which the CD19 binding molecule is expressed; and (b) recovering the CD19-binding molecule from the cell culture.

6. BRIEF DESCRIPTION OF THE FIGURES

[0045] FIGS. 1A-1AH: Exemplary BBM configurations. FIG. 1A illustrates components of the exemplary BBM configurations illustrated in FIGS. 1B-1AH. Not all regions connecting the different domains of each chain are illustrated (*e.g.*, the linker connecting the VH and VL domains of an scFv, the hinge connecting the CH2 and CH3 domains of an Fc domain, *etc.*, are

omitted). FIGS. 1B-1F illustrate bivalent BBMs; FIGS. 1G-1Z illustrate trivalent BBMs; FIGS. 1AA-1AH illustrate tetravalent BBMs.

[0046] FIGS. 2A-2V: Exemplary TBM configurations. FIG. 2A illustrates components of the exemplary TBM configurations illustrated in FIGS. 2B-2V. Not all regions connecting the different domains of each chain are illustrated (*e.g.*, the linker connecting the VH and VL domains of an scFv, the hinge connecting the CH2 and CH3 domains of an Fc, *etc.*, are omitted). FIG. 2B-2P illustrates trivalent TBMs; FIGS. 2Q-2S illustrate tetravalent TBMs; FIG. 2T illustrates a pentavalent TBM, and FIGS. 2U-2V illustrate hexavalent TBMs.

[0047] FIGS. 3A-3C: Schematics of the bispecific (FIG. 3A and FIG. 3C) and trispecific (FIG. 3B) constructs of Example 1.

[0048] FIGS. 4A-4B: Ability of CD19 BBMs to elicit redirected T-cell cytotoxic activity (RTCC) against CD19+ target cells. Both NEG258-based and NEG218-based BBMs mediated RTCC activity against CD19+ target cell lines. Nalm6-luc (FIG. 4A) and Karpas422-luc (FIG. 4B) cells were co-cultured with expanded T cells in the presence of serial diluted BBMs at an effector cell: target cell (E:T) ratio of 3:1. Luminescence signal was measured after 24h of incubation.

[0049] FIGS. 5A-5B: Ability of CD19 BBMs to elicit T-cell proliferation. Both NEG258-based and NEG218-based BBMs induced T cell proliferation. Karpas422-luc (FIG. 5A) and Nalm6-luc (FIG. 5B)cells were co-cultured with expanded T cells in the presence of serial diluted BBMs at an E:T ratio of 1:1. Luminescence signal was measured after 96h of incubation.

[0050] FIGS. 6A-6F: Ability of CD19 TBMs to elicit CD2 dependent T cell activation. CD2 knock out attenuated advantage of trispecific constructs. FIGS. 6A-6B show representative flow cytometry analysis of CD2 expression on JNL CD2 WT (FIG. 6A) and KO (FIG. 6B) cells. Staining by the anti-CD2 mAb (dot filled histogram) is overlaid with that of the mIgG1 isotype control (diagonal line filled histogram) or unstained (open histogram). FIGS. 6C-6F show data for JNL $CD2^+$ (FIG. 6C-6D) and $CD2^-$ (FIG. 6E-6F) cells co-cultured with $CD19^+$ target cells in the presence of serial diluted BBMs and TBMs at an E:T ratio of 3:1. Luminescence signal was measured after 24h of incubation.

[0051] FIGS. 7A-7B: Binding of CD19 TBMs to cyno B cells. FIG. 7A shows data for a TBM with a NEG218-based CD19 binding arm and FIG. 7B shows data for a TBM with a NEG258-based CD19 binding arm.

[0052] FIGS. 8A-8H: Ability of CD19 TBMs to induce T cell activation upon cyno B cell depletion in PBMCs. In FIG. 8A, PBMCs were isolated from cyno monkey whole blood using ficoll gradient centrifugation and were incubated with bi or trispecific constructs for overnight. Samples were harvested and simultaneously stained for CD3 and CD20 to identify B and T

cells within the PBMC population. Percentage of B cell depletion was calculated as described in Section 8.6.1. FIGS. 8B-8H show the results of FACS analysis of CD69 and CD25 expression on $CD3^+$ T cells to determine single ($CD69^+$ $CD25^-$ or $CD69^-CD25^+$) or double-positive cells ($CD69^+CD25^+$). FIG. 8B: untreated (media only); FIGS. 8C-8E: CD3hi TSP1L; FIGS. 8F-8H: CD3hi TSP1.

[0053] FIGS. 9A-9P: Ability of NEG258- and NEG218-based TBMs to induce redirected T cell cytotoxicity by human donor cells against Nalm6 (FIGS. 9A-9H) and Karpas422 (FIGS. 9I-9P) target cells.

[0054] FIGS. 10A-10P: Ability of NEG258- and NEG218-based TBMs with different CD3 affinities to induce redirected T cell cytotoxicity by human donor cells against Nalm6 (FIGS. 10A-10H) and Karpas422 (FIGS. 10I-10P) target cells.

[0055] FIGS. 11A-11L: Ability of NEG258-based TBMs that include a CD2-binding arm and those that include a control lysozyme binding arm to induce redirected T cell cytotoxicity by human donor cells against Nalm6 (FIGS. 11A-11H) and Karpas422 (FIGS. 11I-11L) target cells.

[0056] FIGS. 12A-12C: Induction of T cell cytokine release by NEG258- and NEG218-based TBMs. FIG. 12A: IFN-γ; FIG. 12B: TNF-α; FIG. 12C: IL2.

[0057] FIGS. 13A-13C: Binding of NEG258- and NEG218-based TBMs to murine 300.19 cell lines that overexpress human CD19 (FIG. 13A) or cyno CD19 (FIG. 13B). The TBMs show negligible binding to the wild type 300.19 cell line (FIG. 13C).

[0058] FIG. 14: A schematic representation of CD58.

[0059] FIG. 15: Redirected T cell cytotoxicity by TBMs containing CD58 variant sequences.

[0060] FIG. 16: Antigen-independent T-cell activation by TBMs containing CD58 variant sequences. Data expressed as relative luminescence units (RLU).

[0061] FIGS. 17A-17H: CD19 and CD58 expression on various cell lines: FIGS. 17A-17B: CD19 and CD58 expression, respectively, on OCI-LY-19 cells; FIGS. 17C-17D: CD19 and CD58 expression, respectively, on Karpas-422 cells; FIGS. 17E-17F: CD19 and CD58 expression, respectively, on Toledo cells; FIGS. 17G-17H: CD19 and CD58 expression, respectively, on Nalm-6 cells.

[0062] FIGS. 18A-18B: Ability of NEG258-based TBMs and BBM to induce redirected T cell cytotoxicity by human donor cells against Karpas422 target cells. FIG. 18A and FIG. 18B show data using T cells from two different donors.

[0063] FIGS. 19A-19F: Induction of T cell cytokine release by NEG258-based TBMs and BBM. FIGS. 19A-19B: IFN-γ (donor 1 and donor 2, respectively); FIGS. 19C-19D: IL-2 (donor 1 and

donor 2, respectively); FIGS. 19E-19F: TNF-α (donor 1 and donor 2, respectively). Triangles on X-axis indicate decreasing concentration of constructs from left to right in the figures.

[0064] FIG. 20: NEG258-based TBM and BBM binding to T cells.

[0065] FIGS. 21A-21C: NEG258-based TBM and BBM mediated T cell proliferation. FIG. 21A: T cell proliferation in OC-LY-19 co-culture; FIG. 21B: T cell proliferation in Karpas422 co-culture; FIG. 21C: T cell proliferation in Toledo co-culture.

[0066] FIGS. 22A-22B: Ability of NEG258-based TBMs and BBM to induce redirected T cell cytotoxicity by human donor cells against Karpas422 target cells. FIG. 22A and FIG. 22B show data using T cells from two different donors.

[0067] FIGS. 23A-23J: Ability of NEG258-based TBMs and BBM to induce redirected T cell cytotoxicity by human donor cells against various target cells. FIGS. 23A-23B: OC-LY-19 (donor 1 and donor 2, respectively); FIGS. 23C-23D: Toledo (donor 1 and donor 2, respectively); FIGS. 23E-23F: Nalm6 (donor 1 and donor 2, respectively); FIGS. 23G-23H: Nalm6 KO (donor 1 and donor 2, respectively); FIGS. 23I-23J: K562 (donor 1 and donor 2, respectively).

[0068] FIGS. 24A-24J: Induction of T cell cytokine release by NEG258-based TBMs and BBM in various target cells. FIGS. 24A-24B: TNF-α from OC-LY-19 (donor 1 and donor 2, respectively); FIGS. 24C-24D: TNF-α from Toledo (donor 1 and donor 2, respectively); FIGS. 24E-24F: TNF-α from Nalm6 (donor 1 and donor 2, respectively); FIGS. 24G-24H: TNF-α from Nalm6 KO (donor 1 and donor 2, respectively); FIGS. 24I-24J: TNF-α from K562 (donor 1 and donor 2, respectively).

[0069] FIGS. 25A-25H: Re-challenge RTCC assay with Karpas 422 and OCI-LY-19 cell lines. FIG. 25A: assay set-up. FIGS. 25B-25D: Karpas 422 (post first challenge, post second challenge, and post third challenge, respectively); FIGS. 25E-25H OCI-LY-19 (post first challenge, post second challenge, post third challenge, and post fourth challenge, respectively).

[0070] FIGS. 26A-26P: Re-challenge T cell phenotyping with Karpas 422 and OCI-LY-19 cell lines. FIGS. 26A-26H: Karpas 422 phenotyping; FIGS. 26I-26P: OCI-LY-19 phenotyping. FIGS. 26A and 26I: % IL-2+ CD4 T cells; FIGS. 26B and 26J: % IFNγ + CD4 T cells; FIGS. 26C and 26K: % IL-2+ CD8 T cells; FIGS. 26D and 26L: % IFNγ + CD8 T cells; FIGS. 26E and 26M: CD3 young; FIGS. 26F and 26N: CD4 old; FIGS. 26G and 26O: CD8 young; FIGS. 26H and 26P: CD8 old. Lines in figures represent different T cell donors.

[0071] FIGS. 27A-27D: Ability of CD3hi TSP1 vs. CD3hi BSP1 to elicit T cell proliferation in presence of CD19+ target cells. Nalm6-luc cells were co-cultured for 72h with sorted $CD28^+$ or $CD28^-$ CD8 T cells at an E:T ratio of 1:3 in the presence of 1nM (FIGS. 27A-27B) or 0.1nM

(FIGS. 27C-27D) CD3hi TSP1 or CD3hi BSP1 and in presence (FIGS. 27A and 27C) or absence (FIGS. 27B and 27D) of irradiated autologous PBMCs (T cells depleted). Proliferation was measured as percentage of CFSE-diluted cells among the live cells.

[0072] FIGS. 28A-28L: Ability of CD3hi TSP1 and CD3hi BSP1 to induce T cells' cytokines production in presence of Nalm6 CD19+ target cells (E:T 1:3). FIGS. 28A-28B: median fluorescence intensity (MFI) for GzB (FIG. 28A) and IFN-γ (FIG. 28B) producing $CD28^-$ and $CD28^+$ CD8 T cells, when co-cultured in presence of irradiated PBMCs and 1 nM CD3hi TSP1 or 1 nM CD3hi BSP1. FIGS. 28C-28D: MFI for GzB (FIG. 28C) and IFN-γ (FIG. 28D) producing $CD28^-$ and $CD28^+$ CD8 T cells, when co-cultured in absence of irradiated PBMCs and 1 nM CD3hi TSP1 or 1 nM CD3hi BSP1. FIGS. 28E-28F: MFI for GzB (FIG. 28E) and IFN-γ (FIG. 28F) producing $CD28^-$ and $CD28^+$ CD8 T cells, when co-cultured in presence of irradiated PBMCs and 0.1 nM CD3hi TSP1 or 0.1 nM CD3hi BSP1. FIGS. 28G-28H: MFI for GzB (FIG. 28G) and IFN-γ (FIG. 28H) producing $CD28^-$ and $CD28^+$ CD8 T cells, when co-cultured in absence of irradiated PBMCs and 0.1 nM CD3hi TSP1 or 0.1 nM CD3hi BSP1. FIGS. 28I-28L: proportions of live T cells, when co-cultured in the presence (FIGS. 28I and 28K) or absence (FIGS. 28J and 28L) of irradiated PBMCs and 1 nM (FIGS. 28I and 28J) or 0.1 nM (FIGS. 28K and FIGS. 28L) CD3hi TSP1 or CD3hi BSP1.

[0073] FIGS: 29A-29I: Ability of CD3hi TSP1 vs. CD3hi BSP1 to induce changes in T cell phenotype. FIG. 29A: Representative example of $CD28^-$ and $CD28^+$ T cells sorted for CCR7 and CD45RO expression. FIGS. 29B-29I : distribution of different T cell populations defined according to the combined expression of the two surface markers CD45RO and CCR7 (naive, $CD45RO^-CCR7^+$; central memory (CM), $CD45RO^+CCR7^+$; effector memory (EM), $CD45RO^+CCR7^-$; and terminally differentiated (TEMRA), $CD45RO^-CCR7^-$) following 72 hour co-culture (E:T 1:3) in the presence (FIGS. 29B-29E) or absence (FIGS. 29F-29I) of PBMCs and presence of 1 nM (FIGS. 29B-29C and 29F-29G) or 0.1 nM (FIGS. 29D-29E and 29H-29I) CD3hi TSP1 or CD3hi BSP1. Data for proliferating cells (CFSE-) are shown in FIGS. 29B, 29D, 29F, and 29H. Data for non-proliferating cells (CSFE+) are shown in FIGS. 29C, 29E, 29G, and 29I. Data for CD28- cells are shown on the left side of each figure and data for CD28+ cells are shown on the right side of the figure.

[0074] FIGS. 30A-30D: Ability of CD3hi TSP1 vs. CD3hi BSP1 to elicit redirected T-cell cytotoxic activity (RTCC) against CD19+ target cells. RTCC results from Nalm6-luc cells co-cultured for 72h with sorted $CD28^+$ or $CD28^-$ CD8 T cells at an E:T ratio of 1:3 in the presence of 1nM (FIGS. 30A and 30C) or 0.1nM (FIGS. 30B and 30D) of CD3hi BSP1, CD3hi TSP1, or CD3hi TSP1C and in the presence (FIGS. 30A and 30B) or absence (FIGS. 30C and 30D) of irradiated autologous PBMCs (T cells depleted). (n=3) Luminescence signal was measured at the end of the co-culture incubation. Results are expressed as fold increase vs. untreated

condition, where no antibodies were added in order to evaluate the background signal given by the control antibody.

[0075] FIGS. 31A-31B: Anti-tumor activity of CD3hi TSP1 (FIG. 31A) and CD3med TSP1 (FIG. 31B) in a human PBMC adoptive transfer adaptation of the OCI-LY-19 subcutaneous tumor model.

[0076] FIGS. 32A-32B: Body weight change following treatment with CD3hi TSP1 (FIG. 32A) and CD3med TSP1 (FIG. 32B) in a human PBMC adoptive transfer adaptation of the OCI-LY-19 subcutaneous tumor model.

[0077] FIG. 33: Schematic of the humanization process of a NSG mouse.

[0078] FIGS. 34A-34B: Anti-tumor activity of CD3 TSP1, CD3hi BSP1 and CD3med TSP1 in a DLBCL subcutaneous tumor model in huCD34+ NSG mice (FIG. 34A) and body weight change following treatment with CD3 TSP1, CD3hi BSP1 and CD3med TSP1 in the DLBCL subcutaneous tumor model in huCD34+ NSG mice (FIG. 34B).

[0079] FIGS. 35A-35D: Anti-tumor activity (FIGS. 35A and 35C) and body weight response (FIGS. 35B and FIGS. 35D) following antibody treatment with CD3hi TSP1 (FIGS. 35A and 35B) and CD3med TSP1 (FIGS. 35C and 35D) in a OCI-LY-19 DLBCL subcutaneous tumor model in huCD34+ NSG mice.

[0080] FIGS. 36A-36C: Anti-tumor activity of CD3hi BSP1 (FIG. 36A), CD3hi TSP1 (FIG. 36B), and CD3med TSP1 (FIG. 36C) in a human PBMC adoptive transfer adaptation of the Daudi-Luc subcutaneous tumor model.

[0081] FIGS. 37A-37C: Body weight change following antibody treatment with CD3hi BSP1 (FIG. 37A), CD3hi TSP1 (FIG. 37B), or CD3med TSP1 (FIG. 37C) in a human PBMC adoptive transfer adaptation of the Daudi-Luc subcutaneous tumor model.

[0082] FIGS. 38A-38B: Shows schematic overview of a Biacore measuring cycle.

FIGS. 39A.1-39C.11: Shows representative sensorgrams and response and concentration plots. FIG. 39A.1 to FIG. 39A.11 (collectively, "FIG. 39A") show representative sensorgrams and response plots of WT IgG1, LALAPA-IgG1, LALAGA-IgG1, LALAPG-IgG1, DAPA-IgG1, LALASKPA-IgG1, DAPASK-IgG1, GADAPA-IgG1, GADAPASK-IgG1 and DANAPA-IgG1. (Concentration range: 0.2nM-100nM for human FcγR1A); FIG. 39B.1 to FIG. B.11 (collectively, "FIG. 39B") show sensorgrams and binding kinetics of WT, LALAPA-IgG1, LALAGA-IgG1, LALAPG-IgG1, DAPA-IgG1, LALASKPA-IgG1, DAPASK-IgG1, GADAPA-IgG1, GADAPASK-IgG1 and DANAPA-IgG1 towards FcgammaR3A V158 (Concentration range: 1.95nM-1000nM for human FcγR3A V158); FIG. 39C.1 to FIG. 39C.11 ("collectively, "FIG. 39C") show sensorgrams and binding kinetics of WT, LALAPA-IgG1, LALAGA-IgG1, LALAPG-IgG1, DAPA-

IgG1, LALASKPA-IgG1, DAPASK-IgG1, GADAPA-IgG1, GADAPASK-IgG1 and DANAPA-IgG1 towards C1q. (Concentration range: 0.49nM-250nM for human C1q)

[0083] FIGS. 40A-40B: FIG. 40A shows the nuclear factor of activated T-cells (NFAT) pathway activity of the wild type and mutated antibodies. FIG. 40B shows the NFAT pathway activity of the wild type and mutated antibodies, cells sensitized with addition of INFgamma.

FIGS. 41A-41E: Shows representative sensorgrams and response plots of WT, DANAPA, GADAPASK, LALA and LALASKPA variants. (Concentration range: 0.2nM-25nM for human FcγR1A)

[0084] FIG. 42: Shows the nuclear factor of activated T-cells (NFAT) pathway activity of the wild type and mutated antibodies.

7. DETAILED DESCRIPTION

7.1. Definitions

[0085] As used herein, the following terms are intended to have the following meanings:

[0086] ABM chain: Individual ABMs can exist as one (*e.g.*, in the case of an scFv) polypeptide chain or form through the association of more than one polypeptide chains (*e.g.*, in the case of a Fab). As used herein, the term "ABM chain" refers to all or a portion of an ABM that exists on a single polypeptide chain. The use of the term "ABM chain" is intended for convenience and descriptive purposes only and does not connote a particular configuration or method of production.

[0087] ADCC: By "ADCC" or "antibody dependent cell-mediated cytotoxicity" as used herein is meant the cell-mediated reaction where nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause lysis of the target cell. ADCC is correlated with binding to FcγRIIIa; increased binding to FcγRIIIa leads to an increase in ADCC activity.

[0088] ADCP: By "ADCP" or antibody dependent cell-mediated phagocytosis as used herein is meant the cell-mediated reaction where nonspecific phagocytic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause phagocytosis of the target cell.

[0089] Additional Agent: For convenience, an agent that is used in combination with an antigen-binding molecule of the disclosure is referred to herein as an "additional" agent.

[0090] Antibody: The term "antibody" as used herein refers to a polypeptide (or set of polypeptides) of the immunoglobulin family that is capable of binding an antigen non-covalently, reversibly and specifically. For example, a naturally occurring "antibody" of the IgG type is a tetramer comprising at least two heavy (H) chains and two light (L) chains inter-connected by

disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as VL) and a light chain constant region. The light chain constant region is comprised of one domain (abbreviated herein as CL). The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies can mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and components of the classical complement system. The term "antibody" includes, but is not limited to, monoclonal antibodies, human antibodies, humanized antibodies, camelised antibodies, chimeric antibodies, bispecific or multispecific antibodies and anti-idiotypic (anti-Id) antibodies (including, *e.g.*, anti-Id antibodies to antibodies of the disclosure).

[0091] Both the light and heavy chains are divided into regions of structural and functional homology. The terms "constant" and "variable" are used functionally. In this regard, it will be appreciated that the variable domains of both the light (VL) and heavy (VH) chain portions determine antigen recognition and specificity. Conversely, the constant domains of the light chain (CL) and the heavy chain (CH1, CH2 or CH3) confer important biological properties such as secretion, transplacental mobility, Fc receptor binding, complement binding, and the like. By convention the numbering of the constant region domains increases as they become more distal from the antigen-binding site or amino-terminus of the antibody. In a wild-type antibody, at the N-terminus is a variable region and at the C-terminus is a constant region; the CH3 and CL domains actually comprise the carboxy-terminus of the heavy and light chain, respectively.

[0092] **Antibody fragment:** The term "antibody fragment" of an antibody as used herein refers to one or more portions of an antibody. In some embodiments, these portions are part of the contact domain(s) of an antibody. In some other embodiments, these portion(s) are antigen-binding fragments that retain the ability of binding an antigen non-covalently, reversibly and specifically, sometimes referred to herein as the "antigen-binding fragment", "antigen-binding fragment thereof," "antigen-binding portion", and the like. Examples of binding fragments include, but are not limited to, single-chain Fvs (scFv), a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; a F(ab)2 fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment consisting of the VH and CH1 domains; a Fv fragment consisting of the VL and VH domains of

a single arm of an antibody; a dAb fragment (Ward *et al.*, 1989, Nature 341:544-546), which consists of a VH domain; and an isolated complementarity determining region (CDR). Thus, the term "antibody fragment" encompasses both proteolytic fragments of antibodies (*e.g.*, Fab and F(ab)2 fragments) and engineered proteins comprising one or more portions of an antibody (*e.g.*, an scFv).

[0093] Antibody fragments can also be incorporated into single domain antibodies, maxibodies, minibodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv (see, *e.g.*, Hollinger and Hudson, 2005, Nature Biotechnology 23: 1126-1136). Antibody fragments can be grafted into scaffolds based on polypeptides such as Fibronectin type III (Fn3) (see U.S. Pat. No. 6,703,199, which describes fibronectin polypeptide monobodies).

[0094] Antibody fragments can be incorporated into single chain molecules comprising a pair of tandem Fv segments (for example, VH-CH1-VH-CH1) which, together with complementary light chain polypeptides (for example, VL-VC-VL-VC), form a pair of antigen-binding regions (Zapata *et al.*, 1995, Protein Eng. 8:1057-1062; and U.S. Pat. No. 5,641,870).

[0095] **Antibody Numbering System:** In the present specification, the references to numbered amino acid residues in antibody domains are based on the EU numbering system unless otherwise specified (for example, in Table 1). This system was originally devised by Edelman *et al.*, 1969, Proc. Nat'l Acad. Sci. USA 63:78-85 and is described in detail in Kabat *et al.*, 1991, in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA.

[0096] **Antigen-binding module:** The term "antigen-binding module" or "ABM" as used herein refers to a portion of a MBM that has the ability to bind to an antigen non-covalently, reversibly and specifically. An ABM can be immunoglobulin- or non-immunoglobulin-based. As used herein, the terms "ABM1" and "CD19 ABM" (and the like) refer to an ABM that binds specifically to CD19, the terms "ABM2" and "TCR ABM" (and the like) refer to an ABM that binds specifically to a component of a TCR complex, the term "ABM3" refers to an ABM that binds specifically to CD2 or to a TAA (depending on context), the term "CD2 ABM" (and the like) refers to an ABM that binds specifically to CD2, and the term "TAA ABM" (and the like) refers to an ABM that binds specifically to a TAA. The terms ABM1, ABM2, and ABM3 are used merely for convenience and are not intended to convey any particular configuration of a MBM. In some embodiments, an ABM2 binds to CD3 (referred to herein a "CD3 ABM" or the like). Accordingly, disclosures relating to ABM2 and ABM2s are also applicable to CD3 ABMs.

[0097] **Antigen-binding fragment:** The term "antigen-binding fragment" of an antibody refers to a portion of an antibody that has the ability to bind to an antigen non-covalently, reversibly and specifically.

[0098] **Antigen-binding molecule:** The term "antigen-binding molecule" refers to a molecule comprising one or more antigen-binding domains, for example an antibody. The antigen-binding molecule can comprise one or more polypeptide chains, *e.g.*, one, two, three, four or more polypeptide chains. The polypeptide chains in an antigen-binding molecule can be associated with one another directly or indirectly (for example a first polypeptide chain can be associated with a second polypeptide chain which in turn can be associated with a third polypeptide chain to form an antigen-binding molecule in which the first and second polypeptide chains are directly associated with one another, the second and third polypeptide chains are directly associated with one another, and the first and third polypeptide chains are indirectly associated with one another through the second polypeptide chain).

[0099] **Associated:** The term "associated" in the context of an antigen-binding molecule refers to a functional relationship between two or more polypeptide chains and/or two or more portions of a single polypeptide chain. In particular, the term "associated" means that two or more polypeptides (or portions of a single polypeptide) are associated with one another, *e.g.*, non-covalently through molecular interactions and/or covalently through one or more disulfide bridges or chemical cross-linkages, so as to produce a functional antigen-binding molecule, *e.g.*, a BBM or TBM in which the antigen binding domains can bind their respective targets. Examples of associations that might be present in a MBM include (but are not limited to) associations between Fc regions in an Fc domain (homodimeric or heterodimeric as described in Section 7.4.1.5), associations between VH and VL regions in a Fab or Fv, and associations between CH1 and CL in a Fab.

[00100] **B cell:** As used herein, the term "B cell" refers to a cell of B cell lineage, which is a type of white blood cell of the lymphocyte subtype. Examples of B cells include plasmablasts, plasma cells, lymphoplasmacytoid cells, memory B cells, follicular B cells, marginal zone B cells, B-1 cells, B-2 cells, and regulatory B cells.

[00101] **B cell malignancy:** As used herein, a B cell malignancy refers to an uncontrolled proliferation of B cells. Examples of B cell malignancy include non-Hodgkin's lymphomas (NHL), Hodgkin's lymphomas, leukemia, and myeloma. For example, a B cell malignancy can be, but is not limited to, multiple myeloma, chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL), follicular lymphoma, mantle cell lymphoma (MCL), diffuse large B-cell lymphoma (DLBCL), marginal zone lymphomas, Burkitt lymphoma, lymphoplasmacytic lymphoma (Waldenstrom macroglobulinemia), hairy cell leukemia, primary central nervous system (CNS) lymphoma, primary mediastinal large B-cell lymphoma, mediastinal grey-zone lymphoma (MGZL), splenic marginal zone B-cell lymphoma, extranodal marginal zone B-cell lymphoma of MALT, nodal marginal zone B-cell lymphoma, and primary

effusion lymphoma, and plasmacytic dendritic cell neoplasms.For example DLBCL includes relapsed or refractory DLBCL (RR DLBCL or R/R DLBCL).

[00102] **Binding Sequences:** In reference to Tables 1, 12, 13, 14, 16, or 17 (including subparts thereof), the term "binding sequences" means an ABM having a full set of CDRs, a VH-VL pair, or an scFv set forth in that table.

[00103] **Bispecific binding molecule:** The term "bispecific binding molecule" or "BBM" refers to a molecule that specifically binds to two antigens and comprises two or more ABMs. The BBMs of the disclosure comprise at least one antigen-binding domain which is specific for CD19 and at least one antigen-binding domain which is specific for a different antigen, *e.g.*, component of a TCR complex. Representative BBMs are illustrated in FIG. 1B-1AH. BBMs can comprise one, two, three, four or even more polypeptide chains.

[00104] **Bivalent:** The term "bivalent" as used herein in the context of an antigen-binding molecule refers to an antigen-binding molecule that has two antigen-binding domains. The domains can be the same or different. Accordingly, a bivalent antigen-binding molecule can be monospecific or bispecific. Bivalent BBMs can comprise an ABM that specifically binds to CD19 and another ABM that binds to another antigen, *e.g.*, a component of the TCR complex.

[00105] **Cancer:** The term "cancer" refers to a disease characterized by the uncontrolled (and often rapid) growth of aberrant cells. Cancer cells can spread locally or through the bloodstream and lymphatic system to other parts of the body. Examples of various cancers are described herein and include but are not limited to, leukemia, multiple myeloma, asymptomatic myeloma, Hodgkin's lymphoma and non-Hodgkin's lymphoma, *e.g.*, any CD19-positive cancers of any of the foregoing types. The term "cancerous B cell" refers to a B cell that is undergoing or has undergone uncontrolled proliferation.

[00106] **CD3:** The term "CD3" or "cluster of differentiation 3" refers to the cluster of differentiation 3 co-receptor of the T cell receptor. CD3 helps in activation of both cytotoxic T-cell (*e.g.*, CD8+ naïve T cells) and T helper cells (*e.g.*, CD4+ naïve T cells) and is composed of four distinct chains: one CD3γ chain (*e.g.*, Genbank Accession Numbers NM_000073 and MP_000064 (human)), one CD3δ chain (*e.g.*, Genbank Accession Numbers NM_000732, NM_001040651, NP_00732 and NP_001035741 (human)), and two CD3ε chains (*e.g.*, Genbank Accession Numbers NM_000733 and NP_00724 (human)). The chains of CD3 are highly related cell-surface proteins of the immunoglobulin superfamily containing a single extracellular immunoglobulin domain. The CD3 molecule associates with the T-cell receptor (TCR) and ζ-chain to form the T-cell receptor (TCR) complex, which functions in generating activation signals in T lymphocytes. Unless expressly indicated otherwise, the reference to CD3

in the application can refer to the CD3 co-receptor, the CD3 co-receptor complex, or any polypeptide chain of the CD3 co-receptor complex.

[00107] **CD19: :** The term "CD19" or "cluster of differentiation 19" refers to the Cluster of Differentiation 19 protein, which is an antigenic determinant detectable on leukemia precursor cells. The human and murine amino acid and nucleic acid sequences can be found in a public database, such as GenBank, UniProt and Swiss-Prot. For example, the amino acid sequence of human CD19 can be found as UniProt/Swiss-Prot Accession No. P15391 and the nucleotide sequence encoding of the human CD19 can be found at Accession No. NM_001178098. CD19 is expressed on most B lineage cancers, including, *e.g.*, acute lymphoblastic leukaemia, chronic lymphocyte leukaemia and non-Hodgkin's lymphoma. Other cells with express CD19 are provided below in the definition of "disease associated with expression of CD19." It is also an early marker of B cell progenitors. See, *e.g.*, Nicholson *et al.*, 1997, Mol. Immun. 34 (16-17): 1157-1165.

[00108] **Chimeric Antibody:** The term "chimeric antibody" (or antigen-binding fragment thereof) is an antibody molecule (or antigen-binding fragment thereof) in which (a) the constant region, or a portion thereof, is altered, replaced or exchanged so that the antigen-binding site (variable region) is linked to a constant region of a different or altered class, effector function and/or species, or an entirely different molecule which confers new properties to the chimeric antibody, *e.g.*, an enzyme, toxin, hormone, growth factor, drug, etc.; or (b) the variable region, or a portion thereof, is altered, replaced or exchanged with a variable region having a different or altered antigen specificity. For example, a mouse antibody can be modified by replacing its constant region with the constant region from a human immunoglobulin. Due to the replacement with a human constant region, the chimeric antibody can retain its specificity in recognizing the antigen while having reduced antigenicity in human as compared to the original mouse antibody.

[00109] **Chimeric Antigen Receptor:** The term "Chimeric Antigen Receptor" or alternatively a "CAR" refers to a set of polypeptides, typically two in the simplest embodiments, which when in an immune effector cell, provides the cell with specificity for a target cell, typically a cancer cell, and with intracellular signal generation. In some embodiments, a CAR comprises at least an extracellular antigen binding domain, a transmembrane domain and a cytoplasmic signaling domain (also referred to herein as "an intracellular signaling domain") comprising a functional signaling domain derived from a stimulatory molecule and/or costimulatory molecule as defined below. The set of polypeptides can be contiguous or non-contiguous with each other. Where the polypeptides are not contiguous with one another, the set of polypeptides include a dimerization switch that, upon the presence of a dimerization molecule, can couple the polypeptides to one another, *e.g.*, can couple an antigen binding

domain to an intracellular signaling domain. CAR molecules are typically administered to a subject by way of administration of immune effector cells (*e.g.*, T cells that are preferably autologous to the subject) engineered to express a CAR molecule.

[00110] **In combination:** Administered "in combination," as used herein, means that two (or more) different treatments are delivered to the subject during the course of the subject's affliction with the disorder, *e.g.*, the two or more treatments are delivered after the subject has been diagnosed with the disorder and before the disorder has been cured or eliminated or treatment has ceased for other reasons. The terms "combination" and "in combination" are not limited to the administration of two or more treatments at exactly the same time, but rather it is meant that a pharmaceutical composition comprising an agent (*e.g.*, an anti-CD19 agent) is administered to a subject in a sequence and within a time interval such that the agent can act together with the additional therapy(ies) to provide an increased benefit than if they were administered otherwise.

[00111] **Complementarity Determining Region:** The terms "complementarity determining region" or "CDR," as used herein, refer to the sequences of amino acids within antibody variable regions which confer antigen specificity and binding affinity. For example, in general, there are three CDRs in each heavy chain variable region (*e.g.*, CDR-H1, CDR-H2, and CDR-H3) and three CDRs in each light chain variable region (CDR-L1, CDR-L2, and CDR-L3). The precise amino acid sequence boundaries of a given CDR can be determined using any of a number of well-known schemes, including those described by Kabat *et al.,* 1991, "Sequences of Proteins of Immunological Interest," 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD ("Kabat" numbering scheme), Al-Lazikani *et al.*, 1997, JMB 273:927-948 ("Chothia" numbering scheme) and ImMunoGenTics (IMGT) numbering (Lefranc, 1999, The Immunologist 7:132-136; Lefranc *et al.*, 2003, Dev. Comp. Immunol. 27:55-77 ("IMGT" numbering scheme). For example, for classic formats, under Kabat, the CDR amino acid residues in the heavy chain variable domain (VH) are numbered 31-35 (CDR-H1), 50-65 (CDR-H2), and 95-102 (CDR-H3); and the CDR amino acid residues in the light chain variable domain (VL) are numbered 24-34 (CDR-L1), 50-56 (CDR-L2), and 89-97 (CDR-L3). Under Chothia, the CDR amino acids in the VH are numbered 26-32 (CDR-H1), 52-56 (CDR-H2), and 95-102 (CDR-H3); and the amino acid residues in VL are numbered 26-32 (CDR-L1), 50-52 (CDR-L2), and 91-96 (CDR-L3). By combining the CDR definitions of both Kabat and Chothia, the CDRs consist of amino acid residues 26-35 (CDR-H1), 50-65 (CDR-H2), and 95-102 (CDR-H3) in human VH and amino acid residues 24-34 (CDR-L1), 50-56 (CDR-L2), and 89-97 (CDR-L3) in human VL. Under IMGT the CDR amino acid residues in the VH are numbered approximately 26-35 (CDR-H1), 51-57 (CDR-H2) and 93-102 (CDR-H3), and the CDR amino acid residues in the VL are numbered approximately 27-32 (CDR-L1), 50-52 (CDR-L2), and 89-

97 (CDR-L3) (numbering according to "Kabat"). Under IMGT, the CDR regions of an antibody can be determined using the program IMGT/DomainGap Align.

[00112] **Concurrently:** The term "concurrently" is not limited to the administration of therapies (*e.g.*, prophylactic or therapeutic agents) at exactly the same time, but rather it is meant that a pharmaceutical composition comprising an antigen-binding molecule of the disclosure is administered to a subject in a sequence and within a time interval such that the molecules can act together with the additional therapy(ies) to provide an increased benefit than if they were administered otherwise.

[00113] **Conservative Sequence Modifications:** The term "conservative sequence modifications" refers to amino acid modifications that do not significantly affect or alter the binding characteristics of a CD19 binding molecule or a component thereof (*e.g.*, a CD19-binding domain or an Fc region). Such conservative modifications include amino acid substitutions, additions and deletions. Modifications can be introduced into a binding molecule by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions are ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, one or more amino acid residues within a binding molecule can be replaced with other amino acid residues from the same side chain family and the altered binding molecule can be tested for, *e.g.*, binding to target molecules and/or effective heterodimerization and/or effector function.

[00114] **Diabody:** The term "diabody" as used herein refers to small antibody fragments with two antigen-binding sites, typically formed by pairing of scFv chains. Each scFv comprises a heavy chain variable domain (VH) connected to a light chain variable domain (VL) in the same polypeptide chain (VH-VL, where the VH is either N-terminal or C-terminal to the VL). Unlike a typical scFv in which the VH and VL are separated by a linker that allows the VH and VL on the same polypeptide chain to pair and form an antigen-binding domain, diabodies typically comprise a linker that is too short to allow pairing between the VH and VL domains on the same chain, forcing the VH and VL domains to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger *et al.*, 1993, Proc. Natl. Acad. Sci. USA 90:6444-6448.

[00115] **dsFv:** The term "dsFv" refers to disulfide-stabilized Fv fragments. In a dsFv, a VH and VL are connected by an interdomain disulfide bond. To generate such molecules, one amino acid each in the framework region of in VH and VL are mutated to a cysteine, which in turn form a stable interchain disulfide bond. Typically, position 44 in the VH and position 100 in the VL are mutated to cysteines. See Brinkmann, 2010, Antibody Engineering 181-189, DOI:10.1007/978-3-642-01147-4_14. The term dsFv encompasses both what is known as a dsFv (a molecule in which the VH and VL are connected by an interchain disulfide bond but not a linker peptide) or scdsFv (a molecule in which the VH and VL are connected by a linker as well as an interchain disulfide bond).

[00116] **Effector Function:** The term "effector function" refers to an activity of an antibody molecule that is mediated by binding through a domain of the antibody other than the antigen-binding domain, usually mediated by binding of effector molecules. Effector function includes complement-mediated effector function, which is mediated by, for example, binding of the C1 component of the complement to the antibody. Activation of complement is important in the opsonization and lysis of cell pathogens. The activation of complement also stimulates the inflammatory response and may also be involved in autoimmune hypersensitivity. Effector function also includes Fc receptor (FcR)-mediated effector function, which can be triggered upon binding of the constant domain of an antibody to an Fc receptor (FcR). Binding of antibody to Fc receptors on cell surfaces triggers a number of important and diverse biological responses including engulfment and destruction of antibody-coated particles, clearance of immune complexes, lysis of antibody-coated target cells by killer cells (called antibody-dependent cell-mediated cytotoxicity, or ADCC), release of inflammatory mediators, placental transfer and control of immunoglobulin production. An effector function of an antibody can be altered by altering, *e.g.*, enhancing or reducing, the affinity of the antibody for an effector molecule such as an Fc receptor or a complement component. Binding affinity will generally be varied by modifying the effector molecule binding site, and in this case it is appropriate to locate the site of interest and modify at least part of the site in a suitable way. It is also envisaged that an alteration in the binding site on the antibody for the effector molecule need not alter significantly the overall binding affinity but can alter the geometry of the interaction rendering the effector mechanism ineffective as in non-productive binding. It is further envisaged that an effector function can also be altered by modifying a site not directly involved in effector molecule binding, but otherwise involved in performance of the effector function.

[00117] **Epitope:** An epitope, or antigenic determinant, is a portion of an antigen recognized by an antibody or other antigen-binding moiety as described herein. An epitope can be linear or conformational.

[00118] **Fab:** By "Fab" or "Fab region" as used herein is meant a polypeptide region that comprises the VH, CH1, VL, and CL immunoglobulin domain. These terms can refer to this region in isolation, or this region in the context of an antigen-binding molecule of the disclosure.

[00119] Fab domains are formed by association of a CH1 domain attached to a VH domain with a CL domain attached to a VL domain. The VH domain is paired with the VL domain to constitute the Fv region, and the CH1 domain is paired with the CL domain to further stabilize the binding module. A disulfide bond between the two constant domains can further stabilize the Fab domain.

[00120] Fab regions can be produced by proteolytic cleavage of immunoglobulin molecules (*e.g.*, using enzymes such as papain) or through recombinant expression. In native immunoglobulin molecules, Fabs are formed by association of two different polypeptide chains (*e.g.*, VH-CH1 on one chain associates with VL-CL on the other chain). The Fab regions are typically expressed recombinantly, typically on two polypeptide chains, although single chain Fabs are also contemplated herein.

[00121] **Fc domain:** The term "Fc domain" refers to a pair of associated Fc regions. The two Fc regions dimerize to create the Fc domain. The two Fc regions within the Fc domain can be the same (such an Fc domain being referred to herein as an "Fc homodimer") or different from one another (such an Fc domain being referred to herein as an "Fc heterodimer").

[00122] **Fc region:** The term "Fc region" or "Fc chain" as used herein is meant the polypeptide comprising the CH2-CH3 domains of an IgG molecule, and in some cases, inclusive of the hinge. In EU numbering for human IgG1, the CH2-CH3 domain comprises amino acids 231 to 447, and the hinge is 216 to 230. Thus the definition of "Fc region" includes both (CH2-CH3) or (hinge-CH2-CH3), or fragments thereof. An "Fc fragment" in this context can contain fewer amino acids from either or both of the N- and C-termini but still retains the ability to form a dimer with another Fc region as can be detected using standard methods, generally based on size (*e.g.*, non-denaturing chromatography, size exclusion chromatography).

[00123] **Fv:** The term "Fv" refers to the minimum antibody fragment derivable from an immunoglobulin that contains a complete target recognition and binding site. This region consists of a dimer of one heavy and one light chain variable domain in a tight, noncovalent association (VH-VL dimer). It is in this configuration that the three CDRs of each variable domain interact to define a target binding site on the surface of the VH-VL dimer. Often, the six CDRs confer target binding specificity to the antibody. However, in some instances even a single variable domain (or half of an Fv comprising only three CDRs specific for a target) can have the ability to recognize and bind target. The reference to a VH-VL dimer herein is not

intended to convey any particular configuration. By way of example and not limitation, the VH and VL can come together in any configuration described herein to form a half antibody, or can each be present on a separate half antibody and come together to form an antigen binding domain when the separate half antibodies associate, for example to form a TBM of the disclosure. When present on a single polypeptide chain (*e.g.*, a scFv), the VH and be N-terminal or C-terminal to the VL.

[0100] **Half Antibody:** The term "half antibody" refers to a molecule that comprises at least one ABM or ABM chain and can associate with another molecule comprising an ABM or ABM chain through, *e.g.*, a disulfide bridge or molecular interactions (*e.g.*, knob-in-hole interactions between Fc heterodimers). A half antibody can be composed of one polypeptide chain or more than one polypeptide chains (*e.g.*, the two polypeptide chains of a Fab). In an embodiment, a half-antibody comprises an Fc region.

[0101] An example of a half antibody is a molecule comprising a heavy and light chain of an antibody (*e.g.*, an IgG antibody). Another example of a half antibody is a molecule comprising a first polypeptide comprising a VL domain and a CL domain, and a second polypeptide comprising a VH domain, a CH1 domain, a hinge domain, a CH2 domain, and a CH3 domain, where the VL and VH domains form an ABM. Yet another example of a half antibody is a polypeptide comprising an scFv domain, a CH2 domain and a CH3 domain.

[0102] A half antibody might include more than one ABM, for example a half-antibody comprising (in N- to C-terminal order) an scFv domain, a CH2 domain, a CH3 domain, and another scFv domain.

[0103] Half antibodies might also include an ABM chain that when associated with another ABM chain in another half antibody forms a complete ABM.

[0104] Thus, a MBM can comprise one, more typically two, or even more than two half antibodies, and a half antibody can comprise one or more ABMs or ABM chains.

[0105] In some MBMs, a first half antibody will associate, *e.g.*, heterodimerize, with a second half antibody. In other MBMs, a first half antibody will be covalently linked to a second half antibody, for example through disulfide bridges or chemical crosslinking. In yet other MBMs, a first half antibody will associate with a second half antibody through both covalent attachments and non-covalent interactions, for example disulfide bridges and knob-in-hole interactions.

[0106] The term "half antibody" is intended for descriptive purposes only and does not connote a particular configuration or method of production. Descriptions of a half antibody as a "first" half antibody, a "second" half antibody, a "left" half antibody, a "right" half antibody or the like are merely for convenience and descriptive purposes.

[0107] Hexavalent: The term "hexavalent" as used herein in the context of an antigen-binding molecule (*e.g.*, a TBM) refers to an antigen-binding molecule that has six antigen-binding domains. Hexavalent TBMs of the disclosure generally have three pairs of antigen-binding domains that each bind to the same antigen, although different configurations (*e.g.*, three antigen-binding domains that bind to CD19, two antigen-binding domains that bind to a component of a TCR complex, and one antigen-binding domain that binds to CD2 or a TAA, or three antigen-binding domains that bind to CD19, two antigen-binding domains that bind to CD2 or a TAA, and one antigen-binding domain that binds to a component of a TCR complex) are within the scope of the disclosure. Examples of hexavalent TBMs are shown schematically in FIGS. 1U-1V.

[0108] Hole: In the context of a knob-into-hole, a "hole" refers to at least one amino acid side chain which is recessed from the interface of a first Fc chain and is therefore positionable in a compensatory "knob" on the adjacent interfacing surface of a second Fc chain so as to stabilize the Fc heterodimer, and thereby favor Fc heterodimer formation over Fc homodimer formation, for example.

[0109] Host cell or recombinant host cell: The terms "host cell" or "recombinant host cell" refer to a cell that has been genetically-engineered, *e.g.*, through introduction of a heterologous nucleic acid. It should be understood that such terms are intended to refer not only to the particular subject cell but to the progeny of such a cell. Because certain modifications can occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein. A host cell can carry the heterologous nucleic acid transiently, *e.g.*, on an extrachromosomal heterologous expression vector, or stably, *e.g.*, through integration of the heterologous nucleic acid into the host cell genome. For purposes of expressing an antigen-binding molecule, a host cell can be a cell line of mammalian origin or mammalian-like characteristics, such as monkey kidney cells (COS, *e.g.*, COS-1, COS-7), HEK293, baby hamster kidney (BHK, *e.g.*, BHK21), Chinese hamster ovary (CHO), NSO, PerC6, BSC-1, human hepatocellular carcinoma cells (*e.g.*, Hep G2), SP2/0, HeLa, Madin-Darby bovine kidney (MDBK), myeloma and lymphoma cells, or derivatives and/or engineered variants thereof. The engineered variants include, *e.g.*, glycan profile modified and/or site-specific integration site derivatives.

[0110] Human Antibody: The term "human antibody" as used herein includes antibodies having variable regions in which both the framework and CDR regions are derived from sequences of human origin. Furthermore, if the antibody contains a constant region, the constant region also is derived from such human sequences, *e.g.*, human germline sequences, or mutated versions of human germline sequences or antibody containing consensus

framework sequences derived from human framework sequences analysis, for example, as described in Knappik *et al.*, 2000, J Mol Biol 296, 57-86. The structures and locations of immunoglobulin variable domains, *e.g.*, CDRs, can be defined using well known numbering schemes, *e.g.*, the Kabat numbering scheme, the Chothia numbering scheme, or a combination of Kabat and Chothia (see, *e.g.*, Lazikani *et al.*, 1997, J. Mol. Bio. 273:927 948; Kabat *et al.*, 1991, Sequences of Proteins of Immunological Interest, 5th edit., NIH Publication no. 91-3242 U.S. Department of Health and Human Services; Chothia *et al.*, 1987, J. Mol. Biol. 196:901-917; Chothia *et al.*, 1989, Nature 342:877-883).

[0111] Human antibodies can include amino acid residues not encoded by human sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*, or a conservative substitution to promote stability or manufacturing). However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

[0112] **Humanized:** The term "humanized" forms of non-human (*e.g.*, murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or non-human primate having the desired specificity, affinity, and capacity. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies can comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin lo sequence. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones *et al.*, 1986, Nature 321:522-525; Riechmann *et al.*, 1988, Nature 332:323-329; and Presta, 1992, Curr. Op. Struct. Biol. 2:593-596. See also the following review articles and references cited therein: Vaswani and Hamilton, 1998, Ann. Allergy, Asthma & Immunol. 1:105-115; Harris, 1995, Biochem. Soc. Transactions 23:1035-1038; Hurle and Gross, 1994, Curr. Op. Biotech. 5:428-433.

[0113] **Knob:** In the context of a knob-into-hole, a "knob" refers to at least one amino acid side chain which projects from the interface of a first Fc chain and is therefore positionable in a

compensatory "hole" in the interface with a second Fc chain so as to stabilize the Fc heterodimer, and thereby favor Fc heterodimer formation over Fc homodimer formation, for example.

[0114] Knobs and holes (or knobs-into-holes): One mechanism for Fc heterodimerization is generally referred to in the art as "knobs and holes", or "knob-in-holes", or "knobs-into-holes". These terms refer to amino acid mutations that create steric influences to favor formation of Fc heterodimers over Fc homodimers, as described in, *e.g.*, Ridgway *et al.*, 1996, Protein Engineering 9(7):617; Atwell *et al.*, 1997, J. Mol. Biol. 270:26; and U.S. Patent No. 8,216,805. Knob-in-hole mutations can be combined with other strategies to improve heterodimerization, for example as described in Section 7.4.1.6.

[0115] Monoclonal Antibody: The term "monoclonal antibody" as used herein refers to polypeptides, including antibodies, antibody fragments, molecules (including MBMs), *etc.* that are derived from the same genetic source.

[0116] Monovalent: The term "monovalent" as used herein in the context of an antigen-binding molecule refers to an antigen-binding molecule that has a single antigen-binding domain.

[0117] Multispecific binding molecules: The term "multispecific binding molecules" or "MBMs" refers to molecules that specifically bind to at least two antigens and comprise two or more antigen-binding domains. The antigen-binding domains can each independently be an antibody fragment (*e.g.*, scFv, Fab, nanobody), a ligand, or a non-antibody derived binder (*e.g.*, fibronectin, Fynomer, DARPin).

[0118] Mutation or modification: In the context of the primary amino acid sequence of a polypeptide, the terms "modification" and "mutation" refer to an amino acid substitution, insertion, and/or deletion in the polypeptide sequence relative to a reference polypeptide. Additionally, the term "modification" further encompasses an alteration to an amino acid residue, for example by chemical conjugation (*e.g.*, of a drug or polyethylene glycol moiety) or post-translational modification (*e.g.*, glycosylation).

[0119] Nucleic Acid: The term "nucleic acid" is used herein interchangeably with the term "polynucleotide" and refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. The term encompasses nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, which have similar binding properties as the reference nucleic acid, and which are metabolized in a manner similar to the reference nucleotides. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates,

methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, and peptide-nucleic acids (PNAs).

[0120] Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (*e.g.*, degenerate codon substitutions) and complementary sequences, as well as the sequence explicitly indicated. Specifically, as detailed below, degenerate codon substitutions can be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer *et al.*, 1991, Nucleic Acid Res. 19:5081; Ohtsuka *et al.*, 1985, J. Biol. Chem. 260:2605-2608; and Rossolini *et al.*, 1994, Mol. Cell. Probes 8:91-98).

[0121] **Operably linked:** The term "operably linked" refers to a functional relationship between two or more peptide or polypeptide domains or nucleic acid (*e.g.*, DNA) segments. In the context of a fusion protein or other polypeptide, the term "operably linked" means that two or more amino acid segments are linked so as to produce a functional polypeptide. For example, in the context of an antigen-binding molecule, separate ABMs (or chains of an ABM) can be operably linked through peptide linker sequences. In the context of a nucleic acid encoding a fusion protein, such as a polypeptide chain of an antigen-binding molecule, "operably linked" means that the two nucleic acids are joined such that the amino acid sequences encoded by the two nucleic acids remain in-frame. In the context of transcriptional regulation, the term refers to the functional relationship of a transcriptional regulatory sequence to a transcribed sequence. For example, a promoter or enhancer sequence is operably linked to a coding sequence if it stimulates or modulates the transcription of the coding sequence in an appropriate host cell or other expression system.

[0122] **Pentavalent:** The term "pentavalent" as used herein in the context of an antigen-binding molecule (*e.g.*, a TBM) refers to an antigen-binding molecule that has five antigen-binding domains. Pentavalent TBMs of the disclosure generally have either (a) two pairs of antigen-binding domains that each bind to the same antigen and a single antigen-binding domain that binds to the third antigen or (b) three antigen-binding domains that bind to the same antigen and two antigen-binding domains that each bind to a separate antigen. An example of a pentavalent TBM is shown schematically in FIG. 1T.

[0123] **Polypeptide and Protein:** The terms "polypeptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms encompass amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer. Additionally, the terms encompass amino acid polymers that are derivatized, for example, by synthetic derivatization of one or

more side chains or termini, glycosylation, PEGylation, circular permutation, cyclization, linkers to other molecules, fusion to proteins or protein domains, and addition of peptide tags or labels.

[0124] Recognize: The term "recognize" as used herein refers to an ABM that finds and interacts (*e.g.*, binds) with its epitope.

[0125] Sequence identity: Sequence identity between two similar sequences (*e.g.*, antibody variable domains) can be measured by algorithms such as that of Smith, T.F. & Waterman, M.S. (1981) "Comparison Of Biosequences," Adv. Appl. Math. 2:482 [local homology algorithm]; Needleman, S.B. & Wunsch, CD. (1970) "A General Method Applicable To The Search For Similarities In The Amino Acid Sequence Of Two Proteins," J. Mol. Biol.48:443 [homology alignment algorithm], Pearson, W.R. & Lipman, D.J. (1988) "Improved Tools For Biological Sequence Comparison," Proc. Natl. Acad. Sci. (U.S.A.) 85:2444 [search for similarity method]; or Altschul, S.F. *et al*, 1990, "Basic Local Alignment Search Tool," J. Mol. Biol. 215:403-10 , the "BLAST" algorithm, see blast.ncbi.nlm.nih.gov/Blast.cgi. When using any of the aforementioned algorithms, the default parameters (for Window length, gap penalty, etc.) are used. In one embodiment, sequence identity is done using the BLAST algorithm, using default parameters.

[0126] Optionally, the identity is determined over a region that is at least about 50 nucleotides (or, in the case of a peptide or polypeptide, at least about 10 amino acids) in length, or in some cases over a region that is 100 to 500 or 1000 or more nucleotides (or 20, 50, 200 or more amino acids) in length. In some embodiments, the identity is determined over a defined domain, *e.g.*, the VH or VL of an antibody. Unless specified otherwise, the sequence identity between two sequences is determined over the entire length of the shorter of the two sequences.

[0127] Single Chain Fab or scFab: The terms "single chain Fab" and "scFab" mean a polypeptide comprising an antibody heavy chain variable domain (VH), an antibody constant domain 1 (CH1), an antibody light chain variable domain (VL), an antibody light chain constant domain (CL) and a linker, such that the VH and VL are in association with one another and the CH1 and CL are in association with one another. In some embodiments, the antibody domains and the linker have one of the following orders in N-terminal to C-terminal direction: a) VH-CH1-linker-VL-CL, b) VL-CL-linker-VH-CH1, c) VH-CL-linker-VL-CH1 or d) VL-CH1-linker-VH-CL. The linker can be a polypeptide of at least 30 amino acids, for example between 32 and 50 amino acids. The single chain Fabs are stabilized via the natural disulfide bond between the CL domain and the CH1 domain.

[0128] Single Chain Fv or scFv: The term "single-chain Fv" or "scFv" as used herein refers to antibody fragments that comprise the VH and VL domains of an antibody, where these domains are present in a single polypeptide chain. The Fv polypeptide can further comprise a

polypeptide linker between the VH and VL domains which enables the scFv to form the desired structure for antigen-binding. For a review of scFv see Plückthun in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., 1994, Springer-Verlag, New York, pp. 269-315.

[0129] Specifically (or selectively) binds: The term "specifically (or selectively) binds" to an antigen or an epitope refers to a binding reaction that is determinative of the presence of a cognate antigen or an epitope in a heterogeneous population of proteins and other biologics. The binding reaction can be but need not be mediated by an antibody or antibody fragment, but can also be mediated by, for example, any type of ABM described in Section 7.3, such as a ligand, a DARPin, *etc*. An ABM typically also has a dissociation rate constant (KD) (koff/kon) of less than $5x10^{-2}$M, less than 10^{-2}M, less than $5x10^{-3}$M, less than 10^{-3}M, less than $5x10^{-4}$M, less than 10^{-4}M, less than $5x10^{-5}$M, less than 10^{-5}M, less than $5x10^{-6}$M, less than 10^{-6}M, less than $5x10^{-7}$M, less than 10^{-7}M, less than $5x10^{-8}$M, less than 10^{-8}M, less than $5x10^{-9}$M, or less than 10^{-9}M, and binds to the target antigen with an affinity that is at least two-fold greater than its affinity for binding to a non-specific antigen (*e.g.*, HSA). Binding affinity can be measured using a Biacore, SPR or BLI assay. The term "specifically binds" does not exclude cross-species reactivity. For example, an antigen-binding module (*e.g.*, an antigen-binding fragment of an antibody) that "specifically binds" to an antigen from one species can also "specifically bind" to that antigen in one or more other species. Thus, such cross-species reactivity does not itself alter the classification of an antigen-binding module as a "specific" binder. In certain embodiments, an antigen-binding module that specifically binds to a human antigen has cross-species reactivity with one or more non-human mammalian species, *e.g.*, a primate species (including but not limited to one or more of *Macaca fascicularis*, *Macaca mulatta*, and *Macaca nemestrina*) or a rodent species, *e.g.*, *Mus musculus*. In other embodiments, the antigen-binding module does not have cross-species reactivity.

[0130] Subject: The term "subject" includes human and non-human animals. Non-human animals include all vertebrates, *e.g.*, mammals and non-mammals, such as non-human primates, sheep, dog, cow, chickens, amphibians, and reptiles. Except when noted, the terms "patient" or "subject" are used herein interchangeably.

[0131] Tandem of VH Domains: The term "a tandem of VH domains (or VHs)" as used herein refers to a string of VH domains, consisting of multiple numbers of identical VH domains of an antibody. Each of the VH domains, except the last one at the end of the tandem, has its C-terminus connected to the N-terminus of another VH domain with or without a linker. A tandem has at least 2 VH domains, and in particular embodiments an antigen-binding molecule has 3, 4, 5, 6, 7, 8, 9, or 10 VH domains. The tandem of VH can be produced by joining the encoding nucleic acids of each VH domain in a desired order using recombinant methods with or without

a linker (*e.g.*, as described in Section 7.4.3) that enables them to be made as a single polypeptide chain. The N-terminus of the first VH domain in the tandem is defined as the N-terminus of the tandem, while the C-terminus of the last VH domain in the tandem is defined as the C-terminus of the tandem.

[0132] **Tandem of VL Domains:** The term "a tandem of VL domains (or VLs)" as used herein refers to a string of VL domains, consisting of multiple numbers of identical VL domains of an antibody. Each of the VL domains, except the last one at the end of the tandem, has its C-terminus connected to the N-terminus of another VL with or without a linker. A tandem has at least 2 VL domains, and in particular embodiments an antigen-binding molecule has 3, 4, 5, 6, 7, 8, 9, or 10 VL domains. The tandem of VL can be produced by joining the encoding nucleic acids of each VL domain in a desired order using recombinant methods with or without a linker (*e.g.*, as described in Section 7.4.3) that enables them to be made as a single polypeptide chain. The N-terminus of the first VL domain in the tandem is defined as the N-terminus of the tandem, while the C-terminus of the last VL domain in the tandem is defined as the C-terminus of the tandem.

[0133] **Target Antigen:** By "target antigen" as used herein is meant the molecule that is bound non-covalently, reversibly and specifically by an antigen binding domain.

[0134] **Tetravalent:** The term "tetravalent" as used herein in the context of an antigen-binding molecule (*e.g.*, a BBM or TBM) refers to an antigen-binding molecule that has four antigen-binding domains. Tetravalent TBMs of the disclosure generally have two antigen-binding domains that bind to the same antigen (*e.g.*, CD19) and two antigen-binding domains that each bind to a separate antigen (*e.g.*, a component of a TCR complex and either CD2 or a TAA). Examples of tetravalent BBMs are shown schematically in FIGS. 1AA-1AH and examples of tetravalent TBMs are shown schematically in FIGS. 2Q-2S.

[0135] **Therapeutically effective amount:** A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result.

[0136] **Treat, Treatment, Treating:** As used herein, the terms "treat", "treatment" and "treating" refer to the reduction or amelioration of the progression, severity and/or duration of a disease or disorder (*e.g.*, a proliferative disorder), or the amelioration of one or more symptoms (*e.g.*, one or more discernible symptoms) of a disorder resulting from the administration of one or more CD19 binding molecules of the disclosure. In some embodiments, the terms "treat", "treatment" and "treating" refer to the amelioration of at least one measurable physical parameter of a disorder, such as growth of a tumor, not necessarily discernible by the patient. In other embodiments the terms "treat", "treatment" and "treating" refer to the inhibition of the

progression of a disorder, either physically by, *e.g.*, stabilization of a discernible symptom, physiologically by, *e.g.*, stabilization of a physical parameter, or both. In some embodiments, the terms "treat", "treatment" and "treating" can refer to the reduction or stabilization of tumor size or cancerous cell count.

[0137] Trispecific binding molecules: The term "trispecific binding molecules" or "TBMs" refers to molecules that specifically bind to three antigens and comprise three or more antigen-binding domains. The TBMs of the disclosure comprise at least one antigen-binding domain which is specific for CD19, at least one antigen-binding domain which is specific for a component of a TCR complex, and at least one antigen-binding domain which is specific for CD2 or a TAA. The antigen-binding domains can each independently be an antibody fragment (*e.g.*, scFv, Fab, nanobody), a ligand, or a non-antibody derived binder (*e.g.*, fibronectin, Fynomer, DARPin). Representative TBMs are illustrated in FIG. 1. TBMs can comprise one, two, three, four or even more polypeptide chains. For example, the TBM illustrated in FIG. 1M comprises a single polypeptide chain comprising three scFvs connected by ABM linkers one a single polypeptide chain. The TBM illustrated in FIG. 1K comprises two polypeptide chains comprising three scFvs connected by, *inter alia*, an Fc domain. The TBM illustrated in FIG. 1J comprises three polypeptide chains forming an scFv, a ligand, and a Fab connected by, *inter alia*, an Fc domain. The TBM illustrated in FIG. 1C comprises four polypeptide chains forming three Fabs connected by, *inter alia*, an Fc domain. The TBM illustrated in FIG. 1U comprises 6 polypeptide chains forming four Fabs and two scFvs connected by, *inter alia*, an Fc domain.

[0138] Trivalent: The term "trivalent" as used herein in the context of an antigen-binding molecule (*e.g.*, a MBM) refers to an antigen-binding molecule that has three antigen-binding domains. The MBMs of the disclosure are typically bispecific or trispecific. Bispecific BBMs specifically bind to CD19 and a component of a TCR complex. Trispecific TBMs specifically bind to CD19, a component of a TCR complex, and CD2 or a TAA. Accordingly, the trivalent BBMs have three antigen binding domains, two of which bind to CD19 and one of which binds to a component of the TCR, or vice versa. TBMs have three antigen-binding domains that each bind to a different antigen. Examples of trivalent BBMs are shown schematically in FIGS. 1G-1Z and examples of trivalent TBMs are shown schematically in FIGS. 2B-2V.

[0139] Tumor: The term "tumor" is used interchangeably with the term "cancer" herein, *e.g.*, both terms encompass solid and liquid, *e.g.*, diffuse or circulating, tumors. As used herein, the term "cancer" or "tumor" includes premalignant, as well as malignant cancers and tumors.

[0140] Tumor-Associated Antigen: The term "tumor-associated antigen" or "TAA" refers to a molecule (typically a protein, carbohydrate, lipid or some combination thereof) that is expressed on the surface of a cancer cell, either entirely or as a fragment (*e.g.*, MHC/peptide), and which

is useful for the preferential targeting of a pharmacological agent to the cancer cell. In some embodiments, a TAA is a marker expressed by both normal cells and cancer cells, *e.g.*, a lineage marker, *e.g.*, CD19 on B cells. In some embodiments, a TAA is a cell surface molecule that is overexpressed in a cancer cell in comparison to a normal cell, for instance, 1-fold over expression, 2-fold overexpression, 3-fold overexpression or more in comparison to a normal cell. In some embodiments, a TAA is a cell surface molecule that is inappropriately synthesized in the cancer cell, for instance, a molecule that contains deletions, additions or mutations in comparison to the molecule expressed on a normal cell. In some embodiments, a TAA will be expressed exclusively on the cell surface of a cancer cell, entirely or as a fragment (*e.g.*, MHC/peptide), and not synthesized or expressed on the surface of a normal cell. Accordingly, the term "TAA" encompasses antigens that are specific to cancer cells, sometimes referred to as tumor-specific antigens ("TSAs"). Although CD19 has features of a tumor-associated antigen, the terms "tumor-associated antigen" and "TAA" are used throughout the disclosure to refer to molecules other than CD19.

[0141] **Variable region:** By "variable region" or "variable domain" as used herein is meant the region of an immunoglobulin that comprises one or more Ig domains substantially encoded by any of the Vκ, Vλ, and/or VH genes that make up the kappa, lambda, and heavy chain immunoglobulin genetic loci respectively, and contains the CDRs that confer antigen specificity. A "variable heavy domain" can pair with a "variable light domain" to form an antigen binding domain ("ABD") or antigen-binding module ("ABM"). In addition, each variable domain comprises three hypervariable regions ("complementary determining regions," "CDRs") (CDR-H1, CDR-H2, CDR-H3 for the variable heavy domain and CDR-L1, CDR-L2, CDR-L3 for the variable light domain) and four framework (FR) regions, arranged from amino-terminus to carboxy-terminus in the following order: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4.

[0142] **Vector:** The term "vector" is intended to refer to a polynucleotide molecule capable of transporting another polynucleotide to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, where additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operably linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of

plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the disclosure is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

[0143] **VH:** The term "VH" refers to the variable region of an immunoglobulin heavy chain of an antibody, including the heavy chain of an Fv, scFv, dsFv or Fab.

[0144] **VL:** The term "VL" refers to the variable region of an immunoglobulin light chain, including the light chain of an Fv, scFv, dsFv or Fab.

[0145] **VH-VL or VH-VL Pair:** In reference to a VH-VL pair, whether on the same polypeptide chain or on different polypeptide chains, the terms "VH-VL" and "VH-VL pair" are used for convenience and are not intended to convey any particular orientation, unless the context dictates otherwise. Thus, a scFv comprising a "VH-VL" or "VH-VL pair" can have the VH and VL domains in any orientation, for example the VH N-terminal to the VL or the VL N-terminal to the VH.

7.2. CD19 Binding Molecules

[0146] In one aspect, the disclosure provides CD19 binding molecules, including monospecific and multispecific molecules that bind to human CD19. Typically, the CD19 binding molecules of the disclosure comprise a Fc domain comprising a first variant human IgG1 Fc region and a second variant human IgG1 Fc region having L234A, L235A, and G237A ("LALAGA") substitutions, L234A, L235A, S267K, and P329A ("LALASKPA") substitutions, D265A, P329A, and S267K ("DAPASK") substitutions, G237A, D265A, and P329A ("GADAPA") substitutions, G237A, D265A, P329A, and S267K ("GADAPASK") substitutions, L234A, L235A, and P329G ("LALAPG") substitutions, or L234A, L235A, and P329A ("LALAPA") substitutions.

[0147] In some embodiments, the CD19 binding molecule is a monospecific binding molecule. For example, the monospecific binding molecule can be an antibody or an antigen-binding fragment thereof (*e.g.*, an antibody fragment, an scFv, a dsFv, a Fv, a Fab, an scFab, a (Fab')2, or a single domain antibody (SDAB). In other embodiments, the CD19 binding molecule is a multispecific (*e.g.*, bispecific) CD19 binding molecule (*e.g.*, a bispecific antibody).

[0148] In some embodiments, the CD19 binding molecules are chimeric or humanized monoclonal antibodies. Chimeric and/or humanized antibodies, can be engineered to minimize the immune response by a human patient to antibodies produced in non-human subjects or derived from the expression of non-human antibody genes. Chimeric antibodies comprise a non-human animal antibody variable region and a human antibody constant region. Such antibodies retain the epitope binding specificity of the original monoclonal antibody, but can be less immunogenic when administered to humans, and therefore more likely to be tolerated by

the patient. For example, one or all (*e.g.*, one, two, or three) of the variable regions of the light chain(s) and/or one or all (*e.g.*, one, two, or three) of the variable regions the heavy chain(s) of a mouse antibody (*e.g.*, a mouse monoclonal antibody) can each be joined to a human constant region, such as, without limitation an IgG1 human constant region. Chimeric monoclonal antibodies can be produced by known recombinant DNA techniques. For example, a gene encoding the constant region of a non-human antibody molecule can be substituted with a gene encoding a human constant region (see Robinson *et al.*, PCT Patent Publication PCT/US86/02269; Akira, *et al.*, European Patent Application 184,187; or Taniguchi, M., European Patent Application 171,496). In addition, other suitable techniques that can be used to generate chimeric antibodies are described, for example, in U.S. Patent Nos. 4,816,567; 4,978,775; 4,975,369; and 4,816,397.

[0149] Chimeric or humanized antibodies and antigen binding fragments thereof of the present disclosure can be prepared based on the sequence of a murine monoclonal antibody. DNA encoding the heavy and light chain immunoglobulins can be obtained from a murine hybridoma of interest and engineered to contain non-murine (*e.g.*, human) immunoglobulin sequences using standard molecular biology techniques. For example, to create a chimeric antibody, the murine variable regions can be linked to human constant regions using known methods (see *e.g.*, U.S. Pat. No. 4,816,567 to Cabilly *et al.*). To create a humanized antibody, the murine CDR regions can be inserted into a human framework using known methods. See *e.g.*, U.S. Pat. No. 5,225,539 to Winter, and U.S. Pat. Nos. 5,530,101; 5,585,089; 5,693,762 and 6180370 to Queen *et al.*

[0150] A humanized antibody can be produced using a variety of known techniques, including but not limited to, CDR-grafting (see, *e.g.*, European Patent No. EP 239,400; International Publication No. WO 91/09967; and U.S. Pat. Nos. 5,225,539, 5,530,101, and 5,585,089), veneering or resurfacing (see, *e.g.*, European Patent Nos. EP 592,106 and EP 519,596; Padlan, 1991, Molecular Immunology, 28(4/5):489-498; Studnicka *et al.*, 1994, Protein Engineering, 7(6):805-814; and Roguska *et al.*, 1994, PNAS, 91:969-973), chain shuffling (see, *e.g.*, U.S. Pat. No. 5,565,332), and techniques disclosed in, *e.g.*, U.S. Patent Application Publication No. US2005/0042664, U.S. Patent Application Publication No. US2005/0048617, U.S. Pat. No. 6,407,213, U.S. Pat. No. 5,766,886, International Publication No. WO 9317105, Tan *et al.*, J. Immunol., 169:1119-25 (2002), Caldas *et al.*, Protein Eng., 13(5):353-60 (2000), Morea *et al.*, Methods, 20(3):267-79 (2000), Baca *et al.*, J. Biol. Chem., 272(16):10678-84 (1997), Roguska *et al.*, Protein Eng., 9(10):895-904 (1996), Couto *et al.*, Cancer Res., 55 (23 Supp):5973s-5977s (1995), Couto *et al.*, Cancer Res., 55(8):1717-22 (1995), Sandhu J S, Gene, 150(2):409-10 (1994), and Pedersen *et al.*, J. Mol. Biol., 235(3):959-73 (1994). Often, framework residues in the framework regions will be substituted with the corresponding residue

from the CDR donor antibody to alter, for example improve, antigen binding. These framework substitutions, *e.g.*, conservative substitutions are identified by known methods, *e.g.*, by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, *e.g.*, Queen *et al.*, U.S. Pat. No. 5,585,089; and Riechmann *et al.*, 1988, Nature, 332:323).

[0151] As provided herein, humanized antibodies or antibody fragments can comprise one or more CDRs from nonhuman immunoglobulin molecules and framework regions where the amino acid residues comprising the framework are derived completely or mostly from human germline. Multiple techniques for humanization of antibodies or antibody fragments are well-known and can essentially be performed following the method of Winter and co-workers (Jones *et al.*, Nature, 321:522-525 (1986); Riechmann *et al.*, Nature, 332:323-327 (1988); Verhoeyen *et al.*, Science, 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody, i.e., CDR-grafting (EP 239,400; PCT Publication No. WO 91/09967; and U.S. Pat. Nos. 4,816,567; 6,331,415; 5,225,539; 5,530,101; 5,585,089; 6,548,640). In such humanized antibodies and antibody fragments, substantially less than an intact human variable domain has been substituted by the corresponding sequence from a nonhuman species. Humanized antibodies are often human antibodies in which some CDR residues and possibly some framework (FR) residues are substituted by residues from analogous sites in rodent antibodies. Humanization of antibodies and antibody fragments can also be achieved by veneering or resurfacing (EP 592,106; EP 519,596; Padlan, 1991, Molecular Immunology, 28(4/5):489-498; Studnicka *et al.*, Protein Engineering, 7(6):805-814 (1994); and Roguska *et al.*, PNAS, 91:969-973 (1994)) or chain shuffling (U.S. Pat. No. 5,565,332).

[0152] The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is to reduce antigenicity. According to the so-called “best-fit” method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework (FR) for the humanized antibody (Sims *et al.*, J. Immunol., 151:2296 (1993); Chothia *et al.*, J. Mol. Biol., 196:901 (1987)). Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework can be used for several different humanized antibodies (see, *e.g.*, Nicholson *et al.* Mol. Immun. 34 (16-17): 1157-1165 (1997); Carter *et al.*, Proc. Natl. Acad. Sci. USA, 89:4285 (1992); Presta *et al.*, J. Immunol., 151:2623 (1993). In some embodiments, the framework region, *e.g.*, all four framework regions, of the heavy chain variable region are derived from a VH4_4-59 germline sequence. In one

embodiment, the framework region can comprise, one, two, three, four or five modifications, *e.g.*, substitutions, *e.g.*, conservative substitutions, *e.g.*, from the amino acid at the corresponding murine sequence. In one embodiment, the framework region, *e.g.*, all four framework regions of the light chain variable region are derived from a VK3_1.25 germline sequence. In one embodiment, the framework region can comprise, one, two, three, four or five modifications, *e.g.*, substitutions, *e.g.*, conservative substitutions, *e.g.*, from the amino acid at the corresponding murine sequence.

[0153] In certain embodiments, the CD19 binding molecules comprise a heavy chain variable region from a particular germline heavy chain immunoglobulin gene and/or a light chain variable region from a particular germline light chain immunoglobulin gene. For example, such antibodies can comprise or consist of a human antibody comprising heavy or light chain variable regions that are "the product of" or "derived from" a particular germline sequence. A human antibody that is "the product of" or "derived from" a human germline immunoglobulin sequence can be identified as such by comparing the amino acid sequence of the human antibody to the amino acid sequences of human germline immunoglobulins and selecting the human germline immunoglobulin sequence that is closest in sequence (i.e., greatest % identity) to the sequence of the human antibody (using the methods outlined herein). A human antibody that is "the product of" or "derived from" a particular human germline immunoglobulin sequence can contain amino acid differences as compared to the germline sequence, due to, for example, naturally-occurring somatic mutations or intentional introduction of site-directed mutation. However, a humanized antibody typically is at least 90% identical in amino acids sequence to an amino acid sequence encoded by a human germline immunoglobulin gene and contains amino acid residues that identify the antibody as being derived from human sequences when compared to the germline immunoglobulin amino acid sequences of other species (*e.g.*, murine germline sequences). In certain cases, a humanized antibody can be at least 95, 96, 97, 98 or 99%, or even at least 96%, 97%, 98%, or 99% identical in amino acid sequence to the amino acid sequence encoded by the germline immunoglobulin gene. Typically, a humanized antibody derived from a particular human germline sequence will display no more than 10-20 amino acid differences from the amino acid sequence encoded by the human germline immunoglobulin gene (prior to the introduction of any skew, pI and ablation variants herein; that is, the number of variants is generally low, prior to the introduction of the variants of the disclosure). In certain cases, the humanized antibody can display no more than 5, or even no more than 4, 3, 2, or 1 amino acid difference from the amino acid sequence encoded by the germline immunoglobulin gene (again, prior to the introduction of any skew, pI and ablation variants herein; that is, the number of variants is generally low, prior to the introduction of the variants of the disclosure).

[0154] In one embodiment, the parent antibody has been affinity matured. Structure-based methods can be employed for humanization and affinity maturation, for example as described in USSN 11/004,590. Selection based methods can be employed to humanize and/or affinity mature antibody variable regions, including but not limited to methods described in Wu *et al.*, 1999, J. Mol. Biol. 294:151-162; Baca *et al.*, 1997, J. Biol. Chem. 272(16):10678-10684; Rosok *et al.*, 1996, J. Biol. Chem. 271(37): 22611-22618; Rader *et al.*, 1998, Proc. Natl. Acad. Sci. USA 95: 8910-8915; Krauss *et al.*, 2003, Protein Engineering 16(10):753-759. Other humanization methods can involve the grafting of only parts of the CDRs, including but not limited to methods described in USSN 09/810,510; Tan *et al.*, 2002, J. Immunol. 169:1119-1125; De Pascalis *et al.*, 2002, J. Immunol. 169:3076-3084.

[0155] In some embodiments, the CD19 binding molecule comprises an ABM which is a Fab. Fab domains can be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain, or through recombinant expression. Fab domains typically comprise a CH1 domain attached to a VH domain which pairs with a CL domain attached to a VL domain. In a wild-type immunoglobulin, the VH domain is paired with the VL domain to constitute the Fv region, and the CH1 domain is paired with the CL domain to further stabilize the binding module. A disulfide bond between the two constant domains can further stabilize the Fab domain.

[0156] In some embodiments, the CD19 binding molecule comprises an ABM which is a scFab. In an embodiment, the antibody domains and the linker in the scFab fragment have one of the following orders in N-terminal to C-terminal direction: a) VH-CH1-linker-VL-CL, or b) VL-CL-linker-VH-CH1. In some cases, VL-CL-linker-VH-CH1 is used.

[0157] In another embodiment, the antibody domains and the linker in the scFab fragment have one of the following orders in N-terminal to C-terminal direction: a) VH-CL-linker-VL-CH1 or b) VL-CH1-linker-VH-CL.

[0158] Optionally in the scFab fragment, additionally to the natural disulfide bond between the CL-domain and the CH1 domain, also the antibody heavy chain variable domain (VH) and the antibody light chain variable domain (VL) are disulfide stabilized by introduction of a disulfide bond between the following positions: i) heavy chain variable domain position 44 to light chain variable domain position 100, ii) heavy chain variable domain position 105 to light chain variable domain position 43, or iii) heavy chain variable domain position 101 to light chain variable domain position 100 (numbering according to EU index of Kabat).

[0159] Such further disulfide stabilization of scFab fragments is achieved by the introduction of a disulfide bond between the variable domains VH and VL of the single chain Fab fragments. Techniques to introduce unnatural disulfide bridges for stabilization for a single chain Fv are

described *e.g.* in WO 94/029350, Rajagopal *et al*., 1997, Prot. Engin. 10:1453-59; Kobayashi *et al*., 1998, Nuclear Medicine & Biology, 25:387-393; and Schmidt, *et al*., 1999, Oncogene 18:1711-1721. In one embodiment, the optional disulfide bond between the variable domains of the scFab fragments is between heavy chain variable domain position 44 and light chain variable domain position 100. In one embodiment, the optional disulfide bond between the variable domains of the scFab fragments is between heavy chain variable domain position 105 and light chain variable domain position 43 (numbering according to EU index of Kabat).

[0160] In some embodiments, the CD19 binding molecule comprises an ABM which is a scFv. Single chain Fv antibody fragments comprise the VH and VL domains of an antibody in a single polypeptide chain, are capable of being expressed as a single chain polypeptide, and retain the specificity of the intact antibody from which it is derived. Generally, the scFv polypeptide further comprises a polypeptide linker between the VH and VL domain that enables the scFv to form the desired structure for target binding. Examples of linkers suitable for connecting the VH and VL chains of an scFV are the ABM linkers identified in Section 7.4.3, for example any of the linkers designated L1 through L58.

[0161] Unless specified, as used herein an scFv can have the VL and VH variable regions in either order, *e.g.*, with respect to the N-terminal and C-terminal ends of the polypeptide, the scFv can comprise VL-linker-VH or can comprise VH-linker-VL.

[0162] To create an scFv-encoding nucleic acid, the VH and VL-encoding DNA fragments are operably linked to another fragment encoding a linker, *e.g.*, encoding any of the linkers described in Section 7.4.3 (such as the amino acid sequence (Gly4˜Ser)3 (SEQ ID NO:53)), such that the VH and VL sequences can be expressed as a contiguous single-chain protein, with the VL and VH regions joined by the flexible linker (see *e.g.*, Bird *et al*., 1988, Science 242:423-426; Huston *et al*., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; McCafferty *et al*., 1990, Nature 348:552-554).

[0163] CD19 binding molecules can also comprise an ABM which is a Fv, a dsFv, a (Fab')2, a single domain antibody (SDAB), a VH or VL domain, or a camelid VHH domain (also called a nanobody).

[0164] CD19 binding molecules can comprise a single domain antibody composed of a single VH or VL domain which exhibits sufficient affinity to CD19. In an embodiment, the single domain antibody is a camelid VHH domain (see, *e.g.*, Riechmann, 1999, Journal of Immunological Methods 231:25-38; WO 94/04678).

[0165] Tables 1A and 1B (collectively "Table 1") list the sequences of exemplary CD19 binding sequences that can be included in CD19 binding molecules. The sequences set forth in Table 1A are based on the CD19 antibody NEG258.

TABLE 1A
NEG258-Based Binder Sequences

Chain	Portion	Sequence	SEQ ID NO:
NEG258_VH	CDR-H1 (Combined)	GYTFTTYWIQ	1
	CDR-H2 (Combined)	AVYPGDADTRYTQKFQG	2
	CDR-H3 (Combined)	DAGLEYYALDY	3
	CDR-H1 (Kabat)	TYWIQ	4
	CDR-H2 (Kabat)	AVYPGDADTRYTQKFQG	5
	CDR-H3 (Kabat)	DAGLEYYALDY	6
	CDR-H1 (Chothia)	GYTFTTY	7
	CDR-H2 (Chothia)	YPGDAD	8
	CDR-H3 (Chothia)	DAGLEYYALDY	9
	CDR-H1 (IMGT)	GYTFTTYW	10
	CDR-H2 (IMGT)	VYPGDADT	11
	CDR-H3 (IMGT)	GRDAGLEYYALDY	12
	VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYWIQWVRQ APGQRLEWMGAVYPGDADTRYTQKFQGRVTLTADRSAST AYMELSSLRSEDTAVYYCGRDAGLEYYALDYWGQGTLVT VSS	13
NEG258_VL	CDR-L1 (Combined)	RASQDVGTAVA	14
	CDR-L2 (Combined)	WASTRHT	15
	CDR-L3 (Combined)	QQYANFPLYT	16
	CDR-L1 (Kabat)	RASQDVGTAVA	17
	CDR-L2 (Kabat)	WASTRHT	18
	CDR-L3 (Kabat)	QQYANFPLYT	19
	CDR-L1 (Chothia)	SQDVGTA	20
	CDR-L2 (Chothia)	WAS	21
	CDR-L3 (Chothia)	YANFPLY	22
	CDR-L1 (IMGT)	QDVGTA	23
	CDR-L2 (IMGT)	WAS	24
	CDR-L3 (IMGT)	QQYANFPLYT	25
	VL	EIVMTQSPATLSVSPGERATLSCRASQDVGTAVAWYQQKP GQAPRLLIYWASTRHTGIPARFSGSGSGTEFTLTISSLQSE DFAVYFCQQYANFPLYTFGQGTKLEIK	26

[0166] In some embodiments, a CD19 binding molecule comprises CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 and CDR-H3 sequences of NEG258 as set forth in Table 1A. The CDR-L1,

CDR-L2, CDR-L3, CDR-H1, CDR-H2 and CDR-H3 sequences can be as defined by Kabat (SEQ ID NOs:17-19 and 4-6, respectively), Chothia (SEQ ID NOs:20-22 and 7-9, respectively), or IMGT (SEQ ID NOs: 23-25 and 10-12, respectively), or the combined Chothia and Kabat CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 and CDR-H3 sequences (SEQ ID NOs:14-16 and 1-3, respectively). The CD19 binding molecule can also comprise a light chain variable sequence (SEQ ID NO:26) and/or heavy chain variable sequence (SEQ ID NO:13) of the anti-CD19 antibody NEG258 as set forth in Table 1A.

[0167] The sequences set forth in Table 1B are based on the CD19 antibody NEG218.

NEG218-Based Sequences

Chain	Portion	Sequence	SEQ ID NO:
NEG218_VH	CDR-H1 (Combined)	GYSFTNYWMN	27
	CDR-H2 (Combined)	MIHPSDSEIRLNQKFQG	28
	CDR-H3 (Combined)	WYYLSSPMDY	29
	CDR-H1 (Kabat)	NYWMN	30
	CDR-H2 (Kabat)	MIHPSDSEIRLNQKFQG	31
	CDR-H3 (Kabat)	WYYLSSPMDY	32
	CDR-H1 (Chothia)	GYSFTNY	33
	CDR-H2 (Chothia)	HPSDSE	34
	CDR-H3 (Chothia)	WYYLSSPMDY	35
	CDR-H1 (IMGT)	GYSFTNYW	36
	CDR-H2 (IMGT)	IHPSDSEI	37
	CDR-H3 (IMGT)	SRWYYLSSPMDY	38
	VH	EVQLVQSGAEVKKPGESLKISCKASGYSFTNYWMNWVRQMPGKGLEWMGMIHPSDSEIRLNQKFQGQVTLSVDKSIGTAYMQWSSLKASDTAMYYCSRWYYLSSPMDYWGQGTTVTVSS	39
NEG218_VL	CDR-L1 (Combined)	RASQDVGTAVA	40
	CDR-L2 (Combined)	WASTRHT	41
	CDR-L3 **TABLE 1B**(Combined)	QQYSSYPYT	42
	CDR-L1 (Kabat)	RASQDVGTAVA	43
	CDR-L2 (Kabat)	WASTRHT	44
	CDR-L3 (Kabat)	QQYSSYPYT	45
	CDR-L1 (Chothia)	SQDVGTA	46
	CDR-L2 (Chothia)	WAS	47

NEG218-Based Sequences

Chain	Portion	Sequence	SEQ ID NO:
	CDR-L3 (Chothia)	YSSYPY	48
	CDR-L1 (IMGT)	QDVGTA	49
	CDR-L2 (IMGT)	WAS	50
	CDR-L3 (IMGT)	QQYSSYPYT	51
	VL	EIVMTQSPATLSVSPGERATLSCRASQDVGTAVAWYQQKP GQAPRLLIYWASTRHTGIPARFSGSGSGTEFTLTISSLQSE DFAVYFCQQYSSYPYTFGQGTKLEIK	52

[0168] In some embodiments, a CD19 binding molecule comprises CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 and CDR-H3 sequences of NEG218 as set forth in Table 1B. The CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 and CDR-H3 sequences can be as defined by Kabat (SEQ ID NOs:43-45 and 30-32, respectively), Chothia (SEQ ID NOs:46-48 and 33-35, respectively), or IMGT (SEQ ID NOs:49-51 and 36-38, respectively), or the combined Chothia and Kabat CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 and CDR-H3 sequences (SEQ ID NOs:40-42 and 27-29, respectively). The CD19 binding molecule can also comprise a light chain variable sequence (SEQ ID NO:52) and/or heavy chain variable sequence (SEQ ID NO:39) of the anti-CD19 antibody NEG218 as set forth in Table 1B.

[0169] Other CD19 binding molecules include amino acids that have been mutated, yet have at least 80, 85, 90, 95, 96, 97, 98, or 99 percent identity in the CDR regions with the CDR sequences described in Table 1. In some embodiments, such CD19 binding molecules include mutant amino acid sequences where no more than 1, 2, 3, 4 or 5 amino acids have been mutated in the CDR regions when compared with the CDR sequences described in Table 1.

[0170] Other CD19 binding molecules include VH and/or VL domains comprising amino acid sequences having at least 80, 85, 90, 95, 96, 97, 98, or 99 percent identity to the VH and/or VL sequences described in Table 1. In some embodiments, CD19 binding molecules include VH and/or VL domains where no more than 1, 2, 3, 4 or 5 amino acids have been mutated when compared with the VH and/or VL domains depicted in the sequences described in Table 1, while retaining substantially the same therapeutic activity.

[0171] The CD19 binding molecules can be fused or chemically conjugated (including both covalent and non-covalent conjugations) to a heterologous protein or polypeptide (or fragment thereof, for example to a polypeptide of at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90 or at least 100 amino acids). For example, a CD19 binding molecule can be fused directly or indirectly to a detectable protein, *e.g.*, an enzyme or a fluorescent protein.. Methods for fusing or conjugating proteins, polypeptides, or

peptides to an antibody or an antibody fragment are known and can be used to fuse or conjugate a protein or polypeptide to a CD19 binding molecule of the disclosure. See, *e.g.*, U.S. Patent Nos. 5,336,603, 5,622,929, 5,359,046, 5,349,053, 5,447,851, and 5,112,946; European Patent Nos. EP 307,434 and EP 367,166; International Publication Nos. WO 96/04388 and WO 91/06570; Ashkenazi *et al.*, 1991, Proc. Natl. Acad. Sci. USA 88:10535-10539; Zheng *et al.*, 1995, J. Immunol. 154:5590-5600; and Vil *et al.*, 1992, Proc. Natl. Acad. Sci. USA 89:11337- 11341.

[0172] Additional CD19 binding molecules can be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling can be employed to alter the activities of molecules of the disclosure or fragments thereof (*e.g.*, molecules or fragments thereof with higher affinities and lower dissociation rates). See, generally, U.S. Patent Nos. 5,605,793, 5,811,238, 5,830,721, 5,834,252, and 5,837,458; Patten *et al.*, 1997, Curr. Opinion Biotechnol. 8:724-33; Harayama, 1998, Trends Biotechnol. 16(2):76-82; Hansson *et al.*, 1999, J. Mol. Biol. 287:265-76; and Lorenzo and Blasco, 1998, Biotechniques 24(2):308-313. The CD19 binding molecules described herein or fragments thereof can be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. A polynucleotide encoding a fragment of a CD19 binding molecule described herein can be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

[0173] Moreover, CD19 binding molecules can be fused to marker sequences, such as a peptide to facilitate purification. In some embodiments, the marker amino acid sequence is a hexa-histidine peptide (SEQ ID NO: 54), such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz *et al.*, 1989, Proc. Natl. Acad. Sci. USA 86:821-824, for instance, hexa-histidine (SEQ ID NO: 54) provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the hemagglutinin ("HA") tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson *et al.*, 1984 Cell 37:767), and the "flag" tag.

7.3. Antigen Binding Modules of Multispecific Binding Molecules

[0174] Typically, one or more ABMs of the MBMs comprise immunoglobulin-based antigen-binding domains, for example the sequences of antibody fragments or derivatives. These antibody fragments and derivatives typically include the CDRs of an antibody and can include larger fragments and derivatives thereof, *e.g.*, Fabs, scFabs, Fvs, and scFvs.

[0175] Immunoglobulin-based ABMs can comprise modifications to framework residues within a VH and/or a VL, *e.g.* to improve the properties of a MBM containing the ABM. For example, framework modifications can be made to decrease immunogenicity of a MBM. One approach for making such framework modifications is to "back-mutate" one or more framework residues of the ABM to a corresponding germline sequence. Such residues can be identified by comparing framework sequences to germline sequences from which the ABM is derived. To “match” framework region sequences to desired germline configuration, residues can be "back-mutated" to a corresponding germline sequence by, for example, site-directed mutagenesis. MBMs having such "back-mutated" ABMs are intended to be encompassed by the disclosure.

[0176] Another type of framework modification involves mutating one or more residues within a framework region, or even within one or more CDR regions, to remove T-cell epitopes to thereby reduce potential immunogenicity of a MBM. This approach is also referred to as "deimmunization" and is described in further detail in U.S. Patent Publication 20030153043 by Carr *et al.*

[0177] ABMs can also be modified to have altered glycosylation, which can be useful, for example, to increase the affinity of a MBM for one or more of its antigens. Such carbohydrate modifications can be accomplished by, for example, altering one or more sites of glycosylation within an ABM sequence. For example, one or more amino acid substitutions can be made that result in elimination of one or more variable region framework glycosylation sites to thereby eliminate glycosylation at that site. Such aglycosylation can increase the affinity of the MBM for an antigen. Such an approach is described in, *e.g.*, U.S. Patent Nos. 5,714,350 and 6,350,861 by Co *et al.*

7.3.1. Immunoglobulin Based ABMs

7.3.1.1. Fabs

[0178] In certain aspects, an ABM is a Fab domain.

[0179] For the MBMs of the disclosure, it is advantageous to use Fab heterodimerization strategies to permit the correct association of Fab domains belonging to the same ABM and minimize aberrant pairing of Fab domains belonging to different ABMs. For example, the Fab heterodimerization strategies shown in Table 2 below can be used:

TABLE 2
Fab Heterodimerization Strategies

Name	STRATEGY	VH	CH1	VL	CL	REFERENCE
F1	CrossMabCH1-CL	WT	CL domain	WT	CH1 domain	Schaefer *et al.*, 2011, Cancer Cell 2011; 20:472-86; PMID:22014573.

	TABLE 2 Fab Heterodimerization Strategies					
Name	**STRATEGY**	**VH**	**CH1**	**VL**	**CL**	**REFERENCE**
F2	orthogonal Fab VHVRD1CH1CRD2 - VLVRD1CλCRD2	39K, 62E	H172A, F174G	1R, 38D, (36F)	L135Y, S176W	Lewis *et al.*, 2014, Nat Biotechnol 32:191-8
F3	orthogonal Fab VHVRD2CH1wt - VLVRD2Cλwt	39Y	WT	38R	WT	Lewis *et al.*, 2014, Nat Biotechnol 32:191-8
F4	TCR CαCβ	39K	TCR Cα	38D	TCR Cβ	Wu *et al.*, 2015, MAbs 7:364-76
F5	CR3	WT	T192E	WT	N137K, S114A	Golay *at al.*, 2016, J Immunol 196:3199-211.
F6	MUT4	WT	L143Q, S188V	WT	V133T, S176V	Golay *at al.*, 2016, J Immunol 196:3199-211.
F7	DuetMab	WT	F126C	WT	S121C	Mazor *et al.*, 2015, MAbs 7:377-89; Mazor *et al.*, 2015, MAbs 7:461-669.

[0180] Accordingly, in certain embodiments, correct association between the two polypeptides of a Fab is promoted by exchanging the VL and VH domains of the Fab for each other or exchanging the CH1 and CL domains for each other, *e.g.*, as described in WO 2009/080251.

[0181] Correct Fab pairing can also be promoted by introducing one or more amino acid modifications in the CH1 domain and one or more amino acid modifications in the CL domain of the Fab and/or one or more amino acid modifications in the VH domain and one or more amino acid modifications in the VL domain. The amino acids that are modified are typically part of the VH:VL and CH1:CL interface such that the Fab components preferentially pair with each other rather than with components of other Fabs.

[0182] In one embodiment, the one or amino acid modifications are limited to the conserved framework residues of the variable (VH, VL) and constant (CH1, CL) domains as indicated by the Kabat numbering of residues. Almagro, 2008, Frontiers In Bioscience 13:1619-1633 provides a definition of the framework residues on the basis of Kabat, Chothia, and IMGT numbering schemes.

[0183] In one embodiment, the modifications introduced in the VH and CH1 and/or VL and CL domains are complementary to each other. Complementarity at the heavy and light chain interface can be achieved on the basis of steric and hydrophobic contacts, electrostatic/charge interactions or a combination of the variety of interactions. The complementarity between protein surfaces is broadly described in the literature in terms of lock and key fit, knob into hole, protrusion and cavity, donor and acceptor etc., all implying the nature of structural and chemical match between the two interacting surfaces.

[0184] In one embodiment, the one or more introduced modifications introduce a new hydrogen bond across the interface of the Fab components. In one embodiment, the one or more introduced modifications introduce a new salt bridge across the interface of the Fab components. Exemplary substitutions are described in WO 2014/150973 and WO 2014/082179.

[0185] In some embodiments, the Fab domain comprises a 192E substitution in the CH1 domain and 114A and 137K substitutions in the CL domain, which introduces a salt-bridge between the CH1 and CL domains (see, Golay *et al.*, 2016, J Immunol 196:3199-211).

[0186] In some embodiments, the Fab domain comprises a 143Q and 188V substitutions in the CH1 domain and 113T and 176V substitutions in the CL domain, which serves to swap hydrophobic and polar regions of contact between the CH1 and CL domain (see, Golay *et al.*, 2016, J Immunol 196:3199-211).

[0187] In some embodiments, the Fab domain can comprise modifications in some or all of the VH, CH1, VL, CL domains to introduce orthogonal Fab interfaces which promote correct assembly of Fab domains (Lewis *et al.*, 2014 Nature Biotechnology 32:191–198). In an embodiment, 39K, 62E modifications are introduced in the VH domain, H172A, F174G modifications are introduced in the CH1 domain, 1R, 38D, (36F) modifications are introduced in the VL domain, and L135Y, S176W modifications are introduced in the CL domain. In another embodiment, a 39Y modification is introduced in the VH domain and a 38R modification is introduced in the VL domain.

[0188] Fab domains can also be modified to replace the native CH1:CL disulfide bond with an engineered disulfide bond, thereby increasing the efficiency of Fab component pairing. For example, an engineered disulfide bond can be introduced by introducing a 126C in the CH1 domain and a 121C in the CL domain (see, Mazor *et al.*, 2015, MAbs 7:377-89).

[0189] Fab domains can also be modified by replacing the CH1 domain and CL domain with alternative domains that promote correct assembly. For example, Wu *et al.*, 2015, MAbs 7:364-76, describes substituting the CH1 domain with the constant domain of the α T cell receptor and substituting the CL domain with the β domain of the T cell receptor, and pairing these domain replacements with an additional charge-charge interaction between the VL and VH domains by introducing a 38D modification in the VL domain and a 39K modification in the VH domain.

[0190] ABMs can comprise a single chain Fab fragment, which is a polypeptide consisting of an antibody heavy chain variable domain (VH), an antibody constant domain 1 (CH1), an antibody light chain variable domain (VL), an antibody light chain constant domain (CL) and a linker. In some embodiments, the antibody domains and the linker have one of the following orders in N-terminal to C-terminal direction: a) VH-CH1-linker-VL-CL, b) VL-CL-linker-VH-CH1, c) VH-CL-

linker-VL-CH1 or d) VL-CH1-linker-VH-CL. The linker can be a polypeptide of at least 30 amino acids, *e.g.*, between 32 and 50 amino acids. The single chain Fab domains are stabilized via the natural disulfide bond between the CL domain and the CH1 domain.

[0191] In an embodiment, the antibody domains and the linker in the single chain Fab fragment have one of the following orders in N-terminal to C-terminal direction: a) VH-CH1-linker-VL-CL, or b) VL-CL-linker-VH-CH1. In some cases, VL-CL-linker-VH-CH1 is used.

[0192] In another embodiment, the antibody domains and the linker in the single chain Fab fragment have one of the following orders in N-terminal to C-terminal direction: a) VH-CL-linker-VL-CH1 or b) VL-CH1-linker-VH-CL.

[0193] Optionally in the single chain Fab fragment, additionally to the natural disulfide bond between the CL-domain and the CH1 domain, also the antibody heavy chain variable domain (VH) and the antibody light chain variable domain (VL)ABM are disulfide stabilized by introduction of a disulfide bond between the following positions: i) heavy chain variable domain position 44 to light chain variable domain position 100, ii) heavy chain variable domain position 105 to light chain variable domain position 43, or iii) heavy chain variable domain position 101 to light chain variable domain position 100 (numbering according to EU index of Kabat).

[0194] In one embodiment, the optional disulfide bond between the variable domains of the single chain Fab fragments is between heavy chain variable domain position 44 and light chain variable domain position 100. In one embodiment, the optional disulfide bond between the variable domains of the single chain Fab fragments is between heavy chain variable domain position 105 and light chain variable domain position 43 (numbering according to EU index of Kabat).

7.3.1.2. scFvs

[0195] In certain aspects, an ABM is a single chain Fv or "scFv". Examples of linkers suitable for connecting the VH and VL chains of an scFV are the ABM linkers identified in Section 7.4.3, for example any of the linkers designated L1 through L54.

[0196] To create an scFv-encoding nucleic acid, the VH and VL-encoding DNA fragments are operably linked to another fragment encoding a linker, *e.g.*, encoding any of the ABM linkers described in Section 7.4.3 (such as the amino acid sequence (Gly4˜Ser)3 (SEQ ID NO:53)

7.3.1.3. Other immunoglobulin-based ABMs

[0197] MBMs can also comprise ABMs having an immunoglobulin format which is other than Fab or scFv, for example Fv, dsFv, (Fab')2, a single domain antibody (SDAB), a VH or VL domain, or a camelid VHH domain (also called a nanobody).

[0198] An ABM can be a single domain antibody composed of a single VH or VL domain which exhibits sufficient affinity to the target. In an embodiment, the single domain antibody is a camelid VHH domain (see, *e.g.*, Riechmann, 1999, Journal of Immunological Methods 231:25-38; WO 94/04678).

7.3.2. Non-Immunoglobulin Based ABM

[0199] In certain embodiments, MBMs comprise one or more of the ABMs derived from non-antibody scaffold proteins (including, but not limited to, designed ankyrin repeat proteins (DARPins), Avimers (short for avidity multimers), Anticalin/Lipocalins, Centyrins, Kunitz domains, Adnexins, Affilins, Affitins (also known as Nonfitins), Knottins, Pronectins, Versabodies, Duocalins, and Fynomers), ligands, receptors, cytokines or chemokines.

[0200] Non-immunoglobulin scaffolds that can be used in the MBMs include those listed in Tables 3 and 4 of Mintz and Crea, 2013, Bioprocess International 11(2):40-48; in Figure 1, Table 1 and Figure I of Vazquez-Lombardi *et al.*, 2015, Drug Discovery Today 20(10):1271-83; in Table 1 and Box 2 of Skrlec *et al.*, 2015, Trends in Biotechnology 33(7):408-18. The contents of Tables 3 and 4 of Mintz and Crea, 2013, Bioprocess International 11(2):40-48; in Figure 1, Table 1 and Figure I of Vazquez-Lombardi *et al.*, 2015, Drug Discovery Today 20(10):1271-83; in Table 1 and Box 2 of Skrlec *et al.*, 2015, Trends in Biotechnology 33(7):408-18 (collectively, "Scaffold Disclosures"). In a particular embodiment, the Scaffold Disclosures are incorporated by reference for what they disclose relating to Adnexins. In another embodiment, the Scaffold Disclosures are incorporated by reference for what they disclose relating to Avimers. In another embodiment, the Scaffold Disclosures are incorporated by reference for what they disclose relating to Affibodies. In yet another embodiment, the Scaffold Disclosures are incorporated by reference for what they disclose relating to Anticalins. In yet another embodiment, the Scaffold Disclosures are incorporated by reference for what they disclose relating to DARPins. In yet another embodiment, the Scaffold Disclosures are incorporated by reference for what they disclose relating to Kunitz domains. In yet another embodiment, the Scaffold Disclosures are incorporated by reference for what they disclose relating to Knottins. In yet another embodiment, the Scaffold Disclosures are incorporated by reference for what they disclose relating to Pronectins. In yet another embodiment, the Scaffold Disclosures are incorporated by reference for what they disclose relating to Nanofitins. In yet another embodiment, the Scaffold Disclosures are incorporated by reference for what they disclose relating to Affilins. In yet another embodiment, the Scaffold Disclosures are incorporated by reference for what they disclose relating to Adnectins. In yet another embodiment, the Scaffold Disclosures are incorporated by reference for what they disclose relating to ABMs. In yet another embodiment, the Scaffold Disclosures are incorporated by reference for what they disclose relating to Adhirons. In yet another embodiment, the Scaffold

Disclosures are incorporated by reference for what they disclose relating to Affimers. In yet another embodiment, the Scaffold Disclosures are incorporated by reference for what they disclose relating to Alphabodies. In yet another embodiment, the Scaffold Disclosures are incorporated by reference for what they disclose relating to Armadillo Repeat Proteins. In yet another embodiment, the Scaffold Disclosures are incorporated by reference for what they disclose relating to Atrimers/Tetranectins. In yet another embodiment, the Scaffold Disclosures are incorporated by reference for what they disclose relating to Obodies/OB-folds. In yet another embodiment, the Scaffold Disclosures are incorporated by reference for what they disclose relating to Centyrins. In yet another embodiment, the Scaffold Disclosures are incorporated by reference for what they disclose relating to Repebodies. In yet another embodiment, the Scaffold Disclosures are incorporated by reference for what they disclose relating to Anticalins. In yet another embodiment, the Scaffold Disclosures are incorporated by reference for what they disclose relating to Atrimers. In yet another embodiment, the Scaffold Disclosures are incorporated by reference for what they disclose relating to bicyclic peptides. In yet another embodiment, the Scaffold Disclosures are incorporated by reference for what they disclose relating to cys-knots. In yet another embodiment, the Scaffold Disclosures are incorporated by reference for what they disclose relating to Fn3 scaffolds (including Adnectins, Centryrins, Pronectins, and Tn3).

[0201] In an embodiment, an ABM can be a designed ankyrin repeat protein ("DARPin"). DARPins are antibody mimetic proteins that typically exhibit highly specific and high-affinity target protein binding. They are typically genetically engineered and derived from natural ankyrin proteins and consist of at least three, usually four or five repeat motifs of these proteins. Their molecular mass is about 14 or 18 kDa (kilodaltons) for four- or five-repeat DARPins, respectively. Examples of DARPins can be found, for example in U.S. Pat. No. 7,417,130. Multispecific binding molecules comprising DARPin binding modules and immunoglobulin-based binding modules are disclosed in, for example, U.S. Publication No. 2015/0030596 A1.

[0202] In another embodiment, an ABM can be an Affibody. An Affibody is well known and refers to affinity proteins based on a 58 amino acid residue protein domain, derived from one of the IgG binding domain of staphylococcal protein A.

[0203] In another embodiment, an ABM can be an Anticalin. Anticalins are well known and refer to another antibody mimetic technology, where the binding specificity is derived from Lipocalins. Anticalins can also be formatted as dual targeting protein, called Duocalins.

[0204] In another embodiment, an ABM can be a Versabody. Versabodies are well known and refer to another antibody mimetic technology. They are small proteins of 3-5 kDa with >15%

cysteines, which form a high disulfide density scaffold, replacing the hydrophobic core of typical proteins.

[0205] Other non-immunoglobulin ABMs include "A" domain oligomers (also known as Avimers) (see for example, U.S. Patent Application Publication Nos. 2005/0164301, 2005/0048512, and 2004/017576), Fn3 based protein scaffolds (see for example, U.S. Patent Application Publication 2003/0170753), VASP polypeptides, Avian pancreatic polypeptide (aPP), Tetranectin (based on CTLD3), Affililin (based on γB-crystallin/ubiquitin), Knottins, SH3 domains, PDZ domains, Tendamistat, Neocarzinostatin, Protein A domains, Lipocalins, Transferrin, or Kunitz domains. In one aspect, ABMs useful in the construction of the MBMs comprise fibronectin-based scaffolds as exemplified in WO 2011/130324.

[0206] Moreover, in certain aspects, an ABM comprises a ligand binding domain of a receptor or a receptor binding domain of a ligand.

7.4. Connectors

[0207] It is contemplated that the CD19 binding molecules can in some instances include pairs of ABMs or ABM chains (*e.g.*, the VH-CH1 or VL-CL component of a Fab) connected directly to one another, *e.g.*, as a fusion protein without a linker. For example, the CD19 binding molecules can comprise connector moieties linking individual ABMs or ABM chains. The use of connector moieties can improve target binding, for example by increasing flexibility of the ABMs within a CD19 binding molecule and thus reducing steric hindrance. The ABMs or ABM chains can be connected to one another through, for example, Fc domains (each Fc domain representing a pair of associated Fc regions) and/or ABM linkers. The use of Fc domains will typically require the use of hinge regions as connectors of the ABMs or ABM chains for optimal antigen binding. Thus, the term "connector" encompasses, but is not limited to, Fc regions, Fc domains, and hinge regions.

[0208] Connectors can be selected or modified to, for example, increase or decrease the biological half-life of a CD19 binding molecule. For example, to decrease biological half-life, one or more amino acid mutations can be introduced into a CH2-CH3 domain interface region of an Fc-hinge fragment such that a CD19 binding molecule comprising the fragment has impaired Staphylococcyl Protein A (SpA) binding relative to native Fc-hinge domain SpA binding. This approach is described in further detail in U.S. Patent No. 6,165,745 by Ward *et al.* Alternatively, a CD19 binding molecule can be modified to increase its biological half-life. For example, one or more of the following mutations can be introduced: T252L, T254S, T256F, as described in U.S. Patent No. 6,277,375 to Ward. Alternatively, to increase the biological half-life, a CD19 binding molecule can be altered within a CH1 or CL region to contain a salvage receptor

binding epitope taken from two loops of a CH2 domain of an Fc region of an IgG, as described in U.S. Patent Nos. 5,869,046 and 6,121,022 by Presta *et al.*

[0209] Examples of Fc domains (formed by the pairing of two Fc regions), hinge regions and ABM linkers are described in Sections 7.4.1, 7.4.2, and 7.4.3, respectively.

7.4.1. Fc domains

[0210] The CD19 binding molecules can include an Fc domain derived from any suitable species. In one embodiment, the Fc domain is derived from a human Fc domain.

[0211] The Fc domain can be derived from any suitable class of antibody, including IgA (including subclasses IgA1 and IgA2), IgD, IgE, IgG (including subclasses IgG1, IgG2, IgG3 and IgG4), and IgM. In one embodiment, the Fc domain is derived from IgG1, IgG2, IgG3 or IgG4. In one embodiment, the Fc domain is derived from IgG1. In one embodiment, the Fc domain is derived from IgG4.

[0212] The Fc domain comprises two polypeptide chains, each referred to as a heavy chain Fc region. The two heavy chain Fc regions dimerize to create the Fc domain. The two Fc regions within the Fc domain can be the same or different from one another. In a native antibody the Fc regions are typically identical, but for the purpose of producing multispecific binding molecules of the disclosure, the Fc regions might advantageously be different to allow for heterodimerization, as described in Section 7.4.1.5 below.

[0213] Typically each heavy chain Fc region comprises or consists of two or three heavy chain constant domains.

[0214] In native antibodies, the heavy chain Fc region of IgA, IgD and IgG is composed of two heavy chain constant domains (CH2 and CH3) and that of IgE and IgM is composed of three heavy chain constant domains (CH2, CH3 and CH4). These dimerize to create an Fc domain.

[0215] In the present disclosure, the heavy chain Fc region can comprise heavy chain constant domains from one or more different classes of antibody, for example one, two or three different classes.

[0216] In one embodiment, the heavy chain Fc region comprises CH2 and CH3 domains derived from IgG1. An exemplary sequence of a heavy chain Fc region derived from human IgG1 is given in SEQ ID NO:1109:

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP (SEQ ID NO:1109).

In some embodiments, a CD19 binding molecule of the disclosure comprises a Fc region whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1109 modified with one or more of the substitutions described in Section 7.4.1 and its subparts.

[0217] In one embodiment, the heavy chain Fc region comprises CH2 and CH3 domains derived from IgG2.

[0218] In one embodiment, the heavy chain Fc region comprises CH2 and CH3 domains derived from IgG3.

[0219] In one embodiment, the heavy chain Fc region comprises CH2 and CH3 domains derived from IgG4.

[0220] In one embodiment, the heavy chain Fc region comprises a CH4 domain from IgM. The IgM CH4 domain is typically located at the C-terminus of the CH3 domain.

[0221] In one embodiment, the heavy chain Fc region comprises CH2 and CH3 domains derived from IgG and a CH4 domain derived from IgM.

[0222] It will be appreciated that the heavy chain constant domains for use in producing a heavy chain Fc region for the CD19 binding molecules of the present disclosure can include variants of the naturally occurring constant domains described above. Such variants can comprise one or more amino acid variations compared to wild type constant domains. In one example the heavy chain Fc region of the present disclosure comprises at least one constant domain that varies in sequence from the wild type constant domain. It will be appreciated that the variant constant domains can be longer or shorter than the wild type constant domain. For example, the variant constant domains are at least 60% identical or similar to a wild type constant domain. In another example the variant constant domains are at least 70% identical or similar. In another example the variant constant domains are at least 75% identical or similar. In another example the variant constant domains are at least 80% identical or similar. In another example the variant constant domains are at least 85% identical or similar. In another example the variant constant domains are at least 90% identical or similar. In another example the variant constant domains are at least 95% identical or similar. In another example the variant constant domains are at least 99% identical or similar. Exemplary Fc variants are described in Sections 7.4.1.1 through 7.4.1.5, *infra*.

[0223] IgM and IgA occur naturally in humans as covalent multimers of the common H2L2 antibody unit. IgM occurs as a pentamer when it has incorporated a J-chain, or as a hexamer when it lacks a J-chain. IgA occurs as monomer and dimer forms. The heavy chains of IgM and IgA possess an 18 amino acid extension to the C-terminal constant domain, known as a tailpiece. The tailpiece includes a cysteine residue that forms a disulfide bond between heavy

chains in the polymer, and is believed to have an important role in polymerization. The tailpiece also contains a glycosylation site. In certain embodiments, the CD19 binding molecules of the present disclosure do not comprise a tailpiece.

[0224] The Fc domains that are incorporated into the CD19 binding molecules of the present disclosure can comprise one or more modifications that alter one or more functional properties of the proteins, such as serum half-life, complement fixation, Fc receptor binding, and/or antigen-dependent cellular cytotoxicity. Furthermore, a CD19 binding molecule can be chemically modified (*e.g.*, one or more chemical moieties can be attached to the CD19 binding molecule) or be modified to alter its glycosylation, again to alter one or more functional properties of the CD19 binding molecule.

[0225] Effector function of an antibody molecule includes complement-mediated effector function, which is mediated by, for example, binding of the C1 component of the complement to the antibody. Activation of complement is important in the opsonization and direct lysis of pathogens. In addition, it stimulates the inflammatory response by recruiting and activating phagocytes to the site of complement activation. Effector function includes Fc receptor (FcR)-mediated effector function, which can be triggered upon binding of the constant domains of an antibody to an Fc receptor (FcR). Antigen-antibody complex-mediated crosslinking of Fc receptors on effector cell surfaces triggers a number of important and diverse biological responses including engulfment and destruction of antibody-coated particles, clearance of immune complexes, lysis of antibody-coated target cells by killer cells (called antibody-dependent cell-mediated cytotoxicity, or ADCC), release of inflammatory mediators, placental transfer and control of immunoglobulin production.

[0226] Fc regions can be altered by replacing at least one amino acid residue with a different amino acid residue to alter the effector functions. For example, one or more amino acids can be replaced with a different amino acid residue such that the Fc region has an altered affinity for an effector ligand. The effector ligand to which affinity is altered can be, for example, an Fc receptor or the C1 component of complement. This approach is described in, *e.g.*, U.S. Patent Nos. 5,624,821 and 5,648,260, both by Winter *et al.* Modified Fc regions can also alter C1q binding and/or reduce or abolish complement dependent cytotoxicity (CDC). This approach is described in, *e.g.*, U.S. Patent Nos. 6,194,551 by Idusogie *et al.* Modified Fc regions can also alter the ability of an Fc region to fix complement. This approach is described in, *e.g.*, the PCT Publication WO 94/29351 by Bodmer *et al.* Allotypic amino acid residues include, but are not limited to, constant region of a heavy chain of the IgG1, IgG2, and IgG3 subclasses as well as constant region of a light chain of the kappa isotype as described by Jefferis *et al.*, 2009, MAbs, 1:332-338.

[0227] Fc regions can also be modified to "silence" the effector function, for example, to reduce or eliminate the ability of a CD19 binding molecule to mediate antibody dependent cellular cytotoxicity (ADCC) and/or antibody dependent cellular phagocytosis (ADCP). This can be achieved, for example, by introducing a mutation in an Fc region. Such mutations have been described in the art: LALA and N297A (Strohl, 2009, Curr. Opin. Biotechnol. 20(6):685-691); and D265A (Baudino *et al.*, 2008, J. Immunol. 181: 6664-69; Strohl, *supra*). Examples of silent Fc IgG1 antibodies comprise the so-called LALA mutant comprising L234A and L235A mutation in the IgG1 Fc amino acid sequence. Another example of a silent IgG1 antibody comprises the D265A mutation. Another silent IgG1 antibody comprises the so-called DAPA mutant comprising D265A and P329A mutations in the IgG1 Fc amino acid sequence. Another silent IgG1 antibody comprises the N297A mutation, which results in aglycosylated/non-glycosylated antibodies.

[0228] Fc regions can be modified to increase the ability of a CD19 binding molecule containing the Fc region to mediate antibody dependent cellular cytotoxicity (ADCC) and/or antibody dependent cellular phagocytosis (ADCP), for example, by modifying one or more amino acid residues to increase the affinity of the CD19 binding molecule for an activating Fcγ receptor, or to decrease the affinity of the CD19 binding molecule for an inhibitory Fcγ receptor. Human activating Fcγ receptors include FcγRIa, FcγRIIa, FcγRIIIa, and FcγRIIIb, and human inhibitory Fcγ receptor includes FcγRIIb. This approach is described in, *e.g.*, the PCT Publication WO 00/42072 by Presta. Moreover, binding sites on human IgG1 for FcγRI, FcγRII, FcγRIII and FcRn have been mapped and variants with improved binding have been described (see Shields *et al.*, J. Biol. Chem. 276:6591-6604, 2001). Optimization of Fc-mediated effector functions of monoclonal antibodies such as increased ADCC/ADCP function has been described (see Strohl, 2009, Current Opinion in Biotechnology 20:685–691). Mutations that can enhance ADCC/ADCP function include one or more mutations selected from G236A, S239D, F243L, P247I, D280H, K290S, R292P, S298A, S298D, S298V, Y300L, V305I, A330L, I332E, E333A, K334A, A339D, A339Q, A339T, and P396L (all positions by EU numbering).

[0229] Fc regions can also be modified to increase the ability of a CD19 binding molecule to mediate ADCC and/or ADCP, for example, by modifying one or more amino acids to increase the affinity of the CD19 binding molecule for an activating receptor that would typically not recognize the parent CD19 binding molecule, such as FcαRI. This approach is described in, *e.g.*, Borrok *et al.*, 2015, mAbs. 7(4):743-751.

[0230] Accordingly, in certain aspects, the CD19 binding molecules of the present disclosure can include Fc domains with altered effector function such as, but not limited to, binding to Fc-receptors such as FcRn or leukocyte receptors (for example, as described above or in Section

7.4.1.1), binding to complement (for example as described above or in Section 7.4.1.2), modified disulfide bond architecture (for example as described above or in Section 7.4.1.3), or altered glycosylation patterns (for example as described above or in Section 7.4.1.4). The Fc domains can also be altered to include modifications that improve manufacturability of asymmetric CD19 binding molecules, for example by allowing heterodimerization, which is the preferential pairing of non-identical Fc regions over identical Fc regions. Heterodimerization permits the production of CD19 binding molecules in which different ABMs are connected to one another by an Fc domain containing Fc regions that differ in sequence. Examples of heterodimerization strategies are exemplified in Section 7.4.1.5 (and subsections thereof).

[0231] It will be appreciated that any of the modifications described in Sections 7.4.1.1 through 7.4.1.5 can be combined in any suitable manner to achieve the desired functional properties and/or combined with other modifications to alter the properties of the CD19 binding molecules. In some embodiments, a CD19 binding molecule comprises a IgG1 Fc domain having a mutation at 1, 2, 3, 4, 5, 6, or more than 6 of positions 233, 234, 235, 236, 237, 239, 265, 266, 267, 268, 269, 297, 299, 322, 327, 328, 329, 330, 331 and 332 (EU numbering). For example, a CD19 binding molecule can comprise an IgG1 sequence of SEQ ID NO:1109 with a mutation at 1, 2, 3, 4, 5, 6, or more than 6 of positions 233, 234, 235, 236, 237, 239, 265, 266, 267, 268, 269, 297, 299, 322, 327, 328, 329, 330, 331 and 332.

[0232] In some embodiments, a CD19 binding molecule comprises a first and second human IgG1 Fc region having amino acid substitutions selected from the following combinations of substitutions: substitutions L234A, L235A, and G237A (“LALAGA”); substitutions L234A, L235A, S267K, and P329A (“LALASKPA”); subsitutions D265A, P329A, and S267K (“DAPASK”); substitutions G237A, D265A, and P329A (“GADAPA”); substitutions G237A, D265A, P329A, and S267K (“GADAPASK”); substitutions L234A, L235A, and P329G (“LALAPG”), and substitutions L234A, L235A, and P329A (“LALAPA”), wherein the amino acid residues are numbered according to the EU numbering system. It should be understood that the terms “LALAGA”, “LALASKPA”, “DAPASK”, “GADAPA”, “GADAPASK”, “LALAPG”, and “LALAPA” represent shorthand terminology for the different combinations of subsitutions described in this paragraph rather than contiguous amino acid sequences.

[0233] In another embodiment, a CD19 binding molecule comprises a human IgG1 Fc region having amino acid substitutions selected from the combinations of substitutions L234A, L235A, S267K, P329A (“LALASKPA”), or substitutions G237A, D265A, P329A, S267K (“GADAPASK”), wherein the amino acid residues are numbered according to the EU numbering system.

[0234] In a further embodiment, a CD19 binding molecules comprises a Fc region selected from FCV1-FCV7. (See Table A below)

[0235] In yet a further embodiment, a CD19 binding molecules comprises a Fc region which is FCV4 or FCV7.

[0236] In some aspects, the CD19 binding molecule has reduced or undetectable binding affinity to a Fc gamma receptor or C1q compared to a polypeptide comprising the wild-type human IgG1 Fc region optionally measured by surface plasmon resonance using a Biacore T200 instrument, wherein the Fc gamma receptor is selected from the group consisting of Fc gamma RIA, Fc gamma RIIIa V158 variant and Fc gamma RIIIa F158 variant, and wherein the binding compared to wildtype is reduced by 50%, 80%, 90%, 95%, 98%, 99% or is undetectable.

[0237] In some aspects, the CD19 binding molecule has reduced or undetectable effector function compared to a polypeptide comprising the wild-type human IgG1 Fc region.

[0238] In some aspects, the CD19 binding molecule is capable of binding to an antigen without triggering detectable antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), or complement dependent cytotoxicity (CDC). In some aspects, the effector function to be reduced or diminished is antibody-dependent cell-mediated cytotoxicity (ADCC) in the individual. In some aspects, the effector function to be reduced or diminished is antibody-dependent cellular phagocytosis (ADCP) in the individual. In some aspects, the effector function to be reduced or diminished is complement dependent cytotoxicity (CDC) in the individual. In some aspects, the first and second Fc regions of a Fc domain each comprise a nucleic acid sequence selected from a nucleic acid sequence listed in Table A below, or any sequence having at least about 90%, 91% 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity thereto.

[0239] In an embodiment, a nucleic acid encoding a Fc region comprises the nucleic acid sequence of FCV-7 (see Table A below), or a sequence having at least about 90%, 91% 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity thereto. In an embodiment, a nucleic acid encoding a Fc region comprises the nucleic acid sequence of FCV-4 (see Table A below), or a sequence having at least about 90%, 91% 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity thereto. In some aspects, a Fc domain comprises first and second Fc regions each of which comprises an amino acid sequence selected from an amino acid sequence listed in Table A below, or any sequence having at least about 90%, 91% 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity thereto.

[0240] In an embodiment, a Fc domain comprises first and second Fc regions comprising the amino acid sequence of FCV-7 (see Table A below), or a sequence having at least about 90%, 91% 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity thereto. In an embodiment, a Fc domain comprises first and second Fc regions comprising the amino acid sequence of

FCV-4 (see Table A below), or a sequence having at least about 90%, 91% 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity thereto.

[0241] Additionally provided herein are vectors comprising the polynucleotides encoding CD19 binding molecules comprising a Fc region selected from FCV1-FCV7. (See Table A below)

[0242] Also provided herein are host cells comprising vectors or polynucleotides encoding and capable of expressing CD19 binding molecules comprising a Fc region selected from FCV1-FCV7. (See Table A below).

TABLE A
Sequences of Fc variants

SEQ ID No	Description	Sequence
1144	FCV-1: Human IgG1 Fc variant L234A/L235A/P329A (LALAPA) Amino acid sequence	apeaaggpsvflfppkpkdtlmisrtpevtcvvvdvshedpev kfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlng keykckvsnkalaapiektiskakgqprepqvytlppsrdelt knqvsltclvkgfypsdiavewesngqpennykttppvldsdg sfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslsp gk
1145	FCV-1: Human IgG1 Fc variant L234A/L235A/P329A (LALAPA) Nucleic acid sequence	gcccctgaagccgccggcggaccctccgtgttcctgttccccc caaagcccaaggacaccctgatgatcagccggacccccgaagt gacctgcgtggtggtggacgtgtcccacgaggaccctgaagtg aagttcaattggtacgtggacggcgtggaagtgcacaacgcca agaccaagcccagagaggaacagtacaacagcacctaccgggt ggtgtccgtgctgaccgtgctgcaccaggactggctgaacggc aaagagtacaagtgcaaagtctccaacaaggccctggccgccc ccatcgagaaaaccatcagcaaggccaagggccagccccgcga gccccaggtgtacacactgccccccagccgggacgagctgacc aagaaccaggtgtccctgacctgcctggtcaagggcttctacc ccagcgatatcgccgtggaatgggagagcaacggccagcccga gaacaactacaagaccacccccccctgtgctggacagcgacggc tcattcttcctgtacagcaagctgaccgtggacaagtcccggt ggcagcagggcaacgtgttcagctgcagcgtgatgcacgaggc cctgcacaaccactacacccagaagtccctgagcctgagcccc ggcaaa
1146	FCV-2: Human IgG1 Fc variant	apeaagapsvflfppkpkdtlmisrtpevtcvvvdvshedpev kfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlng keykckvsnkalpapiektiskakgqprepqvytlppsrdelt

TABLE A Sequences of Fc variants		
SEQ ID No	Description	Sequence
	L234A/L235A/G237A (LALAGA) Amino acid sequence	knqvsltclvkgfypsdiavewesngqpennykttppvldsdg sfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslsp gk
1147	FCV-2: Human IgG1 Fc variant L234A/L235A/G237A (LALAGA) Nucleic acid sequence	gcccctgaagccgccggcgccccctccgtgttcctgttccccc caaagcccaaggacaccctgatgatcagccggacccccgaagt gacctgcgtggtggtggacgtgtcccacgaggaccctgaagtg aagttcaattggtacgtggacggcgtggaagtgcacaacgcca agaccaagcccagagaggaacagtacaacagcacctaccgggt ggtgtccgtgctgaccgtgctgcaccaggactggctgaacggc aaagagtacaagtgcaaagtctccaacaaggccctgcctgccc ccatcgagaaaaccatcagcaaggccaagggccagccccgcga gccccaggtgtacacactgcccccagccgggacgagctgacc aagaaccaggtgtccctgacctgcctggtcaagggcttctacc ccagcgatatcgccgtggaatgggagagcaacggccagcccga gaacaactacaagaccacccccccctgtgctggacagcgacggc tcattcttcctgtacagcaagctgaccgtggacaagtcccggt ggcagcagggcaacgtgttcagctgcagcgtgatgcacgaggc cctgcacaaccactacacccagaagtccctgagcctgagcccc ggcaaa
1148	FCV-3: Human IgG1 Fc variant L234A/L235A/P329G (LALAPG) Amino acid sequence	apeaaggpsvflfppkpkdtlmisrtpevtcvvvdvshedpev kfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlng keykckvsnkalgapiektiskakgqprepqvytlppsrdelt knqvsltclvkgfypsdiavewesngqpennykttppvldsdg sfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslsp gk
1149	FCV-3: Human IgG1 Fc variant L234A/L235A/P329G (LALAPG) Nucleic acid sequence	gcccctgaagccgccggcggaccctccgtgttcctgttccccc caaagcccaaggacaccctgatgatcagccggacccccgaagt gacctgcgtggtggtggacgtgtcccacgaggaccctgaagtg aagttcaattggtacgtggacggcgtggaagtgcacaacgcca agaccaagcccagagaggaacagtacaacagcacctaccgggt ggtgtccgtgctgaccgtgctgcaccaggactggctgaacggc aaagagtacaagtgcaaagtctccaacaaggccctgggcgccc ccatcgagaaaaccatcagcaaggccaagggccagccccgcga

TABLE A Sequences of Fc variants		
SEQ ID No	Description	Sequence
		gccccaggtgtacacactgccccccagccgggacgagctgacc aagaaccaggtgtccctgacctgcctggtcaagggcttctacc ccagcgatatcgccgtggaatgggagagcaacggccagcccga gaacaactacaagaccacccccccctgtgctggacagcgacggc tcattcttcctgtacagcaagctgaccgtggacaagtcccggt ggcagcagggcaacgtgttcagctgcagcgtgatgcacgaggc cctgcacaaccactacacccagaagtccctgagcctgagcccc ggcaaa
1150	FCV-4: Human IgG1 Fc variant L234A/L235A/S267K/ P329A (LALASKPA) Amino acid sequence	apeaaggpsvflfppkpkdtlmisrtpevtcvvvdvkhedpev kfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlng keykckvsnkalaapiektiskakgqprepqvytlppsrdelt knqvsltclvkgfypsdiavewesngqpennykttppvldsdg sfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslsp gk
1151	FCV-4: Human IgG1 Fc variant L234A/L235A/S267K/ P329A (LALASKPA) Nucleic acid sequence	gcccctgaagccgccggcggaccctccgtgttcctgttccccc caaagcccaaggacaccctgatgatcagccggacccccgaagt gacctgcgtggtggtggacgtgaagcacgaggaccctgaagtg aagttcaattggtacgtggacggcgtggaagtgcacaacgcca agaccaagcccagagaggaacagtacaacagcacctaccgggt ggtgtccgtgctgaccgtgctgcaccaggactggctgaacggc aaagagtacaagtgcaaagtctccaacaaggccctggccgccc ccatcgagaaaaccatcagcaaggccaagggccagccccgcga gccccaggtgtacacactgccccccagccgggacgagctgacc aagaaccaggtgtccctgacctgcctggtcaagggcttctacc ccagcgatatcgccgtggaatgggagagcaacggccagcccga gaacaactacaagaccacccccccctgtgctggacagcgacggc tcattcttcctgtacagcaagctgaccgtggacaagtcccggt ggcagcagggcaacgtgttcagctgcagcgtgatgcacgaggc cctgcacaaccactacacccagaagtccctgagcctgagcccc ggcaaa
1152	FCV-5: Human IgG1 Fc variant	apellggpsvflfppkpkdtlmisrtpevtcvvvavkhedpev kfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlng keykckvsnkalaapiektiskakgqprepqvytlppsrdelt

TABLE A Sequences of Fc variants		
SEQ ID No	**Description**	**Sequence**
	D265A/P329A/S267K (DAPASK) Amino acid sequence	knqvsltclvkgfypsdiavewesngqpennykttppvldsdg sfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslsp gk
1153	FCV-5: Human IgG1 Fc variant D265A/P329A/S267K (DAPASK) Nucleic acid sequence	gcccctgaactgctgggcggaccctccgtgttcctgttccccc caaagcccaaggacaccctgatgatcagccggacccccgaagt gacctgcgtggtggtggccgtgaagcacgaggaccctgaagtg aagttcaattggtacgtggacggcgtggaagtgcacaacgcca agaccaagcccagagaggaacagtacaacagcacctaccgggt ggtgtccgtgctgaccgtgctgcaccaggactggctgaacggc aaagagtacaagtgcaaagtctccaacaaggccctggccgccc ccatcgagaaaaccatcagcaaggccaagggccagccccgcga gccccaggtgtacacactgcccccagccgggacgagctgacc aagaaccaggtgtccctgacctgcctggtcaagggcttctacc ccagcgatatcgccgtggaatgggagagcaacggccagcccga gaacaactacaagaccacccccctgtgctggacagcgacggc tcattcttcctgtacagcaagctgaccgtggacaagtcccggt ggcagcagggcaacgtgttcagctgcagcgtgatgcacgaggc cctgcacaaccactacacccagaagtccctgagcctgagcccc ggcaaa
1154	FCV-6: Human IgG1 Fc variant G237A/D265A/P329A (GADAPA) Amino acid sequence	apellgapsvflfppkpkdtlmisrtpevtcvvvavshedpev kfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlng keykckvsnkalaapiektiskakgqprepqvytlppsrdelt knqvsltclvkgfypsdiavewesngqpennykttppvldsdg sfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslsp gk
1155	FCV-6: Human IgG1 Fc variant G237A/D265A/P329A (GADAPA) Nucleic acid sequence	gcccctgaactgctgggcgccccctccgtgttcctgttccccc caaagcccaaggacaccctgatgatcagccggacccccgaagt gacctgcgtggtggtggccgtgtcccacgaggaccctgaagtg aagttcaattggtacgtggacggcgtggaagtgcacaacgcca agaccaagcccagagaggaacagtacaacagcacctaccgggt ggtgtccgtgctgaccgtgctgcaccaggactggctgaacggc aaagagtacaagtgcaaagtctccaacaaggccctggccgccc ccatcgagaaaaccatcagcaaggccaagggccagccccgcga

TABLE A Sequences of Fc variants		
SEQ ID No	Description	Sequence
		gccccaggtgtacacactgcccccagccgggacgagctgacc aagaaccaggtgtccctgacctgcctggtcaagggcttctacc ccagcgatatcgccgtggaatgggagagcaacggccagcccga gaacaactacaagaccacccccccctgtgctggacagcgacggc tcattcttcctgtacagcaagctgaccgtggacaagtcccggt ggcagcagggcaacgtgttcagctgcagcgtgatgcacgaggc cctgcacaaccactacacccagaagtccctgagcctgagcccc ggcaaa
1156	FCV-7: Human IgG1 Fc variant G237A/D265A/P329A /S267K (GADAPASK) Amino acid sequence	apellgapsvflfppkpkdtlmisrtpevtcvvvavkhedpev kfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlng keykckvsnkalaapiektiskakgqprepqvytlppsrdelt knqvsltclvkgfypsdiavewesngqpennykttppvldsdg sfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslsp gk
1157	FCV-7: Human IgG1 Fc variant G237A/D265A/P329A /S267K (GADAPASK) Nucleic acid sequence	gcccctgaactgctgggcgccccctccgtgttcctgttccccc caaagcccaaggacaccctgatgatcagccggaccccgaagt gacctgcgtggtggtggccgtgaagcacgaggaccctgaagtg aagttcaattggtacgtggacggcgtggaagtgcacaacgcca agaccaagcccagagaggaacagtacaacagcacctaccgggt ggtgtccgtgctgaccgtgctgcaccaggactggctgaacggc aaagagtacaagtgcaaagtctccaacaaggccctggccgccc ccatcgagaaaaccatcagcaaggccaagggccagccccgcga gccccaggtgtacacactgcccccagccgggacgagctgacc aagaaccaggtgtccctgacctgcctggtcaagggcttctacc ccagcgatatcgccgtggaatgggagagcaacggccagcccga gaacaactacaagaccacccccccctgtgctggacagcgacggc tcattcttcctgtacagcaagctgaccgtggacaagtcccggt ggcagcagggcaacgtgttcagctgcagcgtgatgcacgaggc cctgcacaaccactacacccagaagtccctgagcctgagcccc ggcaaa

TABLE B Sequences of Antibodies and Fc variants		
SEQ ID No	**Description**	**Sequence**
1158	FCV-8: Human IgG1 Fc variant D265A/P329A (DAPA) Amino acid sequence	apellggpsvflfppkpkdtlmisrtpevtcvvvavshedpev kfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlng keykckvsnkalaapiektiskakgqprepqvytlppsrdelt knqvsltclvkgfypsdiavewesngqpennykttppvldsdg sfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslsp gk
1159	FCV-8: Human IgG1 Fc variant D265A/P329A (DAPA) Nucleic acid sequence	gcccctgaactgctgggcggaccctccgtgttcctgttccccc caaagcccaaggacaccctgatgatcagccggacccccgaagt gacctgcgtggtggtggccgtgtcccacgaggaccctgaagtg aagttcaattggtacgtggacggcgtggaagtgcacaacgcca agaccaagcccagagaggaacagtacaacagcacctaccgggt ggtgtccgtgctgaccgtgctgcaccaggactggctgaacggc aaagagtacaagtgcaaagtctccaacaaggccctggccgccc ccatcgagaaaaccatcagcaaggccaagggccagccccgcga gccccaggtgtacacactgccccccagccgggacgagctgacc aagaaccaggtgtccctgacctgcctggtcaagggcttctacc ccagcgatatcgccgtggaatgggagagcaacggccagcccga gaacaactacaagaccacccccccctgtgctggacagcgacggc tcattcttcctgtacagcaagctgaccgtggacaagtcccggt ggcagcagggcaacgtgttcagctgcagcgtgatgcacgaggc cctgcacaaccactacacccagaagtccctgagcctgagcccc ggcaaa
1160	FCV-9: Human IgG1 Fc variant D265A/N297A/P329A (DANAPA) Amino acid sequence	apellggpsvflfppkpkdtlmisrtpevtcvvvavshedpev kfnwyvdgvevhnaktkpreeqyastyrvvsvltvlhqdwlng keykckvsnkalaapiektiskakgqprepqvytlppsrdelt knqvsltclvkgfypsdiavewesngqpennykttppvldsdg sfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslsp gk
1161	FCV-9: Human IgG1 Fc variant D265A/N297A/P329A (DANAPA)	gcccctgaactgctgggaggccctagcgtgttcctgttccccc caaagcccaaggacaccctgatgatcagccggacccccgaagt gacctgtgtggtggtggccgtgtctcacgaggaccctgaagtg aagtttaattggtacgtggacggcgtggaagtgcacaacgcca agaccaagcccagagaggaacagtacgccagcacctaccgggt ggtgtccgtgctgacagtgctgcaccaggactggctgaacggc

TABLE B Sequences of Antibodies and Fc variants		
SEQ ID No	**Description**	**Sequence**
	Nucleic acid sequence	aaagagtacaagtgcaaggtgtccaacaaggccctggccgctc ccatcgagaaaaccatcagcaaggccaagggccagccccgcga accccaggtgtacacactgccccctagcagggacgagctgacc aagaaccaggtgtccctgacctgcctcgtgaagggcttctacc cctccgatatcgccgtggaatgggagagcaacggccagcccga gaacaactacaagaccacccccccctgtgctggactccgacggc tcattcttcctgtacagcaagctgaccgtggacaagtcccggt ggcagcagggcaacgtgttcagctgctccgtgatgcacgaggc cctgcacaaccactacacccagaagtccctgagcctgagcccc ggcaaa
1162	Anti-CD3 human IgG1 Heavy Chain Amino acid sequence	evqlvesggglvqpggslklscaasgftfntyamnwvrqasgk glewvgrirskynnyatyyadsvkdrftisrddskstlylqmn slktedtavyycvrhgnfgnsyvswfaywgqgtlvtvssastk gpsvfplapsskstsggtaalgclvkdyfpepvtvswnsgalt sgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkps ntkvdkrvepkscdkthtcppcpapellggpsvflfppkpkdt lmisrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpre eqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiekti skakgqprepqvytlppsrdeltknqvsltclvkgfypsdiav ewesngqpennykttppvldsdgsfflyskltvdksrwqqgnv fscsvmhealhnhytqkslslspgk
1163	Anti-CD3 human IgG1 Heavy Chain Nucleic acid sequence	gaagtgcagctggtggaatctggcggcggactggtgcagcctg gcggatctctgaagctgagctgtgccgccagcggcttcacctt caacacctacgccatgaactgggtgcgccaggcctctggcaag ggcctggaatgggtgggacggatcagaagcaagtacaacaatt acgccacctactacgccgacagcgtgaaggaccggttcaccat cagccgggacgacagcaagagcaccctgtacctgcagatgaac agcctgaaaaccgaggacaccgccgtgtactactgcgtgcggc acggcaacttcggcaacagctatgtgtcttggtttgcctactg gggccagggcaccctcgtgacagtgagctcagctagcaccaag ggccccagcgtgttcccctggcgcccagcagcaagagcacca gcggcggcacagccgccctgggctgcctggtgaaggactactt ccccgagccagtgaccgtgtcctggaacagcggagccctgacc tccggcgtgcacaccttccccgccgtgctgcagagcagcggcc

TABLE B Sequences of Antibodies and Fc variants		
SEQ ID No	**Description**	**Sequence**
		tgtacagcctgagcagcgtggtgaccgtgcccagcagcagcct gggcacccagacctacatctgcaacgtgaaccacaagcccagc aacaccaaggtggacaagagagtggagcccaagagctgcgaca agacccacacctgccccccctgtcctgcccctgaactgctggg cggaccctccgtgttcctgttcccccaaagcccaaggacacc ctgatgatcagccggaccccgaagtgacctgcgtggtggtgg acgtgtcccacgaggaccctgaagtgaagttcaattggtacgt ggacggcgtggaagtgcacaacgccaagaccaagcccagagag gaacagtacaacagcacctaccgggtggtgtccgtgctgaccg tgctgcaccaggactggctgaacggcaaagagtacaagtgcaa agtctccaacaaggccctgcctgcccccatcgagaaaaccatc agcaaggccaagggccagccccgcgagccccaggtgtacacac tgcccccagccgggacgagctgaccaagaaccaggtgtccct gacctgcctggtcaagggcttctaccccagcgatatcgccgtg gaatgggagagcaacggccagcccgagaacaactacaagacca ccccccctgtgctggacagcgacggctcattcttcctgtacag caagctgaccgtggacaagtcccggtggcagcagggcaacgtg ttcagctgcagcgtgatgcacgaggccctgcacaaccactaca cccagaagtccctgagcctgagccccggcaaa
1164	Anti-CD3 human IgG1 R214K allotype Heavy Chain Amino acid sequence	evqlvesggglvqpggslklscaasgftfntyamnwvrqasgk glewvgrirskynnyatyyadsvkdrftisrddskstlylqmn slktedtavyycvrhgnfgnsyvswfaywgqgtlvtvssastk gpsvfplapsskstsggtaalgclvkdyfpepvtvswnsgalt sgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkps ntkvdkkvepkscdkthtcppcpapellggpsvflfppkpkdt lmisrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpre eqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiekti skakgqprepqvytlppsrdeltknqvsltclvkgfypsdiav ewesngqpennykttppvldsdgsfflyskltvdksrwqqgnv fscsvmhealhnhytqkslslspgk
1165	Anti-CD3 human IgG1 R214K allotype Heavy Chain	gaagtgcagctggtggaatctggcggcggactggtgcagcctg gcggatctctgaagctgagctgtgccgccagcggcttcaccttt caacacctacgccatgaactgggtgcgccaggcctctggcaag ggcctggaatgggtgggacggatcagaagcaagtacaacaatt

TABLE B Sequences of Antibodies and Fc variants		
SEQ ID No	**Description**	**Sequence**
	Nucleic acid sequence	acgccacctactacgccgacagcgtgaaggaccggttcaccat cagccgggacgacagcaagagcaccctgtacctgcagatgaac agcctgaaaaccgaggacaccgccgtgtactactgcgtgcggc acggcaacttcggcaacagctatgtgtcttggtttgcctactg gggccagggcaccctcgtgacagtgagctcagctagcaccaag ggccccagcgtgttccccctggcgcccagcagcaagagcacca gcggcggcacagccgccctgggctgcctggtgaaggactactt ccccgagccagtgaccgtgtcctggaacagcggagccctgacc tccggcgtgcacaccttccccgccgtgctgcagagcagcggcc tgtacagcctgagcagcgtggtgaccgtgcccagcagcagcct gggcacccagacctacatctgcaacgtgaaccacaagcccagc aacaccaaggtggacaagaaggtggagcccaagagctgcgaca agacccacacctgccccccctgtcctgcccctgaactgctggg cggaccctccgtgttcctgttcccccaaagcccaaggacacc ctgatgatcagccggaccccgaagtgacctgcgtggtggtgg acgtgtcccacgaggaccctgaagtgaagttcaattggtacgt ggacggcgtggaagtgcacaacgccaagaccaagcccagagag gaacagtacaacagcacctaccgggtggtgtccgtgctgaccg tgctgcaccaggactggctgaacggcaaagagtacaagtgcaa agtctccaacaaggccctgcctgcccccatcgagaaaaccatc agcaaggccaagggccagccccgcgagccccaggtgtacacac tgcccccagccgggacgagctgaccaagaaccaggtgtccct gacctgcctggtcaagggcttctaccccagcgatatcgccgtg gaatgggagagcaacggccagcccgagaacaactacaagacca ccccccctgtgctggacagcgacggctcattcttcctgtacag caagctgaccgtggacaagtcccggtggcagcagggcaacgtg ttcagctgcagcgtgatgcacgaggccctgcacaaccactaca cccagaagtccctgagcctgagccccggcaaa
1166	Anti-CD3 human Light Chain Amino acid sequence	qavvtqepsltvspggtvtltcrsstgavttsnyanwvqqkpg qaprgliggtnkrapwtparfsgsllgdkaaltlsgaqpedea eyfcalwysnlwvfgggtkltvlgqpkaapsvtlfppsseelq ankatlvclisdfypgavtvawkadsspvkagvetttpskqsn nkyaassylsltpeqwkshrsyscqvthegstvektvaptecs

TABLE B Sequences of Antibodies and Fc variants		
SEQ ID No	**Description**	**Sequence**
1167	Anti-CD3 human Light Chain Nucleic acid sequence	caggctgtcgtgacccaggaacctagcctgaccgtgtctcctg gcggaaccgtgaccctgacctgtagatctagcacaggcgccgt gaccaccagcaactacgccaattgggtgcagcagaagcccggc caggctcctagaggactgatcggcggcaccaacaagagagccc cttggacccctgccagattcagcggctctctgctgggagataa ggccgccctgacactgtctggcgcccagcctgaggatgaggcc gagtacttttgcgccctgtggtacagcaacctgtgggtgttcg gcggaggcaccaagctgaccgtgctgggccagcctaaggccgc tccctccgtgaccctgttcccccccagctccgaggaactgcag gccaacaaggccaccctggtgtgcctgatcagcgacttctacc ctggcgccgtgaccgtggcctggaaggccgacagcagccccgt gaaggccggcgtggagacaaccacccccagcaagcagagcaac aacaagtacgccgccagcagctacctgagcctgaccccgagc agtggaagagccacagaagctacagctgccaggtcacccacga gggcagcaccgtggagaaaaccgtggcccccaccgagtgcagc

7.4.1.1. Fc Domains with Altered FcR Binding

[0243] The Fc domains of the CD19 binding molecules can show altered binding to one or more Fc-receptors (FcRs) in comparison with the corresponding native immunoglobulin. The binding to any particular Fc-receptor can be increased or decreased. In one embodiment, the Fc domain comprises one or more modifications which alter its Fc-receptor binding profile.

[0244] Human cells can express a number of membrane bound FcRs selected from FcαR, FcεR, FcγR, FcRn and glycan receptors. Some cells are also capable of expressing soluble (ectodomain) FcR (Fridman *et al.*, 1993, J Leukocyte Biology 54: 504-512). FcγR can be further divided by affinity of IgG binding (high/low) and biological effect (activating/inhibiting). Human FcγRI is widely considered to be the sole `high affinity` receptor whilst all of the others are considered as medium to low. FcγRIIb is the sole receptor with `inhibitory` functionality by virtue of its intracellular ITIM motif whilst all of the others are considered as `activating` by virtue of ITAM motifs or pairing with the common FcγR--γchain. FcγRIIIb is also unique in that although activatory it associates with the cell via a GPI anchor. In total, humans express six “standard” FcγRs: FcγRI, FcγRIIa, FcγRIIb, FcγRIIc, FcγRIIIa, and FcγRIIIb. In addition to these sequences there are a large number of sequence or allotypic variants spread across these families. Some of these have been found to have important functional consequence and so are

sometimes considered to be receptor sub-types of their own. Examples include FcγRIIa^{H134R}, FcγRIIb^{I190T}, FcγRIIIa^{F158V}, FcγRIIIb^{NA1}, FcγRIIIb^{NA2}, and FcγRIII^{SH}. Each receptor sequence has been shown to have different affinities for the 4 sub-classes of IgG: IgG1, IgG2, IgG3 and IgG4 (Bruhns, 1993, Blood 113:3716-3725). Other species have somewhat different numbers and functionality of FcγR, with the mouse system being the best studied to date and comprising of 4 FcγR, FcγRI FcγRIIb FcγRIII FcγRIV (Bruhns, 2012, Blood 119:5640-5649). Human FcγRI on cells is normally considered to be "occupied" by monomeric IgG in normal serum conditions due to its affinity for IgG1/IgG3/IgG4 (about 10^{-8} M) and the concentration of these IgG in serum (about 10 mg/ml). Hence cells bearing FcγRI on their surface are considered to be capable for "screening" or "sampling" of their antigenic environment vicariously through the bound polyspecific IgG. The other receptors having lower affinities for IgG sub-classes (in the range of about 10^{-5} - 10^{-7} M) are normally considered to be "unoccupied." The low affinity receptors are hence inherently sensitive to the detection of and activation by antibody involved immune complexes. The increased Fc density in an antibody immune complex results in increased functional affinity of binding avidity to low affinity FcγR. This has been demonstrated *in vitro* using a number of methods (Shields *et al.*, 2001, J Biol Chem 276(9):6591-6604; Lux *et al.*, 2013, J Immunol 190:4315-4323). It has also been implicated as being one of the primary modes of action in the use of anti-RhD to treat ITP in humans (Crow, 2008, Transfusion Medicine Reviews 22:103-116).

[0245] Many cell types express multiple types of FcγR and so binding of IgG or antibody immune complex to cells bearing FcγR can have multiple and complex outcomes depending upon the biological context. Most simply, cells can either receive an activatory, inhibitory or mixed signal. This can result in events such as phagocytosis (*e.g.*, macrophages and neutrophils), antigen processing (*e.g.*, dendritic cells), reduced IgG production (*e.g.*, B-cells) or degranulation (*e.g.*, neutrophils, mast cells). There are data to support that the inhibitory signal from FcγRIIb can dominate that of activatory signals (Proulx, 2010, Clinical Immunology 135:422-429).

[0246] There are a number of useful Fc substitutions that can be made to alter binding to one or more of the FcγR receptors. Substitutions that result in increased binding as well as decreased binding can be useful. For example, it is known that increased binding to FcγRIIIa generally results in increased ADCC (antibody dependent cell-mediated cytotoxicity; the cell-mediated reaction where nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause lysis of the target cell). Similarly, decreased binding to FcγRIIb (an inhibitory receptor) can be beneficial as well in some circumstances. Amino acid substitutions that find use in the present disclosure include those listed in US 2006/0024298 (particularly Figure 41), US 2006/0121032, US 2006/0235208, US

2007/0148170, and US 2019/0100587. Particular variants that find use include, but are not limited to, 236A, 239D, 239E, 332E, 332D, 239D/332E, 267D, 267E, 328F, 267E/328F, 236A/332E, 239D/332E/330Y, 239D, 332E/330L, 243A, 243L, 264A, 264V, 299T, 265A/297A/329A, 265N/297D/329G, and 265E/297Q/329S.

[0247] FcRn has a crucial role in maintaining the long half-life of IgG in the serum of adults and children. The receptor binds IgG in acidified vesicles (pH<6.5) protecting the IgG molecule from degradation, and then releasing it at the higher pH of 7.4 in blood.

[0248] FcRn is unlike leukocyte Fc receptors, and instead, has structural similarity to MHC class I molecules. It is a heterodimer composed of a β_2-microglobulin chain, non-covalently attached to a membrane-bound chain that includes three extracellular domains. One of these domains, including a carbohydrate chain, together with β_2-microglobulin interacts with a site between the CH2 and CH3 domains of Fc. The interaction includes salt bridges made to histidine residues on IgG that are positively charged at pH<6.5. At higher pH, the His residues lose their positive charges, the FcRn-IgG interaction is weakened and IgG dissociates.

[0249] In one embodiment, a CD19 binding molecule comprises an Fc domain that binds to human FcRn.

[0250] In one embodiment, the Fc domain has an Fc region(s) (*e.g.*, one or two) comprising a histidine residue at position 310, and in some cases also at position 435. These histidine residues are important for human FcRn binding. In one embodiment, the histidine residues at positions 310 and 435 are native residues, *i.e.*, positions 310 and 435 are not modified. Alternatively, one or both of these histidine residues can be present as a result of a modification.

[0251] The CD19 binding molecules can comprise one or more Fc regions that alter Fc binding to FcRn. The altered binding can be increased binding or decreased binding.

[0252] In one embodiment, the CD19 binding molecule comprises an Fc domain in which at least one (and optionally both) Fc regions comprises one or more modifications such that it binds to FcRn with greater affinity and avidity than the corresponding native immunoglobulin.

[0253] Fc substitutions that increase binding to the FcRn receptor and increase serum half life are described in US 2009/0163699, including, but not limited to, 434S, 434A, 428L, 308F, 259I, 428L/434S, 259I/308F, 436I/428L, 436I or V/434S, 436V/428L and 259I/308F/428L.

[0254] In one embodiment, the Fc region is modified by substituting the threonine residue at position 250 with a glutamine residue (T250Q).

[0255] In one embodiment, the Fc region is modified by substituting the methionine residue at position 252 with a tyrosine residue (M252Y)

[0256] In one embodiment, the Fc region is modified by substituting the serine residue at position 254 with a threonine residue (S254T).

[0257] In one embodiment, the Fc region is modified by substituting the threonine residue at position 256 with a glutamic acid residue (T256E).

[0258] In one embodiment, the Fc region is modified by substituting the threonine residue at position 307 with an alanine residue (T307A).

[0259] In one embodiment, the Fc region is modified by substituting the threonine residue at position 307 with a proline residue (T307P).

[0260] In one embodiment, the Fc region is modified by substituting the valine residue at position 308 with a cysteine residue (V308C).

[0261] In one embodiment, the Fc region is modified by substituting the valine residue at position 308 with a phenylalanine residue (V308F).

[0262] In one embodiment, the Fc region is modified by substituting the valine residue at position 308 with a proline residue (V308P).

[0263] In one embodiment, the Fc region is modified by substituting the glutamine residue at position 311 with an alanine residue (Q311A).

[0264] In one embodiment, the Fc region is modified by substituting the glutamine residue at position 311 with an arginine residue (Q311R).

[0265] In one embodiment, the Fc region is modified by substituting the methionine residue at position 428 with a leucine residue (M428L).

[0266] In one embodiment, the Fc region is modified by substituting the histidine residue at position 433 with a lysine residue (H433K).

[0267] In one embodiment, the Fc region is modified by substituting the asparagine residue at position 434 with a phenylalanine residue (N434F).

[0268] In one embodiment, the Fc region is modified by substituting the asparagine residue at position 434 with a tyrosine residue (N434Y).

[0269] In one embodiment, the Fc region is modified by substituting the methionine residue at position 252 with a tyrosine residue, the serine residue at position 254 with a threonine residue, and the threonine residue at position 256 with a glutamic acid residue (M252Y/S254T/T256E).

[0270] In one embodiment, the Fc region is modified by substituting the valine residue at position 308 with a proline residue and the asparagine residue at position 434 with a tyrosine residue (V308P/N434Y).

[0271] In one embodiment, the Fc region is modified by substituting the methionine residue at position 252 with a tyrosine residue, the serine residue at position 254 with a threonine residue, the threonine residue at position 256 with a glutamic acid residue, the histidine residue at position 433 with a lysine residue and the asparagine residue at position 434 with a phenylalanine residue (M252Y/S254T/T256E/H433K/N434F).

[0272] It will be appreciated that any of the modifications listed above can be combined to alter FcRn binding.

[0273] In one embodiment, the CD19 binding molecule comprises an Fc domain in which one or both Fc regions comprise one or more modifications such that the Fc domain binds to FcRn with lower affinity and avidity than the corresponding native immunoglobulin.

[0274] In one embodiment, the Fc region comprises any amino acid residue other than histidine at position 310 and/or position 435.

[0275] The CD19 binding molecule can comprise an Fc domain in which one or both Fc regions comprise one or more modifications which increase its binding to FcγRIIb. FcγRIIb is the only inhibitory receptor in humans and the only Fc receptor found on B cells.

[0276] In one embodiment, the Fc region is modified by substituting the proline residue at position 238 with an aspartic acid residue (P238D).

[0277] In one embodiment, the Fc region is modified by substituting the glutamic acid residue at position 258 with an alanine residue (E258A).

[0278] In one embodiment, the Fc region is modified by substituting the serine residue at position 267 with an alanine residue (S267A).

[0279] In one embodiment, the Fc region is modified by substituting the serine residue at position 267 with a glutamic acid residue (S267E).

[0280] In one embodiment, the Fc region is modified by substituting the leucine residue at position 328 with a phenylalanine residue (L328F).

[0281] In one embodiment, the Fc region is modified by substituting the glutamic acid residue at position 258 with an alanine residue and the serine residue at position 267 with an alanine residue (E258A/S267A).

[0282] In one embodiment, the Fc region is modified by substituting the serine residue at position 267 with a glutamic acid residue and the leucine residue at position 328 with a phenylalanine residue (S267E/L328F).

[0283] It will be appreciated that any of the modifications listed above can be combined to increase FcγRIIb binding.

[0284] In one embodiment, CD19 binding molecules are provided comprising Fc domains which display decreased binding to FcγR.

[0285] In one embodiment, the CD19 binding molecule comprises an Fc domain in which one or both Fc regions comprise one or more modifications that decrease Fc binding to FcγR.

[0286] The Fc domain can be derived from IgG1.

[0287] In one embodiment, the Fc region is modified by substituting the leucine residue at position 234 with an alanine residue (L234A).

[0288] In one embodiment, the Fc region is modified by substituting the leucine residue at position 235 with an alanine residue (L235A).

[0289] In one embodiment, the Fc region is modified by substituting the glycine residue at position 236 with an arginine residue (G236R).

[0290] In one embodiment, the Fc region is modified by substituting the asparagine residue at position 297 with an alanine residue (N297A) or a glutamine residue (N297Q).

[0291] In one embodiment, the Fc region is modified by substituting the serine residue at position 298 with an alanine residue (S298A).

[0292] In one embodiment, the Fc region is modified by substituting the leucine residue at position 328 with an arginine residue (L328R).

[0293] In one embodiment, the Fc region is modified by substituting the leucine residue at position 234 with an alanine residue and the leucine residue at position 235 with an alanine residue (L234A/L235A).

[0294] In one embodiment, the Fc region is modified by substituting the phenylalanine residue at position 234 with an alanine residue and the leucine residue at position 235 with an alanine residue (F234A/L235A).

[0295] In one embodiment, the Fc region is modified by substituting the glycine residue at position 236 with an arginine residue and the leucine residue at position 328 with an arginine residue (G236R/L328R).

[0296] In one embodiment, the Fc region is modified by substituting the aspartate residue at position 265 with an alanine residue, the asparagine residue at position 297 with an alanine residue and the proline residue at position 329 with an alanine residue (D265A/N297A/P329A).

[0297] In one embodiment, the Fc region is modified by substituting the aspartate residue at position 265 with an asparagine residue, the asparagine residue at position 297 with an aspartate residue and the proline residue at position 329 with a glycine residue (D265N/N297D/P329G).

[0298] In one embodiment, the Fc region is modified by substituting the aspartate residue at position 265 with a glutamate residue, the asparagine residue at position 297 with an glutamine residue and the proline residue at position 329 with a serine residue (D265E/N297Q/P329S).

[0299] It will be appreciated that any of the modifications listed above can be combined to decrease FcγR binding.

[0300] In one embodiment, a CD19 binding molecule comprises an Fc domain in which one or both Fc regions comprise one or more modifications that decrease Fc binding to FcγRIIIa without affecting the Fc's binding to FcγRII.

[0301] In one embodiment, the Fc region is modified by substituting the serine residue at position 239 with an alanine residue (S239A).

[0302] In one embodiment, the Fc region is modified by substituting the glutamic acid residue at position 269 with an alanine residue (E269A).

[0303] In one embodiment, the Fc region is modified by substituting the glutamic acid residue at position 293 with an alanine residue (E293A).

[0304] In one embodiment, the Fc region is modified by substituting the tyrosine residue at position 296 with a phenylalanine residue (Y296F).

[0305] In one embodiment, the Fc region is modified by substituting the valine residue at position 303 with an alanine residue (V303A).

[0306] In one embodiment, the Fc region is modified by substituting the alanine residue at position 327 with a glycine residue (A327G).

[0307] In one embodiment, the Fc region is modified by substituting the lysine residue at position 338 with an alanine residue (K338A).

[0308] In one embodiment, the Fc region is modified by substituting the aspartic acid residue at position 376 with an alanine residue (D376A).

[0309] It will be appreciated that any of the modifications listed above can be combined to decrease FcγRIIIa binding.

[0310] Fc region variants with decreased FcR binding can be referred to as "FcγR ablation variants," "FcγR silencing variants" or "Fc knock out (FcKO or KO)" variants. For some therapeutic applications, it is desirable to reduce or remove the normal binding of an Fc domain to one or more or all of the Fcγ receptors (*e.g.*, FcγR1, FcγRIIa, FcγRIIb, FcγRIIIa) to avoid additional mechanisms of action. That is, for example, in many embodiments, particularly in the use of MBMs that bind CD3 monovalently, it is generally desirable to ablate FcγRIIIa binding to eliminate or significantly reduce ADCC activity. In some embodiments, at least one of the Fc

regions of the MBMs described herein comprises one or more Fcγ receptor ablation variants. In some embodiments, both of the Fc regions comprise one or more Fcγ receptor ablation variants. These ablation variants are depicted in Table 3, and each can be independently and optionally included or excluded, with some aspects utilizing ablation variants selected from the group consisting of G236R/L328R, E233P/L234V/L235A/G236del/S239K, E233P/L234V/L235A/G236del/S267K, E233P/L234V/L235A/G236del/S239K/A327G, E233P/L234V/L235A/G236del/S267K/A327G, E233P/L234V/L235A/G236del, D265A/N297A/P329A, D265N/N297D/P329G, and D265E/N297Q/P329S ("del" connotes a deletion, *e.g.*, G236del refers to a deletion of the glycine at position 236). It should be noted that the ablation variants referenced herein ablate FcγR binding but generally not FcRn binding.

TABLE 3
Ablation Variants

Variant	**Variant(s), cont.**
G236R	P329K
S239G	A330L
S239K	A330S/P331S
S239Q	I332K
S239R	I332R
V266D	V266D/A327Q
S267K	V266D/P329K
S267R	S267R/A327Q
H268K	S267R/P329K
E269R	G236R/L328R
299R	E233P/L234V/L235A/G236del/S239K
299K	E233P/L234V/L235A/G236del/S267K
K322A	E233P/L234V/L235A/G236del/S239K/A327G
A327G	E233P/L234V/L235A/G236del/S267K/A327G
A327L	E233P/L234V/L235A/G236del
A327N	S239K/S267K
A327Q	267K/P329K
L328E	D265A/N297A/P329A
L328R	D265N/N297D/P329G

TABLE 3 Ablation Variants	
Variant	**Variant(s), cont.**
P329A	D265E/N297Q/P329S
P329H	

[0311] In some embodiments, the MBMs of the present disclosure comprises a first Fc region and a second Fc region. In some embodiments, the first Fc region and/or the second Fc region can comprise the following mutations: E233P, L234V, L235A, G236del, and S267K.

[0312] The Fc domain of human IgG1 has the highest binding to the Fcγ receptors, and thus ablation variants can be used when the constant domain (or Fc domain) in the backbone of the heterodimeric antibody is IgG1.

[0313] Alternatively, or in addition to ablation variants in an IgG1 background, mutations at the glycosylation position 297, *e.g.*, substituting the asparagine residue at position 297 with an alanine residue (N297A) or a glutamine residue (N297Q), can significantly ablate binding to FcγRIIIa, for example. Human IgG2 and IgG4 have naturally reduced binding to the Fcγ receptors, and thus those backbones can be used with or without the ablation variants.

7.4.1.2. Fc Domains with Altered Complement Binding

[0314] The CD19 binding molecules can comprise an Fc domain in which one or both Fc regions comprises one or more modifications that alter Fc binding to complement. Altered complement binding can be increased binding or decreased binding.

[0315] In one embodiment, the Fc region comprises one or more modifications which decrease its binding to C1q. Initiation of the classical complement pathway starts with binding of hexameric C1q protein to the CH2 domain of antigen bound IgG and IgM.

[0316] In one embodiment, the CD19 binding molecule comprises an Fc domain in which one or both Fc regions comprises one or more modifications to decrease Fc binding to C1q.

[0317] In one embodiment, the Fc region is modified by substituting the leucine residue at position 234 with an alanine residue (L234A).

[0318] In one embodiment, the Fc region is modified by substituting the leucine residue at position 235 with an alanine residue (L235A).

[0319] In one embodiment, the Fc region is modified by substituting the leucine residue at position 235 with a glutamic acid residue (L235E).

[0320] In one embodiment, the Fc region is modified by substituting the glycine residue at position 237 with an alanine residue (G237A).

[0321] In one embodiment, the Fc region is modified by substituting the lysine residue at position 322 with an alanine residue (K322A).

[0322] In one embodiment, the Fc region is modified by substituting the proline residue at position 331 with an alanine residue (P331A).

[0323] In one embodiment, the Fc region is modified by substituting the proline residue at position 331 with a serine residue (P331S).

[0324] In one embodiment, a CD19 binding molecule comprises an Fc domain derived from IgG4. IgG4 has a naturally lower complement activation profile than IgG1, but also weaker binding of FcγR. Thus, in one embodiment, the CD19 binding molecule comprises an IgG4 Fc domain and also comprises one or more modifications that increase FcγR binding.

[0325] It will be appreciated that any of the modifications listed above can be combined to reduce C1q binding.

7.4.1.3. Fc Domains with Altered Disulfide Architecture

[0326] The CD19 binding molecule can include an Fc domain comprising one or more modifications to create and/or remove a cysteine residue. Cysteine residues have an important role in the spontaneous assembly of Fc-based multispecific binding molecules, by forming disulfide bridges between individual pairs of polypeptide monomers. Thus, by altering the number and/or position of cysteine residues, it is possible to modify the structure of the CD19 binding molecule to produce a protein with improved therapeutic properties.

[0327] A CD19 binding molecule of the present disclosure can comprise an Fc domain in which one or both Fc regions, *e.g.*, both Fc regions, comprise a cysteine residue at position 309. In one embodiment, the cysteine residue at position 309 is created by a modification, *e.g.*, for an Fc domain derived from IgG1, the leucine residue at position 309 is substituted with a cysteine residue (L309C), for an Fc domain derived from IgG2, the valine residue at position 309 is substituted with a cysteine residue (V309C).

[0328] In one embodiment, the Fc region is modified by substituting the valine residue at position 308 with a cysteine residue (V308C).

[0329] In one embodiment, two disulfide bonds in the hinge region are removed by mutating a core hinge sequence CPPC (SEQ ID NO: 55) to SPPS (SEQ ID NO: 56).

7.4.1.4. Fc Domains with Altered Glycosylation

[0330] In certain aspects, CD19 binding molecules with improved manufacturability are provided that comprise fewer glycosylation sites than a corresponding immunoglobulin. These proteins have less complex post translational glycosylation patterns and are thus simpler and less expensive to manufacture.

[0331] In one embodiment a glycosylation site in the CH2 domain is removed by substituting the asparagine residue at position 297 with an alanine residue (N297A) or a glutamine residue (N297Q). In addition to improved manufacturability, these aglycosyl mutants also reduce FcγR binding as described herein above.

[0332] In some embodiments, a CD19 binding molecule can be made that has an altered type of glycosylation, such as a hypofucosylated antibody having reduced amounts of fucosyl residues or an antibody having increased bisecting GlcNac structures. Such altered glycosylation patterns have been demonstrated to increase the ADCC ability of antibodies. Such carbohydrate modifications can be accomplished by, for example, expressing a CD19 binding molecule in a host cell with altered glycosylation machinery. Cells with altered glycosylation machinery have been described in the art and can be used as host cells in which to express CD19 binding molecules to thereby produce CD19 binding molecules with altered glycosylation. For example, EP 1,176,195 by Hang *et al.* describes a cell line with a functionally disrupted FUT8 gene, which encodes a fucosyl transferase, such that antibodies expressed in such a cell line exhibit hypofucosylation. PCT Publication WO 03/035835 by Presta describes a variant CHO cell line, Lecl3 cells, with reduced ability to attach fucose to Asn(297)-linked carbohydrates, also resulting in hypofucosylation of antibodies expressed in that host cell (see also Shields *et al.*, 2002, J. Biol. Chem. 277:26733-26740). PCT Publication WO 99/54342 by Umana *et al.* describes cell lines engineered to express glycoprotein-modifying glycosyl transferases (*e.g.*, beta(1,4)-N acetylglucosaminyltransferase III (GnTIII)) such that antibodies expressed in the engineered cell lines exhibit increased bisecting GlcNac structures which results in increased ADCC activity of the antibodies (see also Umana *et al.*, Nat. Biotech. 17:176-180, 1999).

7.4.1.5. Fc Heterodimerization

[0333] Many multispecific molecule formats entail dimerization between two Fc regions that, unlike a native immunoglobulin, are operably linked to non-identical antigen-binding domains (or portions thereof, *e.g.*, a VH or VH-CH1 of a Fab). Inadequate heterodimerization of two Fc regions to form an Fc domain has always been an obstacle for increasing the yield of desired multispecific molecules and represents challenges for purification. A variety of approaches available in the art can be used in for enhancing dimerization of Fc regions that might be present in the CD19 binding molecules (and particularly in the MBMs of the disclosure), for example as disclosed in EP 1870459A1; U.S. Pat. No. 5,582,996; U.S. Pat. No. 5,731,168; U.S. Pat. No. 5,910,573; U.S. Pat. No. 5,932,448; U.S. Pat. No. 6,833,441; U.S. Pat. No. 7,183,076; U.S. Patent Application Publication No. 2006204493A1; and PCT Publication No. WO2009/089004A1.

[0334] The present disclosure provides CD19 binding molecules comprising Fc heterodimers, *i.e.*, Fc domains comprising heterologous, non-identical Fc regions. Heterodimerization strategies are used to enhance dimerization of Fc regions operably linked to different ABMs (or portions thereof, *e.g.*, a VH or VH-CH1 of a Fab) and reduce dimerization of Fc regions operably linked to the same ABM or portion thereof. Typically, each Fc region in the Fc heterodimer comprises a CH3 domain of an antibody. The CH3 domains are derived from the constant region of an antibody of any isotype, class or subclass, and in some cases, of IgG (IgG1, IgG2, IgG3 and IgG4) class, as described in the preceding section.

[0335] Typically, the MBMs comprise other antibody fragments in addition to CH3 domains, such as, CH1 domains, CH2 domains, hinge domain, VH domain(s), VL domain(s), CDR(s), and/or antigen-binding fragments described herein. In some embodiments, the two hetero-polypeptides are two heavy chains forming a bispecific or multispecific molecules. Heterodimerization of the two different heavy chains at CH3 domains give rise to the desired antibody or antibody-like molecule, while homodimerization of identical heavy chains will reduce yield of the desired antibody or molecule. In an exemplary embodiment, the two or more hetero-polypeptide chains comprise two chains comprising CH3 domains and forming the molecules of any of the multispecific molecule formats described above of the present disclosure. In an embodiment, the two hetero-polypeptide chains comprising CH3 domains comprise modifications that favor heterodimeric association of the polypeptides, relative to unmodified chains. Various examples of modification strategies are provided below in Table 4 and Sections 7.4.1.5.1 to 7.4.1.5.7.

TABLE 4
Fc Heterodimerization Strategies

NO.	STRATEGY	CH3 DOMAIN 1	CH3 DOMAIN 2	REFERENCES
Fc 1	knobs-into-holes (Y-T)	T366Y	Y407T	Ridgway *et al.*, 1996, Protein Eng 9:617-21
Fc 2	knobs-into-holes (CW-CSAV)	S354C, T366W	Y349C, T366S, L368A, Y407V	Atwell *et al.*, 1997, J Mol Biol. 270(1):26-35; Merchant *et al.*, 1998, Nat Biotechnol 16:677-681
Fc 3	HA-TF	S364H, F405A	Y349T, T394F	Moore *et al.*, 2011, MAbs 3(6):546-57
Fc 4	ZW1 (VYAV-VLLW)	T350V, L351Y, F405A, Y407V	T350V, T366L, K392L, T394W	Von Kreudenstein *et al.*, 2013, MAbs 5:646-54
Fc 5	CH3 charge pairs (DD-KK)	K392D, K409D	E356K, D399K	Gunasekaran *et al.*, 2010, J Biol Chem 285:19637-46
Fc 6	IgG1 hingE,CH3 charge pairs (EEE-RRR)	IgG1: D221E, P228E, L368E	IgG1: D221R, P228R, K409R	Strop *et al.*, 2012, J Mol Biol 420:204-19

TABLE 4
Fc Heterodimerization Strategies

NO.	STRATEGY	CH3 DOMAIN 1	CH3 DOMAIN 2	REFERENCES
Fc 7	IgG2 hingE,CH3 charge pairs (EEE-RRRR)	IgG2: C223E, P228E, L368E	IgG2: C223R, E225R, P228R, K409R	Strop *et al.*, 2012, J Mol Biol 420:204-19
Fc 8	EW-RVT	K360E, K409W,	Q347R, D399V, F405T	Choi *et al.*, 2013, Mol Cancer Ther 12:2748-59
Fc 9	EW-RVTS-S	K360E, K409W, Y349C	Q347R, D399V, F405T, S354C	Choi *et al.*, 2015, Mol Immunol 65:377-83
Fc 10	Biclonic	366K (+351K)	351D or E or D at 349, 368, 349, or 349 + 355	Geuijen *et al.*, 2014, Journal of Clinical Oncology 32:suppl:560
Fc 11	DuoBody (L-R)	F405L	K409R	Labrijn *et al.*, 2013, Proc Natl Acad Sci USA 110:5145-50
Fc 12	SEEDbody	IgG/A chimera	IgG/A chimera	Davis *et al.*, 2010, Protein Eng Des Sel 23:195-202
Fc 13	BEAT	residues from TCRα interface	residues from TCRβ interface	Moretti *et al.*, 2013, BMC Proceedings 7(Suppl 6):O9
Fc 14	7.8.60 (DMA-RRVV)	K360D, D399M, Y407A	E345R, Q347R, T366V, K409V	Leaver-Fey *et al.*, Structure 24:641-51
Fc 15	20.8.34 (SYMV-GDQA)	Y349S, K370Y, T366M, K409V	E356G, E357D, S364Q, Y407A	Leaver-Fey *et al.*, Structure 24:641-51
Fc 16	Skew variant 12757	None	None	Figure 34 of US 2016/0355600
Fc 17	Skew variant 12758	L368D, K370S	S364K	Figure 34 of US 2016/0355600
Fc 18	Skew variant 12759	L368D, K370S	S364K, E357L	Figure 34 of US 2016/0355600
Fc 19	Skew variant 12760	L368D, K370S	S364K, E357Q	Figure 34 of US 2016/0355600
Fc 20	Skew variant 12761	T411E, K360E, Q362E	D401K	Figure 34 of US 2016/0355600
Fc 21	Skew variant 12496	L368E, K370S	S364K	Figure 34 of US 2016/0355600
Fc 22	Skew variant 12511	K370S	S364K	Figure 34 of US 2016/0355600
Fc 23	Skew variant 12840	L368E, K370S	S364K, E357Q	Figure 34 of US 2016/0355600
Fc 24	Skew variant 12841	K370S	S364K, E357Q	Figure 34 of US 2016/0355600
Fc 25	Skew variant 12894	L368E, K370S	S364K	Figure 34 of US 2016/0355600
Fc 26	Skew variant 12895	K370S	S364K	Figure 34 of US 2016/0355600
Fc 27	Skew variant 12896	L368E, K370S	S364K, E357Q	Figure 34 of US 2016/0355600

TABLE 4
Fc Heterodimerization Strategies

NO.	STRATEGY	CH3 DOMAIN 1	CH3 DOMAIN 2	REFERENCES
Fc 28	Skew variant 12901	K370S	S364K, E357Q	Figure 34 of US 2016/0355600
Fc 29	pI_ISO(-)	I199T, N203D, K274Q, R355Q, N384S, K392N, V397M, Q419E, DEL447		Figure 31 of US 2016/0355600
Fc 30	pI_(-)_Isosteric_A	N208D, Q295E, N384D, Q418E, N421D		Figure 31 of US 2016/0355600
Fc 31	pI_(-)_isosteric_B	N208D, Q295E, Q418E, N421D		Figure 31 of US 2016/0355600
Fc 32	pI_ISO(+RR)	Q196K, I199T, P217R, P228R, N276K		Figure 31 of US 2016/0355600
Fc 33	pI_ISO(+)	Q196K, I199T, N276K		Figure 31 of US 2016/0355600
Fc 34	pI_(+) isosteric_A	E269Q, E272Q, E283Q, E357Q,		Figure 31 of US 2016/0355600
Fc 35	pI_(+)_isosteric_B	E269Q, E272Q, E283Q		Figure 31 of US 2016/0355600
Fc 36	pI_(+) isosteric_E269Q, E272Q	E269Q, E272Q		Figure 31 of US 2016/0355600
Fc 37	pI_(+)_isosteric_E269Q, E283Q	E269Q, E283Q		Figure 31 of US 2016/0355600
Fc 38	pI_(+) isosteric_E2720, E283Q	E272Q, E283Q		Figure 31 of US 2016/0355600
Fc 39	pI_(+)_isosteric_E269Q	E269Q		Figure 31 of US 2016/0355600
Fc 40	Heterodimerization	F405A	T394F	Figure 30A of US 2016/0355600
Fc 41	Heterodimerization	S364D	Y349K	Figure 30A of US 2016/0355600
Fc 42	Heterodimerization	S364E	L368K	Figure 30A of US 2016/0355600
Fc 43	Heterodimerization	S364E	*Y349K*	Figure 30A of US 2016/0355600
Fc 44	Heterodimerization	S364F	K370G	Figure 30A of US 2016/0355600
Fc 45	Heterodimerization	S364H	Y349K	Figure 30A of US 2016/0355600
Fc 46	Heterodimerization	S364H	Y349T	Figure 30A of US 2016/0355600
Fc 47	Heterodimerization	S364Y	K370G	Figure 30A of US 2016/0355600
Fc 48	Heterodimerization	T411K	K370E	Figure 30A of US 2016/0355600

TABLE 4
Fc Heterodimerization Strategies

NO.	STRATEGY	CH3 DOMAIN 1	CH3 DOMAIN 2	REFERENCES
Fc 49	Heterodimerization	V397S, F405A	T394F	Figure 30A of US 2016/0355600
Fc 50	Heterodimerization	K370R, T411K	K370E, T411E	Figure 30A of US 2016/0355600
Fc 51	Heterodimerization	L351E, S364D	Y349K, L351K	Figure 30A of US 2016/0355600
Fc 52	Heterodimerization	L351E, S364E	Y349K, L351K	Figure 30A of US 2016/0355600
Fc 53	Heterodimerization	L351E, T366D	L351K, T366K	Figure 30A of US 2016/0355600
Fc 54	Heterodimerization	P395T,V397S, F405A	T394F	Figure 30A of US 2016/0355600
Fc 55	Heterodimerization	S364D, K370G	S364Y, K370R	Figure 30A of US 2016/0355600
Fc 56	Heterodimerization	S364D, T394F	Y349K, F405A	Figure 30A of US 2016/0355600
Fc 57	Heterodimerization	S364E, F405A	Y349K, T394F	Figure 30A of US 2016/0355600
Fc 58	Heterodimerization	S364E, F405S	Y349K, T394Y	Figure 30A of US 2016/0355600
Fc 59	Heterodimerization	S364E, T411E	Y349K,D401K	Figure 30A of US 2016/0355600
Fc 60	Heterodimerization	S364H,D401K	Y349T, T411E	Figure 30A of US 2016/0355600
Fc 61	Heterodimerization	S364H, F405A	Y349T, T394F	Figure 30A of US 2016/0355600
Fc 62	Heterodimerization	S364H, T394F	Y349T, F405A	Figure 30A of US 2016/0355600
Fc 63	Heterodimerization	Y349C, S364E	Y349K, S354C	Figure 30A of US 2016/0355600
Fc 64	Heterodimerization	L351E, S364D, F405A	Y349K, L351K, T394F	Figure 30A of US 2016/0355600
Fc 65	Heterodimerization	L351K, S364H, D401K	Y349T, L351E, T411E	Figure 30A of US 2016/0355600
Fc 66	Heterodimerization	S364E, T411E, F405A	Y349K, T394F, D401K	Figure 30A of US 2016/0355600
Fc 67	Heterodimerization	S364H,D401K, F405A	Y349T, T394F, T411E	Figure 30A of US 2016/0355600
Fc 68	Heterodimerization	S364H, F405A, T411E	Y349T, T394F, D401K	Figure 30A of US 2016/0355600
Fc 69	Heterodimerization	T411E, K360E, N390D	D401K	Figure 30C of US 2016/0355600
Fc 70	Heterodimerization	T411E, Q362E, N390D	D401K	Figure 30C of US 2016/0355600
Fc 71	Heterodimerization	T411E, Q347R	D401K, K360D	Figure 30C of US 2016/0355600
Fc 72	Heterodimerization	T411E, Q347R	D401K, K360E	Figure 30C of US 2016/0355600

TABLE 4
Fc Heterodimerization Strategies

NO.	STRATEGY	CH3 DOMAIN 1	CH3 DOMAIN 2	REFERENCES
Fc 73	Heterodimerization	T411E, K360	D401K, Q347K	Figure 30C of US 2016/0355600
Fc 74	Heterodimerization	T411E, K360D	D401K, Q347R	Figure 30C of US 2016/0355600
Fc 75	Heterodimerization	T411E, K360E	D401K, Q347K	Figure 30C of US 2016/0355600
Fc 76	Heterodimerization	T411E, K360E	D401K, Q347R	Figure 30C of US 2016/0355600
Fc 77	Heterodimerization	T411E, S364K	D401K, K370S	Figure 30C of US 2016/0355600
Fc 78	Heterodimerization	T411E, K370S	D401K, S364K	Figure 30C of US 2016/0355600
Fc 79	Heterodimerization	Q347E	E357Q	Figure 30C of US 2016/0355600
Fc 80	Heterodimerization	Q347E	E357Q, Q362K	Figure 30C of US 2016/0355600
Fc 81	Heterodimerization	K360D, Q362E	Q347R	Figure 30C of US 2016/0355600
Fc 82	Heterodimerization	K360D, Q362E	D401K	Figure 30C of US 2016/0355600
Fc 83	Heterodimerization	K360D, Q362E	Q347R, D401K	Figure 30C of US 2016/0355600
Fc 84	Heterodimerization	K360E, Q362E	Q347R	Figure 30C of US 2016/0355600
Fc 85	Heterodimerization	K360E, Q362E	D401K	Figure 30C of US 2016/0355600
Fc 86	Heterodimerization	K360E, Q362E	Q347R, D401K	Figure 30C of US 2016/0355600
Fc 87	Heterodimerization	Q362E, N390D	D401K	Figure 30C of US 2016/0355600
Fc 88	Heterodimerization	Q347E, K360D	D401N	Figure 30C of US 2016/0355600
Fc 89	Heterodimerization	K360D	Q347R, N390K	Figure 30C of US 2016/0355600
Fc 90	Heterodimerization	K360D	N390K, D401N	Figure 30C of US 2016/0355600
Fc 91	Heterodimerization	K360E	Y349H	Figure 30C of US 2016/0355600
Fc 92	Heterodimerization	K370S, Q347E	S364K	Figure 30C of US 2016/0355600
Fc 93	Heterodimerization	K370S, E357L	S364K	Figure 30C of US 2016/0355600
Fc 94	Heterodimerization	K370S, E357Q	S364K	Figure 30C of US 2016/0355600
Fc 95	Heterodimerization	K370S, Q347E, E357L	S364K	Figure 30C of US 2016/0355600
Fc 96	Heterodimerization	K370S, Q347E, E357Q	S364K	Figure 30C of US 2016/0355600

TABLE 4
Fc Heterodimerization Strategies

NO.	STRATEGY	CH3 DOMAIN 1	CH3 DOMAIN 2	REFERENCES
Fc 97	Heterodimerization	L368D, K370S, Q347E	S364K	Figure 30D of US 2016/0355600
Fc 98	Heterodimerization	L368D, K370S, E357L	S364K	Figure 30D of US 2016/0355600
Fc 99	Heterodimerization	L368D, K370S, E357Q	S364K	Figure 30D of US 2016/0355600
Fc 100	Heterodimerization	L368D, K370S, Q347E, E357L	S364K	Figure 30D of US 2016/0355600
Fc 101	Heterodimerization	L368D, K370S, Q347E, E357Q	S364K	Figure 30D of US 2016/0355600
Fc 102	Heterodimerization	L368E, K370S, Q347E	S364K	Figure 30D of US 2016/0355600
Fc 103	Heterodimerization	L368E, K370S, E357L	S364K	Figure 30D of US 2016/0355600
Fc 104	Heterodimerization	L368E, K370S, E357Q	S364K	Figure 30D of US 2016/0355600
Fc 105	Heterodimerization	L368E, K370S, Q347E, E357L	S364K	Figure 30D of US 2016/0355600
Fc 106	Heterodimerization	L368E, K370S, Q347E, E357Q	S364K	Figure 30D of US 2016/0355600
Fc 107	Heterodimerization	L368D, K370T, Q347E	S364K	Figure 30D of US 2016/0355600
Fc 108	Heterodimerization	L368D, K370T, E357L	S364K	Figure 30D of US 2016/0355600
Fc 109	Heterodimerization	L368D, K370T, E357Q	S364K	Figure 30D of US 2016/0355600
Fc 110	Heterodimerization	L368D, K370T, Q347E, E357L	S364K	Figure 30D of US 2016/0355600
Fc 111	Heterodimerization	L368D, K370T, Q347E, E357Q	S364K	Figure 30D of US 2016/0355600
Fc 112	Heterodimerization	L368E, K370T, Q347E	S364K	Figure 30D of US 2016/0355600
Fc 113	Heterodimerization	L368E, K370T, E357L	S364K	Figure 30D of US 2016/0355600
Fc 114	Heterodimerization	L368E, K370T, E357Q	S364K	Figure 30D of US 2016/0355600
Fc 115	Heterodimerization	L368E, K370T, Q347E, E357L	S364K	Figure 30D of US 2016/0355600
Fc 116	Heterodimerization	L368E, K370T, Q347E, E357Q	S364K	Figure 30D of US 2016/0355600
Fc 117	Heterodimerization	T411E, Q362E	D401K, T411K	Figure 30D of US 2016/0355600
Fc 118	Heterodimerization	T411E, N390D	D401K, T411K	Figure 30D of US 2016/0355600
Fc 119	Heterodimerization	T411E, Q362E	D401R, T411R	Figure 30D of US 2016/0355600
Fc 120	Heterodimerization	T411E, N390D	D401R, T411R	Figure 30D of US 2016/0355600

TABLE 4
Fc Heterodimerization Strategies

NO.	STRATEGY	CH3 DOMAIN 1	CH3 DOMAIN 2	REFERENCES
Fc 121	Heterodimerization	Y407T	T366Y	Figure 30D of US 2016/0355600
Fc 122	Heterodimerization	F405A	T394W	Figure 30D of US 2016/0355600
Fc 123	Heterodimerization	T366Y, F405A	T394W, Y407T	Figure 30D of US 2016/0355600
Fc 124	Heterodimerization	T366S, L368A, Y407V	T366W	Figure 30D of US 2016/0355600
Fc 125	Heterodimerization	T366S, L368A, Y407V, Y349C	T366W, S354C	Figure 30D of US 2016/0355600
Fc 126	Heterodimerization	K392D, K409D	E356K,D399K	Figure 30E of US 2016/0355600
Fc 127	Heterodimerization	K370D, K392D, K409D	E356K, E357K, D399K	Figure 30E of US 2016/0355600
Fc 128	Heterodimerization	I199T, N203D, K247Q,R355Q, N384S, K392N, V397M, Q419E, K447	Q196K, L99T, P217R, P228R, N276K	Figure 30E of US 2016/0355600
Fc 129	Heterodimerization	I199T, N203D, K247Q,R355Q, N384S, K392N, V397M, Q419E, K447	Q196K, L99T, N276K	Figure 30E of US 2016/0355600
Fc 130	Heterodimerization	N384S, K392N, V397M, Q419E	N276K	Figure 30E of US 2016/0355600
Fc 131	Heterodimerization	D221E, P228E, L368E	D221R, P228R, K409R	Figure 30E of US 2016/0355600
Fc 132	Heterodimerization	C220E, P228E, L368E	C220R, E224R, P228R, K409R	Figure 30E of US 2016/0355600
Fc 133	Heterodimerization	F405L	K409R	Figure 30E of US 2016/0355600
Fc 134	Heterodimerization	T366I, K392M, T394W	F405A, Y407V	Figure 30E of US 2016/0355600
Fc 135	Heterodimerization	T366V, K409F	L351Y, Y407A	Figure 30E of US 2016/0355600
Fc 136	Heterodimerization	T366A, K392E, K409F, T411E	D399R, S400R, Y407A	Figure 30E of US 2016/0355600
Fc 137	Heterodimerization	L351K	L351E	Figure 30E of US 2016/0355600
Fc 138	Heterodimerization	I199T, N203D, K247Q,R355Q, Q419E, K447	Q196K, L199T, P217R, P228R, N276K	Figure 30E of US 2016/0355600
Fc 139	Heterodimerization	I199T, N203D, K247Q,R355Q, Q419E, K447	Q196K, I199T, N276K	Figure 30E of US 2016/0355600
Fc 140	Heterodimerization	I199T, N203D, K274Q, R355Q, N384S, K392N,		Figure 30E of US 2016/0355600

TABLE 4 Fc Heterodimerization Strategies				
NO.	STRATEGY	CH3 DOMAIN 1	CH3 DOMAIN 2	REFERENCES
		V397M, Q419E DEL447		
Fc 141	Heterodimerization	N208D, Q295E N384D, Q418E N421D		Figure 30E of US 2016/0355600
Fc 142	Heterodimerization	N208D, Q295E Q418E, N421D		Figure 30E of US 2016/0355600
Fc 143	Heterodimerization	Q196K, I199T P217R, P228R N276K		Figure 30E of US 2016/0355600
Fc 144	Heterodimerization	Q196K, I199T N276K		Figure 30E of US 2016/0355600
Fc 145	Heterodimerization	E269Q, E272Q E283Q, E357Q		Figure 30E of US 2016/0355600
Fc 146	Heterodimerization	E269Q, E272Q E283Q,		Figure 30E of US 2016/0355600
Fc 147	Heterodimerization	E269Q, E272Q		Figure 30E of US 2016/0355600
Fc 148	Heterodimerization	E269Q, E283Q		Figure 30E of US 2016/0355600
Fc 149	Heterodimerization	E272Q, E283Q		Figure 30E of US 2016/0355600
Fc 150	Heterodimerization	E269Q		Figure 30E of US 2016/0355600

[0336] Exemplary pairs of heterologous, non-identical Fc sequences that can pair to form a Fc heterodimer, and which can be included in CD19 binding molecules of the disclosure, include (i) SEQ ID NO:1106 and SEQ ID NO:1107, and (ii) SEQ ID NO:1106 and SEQ ID NO:1108.

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVCTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVS
KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:1106)

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS
KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:1107)

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS
KLTVDKSRWQQGNVFSCSVMHEALHNRYTQKSLSLSPGK (SEQ ID NO:1108)

An Fc region having an amino acid sequence of one of SEQ ID NOS: 1106-1108 can be modified to include one or more of the substitutions described in Section 7.4.1 (including its subparts), for example to include the substitution(s) corresponding to an ablation variant set forth in Table 3. In some embodiments, a CD19 binding molecule comprises an Fc region having an amino acid sequence of one of SEQ ID NOs:1106-1108 with a mutation at 1, 2, 3, 4, 5, 6, or more than 6 of positions 233, 234, 235, 236, 237, 239, 265, 266, 267, 268, 269, 297, 299, 322, 327, 328, 329, 330, 331 and 332 (EU numbering), for example mutation(s) described in Section 7.4.1 (including its subparts). For example, a CD19 binding molecule can comprise an Fc region having an amino acid sequence of SEQ ID NO:1106 with a mutation at 1, 2, 3, 4, 5, 6, or more than 6 of positions 233, 234, 235, 236, 237, 239, 265, 266, 267, 268, 269, 297, 299, 322, 327, 328, 329, 330, 331 and 332 and/or an Fc region having an amino acid sequence of SEQ ID NO:1107 with a mutation at 1, 2, 3, 4, 5, 6, or more than 6 of positions 233, 234, 235, 236, 237, 239, 265, 266, 267, 268, 269, 297, 299, 322, 327, 328, 329, 330, 331 and 332 and/or an Fc region having an amino acid sequence of SEQ ID NO:1108 with a mutation at 1, 2, 3, 4, 5, 6, or more than 6 of positions 233, 234, 235, 236, 237, 239, 265, 266, 267, 268, 269, 297, 299, 322, 327, 328, 329, 330, 331 and 332.

7.4.1.5.1. Steric Variants

[0337] CD19 binding molecules can comprise one or more, *e.g.*, a plurality, of modifications to one or more of the constant domains of an Fc domain, *e.g.*, to the CH3 domains. In one example, a CD19 binding molecule of the present disclosure comprises two polypeptides that each comprise a heavy chain constant domain of an antibody, *e.g.*, a CH2 or CH3 domain. In an example, the two heavy chain constant domains, *e.g.*, the CH2 or CH3 domains of the CD19 binding molecule comprise one or more modifications that allow for a heterodimeric association between the two chains. In one aspect, the one or more modifications are disposed on CH2 domains of the two heavy chains. In one aspect, the one or more modifications are disposed on CH3 domains of at least two polypeptides of the CD19 binding molecule.

[0338] One mechanism for Fc heterodimerization is generally referred to as “knobs and holes” or “knobs-into-holes”. These terms refer to amino acid mutations that create steric influences to favor formation of Fc heterodimers over Fc homodimers, as described in, *e.g.*, Ridgway *et al.*, 1996, Protein Engineering 9(7):617; Atwell *et al.*, 1997, J. Mol. Biol. 270:26; U.S. Patent No. 8,216,805. Knob-in-hole mutations can be combined with other strategies to improve heterodimerization.

[0339] In one aspect, the one or more modifications to a first polypeptide of the CD19 binding molecule comprising a heavy chain constant domain can create a “knob” and the one or more modifications to a second polypeptide of the CD19 binding molecule creates a “hole,” such that heterodimerization of the polypeptide of the CD19 binding molecule comprising a heavy chain

constant domain causes the "knob" to interface (*e.g.*, interact, *e.g.*, a CH2 domain of a first polypeptide interacting with a CH2 domain of a second polypeptide, or a CH3 domain of a first polypeptide interacting with a CH3 domain of a second polypeptide) with the "hole." The knob projects from the interface of a first polypeptide of the CD19 binding molecule comprising a heavy chain constant domain and is therefore positionable in a compensatory "hole" in the interface with a second polypeptide of the CD19 binding molecule comprising a heavy chain constant domain so as to stabilize the heteromultimer, and thereby favor heteromultimer formation over homomultimer formation, for example. The knob can exist in the original interface or can be introduced synthetically (*e.g.* by altering nucleic acid encoding the interface). The import residues for the formation of a knob are generally naturally occurring amino acid residues and can be selected from arginine (R), phenylalanine (F), tyrosine (Y) and tryptophan (W). In some cases, tryptophan and tyrosine are selected. In an embodiment, the original residue for the formation of the protuberance has a small side chain volume, such as alanine, asparagine, aspartic acid, glycine, serine, threonine or valine.

[0340] A "hole" comprises at least one amino acid side chain which is recessed from the interface of a second polypeptide of the CD19 binding molecule comprising a heavy chain constant domain and therefore accommodates a corresponding knob on the adjacent interfacing surface of a first polypeptide of the CD19 binding molecule comprising a heavy chain constant domain. The hole can exist in the original interface or can be introduced synthetically (*e.g.* by altering nucleic acid encoding the interface). The import residues for the formation of a hole are usually naturally occurring amino acid residues and are in some embodiments selected from alanine (A), serine (S), threonine (T) and valine (V). In one embodiment, the amino acid residue is serine, alanine or threonine. In another embodiment, the original residue for the formation of the hole has a large side chain volume, such as tyrosine, arginine, phenylalanine or tryptophan.

[0341] In an embodiment, a first CH3 domain is modified at residue 366, 405 or 407 to create either a "knob" or a hole" (as described above), and the second CH3 domain that heterodimerizes with the first CH3 domain is modified at: residue 407 if residue 366 is modified in the first CH3 domain, residue 394 if residue 405 is modified in the first CH3 domain, or residue 366 if residue 407 is modified in the first CH3 domain to create a "hole" or "knob" complementary to the "knob" or "hole" of the first CH3 domain.

[0342] In another embodiment, a first CH3 domain is modified at residue 366, and the second CH3 domain that heterodimerizes with the first CH3 domain is modified at residues 366, 368 and/or 407, to create a "hole" or "knob" complementary to the "knob" or "hole" of the first CH3 domain. In one embodiment, the modification to the first CH3 domain introduces a tyrosine (Y) residue at position 366. In an embodiment, the modification to the first CH3 is T366Y. In one

embodiment, the modification to the first CH3 domain introduces a tryptophan (W) residue at position 366. In an embodiment, the modification to the first CH3 is T366W. In some embodiments, the modification to the second CH3 domain that heterodimerizes with the first CH3 domain modified at position 366 (*e.g.*, has a tyrosine (Y) or tryptophan (W) introduced at position 366, *e.g.*, comprises the modification T366Y or T366W), comprises a modification at position 366, a modification at position 368 and a modification at position 407. In some embodiments, the modification at position 366 introduces a serine (S) residue, the modification at position 368 introduces an alanine (A), and the modification at position 407 introduces a valine (V). In some embodiments, the modifications comprise T366S, L368A and Y407V. In one embodiment, the first CH3 domain of the multispecific molecule comprises the modification T366Y, and the second CH3 domain that heterodimerizes with the first CH3 domain comprises the modifications T366S, L368A and Y407V, or vice versa. In one embodiment, the first CH3 domain of the multispecific molecule comprises the modification T366W, and the second CH3 domain that heterodimerizes with the first CH3 domain comprises the modifications T366S, L368A and Y407V, or vice versa.

[0343] Additional steric or "skew" (*e.g.*, knob in hole) modifications are described in PCT publication no. WO2014/145806 (for example, Figure 3, Figure 4 and Figure 12 of WO2014/145806), PCT publication no. WO2014/110601, and PCT publication no. WO 2016/086186, WO 2016/086189, WO 2016/086196 and WO 2016/182751. An example of a KIH variant comprises a first constant chain comprising a L368D and a K370S modification, paired with a second constant chain comprising a S364K and E357Q modification.

[0344] Additional knob in hole modification pairs suitable for use in any of the CD19 binding molecules of the present disclosure are further described in, for example, WO1996/027011, and Merchant *et al.*, 1998, Nat. Biotechnol., 16:677-681.

[0345] In further embodiments, the CH3 domains can be additionally modified to introduce a pair of cysteine residues. Without being bound by theory, it is believed that the introduction of a pair of cysteine residues capable of forming a disulfide bond provide stability to heterodimerized CD19 binding molecules, *e.g.*, MBMs, comprising paired CH3 domains. In some embodiments, the first CH3 domain comprises a cysteine at position 354, and the second CH3 domain that heterodimerizes with the first CH3 domain comprises a cysteine at position 349. In some embodiments, the first CH3 domain comprises a cysteine at position 354 (*e.g.*, comprises the modification S354C) and a tyrosine (Y) at position 366 (*e.g.*, comprises the modification T366Y), and the second CH3 domain that heterodimerizes with the first CH3 domain comprises a cysteine at position 349 (*e.g.*, comprises the modification Y349C), a serine at position 366 (*e.g.*, comprises the modification T366S), an alanine at position 368 (*e.g.*, comprises the modification L368A), and a valine at position 407 (*e.g.*, comprises the modification Y407V). In

some embodiments, the first CH3 domain comprises a cysteine at position 354 (*e.g.*, comprises the modification S354C) and a tryptophan (W) at position 366 (*e.g.*, comprises the modification T366W), and the second CH3 domain that heterodimerizes with the first CH3 domain comprises a cysteine at position 349 (*e.g.*, comprises the modification Y349C), a serine at position 366 (*e.g.*, comprises the modification T366S), an alanine at position 368 (*e.g.*, comprises the modification L368A), and a valine at position 407 (*e.g.*, comprises the modification Y407V).

[0346] An additional mechanism that finds use in the generation of heterodimers is sometimes referred to as "electrostatic steering" as described in Gunasekaran *et al.*, 2010, J. Biol. Chem. 285(25):19637. This is sometimes referred to herein as "charge pairs". In this embodiment, electrostatics are used to skew the formation towards heterodimerization. As a skilled artisan will appreciate, these can also have an effect on pI, and thus on purification, and thus could in some cases also be considered pI variants. However, as these were generated to force heterodimerization and were not used as purification tools, they are classified as "steric variants". These include, but are not limited to, D221E/P228E/L368E paired with D221R/P228R/K409R and C220E/P228E/368E paired with C220R/E224R/P228R/K409R.

[0347] Additional variants that can be combined with other variants, optionally and independently in any amount, such as pI variants outlined herein or other steric variants that are shown in Figure 37 of US 2012/0149876.

[0348] In some embodiments, the steric variants outlined herein can be optionally and independently incorporated with any pI variant (or other variants such as Fc variants, FcRn variants) into one or both Fc regions, and can be independently and optionally included or excluded from the CD19 binding molecules of the disclosure.

[0349] A list of suitable skew variants is found in Table 5 showing some pairs of particular utility in many embodiments. Of particular use in many embodiments are the pairs of sets including, but not limited to, S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411T/E360E/Q362E : D401K; L368D/K370S : S364K/E357L; and K370S : S364K/E357Q. In terms of nomenclature, the pair "S364K/E357Q : L368D/K370S" means that one of the Fc regions has the double variant set S364K/E357Q and the other has the double variant set L368D/K370S.

TABLE 5
Exemplary skew variants

Fc region 1	Fc region 2
F405A	T394F
S364D	Y349K
S364E	L368K
S364E	Y349K

TABLE 5 Exemplary skew variants	
Fc region 1	**Fc region 2**
S364F	K370G
S364H	Y349K
S364H	Y349T
S364Y	K370G
T411K	K370E
V397S/F405A	T394F
K370R/T411K	K370E/T411E
L351E/S364D	Y349K/L351K
L351E/S364E	Y349K/L351K
L351E/T366D	L351K/T366K
P395T/V397S/F405A	T394F
S364D/K370G	S364Y/K370R
S364D/T394F	Y349K/F405A
S364E/F405A	Y349K/T394F
S364E/F405S	Y349K/T394Y
S364E/T411E	Y349K/D401K
S364H/D401K	Y349T/T411E
S364H/F405A	Y349T/T394F
S364H/T394F	Y349T/F405A
Y349C/S364E	Y349K/S354C
L351E/S364D/F405A	Y349K/L351K/T394F
L351K/S364H/D401K	Y349T/L351E/T411E
S364E/T411E/F405A	Y349K/T394F/D401K
S364H/D401K/F405A	Y349T/T394F/T411E
S364H/F405A/T411E	Y349T/T394F/D401K
K370E/T411D	T411K
L368E/K409E	L368K
Y349T/T394F/S354C	S364H/F405A/Y349C
T411E	D401K
T411E	D401R/T411R
Q347E/K360E	Q347R
L368E	S364K
L368E/K370S	S364K
L368E/K370T	S364K
L368E/D401R	S364K
L368E/D401N	S364K
L368E	E357S/S364K
L368E	S364K/K409E
L368E	S364K/K409V
L368D	S364K
L368D/K370S	S364K
L368D/K370S	S364K/E357L
L368D/K370S	S364K/E357Q
T411E/K360E/Q362E	D401K
K370S	S364K
L368E/K370S	S364K/E357Q
K370S	S364K/E357Q
T411E/K360D	D401K
T411E/K360E	D401K
T411E/Q362E	D401K

TABLE 5 Exemplary skew variants	
Fc region 1	**Fc region 2**
T411E/N390D	D401K
T411E	D401K/Q347K
T411E	D401K/Q347R
T411E/K360D/Q362E	D401K
K392D/K409D	E356K/D399K
K370D/K392D/K409D	E356K/E357K/D399K
I199T/N203D/K247Q/R355Q/N384S/K392N/V397M/Q419E/ K447_	Q196K/I199T/P217R/P228 R /N276K
I199T/N203D/K247Q/R355Q/N384S/K392N/V397M/Q419E/ K447_	Q196K/I199T/N276K
N384S/K392N/V397M/Q419E	N276K
D221E/P228E/L368E	D221R/P228R/K409R
C220E/P228E/L368E	C220R/E224R/P228R/ K409R
F405L	K409R
T366I/K392M/T394W	F405A/Y407V
T366V/K409F	L351Y/Y407A
T366A/K392E/K409F/T411E	D399R/S400R/Y407A
L351K	L351E
I199T/N203D/K247Q/R355Q/Q419E/K447_	Q196K/I199T/P217R/P228 R/ N276K
I199T/N203D/K247Q/R355Q/Q419E/K447_	Q196K/I199T/N276K
I199T N203D K274Q R355Q N384S K392N V397M Q419E DEL447	
N208D Q295E N384D Q418E N421D	
N208D Q295E Q418E N421D	
Q196K I199T P217R P228R N276K	
Q196K I199T N276K	
E269Q E272Q E283Q E357Q	
E269Q E272Q E283Q	
E269Q E272Q	
E269Q E283Q	
E272Q E283Q	
E269Q	
T411E/K360E/N390D	D401K
T411E/Q362E/N390D	D401K
T411E/Q347R	D401K/K360D
T411E/Q347R	D401K/K360E
T411E/K360	D401K/Q347K
T411E/K360D	D401K/Q347R
T411E/K360E	D401K/Q347K
T411E/K360E	D401K/Q347R
T411E/S364K	D401K/K370S
T411E/K370S	D401K/S364K
Q347E	E357Q
Q347E	E357Q/Q362K
K360D/Q362E	Q347R
K360D/Q362E	D401K
K360D/Q362E	Q347R/D401K
K360E/Q362E	Q347R

TABLE 5 Exemplary skew variants	
Fc region 1	**Fc region 2**
K360E/Q362E	D401K
K360E/Q362E	Q347R/D401K
Q362E/N390D	D401K
Q347E/K360D	D401N
K360D	Q347R/N390K
K360D	N390K/D401N
K360E	Y349H
K370S/Q347E	S364K
K370S/E357L	S364K
K370S/E357Q	S364K
K370S/Q347E/E357L	S364K
K370S/Q347E/E357Q	S364K
L368D/K370S/Q347E	S364K
L368D/K370S/E357L	S364K
L368D/K370S/E357Q	S364K
L368D/K370S/Q347E/E357L	S364K
L368D/K370S/Q347E/E357Q	S364K
L368E/K370S/Q347E	S364K
L368E/K370S/E357L	S364K
L368E/K370S/E357Q	S364K
L368E/K370S/Q347E/E357L	S364K
L368E/K370S/Q347E/E357Q	S364K
L368D/K370T/Q347E	S364K
L368D/K370T/E357L	S364K
L368D/K370T/E357Q	S364K
L368D/K370T/Q347E/E357L	S364K
L368D/K370T/Q347E/E357Q	S364K
L368E/K370T/Q347E	S364K
L368E/K370T/E357L	S364K
L368E/K370T/E357Q	S364K
L368E/K370T/Q347E/E357L	S364K
L368E/K370T/Q347E/E357Q	S364K
T411E/Q362E	D401K/T411K
T411E/N390D	D401K/T411K
T411E/Q362E	D401R/T411R
T411E/N390D	D401R/T411R
Y407T	T366Y
F405A	T394W
T366Y/F405A	T394W/Y407T
Y407A	T366W
T366S/L368A/Y407V	T366W
T366S/L368A/Y407V/Y349C	T366W/S354C
K392D/K409D	E356K/D399K
K370D/K392D/K409D	E356K/E357K/D399K
I199T/N203D/K247Q/R355Q/N384S/K392N/V397M/Q419E/ K447_	Q196K/I199T/P217R/P228 R /N276K
I199T/N203D/K247Q/R355Q/N384S/K392N/V397M/Q419E/ K447_	Q196K/I199T/N276K
N384S/K392N/V397M/Q419E	N276K
D221E/P228E/L368E	D221R/P228R/K409R

TABLE 5 Exemplary skew variants	
Fc region 1	**Fc region 2**
C220E/P228E/L368E	C220R/E224R/P228R/ K409R
F405L	K409R
T366I/K392M/T394W	F405A/Y407V
T366V/K409F	L351Y/Y407A
T366A/K392E/K409F/T411E	D399R/S400R/Y407A
L351K	L351E
I199T/N203D/K247Q/R355Q/Q419E/K447_	Q196K/I199T/P217R/P228R /N276K
I199T/N203D/K247Q/R355Q/Q419E/K447_	Q196K/I199T/N276K
I199T N203D K274Q R355Q N384S K392N V397M Q419E DEL447	
N208D Q295E N384D Q418E N421D	
Q295E N384D Q418E N421D	
N208D Q295E Q418E N421D	
Q295E Q418E N421D	
Q196K I199T P217R P228R N276K	
Q196K I199T N276K	
E269Q E272Q E283Q E357Q	
E269Q E272Q E283Q	
E269Q E272Q	
E269Q E283Q	
E272Q E283Q	
E269Q	

[0350] In some embodiments, a CD19 binding molecule comprises a first Fc region and a second Fc region. In some embodiments, the first Fc region comprises the following mutations: L368D and K370S, and the second Fc region comprises the following mutations: S364K and E357Q. In some embodiments, the first Fc region comprises the following mutations: S364K and E357Q, and the second Fc region comprises the following mutations: L368D and K370S.

7.4.1.5.2. Alternative Knob and Hole: IgG Heterodimerization

[0351] Heterodimerization of polypeptide chains of a CD19 binding molecule comprising paired CH3 domains can be increased by introducing one or more modifications in a CH3 domain which is derived from the IgG1 antibody class. In an embodiment, the modifications comprise a K409R modification to one CH3 domain paired with F405L modification in the second CH3 domain. Additional modifications can also, or alternatively, be at positions 366, 368, 370, 399, 405, 407, and 409. In some cases, heterodimerization of polypeptides comprising such modifications is achieved under reducing conditions, *e.g.*, 10-100 mM 2-MEA (*e.g.*, 25, 50, or 100 mM 2-MEA) for 1-10, *e.g.*, 1.5-5, *e.g.*, 5, hours at 25-37C, *e.g.*, 25C or 37C.

[0352] The amino acid replacements described herein can be introduced into the CH3 domains using techniques which are well known (see, *e.g.*, McPherson, ed., 1991, Directed Mutagenesis: a Practical Approach; Adelman *et al.*, 1983, DNA, 2:183).

[0353] The IgG heterodimerization strategy is further described in, for example, WO2008/119353, WO2011/131746, and WO2013/060867.

[0354] In any of the embodiments described in this Section, the CH3 domains can be additionally modified to introduce a pair of cysteine residues as described in Section 7.4.1.3.

7.4.1.5.3. pI (Isoelectric point) Variants

[0355] In general, as will be appreciated by a skilled artisan, there are two general categories of pI variants: those that increase the pI of the protein (basic changes) and those that decrease the pI of the protein (acidic changes). As described herein, all combinations of these variants can be done: one Fc region can be wild type, or a variant that does not display a significantly different pI from wild-type, and the other can be either more basic or more acidic. Alternatively, each Fc region is changed, one to more basic and one to more acidic.

[0356] Exemplary combinations of pI variants are shown in Table 6. As outlined herein and shown in Table 6, these changes are shown relative to IgG1, but all isotypes can be altered this way, as well as isotype hybrids. In the case where the heavy chain constant domain is from IgG2-4, R133E and R133Q can also be used.

TABLE 6
Exemplary pI Variant Combinations

Variant constant region	Substitutions
pI_ISO(-)	I199T N203D K274Q R355Q N384S K392N V397M Q419E DEL447
pI_(-)_isosteric_A	N208D Q295E N384D Q418E N421D
pI_(-)_isosteric A-Fc only	Q295E N384D Q418E N421D
pI_(-)_isosteric_B	N208D Q295E Q418E N421D
pI_(-)_isosteric_B-Fc only	Q295E Q418E N421D
pI_ISO(+RR)	Q196K I199T P217R P228R N276K
pI_ISO(+)	Q196K I199T N276K
pI_(+)_isosteric_A	E269Q E272Q E283Q E357Q
pI_(+)_isosteric_B	E269Q E272Q E283Q
pI_(+)_isosteric_E269Q/E272Q	E269Q E272Q

pI_(+)_isosteric_E269Q/E283Q	E269Q E283Q
pI_(+)_isosteric_E272Q/E283Q	E272Q E283Q
pI_(+)_isosteric_E269Q	E269Q

[0357] In one embodiment, for example in the FIG. 1B-1W, FIG. 1Y-1AH, FIG. 2B-2L, and FIG 2N-2V formats, a combination of pI variants has one Fc region (the negative Fab side) comprising 208D/295E/384D/418E/421D variants (N208D/Q295E/N384D/Q418E/N421D when relative to human IgG1) and a second Fc region (the positive scFv side) comprising a positively charged scFv linker, *e.g.*, L36 (described in Section 7.4.3). However, as will be appreciated by a skilled artisan, the first Fc region includes a CH1 domain, including position 208. Accordingly, in constructs that do not include a CH1 domain (for example for MBMs that do not utilize a CH1 domain as one of the domains, for example in a format depicted in FIG. 2K), a negative pI variant Fc set can include 295E/384D/418E/421D variants (Q295E/N384D/Q418E/N421D when relative to human IgG1).

[0358] In some embodiments, a first Fc region has a set of substitutions from Table 6 and a second Fc region is connected to a charged linker (*e.g.*, selected from those described in Section 7.4.3).

[0359] In some embodiments, the CD19 binding molecule of the present disclosure comprises a first Fc region and a second Fc region. In some embodiments, the first Fc region comprises the following mutations: N208D, Q295E, N384D, Q418E, and N421D. In some embodiments, the second Fc region comprises the following mutations: N208D, Q295E, N384D, Q418E, and N421D.

7.4.1.5.4. Isotopic Variants

[0360] In addition, many embodiments of the disclosure rely on the "importation" of pI amino acids at particular positions from one IgG isotype into another, thus reducing or eliminating the possibility of unwanted immunogenicity being introduced into the variants. A number of these are shown in Figure 21 of US Publ. 2014/0370013. That is, IgG1 is a common isotype for therapeutic antibodies for a variety of reasons, including high effector function. However, the heavy constant region of IgG1 has a higher pI than that of IgG2 (8.10 versus 7.31). By introducing IgG2 residues at particular positions into the IgG1 backbone, the pI of the resulting Fc region is lowered (or increased) and additionally exhibits longer serum half-life. For example, IgG1 has a glycine (pI 5.97) at position 137, and IgG2 has a glutamic acid (pI 3.22); importing the glutamic acid will affect the pI of the resulting protein. As is described below, a number of amino acid substitutions are generally required to significantly affect the pI of the

variant antibody. However, it should be noted as discussed below that even changes in IgG2 molecules allow for increased serum half-life.

[0361] In other embodiments, non-isotypic amino acid changes are made, either to reduce the overall charge state of the resulting protein (*e.g.*, by changing a higher pI amino acid to a lower pI amino acid), or to allow accommodations in structure for stability, as is further described below.

[0362] In addition, by pI engineering both the heavy and light constant domains of a CD19 binding molecule comprising two half antibodies, significant changes in each half antibody can be seen. Having the pIs of the two half antibodies differ by at least 0.5 can allow separation by ion exchange chromatography or isoelectric focusing, or other methods sensitive to isoelectric point.

7.4.1.5.5. Calculating pI

[0363] The pI of a half antibody comprising an Fc region and an ABM or ABM chain can depend on the pI of the variant heavy chain constant domain and the pI of the total half antibody, including the variant heavy chain constant domain and ABM or ABM chain. Thus, in some embodiments, the change in pI is calculated on the basis of the variant heavy chain constant domain, using the chart in the Figure 19 of US Pub. 2014/0370013. As discussed herein, which half antibody to engineer is generally decided by the inherent pI of the half antibodies. Alternatively, the pI of each half antibody can be compared.

7.4.1.5.6. pI Variants that also confer better FcRn in vivo binding

[0364] In the case where a pI variant decreases the pI of an Fc region, it can have the added benefit of improving serum retention in vivo.

[0365] pI variant Fc regions are believed to provide longer half-lives to antigen binding molecules in vivo, because binding to FcRn at pH 6 in an endosome sequesters the Fc (Ghetie and Ward, 1997, Immunol Today. 18(12): 592-598). The endosomal compartment then recycles the Fc to the cell surface. Once the compartment opens to the extracellular space, the higher pH ~7.4, induces the release of Fc back into the blood. In mice, Dall' Acqua *et al*. showed that Fc mutants with increased FcRn binding at pH 6 and pH 7.4 actually had reduced serum concentrations and the same half life as wild-type Fc (Dall' Acqua *et al*,. 2002, J. Immunol. 169:5171-5180). The increased affinity of Fc for FcRn at pH 7.4 is thought to forbid the release of the Fc back into the blood. Therefore, the Fc mutations that will increase Fc's half-life in vivo will ideally increase FcRn binding at the lower pH while still allowing release of Fc at higher pH. The amino acid histidine changes its charge state in the pH range of 6.0 to 7.4. Therefore, it is not surprising to find His residues at important positions in the Fc/FcRn complex.

[0366] It has been suggested that antibodies with variable regions that have lower isoelectric points can also have longer serum half-lives (Igawa *et al.*, 2010, PEDS. 23(5): 385-392). However, the mechanism of this is still poorly understood. Moreover, variable regions differ from antibody to antibody. Constant region variants with reduced pI and extended half-life would provide a more modular approach to improving the pharmacokinetic properties of CD19 binding molecules, as described herein.

7.4.1.5.7. Polar Bridge

[0367] Heterodimerization of polypeptide chains of CD19 binding molecules, *e.g.*, MBMs, comprising an Fc domain can be increased by introducing modifications based on the "polar-bridging" rationale, which is to make residues at the binding interface of the two polypeptide chains to interact with residues of similar (or complimentary) physical property in the heterodimer configuration, while with residues of different physical property in the homodimer configuration. In particular, these modifications are designed so that, in the heterodimer formation, polar residues interact with polar residues, while hydrophobic residues interact with hydrophobic residues. In contrast, in the homodimer formation, residues are modified so that polar residues interact with hydrophobic residues. The favorable interactions in the heterodimer configuration and the unfavorable interactions in the homodimer configuration work together to make it more likely for Fc regions to form heterodimers than to form homodimers.

[0368] In an exemplary embodiment, the above modifications are generated at one or more positions of residues 364, 368, 399, 405, 409, and 411 of a CH3 domain.

[0369] In some embodiments, one or more modifications selected from the group consisting of S364L, T366V, L368Q, N399K, F405S, K409F and R411K are introduced into one of the two CH3 domains. One or more modifications selected from the group consisting of Y407F, K409Q and T411N can be introduced into the second CH3 domain.

[0370] In another embodiment, one or more modifications selected from the group consisting of S364L, T366V, L368Q, D399K, F405S, K409F and T411K are introduced into one CH3 domain, while one or more modifications selected from the group consisting of Y407F, K409Q and T411D are introduced into the second CH3 domain.

[0371] In one exemplary embodiment, the original residue of threonine at position 366 of one CH3 domain is replaced by valine, while the original residue of tyrosine at position 407 of the other CH3 domain is replaced by phenylalanine.

[0372] In another exemplary embodiment, the original residue of serine at position 364 of one CH3 domain is replaced by leucine, while the original residue of leucine at position 368 of the same CH3 domain is replaced by glutamine.

[0373] In yet another exemplary embodiment, the original residue of phenylalanine at position 405 of one CH3 domain is replaced by serine and the original residue of lysine at position 409 of this CH3 domain is replaced by phenylalanine, while the original residue of lysine at position 409 of the other CH3 domain is replaced by glutamine.

[0374] In yet another exemplary embodiment, the original residue of aspartic acid at position 399 of one CH3 domain is replaced by lysine, and the original residue of threonine at position 411 of the same CH3 domain is replaced by lysine, while the original residue of threonine at position 411 of the other CH3 domain is replaced by aspartic acid.

[0375] The amino acid replacements described herein can be introduced into the CH3 domains using techniques which are well known (see, *e.g.*, McPherson, ed., 1991, Directed Mutagenesis: a Practical Approach; Adelman *et al.*, 1983, DNA, 2:183). The polar bridge strategy is described in, for example, WO2006/106905, WO2009/089004 and Gunasekaran *et al.*, 2010, JBC 285:19637-19646.

[0376] Additional polar bridge modifications are described in, for example, PCT publication no. WO2014/145806 (for example, Figure 6 of WO2014/145806), PCT publication no. WO2014/110601, and PCT publication no. WO 2016/086186, WO 2016/086189, WO 2016/086196 and WO 2016/182751. An example of a polar bridge variant comprises a constant chain comprising a N208D, Q295E, N384D, Q418E and N421D modification.

[0377] In any of the embodiments described herein, the CH3 domains can be additionally modified to introduce a pair of cysteine residues as described in Section 7.4.1.3.

[0378] Additional strategies for enhancing heterodimerization are described in, for example, WO2016/105450, WO2016/086186, WO2016/086189, WO2016/086196, WO2016/141378, and WO2014/145806, and WO2014/110601. Any of the strategies can be employed in a CD19 binding molecule described herein.

7.4.1.6. Combination of Heterodimerization Variants and Other Fc Variants

[0379] As will be appreciated by a skilled artisan, all of the recited heterodimerization variants (including skew and/or pI variants) can be optionally and independently combined in any way, as long as the Fc regions of an Fc domain retain their ability to dimerize. In addition, all of these variants can be combined into any of the heterodimerization formats.

[0380] In the case of pI variants, while embodiments finding particular use are shown in the Table 6, other combinations can be generated, following the basic rule of altering the pI difference between two Fc regions in an Fc heterodimer to facilitate purification.

[0381] In addition, any of the heterodimerization variants, skew and pI, are also independently and optionally combined with Fc ablation variants, Fc variants, FcRn variants, as generally outlined herein.

[0382] In some embodiments, a particular combination of skew and pI variants that finds use in the present disclosure is T366S/L368A/Y407V : T366W (optionally including a bridging disulfide, T366S/L368A/Y407V/Y349C : T366W/S354C) with one Fc region comprising Q295E/N384D/Q418E/N481D and the other a positively charged scFv linker (when the format includes an scFv domain). As will be appreciated by a skilled artisan, the "knobs in holes" variants do not change pI, and thus can be used on either one of the Fc regions in an Fc heterodimer.

[0383] In some embodiments, first and second Fc regions that find use the present disclosure include the amino acid substitutions S364K/E357Q : L368D/K370S, where the first and/or second Fc region includes the ablation variant substitutions 233P/L234V/L235A/G236del/S267K, and the first and/or second Fc region comprises the pI variant substitutions N208D/Q295E/N384D/Q418E/N421D (pI_(-)_isosteric_A).

7.4.2. Hinge Regions

[0384] The CD19 binding molecules can also comprise hinge regions, *e.g.*, connecting an antigen-binding domain to an Fc region. The hinge region can be a native or a modified hinge region. Hinge regions are typically found at the N-termini of Fc regions.

[0385] A native hinge region is the hinge region that would normally be found between Fab and Fc domains in a naturally occurring antibody. A modified hinge region is any hinge that differs in length and/or composition from the native hinge region. Such hinges can include hinge regions from other species, such as human, mouse, rat, rabbit, shark, pig, hamster, camel, llama or goat hinge regions. Other modified hinge regions can comprise a complete hinge region derived from an antibody of a different class or subclass from that of the heavy chain Fc region. Alternatively, the modified hinge region can comprise part of a natural hinge or a repeating unit in which each unit in the repeat is derived from a natural hinge region. In a further alternative, the natural hinge region can be altered by converting one or more cysteine or other residues into neutral residues, such as serine or alanine, or by converting suitably placed residues into cysteine residues. By such means the number of cysteine residues in the hinge region can be increased or decreased. This approach is described further in U.S. Patent No. 5,677,425 by Bodmer *et al.*. Altering the number of cysteine residues in a hinge region can, for example, facilitate assembly of light and heavy chains, or increase or decrease the stability of a CD19 binding molecule. Other modified hinge regions can be entirely synthetic and can be designed to possess desired properties such as length, cysteine composition and flexibility.

[0386] A number of modified hinge regions have been described for example, in U.S. Pat. No. 5,677,425, WO9915549, WO2005003170, WO2005003169, WO2005003170, WO9825971 and WO2005003171.

[0387] Examples of suitable hinge sequences are shown in Table 7.

TABLE 7
Hinge Sequences

Hinge Name	Hinge Description	Hinge Sequence	SEQ ID NO:
H1	Human IgA1	VPSTPPTPSPSTPPTPSPS	57
H2	Human IgA2	VPPPPP	58
H3	Human IgD	ESPKAQASSVPTAQPQAEGSLAKATTAPATTRN TGRGGEEKKKEKEKEEQEERETKTP	59
H4	Human IgG1	EPKSCDKTHTCPPCP	60
H5	Human IgG2	ERKCCVECPPCP	61
H6	Human IgG3	ELKTPLGDTTHTCPRCPEPKSCDTPPPCPRCPE PKSCDTPPPCPRCPEPKSCDTPPPCPRCP	62
H7	Human IgG4	ESKYGPPCPSCP	63
H8	Human IgG4(P)	ESKYGPPCPPCP	64
H9	Engineered v1	CPPC	55
H10	Engineered v2	CPSC	65
H11	Engineered v3	CPRC	66
H12	Engineered v4	SPPC	67
H13	Engineered v5	CPPS	68
H14	Engineered v6	SPPS	56
H15	Engineered v7	DKTHTCAA	69
H16	Engineered v8	DKTHTCPPCPA	70
H17	Engineered v9	DKTHTCPPCPATCPPCPA	71
H18	Engineered v10	DKTHTCPPCPATCPPCPATCPPCPA	72
H19	Engineered v11	DKTHTCPPCPAGKPTLYNSLVMSDTAGTCY	73
H20	Engineered v12	DKTHTCPPCPAGKPTHVNVSVVMAEVDGTCY	74
H21	Engineered v13	DKTHTCCVECPPCPA	75
H22	Engineered v14	DKTHTCPRCPEPKSCDTPPPCPRCPA	76
H23	Engineered v15	DKTHTCPSCPA	77

[0388] In one embodiment, the heavy chain Fc region possesses an intact hinge region at its N-terminus.

[0389] In one embodiment, the heavy chain Fc region and hinge region are derived from IgG4 and the hinge region comprises the modified sequence CPPC (SEQ ID NO:55). The core hinge region of human IgG4 contains the sequence CPSC (SEQ ID NO:65) compared to IgG1 which contains the sequence CPPC (SEQ ID NO:55). The serine residue present in the IgG4 sequence leads to increased flexibility in this region, and therefore a proportion of molecules form disulfide bonds within the same protein chain (an intrachain disulfide) rather than bridging to the other heavy chain in the IgG molecule to form the interchain disulfide. (Angel *et al.*, 1993, Mol Immunol 30(1):105-108). Changing the serine residue to a proline to give the same core sequence as IgG1 allows complete formation of inter-chain disulfides in the IgG4 hinge region, thus reducing heterogeneity in the purified product. This altered isotype is termed IgG4P.

7.4.3. ABM Linkers

[0390] In certain aspects, the present disclosure provides CD19 binding molecules where two or more components of an ABM (*e.g.*, a VH and a VL of an scFv), two or more ABMs, or an ABM and a non-ABM domain (*e.g.*, a dimerization domain such as an Fc region) are connected to one another by a peptide linker. Such linkers are referred to herein an “ABM linkers.”,

[0391] A peptide linker can range from 2 amino acids to 60 or more amino acids, and in certain aspects a peptide linker ranges from 3 amino acids to 50 amino acids, from 4 to 30 amino acids, from 5 to 25 amino acids, from 10 to 25 amino acids or from 12 to 20 amino acids. In particular embodiments, a peptide linker is 2 amino acids, 3 amino acids, 4 amino acid, 5 amino acids, 6 amino acids, 7 amino acids, 8 amino acids, 9 amino acids, 10 amino acids, 11 amino acids, 12 amino acids, 13 amino acids, 14 amino acid, 15 amino acids, 16 amino acids, 17 amino acids, 18 amino acids, 19 amino acids, 20 amino acids, 21 amino acids, 22 amino acids, 23 amino acids, 24 amino acid, 25 amino acids, 26 amino acids, 27 amino acids, 28 amino acids, 29 amino acids, 30 amino acids, 31 amino acids, 32 amino acids, 33 amino acids, 34 amino acid, 35 amino acids, 36 amino acids, 37 amino acids, 38 amino acids, 39 amino acids, 40 amino acids, 41 amino acids, 42 amino acids, 43 amino acids, 44 amino acid, 45 amino acids, 46 amino acids, 47 amino acids, 48 amino acids, 49 amino acids, or 50 amino acids in length.

[0392] Charged and/or flexible linkers can be used.

[0393] Examples of flexible ABM linkers that can be used in the CD19 binding molecules include those disclosed by Chen *et al.*, 2013, Adv Drug Deliv Rev. 65(10):1357–1369 and Klein *et al.*, 2014, Protein Engineering, Design & Selection 27(10):325–330. A particularly useful flexible linker is (GGGGS)n (also referred to as (G4S)n) (SEQ ID NO:78). In some embodiments, n is any number between 1 and 10, *i.e.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10, or any

range bounded by any two of the foregoing numbers, *e.g.*, 1 to 5, 2 to 5, 3 to 6, 2 to 4, 1 to 4, and so on and so forth.

[0394] Other examples of suitable ABM linkers for use in the CD19 binding molecules of the present disclosure are shown in Table 8 below:

TABLE 8
ABM Linker Sequences

Linker Name	Linker Sequence	SEQ ID NO:
L1	ADAAP	79
L2	ADAAPTVSIFP	80
L3	ADAAPTVSIFPP	81
L4	AKTTAP	82
L5	AKTTAPSVYPLAP	83
L6	AKTTPKLEEGEFSEARV	84
L7	AKTTPKLGG	85
L8	AKTTPP	86
L9	AKTTPPSVTPLAP	87
L10	ASTKGP	88
L11	ASTKGPSVFPLAP	89
L12	ASTKGPSVFPLAPASTKGPSVFPLAP	90
L13	EGKSSGSGSESKST	91
L14	GEGESGEGESGEGES	92
L15	GEGESGEGESGEGESGEGES	93
L16	GEGGSGEGGSGEGGS	94
L17	GENKVEYAPALMALS	95
L18	GGEGSGGEGSGGEGS	96
L19	GGGESGGEGSGEGGS	97
L20	GGGESGGGESGGGES	98
L21	$(GGGGS)_n$ (also referred to as $(G4S)_n$), where n can be 1-10.	99
L22	GGGGSGGGGS	100
L23	GGGGSGGGGSGGGGS	53
L24	GGGGSGGGGSGGGGSGGGGS	101
L25	GGGKSGGGKSGGGKS	102
L26	GGGKSGGKGSGKGGS	103
L27	GGKGSGGKGSGGKGS	104
L28	GGSGG	105

TABLE 8 ABM Linker Sequences		
Linker Name	**Linker Sequence**	**SEQ ID NO:**
L29	GGSGGGGSG	106
L30	GGSGGGGSGGGGS	107
L31	GHEAAAVMQVQYPAS	108
L32	GKGGSGKGGSGKGGS	109
L33	GKGKSGKGKSGKGKS	110
L34	GKGKSGKGKSGKGKSGKGKS	111
L35	GKPGSGKPGSGKPGS	112
L36	GKPGSGKPGSGKPGSGKPGS	113
L37	GPAKELTPLKEAKVS	114
L38	GSAGSAAGSGEF	115
L39	IRPRAIGGSKPRVA	116
L40	KESGSVSSEQLAQFRSLD	117
L41	KTTPKLEEGEFSEAR	118
L42	QPKAAP	119
L43	QPKAAPSVTLFPP	120
L44	RADAAAA$(G4S)_4$	121
L45	RADAAAAGGPGS	122
L46	RADAAP	123
L47	RADAAPTVS	124
L48	SAKTTP	125
L49	SAKTTPKLEEGEFSEARV	126
L50	SAKTTPKLGG	127
L51	STAGDTHLGGEDFD	128
L52	TVAAP	129
L53	TVAAPSVFIFPP	130
L54	TVAAPSVFIFPPTVAAPSVFIFPP	131
L55	GSTSGSGKPGSGEGSTKG	132
L56	PRGASKSGSASQTGSAPGS	133
L57	GTAAAGAGAAGGAAAGAAG	134
L58	GTSGSSGSGSGGSGSGGGG	135

[0395] In various aspects, the disclosure provides a CD19 binding molecule which comprises one or more ABM linkers. Each of the ABM linkers can be range from 2 amino acids to 60

amino acids in length, *e.g.*, 4 to 30 amino acids, from 5 to 25 amino acids, from 10 to 25 amino acids or from 12 to 20 amino acids in length, optionally selected from Table 8 above. In particular embodiments, the CD19 binding molecule comprises two, three, four, five or six ABM linkers. The ABM linkers can be on one, two, three, four or even more polypeptide chains of the CD19 binding molecule.

7.5. Bispecific Binding Molecule Configurations

[0396] Exemplary BBM configurations are shown in FIG. 1. FIG. 1A shows the components of the BBM configurations shown in FIGS. 1B-1AH. The scFv, Fab, scFab, non-immunoglobulin based ABM, and Fc domains each can have the characteristics described for these components in Sections 7.3 and 7.4. The components of the BBM configurations shown in FIG. 1 can be associated with each other by any of the means described in Sections 7.3 and 7.4 (*e.g.*, by direct bonds, ABM linkers, disulfide bonds, Fc domains with modified with knob in hole interactions, *etc.*). The orientations and associations of the various components shown in FIG. 1 are merely exemplary; as will be appreciated by a skilled artisan, other orientations and associations can be suitable (*e.g.*, as described in Sections 7.3 and 7.4).

[0397] BBMs are not limited to the configurations shown in FIG. 1. Other configurations that can be used are known to those skilled in the art. See, *e.g.*, WO 2014/145806; WO 2017/124002; Liu *et al.*, 2017, Front Immunol. 8:38; Brinkmann & Kontermann, 2017, mAbs 9:2, 182-212; US 2016/0355600; Klein *et al.*, 2016, MAbs 8(6):1010-20; and US 2017/0145116.

7.5.1. Exemplary Bivalent BBMs

[0398] The BBMs can be bivalent, *i.e.*, they have two antigen-binding domains, one of which binds CD19 (ABM1) and one of which binds a second target antigen (ABM2), *e.g.*, a component of a TCR complex.

[0399] Exemplary bivalent BBM configurations are shown in FIGS. 1B-1F.

[0400] As depicted in FIGS. 1B-1D, a BBM can comprise two half antibodies, one comprising one ABM and the other comprising one ABM, the two halves paired through an Fc domain.

[0401] In the embodiment of FIG. 1B, the first (or left) half antibody comprises a Fab and an Fc region, and the second (or right) half antibody comprises a Fab and an Fc region. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0402] In the embodiment of FIG. 1C, the first (or left) half antibody comprises a Fab and an Fc region, and the second (or right) half antibody comprises a scFv and an Fc region. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0403] In the embodiment of FIG. 1D, the first (or left) half antibody comprises an scFv and an Fc region, and the second (or right) half antibody comprises an scFv and an Fc region. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0404] As depicted in FIGS. 1E-1F, a bivalent BBM can comprise two ABMs attached to one Fc region of an Fc domain.

[0405] In the embodiment of FIG. 1E, the BBM comprises a Fab, a scFv and an Fc domain, where the scFv is located between the Fab and the Fc domain.

[0406] In the embodiment of FIG. 1F, (the "one-arm scFv-mAb" configuration) BBM comprises a Fab, a scFv and an Fc domain, where the Fab is located between the scFv and the Fc domain.

[0407] In the configuration shown in FIGS. 1B-1F, each of X and Y represent either ABM1 or ABM2, provided that the BBM comprises one ABM1 and one ABM2. Accordingly, the present disclosure provides a bivalent BBM as shown in any one of FIGS. 1B through 1F, where X is an ABM1 and Y is an ABM2 (this configuration of ABMs designated as "B1" for convenience). The present disclosure also provides a bivalent BBM as shown in any one of FIGS. 1B through 1F, where X is an ABM2 and Y is an ABM1 (this configuration of ABMs designated as "B2" for convenience).

7.5.2. Exemplary Trivalent BBMs

[0408] The BBMs can be trivalent, *i.e.*, they have three antigen-binding domains, one or two of which binds CD19 (ABM1) and one or two of which binds a second target antigen (ABM2), *e.g.*, a component of a TCR complex.

[0409] Exemplary trivalent BBM configurations are shown in FIGS. 1G-1Z.

[0410] As depicted in FIGS. 1G-1N, 1Q-1W, 1Y-1Z a BBM can comprise two half antibodies, one comprising two ABMs and the other comprising one ABM, the two halves paired through an Fc domain.

[0411] In the embodiment of FIG. 1G, the first (or left) half antibody comprises Fab and an Fc region, and the second (or right) half antibody comprises a scFv, a Fab, and an Fc region. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0412] In the embodiment of FIG. 1H, the first (or left) half antibody comprises a Fab and an Fc region, and the second (or right) half antibody comprises a Fab, an scFv, and an Fc region. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0413] In the embodiment of FIG. 1I, the first (or left) half antibody comprises an scFv and an Fc region, and the second (or right) half antibody comprises two Fabs and an Fc region. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0414] In the embodiment of FIG. 1J, the first (or left) half antibody comprises two Fav and an Fc region, and the second (or right) half antibody comprises a Fab and an Fc region. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0415] In the embodiment of FIG. 1K, the first (or left) half antibody comprises an scFv and an Fc region, and the second (or right) half antibody comprises two scFvs and an Fc region. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0416] In the embodiment of FIG. 1L, the first (or left) half antibody comprises an scFv and an Fc region, and the second (or right) half antibody comprises an scFv, a Fab, and an Fc region. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0417] In the embodiment of FIG. 1M, the first (or left) half antibody comprises a scFv and an Fc region, and the second (or right) half antibody comprises a Fab, a scFv and an Fc region. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0418] In the embodiment of FIG. 1N, the first (or left) half antibody comprises a diabody-type binding domain and an Fc region, and the second (or right) half antibody comprises a Fab and an Fc region. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0419] In the embodiment of FIG. 1Q, the first (or left) half antibody comprises a Fab and an Fc region, and the second (or right) half antibody comprises a Fab, an Fc region, and an scFv. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0420] In the embodiment of FIG. 1R, the first (or left) half antibody comprises a scFv and an Fc region, and the second (or right) half antibody comprises a Fab, an Fc region, and an scFv. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0421] In the embodiment of FIG. 1S, the first (or left) half antibody comprises an scFv and an Fc region, and the second (or right) half antibody comprises an scFv, an Fc region, and a second scFv. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0422] In the embodiment of FIG. 1T, the first (or left) half antibody comprises an scFv, an Fc region, and a Fab, and the second (or right) half antibody comprises a Fab and an Fc region. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0423] In the embodiment of FIG. 1U, the first (or left) half antibody comprises two Fab and an Fc region, and the second (or right) half antibody comprises a non-immunoglobulin based ABM and an Fc region. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0424] In the embodiment of FIG. 1V, the first (or left) half antibody comprises a Fab, an scFv, and an Fc region, and the second (or right) half antibody comprises a non-immunoglobulin based ABM and an Fc region. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0425] In the embodiment of FIG. 1W, the first (or left) half antibody comprises a Fab and an Fc region, and the second (or right) half antibody comprises a scFv, a non-immunoglobulin based ABM, and an Fc region. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0426] In the embodiment of FIG. 1Y, the first (or left) half antibody comprises an scFv and an Fc region, and the second (or right) half antibody comprises a Fab, an scFv and an Fc region. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0427] In the embodiment of FIG. 1Z, the first (or left) half antibody comprises a Fab, an Fc region, and a scFab, and the second (or right) half antibody comprises a Fab and an Fc region. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0428] Alternatively, as depicted in FIGS. 1O and 1P, trivalent a BBM can comprise two half antibodies, each comprising one complete ABM (a Fab in FIGS. 1O and 1P) and a portion of another ABM (one a VH, the other a VL). The two half antibodies are paired through an Fc domain, whereupon the VH and the VL associate to form a complete antigen-binding Fv domain.

[0429] The BBM can be a single chain, as shown in FIG. 1X. The BBM of FIG. 1X comprises three scFv domains connected through linkers.

[0430] In the configuration shown in FIGS. 1G-1Z, each of X, Y and A represent either an ABM1 or ABM2, provided that the BBM comprises at least ABM1 and at least one ABM2. Thus, the trivalent MBMs will include one or two ABM1s and one or two ABM2s. In some

embodiments, a trivalent BBM comprises two ABM1s and one ABM2. In other embodiments, a trivalent BBM of the disclosure comprises one ABM1 and two ABM2s.

[0431] Accordingly, in the present disclosure provides a trivalent BBM as shown in any one of FIGS. 1G through 1Z, where X is an ABM1, Y is an ABM1 and A is an ABM2 (this configuration of ABMs designated as "T1" for convenience).

[0432] The disclosure further provides a trivalent BBM as shown in any one of FIGS. 1G through 1Z, where X is an ABM1, Y is an ABM2 and A is an ABM1 (this configuration of ABMs designated as "T2" for convenience).

[0433] The disclosure further provides a trivalent BBM as shown in any one of FIGS. 1G through 1Z, where X is an ABM2, Y is an ABM1 and A is an ABM1 (this configuration of ABMs designated as "T3" for convenience).

[0434] The disclosure further provides a trivalent BBM as shown in any one of FIGS. 1G through 1Z, where X is an ABM1, Y is an ABM2 and A is an ABM2 (this configuration of ABMs designated as "T4" for convenience).

[0435] The disclosure further provides a trivalent BBM as shown in any one of FIGS. 1G through 1Z, where X is an ABM2, Y is an ABM1 and A is an ABM2 (this configuration of ABMs designated as "T5" for convenience).

[0436] The disclosure further provides a trivalent BBM as shown in any one of FIGS. 1G through 1Z, where X is an ABM2, Y is an ABM2 and A is an ABM1 (this configuration of ABMs designated as "T6" for convenience).

7.5.3. Exemplary Tetravalent BBMs

[0437] The BBMs can be tetravalent, *i.e.*, they have four antigen-binding domains, one, two, or three of which binds CD19 (ABM1) and one, two, or three of which binds a second target antigen (ABM2), *e.g.*, a component of a TCR complex.

[0438] Exemplary tetravalent BBM configurations are shown in FIGS. 1AA-1AH.

[0439] As depicted in FIGS. 1AA-1AH, a tetravalent BBM can comprise two half antibodies, each comprising two complete ABMs, the two halves paired through an Fc domain.

[0440] In the embodiment of FIG. 1AA, the first (or left) half antibody comprises a Fab, an Fc region, and an scFv, and the second (or right) half antibody comprises a Fab, an Fc region, and an scFv. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0441] In the embodiment of FIG. 1AB, the first (or left) half antibody comprises a Fab, an scFv, and an Fc region, and the second (or right) half antibody comprises a Fab, an scFv, and an Fc

region. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0442] In the embodiment of FIG. 1AC, the first (or left) half antibody comprises an scFv, a Fab, and an Fc region, and the second (or right) half antibody comprises an scFv, a Fab, and an Fc region. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0443] In the embodiment of FIG. 1AD, the first (or left) half antibody comprises a Fab, an Fc region, and a second Fab, and the second (or right) half antibody comprises a Fab, an Fc region, and a second Fab. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0444] In the embodiment of FIG. 1AE, the first (or left) half antibody comprises an scFv, a second scFv, and an Fc region, and the second (or right) half antibody comprises an scFv, a second scFv, and an Fc region. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0445] In the embodiment of FIG. 1AF, the first (or left) half antibody comprises a Fab, an scFv, and an Fc region, and the second (or right) half antibody comprises a Fab, an scFv, and an Fc region. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0446] In the embodiment of FIG. 1AG, the first (or left) half antibody comprises a Fab, an Fc region, and an scFv, and the second (or right) half antibody comprises a scFv, an Fc region, and a Fab. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0447] In the embodiment of FIG. 1AH, the first (or left) half antibody comprises a scFv, an Fc region, and an Fab, and the second (or right) half antibody comprises a scFv, an Fc region, and a Fab. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0448] In the configuration shown in FIGS. 1AA-1AH, each of X, Y, A, and B represent ABM1 or ABM2, although not necessarily in that order, and provided that the BBM comprises at least one ABM1 and at least one ABM2. Thus, the tetravalent ABMs will include one, two, or three ABM1s and one, two, or ABM2s. In some embodiments, a tetravalent BBM comprises three ABM1s and one ABM2. In other embodiments, a tetravalent BBM comprises two ABM1s two ABM2s. In yet other embodiments, a tetravalent BBM comprises one ABM1 and three ABM2s.

[0449] Accordingly, in the present disclosure provides a tetravalent BBM as shown in any one of FIGS. 1AA-1AH, where X is an ABM1 and each of Y, A, and B are ABM2s (this configuration of ABMs designated as “Tv 1” for convenience).

[0450] The disclosure further provides a tetravalent BBM as shown in any one of FIGS. 1AA-1AH, where Y is an ABM1 and each of X, A, and B are ABM2s (this configuration of ABMs designated as “Tv 2” for convenience).

[0451] The disclosure further provides a tetravalent BBM as shown in any one of FIGS. 1AA-1AH, where A is an ABM1 and each of X, Y, and B are ABM2s (this configuration of ABMs designated as “Tv 3” for convenience).

[0452] The disclosure further provides a tetravalent BBM as shown in any one of FIGS. 1AA-1AH, where B is an ABM1 and each of X, Y, and A are ABM2s (this configuration of ABMs designated as “Tv 4” for convenience).

[0453] The disclosure further provides a tetravalent BBM as shown in any one of FIGS. 1AA-1AH, where X and Y are both ABM1s and both of A and B are ABM2s (this configuration of ABMs designated as “Tv 5” for convenience).

[0454] The disclosure further provides a tetravalent BBM as shown in any one of FIGS. 1AA-1AH, where X and A are both ABM1s and both of Y and B are ABM2s (this configuration of ABMs designated as “Tv 6” for convenience).

[0455] The disclosure further provides a tetravalent BBM as shown in any one of FIGS. 1AA-1AH, where X and B are both ABM1s and both of Y and A are ABM2s (this configuration of ABMs designated as “Tv 7” for convenience).

[0456] The disclosure further provides a tetravalent BBM as shown in any one of FIGS. 1AA-1AH, where Y and A are both ABM1s and both of X and B are ABM2s (this configuration of ABMs designated as “Tv 8” for convenience).

[0457] The disclosure further provides a tetravalent BBM as shown in any one of FIGS. 1AA-1AH, where Y and B are both ABM1s and both of X and A are ABM2s (this configuration of ABMs designated as “Tv 9” for convenience).

[0458] The disclosure further provides a tetravalent BBM as shown in any one of FIGS. 1AA-1AH, where A and B are both ABM1s and both of X and Y are ABM2s (this configuration of ABMs designated as “Tv 10” for convenience).

[0459] The disclosure further provides a tetravalent BBM as shown in any one of FIGS. 1AA-1AH, where each of X, Y, and A is an ABM1 and B is an ABM2 (this configuration of ABMs designated as “Tv 11” for convenience).

[0460] The disclosure further provides a tetravalent BBM as shown in any one of FIGS. 1AA-1AH, where each of X, Y, and B is an ABM1 and A is an ABM2 (this configuration of ABMs designated as "Tv 12" for convenience).

[0461] The disclosure further provides a tetravalent BBM as shown in any one of FIGS. 1AA-1AH, where each of X, A, and B is an ABM1 and Y is an ABM2 (this configuration of ABMs designated as "Tv 13" for convenience).

[0462] The disclosure further provides a tetravalent BBM as shown in any one of FIGS. 1AA-1AH, where each of Y, A, and B is an ABM1 and X is an ABM2 (this configuration of ABMs designated as "Tv 14" for convenience).

7.6. Trispecific Binding Molecule Configurations

[0463] Exemplary TBM configurations are shown in FIG. 2. FIG. 2A shows the components of the TBM configurations shown in FIGS. 2B-1V. The scFv, Fab, non-immunoglobulin based ABM, and Fc each can have the characteristics described for these components in Sections 7.3 and 7.4. The components of the TBM configurations shown in FIG. 2 can be associated with each other by any of the means described in Sections 7.3 and 7.4 (*e.g.*, by direct bonds, ABM linkers, disulfide bonds, Fc domains with modified with knob in hole interactions, *etc.*). The orientations and associations of the various components shown in FIG. 2 are merely exemplary; as will be appreciated by a skilled artisan, other orientations and associations can be suitable (*e.g.*, as described in Sections 7.3 and 7.4).

[0464] TBMs are not limited to the configurations shown in FIG. 2. Other configurations that can be used are known to those skilled in the art. See, *e.g.*, WO 2014/145806; WO 2017/124002; Liu *et al.*, 2017, Front Immunol. 8:38; Brinkmann & Kontermann, 2017, mAbs 9:2, 182-212; US 2016/0355600; Klein *et al.*, 2016, MAbs 8(6):1010-20; and US 2017/0145116.

7.6.1. Exemplary Trivalent TBMs

[0465] The TBMs of the disclosure can be trivalent, *i.e.*, they have three antigen-binding domains, one of which binds CD19, one of which binds a component of a TCR complex, and one of which binds either CD2 or a TAA.

[0466] Exemplary trivalent TBM configurations are shown in FIGS. 2B through 2P.

[0467] As depicted in FIGS. 2B-2K and 2N-2P, a TBM can comprise two half antibodies, one comprising two ABMs and the other comprising one ABM, the two halves paired through an Fc domain.

[0468] In the embodiment of FIG. 2B, the first (or left) half antibody comprises an scFv and an Fc region, and the second (or right) half antibody comprises a Fab, an scFv and an Fc region.

The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0469] In the embodiment of FIG. 2C, the first (or left) half antibody comprises two Fab and an Fc region, and the second (or right) half antibody comprises a Fab and an Fc region. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0470] In the embodiment of FIG. 2D, the first (or left) half antibody comprises a Fab, an scFv and an Fc region, and the second (or right) half antibody comprises a Fab and an Fc region. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0471] In the embodiment of FIG. 2E, the first (or left) half antibody comprises an scFv and an Fc region, and the second (or right) half antibody comprises two Fab and an Fc region. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0472] In the embodiment of FIG. 2F, the first (or left) half antibody comprises an scFv, an Fc region, and a Fab, and the second (or right) half antibody comprises a Fab and an Fc region. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0473] In the embodiment of FIG. 2G, the first (or left) half antibody comprises an scFv and an Fc region, and the second (or right) half antibody comprises a Fab an Fc region, and an scFV. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0474] In the embodiment of FIG. 2H, the first (or left) half antibody comprises two Fab and an Fc region, and the second (or right) half antibody comprises a non-immunoglobulin based ABM and an Fc region. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0475] In the embodiment of FIG. 2I, the first (or left) half antibody comprises a Fab, an scFv, and an Fc region, and the second (or right) half antibody comprises a non-immunoglobulin based ABM and an Fc region. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0476] In the embodiment of FIG. 2J, the first (or left) half antibody comprises a Fab and an Fc region, and the second (or right) half antibody comprises an scFv, a non-immunoglobulin based ABM and an Fc region. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0477] In the embodiment of FIG. 2K, the first (or left) half antibody comprises an scFv and an Fc region, and the second (or right) half antibody comprises an scFv, an Fc region, and a

second scFv. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0478] In the embodiment of FIG. 2N, the first (or left) half antibody comprises a Fab, an Fc region, and an scFv, and the second (or right) half antibody comprises a Fab, and an Fc region. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0479] In the embodiment of FIG. 2O, the first (or left) half antibody comprises a Fab, an Fc region, and a scFab, and the second (or right) half antibody comprises a Fab and an Fc region. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0480] In the embodiment of FIG. 2P, the first (or left) half antibody comprises a Fab, a non-immunoglobulin based ABM, and an Fc region, and the second (or right) half antibody comprises a scFv and an Fc region. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0481] Alternatively, as depicted in FIG. 2L, trivalent a TBM can comprise two half antibodies, each comprising one complete ABM and a portion of another ABM (one a VH, the other a VL). The two half antibodies are paired through an Fc domain, whereupon the VH and the VL associate to form a complete antigen-binding Fv domain.

[0482] The TBM can be a single chain, as shown in FIG. 2M. The TBM of FIG. 2M comprises three scFv domains connected through linkers.

[0483] In each of the configurations shown in FIGS. 2B-2P, each of the domains designated X, Y, and Z represents an ABM1, ABM2, or ABM3, although not necessarily in that order. In other words, X can be ABM1, ABM2, or ABM3, Y can be ABM1, ABM2, or ABM3, and Z can be ABM1, ABM2, or ABM3, provided that the TBM comprises one ABM1, one ABM2, and one ABM3.

[0484] Accordingly, in the present disclosure provides a trivalent TBM as shown in any one of FIGS. 2B through 2P, where X is an ABM1, Y is an ABM3 and Z is an ABM2 (this configuration of ABMs designated as “T1” for convenience).

[0485] The present disclosure also provides a trivalent TBM as shown in any one of FIGS. 2B through 2P, where X is an ABM1, Y is an ABM2, and Z is an ABM3 (this configuration of ABMs designated as “T2” for convenience).

[0486] The present disclosure further provides a trivalent TBM as shown in any one of FIGS. 2B through 2P, where X is an ABM3, Y is an ABM1, and Z is an ABM2 (this configuration of ABMs designated as “T3” for convenience).

[0487] The present disclosure yet further provides a trivalent TBM as shown in any one of FIGS. 2B through 2P, where X is an ABM3, Y is an ABM2, and Z is an ABM1 (this configuration of ABMs designated as "T4" for convenience).

[0488] The present disclosure yet further provides a trivalent TBM as shown in any one of FIGS. 2B through 2P, where X is an ABM2, Y is an ABM1, and Z is an ABM3 (this configuration of ABMs designated as "T5" for convenience).

[0489] The present disclosure yet further provides a trivalent TBM as shown in any one of FIGS. 2B through 2P, where X is an ABM2, Y is an ABM3, and Z is an ABM1 (this configuration of ABMs designated as "T6" for convenience).

7.6.2. Exemplary Tetravalent TBMs

[0490] The TBMs of the disclosure can be tetravalent, *i.e.*, they have four antigen-binding domains, one or two of which binds CD19, one or two of which binds a component of a TCR complex, and one or two of which binds CD2 or a TAA.

[0491] Exemplary tetravalent TBM configurations are shown in FIGS. 2Q-2S.

[0492] As depicted in FIGS. 2Q-2S, a tetravalent TBM can comprise two half antibodies, each comprising two complete ABMs, the two halves paired through an Fc domain.

[0493] In the embodiment of FIG. 2Q, the first (or left) half antibody comprises a Fab, an Fc region, and a second Fab, and the second (or right) half antibody comprises a Fab, an Fc region, and a second Fab. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0494] In the embodiment of FIG. 2R, the first (or left) half antibody comprises a Fab, an Fc region, and an scFv, and the second (or right) half antibody comprises a Fab, an Fc region, and an scFv. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0495] In the embodiment of FIG. 2S, the first (or left) half antibody comprises a Fab, an Fc region, and an scFv, and the second (or right) half antibody comprises an scFv, an Fc region, and a Fab. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0496] In the configuration shown in FIGS. 2Q-2S, each of X, Y, Z, and A represent an ABM1, an ABM2, or an ABM3, although not necessarily in that order, and provided that the TBM comprises at least one ABM1, at least one ABM2, and at least one ABM3. Thus, the tetravalent ABMs will include two ABMs against one of CD19, a component of a TCR complex, and CD2 or a TAA. In some cases, a tetravalent TBM has two CD19 ABMs.

7.6.3. Exemplary Pentavalent TBMs

[0497] The TBMs of the disclosure can be pentavalent, *i.e.*, they have five antigen-binding domains, one, two, or three of which binds CD19, one, two, or three of which binds a component of a TCR complex, and one, two, or three of which binds CD2 or a TAA.

[0498] An exemplary pentavalent TBM configuration is shown in FIG. 2T.

[0499] As depicted in FIG. 2T, a pentavalent TBM can comprise two half antibodies, one of which comprises two complete ABMs and the other of which comprises one complete ABM, the two halves paired through an Fc domain.

[0500] In the embodiment of FIG. 2T, the first (or left) half antibody comprises a Fab, an scFv, and an Fc region, and the second (or right) half antibody comprises a Fab, an Fc region, and an scFv. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0501] In the configuration shown in FIG. 2T, each of X, Y, Z, A, and B represent an ABM1, an ABM2, or an ABM3, although not necessarily in that order, and provided that the TBM comprises at least one ABM1, one ABM2, and one ABM3. Thus, the pentavalent TBMs can include two ABMs against two of CD19, a component of a TCR complex, and CD2 or a TAA, or three ABMs against one of CD19, a component of a TCR complex, and CD2 or a TAA. In some cases, a pentavalent TBM has two or three CD19 ABMs. In some embodiments, a pentavalent TBM has three ABM1s, one ABM2 and one ABM3.

7.6.4. Exemplary Hexavalent TBMs

[0502] The TBMs of the disclosure can be hexavalent, *i.e.*, they have six antigen-binding domains, one, two, three, or four of which binds CD19, one, two, three, or four of which binds a component of a TCR complex, and one, two, three, or four of which binds CD2 or a TAA.

[0503] Exemplary hexavalent TBM configurations are shown in FIGS. 2U-2V.

[0504] As depicted in FIGS. 2U-2V, a pentavalent TBM can comprise two half antibodies, one of which comprises two complete ABMs and the other of which comprises one complete ABM, the two halves paired through an Fc domain.

[0505] In the embodiment of FIG. 2U, the first (or left) half antibody comprises a Fab, a second Fab, an Fc region, and an scFv, and the second (or right) half antibody comprises a Fab, a second Fab, an Fc region, and an scFv. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0506] In the embodiment of FIG. 2V, the first (or left) half antibody comprises a first Fv, a second Fv, a third Fv, and an Fc region, and the second (or right) half antibody comprises a

first Fv, a second Fv, a third Fv, and an Fc region. The first and second half antibodies are associated through the Fc regions forming an Fc domain.

[0507] In the configuration shown in FIGS. 2U-2V, each of X, Y, Z, A, B, and C represent an ABM1, an ABM2, or an ABM3, although not necessarily in that order, and provided that the TBM comprises at least one ABM1, one ABM2, and one ABM3. Thus, the hexavalent TBMs can include (i) two ABMs against each of CD19, a component of a TCR complex, and CD2 or a TAA, (ii) three ABMs against one of CD19, a component of a TCR complex, and CD2 or a TAA, or (iii) four ABMs against one of CD19, a component of a TCR complex, and CD2 or a TAA. For example, a hexavalent ABM can include three ABMs against CD19, two ABMs against CD2 or a TAA and one ABM against a component of a TCR complex. As another example, a hexavalent ABM can include three ABMs against CD19, two ABMs against a component of a TCR complex and one ABM against CD2 or a TAA. In some cases, a hexavalent TBM has two, three, our four CD19 ABMs. In some embodiments, a hexavalent TBM has three CD19 ABMs. In other embodiments, a hexavalent TBM has four CD19 ABMs.

7.7. TCR ABMs

[0508] The MBMs of the disclosure contain an ABM that specifically binds to CD19 and an ABM2 which is specific for a different antigen. In the BBMs, Type 1 TBMs and Type 2 TBMs of the disclosure, ABM2 can bind to a component of a TCR complex. The TCR is a disulfide-linked membrane-anchored heterodimeric protein normally consisting of the highly variable alpha (α) and beta (β) chains expressed as part of a complex with the invariant CD3 chain molecules. T cells expressing this receptor are referred to as α:β (or αβ) T cells, though a minority of T cells express an alternate receptor, formed by variable gamma (γ) and delta (δ) chains, referred as γδ T cells.

[0509] In an embodiment, MBMs contain an ABM that specifically binds to CD3.

7.7.1. CD3 ABMs

[0510] The MBMs can contain an ABM that specifically binds to CD3. The term “CD3” refers to the cluster of differentiation 3 co-receptor (or co-receptor complex, or polypeptide chain of the co-receptor complex) of the T cell receptor. The amino acid sequence of the polypeptide chains of human CD3 are provided in NCBI Accession P04234, P07766 and P09693. CD3 proteins can also include variants. CD3 proteins can also include fragments. CD3 proteins also include post-translational modifications of the CD3 amino acid sequences. Post-translational modifications include, but are not limited to, N-and O-linked glycosylation.

[0511] In some embodiments, a MBM can comprise an ABM which is an anti-CD3 antibody (*e.g.*, as described in US 2016/0355600, WO 2014/110601, and WO 2014/145806) or an antigen-binding domain thereof. Exemplary anti-CD3 VH, VL, and scFV sequences that can be used in a MBM are provided in Table 12A.

TABLE 12A
CD3 Binders – Variable domain sequences

Binding Domain	Chain	Sequence	SEQ ID NO:
CD3-1	VH	QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQG LEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDS AVYYCARYYDDHYCLDYWGQGTTLTVSS	136
	VL	QIVLTQSPAIMSASPGEKVTMTCSASSSVSYMNWYQQKSGTSPKR WIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAATYYCQQW SSNPFTFGSGTKLEIN	137
CD3-2	VH	EVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSQSILYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSA	138
	VL	QAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEKPDHLF TGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCALWY SNLWVFGGGTKLTVL	139
CD3-3	VH	QVQLQQSGAELARPGASVKMSCKASGYTFTSYTMHWVKQRPGQG LEWIGYINPSSGYTKYNQKFKDKATLTADKSSSTAYMQLSSLTSEDS AVYYCARWQDYDVYFDYWGQGTTLTVSS	140
	VL	QIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQQKPGSSPKP WIYATSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS SNPPTFGGGTKLETK	141
CD3-4	VH	QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQG LEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDS AVYYCARYYDDHYCLDYWGQGTTLTVSS	136
	VL	QIVLTQSPAIMSASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKR WIYDTSKVASGVPYRFSGSGSGTSYSLTISSMEAEDAATYYCQQWS SNPLTFGSGTKLEIN	142
CD3-5	VH	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGKG LEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTAFLQMDSLRPEDT GVYFCARYYDDHYCLDYWGQGTPVTVSS	143
	VL	DIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKR WIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQQWS SNPFTFGQGTKLQIT	144
CD3-6	VH	QVQLVESGGGVVQPGRSLRLSCAASGFKFSGYGMHWVRQAPGKG LEWVAVIWYDGSKKYYVDSVKGRFTISRDNSKNTLYLQMNSLRAED TAVYYCARQMGYWHFDLWGRGTLVTVSS	145
	VL	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRL LIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSN WPPLTFGGGTKVEIK	146
CD3-7	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGL EWVGRIRSKYNNYATYYADSVKDRFISRDDSKNSLYLQMNSLKTED TAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS	147
	VL	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA PRGLIGGTNKRAPWTPARFSGSLLGGKAALIGAQAEDEADYYCALW YSNLWVFGGGTKLTVL	148

TABLE 12A
CD3 Binders – Variable domain sequences

Binding Domain	Chain	Sequence	SEQ ID NO:
CD3-8	VH	DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPGQGL EWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSA VYYCARYYDDHYCLDYWGQGTTLTVSS	149
	VL	DIQLTQSPAIMSASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKR WIYDTSKVASGVPYRFSGSGSGTSYSLISSMEAEDAATYYCQQWS SNPLTFGAGTKLELK	150
CD3-9	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKG LEWVARIRSKYNNYATYYADSVKDRFISRDDSKNSLYLQMNSLKTE DTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS	151
	VL	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA PRGLIGGTNKRAPWTPARFSGSLLGGKAALIGAQAEDEADYYCALW YSNLWVFGGGTKLTVL	148
CD3-10	VH	EVKLLESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSQSILYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSA	152
	VL	QAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEKPDHLF TGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCALWY SNLWVFGGGTKLTVL	139
CD3-11	VH	EVQLVESGGGLVQPGGSLKLSCAASGFTFNSYAMNWVRQAPGKG LEWVARIRSKYNNYATYYADSVKGRFTISRDDSKNTAYLQMNNLKT EDTAVYYCVRHGNFGNSYVSWWAYWGQGTLVTVSS	153
	VL	QTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKPGQA PRGLIGGTKFLAPGTPQRFSGSLLGGKAALTLSGVQPEDEAEYYCV LWYSNRWVFGGGTKLTVL	154
CD3-12	VH	EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKG LEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKT EDTAVYYCVRHGNFGNSYISYWAYWGQGTLVTVSS	155
	VL	QTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKPGQA PRGLIGGTKFLAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCVL WYSNRWVFGGGTKLTVL	156
	scFv	EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKG LEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKT EDTAVYYCVRHGNFGNSYISYWAYWGQGTLVTVSSGGGGSGGGG SGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNY PNWVQQKPGQAPRGLIGGTKFLAPGTPARFSGSLLGGKAALTLSGV QPEDEAEYYCVLWYSNRWVFGGGTKLTVL	157
CD3-13	VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAPGQG LEWMGYINPSRGYTNYNQKFKDRVTMTTDTSISTAYMELSRLRSDD TAVYYCARYYDDHYCLDYWGQGTLVTVSS	158
	VL	EIVLTQSPATLSLSPGERATLSCSASSSVSYMNWYQQKPGQAPRLLI YDTSKLASGVPAHFRGSGSGTDFTLTISSLEPEDFAVYYCQQWSSN PFTFGQGTKVEIK	159
CD3-14	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGL EWVSRIRSKYNNYATYYADSVKDRFTISRDDSKNTLYLQMNSLRAE DTAVYYCARHGNFGNSYVSWFAYWGQGTMVTVSS	160
	VL	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA PRGLIGGTNKRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCA LWYSNLWVFGGGTKLTVL	161

TABLE 12A CD3 Binders – Variable domain sequences			
Binding Domain	**Chain**	**Sequence**	**SEQ ID NO:**
CD3-15	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKG LEWVGRIRSKYNNYATYYADSVKDRFTISRDDSKNSLYLQMNSLKT EDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS	162
	VL	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQEKPGQA PRGLIGGTNKRAPWTPARFSGSLLGGKAALTITGAQAEDEADYYCA LWYSNLWVFGGGTKLTVL	163
CD3-16	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKG LEWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRA EDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS	164
	VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQA PRGLIGGTNKRAPGVPARFSGSLLGGKAALTLSGAQPEDEAEYYCA LWYSNLWVFGGGTKLTVL	165
CD3-17	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGL EWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAE DTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSS	166
	VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKS PRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPEDEADYYCA LWYSNHWVFGGGTKLTVL	167
CD3-18	VH	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGKG LEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTAFLQMDSLRPEDT GVYFCARYYDDHYCLDYWGQGTPVTVSS	143
	VL	DIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKR WIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQQWS SNPFTFGQGT	168
CD3-19	VH	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGKG LEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTAFLQMDSLRPEDT GVYFCARYYDDHYSLDYWGQGTPVTVSS	169
	VL	DIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKR WIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQQWS SNPFTFGQGT	168
CD3-20	VH	EVQLQQSGPELVKPGASMKISCKASGYSFTGYTMNWVKQSHGKNL EWMGLINPYKGVSTYNQKFKDKATLTVDKSSSTAYMELLSLTSEDS AVYYCARSGYYGDSDWYFDVWGQGTTLTVFS	170
	VL	DIQMTQTTSSLSASLGDRVTISCRASQDIRNYLNWYQQKPDGTVKLL IYYTSRLHSGVPSKFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTL PWTFAGGTKLEIK	171
CD3-21	VH	EVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQASGKGL EWVGRIRSKYNNYATYYADSVKDRFTISRDDSKSTLYLQMNSLKTE DTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS	172
	VL	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA PRGLIGGTNKRAPWTPARFSGSLLGDKAALTLSGAQPEDEAEYFCA LWYSNLWVFGGGTKLTVL	173
	scFv	EVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQASGKGL EWVGRIRSKYNNYATYYADSVKDRFTISRDDSKSTLYLQMNSLKTE DTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGG SGGGGSGGGGSQAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNY ANWVQQKPGQAPRGLIGGTNKRAPWTPARFSGSLLGDKAALTLSG AQPEDEAEYFCALWYSNLWVFGGGTKLTVL	174

TABLE 12A
CD3 Binders – Variable domain sequences

Binding Domain	Chain	Sequence	SEQ ID NO:
CD3-22	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGL EWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAE DTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSS	166
	VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKS PRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPEDEADYYCA LWYSNHWVFGGGTKLTVL	167
	scFv	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGL EWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAE DTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGKPGSGKPGS GKPGSGKPGSQAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYA NWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGA QPEDEADYYCALWYSNHWVFGGGTKLTVL	175
CD3-23	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGL EWVGRIRSKANNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAE DTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSS	176
	VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKS PRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPEDEADYYCA LWYSNHWVFGGGTKLTVL	167
	scFv	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGL EWVGRIRSKANNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAE DTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGKPGSGKPGS GKPGSGKPGSQAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYA NWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGA QPEDEADYYCALWYSNHWVFGGGTKLTVL	177
CD3-24	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGL EWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAE DTAVYYCVRHGNFGDEYVSWFAYWGQGTLVTVSS	178
	VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKS PRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPEDEADYYCA LWYSNHWVFGGGTKLTVL	167
	scFv	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGL EWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAE DTAVYYCVRHGNFGDEYVSWFAYWGQGTLVTVSSGKPGSGKPGS GKPGSGKPGSQAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYA NWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGA QPEDEADYYCALWYSNHWVFGGGTKLTVL	179
CD3-25	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGL EWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAE DTAVYYCVRHGNFGDPYVSWFAYWGQGTLVTVSS	180
	VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKS PRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPEDEADYYCA LWYSNHWVFGGGTKLTVL	167
	scFv	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGL EWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAE DTAVYYCVRHGNFGDPYVSWFAYWGQGTLVTVSSGKPGSGKPGS GKPGSGKPGSQAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYA NWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGA QPEDEADYYCALWYSNHWVFGGGTKLTVL	181

TABLE 12A CD3 Binders – Variable domain sequences			
Binding Domain	**Chain**	**Sequence**	**SEQ ID NO:**
CD3-26	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGL EWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAE DTAVYYCVRHGNFGDSYVSWFDYWGQGTLVTVSS	182
	VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKS PRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPEDEADYYCA LWYSNHWVFGGGTKLTVL	167
	scFv	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGL EWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAE DTAVYYCVRHGNFGDSYVSWFDYWGQGTLVTVSSGKPGSGKPGS GKPGSGKPGSQAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYA NWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGA QPEDEADYYCALWYSNHWVFGGGTKLTVL	183
CD3-27	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMSWVRQAPGKGL EWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAE DTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSS	184
	VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKS PRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPEDEADYYCA LWYSNHWVFGGGTKLTVL	167
	scFv	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMSWVRQAPGKGL EWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAE DTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGKPGSGKPGS GKPGSGKPGSQAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYA NWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGA QPEDEADYYCALWYSNHWVFGGGTKLTVL	185
CD3-28	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKG LEWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRA EDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS	164
	VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQA PRGLIGGTNKRAPGVPARFSGSLLGGKAALTLSGAQPEDEAEYYCA LWYSNLWVFGGGTKLTVL	165
	scFv	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKG LEWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRA EDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGG GSGGGGSQAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWV QQKPGQAPRGLIGGTNKRAPGVPARFSGSLLGGKAALTLSGAQPE DEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH	186
CD3-129	VH	EVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQASGKGL EWVGRIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNSLKTE DTAVYYCVRHGNFGNSYVSWFAHWGQGTLVTVSS	187
	VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTSSNYANWVQQKPGQA PRGLIGGTNKRAPWTPARFSGSLLGGKAALTLSGAQPEDEAEYYCA LWYSNLWVFGGGTKLTVL	188
	scFv	EVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQASGKGL EWVGRIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNSLKTE DTAVYYCVRHGNFGNSYVSWFAHWGQGTLVTVSSGGGGSGGGG SGGGGSGGGGSQAVVTQEPSLTVSPGGTVTLTCGSSTGAVTSSNY ANWVQQKPGQAPRGLIGGTNKRAPWTPARFSGSLLGGKAALTLSG AQPEDEAEYYCALWYSNLWVFGGGTKLTVL	189

TABLE 12A CD3 Binders – Variable domain sequences			
Binding Domain	**Chain**	**Sequence**	**SEQ ID NO:**
CD3-130	VH	EVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQASGKGL EWVGRIRSKYNNYATYYADSVKDRFTISRDDSKSTAYLQMNSLKTE DTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS	190
	VL	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA PRGLIGGTNKRAPWTPARFSGSLLGDKAALTLSGAQPEDEAEYFCA LWYSNLWVFGGGTKLTVL	173
	scFv	EVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQASGKGL EWVGRIRSKYNNYATYYADSVKDRFTISRDDSKSTAYLQMNSLKTE DTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGG SGGGGSGGGGSQAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNY ANWVQQKPGQAPRGLIGGTNKRAPWTPARFSGSLLGDKAALTLSG AQPEDEAEYFCALWYSNLWVFGGGTKLTVL	191

[0512] CDR sequences for a number of CD3 binders as defined by the Kabat numbering scheme (Kabat *et al*, 1991, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md.), Chothia numbering scheme (Al-Lazikani *et al.*, 1997, J. Mol. Biol 273:927-948), and a combination of Kabat and Chothia numbering are provided in Tables 12B-12D, respectively.

TABLE 12B CD3 Binders – CDR sequences according to Kabat numbering scheme							
Binding Domain	**Chain**	**CDR1**	**SEQ ID NO:**	**CDR2**	**SEQ ID NO:**	**CDR3**	**SEQ ID NO:**
CD3-1	VH	RYTMH	192	YINPSRGYTNYNQKFKD	212	YYDDHYCLDY	236
	VL	SASSSVSYMN	193	DTSKLAS	213	QQWSSNPFT	237
CD3-2	VH	TYAMN	194	RIRSKYNNYATYYADSVKD	214	HGNFGNSYVSWFAY	238
	VL	RSSTGAVTTSNYAN	195	GTNKRAP	215	ALWYSNLWV	239
CD3-3	VH	SYTMH	196	YINPSSGYTKYNQKFKD	216	WQDYDVYFDY	240
	VL	RASSSVSYMH	197	ATSNLAS	217	QQWSSNPPT	241
CD3-4	VH	RYTMH	192	YINPSRGYTNYNQKFKD	212	YYDDHYCLDY	236
	VL	RASSSVSYMN	198	DTSKVAS	218	QQWSSNPLT	242
CD3-5	VH	RYTMH	192	YINPSRGYTNYNQKVKD	219	YYDDHYCLDY	236
	VL	SASSSVSYMN	193	DTSKLAS	213	QQWSSNPFT	237
CD3-6	VH	GYGMH	199	VIWYDGSKKYYVDSVKG	220	QMGYWHFDL	243
	VL	RASQSVSSYLA	200	DASNRAT	221	QQRSNWPPLT	244

TABLE 12B
CD3 Binders – CDR sequences according to Kabat numbering scheme

Binding Domain	Chain	CDR1	SEQ ID NO:	CDR2	SEQ ID NO:	CDR3	SEQ ID NO:
CD3-7	VH	TYAMN	194	RIRSKYNNYATYYAD	222	VRHGNFGNSYVSWFAY	245
	VL	RSSTGAVTTSNYAN	195	GTNKRAP	215	ALWYSNLWV	239
CD3-8	VH	RYTMH	192	YINPSRGYTNYNQKFKD	212	YYDDHYCLDY	236
	VL	RASSSVSYMN	198	DTSKVAS	218	QQWSSNPLT	242
CD3-9	VH	TYAMN	194	RIRSKYNNYATYYAD	222	VRHGNFGNSYVSWFAY	245
	VL	RSSTGAVTTSNYAN	195	GTNKRAP	215	ALWYSNLWV	239
CD3-10	VH	TYAMN	194	RIRSKYNNYATYYADSVKD	214	HGNFGNSYVSWFAY	238
	VL	RSSTGAVTTSNYAN	195	GTNKRAP	215	ALWYSNLWV	239
CD3-11	VH	SYAMN	201	RIRSKYNNYATYYADSVKG	223	HGNFGNSYVSWWAY	246
	VL	GSSTGAVTSGNYPN	202	GTKFLAP	224	VLWYSNRWV	247
CD3-12	VH	KYAMN	203	RIRSKYNNYATYYADSVKD	214	HGNFGNSYISYWAY	248
	VL	GSSTGAVTSGNYPN	202	GTKFLAP	224	VLWYSNRWV	247
CD3-13	VH	RYTMH	192	YINPSRGYTNYNQKFKD	212	YYDDHYCLDY	236
	VL	SASSSVSYMN	193	DTSKLAS	213	QQWSSNPFT	237
CD3-14	VH	TYAMN	194	RIRSKYNNYATYYADSVKD	214	HGNFGNSYVSWFAY	238
	VL	RSSTGAVTTSNYAN	195	GTNKRAP	215	ALWYSNLWV	239
CD3-15	VH	TYAMN	194	RIRSKYNNYATYYADSVKD	214	HGNFGNSYVSWFAY	238
	VL	RSSTGAVTTSNYAN	195	GTNKRAP	215	ALWYSNLWV	239
CD3-16	VH	TYAMN	194	RIRSKYNNYATYYADSVKG	223	HGNFGNSYVSWFAY	238
	VL	GSSTGAVTTSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-17	VH	TYAMN	194	RIRSKYNNYATYYADSVKG	223	HGNFGDSYVSWFAY	249
	VL	GSSTGAVTTSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-18	VH	RYTMH	192	YINPSRGYTNYNQKVKD	219	YYDDHYCLDY	236
	VL	SASSSVSYMN	193	DTSKLAS	213	QQWSSNPFT	237
CD3-19	VH	RYTMH	192	YINPSRGYTNYNQKVKD	219	YYDDHYSLDY	251
	VL	SASSSVSYMN	193	DTSKLAS	213	QQWSSNPFT	237
CD3-20	VH	GYTMN	205	LINPYKGVSTYNQKFKD	225	SGYYGDSDWYFDV	252

TABLE 12B
CD3 Binders – CDR sequences according to Kabat numbering scheme

Binding Domain	Chain	CDR1	SEQ ID NO:	CDR2	SEQ ID NO:	CDR3	SEQ ID NO:
	VL	RASQDIRNYLN	206	YTSRLH	226	QQGNTLPWT	253
CD3-21	VH	TYAMN	194	RIRSKYNNYATYYADSVKD	214	HGNFGNSYVSWFAY	238
	VL	RSSTGAVTTSNYAN	195	GTNKRAP	215	ALWYSNLWV	239
CD3-22	VH	TYAMN	194	RIRSKYNNYATYYADSVKG	223	HGNFGDSYVSWFAY	249
	VL	GSSTGAVTTSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-23	VH	TYAMN	194	RIRSKANNYATYYADSVKG	227	HGNFGDSYVSWFAY	249
	VL	GSSTGAVTTSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-24	VH	TYAMN	194	RIRSKYNNYATYYADSVKG	223	HGNFGDEYVSWFAY	254
	VL	GSSTGAVTTSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-25	VH	TYAMN	194	RIRSKYNNYATYYADSVKG	223	HGNFGDPYVSWFAY	255
	VL	GSSTGAVTTSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-26	VH	TYAMN	194	RIRSKYNNYATYYADSVKG	223	HGNFGDSYVSWFDY	256
	VL	GSSTGAVTTSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-27	VH	TYAMS	207	RIRSKYNNYATYYADSVKG	223	HGNFGDSYVSWFAY	249
	VL	GSSTGAVTTSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-28	VH	TYAMN	194	RIRSKYNNYATYYADSVKG	223	HGNFGNSYVSWFAY	238
	VL	GSSTGAVTTSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-29	VH	TYAMN	194	RIRSKYNNYATYYADSVKG	223	HGNFGNSYVSWFAY	238
	VL	GSSTGAVTTSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-30	VH	TYAMN	194	RIRSKYNNYATYYADSVKG	223	HGNFGNSYVSWFAY	238
	VL	GSSTGAVTTSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-31	VH	TYAMN	194	RIRSKYNNYATYYADSVKG	223	HGNFGNSYVSWFAY	238
	VL	GSSTGAVTTSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-32	VH	TYAMN	194	RIRSKYNNYATYYADSVKG	223	HGNFGNSYVSWFAY	238
	VL	GSSTGAVTTSNYAN	204	GTNKRAP	215	ALWYSNLWV	239

TABLE 12B
CD3 Binders – CDR sequences according to Kabat numbering scheme

Binding Domain	Chain	CDR1	SEQ ID NO:	CDR2	SEQ ID NO:	CDR3	SEQ ID NO:
CD3-33	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-34	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-35	VH	TYAMH	208	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-36	VH	TYAMS	207	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-37	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-38	VH	TYAMN	194	RIRSKANNYYATY YADSVKG	228	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-39	VH	TYAMN	194	RIRSKANSYATYY ADSVKG	229	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-40	VH	TYAMN	194	RIRSKYNNYATAY ADSVKG	230	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-41	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-42	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-43	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-44	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-45	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238

TABLE 12B
CD3 Binders – CDR sequences according to Kabat numbering scheme

Binding Domain	Chain	CDR1	SEQ ID NO:	CDR2	SEQ ID NO:	CDR3	SEQ ID NO:
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-46	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-47	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-48	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-49	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-50	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGDSYVS WFAY	249
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-51	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGQSYVS WFAY	257
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-52	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-53	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFDY	258
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-54	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-55	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-56	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-57	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239

TABLE 12B
CD3 Binders – CDR sequences according to Kabat numbering scheme

Binding Domain	Chain	CDR1	SEQ ID NO:	CDR2	SEQ ID NO:	CDR3	SEQ ID NO:
CD3-58	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-59	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-60	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT SSNYAN	209	GTNKRAP	215	ALWYSNLWV	239
CD3-61	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT SGHYAN	210	GTNKRAP	215	ALWYSNLWV	239
CD3-62	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	DTNKRAP	231	ALWYSNLWV	239
CD3-63	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNNRAP	232	ALWYSNLWV	239
CD3-64	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAS	233	ALWYSNLWV	239
CD3-65	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTSNKHS	234	ALWYSNLWV	239
CD3-66	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-67	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-68	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-69	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-70	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238

TABLE 12B
CD3 Binders – CDR sequences according to Kabat numbering scheme

Binding Domain	Chain	CDR1	SEQ ID NO:	CDR2	SEQ ID NO:	CDR3	SEQ ID NO:
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-71	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-72	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-73	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	LLWYSNLWV	259
CD3-74	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-75	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-76	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	RSSTGAVT TSNYAN	195	GTNKRAP	215	ALWYSNLWV	239
CD3-77	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	KSSTGAVT TSNYAN	211	GTNKRAP	215	ALWYSNLWV	239
CD3-78	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-79	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-80	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-81	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-82	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239

TABLE 12B
CD3 Binders – CDR sequences according to Kabat numbering scheme

Binding Domain	Chain	CDR1	SEQ ID NO:	CDR2	SEQ ID NO:	CDR3	SEQ ID NO:
CD3-83	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-84	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-85	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-86	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-87	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGDSYVS WFAY	249
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-88	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGDSYVS WFAY	249
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-89	VH	TYAMN	194	RIRSKANNYATYY ADSVKG	227	HGNFGDSYVS WFAY	249
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-90	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGDSYVS WFDY	256
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-91	VH	TYAMS	207	RIRSKANNYATYY ADSVKG	227	HGNFGDSYVS WFDY	256
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-92	VH	TYAMN	194	RIRSNGGYSTYYA DSVKG	235	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-93	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-94	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-95	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238

TABLE 12B
CD3 Binders – CDR sequences according to Kabat numbering scheme

Binding Domain	Chain	CDR1	SEQ ID NO:	CDR2	SEQ ID NO:	CDR3	SEQ ID NO:
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-96	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-97	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGDSYVS WFAY	249
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-98	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-99	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-100	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGDSYVS WFAY	249
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-101	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGDSYVS WFAY	249
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-102	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-103	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-104	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGDSYVS WFAY	249
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-105	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGDSYVS WFAY	249
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-106	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-107	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNHWV	250

TABLE 12B
CD3 Binders – CDR sequences according to Kabat numbering scheme

Binding Domain	Chain	CDR1	SEQ ID NO:	CDR2	SEQ ID NO:	CDR3	SEQ ID NO:
CD3-108	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGDSYVS WFAY	249
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-109	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGDSYVS WFAY	249
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-110	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-111	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-112	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGDSYVS WFAY	249
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-113	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGDSYVS WFAY	249
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-114	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-115	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-116	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGDSYVS WFAY	249
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-117	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGDSYVS WFAY	249
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-118	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-119	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGNSYVS WFAY	238
	VL	GSSTGAVT TSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-120	VH	TYAMN	194	RIRSKYNNYATYY ADSVKG	223	HGNFGDSYVS WFAY	249

TABLE 12B
CD3 Binders – CDR sequences according to Kabat numbering scheme

Binding Domain	Chain	CDR1	SEQ ID NO:	CDR2	SEQ ID NO:	CDR3	SEQ ID NO:
	VL	GSSTGAVTTSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-121	VH	TYAMN	194	RIRSKYNNYATYYADSVKG	223	HGNFGDSYVSWFAY	249
	VL	GSSTGAVTTSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-122	VH	TYAMN	194	RIRSKYNNYATYYADSVKG	223	HGNFGNSYVSWFAY	238
	VL	GSSTGAVTTSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-123	VH	TYAMN	194	RIRSKYNNYATYYADSVKG	223	HGNFGNSYVSWFAY	238
	VL	GSSTGAVTTSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-124	VH	TYAMN	194	RIRSKYNNYATYYADSVKG	223	HGNFGDSYVSWFAY	249
	VL	GSSTGAVTTSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-125	VH	TYAMN	194	RIRSKYNNYATYYADSVKG	223	HGNFGDSYVSWFAY	249
	VL	GSSTGAVTTSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-126	VH	TYAMN	194	RIRSKYNNYATYYADSVKG	223	HGNFGNSYVSWFAY	238
	VL	GSSTGAVTTSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-127	VH	TYAMN	194	RIRSKYNNYATYYADSVKG	223	HGNFGNSYVSWFAY	238
	VL	GSSTGAVTTSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-128	VH	TYAMN	194	RIRSKYNNYATYYADSVKG	223	HGNFGDSYVSWFAY	249
	VL	GSSTGAVTTSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-129	VH	TYAMN	194	RIRSKYNNYATYYADSVKD	214	HGNFGNSYVSWFAH	260
	VL	GSSTGAVTSSNYAN	209	GTNKRAP	215	ALWYSNLWV	239
CD3-130	VH	TYAMN	194	RIRSKYNNYATYYADSVKD	214	HGNFGNSYVSWFAY	238
	VL	RSSTGAVTTSNYAN	195	GTNKRAP	215	ALWYSNLWV	239

TABLE 12C
CD3 Binders – CDR sequences according to Chothia numbering scheme

Binding Domain	Chain	CDR1	SEQ ID NO:	CDR2	SEQ ID NO:	CDR3	SEQ ID NO:
CD3-1	VH	GYTFTRY	261	NPSRGY	274	YYDDHYCLDY	236
	VL	SSSVSY	262	DTS	275	WSSNPF	286

TABLE 12C
CD3 Binders – CDR sequences according to Chothia numbering scheme

Binding Domain	Chain	CDR1	SEQ ID NO:	CDR2	SEQ ID NO:	CDR3	SEQ ID NO:
CD3-2	VH	GFTFNTY	263	RSKYNN YA	276	HGNFGNSYVS WFAY	238
	VL	STGAVTTSNY	264	GTN	277	WYSNLW	287
CD3-3	VH	GYTFTSY	265	NPSSGY	278	WQDYDVYFDY	240
	VL	SSSVSY	262	ATS	279	WSSNPP	288
CD3-4	VH	GYTFTRY	261	NPSRGY	274	YYDDHYCLDY	236
	VL	SSSVSY	262	DTS	275	WSSNPL	289
CD3-5	VH	GYTFTRY	261	NPSRGY	274	YYDDHYCLDY	236
	VL	SSSVSY	262	DTS	275	WSSNPF	286
CD3-6	VH	GFKFSGY	266	WYDGSK	280	QMGYWHFDL	243
	VL	SQSVSSY	267	DAS	281	RSNWPPL	290
CD3-7	VH	GFTFSTY	268	RSKYNN YAT	282	HGNFGNSYVS WFA	291
	VL	STGAVTTSNY	264	GTN	277	WYSNLW	287
CD3-8	VH	GYTFTRY	261	NPSRGY	274	YYDDHYCLDY	236
	VL	SSSVSY	262	DTS	275	WSSNPL	289
CD3-9	VH	GFTFNTY	263	RSKYNN YAT	282	HGNFGNSYVS WFA	291
	VL	STGAVTTSNY	264	GTN	277	WYSNLW	287
CD3-10	VH	GFTFNTY	263	RSKYNN YA	276	HGNFGNSYVS WFAY	238
	VL	STGAVTTSNY	264	GTN	277	WYSNLW	287
CD3-11	VH	GFTFNSY	269	RSKYNN YA	276	HGNFGNSYVS WWAY	246
	VL	STGAVTSGNY	270	GTK	283	WYSNRW	292
CD3-12	VH	GFTFNKY	271	RSKYNN YA	276	HGNFGNSYISY WAY	248
	VL	STGAVTSGNY	270	GTK	283	WYSNRW	292
CD3-13	VH	GYTFTRY	261	NPSRGY	274	YYDDHYCLDY	236
	VL	SSSVSY	262	DTS	275	WSSNPF	286
CD3-14	VH	GFTFSTY	268	RSKYNN YA	276	HGNFGNSYVS WFAY	238
	VL	STGAVTTSNY	264	GTN	277	WYSNLW	287
CD3-15	VH	GFTFNTY	263	RSKYNN YA	276	HGNFGNSYVS WFAY	238
	VL	STGAVTTSNY	264	GTN	277	WYSNLW	287
CD3-16	VH	GFTFNTY	263	RSKYNN YA	276	HGNFGNSYVS WFAY	238
	VL	STGAVTTSNY	264	GTN	277	WYSNLW	287
CD3-17	VH	GFTFSTY	268	RSKYNN YA	276	HGNFGDSYVS WFAY	249
	VL	STGAVTTSNY	264	GTN	277	WYSNHW	293
CD3-18	VH	GYTFTRY	261	NPSRGY	274	YYDDHYCLDY	236
	VL	SSSVSY	262	DTS	275	WSSNPF	286
CD3-19	VH	GYTFTRY	261	NPSRGY	274	YYDDHYSLDY	251

TABLE 12C CD3 Binders – CDR sequences according to Chothia numbering scheme							
Binding Domain	**Chain**	**CDR1**	**SEQ ID NO:**	**CDR2**	**SEQ ID NO:**	**CDR3**	**SEQ ID NO:**
	VL	SSSVSY	262	DTS	275	WSSNPF	286
CD3-20	VH	GYSFTGY	272	NPYKGV	284	SGYYGDSDWY FDV	252
	VL	SQDIRNY	273	YTS	285	GNTLPW	294
CD3-21	VH	GFTFNTY	263	RSKYNN YA	276	HGNFGNSYVS WFAY	238
	VL	RSSTGAVTTS NYAN	195	GTNKRA P	215	ALWYSNLWV	239

TABLE 12D CD3 Binders – CDR sequences according to combination of Kabat and Chothia numbering schemes							
Binding Domain	**Chain**	**CDR1**	**SEQ ID NO:**	**CDR2**	**SEQ ID NO:**	**CDR3**	**SEQ ID NO:**
CD3-1	VH	GYTFTRYT MH	295	YINPSRGYTNYN QKFKD	212	YYDDHYCLDY	236
	VL	SASSSVSY MN	193	DTSKLAS	213	QQWSSNPFT	237
CD3-2	VH	GFTFNTYA MN	296	RIRSKYNNYATYY ADSVKD	214	HGNFGNSYV SWFAY	238
	VL	RSSTGAVT TSNYAN	195	GTNKRAP	215	ALWYSNLWV	239
CD3-3	VH	GYTFTSYT MH	297	YINPSSGYTKYN QKFKD	216	WQDYDVYFD Y	240
	VL	RASSSVSY MH	197	ATSNLAS	217	QQWSSNPPT	241
CD3-4	VH	GYTFTRYT MH	295	YINPSRGYTNYN QKFKD	212	YYDDHYCLDY	236
	VL	RASSSVSY MN	198	DTSKVAS	218	QQWSSNPLT	242
CD3-5	VH	GYTFTRYT MH	295	YINPSRGYTNYN QKVKD	219	YYDDHYCLDY	236
	VL	SASSSVSY MN	193	DTSKLAS	213	QQWSSNPFT	237
CD3-6	VH	GFKFSGY GMH	298	VIWYDGSKKYYV DSVKG	220	QMGYWHFDL	243
	VL	RASQSVS SYLA	200	DASNRAT	221	QQRSNWPPL T	244
CD3-7	VH	GFTFSTYA MN	299	RIRSKYNNYATYY ADSVK	303	HGNFGNSYV SWFAY	238
	VL	RSSTGAVT TSNYAN	195	GTNKRAP	215	ALWYSNLWV	239
CD3-8	VH	GYTFTRYT MH	295	YINPSRGYTNYN QKFKD	212	YYDDHYCLDY	236
	VL	RASSSVSY MN	198	DTSKVAS	218	QQWSSNPLT	242
CD3-9	VH	GFTFNTYA MN	296	RIRSKYNNYATYY ADSVK	303	HGNFGNSYV SWFAY	238

TABLE 12D
CD3 Binders – CDR sequences according to combination of Kabat and Chothia numbering schemes

Binding Domain	Chain	CDR1	SEQ ID NO:	CDR2	SEQ ID NO:	CDR3	SEQ ID NO:
	VL	RSSTGAVTTSNYAN	195	GTNKRAP	215	ALWYSNLWV	239
CD3-10	VH	GFTFNTYAMN	296	RIRSKYNNYATYYADSVKD	214	HGNFGNSYVSWFAY	238
	VL	RSSTGAVTTSNYAN	195	GTNKRAP	215	ALWYSNLWV	239
CD3-11	VH	GFTFNSYAMN	300	RIRSKYNNYATYYADSVKG	223	HGNFGNSYVSWWAY	246
	VL	GSSTGAVTSGNYPN	202	GTKFLAP	224	VLWYSNRWV	247
CD3-12	VH	GFTFNKYAMN	301	RIRSKYNNYATYYADSVKD	214	HGNFGNSYISYWAY	248
	VL	GSSTGAVTSGNYPN	202	GTKFLAP	224	VLWYSNRWV	247
CD3-13	VH	GYTFTRYTMH	295	YINPSRGYTNYNQKFKD	212	YYDDHYCLDY	236
	VL	SASSSVSYMN	193	DTSKLAS	213	QQWSSNPFT	237
CD3-14	VH	GFTFSTYAMN	299	RIRSKYNNYATYYADSVKD	214	HGNFGNSYVSWFAY	238
	VL	RSSTGAVTTSNYAN	195	GTNKRAP	215	ALWYSNLWV	239
CD3-15	VH	GFTFNTYAMN	296	RIRSKYNNYATYYADSVKD	214	HGNFGNSYVSWFAY	238
	VL	RSSTGAVTTSNYAN	195	GTNKRAP	215	ALWYSNLWV	239
CD3-16	VH	GFTFNTYAMN	296	RIRSKYNNYATYYADSVKG	223	HGNFGNSYVSWFAY	238
	VL	GSSTGAVTTSNYAN	204	GTNKRAP	215	ALWYSNLWV	239
CD3-17	VH	GFTFSTYAMN	299	RIRSKYNNYATYYADSVKG	223	HGNFGDSYVSWFAY	249
	VL	GSSTGAVTTSNYAN	204	GTNKRAP	215	ALWYSNHWV	250
CD3-18	VH	GYTFTRYTMH	295	YINPSRGYTNYNQKVKD	219	YYDDHYCLDY	236
	VL	SASSSVSYMN	193	DTSKLAS	213	QQWSSNPFT	237
CD3-19	VH	GYTFTRYTMH	295	YINPSRGYTNYNQKVKD	219	YYDDHYSLDY	251
	VL	SASSSVSYMN	193	DTSKLAS	213	QQWSSNPFT	237
CD3-20	VH	GYSFTGYTMN	302	LINPYKGVSTYNQKFKD	225	SGYYGDSDWYFDV	252
	VL	RASQDIRNYLN	206	YTSRLHS	304	QQGNTLPWT	253

[0513] In some embodiments, a MBM can comprise a CD3 ABM which comprises the CDRs of any of CD3-1 to CD3-130 as defined by Kabat numbering (*e.g.*, as set forth in Table 12B). In other embodiments, a MBM can comprise a CD3 ABM which comprises the CDRs of any of CD3-1 to CD3-130 as defined by Chothia numbering (*e.g.*, as set forth in Table 12C). In yet other embodiments, a MBM can comprise a CD3 ABM which comprises the CDRs of any of CD3-1 to CD3-130 as defined by a combination of Kabat and Chothia numbering (*e.g.*, as set forth in Table 12D).

[0514] In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-1. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-2. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-3. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-4. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-5. In some embodiments a CD3 ABM comprises the CDR sequences of CD3-6. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-7. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-8. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-9. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-10. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-11. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-12. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-13. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-14. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-15. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-16. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-17. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-18. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-19. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-20. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-21. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-22. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-23. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-24. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-25. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-26. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-27. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-28. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-29. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-30. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-31. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-32. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-33. In some embodiments, a

CD3 ABM comprises the CDR sequences of CD3-34. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-35. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-36. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-37. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-38. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-39. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-40. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-41. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-42. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-43. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-44. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-45. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-46. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-47. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-48. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-49. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-50. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-51. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-52. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-53. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-54. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-55. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-56. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-57. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-58. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-59. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-60. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-61. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-62. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-63. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-64. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-65. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-66. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-67. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-68. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-69. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-70. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-71. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-72. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-73. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-74. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-75. In some embodiments, a CD3 ABM comprises the

CDR sequences of CD3-76. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-77. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-78. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-79. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-80. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-81. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-82. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-83. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-84. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-85. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-86. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-87. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-88. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-89. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-90. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-91. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-92. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-93. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-94. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-95. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-96. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-97. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-98. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-99. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-100. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-101. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-102. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-103. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-104. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-105. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-106. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-107. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-108. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-109. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-110. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-111. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-112. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-113. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-114. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-115. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-116. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-117. In some embodiments, a CD3 ABM comprises the CDR sequences of

CD3-118. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-119. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-120. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-121. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-122. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-123. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-124. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-125. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-126. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-127. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-126. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-127. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-128. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-129. In some embodiments, a CD3 ABM comprises the CDR sequences of CD3-130.

[0515] A MBM can comprise the complete heavy and light variable sequences of any of CD3-1 to CD3-130. In some embodiments, a MBM comprises a CD3 ABM which comprises the VH and VL sequences of CD3-1. In some embodiments, a MBM comprises a CD3 ABM which comprises the VH and VL sequences of CD3-1. In some embodiments, a MBM comprises a CD3 ABM which comprises the VH and VL sequences of CD3-2. In some embodiments, a MBM comprises a CD3 ABM which comprises the VH and VL sequences of CD3-3. In some embodiments, a MBM comprises a CD3 ABM which comprises the VH and VL sequences of CD3-4. In some embodiments, a MBM comprises a CD3 ABM which comprises the VH and VL sequences of CD3-5. In some embodiments, a MBM comprises a CD3 ABM which comprises the VH and VL sequences of CD3-6. In some embodiments, a MBM comprises a CD3 ABM which comprises the VH and VL sequences of CD3-7. In some embodiments, a MBM comprises a CD3 ABM which comprises the VH and VL sequences of CD3-8. In some embodiments, a MBM comprises a CD3 ABM which comprises the VH and VL sequences of CD3-9. In some embodiments, a MBM comprises a CD3 ABM which comprises the VH and VL sequences of CD3-10. In some embodiments, a MBM comprises a CD3 ABM which comprises the VH and VL sequences of CD3-11. In some embodiments, a MBM comprises a CD3 ABM which comprises the VH and VL sequences of CD3-12. In some embodiments, a MBM comprises a CD3 ABM which comprises the VH and VL sequences of CD3-13. In some embodiments, a MBM comprises a CD3 ABM which comprises the VH and VL sequences of CD3-14. In some embodiments, a MBM comprises a CD3 ABM which comprises the VH and VL sequences of CD3-15. In some embodiments, a MBM comprises a CD3 ABM which comprises the VH and VL sequences of CD3-16. In some embodiments, a MBM comprises a CD3 ABM which comprises the VH and VL sequences of CD3-17. In some embodiments, a

MBM comprises a CD3 ABM which comprises the VH and VL sequences of CD3-18. In some embodiments, a MBM comprises a CD3 ABM which comprises the VH and VL sequences of CD3-19. In some embodiments, a MBM comprises a CD3 ABM which comprises the VH and VL sequences of CD3-20. In some embodiments, a MBM comprises a CD3 ABM which comprises the VH and VL sequences of CD3-21. In some embodiments, a MBM comprises a CD3 ABM which comprises the VH and VL sequences of CD3-22. In some embodiments, a MBM comprises a CD3 ABM which comprises the VH and VL sequences of CD3-23. In some embodiments, a MBM comprises a CD3 ABM which comprises the VH and VL sequences of CD3-24. In some embodiments, a MBM comprises a CD3 ABM which comprises the VH and VL sequences of CD3-25. In some embodiments, a MBM comprises a CD3 ABM which comprises the VH and VL sequences of CD3-26. In some embodiments, a MBM comprises a CD3 ABM which comprises the VH and VL sequences of CD3-27. In some embodiments, a MBM comprises a CD3 ABM which comprises the VH and VL sequences of CD3-28. In some embodiments, a MBM comprises a CD3 ABM which comprises the VH and VL sequences of CD3-129. In some embodiments, a MBM comprises a CD3 ABM which comprises the VH and VL sequences of CD3-130.

[0516] In addition to the CDR sets described in Tables 12B-12D (*i.e.*, the set of six CDRs for each of CD3-1 to CD3-130), the present disclosure provides variant CDR sets. In one embodiment, a set of 6 CDRs can have 1, 2, 3, 4 or 5 amino acid changes from a CDR set described in Tables 12B-12D, as long as the CD3 ABM is still able to bind to the target antigen, as measured by at least one of a Biacore, surface plasmon resonance (SPR) and/or BLI (biolayer interferometry, *e.g.*, Octet assay) assay.

[0517] In addition to the variable heavy and variable light domains disclosed in Table 12A that form an ABM to CD3, the present disclosure provides variant VH and VL domains. In one embodiment, the variant VH and VL domains each can have from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid changes from the VH and VL domain set forth in Table 12A, as long as the ABM is still able to bind to the target antigen, as measured at least one of a Biacore, surface plasmon resonance (SPR) and/or BLI (biolayer interferometry, *e.g.*, Octet assay) assay. In another embodiment, the variant VH and VL are at least 90, 95, 97, 98 or 99% identical to the respective VH or VL disclosed in Table 12A, as long as the ABM is still able to bind to the target antigen, as measured by at least one of a Biacore, surface plasmon resonance (SPR) and/or BLI (biolayer interferometry, *e.g.*, Octet assay) assay.

[0518] In some embodiments, a MBM can comprise an ABM which is a CD3 binding molecule as described in WO 2020/052692 or an antigen-binding domain thereof.

[0519] VH and VL sequences (amino acid sequences and the nucleotide sequences encoding the amino acid sequences) can be "mixed and matched" to create other CD3 ABMs. Such "mixed and matched" CD3 ABMs can be tested using binding assays known in the art (*e.g.*, FACS assays). When chains are mixed and matched, a VH sequence from a particular VH/VL pairing should be replaced with a structurally similar VH sequence. A VL sequence from a particular VH/VL pairing should be replaced with a structurally similar VL sequence.

[0520] In some embodiments, the antigen-binding domain that specifically binds to human CD3 is non-immunoglobulin based and is instead derived from a non-antibody scaffold protein, for example one of the non-antibody scaffold proteins described in Section 7.3.2. In an embodiment, the antigen-binding domain that specifically binds to human CD3 comprises Affilin-144160, which is described in WO 2017/013136. Affilin-144160 has the following amino acid sequence:

MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQWLWFAGKQLEDGRTLSDYNIQKES TLKLWLVDKAAMQIFVYTRTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIWAGKQLEDGR TLSDYNIALESGLHLVLRLRAA (SEQ ID NO:305)

7.7.2. TCR-α/β ABMs

[0521] The MBMs can contain an ABM that specifically binds to the TCR-α chain, the TCR-β chain, or the TCR-αβ dimer. Exemplary anti-TCR-α/β antibodies are known (see, *e.g.*, US 2012/0034221; Borst *et* al., 1990, Hum Immunol. 29(3):175-88 (describing antibody BMA031)). The VH, VL, and Kabat CDR sequences of antibody BMA031 are provided in Table 13.

TABLE 13
BMA031 sequences

Domain	Sequence	SEQ ID NO:
BMA031 CDR-H1	KASGYKFTSYVMH	306
BMA031 CDR-H2	YINPYNDVTKYNEKFK	307
BMA031 CDR-H3	GSYYDYDGFVY	308
BMA031 CDR-L1	SATSSVSYMH	309
BMA031 CDR-L2	DTSKLAS	213
BMA031 CDR-L3	QQWSSNPLT	242

TABLE 13 BMA031 sequences		
Domain	**Sequence**	**SEQ ID NO:**
BMA031 VH	EVQLQQSGPELVKPGASVKMSCKASGYKFTSYVMHWVKQKPGQGLE WIGYINPYNDVTKYNEKFKGKATLTSDKSSSTAYMELSSLTSEDSAVH YCARGSYYDYDGFVYWGQGTLVTVSA	310
BMA031 VL	QIVLTQSPAIMSASPGEKVTMTCSATSSVSYMHWYQQKSGTSPKRWI YDTSKLASGVPARFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSNP LTFGAGTKLELK	311

[0522] In an embodiment, a TCR ABM can comprise the CDR sequences of antibody BMA031. In other embodiments, a TCR ABM can comprise the VH and VL sequences of antibody BMA031.

7.7.3. TCR- γ/δ ABMs

[0523] The MBMs can contain an ABM that specifically binds to the TCR- γ chain, the TCR- δ chain, or the TCR- γδ dimer. Exemplary anti-TCR-γ/δ antibodies are known (see, *e.g.*, US Pat. No. 5,980,892 (describing δTCS1, produced by the hybridoma deposited with the ATCC as accession number HB 9578)).

7.8. CD2 ABMs

7.8.1. Immunoglobulin-Based CD2 ABMs

[0524] A Type 1 TBM can comprise an ABM which is an anti-CD2 antibody or an antigen-binding domain thereof. Exemplary anti-CD2 antibodies are known (see, *e.g.*, US 6,849,258, CN102827281A, US 2003/0139579 A1, and US 5,795,572). Table 14 provides exemplary CDR, VH, and VL sequences that can be included in anti-CD2 antibodies or antigen-binding fragments thereof, for use in MBMs of the disclosure.

TABLE 14 Immunoglobulin Based CD2 Binders			
Name	**Domain**	**Sequence**	**SEQ ID NO:**
CD2-1	CDR-H1	EYYMY (Rat Lo-CD2a = BTI-322 from Fig. 33 of USP 6,849,258)	312
CD2-1	CDR-H2	RIDPEDGSIDYVEKFKK (Rat Lo-CD2a = BTI-322 from Fig. 33 of USP 6,849,258)	313
CD2-1	CDR-H3	GKFNYRFAY (Rat Lo-CD2a = BTI-322 from Fig. 33 of USP 6,849,258)	314
CD2-1	CDR-L1	RSSQSLLHSSGNTYLN (Rat Lo-CD2a = BTI-322 from Fig. 31 of USP 6,849,258)	315
CD2-1	CDR-L2	LVSKLES (Rat Lo-CD2a = BTI-322 from Fig. 31 of USP 6,849,258)	316
CD2-1	CDR-L3	QFTHYPYT (Rat Lo-CD2a = BTI-322 from Fig. 31 of USP 6,849,258)	317

TABLE 14
Immunoglobulin Based CD2 Binders

Name	Domain	Sequence	SEQ ID NO:
CD2-1	VH	EVQLQQSGPELQRPGASVKLSCKASGYIFT**EYYMY**WVKQR PKQGLELVG**RIDPEDGSIDYVEKFK**KKATLTADTSSNTAYM QLSSLTSEDTATYFCARGKFNYRFAYWGQGTLVTVSS (SEQ ID NO:100 of USP 6,849,258)	318
CD2-1	VL	DVVLTQTPPTLLATIGQSVSISC**RSSQSLLHSSGNTYLN**WLL QRTGQSPQPLIYLVSKLESGVPNRFSGSGSGTDFTLKISGV EAEDLGVYYCMQFTHYPYTFGAGTKLELK (Rat Lo-CD2a Vk from SEQ ID NO:92, without signal sequence as shown in Fig. 31 of USP 6,849,258)	319
hu1CD2-1	VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFT**EYYMY**WVRQ APGQGLELMG**RIDPEDGSIDYVEKFKK**KVTLTADTSSSTAY MELSSLTSDDTAVYYCARGKFNYRFAYWGQGTLVTVSS (SEQ ID NO:101 of USP 6,849,258)	320
	VL	DVVMTQSPPSLLVTLGQPASISC**RSSQSLLHSSGNTYLN**WL LQRPGQSPQPLIYLVSKLESGVPDRFSGSGSGTDFTLKISG VEAEDVGVYYCMQFTHYPYTFGQGTKLEIK (SEQ ID NO:96 of USP 6,849,258)	321
hu2CD2-1	VH	EVQLQQSGPELQRPGASVKLSCKASGYIFT**EYYMY**WVKQR PKQGLELVG**RIDPEDGSIDYVEKFKK**KATLTADTSSNTAYM QLSSLTSEDTATYFCARGKFNYRFAYWGQGTLVTVSS (Vh of MEDI-507; SEQ ID NO:105 of USP 6,849,258)	318
	VL	DVVMTQSPPSLLVTLGQPASISC**RSSQSLLHSSGNTYLN**WL LQRPGQSPQPLIYLVSKLESGVPDRFSGSGSGTDFTLKISG VEAEDVGVYYCMQFTHYPYTFGQGTKLEIK (SEQ ID NO:96 of USP 6,849,258)(same as hu1CD2-1)	321

[0525] In some embodiments, a CD2 ABM comprises the CDR sequences of CD2-1 (SEQ ID NOS:312-317). In some embodiments, a CD2 ABM comprises the heavy and light chain variable sequences of CD2-1 (SEQ ID NOS: 318 and 319, respectively). In some embodiments, a CD2 ABM comprises the heavy and light chain variable sequences of hu1CD2-1 (SEQ ID NOS: 320 and 321, respectively). In some embodiments, a CD2 ABM comprises the heavy and light chain variable sequences of hu2CD2-1 (SEQ ID NOS: 318 and 321, respectively).

[0526] In other embodiments, a CD2 ABM can comprise the CDR sequences of antibody 9D1 produced by the hybridoma deposited with the Chinese Culture Collection Committee General Microbiology Center on May 16, 2012 with accession no. CGMCC 6132, and which is described in CN102827281A. In other embodiments, a CD2 ABM can comprise the CDR sequences of antibody LO-CD2b produced by the hybridoma deposited with the American Type Culture Collection on June 22, 1999 with accession no. PTA-802, and which is described in US 2003/0139579 A1. In yet other embodiments, a CD2 ABM can comprise the CDR sequences of the CD2 SFv-Ig produced by expression of the construct cloned in the recombinant *E. coli*

deposited with the ATCC on April 9, 1993 with accession no. 69277, and which is described in US 5,795,572.

[0527] In other embodiments, a CD2 ABM can comprise the VH and VL sequences of antibody 9D1. In other embodiments, a CD2 ABM can comprise the VH and VL sequences of antibody LO-CD2b. In yet other embodiments, a CD2 ABM can comprise the VH and VL sequences of the CD2 SFv-Ig produced by expression of the construct cloned in the recombinant *E. coli* having ATCC accession no. 69277.

7.8.2. CD58-based CD2 ABMs

[0528] In certain aspects the present disclosure provides a Type 1 TBM comprising a CD2 ABM which is a ligand. The CD2 ABM specifically binds to human CD2, whose natural ligand is CD58, also known as LFA-3. CD58/LFA-3 proteins are glycoproteins that are expressed on the surfaces of a variety of cell types (Dustin *et al.*, 1991, Annu. Rev. Immunol. 9:27) and play roles in mediating T-cell interactions with APCs in both antigen-dependent and antigen-independent manners (Wallner *et al.*, 1987, J. Exp. Med. 166:923). Accordingly, in certain aspects, the CD2 ABM is a CD58 moiety. As used herein, a CD58 moiety comprises an amino acid sequence comprising at least 70% sequence identity to a CD2-binding portion of CD58, *e.g.*, at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%,81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to a CD2-binding portion of CD58. The sequence of human CD58 has the Uniprot identifier P19256 (www.uniprot.org/uniprot/P19256). It has been established that CD58 fragments containing amino acid residues 30-123 of full length CD58 (*i.e.*, the sequence designated as CD58-6 in Table 15 below) are sufficient for binding to CD2. Wang *et al.*, 1999, Cell 97:791-803. Accordingly, in certain aspects, a CD58 moiety comprises an amino acid sequence comprising at least 70% sequence identity to amino acids 30-123 of CD58, *e.g.*, at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%,81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence designated CD58-6.

[0529] The interactions between CD58 and CD2 have been mapped through x-ray crystallography and molecular modeling. The substitution of residues E25, K29, K30, K32, D33, K34, E37, D84 and K87 (with numbering referring to the in the mature polypeptide) reduces binding to CD2. Ikemizu *et al.*, 1999, Proc. Natl. Acad. Sci. USA 96:4289-94. Accordingly, in some embodiments the CD58 moiety retains the wild type residues at E25, K29, K30, K32, D33, K34, E37, D84 and K87.

[0530] In contrast, the following substitutions (with numbering referring to the full length polypeptide) did not impact binding to CD2: F29S; V37K; V49Q; V86K; T113S; and L121G.

Accordingly, a CD58 moiety can include one, two, three, four, five or all six of the foregoing substitutions.

[0531] In some embodiments, the CD58 moiety is engineered to include a pair of cysteine substitutions that upon recombinant expression create a disulfide bridge. Exemplary amino acid pairs that can be substituted with cysteines in order to form a disulfide bridge upon expression (with numbering referring to the full length polypeptide) are (a) a V45C substitution and a M105C substitution; (b) a V54C substitution and a G88C substitution; (c) a V45C substitution and a M114C substitution; and (d) a W56C substitution and a L90C substitution.

[0532] Exemplary CD58 moieties are provided in Table 15 below:

TABLE 15
CD58 sequences

Name	Description	Sequence	SEQ ID NO:
CD58-1	Full length CD58, including signal sequence and full intracellular domain (P19256)	MVAGSDAGRALGVLSVVCLLHCFGFISCFSQQIYGVVY GNVTFHVPSNVPLKEVLWKKQKDKVAELENSEFRAFS SFKNRVYLDTVSGSLTIYNLTSSDEDEYEMESPNITDTM KFFLYVLESLPSPTLTCALTNGSIEVQCMIPEHYNSHRG LIMYSWDCPMEQCKRNSTSIYFKMENDLPQKIQCTLSN PLFNTTSSIILTTCIPSSGHSRHRYALIPIPLAVITTCIVLY MNGILKCDRKPDRTNSN	322
CD58-2	Full length CD58, including signal sequence and but no intracellular domain (P19256-2)	MVAGSDAGRALGVLSVVCLLHCFGFISCFSQQIYGVVY GNVTFHVPSNVPLKEVLWKKQKDKVAELENSEFRAFS SFKNRVYLDTVSGSLTIYNLTSSDEDEYEMESPNITDTM KFFLYVLESLPSPTLTCALTNGSIEVQCMIPEHYNSHRG LIMYSWDCPMEQCKRNSTSIYFKMENDLPQKIQCTLSN PLFNTTSSIILTTCIPSSGHSRHRYALIPIPLAVITTCIVLY MNVL	323
CD58-3	Full length CD58, including signal sequence and variant intracellular domain (P19256-3)	MVAGSDAGRALGVLSVVCLLHCFGFISCFSQQIYGVVY GNVTFHVPSNVPLKEVLWKKQKDKVAELENSEFRAFS SFKNRVYLDTVSGSLTIYNLTSSDEDEYEMESPNITDTM KFFLYVLESLPSPTLTCALTNGSIEVQCMIPEHYNSHRG LIMYSWDCPMEQCKRNSTSIYFKMENDLPQKIQCTLSN PLFNTTSSIILTTCIPSSGHSRHRYALIPIPLAVITTCIVLY MNGILKCDRKPDRTK	324
CD58-4	Extracellular domain of CD58, corresponding to amino acids 29-215 of CD58 (WT)	FSQQIYGVVYGNVTFHVPSNVPLKEVLWKKQKDKVAE LENSEFRAFSSFKNRVYLDTVSGSLTIYNLTSSDEDEYE MESPNITDTMKFFLYVLESLPSPTLTCALTNGSIEVQCM IPEHYNSHRGLIMYSWDCPMEQCKRNSTSIYFKMENDL PQKIQCTLSNPLFNTTSSIILTTCIPSSGHSRHR	325
CD58-5	Extracellular domain of CD58, corresponding to amino acids 29-215 of CD58 (with permitted substitutions)	BSQQIYGVJYGNVTFHVPSNOPLKEVLWKKQKDK VAELENSEFRAFSSFKNRVYLDTUSGSLTIYNLTS SDEDEYEMESPNITDXMKFFLYVZESLPSPTLTCA LTNGSIEVQCMIPEHYNSHRGLIMYSWDCPMEQC KRNSTSIYFKMENDLPQKIQCTLSNPLFNTTSSIILT TCIPSSGHSRHR B = F or S J= V or K	326

	TABLE 15 CD58 sequences		
Name	**Description**	**Sequence**	**SEQ ID NO:**
		O = V or Q U = V or K X= T or S Z= L or G	
CD58-6	Amino acids 30-123 (WT) Ig-V like domain	SQQIYGVVYGNVTFHVPSNVPLKEVLWKKQKDKVAEL ENSEFRAFSSFKNRVYLDTVSGSLTIYNLTSSDEDEYE MESPNITDTMKFFLYVLES	327
CD58-7	Amino acids 30-123 (with permitted substitutions) Ig-V like domain	SQQIYGVJYGNVTFHVPSNOPLKEVLWKKQKDKVAEL ENSEFRAFSSFKNRVYLDTVSGSLTIYNLTSSDEDEYE MESPNITDTMKFFLYVLES J= V or K O = V or Q	328
CD58-8	Amino acids 30-123 (V45C_M105C) Ig-V like domain	SQQIYGVVYGNVTFHCPSNVPLKEVLWKKQKDKVAEL ENSEFRAFSSFKNRVYLDTVSGSLTIYNLTSSDEDEYE CESPNITDTMKFFLYVLES	329
CD58-9	Amino acids 30-123 (V54C_G88C) Ig-V like domain	SQQIYGVVYGNVTFHVPSNVPLKECLWKKQKDKVAEL ENSEFRAFSSFKNRVYLDTVSCSLTIYNLTSSDEDEYE MESPNITDTMKFFLYVLES	330
CD58-10	Amino acids 30-123 (V45C_M114C) Ig-V like domain	SQQIYGVVYGNVTFHCPSNVPLKEVLWKKQKDKVAEL ENSEFRAFSSFKNRVYLDTVSGSLTIYNLTSSDEDEYE MESPNITDTCKFFLYVLES	331
CD58-11	Amino acids 30-123 (W56C_L90C) Ig-V like domain	SQQIYGVVYGNVTFHVPSNVPLKEVLCKKQKDKVAELE NSEFRAFSSFKNRVYLDTVSGSCTIYNLTSSDEDEYEM ESPNITDTMKFFLYVLES	332

7.8.3. CD48-based CD2 ABMs

[0533] In certain aspects the present disclosure provides a MBM comprising a CD2 ABM which is CD48 moiety. As used herein, a CD48 moiety comprises an amino acid sequence comprising at least 70% sequence identity to a CD2-binding portion of CD48, *e.g.*, at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%,81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to a CD2-binding portion of CD48. The sequence of human CD48 has the Uniprot identifier P09326 (www.uniprot.org/uniprot/P09326), which includes a signal peptide (amino acids 1-26) and a GPI anchor (amino acids 221-243). In certain aspects, a CD48 moiety comprises an amino

acid sequence comprising at least 70% sequence identity (*e.g.*, at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%,81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity) to the amino acid sequence consisting of amino acids 27-220 of Uniprot identifier P09326. Human CD48 has an Ig-like C2-type I domain (amino acids 29-127 of Uniprot identifier P09326) and a Ig-like C2 type 2 domain (amino acids 132-212 of Uniprot identifier P09326). Accordingly, in some embodiments, a CD48 moiety comprises an amino acid sequence comprising at least 70% sequence identity (*e.g.*, at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%,81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity) to the amino acid sequence consisting of amino acids 29-212 of Uniprot identifier P09326, to the C2-type I domain (amino acids 29-127 of Uniprot identifier P09326) and/or to the Ig-like C2 type 2 domain (amino acids 132-212 of Uniprot identifier P09326). A CD48 moiety can in some embodiments comprise one or more natural variants relative to the sequence of Uniprot identifier P09326. For example, a CD48 moiety can include a E102Q substitution. As another example, a CD48 moiety can comprise an amino acid sequence corresponding to a CD-48 isoform or a CD2 binding portion thereof, *e.g.*, the isoform having Uniprot identifier P09326-2 or a CD2 binding portion thereof.

7.9. Tumor-Associated Antigen ABMs

[0534] The Type 2 TBMs can comprise an ABM that binds specifically to a tumor-associated antigen (TAA). In some embodiments, the TAA is a human TAA. The antigen may or may not be present on normal cells. In certain embodiments, the TAA is preferentially expressed or upregulated on tumor cells as compared to normal cells. In other embodiments, the TAA is a lineage marker.

[0535] In certain embodiments, the TAA is expressed or upregulated on cancerous B cells as compared to normal B cells. In other embodiments, the TAA is a B cell lineage marker.

[0536] It is anticipated that any type of B cell malignancy can be targeted by the MBMs of the disclosure. Exemplary types of B cell malignancies that can be targeted include Hodgkin's lymphomas, non-Hodgkin's lymphomas (NHLs), and multiple myeloma. Examples of NHLs include diffuse large B-cell lymphoma (DLBCL), follicular lymphoma, chronic lymphocytic leukemia (CLL) /small lymphocytic lymphoma (SLL), mantle cell lymphoma (MCL), marginal zone lymphomas, Burkitt lymphoma, lymphoplasmacytic lymphoma (Waldenstrom macroglobulinemia), hairy cell leukemia, primary central nervous system (CNS) lymphoma, primary mediastinal large B-cell lymphoma, mediastinal grey-zone lymphoma (MGZL), splenic marginal zone B-cell lymphoma, extranodal marginal zone B-cell lymphoma of MALT, nodal marginal zone B-cell lymphoma, and primary effusion lymphoma.

[0537] Examples of TAAs other than CD19 that can be targeted by the MBMs (*e.g.*, TBMs) include BCMA, CD20, CD22, CD123, CD33, CLL1, CD138 (also known as Syndecan-1, SDC1), CS1, CD38, CD133, FLT3, CD52, TNFRSF13C (TNF Receptor Superfamily Member 13C, also referred to in the art as BAFFR: B-Cell-Activating Factor Receptor), TNFRSF13B (TNF Receptor Superfamily Member 13B, also referred to in the art as TACI: Transmembrane Activator And CAML Interactor), CXCR4 (C-X-C Motif Chemokine Receptor 4), PD-L1 (programmed death-ligand 1), LY9 (lymphocyte antigen 9, also referred to in the art as CD229), CD200, FCGR2B (Fc fragment of IgG receptor IIb, also referred to in the art as CD32b), CD21, CD23, CD24, CD40L, CD72, CD79a, and CD79b. In some embodiments, the TAA is BCMA. In some embodiments, the TAA is CD20. In some embodiments, the TAA is CD22. In some embodiments, the TAA is CD123. In some embodiments, the TAA is CD33. In some embodiments, the TAA is CLL1. In some embodiments, the TAA is CD138. In some embodiments, the TAA is CS1. In some embodiments, the TAA is CD38. In some embodiments, the TAA is CD133. In some embodiments, the TAA is FLT3. In some embodiments, the TAA is CD52. In some embodiments, the TAA is TNFRSF13C. In some embodiments, the TAA is TNFRSF13B. In some embodiments, the TAA is CXCR4. In some embodiments, the TAA is PD-L1. In some embodiments, the TAA is LY9. In some embodiments, the TAA is CD200. In some embodiments, the TAA is CD21. In some embodiments, the TAA is CD23. In some embodiments, the TAA is CD24. In some embodiments, the TAA is CD40L. In some embodiments, the TAA is CD72. In some embodiments, the TAA is CD79a. In some embodiments, the TAA is CD79b.

[0538] A TAA-binding ABM can comprise, for example, an anti-TAA antibody or an antigen-binding fragment thereof. The anti-TAA antibody or antigen-binding fragment can comprise, for example, the CDR sequences of an antibody set forth in Table 16. In some embodiments, the anti-TAA antibody or antigen-binding domain thereof has the heavy and light chain variable region sequences of an antibody set forth in Table 16.

TABLE 16
Exemplary Anti-Tumor-Associated Antigen Antibodies

Target	Examples of Antibody Name and/or Reference(s) and/or Source
CD123	Any CD123 antibody described in US Pat. No. 8,852,551, EP2426148, WO 2014/138819, WO 2016/028896, or WO 2014/130635
BCMA	Any BCMA antibody described in WO2012163805, WO200112812, or WO2003062401.
CD20	Rituximab, Ofatumumab, Ocrelizumab, Veltuzumab, or GA101
CD22	Any CD22 antibody described in Haso *et al.*, 2013, Blood, 121(7): 1165-1174, Wayne *et al.*, 2010, Clin Cancer Res 16(6): 1894-1903, Kato *et al.*, 2013, Leuk Res 37(1):83-88, or Creative BioMart (creativebiomart.net): MOM-18047-S(P).
CD33	Any CD33 antibody described in Bross *et al.*, 2001, Clin Cancer Res 7(6):1490-1496 (Gemtuzumab Ozogamicin, hP67.6),Caron *et al.*, 1992, Cancer Res 52(24):6761-6767 (Lintuzumab, HuM195), Lapusan *et al.*, 2012, Invest New

TABLE 16
Exemplary Anti-Tumor-Associated Antigen Antibodies

Target	Examples of Antibody Name and/or Reference(s) and/or Source
	Drugs 30(3):1121-1131 (AVE9633), Aigner *et al.*, 2013, Leukemia 27(5): 1107-1115 (AMG330, CD33 BiTE), Dutour *et al.*, 2012, Adv Hematol 2012:683065, or Pizzitola *et al.*, 2014, Leukemia doi:10.1038/Lue.2014.62.
CD38	Daratumumab (see, *e.g.*, Groen *et al.*, 2010, Blood 116(21):1261-1262; MOR202 (see, *e.g.*, US Pat. No. 8,263,746); or any CD38 antibody described in US Pat. No. 8,362,211.
CLL-1	PE-CLL1-hu Cat# 353604 (BioLegend); PE-CLL1 (CLEC12A) Cat# 562566 (BD); Any CLL-1 antibody described in WO 2014/051433 A1, US 2016/0368994 A1, US 2013/0295118 A1, US Pat. No. 8,536,310 B2, Lu *et al.*, 2014, Angewandte Chemie International Edition 53(37):9841–9845, or Leong *et al.*, 2017, Blood 129(5):609-618
CS1	Elotuzumab (BMS), see *e.g.*, Tai *et al.*, 2008, Blood 112(4):1329-37; Tai *et al.*, 2007, Blood. 110(5):1656-63.
FLT3	Any FLT3 antibody described in WO 2011/076922, US Pat. No. 5777084, EP0754230, or US 2009/0297529.
CD133	Any CD133 antibody described in US Pat No. 9,624,303, WO 2016/154623, or WO 2011/089211; 5E3 (ThermoFisher); MAB11331 (R&D Systems); MAB4310 (Millipore Sigma)
CD138	Any CD138 antibody described in WO/2009/080829, WO/2017/014679, or US Pat. No. 9,289,509; nBT062 (Biotest AG); MI15, B-A38, SP152, DL-101 (ThermoFisher)
CD52	alemtuzumab (Genzyme); ANT1034 (see, Holgate *et al.*, 2015, PLOS ONE 10(9): e0138123; any CD52 antibody described in WO/2010/132659; any CD52 antibody described in U.S. Pat No. 9708407; any CD52 antibody described in WO/2010/132659
TNFRSF13C	Any TNFRSF13C antibody described in WO 2010/007082, US Pat. No. 9,382,326
TNFRSF13B	Any TNFRSF13B antibody described in WO 2004/011611; LS-C89973 (Lifespan Biosciences, Inc.) M02952-1 (Boster Biological Technology); MAB1041, MAB1741, and MAB174 (R&D Systems)
CXCR4	Any CXCR4 antibody described in US Pat. Nos. 7,138,496, 8,329,178, 8,450,464, 9,249,223, or 9,260,527
PD-L1	Any PD-L1 antibody described in US 2015/0203580, US 2017/0058033, US 2017/0204184, US Pat. No. 8,741,295, US Pat. No. 9,789,183, or US Pat. No. 9,637,546
LY9	HLy9.25 (*e.g.*, Lifespan Biosciences, Inc. cat. no. LS-C112605); MAB1898 (R&D Systems)
CD200	Any CD200 antibody described in US Pat. No. 7,887,798; ab23552 (Abcam); Ox104 (ThermoFisher)
FCGR2B	Any FCGR2B antibody described in US Pat No. 8,802,089 or WO 2017/103895; ab45143 (Abcam); AT130-2 (ThermoFisher); 2E10 (Millipore Sigma)
CD21	ab75985 (Abcam); ab9492 (Abcam); 2G9 (ThermoFisher); HB5 (ThermoFisher); MAB4909 (R&D Systems)
CD23	Any CD23 antibody described in US Pat. No. 7,008,623 or US Pat. No. 6,011,138; lumiliximab (Biogen); ab16702 (Abcam); SP23 (ThermoFisher)
CD24	Any CD24 antibody described in US Pat. No. 8,614,301; SN3 (ThermoFisher); SN3b (ThermoFisher); 2Q1282 (Santa Cruz Biotechnology); 3H1143 (Santa Cruz Biotechnology); ALB9 (Santa Cruz Biotechnology); MAB5248 (R&D Systems)

TABLE 16 Exemplary Anti-Tumor-Associated Antigen Antibodies	
Target	**Examples of Antibody Name and/or Reference(s) and/or Source**
CD40L	Any CD40L antibody described in US Pat. No. 9,228,018 or US 2003/0099642; 24-31 (Biolegend); ab52750 (Abcam); ab47204 (Abcam); CDP7657 (UCB Pharma); 5c8 (Biogen)
CD72	3F3 (Biolegend); Bu40 (ThermoFisher); H-7 (Santa Cruz Biotechnology); H-96 (Santa Cruz Biotechnology); G-5 (Santa Cruz Biotechnology); ab92509 (Abcam)
CD79a	ab62650 (Abcam); ab79414 (Abcam); MAB69201 (R&D Systems); HM57 (Bio-Rad)
CD79b	Any CD79b antibody described in WO 2014/011521; ab130422 (Abcam); ab134147 (Abcam); polatuzumab (Genentech)

[0539] In certain embodiments, the TAA is selected from BCMA and CD20. In some embodiments, the TAA is BCMA. "BCMA" refers to B-cell maturation antigen. BCMA (also known as TNFRSF17, BCM or CD269) is a member of the tumor necrosis receptor (TNFR) family and is predominantly expressed on terminally differentiated B cells, *e.g.*, memory B cells and plasma cells. Its ligands include B-cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL). The protein BCMA is encoded by the gene TNFRSF17. Exemplary BCMA sequences are available at the Uniprot database under accession number Q02223.

[0540] In certain aspects, a Type 2 TBM comprises an ABM3 that specifically binds to BCMA, for example, an anti-BCMA antibody or an antigen-binding domain thereof. The anti-BCMA antibody or antigen-binding domain thereof can comprise, for example, CDR, VH, VL, or scFV sequences set forth in Tables 17A-17G.

TABLE 17A BCMA Binders – Variable domain and scFv sequences			
Antibody	**Domain**	**Sequence**	**SEQ ID NO.**
BCMA-1	VH	EVQLVESGGGLVQPGGSLRLSCAVSGFALSNHGMSWVRRAPG KGLEWVSGIVYSGSTYYAASVKGRFTISRDNSRNTLYLQMNSLR PEDTAIYYCSAHGGESDVWGQGTTVTVSS	333
	VL	DIQLTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPK LLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ SYSTPYTFGQGTKVEIK	334
	scFv	EVQLVESGGGLVQPGGSLRLSCAVSGFALSNHGMSWVRRAPG KGLEWVSGIVYSGSTYYAASVKGRFTISRDNSRNTLYLQMNSLR PEDTAIYYCSAHGGESDVWGQGTTVTVSSASGGGGSGGRASG GGGSDIQLTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKP GKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFAT YYCQQSYSTPYTFGQGTKVEIK	335
BCMA-2	VH	QVQLVESGGGLVQPGRSLRLSCAASGFTFSNYAMSWVRQAPG KGLGWVSGISRSGENTYYADSVKGRFTISRDNSKNTLYLQMNSL RDEDTAVYYCARSPAHYYGGMDVWGQGTTVTVSS	336
	VL	DIVLTQSPGTLSLSPGERATLSCRASQSISSSFLAWYQQKPGQA PRLLIYGASRRATGIPDRFSGSGSGTDFTLTISRLEPEDSAVYYC QQYHSSPSWTFGQGTKLEIK	337

TABLE 17A
BCMA Binders – Variable domain and scFv sequences

Antibody	Domain	Sequence	SEQ ID NO.
	scFv	QVQLVESGGGLVQPGRSLRLSCAASGFTFSNYAMSWVRQAPG KGLGWVSGISRSGENTYYADSVKGRFTISRDNSKNTLYLQMNSL RDEDTAVYYCARSPAHYYGGMDVWGQGTTVTVSSASGGGGSG GRASGGGGSDIVLTQSPGTLSLSPGERATLSCRASQSISSSFLA WYQQKPGQAPRLLIYGASRRATGIPDRFSGSGSGTDFTLTISRLE PEDSAVYYCQQYHSSPSWTFGQGTKLEIK	338
BCMA-3	VH	QVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPG KGLEWVSGISWNSGSIGYADSVKGRFTISRDNAKNSLYLQMNSL RAEDTALYYCSVHSFLAYWGQGTLVTVSS	339
	VL	DIVMTQTPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQK PGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDV GVYYCMQALQTPYTFGQGTKVEIK	340
	scFv	QVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPG KGLEWVSGISWNSGSIGYADSVKGRFTISRDNAKNSLYLQMNSL RAEDTALYYCSVHSFLAYWGQGTLVTVSSASGGGGSGGRASG GGGSDIVMTQTPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDW YLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVE AEDVGVYYCMQALQTPYTFGQGTKVEIK	341
BCMA-4	VH	EVQLLESGGGLVQPGGSLRLSCAVSGFALSNHGMSWVRRAPG KGLEWVSGIVYSGSTYYAASVKGRFTISRDNSRNTLYLQMNSLR PEDTAIYYCSAHGGESDVWGQGTTVTVSS	342
	VL	DIVMTQTPLSLSVTPGQPASISCKSSQSLLRNDGKTPLYWYLQKA GQPPQLLIYEVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVG AYYCMQNIQFPSFGGGTKLEIK	343
	scFv	EVQLLESGGGLVQPGGSLRLSCAVSGFALSNHGMSWVRRAPG KGLEWVSGIVYSGSTYYAASVKGRFTISRDNSRNTLYLQMNSLR PEDTAIYYCSAHGGESDVWGQGTTVTVSSASGGGGSGGRASG GGGSDIVMTQTPLSLSVTPGQPASISCKSSQSLLRNDGKTPLYW YLQKAGQPPQLLIYEVSNRFSGVPDRFSGSGSGTDFTLKISRVEA EDVGAYYCMQNIQFPSFGGGTKLEIK	344
BCMA-5	VH	QVQLVQSGAEVRKTGASVKVSCKASGYIFDNFGINWVRQAPGQ GLEWMGWINPKNNNTNYAQKFQGRVTITADESTNTAYMEVSSL RSEDTAVYYCARGPYYYQSYMDVWGQGTMVTVSS	345
	VL	DIVMTQTPLSLPVTPGEPASISCRSSQSLLHSNGYNYLNWYLQK PGQSPQLLIYLGSKRASGVPDRFSGSGSGTDFTLHITRVGAEDV GVYYCMQALQTPYTFGQGTKLEIK	346
	scFv	QVQLVQSGAEVRKTGASVKVSCKASGYIFDNFGINWVRQAPGQ GLEWMGWINPKNNNTNYAQKFQGRVTITADESTNTAYMEVSSL RSEDTAVYYCARGPYYYQSYMDVWGQGTMVTVSSASGGGGS GGRASGGGGSDIVMTQTPLSLPVTPGEPASISCRSSQSLLHSNG YNYLNWYLQKPGQSPQLLIYLGSKRASGVPDRFSGSGSGTDFTL HITRVGAEDVGVYYCMQALQTPYTFGQGTKLEIK	347
BCMA-6	VH	QVQLQESGGGLVQPGGSLRLSCAASGFTFSSDAMTWVRQAPG KGLEWVSVISGSGGTTYYADSVKGRFTISRDNSKNTLYLQMNSL RAEDTAVYYCAKLDSSGYYYARGPRYWGQGTLVTVSS	348
	VL	DIQLTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPK LLIYGASTLASGVPARFSGSGSGTHFTLTINSLQSEDSATYYCQQ SYKRASFGQGTKVEIK	349
	scFv	QVQLQESGGGLVQPGGSLRLSCAASGFTFSSDAMTWVRQAPG KGLEWVSVISGSGGTTYYADSVKGRFTISRDNSKNTLYLQMNSL RAEDTAVYYCAKLDSSGYYYARGPRYWGQGTLVTVSSASGGG GSGGRASGGGGSDIQLTQSPSSLSASVGDRVTITCRASQSISSY LNWYQQKPGKAPKLLIYGASTLASGVPARFSGSGSGTHFTLTINS LQSEDSATYYCQQSYKRASFGQGTKVEIK	350

TABLE 17A
BCMA Binders – Variable domain and scFv sequences

Antibody	Domain	Sequence	SEQ ID NO.
BCMA-7	VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFSNYGITWVRQAPGQ GLEWMGWISAYNGNTNYAQKFQGRVTMTRNTSISTAYMELSSL RSEDTAVYYCARGPYYYYMDVWGKGTMVTVSS	351
	VL	EIVMTQSPLSLPVTPGEPASISCRSSQSLLYSNGYNYVDWYLQK PGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFKLQISRVEAEDV GIYYCMQGRQFPYSFGQGTKVEIK	352
	scFv	QVQLVQSGAEVKKPGASVKVSCKASGYTFSNYGITWVRQAPGQ GLEWMGWISAYNGNTNYAQKFQGRVTMTRNTSISTAYMELSSL RSEDTAVYYCARGPYYYYMDVWGKGTMVTVSSASGGGGSGG RASGGGGSEIVMTQSPLSLPVTPGEPASISCRSSQSLLYSNGYN YVDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFKLQI SRVEAEDVGIYYCMQGRQFPYSFGQGTKVEIK	353
BCMA-8	VH	EVQLLETGGGLVQPGGSLRLSCAVSGFALSNHGMSWVRRAPG KGLEWVSGIVYSGSTYYAASVKGRFTISRDNSRNTLYLQMNSLR PEDTAIYYCSAHGGESDVWGQGTTVTVSS	354
	VL	EIVLTQSPATLSVSPGESATLSCRASQSVSSNLAWYQQKPGQAP RLLIYGASTRASGIPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQ QYGSSLTFGGGTKVEIK	355
	scFv	EVQLLETGGGLVQPGGSLRLSCAVSGFALSNHGMSWVRRAPG KGLEWVSGIVYSGSTYYAASVKGRFTISRDNSRNTLYLQMNSLR PEDTAIYYCSAHGGESDVWGQGTTVTVSSASGGGGSGGRASG GGGSEIVLTQSPATLSVSPGESATLSCRASQSVSSNLAWYQQKP GQAPRLLIYGASTRASGIPDRFSGSGSGTDFTLTISSLQAEDVAV YYCQQYGSSLTFGGGTKVEIK	356
BCMA-9	VH	EVQLVETGGGLVQPGGSLRLSCAVSGFALSNHGMSWVRRAPG KGLEWVSGIVYSGSTYYAASVKGRFTISRDNSRNTLYLQMNSLR PEDTAIYYCSAHGGESDVWGQGTTVTVSS	357
	VL	EIVMTQSPATLSVSPGERATLSCRASQSVSSKLAWYQQKPGQA PRLLMYGASIRATGIPDRFSGSGSGTEFTLTISSLEPEDFAVYYC QQYGSSSWTFGQGTKVEIK	358
	scFv	EVQLVETGGGLVQPGGSLRLSCAVSGFALSNHGMSWVRRAPG KGLEWVSGIVYSGSTYYAASVKGRFTISRDNSRNTLYLQMNSLR PEDTAIYYCSAHGGESDVWGQGTTVTVSSASGGGGSGGRASG GGGSEIVMTQSPATLSVSPGERATLSCRASQSVSSKLAWYQQK PGQAPRLLMYGASIRATGIPDRFSGSGSGTEFTLTISSLEPEDFA VYYCQQYGSSSWTFGQGTKVEIK	359
BCMA-10	VH	EVQLVETGGGVVQPGGSLRLSCAVSGFALSNHGMSWVRRAPG KGLEWVSGIVYSGSTYYAASVKGRFTISRDNSRNTLYLQMNSLR PEDTAIYYCSAHGGESDVWGQGTTVTVSS	360
	VL	EIVLTQSPGTLSLSPGERATLSCRASQSVGSTNLAWYQQKPGQA PRLLIYDASNRATGIPDRFSGGGSGTDFTLTISRLEPEDFAVYYC QQYGSSPPWTFGQGTKVEIK	361
	scFv	EVQLVETGGGVVQPGGSLRLSCAVSGFALSNHGMSWVRRAPG KGLEWVSGIVYSGSTYYAASVKGRFTISRDNSRNTLYLQMNSLR PEDTAIYYCSAHGGESDVWGQGTTVTVSSASGGGGSGGRASG GGGSEIVLTQSPGTLSLSPGERATLSCRASQSVGSTNLAWYQQ KPGQAPRLLIYDASNRATGIPDRFSGGGSGTDFTLTISRLEPEDF AVYYCQQYGSSPPWTFGQGTKVEIK	362
BCMA-11	VH	QVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGK GLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRA EDTAVYYCARESGDGMDVWGQGTTVTVSS	363
	VL	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAP KLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ QSYTLAFGQGTKVDIK	364

TABLE 17A
BCMA Binders – Variable domain and scFv sequences

Antibody	Domain	Sequence	SEQ ID NO.
	scFv	QVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGK GLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRA EDTAVYYCARESGDGMDVWGQGTTVTVSSASGGGGSGGRAS GGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQK PGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFA TYYCQQSYTLAFGQGTKVDIK	365
BCMA-12	VH	QVQLVQSGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGK GLEWVSYISSSGNTIYYADSVKGRFTISRDNAKNSLYLQMNSLRA EDTAVYYCARSTMVREDYWGQGTLVTVSS	366
	VL	DIVLTQSPLSLPVTLGQPASISCKSSESLVHNSGKTYLNWFHQRP GQSPRRLIYEVSNRDSGVPDRFTGSGSGTDFTLKISRVEAEDVG VYYCMQGTHWPGTFGQGTKLEIK	367
	scFv	QVQLVQSGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGK GLEWVSYISSSGNTIYYADSVKGRFTISRDNAKNSLYLQMNSLRA EDTAVYYCARSTMVREDYWGQGTLVTVSSASGGGGSGGRASG GGGSDIVLTQSPLSLPVTLGQPASISCKSSESLVHNSGKTYLNWF HQRPGQSPRRLIYEVSNRDSGVPDRFTGSGSGTDFTLKISRVEA EDVGVYYCMQGTHWPGTFGQGTKLEIK	368
BCMA-13	VH	QVQLVESGGGLVQPGGSLRLSCAVSGFALSNHGMSWVRRAPG KGLEWVSGIVYSGSTYYAASVKGRFTISRDNSRNTLYLQMNSLR PEDTAIYYCSAHGGESDVWGQGTTVTVSS	369
	VL	DIRLTQSPSPLSASVGDRVTITCQASEDINKFLNWYHQTPGKAPK LLIYDASTLQTGVPSRFSGSGSGTDFTLTINSLQPEDIGTYYCQQ YESLPLTFGGGTKVEIK	370
	scFv	QVQLVESGGGLVQPGGSLRLSCAVSGFALSNHGMSWVRRAPG KGLEWVSGIVYSGSTYYAASVKGRFTISRDNSRNTLYLQMNSLR PEDTAIYYCSAHGGESDVWGQGTTVTVSSASGGGGSGGRASG GGGSDIRLTQSPSPLSASVGDRVTITCQASEDINKFLNWYHQTP GKAPKLLIYDASTLQTGVPSRFSGSGSGTDFTLTINSLQPEDIGTY YCQQYESLPLTFGGGTKVEIK	371
BCMA-14	VH	EVQLVETGGGLVQPGGSLRLSCAVSGFALSNHGMSWVRRAPG KGLEWVSGIVYSGSTYYAASVKGRFTISRDNSRNTLYLQMNSLR PEDTAIYYCSAHGGESDVWGQGTTVTVSS	357
	VL	ETTLTQSPATLSVSPGERATLSCRASQSVGSNLAWYQQKPGQG PRLLIYGASTRATGIPARFSGSGSGTEFTLTISSLQPEDFAVYYCQ QYNDWLPVTFGQGTKVEIK	372
	scFv	EVQLVETGGGLVQPGGSLRLSCAVSGFALSNHGMSWVRRAPG KGLEWVSGIVYSGSTYYAASVKGRFTISRDNSRNTLYLQMNSLR PEDTAIYYCSAHGGESDVWGQGTTVTVSSASGGGGSGGRASG GGGSETTLTQSPATLSVSPGERATLSCRASQSVGSNLAWYQQK PGQGPRLLIYGASTRATGIPARFSGSGSGTEFTLTISSLQPEDFA VYYCQQYNDWLPVTFGQGTKVEIK	373
BCMA-15	VH	EVQLVESGGGLVQPGGSLRLSCAVSGFALSNHGMSWVRRAPG KGLEWVSGIVYSGSTYYAASVKGRFTISRDNSRNTLYLQMNSLR PEDTAIYYCSAHGGESDVWGQGTTVTVSS	333
	VL	EIVLTQSPGTLSLSPGERATLSCRASQSIGSSSLAWYQQKPGQA PRLLMYGASSRASGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC QQYAGSPPFTFGQGTKVEIK	374
	scFv	EVQLVESGGGLVQPGGSLRLSCAVSGFALSNHGMSWVRRAPG KGLEWVSGIVYSGSTYYAASVKGRFTISRDNSRNTLYLQMNSLR PEDTAIYYCSAHGGESDVWGQGTTVTVSSASGGGGSGGRASG GGGSEIVLTQSPGTLSLSPGERATLSCRASQSIGSSSLAWYQQK PGQAPRLLMYGASSRASGIPDRFSGSGSGTDFTLTISRLEPEDF AVYYCQQYAGSPPFTFGQGTKVEIK	375

TABLE 17A
BCMA Binders – Variable domain and scFv sequences

Antibody	Domain	Sequence	SEQ ID NO.
BCMA-16	VH	QVQLQESGPGLVKPSETLSLTCTVSGGSISSSYYYWGWIRQPPG KGLEWIGSIYYSGSAYYNPSLKSRVTISVDTSKNQFSLRLSSVTA ADTAVYYCARHWQEWPDAFDIWGQGTMVTVSS	376
	VL	ETTLTQSPAFMSATPGDKVIISCKASQDIDDAMNWYQQKPGEAP LFIIQSATSPVPGIPPRFSGSGFGTDFSLTINNIESEDAAYYFCLQH DNFPLTFGQGTKLEIK	377
	scFv	QVQLQESGPGLVKPSETLSLTCTVSGGSISSSYYYWGWIRQPPG KGLEWIGSIYYSGSAYYNPSLKSRVTISVDTSKNQFSLRLSSVTA ADTAVYYCARHWQEWPDAFDIWGQGTMVTVSSGGGGSGGGG SGGGGSETTLTQSPAFMSATPGDKVIISCKASQDIDDAMNWYQQ KPGEAPLFIIQSATSPVPGIPPRFSGSGFGTDFSLTINNIESEDAAY YFCLQHDNFPLTFGQGTKLEIK	378
BCMA-17	VH	QVNLRESGPALVKPTQTLTLTCTFSGFSLRTSGMCVSWIRQPPG KALEWLARIDWDEDKFYSTSLKTRLTISKDTSDNQVVLRMTNMD PADTATYYCARSGAGGTSATAFDIWGPGTMVTVSS	379
	VL	DIQMTQSPSSLSASVGDRVTITCRASQDIYNNLAWFQLKPGSAP RSLMYAANKSQSGVPSRFSGSASGTDFTLTISSLQPEDFATYYC QHYYRFPYSFGQGTKLEIK	380
	scFv	VNLRESGPALVKPTQTLTLTCTFSGFSLRTSGMCVSWIRQPPGK ALEWLARIDWDEDKFYSTSLKTRLTISKDTSDNQVVLRMTNMDP ADTATYYCARSGAGGTSATAFDIWGPGTMVTVSSGGGGSGGG GSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQDIYNNLAWFQ LKPGSAPRSLMYAANKSQSGVPSRFSGSASGTDFTLTISSLQPE DFATYYCQHYYRFPYSFGQGTKLEIK	381
BCMA-18	VH	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGK GLEWVSSISSSSSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRA EDTAVYYCAKTIAAVYAFDIWGQGTTVTVSS	382
	VL	EIVLTQSPLSLPVTPEEPASISCRSSQSLLHSNGYNYLDWYLQKP GQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVG VYYCMQALQTPYTFGQGTKLEIK	383
	scFv	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGK GLEWVSSISSSSSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRA EDTAVYYCAKTIAAVYAFDIWGQGTTVTVSSGGGGSGGGGSGG GGSEIVLTQSPLSLPVTPEEPASISCRSSQSLLHSNGYNYLDWYL QKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAE DVGVYYCMQALQTPYTFGQGTKLEIK	384
BCMA-19	VH	EVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGK GLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRA EDTAVYYCARDLRGAFDIWGQGTMVTVSS	385
	VL	SYVLTQSPSVSAAPGYTATISCGGNNIGTKSVHWYQQKPGQAPL LVIRDDSVRPSKIPGRFSGSNSGNMATLTISGVQAGDEADFYCQ VWDSDSEHVVFGGGTKLTVL	386
	scFv	EVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGK GLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRA EDTAVYYCARDLRGAFDIWGQGTMVTVSSGGGGSGGGGSGGG GSSYVLTQSPSVSAAPGYTATISCGGNNIGTKSVHWYQQKPGQ APLLVIRDDSVRPSKIPGRFSGSNSGNMATLTISGVQAGDEADFY CQVWDSDSEHVVFGGGTKLTVL	387
BCMA-20	VH	QVQLVQSGAEVKKPGASVKVSCKPSGYTVTSHYIHWVRRAPGQ GLEWMGMINPSGGVTAYSQTLQGRVTMTSDTSSSTVYMELSSL RSEDTAMYYCAREGSGSGWYFDFWGRGTLVTVSS	388
	VL	SYVLTQPPSVSVSPGQTASITCSGDGLSKKYVSWYQQKAGQSP VVLISRDKERPSGIPDRFSGSNSADTATLTISGTQAMDEADYYCQ AWDDTTVVFGGGTKLTVL	389

TABLE 17A
BCMA Binders – Variable domain and scFv sequences

Antibody	Domain	Sequence	SEQ ID NO.
	scFv	QVQLVQSGAEVKKPGASVKVSCKPSGYTVTSHYIHWVRRAPGQ GLEWMGMINPSGGVTAYSQTLQGRVTMTSDTSSSTVYMELSSL RSEDTAMYYCAREGSGSGWYFDFWGRGTLVTVSSGGGGSGG GGSGGGGSSYVLTQPPSVSVSPGQTASITCSGDGLSKKYVSWY QQKAGQSPVVLISRDKERPSGIPDRFSGSNSADTATLTISGTQA MDEADYYCQAWDDTTVVFGGGTKLTVL	390
BCMA-21	VH	QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGGYYWSWIRQHP GKGLEWIGYIYYSGSTYYNPSLKSRVTISVDTSKNQFSLKLSSVT AADTAVYYCARAGIAARLRGAFDIWGQGTMVTVSS	391
	VL	DIVMTQSPSSVSASVGDRVIITCRASQGIRNWLAWYQQKPGKAP NLLIYAASNLQSGVPSRFSGSGSGADFTLTISSLQPEDVATYYCQ KYNSAPFTFGPGTKVDIK	392
	scFv	QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGGYYWSWIRQHP GKGLEWIGYIYYSGSTYYNPSLKSRVTISVDTSKNQFSLKLSSVT AADTAVYYCARAGIAARLRGAFDIWGQGTMVTVSSGGGGSGGG GSGGGGSDIVMTQSPSSVSASVGDRVIITCRASQGIRNWLAWY QQKPGKAPNLLIYAASNLQSGVPSRFSGSGSGADFTLTISSLQPE DVATYYCQKYNSAPFTFGPGTKVDIK	393
BCMA-22	VH	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQ GLEWMGGIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRS EDTAVYYCARRGGYQLLRWDVGLLRSAFDIWGQGTMVTVSS	394
	VL	SYVLTQPPSVSVAPGQTARITCGGNNIGSKSVHWYQQKPGQAP VLVLYGKNNRPSGVPDRFSGSRSGTTASLTITGAQAEDEADYYC SSRDSSGDHLRVFGTGTKVTVL	395
	scFv	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQ GLEWMGGIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRS EDTAVYYCARRGGYQLLRWDVGLLRSAFDIWGQGTMVTVSSG GGGSGGGGSGGGGSSYVLTQPPSVSVAPGQTARITCGGNNIGS KSVHWYQQKPGQAPVLVLYGKNNRPSGVPDRFSGSRSGTTASL TITGAQAEDEADYYCSSRDSSGDHLRVFGTGTKVTVL	396
BCMA-23	VH	EVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWIRQSPS RGLEWLGRTYYRSKWYSFYAISLKSRIIINPDTSKNQFSLQLKSVT PEDTAVYYCARSSPEGLFLYWFDPWGQGTLVTVSS	397
	VL	SSELTQDPAVSVALGQTIRITCQGDSLGNYYATWYQQKPGQAPV LVIYGTNNRPSGIPDRFSASSSGNTASLTITGAQAEDEADYYCNS RDSSGHHLLFGTGTKVTVL	398
	ScFv	EVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWIRQSPS RGLEWLGRTYYRSKWYSFYAISLKSRIIINPDTSKNQFSLQLKSVT PEDTAVYYCARSSPEGLFLYWFDPWGQGTLVTVSSGGDGSGG GGSGGGGSSSELTQDPAVSVALGQTIRITCQGDSLGNYYATWY QQKPGQAPVLVIYGTNNRPSGIPDRFSASSSGNTASLTITGAQAE DEADYYCNSRDSSGHHLLFGTGTKVTVL	399
BCMA-24	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPG KGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSL RAEDTAVYYCAKVEGSGSLDYWGQGTLVTVSS	400
	VL	EIVMTQSPGTLSLSPGERATLSCRASQSVSSAYLAWYQQKPGQ PPRLLISGASTRATGIPDRFGGSGSGTDFTLTISRLEPEDFAVYYC QHYGSSFNGSSLFTFGQGTRLEIK	401
	scFv	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPG KGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSL RAEDTAVYYCAKVEGSGSLDYWGQGTLVTVSSGGGGSGGGGS GGGGSEIVMTQSPGTLSLSPGERATLSCRASQSVSSAYLAWYQ QKPGQPPRLLISGASTRATGIPDRFGGSGSGTDFTLTISRLEPED FAVYYCQHYGSSFNGSSLFTFGQGTRLEIK	402

TABLE 17A
BCMA Binders – Variable domain and scFv sequences

Antibody	Domain	Sequence	SEQ ID NO.
	VH	EVQLVETGGGLVQPGGSLRLSCAASGITFSRYPMSWVRQAPGK GLEWVSGISDSGVSTYYADSAKGRFTISRDNSKNTLFLQMSSLR DEDTAVYYCVTRAGSEASDIWGQGTMVTVSS	403
BCMA-25	VL	EIVLTQSPATLSLSPGERATLSCRASQSVSNSLAWYQQKPGQAP RLLIYDASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAIYYCQQ FGTSSGLTFGGGTKLEIK	404
	scFv	EVQLVETGGGLVQPGGSLRLSCAASGITFSRYPMSWVRQAPGK GLEWVSGISDSGVSTYYADSAKGRFTISRDNSKNTLFLQMSSLR DEDTAVYYCVTRAGSEASDIWGQGTMVTVSSGGGGSGGGGSG GGGSEIVLTQSPATLSLSPGERATLSCRASQSVSNSLAWYQQKP GQAPRLLIYDASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAIY YCQQFGTSSGLTFGGGTKLEIK	405
BCMA-26	VH	QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPG KGLEWVSAISGSGGSTYYADSVKGRFTISRDNAKNSLYLQMNSL RAEDTAIYYCARATYKRELRYYYGMDVWGQGTMVTVSS	406
	VL	EIVMTQSPGTVSLSPGERATLSCRASQSVSSSFLAWYQQKPGQ APRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDSAVYYC QQYHSSPSWTFGQGTRLEIK	407
	scFv	QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPG KGLEWVSAISGSGGSTYYADSVKGRFTISRDNAKNSLYLQMNSL RAEDTAIYYCARATYKRELRYYYGMDVWGQGTMVTVSSGGGG SGGGGSGGGGSEIVMTQSPGTVSLSPGERATLSCRASQSVSSS FLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTIS RLEPEDSAVYYCQQYHSSPSWTFGQGTRLEIK	408
BCMA-27	VH	EVQLVETGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGK GLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNTLK AEDTAVYYCARATYKRELRYYYGMDVWGQGTTVTVSS	409
	VL	EIVLTQSPSTLSLSPGESATLSCRASQSVSTTFLAWYQQKPGQA PRLLIYGSSNRATGIPDRFSGSGSGTDFTLTIRRLEPEDFAVYYC QQYHSSPSWTFGQGTKVEIK	410
	scFv	EVQLVETGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGK GLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNTLK AEDTAVYYCARATYKRELRYYYGMDVWGQGTTVTVSSGGGGS GGGGSGGGGSEIVLTQSPSTLSLSPGESATLSCRASQSVSTTFL AWYQQKPGQAPRLLIYGSSNRATGIPDRFSGSGSGTDFTLTIRR LEPEDFAVYYCQQYHSSPSWTFGQGTKVEIK	411
BCMA-28	VH	EVQLVETGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPG KGLEWVSGISWNSGSIGYADSVKGRFTISRDNAKNSLYLQMNSL RDEDTAVYYCARVGKAVPDVWGQGTTVTVSS	412
	VL	DIVMTQTPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAP KLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ QSYSTPYSFGQGTRLEIK	413
	scFv	EVQLVETGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPG KGLEWVSGISWNSGSIGYADSVKGRFTISRDNAKNSLYLQMNSL RDEDTAVYYCARVGKAVPDVWGQGTTVTVSSGGGGSGGGGS GGGGSDIVMTQTPSSLSASVGDRVTITCRASQSISSYLNWYQQK PGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFA TYYCQQSYSTPYSFGQGTRLEIK	414
BCMA-29	VH	EVQLVESGGGLVQPGRSLRLSCTASGFTFDDYAMHWVRQRPG KGLEWVASINWKGNSLAYGDSVKGRFAISRDNAKNTVFLQMNSL RTEDTAVYYCASHQGVAYYNYAMDVWGRGTLVTVSS	415
	VL	EIVLTQSPGTLSLSPGERATLSCRATQSIGSSFLAWYQQRPGQA PRLLIYGASQRATGIPDRFSGRGSGTDFTLTISRVEPEDSAVYYC QHYESSPSWTFGQGTKVEIK	416

TABLE 17A
BCMA Binders – Variable domain and scFv sequences

Antibody	Domain	Sequence	SEQ ID NO.
	scFv	EVQLVESGGGLVQPGRSLRLSCTASGFTFDDYAMHWVRQRPG KGLEWVASINWKGNSLAYGDSVKGRFAISRDNAKNTVFLQMNSL RTEDTAVYYCASHQGVAYYNYAMDVWGRGTLVTVSSGGGGSG GGGSGGGGSEIVLTQSPGTLSLSPGERATLSCRATQSIGSSFLA WYQQRPGQAPRLLIYGASQRATGIPDRFSGRGSGTDFTLTISRV EPEDSAVYYCQHYESSPSWTFGQGTKVEIK	417
BCMA-30	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPG KGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSL RAEDTAVYYCAKVVRDGMDVWGQGTTVTVSS	418
	VL	EIVLTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQA PRLLIYGASSRATGIPDRFSGNGSGTDFTLTISRLEPEDFAVYYC QQYGSPPRFTFGPGTKVDIK	419
	scFv	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPG KGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSL RAEDTAVYYCAKVVRDGMDVWGQGTTVTVSSGGGGSGGGGS GGGGSEIVLTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQ KPGQAPRLLIYGASSRATGIPDRFSGNGSGTDFTLTISRLEPEDF AVYYCQQYGSPPRFTFGPGTKVDIK	420
BCMA-31	VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGK GLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLR AEDTAVYYCAKIPQTGTFDYWGQGTLVTVSS	421
	VL	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQRPGQA PRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC QHYGSSPSWTFGQGTRLEIK	422
	scFv	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGK GLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLR AEDTAVYYCAKIPQTGTFDYWGQGTLVTVSSGGGGSGGGGSG GGGSEIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQR PGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFA VYYCQHYGSSPSWTFGQGTRLEIK	423
BCMA-32	VH	EVQLVETGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGK GLEWVSAISGSGGSTYYADSVKGRFTMSRENDKNSVFLQMNSL RVEDTGVYYCARANYKRELRYYYGMDVWGQGTMVTVSS	424
	VL	EIVMTQSPGTLSLSPGESATLSCRASQRVASNYLAWYQHKPGQ APSLLISGASSRATGVPDRFSGSGSGTDFTLAISRLEPEDSAVYY CQHYDSSPSWTFGQGTKVEIK	425
	scFv	EVQLVETGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGK GLEWVSAISGSGGSTYYADSVKGRFTMSRENDKNSVFLQMNSL RVEDTGVYYCARANYKRELRYYYGMDVWGQGTMVTVSSGGG GSGGGGSGGGGSEIVMTQSPGTLSLSPGESATLSCRASQRVAS NYLAWYQHKPGQAPSLLISGASSRATGVPDRFSGSGSGTDFTLA ISRLEPEDSAVYYCQHYDSSPSWTFGQGTKVEIK	426
BCMA-33	VH	EVQLLETGGGLVQPGGSLRLSCAASGFSFSSYAMSWVRQAPGK GLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLR AEDTAVYYCAKALVGATGAFDIWGQGTLVTVSS	427
	VL	EIVLTQSPGTLSLSPGERATLSCRASQSLSSNFLAWYQQKPGQA PGLLIYGASNWATGTPDRFSGSGSGTDFTLTITRLEPEDFAVYYC QYYGTSPMYTFGQGTKVEIK	428
	scFv	EVQLLETGGGLVQPGGSLRLSCAASGFSFSSYAMSWVRQAPGK GLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLR AEDTAVYYCAKALVGATGAFDIWGQGTLVTVSSGGGGSGGGGS GGGGSEIVLTQSPGTLSLSPGERATLSCRASQSLSSNFLAWYQQ KPGQAPGLLIYGASNWATGTPDRFSGSGSGTDFTLTITRLEPED FAVYYCQYYGTSPMYTFGQGTKVEIK	429

TABLE 17A
BCMA Binders – Variable domain and scFv sequences

Antibody	Domain	Sequence	SEQ ID NO.
BCMA-34	VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGK GLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLR AEDTAVYYCVLWFGEGFDPWGQGTLVTVSS	430
	VL	DIVLTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQKP GQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVG VYYCMQALQTPLTFGGGTKVDIK	431
	scFv	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGK GLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLR AEDTAVYYCVLWFGEGFDPWGQGTLVTVSSGGGGSGGGGSG GGGSDIVLTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDW YLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVE AEDVGVYYCMQALQTPLTFGGGTKVDIK	432
BCMA-35	VH	QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPG KGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSL RAEDTAVYYCAKVGYDSSGYYRDYYGMDVWGQGTTVTVSS	433
	VL	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQA PRLLIYGTSSRATGISDRFSGSGSGTDFTLTISRLEPEDFAVYYCQ HYGNSPPKFTFGPGTKLEIK	434
	scFv	QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPG KGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSL RAEDTAVYYCAKVGYDSSGYYRDYYGMDVWGQGTTVTVSSGG GGSGGGGSGGGGSEIVLTQSPGTLSLSPGERATLSCRASQSVS SSYLAWYQQKPGQAPRLLIYGTSSRATGISDRFSGSGSGTDFTL TISRLEPEDFAVYYCQHYGNSPPKFTFGPGTKLEIK	435
BCMA-36	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPG KGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSL RAEDTAVYYCAKMGWSSGYLGAFDIWGQGTTVTVSS	436
	VL	EIVLTQSPGTLSLSPGERATLSCRASQSVASSFLAWYQQKPGQA PRLLIYGASGRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC QHYGGSPRLTFGGGTKVDIK	437
	scFv	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPG KGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSL RAEDTAVYYCAKMGWSSGYLGAFDIWGQGTTVTVSSGGGGSG GGGSGGGGSEIVLTQSPGTLSLSPGERATLSCRASQSVASSFLA WYQQKPGQAPRLLIYGASGRATGIPDRFSGSGSGTDFTLTISRL EPEDFAVYYCQHYGGSPRLTFGGGTKVDIK	438
BCMA-37	VH	QIQLVQSGPDLKKPGETVKLSCKASGYTFTNFGMNWVKQAPGK GFKWMAWINTYTGESYFADDFKGRFAFSVETSATTAYLQINNLK TEDTATYFCARGEIYYGYDGGFAYWGQGTLVTVSA	439
	VL	DVVMTQSHRFMSTSVGDRVSITCRASQDVNTAVSWYQQKPGQ SPKLLIFSASYRYTGVPDRFTGSGSGADFTLTISSVQAEDLAVYY CQQHYSTPWTFGGGTKLDIK	440
	scFv	QIQLVQSGPDLKKPGETVKLSCKASGYTFTNFGMNWVKQAPGK GFKWMAWINTYTGESYFADDFKGRFAFSVETSATTAYLQINNLK TEDTATYFCARGEIYYGYDGGFAYWGQGTLVTVSAGGGGSGG GGSGGGGSDVVMTQSHRFMSTSVGDRVSITCRASQDVNTAVS WYQQKPGQSPKLLIFSASYRYTGVPDRFTGSGSGADFTLTISSV QAEDLAVYYCQQHYSTPWTFGGGTKLDIK	441
BCMA-38	VH	QIQLVQSGPELKKPGETVKISCKASGYTFTDYSINWVKRAPGKGL KWMGWINTETREPAYAYDFRGRFAFSLETSASTAYLQINNLKYE DTATYFCALDYSYAMDYWGQGTSVTVSS	442

TABLE 17A
BCMA Binders – Variable domain and scFv sequences

Antibody	Domain	Sequence	SEQ ID NO.
	VL	DIVLTQSPASLAMSLGKRATISCRASESVSVIGAHLIHWYQQKPG QPPKLLIYLASNLETGVPARFSGSGSGTDFTLTIDPVEEDDVAIYS CLQSRIFPRTFGGGTKLEIK	443
	scFv	QIQLVQSGPELKKPGETVKISCKASGYTFTDYSINWVKRAPGKGL KWMGWINTETREPAYAYDFRGRFAFSLETSASTAYLQINNLKYE DTATYFCALDYSYAMDYWGQGTSVTVSSGGGGSGGGGSGGG GSQIQLVQSGPELKKPGETVKISCKASGYTFTDYSINWVKRAPG KGLKWMGWINTETREPAYAYDFRGRFAFSLETSASTAYLQINNL KYEDTATYFCALDYSYAMDYWGQGTSVTVSS	444
BCMA-39	VH	QIQLVQSGPELKKPGETVKISCKASGYTFRHYSMNWVKQAPGK GLKWMGRINTESGVPIYADDFKGRFAFSVETSASTAYLVINNLKD EDTASYFCSNDYLYSLDFWGQGTALTVSS	445
	VL	DIVLTQSPPSLAMSLGKRATISCRASESVTILGSHLIYWYQQKPG QPPTLLIQLASNVQTGVPARFSGSGSRTDFTLTIDPVEEDDVAVY YCLQSRTIPRTFGGGTKLEIK	446
	scFv	QIQLVQSGPELKKPGETVKISCKASGYTFRHYSMNWVKQAPGK GLKWMGRINTESGVPIYADDFKGRFAFSVETSASTAYLVINNLKD EDTASYFCSNDYLYSLDFWGQGTALTVSSGGGGSGGGGSGGG GSDIVLTQSPPSLAMSLGKRATISCRASESVTILGSHLIYWYQQK PGQPPTLLIQLASNVQTGVPARFSGSGSRTDFTLTIDPVEEDDVA VYYCLQSRTIPRTFGGGTKLEIK	447
BCMA-40	VH	QIQLVQSGPELKKPGETVKISCKASGYTFTHYSMNWVKQAPGKG LKWMGRINTETGEPLYADDFKGRFAFSLETSASTAYLVINNLKNE DTATFFCSNDYLYSCDYWGQGTTLTVSS	448
	VL	DIVLTQSPPSLAMSLGKRATISCRASESVTILGSHLIYWYQQKPG QPPTLLIQLASNVQTGVPARFSGSGSRTDFTLTIDPVEEDDVAVY YCLQSRTIPRTFGGGTKLEIK	446
	scFv	QIQLVQSGPELKKPGETVKISCKASGYTFTHYSMNWVKQAPGKG LKWMGRINTETGEPLYADDFKGRFAFSLETSASTAYLVINNLKNE DTATFFCSNDYLYSCDYWGQGTTLTVSSGGGGSGGGGSGGGG SDIVLTQSPPSLAMSLGKRATISCRASESVTILGSHLIYWYQQKP GQPPTLLIQLASNVQTGVPARFSGSGSRTDFTLTIDPVEEDDVAV YYCLQSRTIPRTFGGGTKLEIK	449

TABLE 17B
BCMA Binders – Light chain CDR sequences according to Kabat numbering scheme

Antibody	CDR-L1	SEQ ID NO:	CDR-L2	SEQ ID NO:	CDR-L3	SEQ ID NO:
BCMA-1	RASQSISSYLN	450	AASSLQS	482	QQSYSTPYT	511
BCMA-2	RASQSISSSFLA	451	GASRRAT	483	QQYHSSPSWT	512

TABLE 17B
BCMA Binders – Light chain CDR sequences according to Kabat numbering scheme

Antibody	CDR-L1	SEQ ID NO:	CDR-L2	SEQ ID NO:	CDR-L3	SEQ ID NO:
BCMA-3	RSSQSLLHSNGYNYLD	452	LGSNRAS	484	MQALQTPYT	513
BCMA-4	KSSQSLLRNDGKTPLY	453	EVSNRFS	485	MQNIQFPS	514
BCMA-5	RSSQSLLHSNGYNYLN	454	LGSKRAS	486	MQALQTPYT	513
BCMA-6	RASQSISSYLN	450	GASTLAS	487	QQSYKRAS	515
BCMA-7	RSSQSLLYSNGYNYVD	455	LGSNRAS	484	MQGRQFPYS	516
BCMA-8	RASQSVSSNLA	456	GASTRAS	488	QQYGSSLT	517
BCMA-9	RASQSVSSKLA	457	GASIRAT	489	QQYGSSSWT	518
BCMA-10	RASQSVGSTNLA	458	DASNRAT	221	QQYGSSPPWT	519
BCMA-11	RASQSISSYLN	450	AASSLQS	482	QQSYTLA	520
BCMA-12	KSSESLVHNSGKTYLN	459	EVSNRDS	490	MQGTHWPGT	521
BCMA-13	QASEDINKFLN	460	DASTLQT	491	QQYESLPLT	522
BCMA-14	RASQSVGSNLA	461	GASTRAT	492	QQYNDWLPVT	523
BCMA-15	RASQSIGSSSLA	462	GASSRAS	493	QQYAGSPPFT	524
BCMA-16	KASQDIDDAMN	463	SATSPVP	494	LQHDNFPLT	525
BCMA-17	RASQDIYNNLA	464	AANKSQS	495	QHYYRFPYS	526
BCMA-18	RSSQSLLHSNGYNYLD	452	LGSNRAS	484	MQALQTPYT	513
BCMA-19	GGNNIGTKSVH	465	DDSVRPS	496	QVWDSDSEHVV	527
BCMA-20	SGDGLSKKYVS	466	RDKERPS	497	QAWDDTTVV	528
BCMA-21	RASQGIRNWLA	467	AASNLQS	498	QKYNSAPFT	529
BCMA-22	GGNNIGSKSVH	468	GKNNRPS	499	SSRDSSGDHLRV	530
BCMA-23	QGDSLGNYYAT	469	GTNNRPS	500	NSRDSSGHHLL	531
BCMA-24	RASQSVSSAYLA	470	GASTRAT	492	QHYGSSFNGSSLFT	532
BCMA-25	RASQSVSNSLA	471	DASSRAT	501	QQFGTSSGLT	533
BCMA-26	RASQSVSSSFLA	472	GASSRAT	502	QQYHSSPSWT	512
BCMA-27	RASQSVSTTFLA	473	GSSNRAT	503	QQYHSSPSWT	512
BCMA-28	RASQSISSYLN	450	AASSLQS	482	QQSYSTPYS	534
BCMA-29	RATQSIGSSFLA	474	GASQRAT	504	QHYESSPSWT	535
BCMA-30	RASQSVSSSYLA	475	GASSRAT	502	QQYGSPPRFT	536
BCMA-31	RASQSVSSSYLA	475	GASSRAT	502	QHYGSSPSWT	537
BCMA-32	RASQRVASNYLA	476	GASSRAT	502	QHYDSSPSWT	538
BCMA-33	RASQSLSSNFLA	477	GASNWAT	505	QYYGTSPMYT	539
BCMA-34	RSSQSLLHSNGYNYLD	452	LGSNRAS	484	MQALQTPLT	540
BCMA-35	RASQSVSSSYLA	475	GTSSRAT	506	QHYGNSPPKFT	541
BCMA-36	RASQSVASSFLA	478	GASGRAT	507	QHYGGSPRLT	542

TABLE 17B BCMA Binders – Light chain CDR sequences according to Kabat numbering scheme						
Antibody	**CDR-L1**	**SEQ ID NO:**	**CDR-L2**	**SEQ ID NO:**	**CDR-L3**	**SEQ ID NO:**
BCMA-37	RASQDVNTAVS	479	SASYRYT	508	QQHYSTPWT	543
BCMA-38	RASESVSVIGAHLIH	480	LASNLET	509	LQSRIFPRT	544
BCMA-39	RASESVTILGSHLIY	481	LASNVQT	510	LQSRTIPRT	545
BCMA-40	RASESVTILGSHLIY	481	LASNVQT	510	LQSRTIPRT	545

TABLE 17C BCMA Binders – Light chain CDR sequences according to Chothia numbering scheme						
Antibody	**CDR-L1**	**SEQ ID NO:**	**CDR-L2**	**SEQ ID NO:**	**CDR-L3**	**SEQ ID NO:**
BCMA-1	SQSISSY	546	AAS	577	SYSTPY	590
BCMA-2	SQSISSSF	547	GAS	578	YHSSPSW	591
BCMA-3	SQSLLHSNGYNY	548	LGS	579	ALQTPY	592
BCMA-4	SQSLLRNDGKTP	549	EVS	580	NIQFP	593
BCMA-5	SQSLLHSNGYNY	548	LGS	579	ALQTPY	592
BCMA-6	SQSISSY	546	GAS	578	SYKRA	594
BCMA-7	SQSLLYSNGYNY	550	LGS	579	GRQFPY	595
BCMA-8	SQSVSSN	551	GAS	578	YGSSL	596
BCMA-9	SQSVSSK	552	GAS	578	YGSSSW	597
BCMA-10	SQSVGSTN	553	DAS	281	YGSSPPW	598
BCMA-11	SQSISSY	546	AAS	577	SYTL	599
BCMA-12	SESLVHNSGKTY	554	EVS	580	GTHWPG	600
BCMA-13	SEDINKF	555	DAS	281	YESLPL	601
BCMA-14	SQSVGSN	556	GAS	578	YNDWLPV	602
BCMA-15	SQSIGSSS	557	GAS	578	YAGSPPF	603
BCMA-16	SQDIDDA	558	SAT	581	HDNFPL	604
BCMA-17	SQDIYNN	559	AAN	582	YYRFPY	605
BCMA-18	SQSLLHSNGYNY	548	LGS	579	ALQTPY	592
BCMA-19	NNIGTKS	560	DDS	583	WDSDSEHV	606
BCMA-20	DGLSKKY	561	RDK	584	WDDTTV	607

TABLE 17C
BCMA Binders – Light chain CDR sequences according to Chothia numbering scheme

Antibody	CDR-L1	SEQ ID NO:	CDR-L2	SEQ ID NO:	CDR-L3	SEQ ID NO:
BCMA-21	SQGIRNW	562	AAS	577	YNSAPF	608
BCMA-22	NNIGSKS	563	GKN	585	RDSSGDHLR	609
BCMA-23	DSLGNYY	564	GTN	277	RDSSGHHL	610
BCMA-24	SQSVSSAY	565	GAS	578	YGSSFNGSSLF	611
BCMA-25	SQSVSNS	566	DAS	281	FGTSSGL	612
BCMA-26	SQSVSSSF	567	GAS	578	YHSSPSW	591
BCMA-27	SQSVSTTF	568	GSS	586	YHSSPSW	591
BCMA-28	SQSISSY	546	AAS	577	SYSTPY	590
BCMA-29	TQSIGSSF	569	GAS	578	YESSPSW	613
BCMA-30	SQSVSSSY	570	GAS	578	YGSPPRF	614
BCMA-31	SQSVSSSY	570	GAS	578	YGSSPSW	615
BCMA-32	SQRVASNY	571	GAS	578	YDSSPSW	616
BCMA-33	SQSLSSNF	572	GAS	578	YGTSPMY	617
BCMA-34	SQSLLHSNGYNY	548	LGS	579	ALQTPL	618
BCMA-35	SQSVSSSY	570	GTS	587	YGNSPPKF	619
BCMA-36	SQSVASSF	573	GAS	578	YGGSPRL	620
BCMA-37	SQDVNTA	574	SAS	588	HYSTPW	621
BCMA-38	SESVSVIGAHL	575	LAS	589	SRIFPR	622
BCMA-39	SESVTILGSHL	576	LAS	589	SRTIPR	623
BCMA-40	SESVTILGSHL	576	LAS	589	SRTIPR	623

TABLE 17D
BCMA Binders – Light chain CDR sequences according to combination of Kabat and Chothia numbering schemes

Antibody	CDR-L1	SEQ ID NO:	CDR-L2	SEQ ID NO:	CDR-L3	SEQ ID NO:
BCMA-1	RASQSISSYLN	450	AASSLQS	482	QQSYSTPYT	511
BCMA-2	RASQSISSSFLA	451	GASRRAT	483	QQYHSSPSWT	512

TABLE 17D
BCMA Binders – Light chain CDR sequences according to combination of Kabat and Chothia numbering schemes

Antibody	CDR-L1	SEQ ID NO:	CDR-L2	SEQ ID NO:	CDR-L3	SEQ ID NO:
BCMA-3	RSSQSLLHSNGYNYLD	452	LGSNRAS	484	MQALQTPYT	513
BCMA-4	KSSQSLLRNDGKTPLY	453	EVSNRFS	485	MQNIQFPS	514
BCMA-5	RSSQSLLHSNGYNYLN	454	LGSKRAS	486	MQALQTPYT	513
BCMA-6	RASQSISSYLN	450	GASTLAS	487	QQSYKRAS	515
BCMA-7	RSSQSLLYSNGYNYVD	455	LGSNRAS	484	MQGRQFPYS	516
BCMA-8	RASQSVSSNLA	456	GASTRAS	488	QQYGSSLT	517
BCMA-9	RASQSVSSKLA	457	GASIRAT	489	QQYGSSSWT	518
BCMA-10	RASQSVGSTNLA	458	DASNRAT	221	QQYGSSPPWT	519
BCMA-11	RASQSISSYLN	450	AASSLQS	482	QQSYTLA	520
BCMA-12	KSSESLVHNSGKTYLN	459	EVSNRDS	490	MQGTHWPGT	521
BCMA-13	QASEDINKFLN	460	DASTLQT	491	QQYESLPLT	522
BCMA-14	RASQSVGSNLA	461	GASTRAT	492	QQYNDWLPVT	523
BCMA-15	RASQSIGSSSLA	462	GASSRAS	493	QQYAGSPPFT	524
BCMA-16	KASQDIDDAMN	463	SATSPVP	494	LQHDNFPLT	525
BCMA-17	RASQDIYNNLA	464	AANKSQS	495	QHYYRFPYS	526
BCMA-18	RSSQSLLHSNGYNYLD	452	LGSNRAS	484	MQALQTPYT	513
BCMA-19	GGNNIGTKSVH	465	DDSVRPS	496	QVWDSDSEHVV	527
BCMA-20	SGDGLSKKYVS	466	RDKERPS	497	QAWDDTTVV	528
BCMA-21	RASQGIRNWLA	467	AASNLQS	498	QKYNSAPFT	529
BCMA-22	GGNNIGSKSVH	468	GKNNRPS	499	SSRDSSGDHLRV	530
BCMA-23	QGDSLGNYYAT	469	GTNNRPS	500	NSRDSSGHHLL	531
BCMA-24	RASQSVSSAYLA	470	GASTRAT	492	QHYGSSFNGSSLFT	532
BCMA-25	RASQSVSNSLA	471	DASSRAT	501	QQFGTSSGLT	533
BCMA-26	RASQSVSSSFLA	472	GASSRAT	502	QQYHSSPSWT	512
BCMA-27	RASQSVSTTFLA	473	GSSNRAT	503	QQYHSSPSWT	512
BCMA-28	RASQSISSYLN	450	AASSLQS	482	QQSYSTPYS	534
BCMA-29	RATQSIGSSFLA	474	GASQRAT	504	QHYESSPSWT	535
BCMA-30	RASQSVSSSYLA	475	GASSRAT	502	QQYGSPPRFT	536

TABLE 17D
BCMA Binders – Light chain CDR sequences according to combination of Kabat and Chothia numbering schemes

Antibody	CDR-L1	SEQ ID NO:	CDR-L2	SEQ ID NO:	CDR-L3	SEQ ID NO:
BCMA-31	RASQSVSSSYLA	475	GASSRAT	502	QHYGSSPSWT	537
BCMA-32	RASQRVASNYLA	476	GASSRAT	502	QHYDSSPSWT	538
BCMA-33	RASQSLSSNFLA	477	GASNWAT	505	QYYGTSPMYT	539
BCMA-34	RSSQSLLHSNGYNYLD	452	LGSNRAS	484	MQALQTPLT	540
BCMA-35	RASQSVSSSYLA	475	GTSSRAT	506	QHYGNSPPKFT	541
BCMA-36	RASQSVASSFLA	478	GASGRAT	507	QHYGGSPRLT	542
BCMA-37	RASQDVNTAVS	479	SASYRYT	508	QQHYSTPWT	543
BCMA-38	RASESVSVIGAHLIH	480	LASNLET	509	LQSRIFPRT	544
BCMA-39	RASESVTILGSHLIY	481	LASNVQT	510	LQSRTIPRT	545
BCMA-40	RASESVTILGSHLIY	481	LASNVQT	510	LQSRTIPRT	545

TABLE 17E
BCMA Binders – Heavy chain CDR sequences according to Kabat numbering scheme

Antibody	CDR-H1	SEQ ID NO:	CDR-H2	SEQ ID NO:	CDR-H3	SEQ ID NO:
BCMA-1	NHGMS	624	GIVYSGSTYYAASVKG	639	HGGESDV	656
BCMA-2	NYAMS	1073	GISRSGENTYYADSVKG	750	SPAHYYGGMDV	722
BCMA-3	DYAMH	635	GISWNSGSIGYADSVKG	650	HSFLAY	723
BCMA-4	NHGMS	624	GIVYSGSTYYAASVKG	639	HGGESDV	656
BCMA-5	NFGIN	1074	WINPKNNNTNYAQKFQG	751	GPYYYQSYMDV	724
BCMA-6	SDAMT	1075	VISGSGGTTYYADSVKG	752	LDSSGYYYARGPRY	725
BCMA-7	NYGIT	1076	WISAYNGNTNYAQKFQG	753	GPYYYYMDV	726
BCMA-8	NHGMS	624	GIVYSGSTYYAASVKG	639	HGGESDV	656
BCMA-9	NHGMS	624	GIVYSGSTYYAASVKG	639	HGGESDV	656
BCMA-10	NHGMS	624	GIVYSGSTYYAASVKG	639	HGGESDV	656
BCMA-11	DYYMS	628	YISSSGSTIYYADSVKG	643	ESGDGMDV	727

TABLE 17E
BCMA Binders – Heavy chain CDR sequences according to Kabat numbering scheme

Antibody	CDR-H1	SEQ ID NO:	CDR-H2	SEQ ID NO:	CDR-H3	SEQ ID NO:
BCMA-12	DYYMS	628	YISSSGNTIYYADSVKG	754	STMVREDY	728
BCMA-13	NHGMS	624	GIVYSGSTYYAASVKG	639	HGGESDV	656
BCMA-14	NHGMS	624	GIVYSGSTYYAASVKG	639	HGGESDV	656
BCMA-15	NHGMS	624	GIVYSGSTYYAASVKG	639	HGGESDV	656
BCMA-16	SSYYYWG	625	SIYYSGSAYYNPSLKS	640	HWQEWPDAFDI	657
BCMA-17	TSGMCVS	626	RIDWDEDKFYSTSLKT	641	SGAGGTSATAFDI	658
BCMA-18	SYSMN	627	SISSSSSYIYYADSVKG	642	TIAAVYAFDI	659
BCMA-19	DYYMS	628	YISSSGSTIYYADSVKG	643	DLRGAFDI	660
BCMA-20	SHYIH	629	MINPSGGVTAYSQTLQG	644	EGSGSGWYFDF	661
BCMA-21	SGGYYWS	630	YIYYSGSTYYNPSLKS	645	AGIAARLRGAFDI	662
BCMA-22	SYAIS	631	GIIPIFGTANYAQKFQG	646	RGGYQLLRWDVGLLRSAFDI	663
BCMA-23	SNSAAWN	632	RTYYRSKWYSFYAISLKS	647	SSPEGLFLYWFDP	664
BCMA-24	SYAMS	633	AISGSGGSTYYADSVKG	648	VEGSGSLDY	665
BCMA-25	RYPMS	634	GISDSGVSTYYADSAKG	649	RAGSEASDI	666
BCMA-26	SYAMS	633	AISGSGGSTYYADSVKG	648	ATYKRELRYYYGMDV	667
BCMA-27	SYAMS	633	AISGSGGSTYYADSVKG	648	ATYKRELRYYYGMDV	667
BCMA-28	DYAMH	635	GISWNSGSIGYADSVKG	650	VGKAVPDV	668
BCMA-29	DYAMH	635	SINWKGNSLAYGDSVKG	651	HQGVAYYNYAMDV	669
BCMA-30	SYAMS	633	AISGSGGSTYYADSVKG	648	VVRDGMDV	670
BCMA-31	SYAMS	633	AISGSGGSTYYADSVKG	648	IPQTGTFDY	671
BCMA-32	SYAMS	633	AISGSGGSTYYADSVKG	648	ANYKRELRYYYGMDV	672
BCMA-33	SYAMS	633	AISGSGGSTYYADSVKG	648	ALVGATGAFDI	673
BCMA-34	SYAMS	633	AISGSGGSTYYADSVKG	648	WFGEGFDP	674
BCMA-35	SYAMS	633	AISGSGGSTYYADSVKG	648	VGYDSSGYYRDYYGMDV	675

TABLE 17E
BCMA Binders – Heavy chain CDR sequences according to Kabat numbering scheme

Antibody	CDR-H1	SEQ ID NO:	CDR-H2	SEQ ID NO:	CDR-H3	SEQ ID NO:
BCMA-36	SYAMS	633	AISGSGGSTYYADSVKG	648	MGWSSGYLGAFDI	676
BCMA-37	NFGMN	636	WINTYTGESYFADDFKG	652	GEIYYGYDGGFAY	677
BCMA-38	DYSIN	637	WINTETREPAYAYDFRG	653	DYSYAMDY	678
BCMA-39	HYSMN	638	RINTESGVPIYADDFKG	654	DYLYSLDF	679
BCMA-40	HYSMN	638	RINTETGEPLYADDFKG	655	DYLYSCDY	680

TABLE 17F
BCMA Binders – Heavy chain CDR sequences according to Chothia numbering scheme

Antibody	CDR-H1	SEQ ID NO:	CDR-H2	SEQ ID NO:	CDR-H3	SEQ ID NO:
BCMA-1	GFALSNH	681	VYSGS	701	HGGESDV	656
BCMA-2	GFTFSNY	682	SRSGEN	702	SPAHYYGGMDV	722
BCMA-3	GFTFDDY	683	SWNSGS	703	HSFLAY	723
BCMA-4	GFALSNH	681	VYSGS	701	HGGESDV	656
BCMA-5	GYIFDNF	684	NPKNNN	704	GPYYYQSYMDV	724
BCMA-6	GFTFSSD	685	SGSGGT	705	LDSSGYYYARGPRY	725
BCMA-7	GYTFSNY	686	SAYNGN	706	GPYYYYMDV	726
BCMA-8	GFALSNH	681	VYSGS	701	HGGESDV	656
BCMA-9	GFALSNH	681	VYSGS	701	HGGESDV	656
BCMA-10	GFALSNH	681	VYSGS	701	HGGESDV	656
BCMA-11	GFTFSDY	687	SSSGST	707	ESGDGMDV	727
BCMA-12	GFTFSDY	687	SSSGNT	708	STMVREDY	728
BCMA-13	GFALSNH	681	VYSGS	701	HGGESDV	656
BCMA-14	GFALSNH	681	VYSGS	701	HGGESDV	656
BCMA-15	GFALSNH	681	VYSGS	701	HGGESDV	656
BCMA-16	GGSISSSYY	688	YYSGS	709	HWQEWPDAFDI	657

TABLE 17F
BCMA Binders – Heavy chain CDR sequences according to Chothia numbering scheme

Antibody	CDR-H1	SEQ ID NO:	CDR-H2	SEQ ID NO:	CDR-H3	SEQ ID NO:
BCMA-17	GFSLRTSGM	689	DWDED	710	SGAGGTSATAFDI	658
BCMA-18	GFTFSSY	690	SSSSSY	711	TIAAVYAFDI	659
BCMA-19	GFTFSDY	687	SSSGST	707	DLRGAFDI	660
BCMA-20	GYTVTSH	691	NPSGGV	712	EGSGSGWYFDF	661
BCMA-21	GGSISSGGY	692	YYSGS	709	AGIAARLRGAFDI	662
BCMA-22	GGTFSSY	693	IPIFGT	713	RGGYQLLRWDVGLLRSAFDI	663
BCMA-23	GDSVSSNSA	694	YYRSKWY	714	SSPEGLFLYWFDP	664
BCMA-24	GFTFSSY	690	SGSGGS	715	VEGSGSLDY	665
BCMA-25	GITFSRY	695	SDSGVS	716	RAGSEASDI	666
BCMA-26	GFTFSSY	690	SGSGGS	715	ATYKRELRYYYGMDV	667
BCMA-27	GFTFSSY	690	SGSGGS	715	ATYKRELRYYYGMDV	667
BCMA-28	GFTFDDY	683	SWNSGS	703	VGKAVPDV	668
BCMA-29	GFTFDDY	683	NWKGNS	717	HQGVAYYNYAMDV	669
BCMA-30	GFTFSSY	690	SGSGGS	715	VVRDGMDV	670
BCMA-31	GFTFSSY	690	SGSGGS	715	IPQTGTFDY	671
BCMA-32	GFTFSSY	690	SGSGGS	715	ANYKRELRYYYGMDV	672
BCMA-33	GFSFSSY	696	SGSGGS	715	ALVGATGAFDI	673
BCMA-34	GFTFSSY	690	SGSGGS	715	WFGEGFDP	674
BCMA-35	GFTFSSY	690	SGSGGS	715	VGYDSSGYYRDYYGMDV	675
BCMA-36	GFTFSSY	690	SGSGGS	715	MGWSSGYLGAFDI	676
BCMA-37	GYTFTNF	697	NTYTGE	718	GEIYYGYDGGFAY	677
BCMA-38	GYTFTDY	698	NTETRE	719	DYSYAMDY	678
BCMA-39	GYTFRHY	699	NTESGV	720	DYLYSLDF	679
BCMA-40	GYTFTHY	700	NTETGE	721	DYLYSCDY	680

TABLE 17G
BCMA Binders – Heavy chain CDR sequences according to combination of Kabat and Chothia numbering schemes

Antibody	CDR-H1	SEQ ID NO:	CDR-H2	SEQ ID NO:	CDR-H3	SEQ ID NO:
BCMA-1	GFALSNHGMS	729	GIVYSGSTYYAASVKG	639	HGGESDV	656
BCMA-2	GFTFSNYAMS	730	GISRSGENTYYADSVKG	750	SPAHYYGGMDV	722
BCMA-3	GFTFDDYAMH	731	GISWNSGSIGYADSVKG	650	HSFLAY	723
BCMA-4	GFALSNHGMS	729	GIVYSGSTYYAASVKG	639	HGGESDV	656
BCMA-5	GYIFDNFGIN	732	WINPKNNNTNYAQKFQG	751	GPYYYQSYMDV	724
BCMA-6	GFTFSSDAMT	733	VISGSGGTTYYADSVKG	752	LDSSGYYYARGPRY	725
BCMA-7	GYTFSNYGIT	734	WISAYNGNTNYAQKFQG	753	GPYYYYMDV	726
BCMA-8	GFALSNHGMS	729	GIVYSGSTYYAASVKG	639	HGGESDV	656
BCMA-9	GFALSNHGMS	729	GIVYSGSTYYAASVKG	639	HGGESDV	656
BCMA-10	GFALSNHGMS	729	GIVYSGSTYYAASVKG	639	HGGESDV	656
BCMA-11	GFTFSDYYMS	735	YISSSGSTIYYADSVKG	643	ESGDGMDV	727
BCMA-12	GFTFSDYYMS	735	YISSSGNTIYYADSVKG	754	STMVREDY	728
BCMA-13	GFALSNHGMS	729	GIVYSGSTYYAASVKG	639	HGGESDV	656
BCMA-14	GFALSNHGMS	729	GIVYSGSTYYAASVKG	639	HGGESDV	656
BCMA-15	GFALSNHGMS	729	GIVYSGSTYYAASVKG	639	HGGESDV	656
BCMA-16	GGSISSSYYYWG	736	SIYYSGSAYYNPSLKS	640	HWQEWPDAFDI	657

TABLE 17G
BCMA Binders – Heavy chain CDR sequences according to combination of Kabat and Chothia numbering schemes

Antibody	CDR-H1	SEQ ID NO:	CDR-H2	SEQ ID NO:	CDR-H3	SEQ ID NO:
BCMA-17	GFSLRTSGMCVS	737	RIDWDEDKFYSTSLKT	641	SGAGGTSATAFDI	658
BCMA-18	GFTFSSYSMN	738	SISSSSSYIYYADSVKG	642	TIAAVYAFDI	659
BCMA-19	GFTFSDYYMS	735	YISSSGSTIYYADSVKG	643	DLRGAFDI	660
BCMA-20	GYTVTSHYIH	739	MINPSGGVTAYSQTLQG	644	EGSGSGWYFDF	661
BCMA-21	GGSISSGGYYWS	740	YIYYSGSTYYNPSLKS	645	AGIAARLRGAFDI	662
BCMA-22	GGTFSSYAIS	741	GIIPIFGTANYAQKFQG	646	RGGYQLLRWDVGLLRSAFDI	663
BCMA-23	GDSVSSNSAAWN	742	RTYYRSKWYSFYAISLKS	647	SSPEGLFLYWFDP	664
BCMA-24	GFTFSSYAMS	743	AISGSGGSTYYADSVKG	648	VEGSGSLDY	665
BCMA-25	GITFSRYPMS	744	GISDSGVSTYYADSAKG	649	RAGSEASDI	666
BCMA-26	GFTFSSYAMS	743	AISGSGGSTYYADSVKG	648	ATYKRELRYYYGMDV	667
BCMA-27	GFTFSSYAMS	743	AISGSGGSTYYADSVKG	648	ATYKRELRYYYGMDV	667
BCMA-28	GFTFDDYAMH	731	GISWNSGSIGYADSVKG	650	VGKAVPDV	668
BCMA-29	GFTFDDYAMH	731	SINWKGNSLAYGDSVKG	651	HQGVAYYNYAMDV	669
BCMA-30	GFTFSSYAMS	743	AISGSGGSTYYADSVKG	648	VVRDGMDV	670
BCMA-31	GFTFSSYAMS	743	AISGSGGSTYYADSVKG	648	IPQTGTFDY	671
BCMA-32	GFTFSSYAMS	743	AISGSGGSTYYADSVKG	648	ANYKRELRYYYGMDV	672

TABLE 17G
BCMA Binders – Heavy chain CDR sequences according to combination of Kabat and Chothia numbering schemes

Antibody	CDR-H1	SEQ ID NO:	CDR-H2	SEQ ID NO:	CDR-H3	SEQ ID NO:
BCMA-33	GFSFSSYAMS	745	AISGSGGSTYYADSVKG	648	ALVGATGAFDI	673
BCMA-34	GFTFSSYAMS	743	AISGSGGSTYYADSVKG	648	WFGEGFDP	674
BCMA-35	GFTFSSYAMS	743	AISGSGGSTYYADSVKG	648	VGYDSSGYYRDYYGMDV	675
BCMA-36	GFTFSSYAMS	743	AISGSGGSTYYADSVKG	648	MGWSSGYLGAFDI	676
BCMA-37	GYTFTNFGMN	746	WINTYTGESYFADDFKG	652	GEIYYGYDGGFAY	677
BCMA-38	GYTFTDYSIN	747	WINTETREPAYAYDFRG	653	DYSYAMDY	678
BCMA-39	GYTFRHYSMN	748	RINTESGVPIYADDFKG	654	DYLYSLDF	679
BCMA-40	GYTFTHYSMN	749	RINTETGEPLYADDFKG	655	DYLYSCDY	680

[0541] In some embodiments, the ABM comprises the CDR sequences of BCMA-1. In some embodiments, the ABM comprises the CDR sequences of BCMA-2. In some embodiments, the ABM comprises the CDR sequences of BCMA-3. In some embodiments, the ABM comprises the CDR sequences of BCMA-4. In some embodiments, the ABM comprises the CDR sequences of BCMA-5. In some embodiments, the ABM comprises the CDR sequences of BCMA-6. In some embodiments, the ABM comprises the CDR sequences of BCMA-7. In some embodiments, the ABM comprises the CDR sequences of BCMA-8. In some embodiments, the ABM comprises the CDR sequences of BCMA-9. In some embodiments, the ABM comprises the CDR sequences of BCMA-10. In some embodiments, the ABM comprises the CDR sequences of BCMA-11. In some embodiments, the ABM comprises the CDR sequences of BCMA-12. In some embodiments, the ABM comprises the CDR sequences of BCMA-13. In some embodiments, the ABM comprises the CDR sequences of BCMA-14. In some embodiments, the ABM comprises the CDR sequences of BCMA-15. In some embodiments, the ABM comprises the CDR sequences of BCMA-16. In some embodiments, the ABM comprises the CDR sequences of BCMA-17. In some embodiments, the ABM comprises the

CDR sequences of BCMA-18. In some embodiments, the ABM comprises the CDR sequences of BCMA-19. In some embodiments, the ABM comprises the CDR sequences of BCMA-20. In some embodiments, the ABM comprises the CDR sequences of BCMA-21. In some embodiments, the ABM comprises the CDR sequences of BCMA-22. In some embodiments, the ABM comprises the CDR sequences of BCMA-23. In some embodiments, the ABM comprises the CDR sequences of BCMA-24. In some embodiments, the ABM comprises the CDR sequences of BCMA-25. In some embodiments, the ABM comprises the CDR sequences of BCMA-26. In some embodiments, the ABM comprises the CDR sequences of BCMA-27. In some embodiments, the ABM comprises the CDR sequences of BCMA-28. In some embodiments, the ABM comprises the CDR sequences of BCMA-29. In some embodiments, the ABM comprises the CDR sequences of BCMA-30. In some embodiments, the ABM comprises the CDR sequences of BCMA-31. In some embodiments, the ABM comprises the CDR sequences of BCMA-32. In some embodiments, the ABM comprises the CDR sequences of BCMA-33. In some embodiments, the ABM comprises the CDR sequences of BCMA-34. In some embodiments, the ABM comprises the CDR sequences of BCMA-35. In some embodiments, the ABM comprises the CDR sequences of BCMA-36. In some embodiments, the ABM comprises the CDR sequences of BCMA-37. In some embodiments, the ABM comprises the CDR sequences of BCMA-38. In some embodiments, the ABM comprises the CDR sequences of BCMA-39. In some embodiments, the ABM comprises the CDR sequences of BCMA-40.

[0542] In some embodiments, the CDRs are defined by Kabat numbering, as set forth in Tables 17B and 17E. In other embodiments, the CDRs are defined by Chothia numbering, as set forth in Tables 17C and 17F. In yet other embodiments, the CDRs are defined by a combination of Kabat and Chothia numbering, as set forth in Tables 17D and 17G.

[0543] In some embodiments, the Type 2 TBMs in which ABM3 binds to BCMA can comprise the heavy and light chain variable sequences of any of BCMA-1 to BCMA-40.

[0544] In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-1, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-2, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-3, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-4, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-5, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-6, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-7, as set forth in Table 17A. In some embodiments, the

ABM comprises the heavy and light chain variable sequences of BCMA-8, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-9, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-10, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-11, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-12, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-13, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-14, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-15, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-16, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-17, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-18, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-19, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-20, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-21, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-22, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-23, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-24, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-25, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-26, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-27, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-28, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-29, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-30, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-31, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-32, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-33, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-34, as set forth in Table 17A. In some

embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-35, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-36, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-37, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-38, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-39, as set forth in Table 17A. In some embodiments, the ABM comprises the heavy and light chain variable sequences of BCMA-40, as set forth in Table 17A.

7.10. Nucleic Acids and Host Cells

[0545] In another aspect, the disclosure provides nucleic acids (*i.e.*, polynucleotides) encoding the CD19 binding molecules of the disclosure. In some embodiments, the CD19 binding molecules are encoded by a single nucleic acid. In other embodiments, the CD19 binding molecules are encoded by a plurality of (*e.g.*, two, three, four or more) nucleic acids.

[0546] A single nucleic acid can encode a CD19 binding molecule that comprises a single polypeptide chain, a CD19 binding molecule that comprises two or more polypeptide chains, or a portion of a CD19 binding molecule that comprises more than two polypeptide chains (for example, a single nucleic acid can encode two polypeptide chains of a CD19 binding molecule comprising three, four or more polypeptide chains, or three polypeptide chains of a CD19 binding molecule comprising four or more polypeptide chains). For separate control of expression, the open reading frames encoding two or more polypeptide chains can be under the control of separate transcriptional regulatory elements (*e.g.*, promoters and/or enhancers). The open reading frames encoding two or more polypeptides can also be controlled by the same transcriptional regulatory elements, and separated by internal ribosome entry site (IRES) sequences allowing for translation into separate polypeptides.

[0547] In some embodiments, a CD19 binding molecule comprising two or more polypeptide chains is encoded by two or more nucleic acids. The number of nucleic acids encoding a CD19 binding molecule can be equal to or less than the number of polypeptide chains in the CD19 binding molecule (for example, when more than one polypeptide chains are encoded by a single nucleic acid).

[0548] The nucleic acids can be DNA or RNA (*e.g.*, mRNA).

[0549] In another aspect, the disclosure provides host cells and vectors containing the nucleic acids of the disclosure. The nucleic acids can be present in a single vector or separate vectors present in the same host cell or separate host cell, as described in more detail herein below.

7.10.1. Vectors

[0550] The disclosure provides vectors comprising nucleotide sequences encoding a CD19 binding molecule or a CD19 binding molecule component described herein. In one embodiment, the vectors comprise nucleotides encoding an immunoglobulin-based ABM described herein. In one embodiment, the vectors comprise nucleotides encoding an Fc domain described herein. In one embodiment, the vectors comprise nucleotides encoding a recombinant non-immunoglobulin based ABM described herein. A vector can encode one or more ABMs, one or more Fc domains, one or more non-immunoglobulin based ABM, or any combination thereof (*e.g.*, when multiple components or sub-components are encoded as a single polypeptide chain). In one embodiment, the vectors comprise the nucleotide sequences described herein. The vectors include, but are not limited to, a virus, plasmid, cosmid, lambda phage or a yeast artificial chromosome (YAC).

[0551] Numerous vector systems can be employed. For example, one class of vectors utilizes DNA elements which are derived from animal viruses such as, for example, bovine papilloma virus, polyoma virus, adenovirus, vaccinia virus, baculovirus, retroviruses (Rous Sarcoma Virus, MMTV or MOMLV) or SV40 virus. Another class of vectors utilizes RNA elements derived from RNA viruses such as Semliki Forest virus, Eastern Equine Encephalitis virus and Flaviviruses.

[0552] Additionally, cells which have stably integrated the DNA into their chromosomes can be selected by introducing one or more markers which allow for the selection of transfected host cells. The marker can provide, for example, prototropy to an auxotrophic host, biocide resistance (*e.g.,* antibiotics), or resistance to heavy metals such as copper, or the like. The selectable marker gene can be either directly linked to the DNA sequences to be expressed, or introduced into the same cell by cotransformation. Additional elements may also be needed for optimal synthesis of mRNA. These elements can include splice signals, as well as transcriptional promoters, enhancers, and termination signals.

[0553] Once the expression vector or DNA sequence containing the constructs has been prepared for expression, the expression vectors can be transfected or introduced into an appropriate host cell. Various techniques can be employed to achieve this, such as, for example, protoplast fusion, calcium phosphate precipitation, electroporation, retroviral transduction, viral transfection, gene gun, lipid based transfection or other conventional techniques. Methods and conditions for culturing the resulting transfected cells and for recovering the expressed polypeptides are known to those skilled in the art, and can be varied or optimized depending upon the specific expression vector and mammalian host cell employed, based upon the present description.

7.10.2. Cells

[0554] The disclosure also provides host cells comprising a nucleic acid of the disclosure.

[0555] In one embodiment, the host cells are genetically engineered to comprise one or more nucleic acids described herein.

[0556] In one embodiment, the host cells are genetically engineered by using an expression cassette. The phrase "expression cassette," refers to nucleotide sequences, which are capable of affecting expression of a gene in hosts compatible with such sequences. Such cassettes can include a promoter, an open reading frame with or without introns, and a termination signal. Additional factors necessary or helpful in effecting expression can also be used, such as, for example, an inducible promoter.

[0557] The disclosure also provides host cells comprising the vectors described herein.

[0558] The cell can be, but is not limited to, a eukaryotic cell, a bacterial cell, an insect cell, or a human cell. Suitable eukaryotic cells include, but are not limited to, Vero cells, HeLa cells, COS cells, CHO cells, HEK293 cells, BHK cells and MDCKII cells. Suitable insect cells include, but are not limited to, Sf9 cells.

7.11. CD19 Binding Molecules with Extended *in vivo* Half-Life

[0559] The CD19 binding molecules of the disclosure can be modified to have an extended half-life *in vivo*.

[0560] A variety of strategies can be used to extend the half life of CD19 binding molecules of the disclosure. For example, by chemical linkage to polyethyleneglycol (PEG), reCODE PEG, antibody scaffold, polysialic acid (PSA), hydroxyethyl starch (HES), albumin-binding ligands, and carbohydrate shields; by genetic fusion to proteins binding to serum proteins, such as albumin, IgG, FcRn, and transferring; by coupling (genetically or chemically) to other binding moieties that bind to serum proteins, such as nanobodies, Fabs, DARPins, avimers, affibodies, and anticalins; by genetic fusion to rPEG, albumin, domain of albumin, albumin-binding proteins, and Fc; or by incorporation into nanocarriers, slow release formulations, or medical devices.

[0561] To prolong the serum circulation of CD19 binding molecules *in vivo*, inert polymer molecules such as high molecular weight PEG can be attached to the CD19 binding molecules with or without a multifunctional linker either through site-specific conjugation of the PEG to the N- or C-terminus of a polypeptide comprising the CD19 binding molecule or via epsilon-amino groups present on lysine residues. To pegylate a CD19 binding molecule, the molecule can be reacted with polyethylene glycol (PEG), such as a reactive ester or aldehyde derivative of PEG, under conditions in which one or more PEG groups become attached to the CD19 binding

molecules. The pegylation can be carried out by an acylation reaction or an alkylation reaction with a reactive PEG molecule (or an analogous reactive water-soluble polymer). As used herein, the term "polyethylene glycol" is intended to encompass any one of the forms of PEG that have been used to derivatize other proteins, such as mono (C1-C10)alkoxy- or aryloxy-polyethylene glycol or polyethylene glycol-maleimide. In one embodiment, the CD19 binding molecule to be pegylated is an aglycosylated antibody. Linear or branched polymer derivatization that results in minimal loss of biological activity will be used. The degree of conjugation can be closely monitored by SDS-PAGE and mass spectrometry to ensure proper conjugation of PEG molecules to the antibodies. Unreacted PEG can be separated from antibody-PEG conjugates by size-exclusion or by ion-exchange chromatography. PEG-derivatized antibodies can be tested for binding activity as well as for in vivo efficacy using methods well-known to those of skill in the art, for example, by immunoassays described herein. Methods for pegylating proteins are known and can be applied to CD19 binding molecules of the disclosure. See for example, EP 0154316 by Nishimura *et al.* and EP 0401384 by Ishikawa *et al.*

[0562] Other modified pegylation technologies include reconstituting chemically orthogonal directed engineering technology (ReCODE PEG), which incorporates chemically specified side chains into biosynthetic proteins via a reconstituted system that includes tRNA synthetase and tRNA. This technology enables incorporation of more than 30 new amino acids into biosynthetic proteins in E. coli, yeast, and mammalian cells. The tRNA incorporates a normative amino acid any place an amber codon is positioned, converting the amber from a stop codon to one that signals incorporation of the chemically specified amino acid.

[0563] Recombinant pegylation technology (rPEG) can also be used for serum half life extension. This technology involves genetically fusing a 300-600 amino acid unstructured protein tail to an existing pharmaceutical protein. Because the apparent molecular weight of such an unstructured protein chain is about 15-fold larger than its actual molecular weight, the serum half life of the protein is greatly increased. In contrast to traditional PEGylation, which requires chemical conjugation and repurification, the manufacturing process is greatly simplified and the product is homogeneous.

[0564] Polysialytion is another technology, which uses the natural polymer polysialic acid (PSA) to prolong the active life and improve the stability of therapeutic peptides and proteins. PSA is a polymer of sialic acid (a sugar). When used for protein and therapeutic peptide drug delivery, polysialic acid provides a protective microenvironment on conjugation. This increases the active life of the therapeutic protein in the circulation and prevents it from being recognized by the immune system. The PSA polymer is naturally found in the human body. It was adopted by certain bacteria which evolved over millions of years to coat their walls with it. These naturally

polysialylated bacteria were then able, by virtue of molecular mimicry, to foil the body's defense system. PSA, nature's ultimate stealth technology, can be easily produced from such bacteria in large quantities and with predetermined physical characteristics. Bacterial PSA is completely non-immunogenic, even when coupled to proteins, as it is chemically identical to PSA in the human body.

[0565] Another technology include the use of hydroxyethyl starch ("HES") derivatives linked to CD19 binding molecules. HES is a modified natural polymer derived from waxy maize starch and can be metabolized by the body's enzymes. HES solutions are usually administered to substitute deficient blood volume and to improve the rheological properties of the blood. Hesylation of a CD19 binding molecule enables the prolongation of the circulation half-life by increasing the stability of the molecule, as well as by reducing renal clearance, resulting in an increased biological activity. By varying different parameters, such as the molecular weight of HES, a wide range of HES CD19 binding molecule conjugates can be customized.

[0566] CD19 binding molecules having an increased half-life in vivo can also be generated introducing one or more amino acid modifications (*i.e.*, substitutions, insertions or deletions) into an IgG constant domain, or FcRn binding fragment thereof (*e.g.*, an Fc or hinge Fc domain fragment). See, *e.g.*, International Publication No. WO 98/23289; International Publication No. WO 97/34631; and U.S. Pat. No. 6,277,375.

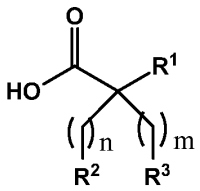
[0567] Furthermore, the CD19 binding molecules can be conjugated to albumin, a domain of albumin, an albumin-binding protein, or an albumin-binding antibody or antibody fragments thereof, in order to make the molecules more stable *in vivo* or have a longer half life in *vivo*. The techniques are well-known, see, *e.g.*, International Publication Nos. WO 93/15199, WO 93/15200, and WO 01/77137; and European Patent No. EP 413,622.

[0568] The CD19 binding molecules of the present disclosure can also be fused to one or more human serum albumin (HSA) polypeptides, or a portion thereof. The use of albumin as a component of an albumin fusion protein as a carrier for various proteins has been suggested in WO 93/15199, WO 93/15200, and EP 413 622. The use of N-terminal fragments of HSA for fusions to polypeptides has also been proposed (EP 399 666). Accordingly, by genetically or chemically fusing or conjugating the molecules to albumin, can stabilize or extend the shelf-life, and/or to retain the molecule's activity for extended periods of time in solution, in vitro and/or in vivo. Additional methods pertaining to HSA fusions can be found, for example, in WO 2001077137 and WO 200306007. In an embodiment, the expression of the fusion protein is performed in mammalian cell lines, for example, CHO cell lines.

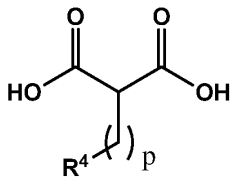
[0569] The CD19 binding molecules of the present disclosure can also be fused to an antibody or antibody fragment thereof that binds to albumin, *e.g.*, human serum albumin (HSA). The

albumin-binding antibody or antibody fragment thereof can be a Fab, a scFv, a Fv, an scFab, a (Fab')2, a single domain antibody, a camelid VHH domain, a VH or VL domain, or a full-length monoclonal antibody (mAb).

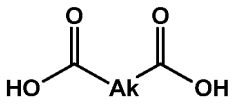
[0570] The CD19 binding molecules of the present disclosure can also be fused to a fatty acid to extend their half-life. Fatty acids suitable for linking to a biomolecule have been described in the art, *e.g.*, WO2015/200078, WO2015/191781, US2013/0040884. Suitable half-life extending fatty acids include those defined as a C6-70alkyl, a C6-70alkenyl or a C6-70alkynyl chain, each of which is substituted with at least one carboxylic acid (for example 1, 2, 3 or 4 CO2H) and optionally further substituted with hydroxyl group. For example, the CD19 binding molecules described herein can be linked to a fatty acid having any of the following Formulae A1, A2 or A3:



A1



A2



or A3

R^1 is CO_2H or H;

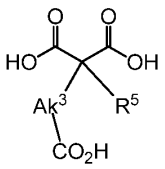
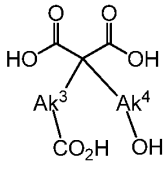
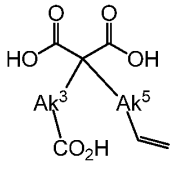
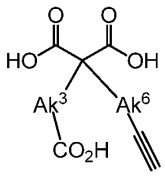
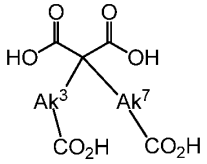
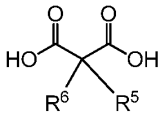
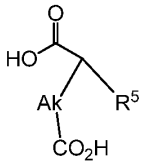
R^2, R^3 and R^4 are independently of each other H, OH, CO_2H, -CH=CH_2 or –C≡CH;

Ak is a branched C_6-C_{30} alkylene;

n, m and p are independently of each other an integer between 6 and 30; or an amide, ester or pharmaceutically acceptable salt thereof.

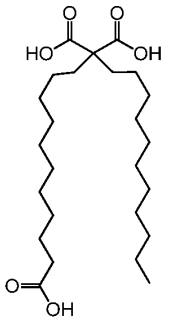
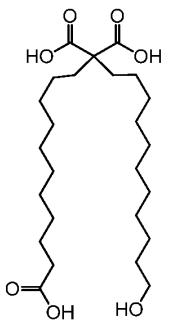
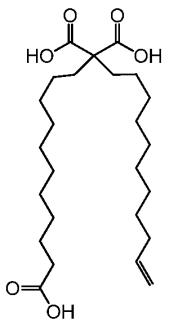
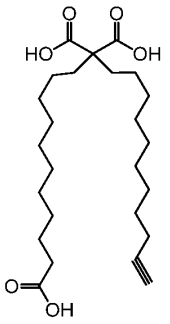
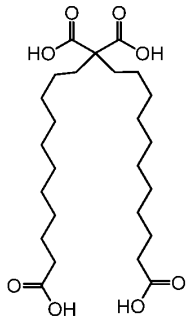
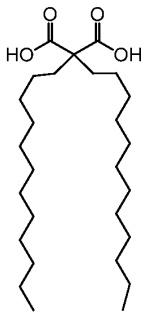
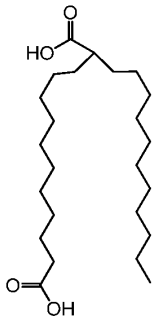
[0571] In some embodiments, the fatty acid is of Formula A1, *e.g.*, a fatty acid of Formula A1 where n and m are independently 8 to 20, *e.g.*, 10 to 16. In another embodiment, the fatty acid moiety is of Formula A1 and where at least one of R^2 and R^3 is CO_2H.

[0572] In some embodiments, the fatty acid is selected from the following Formulae:

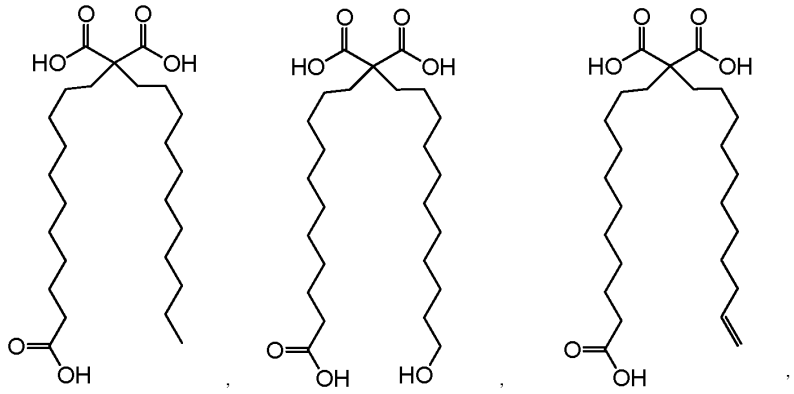






and


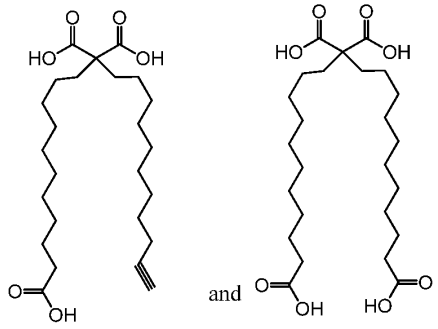
where Ak^3, Ak^4, Ak^5, Ak^6 and Ak^7 are independently a (C_{8-20})alkylene, R^5 and R^6 are independently (C_{8-20})alkyl.

[0573] In some embodiments, the fatty acid is selected from the following Formulae:







and


[0574] In some embodiments, the fatty acid is selected from the following Formulae:





[0575] In some embodiments, the fatty acid is of Formula A2 or A3. In a particular embodiment, the conjugate comprises a fatty acid moiety of Formula A2 where p is 8 to 20, or a fatty acid moiety of Formula A3 where Ak is C_{8-20}alkylene.

7.12. Antibody-Drug Conjugates

[0576] The CD19 binding molecules of the disclosure can be conjugated, *e.g.*, via a linker, to a drug moiety. Such conjugates are referred to herein as antibody-drug conjugates (or "ADCs") for convenience, notwithstanding the fact that one or more of the ABMs might be based on non-immunoglobulin scaffolds, *e.g.*, a MBM comprising one or more non-immunoglobulin based ABMs, such as a TCR ABM comprising Affilin-144160).

[0577] In certain aspects, the drug moiety exerts a cytotoxic or cytostatic activity. In one embodiment, the drug moiety is chosen from a maytansinoid, a kinesin-like protein KIF11 inhibitor, a V-ATPase (vacuolar-type H+ -ATPase) inhibitor, a pro-apoptotic agent, a Bcl2 (B-cell lymphoma 2) inhibitor, an MCL1 (myeloid cell leukemia 1) inhibitor, a HSP90 (heat shock protein 90) inhibitor, an IAP (inhibitor of apoptosis) inhibitor, an mTOR (mechanistic target of rapamycin) inhibitor, a microtubule stabilizer, a microtubule destabilizer, an auristatin, a

dolastatin, a MetAP (methionine aminopeptidase), a CRM1 (chromosomal maintenance 1) inhibitor, a DPPIV (dipeptidyl peptidase IV) inhibitor, a proteasome inhibitor, an inhibitor of a phosphoryl transfer reaction in mitochondria, a protein synthesis inhibitor, a kinase inhibitor, a CDK2 (cyclin-dependent kinase 2) inhibitor, a CDK9 (cyclin-dependent kinase 9) inhibitor, a kinesin inhibitor, an HDAC (histone deacetylase) inhibitor, a DNA damaging agent, a DNA alkylating agent, a DNA intercalator, a DNA minor groove binder, a RNA polymerase inhibitor, a topoisomerase inhibitor, or a DHFR (dihydrofolate reductase) inhibitor. In some embodiments, the drug moiety is a radioactive metal ion, such as alpha-emitters such as 213Bi or macrocyclic chelators useful for conjugating radiometal ions, including but not limited to, 131In, 131LU, 131Y, 131Ho, 131Sm, to polypeptides. In one embodiment, the macrocyclic chelator is 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA).

[0578] In one embodiment, the linker is chosen from a cleavable linker, a non-cleavable linker, a hydrophilic linker, a procharged linker, or a dicarboxylic acid based linker.

[0579] In some embodiments, the ADCs are compounds according to structural formula (I):

$$[D\text{-}L\text{-}XY]_n\text{-}Ab$$

or salts thereof, where each “D” represents, independently of the others, a cytotoxic and/or cytostatic agent (“drug”); each “L” represents, independently of the others, a linker; “Ab” represents a CD19 binding molecule described herein; each “XY” represents a linkage formed between a functional group R^x on the linker and a “complementary” functional group R^y on the antibody, and n represents the number of drugs linked to, or drug-to-antibody ratio (DAR), of the ADC.

[0580] Some embodiments of the various antibodies (Ab) that can comprise the ADCs include the various embodiments of CD19 binding molecules described above.

[0581] In some embodiments of the ADCs and/or salts of structural formula (I), each D is the same and/or each L is the same.

[0582] Cytotoxic and/or cytostatic agents used in the conjugates of the disclosure can be any agents known to inhibit the growth and/or replication of and/or kill cells, and in particular cancer and/or tumor cells. Numerous agents having cytotoxic and/or cytostatic properties are known in the literature. Non-limiting examples of classes of cytotoxic and/or cytostatic agents include, by way of example and not limitation, radionuclides, alkylating agents, topoisomerase I inhibitors, topoisomerase II inhibitors, DNA intercalating agents (*e.g.*, groove binding agents such as minor groove binders), RNA/DNA antimetabolites, cell cycle modulators, kinase inhibitors, protein synthesis inhibitors, histone deacetylase inhibitors, mitochondria inhibitors, and antimitotic agents.

7.13. CD19 Binding Molecules Conjugated to Detectable Agents

[0583] CD19 binding molecules of the disclosure can be conjugated to a diagnostic or detectable agent. Such molecules can be useful for monitoring or prognosing the onset, development, progression and/or severity of a disease or disorder as part of a clinical testing procedure, such as determining the efficacy of a particular therapy. Such diagnosis and detection can accomplished by coupling the CD19 binding molecules to detectable substances including, but not limited to, various enzymes, such as, but not limited to, horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; prosthetic groups, such as, but not limited to, streptavidin/biotin and avidin/biotin; fluorescent materials, such as, but not limited to, umbelliferone, fluorescein, fluorescein isothiocynate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; luminescent materials, such as, but not limited to, luminol; bioluminescent materials, such as but not limited to, luciferase, luciferin, and aequorin; radioactive materials, such as, but not limited to, iodine (^{131}I, ^{125}I, ^{123}I, and ^{121}I,), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{115}In, ^{113}In, ^{112}In, and ^{111}In,), technetium (^{99}Tc), thallium (^{201}Ti), gallium (^{68}Ga, ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm, ^{177}Lu, ^{159}Gd, ^{149}Pm, ^{140}La, ^{175}Yb, ^{166}Ho, ^{90}Y, 47Sc, ^{186}Re, ^{188}Re, ^{142} Pr, ^{105}Rh, ^{97}Ru, ^{68}Ge, ^{57}Co, ^{65}Zn, ^{85}Sr, ^{32}P, ^{153}Gd, ^{169}Yb, ^{51}Cr, ^{54}Mn, ^{75}Se, ^{113}Sn, and ^{117}Tin; and positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions.

7.14. CD19 Binding Molecules Attached to Solid Supports

[0584] The CD19 binding molecules can also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen(s). Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

7.15. Pharmaceutical Compositions

[0585] The CD19 binding molecules of the disclosure (as well as their conjugates; references to CD19 binding molecules in this disclosure also refers to conjugates comprising the CD19 binding molecules, such as ADCs, unless the context dictates otherwise) can be formulated as pharmaceutical compositions comprising the CD19 binding molecules, for example containing one or more pharmaceutically acceptable excipients or carriers. To prepare pharmaceutical or sterile compositions comprising the CD19 binding molecules of the present disclosure a CD19 binding molecule preparation can be combined with one or more pharmaceutically acceptable excipient or carrier.

[0586] For example, formulations of CD19 binding molecules can be prepared by mixing CD19 binding molecules with physiologically acceptable carriers, excipients, or stabilizers in the form

of, *e.g.*, lyophilized powders, slurries, aqueous solutions, lotions, or suspensions (see, *e.g.*, Hardman *et al.*, 2001, Goodman and Gilman's The Pharmacological Basis of Therapeutics, McGraw-Hill, New York, N.Y.; Gennaro, 2000, Remington: The Science and Practice of Pharmacy, Lippincott, Williams, and Wilkins, New York, N.Y.; Avis, *et al.* (eds.),1993, Pharmaceutical Dosage Forms: General Medications, Marcel Dekker, NY; Lieberman, *et al.* (eds.), 1990, Pharmaceutical Dosage Forms: Tablets, Marcel Dekker, NY; Lieberman, *et al.* (eds.), 1990, Pharmaceutical Dosage Forms: Disperse Systems, Marcel Dekker, NY; Weiner and Kotkoskie, 2000, Excipient Toxicity and Safety, Marcel Dekker, Inc., New York, N.Y.).

[0587] Selecting an administration regimen for a CD19 binding molecule depends on several factors, including the serum or tissue turnover rate of the CD19 binding molecule, the level of symptoms, the immunogenicity of the CD19 binding molecule, and the accessibility of the target cells. In certain embodiments, an administration regimen maximizes the amount of CD19 binding molecule delivered to the subject consistent with an acceptable level of side effects. Accordingly, the amount of CD19 binding molecule delivered depends in part on the particular CD19 binding molecule and the severity of the condition being treated. Guidance in selecting appropriate doses of antibodies and small molecules are available (see, *e.g.*, Wawrzynczak, 1996, Antibody Therapy, Bios Scientific Pub. Ltd, Oxfordshire, UK; Kresina (ed.), 1991, Monoclonal Antibodies, Cytokines and Arthritis, Marcel Dekker, New York, N.Y.; Bach (ed.), 1993, Monoclonal Antibodies and Peptide Therapy in Autoimmune Diseases, Marcel Dekker, New York, N.Y.; Baert *et al.*, 2003, New Engl. J. Med. 348:601-608; Milgrom *et al.*, 1999, New Engl. J. Med. 341:1966-1973; Slamon *et al.*, 2001, New Engl. J. Med. 344:783-792; Beniaminovitz *et al.*, 2000, New Engl. J. Med. 342:613-619; Ghosh *et al.*, 2003, New Engl. J. Med. 348:24-32; Lipsky *et al.*, 2000, New Engl. J. Med. 343:1594-1602).

[0588] Determination of the appropriate dose is made by the clinician, *e.g.*, using parameters or factors known or suspected in the art to affect treatment or predicted to affect treatment. Generally, the dose begins with an amount somewhat less than the optimum dose and it is increased by small increments thereafter until the desired or optimum effect is achieved relative to any negative side effects. Important diagnostic measures include those of symptoms of, *e.g.*, the inflammation or level of inflammatory cytokines produced.

[0589] Actual dosage levels of the CD19 binding molecules in the pharmaceutical compositions of the present disclosure can be varied so as to obtain an amount of the CD19 binding molecule which is effective to achieve the desired therapeutic response for a particular subject, composition, and mode of administration, without being toxic to the subject. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular CD19 binding molecule, the route of administration, the time of administration, the rate of excretion of the particular CD19 binding molecule being employed, the duration of the

treatment, other agents (*e.g.*, active agents such as therapeutic drugs or compounds and/or inert materials used as carriers) in combination with the particular CD19 binding molecule employed, the age, sex, weight, condition, general health and prior medical history of the subject being treated, and like factors known in the medical arts.

[0590] Compositions comprising the CD19 binding molecules can be provided by continuous infusion, or by doses at intervals of, *e.g.*, one day, one week, or 1-7 times per week. Doses can be provided intravenously, subcutaneously, topically, orally, nasally, rectally, intramuscular, intracerebrally, or by inhalation. A specific dose protocol is one involving the maximal dose or dose frequency that avoids significant undesirable side effects.

[0591] An effective amount for a particular subject can vary depending on factors such as the condition being treated, the overall health of the subject, the method route and dose of administration and the severity of side effects (see, *e.g.*, Maynard, *et al.* (1996) A Handbook of SOPs for Good Clinical Practice, Interpharm Press, Boca Raton, Fla.; Dent (2001) Good Laboratory and Good Clinical Practice, Urch Publ., London, UK).

[0592] The route of administration can be by, *e.g.*, topical or cutaneous application, injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial, intracerebrospinal, intralesional, or by sustained release systems or an implant (see, *e.g.*, Sidman *et al.*, 1983, Biopolymers 22:547-556; Langer *et al.*, 1981, J. Biomed. Mater. Res. 15:167-277; Langer, 1982, Chem. Tech. 12:98-105; Epstein *et al.*, 1985, Proc. Natl. Acad. Sci. USA 82:3688-3692; Hwang *et al.*, 1980, Proc. Natl. Acad. Sci. USA 77:4030-4034; U.S. Pat. Nos. 6,350,466 and 6,316,024). Where necessary, the composition can also include a solubilizing agent and a local anesthetic such as lidocaine to ease pain at the site of the injection. In addition, pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. See, *e.g.*, U.S. Pat. Nos. 6,019,968, 5,985,320, 5,985,309, 5,934,272, 5,874,064, 5,855,913, 5,290,540, and 4,880,078; and PCT Publication Nos. WO 92/19244, WO 97/32572, WO 97/44013, WO 98/31346, and WO 99/66903.

[0593] A composition of the present disclosure can also be administered via one or more routes of administration using one or more of a variety of known methods. As will be appreciated by a skilled artisan, the route and/or mode of administration will vary depending upon the desired results. Selected routes of administration for CD19 binding molecules include intravenous, intramuscular, intradermal, intraperitoneal, subcutaneous, spinal or other general routes of administration, for example by injection or infusion. General administration can represent modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal,

intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion. Alternatively, a composition of the disclosure can be administered via a non-general route, such as a topical, epidermal or mucosal route of administration, for example, intranasally, orally, vaginally, rectally, sublingually or topically. In one embodiment, the CD19 binding molecule is administered by infusion. In another embodiment, the CD19 binding molecule is administered subcutaneously.

[0594] If the CD19 binding molecules are administered in a controlled release or sustained release system, a pump can be used to achieve controlled or sustained release (see Langer, *supra*; Sefton, 1987, CRC Crit. Ref Biomed. Eng. 14:20; Buchwald *et al.*, 1980, Surgery 88:507; Saudek *et al.*, 1989, N. Engl. J. Med. 321:574). Polymeric materials can be used to achieve controlled or sustained release of the therapies of the disclosure (see, *e.g.*, Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Fla. (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, 1983, J., Macromol. Sci. Rev. Macromol. Chem. 23:61; see also Levy *et al.*, 1985, Science 228:190; During *et al.*, 1989, Ann. Neurol. 25:351; Howard *et al.*, 1989, J. Neurosurg. 71:105); U.S. Pat. No. 5,679,377; U.S. Pat. No. 5,916,597; U.S. Pat. No. 5,912,015; U.S. Pat. No. 5,989,463; U.S. Pat. No. 5,128,326; PCT Publication No. WO 99/15154; and PCT Publication No. WO 99/20253. Examples of polymers used in sustained release formulations include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N-vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. In one embodiment, the polymer used in a sustained release formulation is inert, free of leachable impurities, stable on storage, sterile, and biodegradable. A controlled or sustained release system can be placed in proximity of the prophylactic or therapeutic target, thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)).

[0595] Controlled release systems are discussed in the review by Langer (1990, Science 249:1527-1533). Any technique known to one of skill in the art can be used to produce sustained release formulations comprising one or more CD19 binding molecules of the disclosure. See, *e.g.*, U.S. Pat. No. 4,526,938, PCT publication WO 91/05548, PCT publication WO 96/20698, Ning *et al.*, 1996, Radiotherapy & Oncology 39:179-189, Song *et al.*, 1995, PDA Journal of Pharmaceutical Science & Technology 50:372-397, Cleek *et al.*, 1997, Pro. Int'l. Symp. Control. Rel. Bioact. Mater. 24:853-854, and Lam *et al.*, 1997, Proc. Int'l. Symp. Control Rel. Bioact. Mater. 24:759-760.

[0596] If the CD19 binding molecules are administered topically, they can be formulated in the form of an ointment, cream, transdermal patch, lotion, gel, shampoo, spray, aerosol, solution, emulsion, or other form well-known to one of skill in the art. See, *e.g.*, Remington's Pharmaceutical Sciences and Introduction to Pharmaceutical Dosage Forms, 19th ed., Mack Pub. Co., Easton, Pa. (1995). For non-sprayable topical dosage forms, viscous to semi-solid or solid forms comprising a carrier or one or more excipients compatible with topical application and having a dynamic viscosity, in some embodiments, greater than water are typically employed. Suitable formulations include, without limitation, solutions, suspensions, emulsions, creams, ointments, powders, liniments, salves, and the like, which are, if desired, sterilized or mixed with auxiliary agents (*e.g.*, preservatives, stabilizers, wetting agents, buffers, or salts) for influencing various properties, such as, for example, osmotic pressure. Other suitable topical dosage forms include sprayable aerosol preparations where the active ingredient, in some embodiments, in combination with a solid or liquid inert carrier, is packaged in a mixture with a pressurized volatile (*e.g.*, a gaseous propellant, such as freon) or in a squeeze bottle. Moisturizers or humectants can also be added to pharmaceutical compositions and dosage forms if desired. Examples of such additional ingredients are well-known.

[0597] If the compositions comprising the CD19 binding molecules are administered intranasally, the CD19 binding molecules can be formulated in an aerosol form, spray, mist or in the form of drops. In particular, prophylactic or therapeutic agents for use according to the present disclosure can be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant (*e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas). In the case of a pressurized aerosol the dosage unit can be determined by providing a valve to deliver a metered amount. Capsules and cartridges (composed of, *e.g.*, gelatin) for use in an inhaler or insufflator can be formulated containing a powder mix of the CD19 binding molecule and a suitable powder base such as lactose or starch.

[0598] The CD19 binding molecules of the disclosure can be administered in combination therapy regimens, as described in Section 7.17, *infra*.

[0599] In certain embodiments, the CD19 binding molecules can be formulated to ensure proper distribution in vivo. For example, the blood-brain barrier (BBB) excludes many highly hydrophilic compounds. To ensure that the therapeutic compounds of the disclosure cross the BBB (if desired), they can be formulated, for example, in liposomes. For methods of manufacturing liposomes, see, *e.g.*, U.S. Pat. Nos. 4,522,811; 5,374,548; and 5,399,331. The liposomes can comprise one or more moieties which are selectively transported into specific cells or organs, thus enhance targeted drug delivery (see, *e.g.*, Ranade, 1989, J. Clin. Pharmacol. 29:685). Exemplary targeting moieties include folate or biotin (see, *e.g.*, U.S. Pat.

No. 5,416,016 to Low *et al.*); mannosides (Umezawa *et al.*, 1988, Biochem. Biophys. Res. Commun. 153:1038); antibodies (Bloeman *et al.*, 1995, FEBS Lett. 357:140; Owais *et al.*, 1995, Antimicrob. Agents Chemother. 39:180); surfactant protein A receptor (Briscoe *et al.*, 1995, Am. J. Physiol. 1233:134); p 120 (Schreier *et al.*, 1994, J. Biol. Chem. 269:9090); see also Keinanen and Laukkanen, 1994, FEBS Lett. 346:123; Killion and Fidler, 1994, Immunomethods 4:273.

[0600] When used in combination therapy, *e.g.*, as described in Section 7.17, *infra*, a CD19 binding molecule and one or more additional agents can be administered to a subject in the same pharmaceutical composition. Alternatively, the CD19 binding molecule and the additional agent(s) of the combination therapies can be administered concurrently to a subject in separate pharmaceutical compositions.

[0601] The therapeutic methods described herein can further comprise carrying a "companion diagnostic" test whereby a sample from a subject who is a candidate for therapy with a CD19 binding molecule is tested for the expression of CD19. The companion diagnostic test can be performed prior to initiating therapy with a CD19 binding molecule and/or during a therapeutic regimen with a CD19 binding molecule to monitor the subject's continued suitability for CD19 binding molecule therapy. The agent used in the companion diagnostic can be the CD19 binding molecule itself or another diagnostic agent, for example a labeled monospecific antibody against CD19 or a nucleic acid probe to detect CD19 RNA. The sample that can be tested in a companion diagnostic assay can be any sample in which the cells targeted by the CD19 binding molecule can be present, from example a tumor (*e.g.*, a solid tumor) biopsy, lymph, stool, urine, blood or any other bodily fluid that might contain circulating tumor cells.

7.16. Therapeutic Indications

[0602] The CD19 binding molecules of the disclosure can be used in the treatment of any disease associated with CD19 expression. The phrase "disease associated with CD19 expression" includes, but is not limited to, a disease associated with expression of CD19 or condition associated with cells which express CD19 including, *e.g.*, proliferative diseases such as a cancer or malignancy or a precancerous condition such as a myelodysplasia, a myelodysplastic syndrome or a preleukemia; or a noncancer related indication associated with cells which express CD19. In one aspect, a cancer associated with expression of CD19 is a hematological cancer. In one aspect, the hematological cancer is a leukemia or a lymphoma. In one aspect, a cancer associated with expression of CD19 includes cancers and malignancies including, but not limited to, *e.g.*, one or more acute leukemias including but not limited to, *e.g.*, B-cell acute Lymphoid Leukemia ("BALL"), T-cell acute Lymphoid Leukemia ("TALL"), acute lymphoid leukemia (ALL); one or more chronic leukemias including but not limited to, *e.g.*, chronic myelogenous leukemia (CML), Chronic Lymphoid Leukemia (CLL). Additional cancers

or hematologic conditions associated with expression of CD19 comprise, but are not limited to, *e.g.*, B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma, Follicular lymphoma, Hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma, mantle cell lymphoma, Marginal zone lymphoma, multiple myeloma, myelodysplasia and myelodysplastic syndrome, non-Hodgkin's lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, and “preleukemia” which are a diverse collection of hematological conditions united by ineffective production (or dysplasia) of myeloid blood cells, and the like. Further diseases associated with expression of CD19expression include, but not limited to, *e.g.*, atypical and/or non-classical cancers, malignancies, precancerous conditions or proliferative diseases associated with expression of CD19. Non-cancer related indications associated with expression of CD19 include, but are not limited to, *e.g.*, autoimmune disease, (*e.g.*, lupus), inflammatory disorders (allergy and asthma) and transplantation.

[0603] For example, a CD19 binding molecule can be used to treat a subject who has undergone treatment for a disease associated with elevated expression of CD19, where the subject who has undergone treatment for elevated levels of CD19 exhibits a disease associated with elevated levels of CD19.

[0604] In one aspect, the disclosure provides a method of inhibiting growth of a CD19-expressing tumor cell, comprising contacting the tumor cell with a CD19 binding molecule such that the growth of the tumor cell is inhibited.

[0605] In one aspect, the disclosure provides a method of treating and/or preventing a disease that arises in individuals who are immunocompromised, comprising administering a CD19 binding molecule. In particular, disclosed herein is a method of treating diseases, disorders and conditions associated with expression of CD19, comprising administering a CD19 binding molecule.

[0606] In certain aspects, disclosed herein is a method of treating patients at risk for developing diseases, disorders and conditions associated with expression of CD19, comprising administering a CD19 binding molecule.

[0607] Thus, the present disclosure provides methods for the treatment or prevention of diseases, disorders and conditions associated with expression of CD19 comprising administering to a subject in need thereof, a therapeutically effective amount of a CD19 binding molecule.

[0608] The present disclosure also provides methods for preventing, treating and/or managing a disease associated with CD19-expressing cells (*e.g.*, a hematologic cancer or atypical cancer

expressing CD19), the methods comprising administering to a subject in need a CD19 binding molecule. In one aspect, the subject is a human. Non-limiting examples of disorders associated with CD19-expressing cells include viral or fungal infections, and disorders related to mucosal immunity.

7.16.1. Cancer and cancer-related diseases and disorders and patient populations

[0609] In one aspect, the disclosure provides a method of treating cancer in a subject. The method comprises administering to the subject a CD19 binding molecule such that the cancer is treated in the subject. An example of a cancer that is treatable by the CD19-targeting agent is a cancer associated with expression of CD19.

[0610] In one aspect, the disclosure provides methods for treating a cancer where part of the tumor is negative for CD19 and part of the tumor is positive for CD19.

[0611] In one aspect, the disclosure provides methods for treating a cancer where CD19 is expressed on both normal cells and cancers cells, but is expressed at lower levels on normal cells, using a CD19 binding molecule of the disclosure. In one embodiment, the method further comprises selecting a CD19 binding molecule that binds with an affinity that allows the CD19 binding molecule to bind and kill the cancer cells expressing CD19 but kill less than 30%, 25%, 20%, 15%, 10%, 5% or less of the normal cells expressing CD19, *e.g.*, as determined by an assay described herein. For example, a killing assay such as flow cytometry based on Cr51 CTL can be used. In one embodiment, the CD19 binding molecule has an antigen binding domain that has a binding affinity K_D of 10^{-4} M to 10^{-8} M, *e.g.*, 10^{-5} M to 10^{-7} M, *e.g.*, 10^{-6} M or 10^{-7} M, for CD19.

[0612] In one aspect, disclosed herein is a method of treating a proliferative disease such as a cancer or malignancy or a precancerous condition such as a myelodysplasia, a myelodysplastic syndrome or a preleukemia, comprising administering CD19 binding molecule. In one aspect, the cancer is a hematological cancer. Hematological cancer conditions are the types of cancer such as leukemia and malignant lymphoproliferative conditions that affect blood, bone marrow and the lymphatic system. In one aspect, the hematological cancer is a leukemia. An example of a disease or disorder associated with CD19 is multiple myeloma (also known as MM) (See Claudio *et al.*, *Blood.* 2002, 100(6):2175-86; and Novak *et al.*, *Blood.* 2004, 103(2):689-94). Multiple myeloma, also known as plasma cell myeloma or Kahler's disease, is a cancer characterized by an accumulation of abnormal or malignant plasma B-cells in the bone marrow. Frequently, the cancer cells invade adjacent bone, destroying skeletal structures and resulting in bone pain and fractures. Most cases of myeloma also feature the production of a paraprotein (also known as M proteins or myeloma proteins), which is an abnormal immunoglobulin produced in excess by the clonal proliferation of the malignant plasma cells. Blood serum

paraprotein levels of more than 30g/L is diagnostic of multiple myeloma, according to the diagnostic criteria of the International Myeloma Working Group (IMWG) (*See* Kyle *et al*. (2009), Leukemia. 23:3-9). Other symptoms or signs of multiple myeloma include reduced kidney function or renal failure, bone lesions, anemia, hypercalcemia, and neurological symptoms.

[0613] Other plasma cell proliferative disorders that can be treated by the compositions and methods described herein include, but are not limited to, asymptomatic myeloma (smoldering multiple myeloma or indolent myeloma), monoclonal gammapathy of undetermined significance (MGUS), Waldenstrom's macroglobulinemia, plasmacytomas (*e.g.*, plasma cell dyscrasia, solitary myeloma, solitary plasmacytoma, extramedullary plasmacytoma, and multiple plasmacytoma), systemic amyloid light chain amyloidosis, and POEMS syndrome (also known as Crow-Fukase syndrome, Takatsuki disease, and PEP syndrome).

[0614] Another example of a disease or disorder associated with CD19 is Hodgkin's lymphoma and non-Hodgkin's lymphoma (*See* Chiu *et al*., *Blood*. 2007, 109(2):729-39; He *et al*., *J Immunol.* 2004, 172(5):3268-79).

[0615] Hodgkin's lymphoma (HL), also known as Hodgkin's disease, is a cancer of the lymphatic system that originates from white blood cells, or lymphocytes. The abnormal cells that comprise the lymphoma are called Reed-Sternberg cells. In Hodgkin's lymphoma, the cancer spreads from one lymph node group to another. Hodgkin's lymphoma can be subclassified into four pathologic subtypes based upon Reed-Sternberg cell morphology and the cell composition around the Reed-Sternberg cells (as determined through lymph node biopsy): nodular sclerosing HL, mixed-cellularity subtype, lymphocyte-rich or lymphocytic predominance, lymphocyte depleted. Some Hodgkin's lymphoma can also be nodular lymphocyte predominant Hodgkin's lymphoma, or can be unspecified. Symptoms and signs of Hodgkin's lymphoma include painless swelling in the lymph nodes in the neck, armpits, or groin, fever, night sweats, weight loss, fatigue, itching, or abdominal pain.

[0616] Non-Hodgkin's lymphoma (NHL) comprises a diverse group of blood cancers that include any kind of lymphoma other than Hodgkin's lymphoma. Subtypes of non-Hodgkin's lymphoma are classified primarily by cell morphology, chromosomal aberrations, and surface markers. NHL subtypes (or NHL-associated cancers) include B cell lymphomas such as, but not limited to, Burkitt's lymphoma, B-cell chronic lymphocytic leukemia (B-CLL), B-cell prolymphocytic leukemia (B-PLL), chronic lymphocytic leukemia (CLL), diffuse large B-cell lymphoma (DLBCL) (*e.g.*, intravascular large B-cell lymphoma and primary mediastinal B-cell lymphoma), follicular lymphoma (*e.g.*, follicle center lymphoma, follicular small cleaved cell), hair cell leukemia, high grade B-cell lymphoma (Burkitt's like), lymphoplasmacytic lymphoma (Waldenstrom's macroglublinemia), mantle cell lymphoma, marginal zone B-cell lymphomas

(*e.g.*, extranodal marginal zone B-cell lymphoma or mucosa-associated lymphoid tissue (MALT) lymphoma, nodal marginal zone B-cell lymphoma, and splenic marginal zone B-cell lymphoma), plasmacytoma/myeloma, precursor B-lymphoblastic leukemia/lymphoma (PB-LBL/L), primary central nervous system (CNS) lymphoma, primary intraocular lymphoma, small lymphocytic lymphoma (SLL); and T cell lymphomas, such as, but not limited to, anaplastic large cell lymphoma (ALCL), adult T-cell lymphoma/leukemia (*e.g.*, smoldering, chronic, acute and lymphomatous), angiocentric lymphoma, angioimmunoblastic T-cell lymphoma, cutaneous T-cell lymphomas (*e.g.*, mycosis fungoides, Sezary syndrome, etc.), extranodal natural killer/T-cell lymphoma (nasal-type), enteropathy type intestinal T-cell lymphoma, large granular lymphocyte leukemia, precursor T-lymphoblastic lymphoma/leukemia (T-LBL/L), T-cell chronic lymphocytic leukemia/prolymphocytic leukemia (T-CLL/PLL), and unspecified peripheral T-cell lymphoma. Symptoms and signs of Hodgkin's lymphoma include painless swelling in the lymph nodes in the neck, armpits, or groin, fever, night sweats, weight loss, fatigue, itching, abdominal pain, coughing, or chest pain.

[0617] CD19 expression has also been associated with Waldenstrom's macroglobulinemia (WM), also known as lymphoplasmacytic lymphoma (LPL). (See Elsawa *et al.*, *Blood*. 2006, 107(7):2882-8). Waldenstrom's macroglobulinemia was previously considered to be related to multiple myeloma, but has more recently been classified as a subtype of non-Hodgkin's lymphoma. WM is characterized by uncontrolled B-cell lymphocyte proliferation, resulting in anemia and production of excess amounts of paraprotein, or immunoglobulin M (IgM), which thickens the blood and results in hyperviscosity syndrome. Other symptoms or signs of WM include fever, night sweats, fatigue, anemia, weight loss, lymphadenopathy or splenomegaly, blurred vision, dizziness, nose bleeds, bleeding gums, unusual bruises, renal impairment or failure, amyloidosis, or peripheral neuropathy.

[0618] Another example of a disease or disorder associated with CD19 expression is brain cancer. Specifically, expression of CD19 has been associated with astrocytoma or glioblastoma (*See* Deshayes *et al*, *Oncogene*. 2004, 23(17):3005-12, Pelekanou *et al.*, *PLoS One*. 2013, 8(12):e83250). Astrocytomas are tumors that arise from astrocytes, which are a type of glial cell in the brain. Glioblastoma (also known as glioblastoma multiforme or GBM) is the most malignant form of astrocytoma, and is considered the most advanced stage of brain cancer (stage IV). There are two variants of glioblastoma: giant cell glioblastoma and gliosarcoma. Other astrocytomas include juvenile pilocytic astrocytoma (JPA), fibrillary astrocytoma, pleomorphic xantroastrocytoma (PXA), desembryoplastic neuroepithelial tumor (DNET), and anaplastic astrocytoma (AA).

[0619] Symptoms or signs associated with glioblastoma or astrocytoma include increased pressure in the brain, headaches, seizures, memory loss, changes in behavior, loss in

movement or sensation on one side of the body, language dysfunction, cognitive impairments, visual impairment, nausea, vomiting, and weakness in the arms or legs.

[0620] Surgical removal of the tumor (or resection) is the standard treatment for removal of as much of the glioma as possible without damaging or with minimal damage to the normal, surrounding brain. Radiation therapy and/or chemotherapy are often used after surgery to suppress and slow recurrent disease from any remaining cancer cells or satellite lesions. Radiation therapy includes whole brain radiotherapy (conventional external beam radiation), targeted three-dimensional conformal radiotherapy, and targeted radionuclides. Chemotherapeutic agents commonly used to treat glioblastoma include temozolomide, gefitinib or erlotinib, and cisplatin. Angiogenesis inhibitors, such as Bevacizumab (Avastin®), are also commonly used in combination with chemotherapy and/or radiotherapy.

[0621] Supportive treatment is also frequently used to relieve neurological symptoms and improve neurologic function, and is administered in combination any of the cancer therapies described herein. The primary supportive agents include anticonvulsants and corticosteroids. Thus, the compositions and methods of the present disclosure can be used in combination with any of the standard or supportive treatments to treat a glioblastoma or astrocytoma.

[0622] The present disclosure provides for compositions and methods for treating cancer. In one aspect, the cancer is a hematologic cancer including but not limited to a leukemia or a lymphoma. In one aspect, disclosed herein are methods of treating cancers and malignancies including, but not limited to, *e.g.*, acute leukemias including but not limited to, *e.g.*, B-cell acute lymphoid leukemia ("BALL"), T-cell acute lymphoid leukemia ("TALL"), acute lymphoid leukemia (ALL); one or more chronic leukemias including but not limited to, *e.g.*, chronic myelogenous leukemia (CML), chronic lymphocytic leukemia (CLL); additional hematologic cancers or hematologic conditions including, but not limited to, *e.g.*, B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma, Follicular lymphoma, Hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma, mantle cell lymphoma, Marginal zone lymphoma, multiple myeloma, myelodysplasia and myelodysplastic syndrome, non-Hodgkin's lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, and "preleukemia" which are a diverse collection of hematological conditions united by ineffective production (or dysplasia) of myeloid blood cells, and the like. Further diseases associated with CD19 expression include, but are not limited to, *e.g.*, atypical and/or non-classical cancers, malignancies, precancerous conditions or proliferative diseases expressing CD19.

[0623] In some embodiments, a CD19 binding molecule can be used to treat a disease including but not limited to a plasma cell proliferative disorder, *e.g.*, asymptomatic myeloma (smoldering multiple myeloma or indolent myeloma), monoclonal gammapathy of undetermined significance (MGUS), Waldenstrom's macroglobulinemia, plasmacytomas (*e.g.*, plasma cell dyscrasia, solitary myeloma, solitary plasmacytoma, extramedullary plasmacytoma, and multiple plasmacytoma), systemic amyloid light chain amyloidosis, and POEMS syndrome (also known as Crow-Fukase syndrome, Takatsuki disease, and PEP syndrome).

[0624] In some embodiments, the CD19 binding molecules are used to treat B cell malignancies.

[0625] In some embodiments, the B cell malignancy is a hematological cancer.

[0626] In some embodiments, the B cell malignancy is a malignant lymphoproliferative condition.

[0627] In some embodiments, the B cell malignancy is a plasma cell dyscrasia.

[0628] In some embodiments, the B cell malignancy is an acute leukemia. In some embodiments, the B cell malignancy is B cell acute lymphocytic leukemia (also known as B cell acute lymphoblastic leukaemia or B cell acute lymphoid leukemia) (ALL or B-ALL), *e.g.*, relapsed and/or refractory B-ALL.

[0629] In some embodiments, the B cell malignancy is a non-Hodgkin's lymphoma (NHL), for example, chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL), follicular lymphoma (FL), mantle cell lymphoma (MCL), diffuse large B-cell lymphoma (DLBCL), Burkitt lymphoma, lymphoplasmacytic lymphoma (Waldenstrom macroglobulinemia), MALT lymphoma (mucosa-associated lymphoid tissue lymphoma) marginal zone lymphoma (MZL) (*e.g.*, extranodal marginal zone lymphoma (EMZL) or nodal marginal zone B-cell lymphoma (NZML)).

[0630] In some embodiments, the B cell malignancy is a relapsed and/or refractory non-Hodgkin's lymphoma (NHL).

[0631] In some embodiments, the B cell malignancy is chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL), *e.g.*, relapsed and/or refractory CLL/SLL.

[0632] In some embodiments, the B cell malignancy is follicular lymphoma (FL), *e.g.*, relapsed and/or refractory FL. In some embodiments, the FL is small cell FL. In other embodiments, the FL is large cell FL.

[0633] In some embodiments, the B cell malignancy is mantle cell lymphoma (MCL), *e.g.*, relapsed and/or refractory MCL.

[0634] In some embodiments, the B cell malignancy is diffuse large B-cell lymphoma (DLBCL), *e.g.*, relapsed and/or refractory DLBCL.

[0635] In some embodiments, the B cell malignancy is Burkitt lymphoma.

[0636] In some embodiments, the B cell malignancy is lymphoplasmacytic lymphoma (Waldenstrom macroglobulinemia).

[0637] In some embodiments, the B cell malignancy is MALT lymphoma (mucosa-associated lymphoid tissue lymphoma).

[0638] In some embodiments, the B cell malignancy is marginal zone lymphoma (MZL).

[0639] In some embodiments, the B cell malignancy is extranodal marginal zone lymphoma (EMZL).

[0640] In some embodiments, the B cell malignancy is nodal marginal zone B-cell lymphoma (NZML).

[0641] In some embodiments, the B cell malignancy is splenic marginal zone B-cell lymphoma (SMZL).

[0642] In some embodiments, the B cell malignancy is a Hodgkin's lymphoma.

[0643] In some embodiments, the B cell malignancy is multiple myeloma.

[0644] In some embodiments, the B cell malignancy is hairy cell leukemia.

[0645] In some embodiments, the B cell malignancy is primary effusion lymphoma.

[0646] In some embodiments, the B cell malignancy is B cell prolymphocytic leukemia.

[0647] In some embodiments, the B cell malignancy is plasmablastic lymphoma.

[0648] In some embodiments, the B cell malignancy is follicle center lymphoma.

[0649] In some embodiments, the B cell malignancy is precursor B-lymphoblastic leukemia.

[0650] In some embodiments, the B cell malignancy is high-grade B-cell lymphoma.

[0651] In some embodiments, the B cell malignancy is primary mediastinal large B-cell lymphoma.

[0652] Certain aspects of the foregoing embodiments relate to subjects having an NHL and who (i) have failed at least one prior line (and optionally up to five prior lines) of standard of care therapy, e.g., an anti-CD20 therapy such as rituximab and/or (ii) is intolerant to or ineligible for one or more other approved therapies, e.g., autologous stem cell transplant (ASCT) and/or (iii) is a non-responder to a chimeric antigen receptor (CAR) T cell therapy. The NHL can be chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL), follicular lymphoma

(FL), mantle cell lymphoma (MCL), diffuse large B-cell lymphoma (DLBCL), Burkitt lymphoma, lymphoplasmacytic lymphoma (Waldenstrom macroglobulinemia), MALT lymphoma (mucosa-associated lymphoid tissue lymphoma) marginal zone lymphoma (MZL) (e.g., extranodal marginal zone lymphoma (EMZL) or nodal marginal zone B-cell lymphoma (NZML)). In some embodiments, the NHL can relapsed and/or refractory, such as relapsed and/or refractory DLBCL or MCL.

[0653] Thus, in certain aspects, a subject having an NHL to whom a CD19 binding molecule of the disclosure is administered has failed at least one prior line of standard of care therapy and optionally up to five standard of care therapies. In various embodiments, the subject has failed one, two, three, four or five standard of care therapies. Exemplary standard of care therapies for B cell malignancies include anti-CD20 therapies such as rituximab.

[0654] In further aspects, a subject having an NHL to whom a CD19 binding molecule of the disclosure is administered is intolerant to or ineligible for one or more other approved therapies, *e.g.*, autologous stem cell transplant (ASCT).

[0655] In yet further aspects, a subject having an NHL to whom a CD19 binding molecule of the disclosure is administered is a non-responder to chimeric antigen receptor (CAR) T cell therapy composition ("CAR composition"), *e.g.*, an anti-CD19 CAR composition. In certain embodiments, the CAR composition comprises CTL019. In other embodiments, the CAR composition has the USAN or INN designation tisagenlecleucel. Tisagenlecleucel is marketed as KYMRIAH®. See, *e.g.*, KYMRIAH® prescribing information, available at www.pharma.us.novartis.com/sites/www.pharma.us.novartis.com/files/kymriah.pdf. In other embodiments, the CAR composition has the USAN or INN designation axicabtagene ciloleucel. Axicabtagene ciloleucel is marketed as YESCARTA®. See, *e.g.*, YESCARTA® prescribing information, available at www.yescarta.com/files/yescarta-pi.pdf. In other aspects, the CAR composition has the USAN designation brexucabtagene autoleucel. Brexucabtagene autoleucel is marketed as TECARTUS™. See, *e.g.*, TECARTUS™ prescribing information, available at www.gilead.com/-/media/files/pdfs/medicines/oncology/tecartus/tecartus-pi.pdf. In yet other embodiments, the CAR composition has the USAN or INN designation lisocabtagene maraleucel. Lisocabtagene maraleucel is marketed as BREYANZI®. See, *e.g.*, BREYANZI® prescribing information, available at packageinserts.bms.com/pi/pi_breyanzi.pdf.

[0656] In some embodiments, a CD19 binding molecule can be used to treat a disease including but not limited to a cancer, *e.g.*, a cancer described herein, *e.g.*, a prostate cancer (*e.g.*, castrate-resistant or therapy-resistant prostate cancer, or metastatic prostate cancer), pancreatic cancer, or lung cancer.

[0657] The present disclosure also provides methods for inhibiting the proliferation or reducing a CD19-expressing cell population, the methods comprising contacting a population of cells comprising a CD19-expressing cell with a CD19 binding molecule. In a specific aspect, the present disclosure provides methods for inhibiting the proliferation or reducing the population of cancer cells expressing CD19, the methods comprising contacting the CD19-expressing cancer cell population with a CD19 binding molecule. In one aspect, the disclosure provides methods for inhibiting the proliferation or reducing the population of cancer cells expressing CD19, the methods comprising contacting the BMCA-expressing cancer cell population with a CD19 binding molecule. In certain aspects, the methods reduce the quantity, number, amount or percentage of cells and/or cancer cells by at least 25%, at least 30%, at least 40%, at least 50%, at least 65%, at least 75%, at least 85%, at least 95%, or at least 99% in a subject with or an animal model for myeloid leukemia or another cancer associated with CD19-expressing cells relative to a negative control. In one aspect, the subject is a human.

[0658] The present disclosure provides methods for preventing relapse of cancer associated with CD19-expressing cells, the methods comprising administering to a subject in need thereof a CD19 binding molecule.

7.16.2. Non-cancer related diseases and disorders

[0659] Non-cancer related diseases and disorders associated with CD19 expression, for example immune conditions, can also be treated by the compositions and methods disclosed herein. Such immune conditions can be characterized by inappropriate activation of immune cells and are typically classified into four types: anaphylactic reactions, cytotoxic (cytolytic) reactions, immune complex reactions, or cell-mediated immunity (CMI) reactions (also referred to as delayed-type hypersensitivity (DTH) reactions). (See, *e.g.*, Fundamental Immunology, William E. Paul ed., Raven Press, N.Y., 3rd ed. 1993.)

[0660] Specific examples of such immunological diseases include, but are not limited to, rheumatoid arthritis, multiple sclerosis, endocrine ophthalmopathy, uveoretinitis, systemic lupus erythematosus, myasthenia gravis, Grave's disease, glomerulonephritis, autoimmune hepatological disorder, autoimmune inflammatory bowel disease, anaphylaxis, allergic reaction, Sjogren's syndrome, juvenile onset (Type I) diabetes mellitus, primary biliary cirrhosis, Wegener's granulomatosis, fibromyalgia, inflammatory bowel disease, polymyositis, dermatomyositis, multiple endocrine failure, Schmidt's syndrome, autoimmune uveitis, Addison's disease, adrenalitis, thyroiditis, Hashimoto's thyroiditis, autoimmune thyroid disease, pernicious anemia, gastric atrophy, chronic hepatitis, lupoid hepatitis, atherosclerosis, presenile dementia, demyelinating diseases, subacute cutaneous lupus erythematosus, hypoparathyroidism, Dressler's syndrome, autoimmune thrombocytopenia, idiopathic thrombocytopenic purpura, hemolytic anemia, pemphigus vulgaris, pemphigus, dermatitis

herpetiformis, alopecia arcata, pemphigoid, scleroderma, progressive systemic sclerosis, CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia), adult onset diabetes mellitus (Type II diabetes), male and female autoimmune infertility, ankylosing spondolytis, ulcerative colitis, Crohn's disease, mixed connective tissue disease, polyarteritis nedosa, systemic necrotizing vasculitis, juvenile onset rheumatoid arthritis, atopic dermatitis, atopic rhinitis, Goodpasture's syndrome, Chagas' disease, sarcoidosis, rheumatic fever, asthma, recurrent abortion, anti-phospholipid syndrome, farmer's lung, erythema multiforme, post cardiotomy syndrome, Cushing's syndrome, autoimmune chronic active hepatitis, bird-fancier's lung, allergic encephalomyelitis, toxic epidermal necrolysis, Alport's syndrome, alveolitis, allergic alveolitis, fibrosing alveolitis, interstitial lung disease, erythema nodosum, pyoderma gangrenosum, transfusion reaction, leprosy, malaria, leishmaniasis, trypanosomiasis, Takayasu's arteritis, polymyalgia rheumatica, temporal arteritis, schistosomiasis, giant cell arteritis, ascariasis, aspergillosis, Sampter's syndrome, eczema, lymphomatoid granulomatosis, Behcet's disease, Caplan's syndrome, Kawasaki's disease, dengue, encephalomyelitis, endocarditis, endomyocardial fibrosis, endophthalmitis, erythema elevatum et diutinum, psoriasis, erythroblastosis fetalis, eosinophilic faciitis, Shulman's syndrome, Felty's syndrome, filariasis, cyclitis, chronic cyclitis, heterochronic cyclitis, Fuch's cyclitis, IgA nephropathy, Henoch-Schonlein purpura, graft versus host disease, transplantation rejection, human immunodeficiency virus infection, echovirus infection, cardiomyopathy, Alzheimer's disease, parvovirus infection, rubella virus infection, post vaccination syndromes, congenital rubella infection, Eaton-Lambert syndrome, relapsing polychondritis, cryoglobulinemia, Waldenstrom's macroglobulemia, Epstein-Barr virus infection, mumps, Evan's syndrome, and autoimmune gonadal failure.

[0661] Accordingly, the methods described herein encompass treatment of disorders of B lymphocytes (*e.g.*, systemic lupus erythematosus, Goodpasture's syndrome, rheumatoid arthritis, and type I diabetes), Th_1-lymphocytes (*e.g.*, rheumatoid arthritis, multiple sclerosis, psoriasis, Sjorgren's syndrome, Hashimoto's thyroiditis, Grave's disease, primary biliary cirrhosis, Wegener's granulomatosis, tuberculosis, or graft versus host disease), or Th_2-lymphocytes (*e.g.*, atopic dermatitis, systemic lupus erythematosus, atopic asthma, rhinoconjunctivitis, allergic rhinitis, or chronic graft versus host disease). Generally, disorders involving dendritic cells involve disorders of Th_1-lymphocytes or Th_2-lymphocytes.

[0662] In some embodiments, the CD19 binding molecules are used to treat an autoimmune disease, for example an autoimmune disease that is mediated at least in part by B cells. Examples of autoimmune diseases include acute necrotizing hemorrhagic leukoencephalitis; Addison's disease; Agammaglobulinemia; Allergic asthma; Allergic rhinitis; Alopecia areata; Amyloidosis; Ankylosing spondylitis; Anti-GBM/Anti-TBM nephritis; Antiphospholipid syndrome;

Autoimmune aplastic anemia; Autoimmune dysautonomia; Autoimmune hepatitis; Autoimmune hyperlipidemia; Autoimmune immunodeficiency; Autoimmune inner ear disease; Autoimmune myocarditis; Autoimmune thrombocytopenic purpura; Axonal & neuronal neuropathies; Balo disease; Behcet's disease; Bullous pemphigoid; Cardiomyopathy; Castleman disease; Celiac sprue (nontropical); Chagas disease; Chronic fatigue syndrome; Chronic inflammatory demyelinating polyneuropathy; Churg-Strauss syndrome; Cicatricial pemphigoid/benign mucosal pemphigoid; Crohn's disease; Cogans syndrome; Cold agglutinin disease; Congenital heart block; Coxsackie myocarditis; CREST disease; Essential mixed cryoglobulinemia; Demyelinating neuropathies; Dermatomyositis; Devic disease; Discoid lupus; Dressler's syndrome; Endometriosis; Eosinophilic fasciitis; Erythema nodosum; Experimental allergic encephalomyelitis; Evans syndrome; Fibromyalgia; Fibrosing alveolitis; Giant cell arteritis (temporal arteritis); Goodpasture's syndrome; Graves' disease; Guillain-Barre syndrome; Hashimoto's disease; Hemolytic anemia; Henoch-Schonlein purpura; Herpes gestationis; Hypogammaglobulinemia; Idiopathic thrombocytopenic purpura; IgA nephropathy; Immunoregulatory lipoproteins; Inclusion body myositis; Insulin-dependent diabetes (type1); Interstitial cystitis; Juvenile arthritis; Juvenile diabetes; Kawasaki syndrome; Lambert-Eaton syndrome; Leukocytoclastic vasculitis; Lichen planus; Lichen sclerosus; Ligneous conjunctivitis; Linear IgA disease (LAD); Lupus (SLE); Lyme disease; Meniere's disease; Microscopic polyangiitis; Mixed connective tissue disease; Mooren's ulcer; Mucha-Habermann disease; Multiple sclerosis; Myasthenia gravis; Myositis; Narcolepsy; Neutropenia; Ocular cicatricial pemphigoid; Osteoarthritis; Palindromic rheumatism; Paraneoplastic cerebellar degeneration; Paroxysmal nocturnal hemoglobinuria; Parsonnage-Turner syndrome; Pars planitis (peripheral uveitis); Pemphigus; Peripheral neuropathy; Perivenous encephalomyelitis; Pernicious anemia; POEMS syndrome; Polyarteritis nodosa; Type I, II, & III autoimmune polyglandular syndromes; Polymyalgia rheumatica; Polymyositis; Postmyocardial infarction syndrome; Postpericardiotomy syndrome; Progesterone dermatitis; Primary biliary cirrhosis; Psoriasis; Psoriatic arthritis; Idiopathic pulmonary fibrosis; Pyoderma gangrenosum; Pure red cell aplasia; Raynauds phenomenon; Reflex sympathetic dystrophy; Reiter's syndrome; Relapsing polychondritis; Restless legs syndrome; Rheumatic fever; Rheumatoid arthritis; Sarcoidosis; Schmidt syndrome; Scleritis; Scleroderma; Sjogren's syndrome; Sperm & testicular autoimmunity; Stiff person syndrome; Subacute bacterial endocarditis; Sympathetic ophthalmia; Takayasu's arteritis; Temporal arteritis/Giant cell arteritis; Thrombocytopenic purpura; Autoimmune thyroid disease; Tolosa-Hunt syndrome; Transverse myelitis & necrotizing myelopathy; Ulcerative colitis; Undifferentiated connective tissue disease; Uveitis; Vasculitis; Vesiculobullous dermatosis; Vitiligo; and Wegener's granulomatosis. The more common autoimmune diseases that are of especial interest include (a) connective tissue diseases such as systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosos (scleroderma), Sjogren's syndrome, (b)

neuromuscular diseases such as multiple sclerosos, myasthenis gravis, Guillain-Barre syndrome, (c) endocrine diseases such as Hashimoto's thryoiditis, Grave's disease, insulin-dependent (type 1) diabetes, and (d) gastrointestinal diseases such as inflammatory bowel disease (including Crohn's disease and ulcerative colitis), and (e) other diseases such as vasculitis syndromes, hematologic autoimmune diseases, and autoimmune skin diseases.

[0663] The autoimmune disease can be characterized by the presence of autoantibodies. The autoantibody can bind specifically to host targets or antigens, for example rheumatoid factor (*e.g.*, in rheumatoid arthritis); topoisomerase (*e.g.*, in scleroderma); myelin basic protein (*e.g.*, in multiple sclerosis); basement membrane collagen type iv protein (*e.g.*, in Goodpasture's syndrome); ganglioside (*e.g.*, in Guillain-Barre syndrome); platelets (*e.g.*, chronic idiopathic thrombocytopenia); smooth muscle actin (*e.g.*, in autoimmune hepatitis); bullous pemphigoid antigen 1 and 2; also called hemidesmosome antigens (*e.g.*, in bullous pemphigoid); transglutaminase (*e.g.*, in coeliac disease); desmogein 3 (*e.g.*, in pemphigus vulgaris); p62 or sp100 or mitochondrial (m2) antigens (*e.g.*, in primary biliary cirrhosis); neutrophil cytoplasmic c-ANCA (*e.g.*, in Wegener's granulomatosis); neutrophil perinuclear p-ANCA (*e.g.*, Polyarteritis nodosa, Microscopic polyangiitis, Churg-Strauss syndrome, Systemic vasculitides (non-specific)); double-stranded-DNA (*e.g.*, in systemic lupus erythematosus); exosome complex (*e.g.*, in Scleromyositis); Ro or La antigen (*e.g.*, in systemic lupus erythematosus and neonatal heart block, or primary Sjogren's syndrome); Smith antigen (*e.g.*, in systemic lupus erythematosus); phospholipid antigen (*e.g.*, in antiphospholipid syndrome); SSA or SSB antigen (*e.g.*, in Sjogren's syndrome); centromere (*e.g.*, in CREST syndrome; mitochondria (*e.g.*, in primary biliary cirrhosis); nicotinic acetylcholine receptor (*e.g.*, in myasthenia gravis); voltage-gated calcium channel (*e.g.*, in Lambert-Eaton syndrome); thyroid peroxidase (*e.g.*, in Hashimoto's thyroiditis); TSH receptor (*e.g.*, in Grave's disease); Hu antigen (*e.g.*, in paraneoplastic cerebellar syndrome); voltage-gated potassium channel (*e.g.*, in limbic encephalitis and N-methyl-D-aspartate receptor (*e.g.*, in encephalitis). More than one type of autoantibody can be associated with an immunological disorder or visa versa, and this list in not exhaustive. For example, autoantigens that have been identified in rheumatoid arthritis include joint-associated proteins such as collagen type II, human chondrocyte glycoprotein 39, and proteoglycans; as well as heat shock proteins, citrullinated filaggrin, immunoglobulin, glucose-6-phosphate isomerase, p205, and BiP.

[0664] Autoimmune disorders that can be treated with the CD19 binding molecules of the disclosure include systemic lupus erythematosus (SLE), Sjögren's syndrome, scleroderma, rheumatoid arthritis (RA), juvenile idiopathic arthritis, graft versus host disease, dermatomyositis, type I diabetes mellitus, Hashimoto's thyroiditis, Graves's disease, Addison's disease, celiac disease, disorders related to mucosal immunity, irritable bowel diseases (*e.g.*,

Crohn's Disease, ulcerative colitis), pernicious anaemia, pemphigus vulgaris, vitiligo, autoimmune haemolytic anaemia, idiopathic thrombocytopenic purpura, giant cell arteritis, myasthenia gravis, multiple sclerosis (MS) (*e.g.*, relapsing-remitting MS (RRMS)), glomerulonephritis, Goodpasture's syndrome, bullous pemphigoid, colitis ulcerosa, Guillain-Barré syndrome, chronic inflammatory demyelinating polyneuropathy, anti-phospholipid syndrome, narcolepsy, sarcoidosis, and Wegener's granulomatosis.

[0665] In some embodiments, the CD19 binding molecules are used to treat systemic lupus erythematosus (SLE).

[0666] In some embodiments, the CD19 binding molecules are used to treat Sjögren's syndrome.

[0667] In some embodiments, the CD19 binding molecules are used to treat scleroderma.

[0668] In some embodiments, the CD19 binding molecules are used to treat rheumatoid arthritis (RA).

[0669] In some embodiments, the CD19 binding molecules are used to treat juvenile idiopathic arthritis.

[0670] In some embodiments, the CD19 binding molecules are used to treat graft versus host disease.

[0671] In some embodiments, the CD19 binding molecules are used to treat dermatomyositis.

[0672] In some embodiments, the CD19 binding molecules are used to treat type I diabetes mellitus.

[0673] In some embodiments, the CD19 binding molecules are used to treat Hashimoto's thyroiditis.

[0674] In some embodiments, the CD19 binding molecules are used to treat Graves's disease.

[0675] In some embodiments, the CD19 binding molecules are used to treat Addison's disease.

[0676] In some embodiments, the CD19 binding molecules are used to treat celiac disease.

[0677] In some embodiments, the CD19 binding molecules are used to treat Crohn's Disease.

[0678] In some embodiments, the CD19 binding molecules are used to treat pernicious anaemia.

[0679] In some embodiments, the CD19 binding molecules are used to treat pemphigus vulgaris.

[0680] In some embodiments, the CD19 binding molecules are used to treat vitiligo.

[0681] In some embodiments, the CD19 binding molecules are used to treat autoimmune haemolytic anaemia.

[0682] In some embodiments, the CD19 binding molecules are used to treat idiopathic thrombocytopenic purpura.

[0683] In some embodiments, the CD19 binding molecules are used to treat giant cell arteritis.

[0684] In some embodiments, the CD19 binding molecules are used to treat myasthenia gravis.

[0685] In some embodiments, the CD19 binding molecules are used to treat multiple sclerosis (MS). In some embodiments, the MS is relapsing-remitting MS (RRMS).

[0686] In some embodiments, the CD19 binding molecules are used to treat glomerulonephritis.

[0687] In some embodiments, the CD19 binding molecules are used to treat Goodpasture's syndrome.

[0688] In some embodiments, the CD19 binding molecules are used to treat bullous pemphigoid.

[0689] In some embodiments, the CD19 binding molecules are used to treat colitis ulcerosa.

[0690] In some embodiments, the CD19 binding molecules are used to treat Guillain-Barré syndrome.

[0691] In some embodiments, the CD19 binding molecules are used to treat chronic inflammatory demyelinating polyneuropathy.

[0692] In some embodiments, the CD19 binding molecules are used to treat anti-phospholipid syndrome.

[0693] In some embodiments, the CD19 binding molecules are used to treat narcolepsy.

[0694] In some embodiments, the CD19 binding molecules are used to treat sarcoidosis.

[0695] In some embodiments, the CD19 binding molecules are used to treat Wegener's granulomatosis.

[0696] Examples of other non-cancer related diseases and disorders associated with CD19 expression include, but are not limited to: viral, *e.g.*, HIV, infections and fungal, *e.g.*, *C. neoformans*, infections.

7.17. Combination Therapy

[0697] A CD19 binding molecule of the disclosure can be used in combination other known agents and therapies. For example, the CD19 binding molecules can be used in treatment

regimens in combination with surgery, chemotherapy, antibodies, radiation, peptide vaccines, steroids, cytoxins, proteasome inhibitors, immunomodulatory drugs (*e.g.*, IMiDs), BH3 mimetics, cytokine therapies, stem cell transplant or any combination thereof.

[0698] For convenience, an agent that is used in combination with a CD19 binding molecule is referred to herein as an "additional" agent.

[0699] Administered "in combination," as used herein, means that two (or more) different treatments are delivered to the subject during the course of the subject's affliction with the disorder, *e.g.*, the two or more treatments are delivered after the subject has been diagnosed with the disorder and before the disorder has been cured or eliminated or treatment has ceased for other reasons. In some embodiments, the delivery of one treatment is still occurring when the delivery of the second begins, so that there is overlap in terms of administration. This is sometimes referred to herein as "simultaneous" or "concurrent delivery". For example, each therapy can be administered to a subject at the same time or sequentially in any order at different points in time; however, if not administered at the same time, they should be administered sufficiently close in time so as to provide the desired therapeutic effect.

[0700] A CD19 binding molecule and one or more additional agents can be administered simultaneously, in the same or in separate compositions, or sequentially. For sequential administration, the CD19 binding molecule can be administered first, and the additional agent can be administered second, or the order of administration can be reversed.

[0701] The CD19 binding molecule and the additional agent(s) can be administered to a subject in any appropriate form and by any suitable route. In some embodiments, the routes of administration are the same. In other embodiments the routes of administration are different.

[0702] In other embodiments, the delivery of one treatment ends before the delivery of the other treatment begins.

[0703] In some embodiments of either case, the treatment is more effective because of combined administration. For example, the second treatment is more effective, *e.g.*, an equivalent effect is seen with less of the second treatment, or the second treatment reduces symptoms to a greater extent, than would be seen if the second treatment were administered in the absence of the first treatment, or the analogous situation is seen with the first treatment. In some embodiments, delivery is such that the reduction in a symptom, or other parameter related to the disorder is greater than what would be observed with one treatment delivered in the absence of the other. The effect of the two treatments can be partially additive, wholly additive, or greater than additive. The delivery can be such that an effect of the first treatment delivered is still detectable when the second is delivered.

[0704] The CD19 binding molecules and/or additional agents can be administered during periods of active disorder, or during a period of remission or less active disease. A CD19 binding molecule can be administered before the treatment with the additional agent(s), concurrently with the treatment with the additional agent(s), post-treatment with the additional agent(s), or during remission of the disorder.

[0705] When administered in combination, the CD19 binding molecule and/or the additional agent(s) can be administered in an amount or dose that is higher, lower or the same than the amount or dosage of each agent used individually, *e.g.*, as a monotherapy.

[0706] The additional agent(s) of the combination therapies of the disclosure can be administered to a subject concurrently. The term "concurrently" is not limited to the administration of therapies (*e.g.*, prophylactic or therapeutic agents) at exactly the same time, but rather it is meant that a pharmaceutical composition comprising a CD19 binding molecule is administered to a subject in a sequence and within a time interval such that the molecules of the disclosure can act together with the additional therapy(ies) to provide an increased benefit than if they were administered otherwise. For example, each therapy can be administered to a subject at the same time or sequentially in any order at different points in time; however, if not administered at the same time, they should be administered sufficiently close in time so as to provide the desired therapeutic or prophylactic effect. Each therapy can be administered to a subject separately, in any appropriate form and by any suitable route.

[0707] The CD19 binding molecule and the additional agent(s) can be administered to a subject by the same or different routes of administration.

[0708] The CD19 binding molecules and the additional agent(s) can be cyclically administered. Cycling therapy involves the administration of a first therapy (*e.g.*, a first prophylactic or therapeutic agent) for a period of time, followed by the administration of a second therapy (*e.g.*, a second prophylactic or therapeutic agent) for a period of time, optionally, followed by the administration of a third therapy (*e.g.*, prophylactic or therapeutic agent) for a period of time and so forth, and repeating this sequential administration, *i.e.*, the cycle in order to reduce the development of resistance to one of the therapies, to avoid or reduce the side effects of one of the therapies, and/or to improve the efficacy of the therapies.

[0709] In certain embodiments, the one or more additional agents, are other anti-cancer agents, anti-allergic agents, anti-nausea agents (or anti-emetics), pain relievers, cytoprotective agents, and combinations thereof.

[0710] In one embodiment, a CD19 binding molecule can be used in combination with an anti-cancer agent (*e.g.*, a chemotherapeutic agent). Exemplary chemotherapeutic agents include an anthracycline (*e.g.*, doxorubicin (*e.g.*, liposomal doxorubicin)), a vinca alkaloid (*e.g.*,

vinblastine, vincristine, vindesine, vinorelbine), an alkylating agent (*e.g.*, cyclophosphamide, decarbazine, melphalan, ifosfamide, temozolomide), an immune cell antibody (*e.g.*, alemtuzamab, gemtuzumab, rituximab, tositumomab, obinutuzumab, ofatumumab, daratumumab, elotuzumab), an antimetabolite (including, *e.g.*, folic acid antagonists, pyrimidine analogs, purine analogs and adenosine deaminase inhibitors (*e.g.*, fludarabine)), an mTOR inhibitor, a TNFR glucocorticoid induced TNFR related protein (GITR) agonist, a proteasome inhibitor (*e.g.*, aclacinomycin A, gliotoxin or bortezomib), an immunomodulator such as an immunomodulatory imide drug (IMiD), including thalidomide or a thalidomide derivative (*e.g.*, lenalidomide, pomalidomide, or iberdomide). In some embodiments, the anti-cancer agent is lenalidomide.

[0711] General chemotherapeutic agents considered for use in combination therapies include anastrozole (Arimidex®), bicalutamide (Casodex®), bleomycin sulfate (Blenoxane®), busulfan (Myleran®), busulfan injection (Busulfex®), capecitabine (Xeloda®), N4-pentoxycarbonyl-5-deoxy-5-fluorocytidine, carboplatin (Paraplatin®), carmustine (BiCNU®), chlorambucil (Leukeran®), cisplatin (Platinol®), cladribine (Leustatin®), cyclophosphamide (Cytoxan® or Neosar®), cytarabine, cytosine arabinoside (Cytosar-U®), cytarabine liposome injection (DepoCyt®), dacarbazine (DTIC-Dome®), dactinomycin (Actinomycin D, Cosmegan), daunorubicin hydrochloride (Cerubidine®), daunorubicin citrate liposome injection (DaunoXome®), dexamethasone, docetaxel (Taxotere®), doxorubicin hydrochloride (Adriamycin®, Rubex®), etoposide (Vepesid®), fludarabine phosphate (Fludara®), 5-fluorouracil (Adrucil®, Efudex®), flutamide (Eulexin®), tezacitibine, Gemcitabine (difluorodeoxycitidine), hydroxyurea (Hydrea®), Idarubicin (Idamycin®), ifosfamide (IFEX®), irinotecan (Camptosar®), L-asparaginase (ELSPAR®), leucovorin calcium, melphalan (Alkeran®), 6-mercaptopurine (Purinethol®), methotrexate (Folex®), mitoxantrone (Novantrone®), mylotarg, paclitaxel (Taxol®), phoenix (Yttrium90/MX-DTPA), pentostatin, polifeprosan 20 with carmustine implant (Gliadel®), tamoxifen citrate (Nolvadex®), teniposide (Vumon®), 6-thioguanine, thiotepa, tirapazamine (Tirazone®), topotecan hydrochloride for injection (Hycamptin®), vinblastine (Velban®), vincristine (Oncovin®), and vinorelbine (Navelbine®).

[0712] Anti-cancer agents of particular interest for combinations with the CD19 binding molecules of the present disclosure include: anthracyclines; alkylating agents; antimetabolites; drugs that inhibit either the calcium dependent phosphatase calcineurin or the p70S6 kinase FK506) or inhibit the p70S6 kinase; mTOR inhibitors; immunomodulators; anthracyclines; vinca alkaloids; proteasome inhibitors; GITR agonists (*e.g.*, GWN323); protein tyrosine phosphatase inhibitors; a CDK4 kinase inhibitor; a BTK inhibitor; a MKN kinase inhibitor; a DGK kinase inhibitor; an oncolytic virus; a BH3 mimetic; and cytokine therapies.

[0713] Exemplary alkylating agents include, without limitation, nitrogen mustards, ethylenimine derivatives, alkyl sulfonates, nitrosoureas and triazenes): uracil mustard (Aminouracil Mustard®, Chlorethaminacil®, Demethyldopan®, Desmethyldopan®, Haemanthamine®, Nordopan®, Uracil nitrogen mustard®, Uracillost®, Uracilmostaza®, Uramustin®, Uramustine®), chlormethine (Mustargen®), cyclophosphamide (Cytoxan®, Neosar®, Clafen®, Endoxan®, Procytox®, RevimmuneTM), ifosfamide (Mitoxana®), melphalan (Alkeran®), Chlorambucil (Leukeran®), pipobroman (Amedel®, Vercyte®), triethylenemelamine (Hemel®, Hexalen®, Hexastat®), triethylenethiophosphoramine, Temozolomide (Temodar®), thiotepa (Thioplex®), busulfan (Busilvex®, Myleran®), carmustine (BiCNU®), lomustine (CeeNU®), streptozocin (Zanosar®), and Dacarbazine (DTIC-Dome®). Additional exemplary alkylating agents include, without limitation, Oxaliplatin (Eloxatin®); Temozolomide (Temodar® and Temodal®); Dactinomycin (also known as actinomycin-D, Cosmegen®); Melphalan (also known as L-PAM, L-sarcolysin, and phenylalanine mustard, Alkeran®); Altretamine (also known as hexamethylmelamine (HMM), Hexalen®); Carmustine (BiCNU®); Bendamustine (Treanda®); Busulfan (Busulfex® and Myleran®); Carboplatin (Paraplatin®); Lomustine (also known as CCNU, CeeNU®); Cisplatin (also known as CDDP, Platinol® and Platinol®-AQ); Chlorambucil (Leukeran®); Cyclophosphamide (Cytoxan® and Neosar®); Dacarbazine (also known as DTIC, DIC and imidazole carboxamide, DTIC-Dome®); Altretamine (also known as hexamethylmelamine (HMM), Hexalen®); Ifosfamide (Ifex®); Prednumustine; Procarbazine (Matulane®); Mechlorethamine (also known as nitrogen mustard, mustine and mechloroethamine hydrochloride, Mustargen®); Streptozocin (Zanosar®); Thiotepa (also known as thiophosphoamide, TESPA and TSPA, Thioplex®); Cyclophosphamide (Endoxan®, Cytoxan®, Neosar®, Procytox®, Revimmune®); and Bendamustine HCl (Treanda®).

[0714] Exemplary mTOR inhibitors include, *e.g.*, temsirolimus; ridaforolimus (formally known as deferolimus, (1R,2R,4S)-4-[(2R)-2 [(1R,9S,12S,15R,16E,18R,19R,21R, 23S,24E,26E,28Z,30S,32S,35R)-1,18-dihydroxy-19,30-dimethoxy-15,17,21,23, 29,35-hexamethyl-2,3,10,14,20-pentaoxo-11,36-dioxa-4-azatricyclo[30.3.1.04,9] hexatriaconta-16,24,26,28-tetraen-12-yl]propyl]-2-methoxycyclohexyl dimethylphosphinate, also known as AP23573 and MK8669, and described in PCT Publication No. WO 03/064383); everolimus (Afinitor® or RAD001); rapamycin (AY22989, Sirolimus®); simapimod (CAS 164301-51-3); emsirolimus, (5-{2,4-Bis[(3S)-3-methylmorpholin-4-yl]pyrido[2,3-d]pyrimidin-7-yl}-2-methoxyphenyl)methanol (AZD8055); 2-Amino-8-[trans-4-(2-hydroxyethoxy)cyclohexyl]-6-(6-methoxy-3-pyridinyl)-4-methyl-pyrido[2,3-d]pyrimidin-7(8H)-one (PF04691502, CAS 1013101-36-4); and N2-[1,4-dioxo-4-[[4-(4-oxo-8-phenyl-4H-1-benzopyran-2-yl)morpholinium-4-yl]methoxy]butyl]-L-arginylglycyl-L-α-aspartylL-serine- (SEQ ID NO: 757), inner salt (SF1126, CAS 936487-67-1), and XL765.

[0715] Exemplary immunomodulators include, *e.g.*, afutuzumab (available from Roche®); pegfilgrastim (Neulasta®); lenalidomide (CC-5013, Revlimid®); IMIDs (such as thalidomide (Thalomid®), lenalidomide, pomalidomide, and apremilast), actimid (CC4047); and IRX-2 (mixture of human cytokines including interleukin 1, interleukin 2, and interferon γ, CAS 951209-71-5, available from IRX Therapeutics).

[0716] Exemplary anthracyclines include, *e.g.*, doxorubicin (Adriamycin® and Rubex®); bleomycin (lenoxane®); daunorubicin (dauorubicin hydrochloride, daunomycin, and rubidomycin hydrochloride, Cerubidine®); daunorubicin liposomal (daunorubicin citrate liposome, DaunoXome®); mitoxantrone (DHAD, Novantrone®); epirubicin (Ellence™); idarubicin (Idamycin®, Idamycin PFS®); mitomycin C (Mutamycin®); geldanamycin; herbimycin; ravidomycin; and desacetylravidomycin.

[0717] Exemplary vinca alkaloids include, *e.g.*, vinorelbine tartrate (Navelbine®), Vincristine (Oncovin®), and Vindesine (Eldisine®)); vinblastine (also known as vinblastine sulfate, vincaleukoblastine and VLB, Alkaban-AQ® and Velban®); and vinorelbine (Navelbine®).

[0718] Exemplary proteasome inhibitors include bortezomib (Velcade®); carfilzomib (PX-171-007, (S)-4-Methyl-N-((S)-1-(((S)-4-methyl-1-((R)-2-methyloxiran-2-yl)-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)-2-((S)-2-(2-morpholinoacetamido)-4-phenylbutanamido)-pentanamide); marizomib (NPI-0052); ixazomib citrate (MLN-9708); delanzomib (CEP-18770); and O-Methyl-N-[(2-methyl-5-thiazolyl)carbonyl]-L-seryl-O-methyl-N-[(1S)-2-[(2R)-2-methyl-2-oxiranyl]-2-oxo-1-(phenylmethyl)ethyl]- L-serinamide (ONX-0912).

[0719] Exemplary BH3 mimetics include venetoclax, ABT-737 (4-{4-[(4'-Chloro-2-biphenylyl)methyl]-1-piperazinyl}-N-[(4-{[(2R)-4-(dimethylamino)-1-(phenylsulfanyl)-2-butanyl]amino}-3-nitrophenyl)sulfonyl]benzamide and navitoclax (formerly ABT-263).

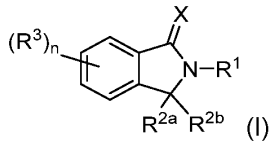
[0720] Exemplary cytokine therapies include interleukin 2 (IL-2) and interferon-alpha (IFN-alpha).

[0721] In certain aspects, "cocktails" of different chemotherapeutic agents are administered as the additional agent(s).

[0722] In some embodiments, a CD19 binding molecule can be used in combination with a member of the thalidomide class of compounds. Members of the thalidomide class of compounds include, but are not limited to, lenalidomide (CC-5013), pomalidomide (CC-4047 or ACTIMID), thalidomide, and salts and derivatives thereof. In some embodiments, the CD19 binding molecule is used in combination with a mixture of one, two, three, or more members of the thalidomide class of compounds. Thalidomide analogs and immunomodulatory properties of thalidomide analogs are described in Bodera and Stankiewicz, Recent Pat Endocr Metab

Immune Drug Discov. 2011 Sep;5(3):192-6. The structural complex of thalidomide analogs and the E3 ubiquitin is described in Gandhi *et al.*, Br J Haematol. 2014 Mar;164(6):811-21. The modulation of the E3 ubiquitin ligase by thalidomide analogs is described in Fischer *et al.*, Nature. 2014 Aug 7;512(7512):49-53.

[0723] In some embodiments, the member of the thalidomide class of compounds comprises a compound of Formula (I):



(I)

or a pharmaceutically acceptable salt, ester, hydrate, solvate, or tautomer thereof, where:

X is O or S;

R^1 is C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 heteroalkyl, carbocyclyl, heterocyclyl, aryl, or heteroaryl, each of which is optionally substituted by one or more R^4;

each of R^{2a} and R^{2b} is independently hydrogen or C_1-C_6 alkyl; or R^{2a} and R^{2b} together with the carbon atom to which they are attached form a carbonyl group or a thiocarbonyl group;

each of R^3 is independently C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 heteroalkyl, halo, cyano, -$C(O)R^A$, -$C(O)OR^B$, -OR^B, -$N(R^C)(R^D)$, -$C(O)N(R^C)(R^D)$, -$N(R^C)C(O)R^A$, -$S(O)_xR^E$, -$S(O)_xN(R^C)(R^D)$, or -$N(R^C)S(O)_xR^E$, where each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more R^6;

each R^4 is independently C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 heteroalkyl, halo, cyano, oxo, -$C(O)R^A$, -$C(O)OR^B$, -OR^B, -$N(R^C)(R^D)$, -$C(O)N(R^C)(R^D)$, -$N(R^C)C(O)R^A$, -$S(O)_xR^E$, -$S(O)_xN(R^C)(R^D)$, -$N(R^C)S(O)_xR^E$, carbocyclyl, heterocyclyl, aryl, or heteroaryl, where each alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently and optionally substituted with one or more R^7;

each of R^A, R^B, R^C, R^D, and R^E is independently hydrogen or C_1-C_6 alkyl;

each R^6 is independently C_1-C_6 alkyl, oxo, cyano, -OR^B, -$N(R^C)(R^D)$, -$C(O)N(R^C)(R^D)$, -$N(R^C)C(O)R^A$, aryl, or heteroaryl, where each aryl and heteroaryl is independently and optionally substituted with one or more R^8;

each R^7 is independently halo, oxo, cyano, -OR^B, -$N(R^C)(R^D)$, -$C(O)N(R^C)(R^D)$, or -$N(R^C)C(O)R^A$;

each R^8 is independently C_1-C_6 alkyl, cyano, -OR^B, -$N(R^C)(R^D)$, -$C(O)N(R^C)(R^D)$, or -$N(R^C)C(O)R^A$;

n is 0, 1, 2, 3 or 4; and

x is 0, 1, or 2.

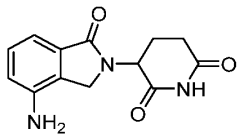
[0724] In some embodiments, X is O.

[0725] In some embodiments, R^1 is heterocyclyl. In some embodiments, R^1 is a 6-membered heterocyclyl or a 5-membered heterocyclyl. In some embodiments, R^1 is a nitrogen-containing heterocyclyl. In some embodiments, R^1 is piperidinyl (*e.g.*, piperidine-2,6-dionyl).

[0726] In some embodiments, each of R^{2a} and R^{2b} is independently hydrogen. In some embodiments, R^{2a} and R^{2b} together with the carbon to which they are attached form a carbonyl group.

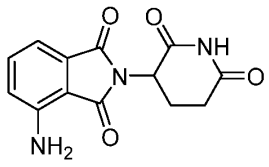
[0727] In some embodiments, R^3 is C_1-C_6 heteroalkyl, -$N(R^C)(R^D)$ or -$N(R^C)C(O)R^A$. In some embodiments, R^3 is C_1-C_6 heteroalkyl (*e.g.*, $CH_2NHC(O)CH_2$-phenyl-t-butyl), -$N(R^C)(R^D)$ (*e.g.*, NH_2), or -$N(R^C)C(O)R^A$ (*e.g.*, $NHC(O)CH_3$).

[0728] In an embodiment, X is O. In an embodiment, R^1 is heterocyclyl (*e.g.*, piperidine-2,6-dionyl). In an embodiment, each of R^{2a} and R^{2b} is independently hydrogen. In an embodiment, n is 1. In an embodiment, R^3 is -$N(R^C)(R^D)$ (*e.g.*, -NH_2). In an embodiment, the compound comprises lenalidomide, *e.g.*, 3-(4-amino-1-oxoisoindolin-2-yl)piperidine-2,6-dione, or a pharmaceutically acceptable salt thereof. In an embodiment, the compound is lenalidomide, *e.g.*, according to the following formula:



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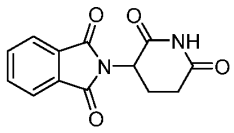
[0729] In an embodiment, X is O. In an embodiment, R^1 is heterocyclyl (*e.g.*, piperidinyl-2,6-dionyl). In some embodiments, R^{2a} and R^{2b} together with the carbon to which they are attached form a carbonyl group. In an embodiment, n is 1. In an embodiment, R^3 is -$N(R^C)(R^D)$ (*e.g.*, -NH_2). In an embodiment, the compound comprises pomalidomide, *e.g.*, 4-amino-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione, or a pharmaceutically acceptable salt thereof. In an embodiment, the compound is pomalidomide, *e.g.*, according to the following formula:



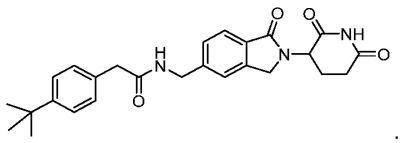
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[0730] In an embodiment, X is O. In an embodiment, R^1 is heterocyclyl (*e.g.*, piperidinyl-2,6-dionyl). In an embodiment, R^{2a} and R^{2b} together with the carbon to which they are attached

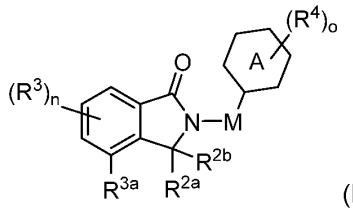
form a carbonyl group. In an embodiment, n is 0. In an embodiment, the compound comprises thalidomide, *e.g.*, 2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione, or a pharmaceutically acceptable salt thereof. In an embodiment, the product is thalidomide, *e.g.*, according to the following formula:



[0731] In an embodiment, X is O. In an embodiment, R^1 is heterocyclyl (*e.g.*, piperidine-2,6-dionyl). In an embodiment, each of R^{2a} and R^{2b} is independently hydrogen. In an embodiment, n is 1. In an embodiment, R^3 is C_1-C_6 heteroalkyl (*e.g.*, $CH_2NHC(O)CH_2$-phenyl-t-butyl) In an embodiment, the compound comprises 2-(4-(tert-butyl)phenyl)-N-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-5-yl)methyl)acetamide, or a pharmaceutically acceptable salt thereof. In an embodiment, the compound has the structure as shown in the following formula:



[0732] In some embodiments, the compound is a compound of Formula (I-a):



(I-a)

or a pharmaceutically acceptable salt, ester, hydrate, or tautomer thereof, where:

Ring A is carbocyclyl, heterocyclyl, aryl, or heteroaryl, each of which optionally substituted with one or more R^4;

M is absent, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, or C_1-C_6 heteroalkyl, where each alkyl, alkenyl, alkynyl, and heteroalkyl is optionally substituted with one or more R^4;

each of R^{2a} and R^{2b} is independently hydrogen or C_1-C_6 alkyl; or R^{2a} and R^{2b} together with the carbon atom to which they are attached to form a carbonyl group or thiocarbonyl group;

R^{3a} is hydrogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 heteroalkyl, halo, cyano, -$C(O)R^A$, -$C(O)OR^B$, -OR^B, -$N(R^C)(R^D)$, -$C(O)N(R^C)(R^D)$, -$N(R^C)C(O)R^A$, -$S(O)_xR^E$, -

$S(O)_xN(R^C)(R^D)$, or $-N(R^C)S(O)_xR^E$, where each alkyl, alkenyl, alkynyl, and heteroalkyl is optionally substituted with one or more R^6;

each of R^3 is independently C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 heteroalkyl, halo, cyano, $-C(O)R^A$, $-C(O)OR^B$, $-OR^B$, $-N(R^C)(R^D)$, $-C(O)N(R^C)(R^D)$, $-N(R^C)C(O)R^A$, $-S(O)_xR^E$, $-S(O)_xN(R^C)(R^D)$, or $-N(R^C)S(O)_xR^E$, where each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more R^6;

each R^4 is independently C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 heteroalkyl, halo, cyano, oxo, $-C(O)R^A$, $-C(O)OR^B$, $-OR^B$, $-N(R^C)(R^D)$, $-C(O)N(R^C)(R^D)$, $-N(R^C)C(O)R^A$, $S(O)_xR^E$, $-S(O)_xN(R^C)(R^D)$, $-N(R^C)S(O)_xR^E$, carbocyclyl, heterocyclyl, aryl, or heteroaryl, where each alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, or heteroaryl is independently and optionally substituted with one or more R^7;

each of R^A, R^B, R^C, R^D, and R^E is independently hydrogen or C_1-C_6 alkyl;

each R^6 is independently C_1-C_6 alkyl, oxo, cyano, $-OR^B$, $-N(R^C)(R^D)$, $-C(O)N(R^C)(R^D)$, $-N(R^C)C(O)R^A$, aryl, or heteroaryl, where each aryl or heteroaryl is independently and optionally substituted with one or more R^8;

each R^7 is independently halo, oxo, cyano, $-OR^B$, $-N(R^C)(R^D)$, $-C(O)N(R^C)(R^D)$, or $-N(R^C)C(O)R^A$;

each R^8 is independently C_1-C_6 alkyl, cyano, $-OR^B$, $-N(R^C)(R^D)$, $-C(O)N(R^C)(R^D)$, or $-N(R^C)C(O)R^A$;

n is 0, 1, 2, or 3;

o is 0, 1, 2, 3, 4, or 5; and

x is 0, 1, or 2.

[0733] In some embodiments, X is O.

[0734] In some embodiments, M is absent.

[0735] In some embodiments, Ring A is heterocyclyl. In some embodiments, Ring A is heterocyclyl, *e.g.*, a 6-membered heterocyclyl or a 5-membered heterocyclyl. In some embodiments, Ring A is a nitrogen-containing heterocyclyl. In some embodiments, Ring A is piperidinyl (*e.g.*, piperidine-2,6-dionyl).

[0736] In some embodiments, M is absent and Ring A is heterocyclyl (*e.g.*, piperidinyl, *e.g.*, piperidine-2,6-dionyl).

[0737] In some embodiments, each of R^{2a} and R^{2b} is independently hydrogen. In some embodiments, R^{2a} and R^{2b} together with the carbon to which they are attached form a carbonyl group.

[0738] In some embodiments, R^{3a} is hydrogen, $-N(R^C)(R^D)$ or $-N(R^C)C(O)R^A$. In some embodiments, R^{3a} is hydrogen. In some embodiments, R^{3a} is $-N(R^C)(R^D)$ (*e.g.*, $-NH_2$). In some embodiments, R^{3a} is $-N(R^C)C(O)R^A$ (*e.g*, $NHC(O)CH_3$).

[0739] In some embodiments, R^3 is C_1-C_6 heteroalkyl (*e.g.*, $CH_2NHC(O)CH_2$-phenyl-t-butyl). In some embodiments, n is 0 or 1. In some embodiments, n is 0. In some embodiments, n is 1.

[0740] The compound can comprise one or more chiral centers or exist as one or more stereoisomers. In some embodiments, the compound comprises a single chiral center and is a mixture of stereoisomers, *e.g.*, an R stereoisomer and an S stereoisomer. In some embodiments, the mixture comprises a ratio of R stereoisomers to S stereoisomers, for example, about a 1:1 ratio of R stereoisomers to S stereoisomers (i.e., a racemic mixture). In some embodiments, the mixture comprises a ratio of R stereoisomers to S stereoisomers of about 51:49, about 52: 48, about 53:47, about 54:46, about 55:45, about 60:40, about 65:35, about 70:30, about 75:25, about 80:20, about 85:15, about 90:10, about 95:5, or about 99:1. In some embodiments, the mixture comprises a ratio of S stereoisomers to R stereoisomers of about 51:49, about 52: 48, about 53:47, about 54:46, about 55:45, about 60:40, about 65:35, about 70:30, about 75:25, about 80:20, about 85:15, about 90:10, about 95:5, or about 99:1. In some embodiments, the compound is a single stereoisomer of Formula (I) or Formula (I-a), *e.g.*, a single R stereoisomer or a single S stereoisomer.

[0741] In some embodiments, the CD19 binding molecule is administered in combination with a kinase inhibitor. In one embodiment, the kinase inhibitor is a PI3-kinase inhibitor, *e.g.*, CLR457, BGT226, or BYL719. In one embodiment, the kinase inhibitor is a CDK4 inhibitor, *e.g.*, a CDK4 inhibitor described herein, *e.g.*, a CDK4/6 inhibitor, such as, *e.g.*, 6-Acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one, hydrochloride (also referred to as palbociclib or PD0332991). In one embodiment, the kinase inhibitor is a BTK inhibitor, *e.g.*, a BTK inhibitor described herein, such as, *e.g.*, ibrutinib. In one embodiment, the kinase inhibitor is an mTOR inhibitor, *e.g.*, an mTOR inhibitor described herein, such as, *e.g.*, rapamycin, a rapamycin analog, OSI-027. The mTOR inhibitor can be, *e.g.*, an mTORC1 inhibitor and/or an mTORC2 inhibitor, *e.g.*, an mTORC1 inhibitor and/or mTORC2 inhibitor described herein. In one embodiment, the kinase inhibitor is a MNK inhibitor, *e.g.*, a MNK inhibitor described herein, such as, *e.g.*, 4-amino-5-(4-fluoroanilino)-pyrazolo [3,4-*d*] pyrimidine. The MNK inhibitor can be, *e.g.*, a MNK1a, MNK1b, MNK2a and/or MNK2b inhibitor. In one embodiment, the kinase inhibitor is a dual PI3K/mTOR inhibitor

described herein, such as, *e.g.*, PF-04695102. In one embodiment, the kinase inhibitor is a DGK inhibitor, *e.g.*, a DGK inhibitor described herein, such as, *e.g.*, DGKinh1 (D5919) or DGKinh2 (D5794).

[0742] In one embodiment, the kinase inhibitor is a BTK inhibitor selected from ibrutinib (PCI-32765); GDC-0834; RN-486; CGI-560; CGI-1764; HM-71224; CC-292; ONO-4059; CNX-774; and LFM-A13. In an embodiment, the BTK inhibitor does not reduce or inhibit the kinase activity of interleukin-2-inducible kinase (ITK), and is selected from GDC-0834; RN-486; CGI-560; CGI-1764; HM-71224; CC-292; ONO-4059; CNX-774; and LFM-A13.

[0743] In one embodiment, the kinase inhibitor is a BTK inhibitor, *e.g.*, ibrutinib (PCI-32765). In some embodiments, a CD19 binding molecule is administered to a subject in combination with a BTK inhibitor (*e.g.*, ibrutinib). In embodiments, a CD19 binding molecule is administered to a subject in combination with ibrutinib (also called PCI-32765) (*e.g.*, to a subject having CLL, mantle cell lymphoma (MCL), or small lymphocytic lymphoma (SLL). For example, the subject can have a deletion in the short arm of chromosome 17 (del(17p), *e.g.*, in a leukemic cell). In other examples, the subject does not have a del(17p). In some embodiments, the subject has relapsed CLL or SLL, *e.g.*, the subject has previously been administered a cancer therapy (*e.g.*, previously been administered one, two, three, or four prior cancer therapies). In some embodiments, the subject has refractory CLL or SLL. In other embodiments, the subject has follicular lymphoma, *e.g.*, relapse or refractory follicular lymphoma. In some embodiments, ibrutinib is administered at a dosage of about 300-600 mg/day (*e.g.*, about 300-350, 350-400, 400-450, 450-500, 500-550, or 550-600 mg/day, *e.g.*, about 420 mg/day or about 560 mg/day), *e.g.*, orally. In some embodiments, the ibrutinib is administered at a dose of about 250 mg, 300 mg, 350 mg, 400 mg, 420 mg, 440 mg, 460 mg, 480 mg, 500 mg, 520 mg, 540 mg, 560 mg, 580 mg, 600 mg (*e.g.*, 250 mg, 420 mg or 560 mg) daily for a period of time, *e.g.*, daily for 21 day cycle, or daily for 28 day cycle. In one embodiment, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or more cycles of ibrutinib are administered. In some embodiments, ibrutinib is administered in combination with rituximab. See, *e.g.*, Burger *et al.*, 2013. Ibrutinib In Combination With Rituximab (iR) Is Well Tolerated and Induces a High Rate Of Durable Remissions In Patients With High-Risk Chronic Lymphocytic Leukemia (CLL): New, Updated Results Of a Phase II Trial In 40 Patients, Abstract 675 presented at 55th ASH Annual Meeting and Exposition, New Orleans, LA 7-10 Dec. Without being bound by theory, it is thought that the addition of ibrutinib enhances the T cell proliferative response and can shift T cells from a T-helper-2 (Th2) to T-helper-1 (Th1) phenotype. Th1 and Th2 are phenotypes of helper T cells, with Th1 versus Th2 directing different immune response pathways. A Th1 phenotype is associated with proinflammatory responses, *e.g.*, for killing cells, such as intracellular pathogens/viruses or

cancerous cells, or perpetuating autoimmune responses. A Th2 phenotype is associated with eosinophil accumulation and anti-inflammatory responses.

[0744] In some embodiments, the CD19 binding molecule is administered in combination with an inhibitor of Epidermal Growth Factor Receptor (EGFR).

[0745] In some embodiments, the EGFR inhibitor is (R,E)-N-(7-chloro-1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide (Compound A40) or a compound disclosed in PCT Publication No. WO 2013/184757.

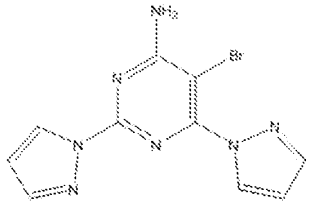
[0746] In some embodiments, the EGFR inhibitor, *e.g.,* (R,E)-N-(7-chloro-1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide (Compound A40) or a compound disclosed in PCT Publication No. WO 2013/184757, is administered at a dose of 150-250 mg, *e.g.,* per day. In some embodiments, the EGFR inhibitor, *e.g.,* (R,E)-N-(7-chloro-1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide (Compound A40) or a compound disclosed in PCT Publication No. WO 2013/184757, is administered at a dose of about 150, 200, or 250 mg, or about 150-200 or 200-250 mg.

[0747] In some embodiments, the EGFR inhibitor, (R,E)-N-(7-chloro-1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide (Compound A40), or a compound disclosed in PCT Publication No. WO 2013/184757, is a covalent, irreversible tyrosine kinase inhibitor. In certain embodiments, the EGFR inhibitor, (R,E)-N-(7-chloro-1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide (Compound A40), or a compound disclosed in PCT Publication No. WO 2013/184757 inhibits activating EGFR mutations (L858R, ex19del). In other embodiments, the EGFR inhibitor, (R,E)-N-(7-chloro-1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide (Compound A40), or a compound disclosed in PCT Publication No. WO 2013/184757 does not inhibit, or does not substantially inhibit, wild-type (wt) EGFR. Compound A40 has shown efficacy in EGFR mutant NSCLC patients. In some embodiments, the EGFR inhibitor, (R,E)-N-(7-chloro-1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide (Compound A40), or a compound disclosed in PCT Publication No. WO 2013/184757 also inhibits one or more kinases in the TEC family of kinases. The Tec family kinases include, *e.g.,* ITK, BMX, TEC, RLK, and BTK, and are central in the propagation of T-cell receptor and chemokine receptor signaling (Schwartzberg *et al.* (2005) *Nat. Rev. Immunol.* p. 284-95). For example, Compound A40 can inhibit ITK with a biochemical IC50 of 1.3 nM. ITK is a critical enzyme for the survival of Th2 cells and its inhibition results in a shift in the balance between Th2 and Th1 cells.

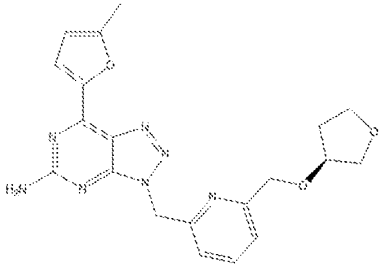
[0748] In some embodiments, the EGFR inhibitor is chosen from one of more of erlotinib, gefitinib, cetuximab, panitumumab, necitumumab, PF-00299804, nimotuzumab, or RO5083945.

[0749] In some embodiments, the CD19 binding molecule is administered in combination with an adenosine A2A receptor (A2AR) antagonist. Exemplary A2AR antagonists include, *e.g.*, PBF509 (Palobiofarma/Novartis), CPI444/V81444 (Corvus/Genentech), AZD4635/HTL-1071 (AstraZeneca/Heptares), Vipadenant (Redox/Juno), GBV-2034 (Globavir), AB928 (Arcus Biosciences), Theophylline, Istradefylline (Kyowa Hakko Kogyo), Tozadenant/SYN-115 (Acorda), KW-6356 (Kyowa Hakko Kogyo), ST-4206 (Leadiant Biosciences), Preladenant/SCH 420814 (Merck/Schering), and NIR178 (Novartis).

[0750] In certain embodiments, the A2AR antagonist is PBF509. PBF509 and other A2AR antagonists are disclosed in US 8,796,284 and WO 2017/025918. In certain embodiments, the A2AR antagonist is 5-bromo-2,6-di-(1H-pyrazol-1-yl)pyrimidine-4-amine. In certain embodiments, the A2AR antagonist has the following structure:

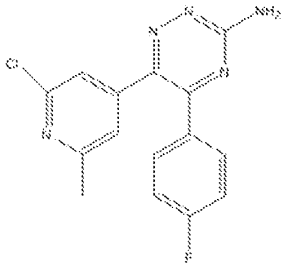


[0751] In certain embodiments, the A2AR antagonist is CPI444/V81444. CPI-444 and other A2AR antagonists are disclosed in WO 2009/156737. In certain embodiments, the A2AR antagonist is (*S*)-7-(5-methylfuran-2-yl)-3-((6-(((tetrahydrofuran-3-yl)oxy)methyl)pyridin-2-yl)methyl)-3*H*-[1,2,3]triazolo[4,5-d]pyrimidin-5-amine. In certain embodiments, the A2AR antagonist is (*R*)-7-(5-methylfuran-2-yl)-3-((6-(((tetrahydrofuran-3-yl)oxy)methyl)pyridin-2-yl)methyl)-3*H*-[1,2,3]triazolo[4,5-d]pyrimidin-5-amine, or racemate thereof. In certain embodiments, the A2AR antagonist is 7-(5-methylfuran-2-yl)-3-((6-(((tetrahydrofuran-3-yl)oxy)methyl)pyridin-2-yl)methyl)-3*H*-[1,2,3]triazolo[4,5-d]pyrimidin-5-amine. In certain embodiments, the A2AR antagonist has the following structure:

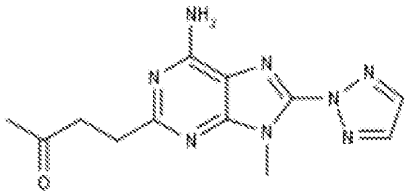


[0752] In certain embodiments, the A2AR antagonist is AZD4635/HTL-1071. A2AR antagonists are disclosed in WO 2011/095625. In certain embodiments, the A2AR antagonist

is 6-(2-chloro-6-methylpyridin-4-yl)-5-(4-fluorophenyl)-1,2,4-triazin-3-amine. In certain embodiments, the A2AR antagonist has the following structure:



[0753] In certain embodiments, the A2AR antagonist is ST-4206 (Leadiant Biosciences). In certain embodiments, the A2AR antagonist is an A2AR antagonist described in US 9,133,197. In certain embodiments, the A2AR antagonist has the following structure:



[0754] In certain embodiments, the A2AR antagonist is an A2AR antagonist described in US8114845, US9029393, US20170015758, or US20160129108.

[0755] In certain embodiments, the A2AR antagonist is istradefylline (CAS Registry Number: 155270-99-8). Istradefylline is also known as KW-6002 or 8-[(E)-2-(3,4-dimethoxyphenyl)vinyl]-1,3-diethyl-7-methyl-3,7-dihydro-1H-purine-2,6-dione. Istradefylline is disclosed, *e.g.,* in LeWitt *et al.* (2008) *Annals of Neurology* 63 (3): 295–302).

[0756] In certain embodiments, the A2aR antagonist is tozadenant (Biotie). Tozadenant is also known as SYN115 or 4-hydroxy-N-(4-methoxy-7-morpholin-4-yl-1,3-benzothiazol-2-yl)-4-methylpiperidine-1-carboxamide. Tozadenant blocks the effect of endogenous adenosine at the A2a receptors, resulting in the potentiation of the effect of dopamine at the D2 receptor and inhibition of the effect of glutamate at the mGluR5 receptor. In some embodiments, the A2aR antagonist is preladenant (CAS Registry Number: 377727-87-2). Preladenant is also known as SCH 420814 or 2-(2-Furanyl)-7-[2-[4-[4-(2-methoxyethoxy)phenyl]-1-piperazinyl]ethyl]7H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidine-5-amine. Preladenant was developed as a drug that acted as a potent and selective antagonist at the adenosine A2A receptor.

[0757] In certain embodiments, the A2aR antagonist is vipadenan. Vipadenan is also known as BIIB014, V2006, or 3-[(4-amino-3-methylphenyl)methyl]-7-(furan-2-yl)triazolo[4,5-d]pyrimidin-5-amine.

[0758] Other exemplary A2aR antagonists include, *e.g.,* ATL-444, MSX-3, SCH-58261, SCH-412,348, SCH-442,416, VER-6623, VER-6947, VER-7835, CGS-15943, or ZM-241,385.

[0759] In some embodiments, the A2aR antagonist is an A2aR pathway antagonist (*e.g.,* a CD-73 inhibitor, *e.g.,* an anti-CD73 antibody) is MEDI9447. MEDI9447 is a monoclonal antibody specific for CD73. Targeting the extracellular production of adenosine by CD73 can reduce the immunosuppressive effects of adenosine. MEDI9447 was reported to have a range of activities, *e.g.,* inhibition of CD73 ectonucleotidase activity, relief from AMP-mediated lymphocyte suppression, and inhibition of syngeneic tumor growth. MEDI9447 can drive changes in both myeloid and lymphoid infiltrating leukocyte populations within the tumor microenvironment. These changes include, *e.g.,* increases in CD8 effector cells and activated macrophages, as well as a reduction in the proportions of myeloid-derived suppressor cells (MDSC) and regulatory T lymphocytes.

[0760] In some embodiments, the CD19 binding molecule is administered in combination with a CD20 inhibitor.

[0761] In one embodiment, the CD20 inhibitor is an anti-CD20 antibody or fragment thereof. In an embodiment, the antibody is a monospecific antibody and in another embodiment, the antibody is a bispecific antibody. In an embodiment, the CD20 inhibitor is a chimeric mouse/human monoclonal antibody, *e.g.*, rituximab. In an embodiment, the CD20 inhibitor is a human monoclonal antibody such as ofatumumab. In an embodiment, the CD20 inhibitor is a humanized antibody such as ocrelizumab, veltuzumab, obinutuzumab, ocaratuzumab, or PRO131921 (Genentech). In an embodiment, the CD20 inhibitor is a fusion protein comprising a portion of an anti-CD20 antibody, such as TRU-015 (Trubion Pharmaceuticals).

[0762] In some embodiments, the CD19 binding molecule is administered in combination with a CD22 inhibitor. In some embodiments, the CD22 inhibitor is a small molecule or an anti-CD22 antibody molecule. In some embodiments, the antibody is a monospecific antibody, optionally conjugated to a second agent such as a chemotherapeutic agent. For instance, in an embodiment, the antibody is an anti-CD22 monoclonal antibody-MMAE conjugate (*e.g.*, DCDT2980S). In an embodiment, the antibody is an scFv of an anti-CD22 antibody, *e.g.*, an scFv of antibody RFB4. This scFv can be fused to all of or a fragment of Pseudomonas exotoxin-A (*e.g.*, BL22). In an embodiment, the antibody is a humanized anti-CD22 monoclonal antibody (*e.g.*, epratuzumab). In an embodiment, the antibody or fragment thereof comprises the Fv portion of an anti-CD22 antibody, which is optionally covalently fused to all or

a fragment or (*e.g.*, a 38 KDa fragment of) Pseudomonas exotoxin-A (*e.g.*, moxetumomab pasudotox). In an embodiment, the anti-CD22 antibody is an anti-CD19/CD22 bispecific antibody, optionally conjugated to a toxin. For instance, in one embodiment, the anti-CD22 antibody comprises an anti-CD19/CD22 bispecific portion, (*e.g.*, two scFv ligands, recognizing human CD19 and CD22) optionally linked to all of or a portion of diphtheria toxin (DT), *e.g.*, first 389 amino acids of diphtheria toxin (DT), DT 390, *e.g.*, a ligand-directed toxin such as DT2219ARL). In another embodiment, the bispecific portion (*e.g.*, anti-CD19/anti-CD22) is linked to a toxin such as deglycosylated ricin A chain (*e.g.*, Combotox).

[0763] In some embodiments, the CD22 inhibitor is a multispecific antibody molecule, *e.g.*, a bispecific antibody molecule, *e.g.*, a bispecific antibody molecule that binds to CD20 and CD3. Exemplary bispecific antibody molecules that bind to CD20 and CD3 are disclosed in WO2016086189 and WO2016182751. In some embodiments, the bispecific antibody molecule that binds to CD20 and CD3 is XENP13676 as disclosed in Figure 74, SEQ ID NOs: 323, 324, and 325 of WO2016086189.

[0764] In some embodiments, the CD19 binding molecule is administered in combination with a FCRL2 or FCRL5 inhibitor. In some embodiments, the FCRL2 or FCRL5 inhibitor is an anti-FCRL2 antibody molecule, *e.g.*, a bispecific antibody molecule, *e.g.*, a bispecific antibody that binds to FCRL2 and CD3. In some embodiments, the FCRL2 or FCRL5 inhibitor is an anti-FCRL5 antibody molecule, *e.g.*, a bispecific antibody molecule, *e.g.*, a bispecific antibody that binds to FCRL5 and CD3.

[0765] Exemplary anti-FCRL5 antibody molecules are disclosed in US20150098900, US20160368985, WO2017096120 (*e.g.*, antibodies ET200-001, ET200-002, ET200-003, ET200-006, ET200-007, ET200-008, ET200-009, ET200-010, ET200-011, ET200-012, ET200-013, ET200-014, ET200-015, ET200-016, ET200-017, ET200-018, ET200-019, ET200-020, ET200-021, ET200-022, ET200-023, ET200-024, ET200-025, ET200-026, ET200-027, ET200-028, ET200-029, ET200-030, ET200-031, ET200-032, ET200-033, ET200-034, ET200-035, ET200-037, ET200-038, ET200-039, ET200-040, ET200-041, ET200-042, ET200-043, ET200-044, ET200-045, ET200-069, ET200-078, ET200-079, ET200-081, ET200-097, ET200-098, ET200-099, ET200-100, ET200-101, ET200-102, ET200-103, ET200-104, ET200-105, ET200-106, ET200-107, ET200-108, ET200-109, ET200-110, ET200-111, ET200-112, ET200-113, ET200-114, ET200-115, ET200-116, ET200-117, ET200-118, ET200-119, ET200-120, ET200-121, ET200-122, ET200-123, ET200-125, ET200-005 and ET200-124 disclosed in WO2017096120).

[0766] In some embodiments, the CD19 binding molecule is administered in combination with an IL15/IL-15Ra complex. In some embodiments, the IL-15/IL-15Ra complex is chosen from NIZ985 (Novartis), ATL-803 (Altor) or CYP0150 (Cytune).

[0767] In some embodiments, the IL-15/IL-15Ra complex comprises human IL-15 complexed with a soluble form of human IL-15Ra. The complex can comprise IL-15 covalently or noncovalently bound to a soluble form of IL-15Ra. In a particular embodiment, the human IL-15 is noncovalently bonded to a soluble form of IL-15Ra. In a particular embodiment, the human IL-15 of the composition comprises an amino acid sequence as described in WO 2014/066527and the soluble form of human IL-15Ra comprises an amino acid sequence as described in WO 2014/066527. The molecules described herein can be made by vectors, host cells, and methods described in WO 2007/084342.

[0768] In some embodiments, the IL-15/IL-15Ra complex is ALT-803, an IL-15/IL-15Ra Fc fusion protein (IL-15N72D:IL-15RaSu/Fc soluble complex). ALT-803 is disclosed in WO 2008/143794.

[0769] In some embodiments, the IL-15/IL-15Ra complex comprises IL-15 fused to the sushi domain of IL-15Ra (CYP0150, Cytune). The sushi domain of IL-15Ra refers to a domain beginning at the first cysteine residue after the signal peptide of IL-15Ra, and ending at the fourth cysteine residue after the signal peptide. The complex of IL-15 fused to the sushi domain of IL-15Ra is disclosed in WO 2007/04606 and WO 2012/175222.

[0770] In some embodiments, the CD19 binding molecule is administered in combination with a PD-1 inhibitor. In some embodiments, the PD-1 inhibitor is chosen from PDR001 (Novartis), Nivolumab (Bristol-Myers Squibb), Pembrolizumab (Merck & Co), Pidilizumab (CureTech), MEDI0680 (Medimmune), REGN2810 (Regeneron), TSR-042 (Tesaro), PF-06801591 (Pfizer), BGB-A317 (Beigene), BGB-108 (Beigene), INCSHR1210 (Incyte), or AMP-224 (Amplimmune). In one embodiment, the PD-1 inhibitor is an anti-PD-1 antibody molecule. In one embodiment, the PD-1 inhibitor is an anti-PD-1 antibody molecule as described in US 2015/0210769.

[0771] In one embodiment, the anti-PD-1 antibody molecule is Nivolumab (Bristol-Myers Squibb), also known as MDX-1106, MDX-1106-04, ONO-4538, BMS-936558, or OPDIVO®. Nivolumab (clone 5C4) and other anti-PD-1 antibodies are disclosed in US 8,008,449 and WO 2006/121168. In one embodiment, the anti-PD-1 antibody molecule comprises one or more of the CDR sequences (or collectively all of the CDR sequences), the heavy chain or light chain variable region sequence, or the heavy chain or light chain sequence of Nivolumab.

[0772] In one embodiment, the anti-PD-1 antibody molecule is Pembrolizumab (Merck & Co), also known as Lambrolizumab, MK-3475, MK03475, SCH-900475, or KEYTRUDA®. Pembrolizumab and other anti-PD-1 antibodies are disclosed in Hamid, O. *et al*. (2013) *New*

England Journal of Medicine 369 (2): 134–44, US 8,354,509, and WO 2009/114335. In one embodiment, the anti-PD-1 antibody molecule comprises one or more of the CDR sequences (or collectively all of the CDR sequences), the heavy chain or light chain variable region sequence, or the heavy chain or light chain sequence of Pembrolizumab.

[0773] In one embodiment, the anti-PD-1 antibody molecule is Pidilizumab (CureTech), also known as CT-011. Pidilizumab and other anti-PD-1 antibodies are disclosed in Rosenblatt, J. *et al.* (2011) *J Immunotherapy* 34(5): 409-18, US 7,695,715, US 7,332,582, and US 8,686,119. In one embodiment, the anti-PD-1 antibody molecule comprises one or more of the CDR sequences (or collectively all of the CDR sequences), the heavy chain or light chain variable region sequence, or the heavy chain or light chain sequence of Pidilizumab.

[0774] In one embodiment, the anti-PD-1 antibody molecule is MEDI0680 (Medimmune), also known as AMP-514. MEDI0680 and other anti-PD-1 antibodies are disclosed in US 9,205,148 and WO 2012/145493. In one embodiment, the anti-PD-1 antibody molecule comprises one or more of the CDR sequences (or collectively all of the CDR sequences), the heavy chain or light chain variable region sequence, or the heavy chain or light chain sequence of MEDI0680.

[0775] In one embodiment, the anti-PD-1 antibody molecule is REGN2810 (Regeneron). In one embodiment, the anti-PD-1 antibody molecule comprises one or more of the CDR sequences (or collectively all of the CDR sequences), the heavy chain or light chain variable region sequence, or the heavy chain or light chain sequence of REGN2810.

[0776] In one embodiment, the anti-PD-1 antibody molecule is PF-06801591 (Pfizer). In one embodiment, the anti-PD-1 antibody molecule comprises one or more of the CDR sequences (or collectively all of the CDR sequences), the heavy chain or light chain variable region sequence, or the heavy chain or light chain sequence of PF-06801591.

[0777] In one embodiment, the anti-PD-1 antibody molecule is BGB-A317 or BGB-108 (Beigene). In one embodiment, the anti-PD-1 antibody molecule comprises one or more of the CDR sequences (or collectively all of the CDR sequences), the heavy chain or light chain variable region sequence, or the heavy chain or light chain sequence of BGB-A317 or BGB-108.

[0778] In one embodiment, the anti-PD-1 antibody molecule is INCSHR1210 (Incyte), also known as INCSHR01210 or SHR-1210. In one embodiment, the anti-PD-1 antibody molecule comprises one or more of the CDR sequences (or collectively all of the CDR sequences), the heavy chain or light chain variable region sequence, or the heavy chain or light chain sequence of INCSHR1210.

[0779] In one embodiment, the anti-PD-1 antibody molecule is TSR-042 (Tesaro), also known as ANB011. In one embodiment, the anti-PD-1 antibody molecule comprises one or more of the CDR sequences (or collectively all of the CDR sequences), the heavy chain or light chain variable region sequence, or the heavy chain or light chain sequence of TSR-042.

[0780] Further known anti-PD-1 antibodies include those described, *e.g.*, in WO 2015/112800, WO 2016/092419, WO 2015/085847, WO 2014/179664, WO 2014/194302, WO 2014/209804, WO 2015/200119, US 8,735,553, US 7,488,802, US 8,927,697, US 8,993,731, and US 9,102,727.

[0781] In one embodiment, the anti-PD-1 antibody is an antibody that competes for binding with, and/or binds to the same epitope on PD-1 as, one of the anti-PD-1 antibodies described herein.

[0782] In one embodiment, the PD-1 inhibitor is a peptide that inhibits the PD-1 signaling pathway, *e.g.*, as described in US 8,907,053. In one embodiment, the PD-1 inhibitor is an immunoadhesin (*e.g.*, an immunoadhesin comprising an extracellular or PD-1 binding portion of PD-L1 or PD-L2 fused to a constant region (*e.g.*, an Fc region of an immunoglobulin sequence). In one embodiment, the PD-1 inhibitor is AMP-224 (B7-DCIg (Amplimmune), *e.g.*, disclosed in WO 2010/027827 and WO 2011/066342).

[0783] In some embodiments, the CD19 binding molecule is administered in combination with a PD-L1 inhibitor. In some embodiments, the PD-L1 inhibitor is chosen from FAZ053 (Novartis), Atezolizumab (Genentech/Roche), Avelumab (Merck Serono and Pfizer), Durvalumab (MedImmune/AstraZeneca), or BMS-936559 (Bristol-Myers Squibb).

[0784] In one embodiment, the PD-L1 inhibitor is an anti-PD-L1 antibody molecule. In one embodiment, the PD-L1 inhibitor is an anti-PD-L1 antibody molecule as disclosed in US 2016/0108123.

[0785] In one embodiment, the anti-PD-L1 antibody molecule is Atezolizumab (Genentech/Roche), also known as MPDL3280A, RG7446, RO5541267, YW243.55.S70, or TECENTRIQ™. Atezolizumab and other anti-PD-L1 antibodies are disclosed in US 8,217,149. In one embodiment, the anti-PD-L1 antibody molecule comprises one or more of the CDR sequences (or collectively all of the CDR sequences), the heavy chain or light chain variable region sequence, or the heavy chain or light chain sequence of Atezolizumab.

[0786] In one embodiment, the anti-PD-L1 antibody molecule is Avelumab (Merck Serono and Pfizer), also known as MSB0010718C. Avelumab and other anti-PD-L1 antibodies are disclosed in WO 2013/079174. In one embodiment, the anti-PD-L1 antibody molecule comprises one or more of the CDR sequences (or collectively all of the CDR sequences), the

heavy chain or light chain variable region sequence, or the heavy chain or light chain sequence of Avelumab.

[0787] In one embodiment, the anti-PD-L1 antibody molecule is Durvalumab (MedImmune/AstraZeneca), also known as MEDI4736. Durvalumab and other anti-PD-L1 antibodies are disclosed in US 8,779,108. In one embodiment, the anti-PD-L1 antibody molecule comprises one or more of the CDR sequences (or collectively all of the CDR sequences), the heavy chain or light chain variable region sequence, or the heavy chain or light chain sequence of Durvalumab.

[0788] In one embodiment, the anti-PD-L1 antibody molecule is BMS-936559 (Bristol-Myers Squibb), also known as MDX-1105 or 12A4. BMS-936559 and other anti-PD-L1 antibodies are disclosed in US 7,943,743 and WO 2015/081158. In one embodiment, the anti-PD-L1 antibody molecule comprises one or more of the CDR sequences (or collectively all of the CDR sequences), the heavy chain or light chain variable region sequence, or the heavy chain or light chain sequence of BMS-936559.

[0789] Further known anti-PD-L1 antibodies include those described, *e.g.*, in WO 2015/181342, WO 2014/100079, WO 2016/000619, WO 2014/022758, WO 2014/055897, WO 2015/061668, WO 2013/079174, WO 2012/145493, WO 2015/112805, WO 2015/109124, WO 2015/195163, US 8,168,179, US 8,552,154, US 8,460,927, and US 9,175,082.

[0790] In some embodiments, the CD19 binding molecule is administered in combination with a LAG-3 inhibitor. In some embodiments, the LAG-3 inhibitor is chosen from LAG525 (Novartis), BMS-986016 (Bristol-Myers Squibb), or TSR-033 (Tesaro).

[0791] In one embodiment, the LAG-3 inhibitor is an anti-LAG-3 antibody molecule. In one embodiment, the LAG-3 inhibitor is an anti-LAG-3 antibody molecule as disclosed in US 2015/0259420.

[0792] In one embodiment, the anti-LAG-3 antibody molecule is BMS-986016 (Bristol-Myers Squibb), also known as BMS986016. BMS-986016 and other anti-LAG-3 antibodies are disclosed in WO 2015/116539 and US 9,505,839. In one embodiment, the anti-LAG-3 antibody molecule comprises one or more of the CDR sequences (or collectively all of the CDR sequences), the heavy chain or light chain variable region sequence, or the heavy chain or light chain sequence of BMS-986016.

[0793] In one embodiment, the anti-LAG-3 antibody molecule is TSR-033 (Tesaro). In one embodiment, the anti-LAG-3 antibody molecule comprises one or more of the CDR sequences (or collectively all of the CDR sequences), the heavy chain or light chain variable region sequence, or the heavy chain or light chain sequence of TSR-033.

[0794] In one embodiment, the anti-LAG-3 antibody molecule is IMP731 or GSK2831781 (GSK and Prima BioMed). IMP731 and other anti-LAG-3 antibodies are disclosed in WO 2008/132601 and US 9,244,059. In one embodiment, the anti-LAG-3 antibody molecule comprises one or more of the CDR sequences (or collectively all of the CDR sequences), the heavy chain or light chain variable region sequence, or the heavy chain or light chain sequence of IMP731. In one embodiment, the anti-LAG-3 antibody molecule comprises one or more of the CDR sequences (or collectively all of the CDR sequences), the heavy chain or light chain variable region sequence, or the heavy chain or light chain sequence of GSK2831781.

[0795] Further known anti-LAG-3 antibodies include those described, *e.g.*, in WO 2008/132601, WO 2010/019570, WO 2014/140180, WO 2015/116539, WO 2015/200119, WO 2016/028672, US 9,244,059, US 9,505,839.

[0796] In one embodiment, the anti-LAG-3 inhibitor is a soluble LAG-3 protein, *e.g.*, IMP321 (Prima BioMed), *e.g.*, as disclosed in WO 2009/044273.

[0797] In some embodiments, the CD19 binding molecule is administered in combination with a TIM-3 inhibitor. In some embodiments, the TIM-3 inhibitor is MBG453 (Novartis) or TSR-022 (Tesaro).

[0798] In one embodiment, the TIM-3 inhibitor is an anti-TIM-3 antibody molecule. In one embodiment, the TIM-3 inhibitor is an anti-TIM-3 antibody molecule as disclosed in US 2015/0218274.

[0799] In one embodiment, the anti-TIM-3 antibody molecule is TSR-022 (AnaptysBio/Tesaro). In one embodiment, the anti-TIM-3 antibody molecule comprises one or more of the CDR sequences (or collectively all of the CDR sequences), the heavy chain or light chain variable region sequence, or the heavy chain or light chain sequence of TSR-022. In one embodiment, the anti-TIM-3 antibody molecule comprises one or more of the CDR sequences (or collectively all of the CDR sequences), the heavy chain or light chain variable region sequence, or the heavy chain or light chain sequence of APE5137 or APE5121. APE5137, APE5121, and other anti-TIM-3 antibodies are disclosed in WO 2016/161270.

[0800] In one embodiment, the anti-TIM-3 antibody molecule is the antibody clone F38-2E2. In one embodiment, the anti-TIM-3 antibody molecule comprises one or more of the CDR sequences (or collectively all of the CDR sequences), the heavy chain or light chain variable region sequence, or the heavy chain or light chain sequence of F38-2E2.

[0801] Further known anti-TIM-3 antibodies include those described, *e.g.*, in WO 2016/111947, WO 2016/071448, WO 2016/144803, US 8,552,156, US 8,841,418, and US 9,163,087.

[0802] In one embodiment, the anti-TIM-3 antibody is an antibody that competes for binding with, and/or binds to the same epitope on TIM-3 as, one of the anti-TIM-3 antibodies described herein.

[0803] In some embodiments, the CD19 binding molecule is administered in combination with a transforming growth factor beta (TGF-β) inhibitor. In some embodiments, the TGF-β inhibitor is fresolimumab (CAS Registry Number: 948564-73-6). Fresolimumab is also known as GC1008. Fresolimumab is a human monoclonal antibody that binds to and inhibits TGF-beta isoforms 1, 2 and 3. Fresolimumab is disclosed, *e.g.*, in WO 2006/086469, US 8,383,780, and US 8,591,901.

[0804] In some embodiments, the TGF-β inhibitor is XOMA 089. XOMA 089 is also known as XPA.42.089. XOMA 089 is a fully human monoclonal antibody that binds and neutralizes TGF-beta 1 and 2 ligands, and is disclosed in PCT Publication No. WO 2012/167143.

[0805] In some embodiments, the CD19 binding molecule is administered in combination with an anti-CD73 antibody molecule. In one embodiment, an anti-CD73 antibody molecule is a full antibody molecule or an antigen-binding fragment thereof. In certain embodiments, the anti-CD73 antibody molecule binds to a CD73 protein and reduces, *e.g.*, inhibits or antagonizes, an activity of CD73, *e.g.*, human CD73.

[0806] In one embodiment, the anti-CD73 antibody molecule is an anti-CD73 antibody disclosed in WO2016/075099. In one embodiment, the anti-CD73 antibody molecule is MEDI 9447, *e.g.*, as disclosed in WO2016/075099. Alternative names for MEDI 9447 include clone 10.3 or 73combo3. MEDI 9447 is an IgG1 antibody that inhibits, *e.g.*, antagonizes, an activity of CD73. MEDI 9447 and other anti-CD73 antibody molecules are also disclosed in WO2016/075176 and US2016/0129108.

[0807] In one embodiment, the anti-CD73 antibody molecule comprises a heavy chain variable domain, a light chain variable domain, or both, of MEDI 9477.

[0808] In one embodiment, the anti-CD73 antibody molecule is an anti-CD73 antibody disclosed in WO2016/081748. In one embodiment, the anti-CD73 antibody molecule is 11F11, *e.g.*, as disclosed in WO2016/081748. 11F11 is an IgG2 antibody that inhibits, *e.g.*, antagonizes, an activity of CD73. Antibodies derived from 11F11, *e.g.*, CD73.4, and CD73.10; clones of 11F11, *e.g.*, 11F11-1 and 11F11-2; and other anti-CD73 antibody molecules are disclosed in WO2016/081748 and US 9,605,080.

[0809] In one embodiment, the anti-CD73 antibody molecule comprises a heavy chain variable domain, a light chain variable domain, or both, of 11F11-1 or 11F11-2.

[0810] In one embodiment, the anti-CD73 antibody molecule is an anti-CD73 antibody disclosed in *e.g.*, US 9,605,080.

[0811] In one embodiment, the anti-CD73 antibody molecule is CD73.4, *e.g.*, as disclosed in US 9,605,080. In one embodiment, the anti-CD73 antibody molecule comprises a heavy chain variable domain, a light chain variable domain, or both, of CD73.4.

[0812] In one embodiment, the anti-CD73 antibody molecule is CD73.10, *e.g.*, as disclosed in US 9,605,080. In one embodiment, the anti-CD73 antibody molecule comprises a heavy chain variable domain, a light chain variable domain, or both, of CD73.10.

[0813] In one embodiment, the anti-CD73 antibody molecule is an anti-CD73 antibody disclosed in WO2009/0203538. In one embodiment, the anti-CD73 antibody molecule is 067-213, *e.g.*, as disclosed in WO2009/0203538.

[0814] In one embodiment, the anti-CD73 antibody molecule comprises a heavy chain variable domain, a light chain variable domain, or both, of 067-213.

[0815] In one embodiment, the anti-CD73 antibody molecule is an anti-CD73 antibody disclosed in US 9,090,697. In one embodiment, the anti-CD73 antibody molecule is TY/23, *e.g.*, as disclosed in US 9,090,697. In one embodiment, the anti-CD73 antibody molecule comprises a heavy chain variable domain, a light chain variable domain, or both, of TY/23.

[0816] In one embodiment, the anti-CD73 antibody molecule is an anti-CD73 antibody disclosed in WO2016/055609. In one embodiment, the anti-CD73 antibody molecule comprises a heavy chain variable domain, a light chain variable domain, or both, of an anti-CD73 antibody disclosed in WO2016/055609.

[0817] In one embodiment, the anti-CD73 antibody molecule is an anti-CD73 antibody disclosed in WO2016/146818. In one embodiment, the anti-CD73 antibody molecule comprises a heavy chain variable domain, a light chain variable domain, or both, of an anti-CD73 antibody disclosed in WO2016/146818.

[0818] In one embodiment, the anti-CD73 antibody molecule is an anti-CD73 antibody disclosed in WO2004/079013. In one embodiment, the anti-CD73 antibody molecule comprises a heavy chain variable domain, a light chain variable domain, or both, of an anti-CD73 antibody disclosed in WO2004/079013.

[0819] In one embodiment, the anti-CD73 antibody molecule is an anti-CD73 antibody disclosed in WO2012/125850. In one embodiment, the anti-CD73 antibody molecule comprises a heavy chain variable domain, a light chain variable domain, or both, of an anti-CD73 antibody disclosed in WO2012/125850.

[0820] In one embodiment, the anti-CD73 antibody molecule is an anti-CD73 antibody disclosed in WO2015/004400. In one embodiment, the anti-CD73 antibody molecule comprises a heavy chain variable domain, a light chain variable domain, or both, of an anti-CD73 antibody disclosed in WO2015/004400.

[0821] In one embodiment, the anti-CD73 antibody molecule is an anti-CD73 antibody disclosed in WO2007/146968. In one embodiment, the anti-CD73 antibody molecule comprises a heavy chain variable domain, a light chain variable domain, or both, of an anti-CD73 antibody disclosed in WO2007146968.

[0822] In one embodiment, the anti-CD73 antibody molecule is an anti-CD73 antibody disclosed in US2007/0042392. In one embodiment, the anti-CD73 antibody molecule comprises a heavy chain variable domain, a light chain variable domain, or both, of an anti-CD73 antibody disclosed in US2007/0042392.

[0823] In one embodiment, the anti-CD73 antibody molecule is an anti-CD73 antibody disclosed in US2009/0138977. In one embodiment, the anti-CD73 antibody molecule comprises a heavy chain variable domain, a light chain variable domain, or both, of an anti-CD73 antibody disclosed in US2009/0138977.

[0824] In one embodiment, the anti-CD73 antibody molecule is an anti-CD73 antibody disclosed in Flocke *et al.*, Eur J Cell Biol. 1992 Jun;58(1):62-70. In one embodiment, the anti-CD73 antibody molecule comprises a heavy chain variable domain, a light chain variable domain, or both, of an anti-CD73 antibody disclosed in Flocke *et al.*, Eur J Cell Biol. 1992 Jun;58(1):62-70.

[0825] In one embodiment, the anti-CD73 antibody molecule is an anti-CD73 antibody disclosed in Stagg *et al.*, PNAS. 2010 Jan 107(4): 1547-1552. In some embodiments, the anti-CD73 antibody molecule is TY/23 or TY11.8, as disclosed in Stagg *et al.* In one embodiment, the anti-CD73 antibody molecule comprises a heavy chain variable domain, a light chain variable domain, or both, of an anti-CD73 antibody disclosed in Stagg *et al.*

[0826] In some embodiments, the CD19 binding molecule is administered in combination with an interleukine-17 (IL-17) inhibitor.

[0827] In some embodiments, the IL-17 inhibitor is secukinumab (CAS Registry Numbers: 875356-43-7 (heavy chain) and 875356-44-8 (light chain)). Secukinumab is also known as AIN457 and COSENTYX®. Secukinumab is a recombinant human monoclonal IgG1/κ antibody that binds specifically to IL-17A. It is expressed in a recombinant Chinese Hamster Ovary (CHO) cell line. Secukinumab is described, *e.g.*, in WO 2006/013107, US 7,807,155, US 8,119,131, US 8,617,552, and EP 1776142.

[0828] In some embodiments, the IL-17 inhibitor is CJM112. CJM112 is also known as XAB4. CJM112 is a fully human monoclonal antibody (*e.g.*, of the IgG1/κ isotype) that targets IL-17A. CJM112 is disclosed, *e.g.*, in WO 2014/122613.

[0829] CJM112 can bind to human, cynomolgus, mouse and rat IL-17A and neutralize the bioactivity of these cytokines in vitro and in vivo. IL-17A, a member of the IL-17 family, is a major proinflammatory cytokine that has been indicated to play important roles in many immune mediated conditions, such as psoriasis and cancers (Witowski *et al*. (2004) Cell Mol. Life Sci. p. 567-79; Miossec and Kolls (2012) Nat. Rev. Drug Discov. p. 763-76).

[0830] In some embodiments, the IL-17 inhibitor is ixekizumab (CAS Registry Number: 1143503-69-8). Ixekizumab is also known as LY2439821. Ixekizumab is a humanized IgG4 monoclonal antibody that targets IL-17A. Ixekizumab is described, *e.g.*, in WO 2007/070750, US 7,838,638, and US 8,110,191.

[0831] In some embodiments, the IL-17 inhibitor is brodalumab (CAS Registry Number: 1174395-19-7). Brodalumab is also known as AMG 827 or AM-14. Brodalumab binds to the interleukin-17 receptor A (IL-17RA) and prevents IL-17 from activating the receptor. Brodalumab is disclosed, *e.g.*, in WO 2008/054603, US 7,767,206, US 7,786,284, US 7,833,527, US 7,939,070, US 8,435,518, US 8,545,842, US 8,790,648, and US 9,073,999.

[0832] In some embodiments, the CD19 binding molecule is administered in combination with an interleukine-1 beta (IL-1β) inhibitor.

[0833] In some embodiments, the IL-1β inhibitor is canakinumab. Canakinumab is also known as ACZ885 or ILARIS®. Canakinumab is a human monoclonal IgG1/κ antibody that neutralizes the bioactivity of human IL-1β. Canakinumab is disclosed, *e.g.*, in WO 2002/16436, US 7,446,175, and EP 1313769.

[0834] In some embodiments, the CD19 binding molecule is administered in combination with a CD32B inhibitor. In some embodiments, the CD32B inhibitor is an anti-CD32B antibody molecule. Exemplary anti-CD32B antibody molecules are disclosed in US8187593, US8778339, US8802089, US20060073142, US20170198040, and US20130251706.

[0835] In some embodiments, the CD19 binding molecule is administered in combination with one of the compounds listed in Table 18.

TABLE 18

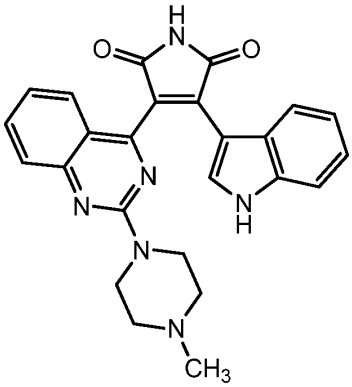
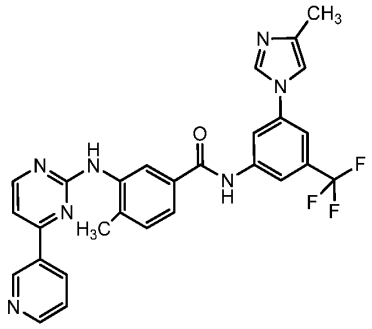
Compound Desig-nation	Generic Name Tradename	Compound Structure	Patents / Patent Application Publications
A1	Sotrastaurin		EP 1682103 US 2007/142401 WO 2005/039549
A2	Nilotinib HCl monohydrate TASIGNA®	 HCl • H_20	WO 2004/005281 US 7,169,791

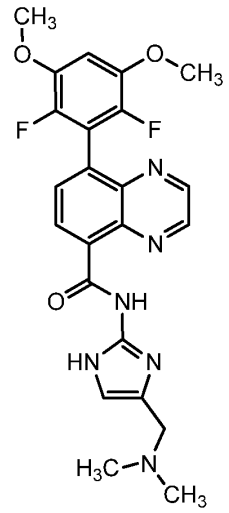
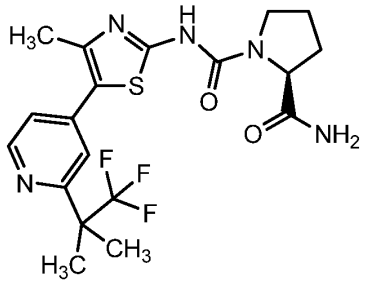
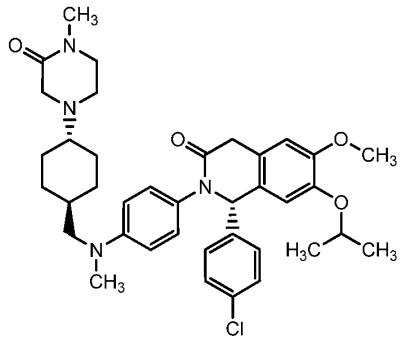
TABLE 18			
Compound Desig-nation	**Generic Name** **Tradename**	**Compound Structure**	**Patents / Patent Application Publications**
A3			WO 2009/141386 US 2010/0105667
A4			WO 2010/029082
A5			WO 2011/076786

TABLE 18

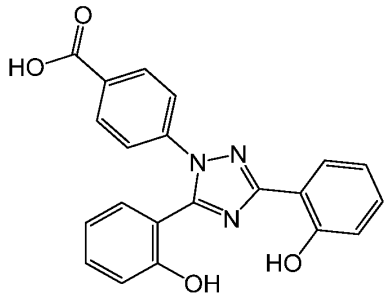
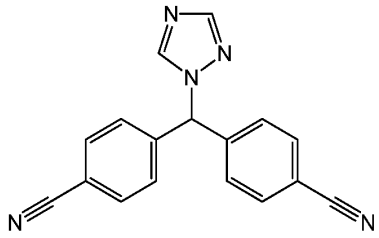
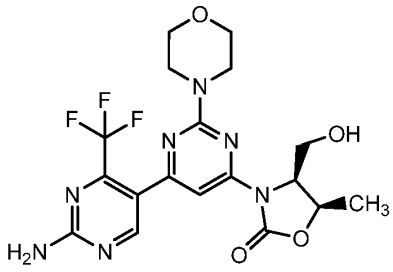
Compound Desig-nation	Generic Name Tradename	Compound Structure	Patents / Patent Application Publications
A6	Deferasirox EXJADE®		WO 1997/049395
A7	Letrozole FEMARA®		US 4,978,672
A8			WO 2013/124826 US 2013/0225574

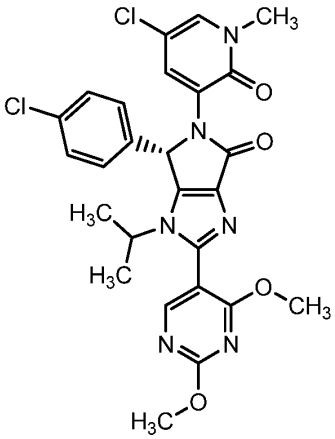
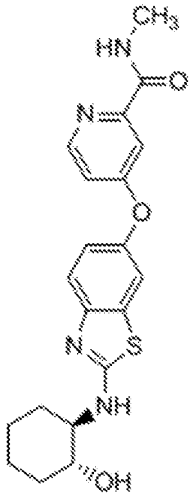
TABLE 18			
Compound Desig-nation	**Generic Name** **Tradename**	**Compound Structure**	**Patents / Patent Application Publications**
A9			WO 2013/111105
A10	BLZ945		WO 2007/121484

TABLE 18

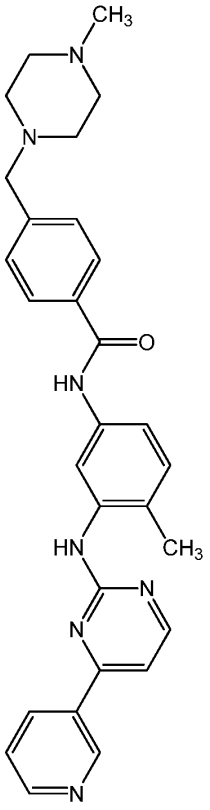
Compound Desig-nation	Generic Name Tradename	Compound Structure	Patents / Patent Application Publications
A11	Imatinib mesylate GLEEVEC®	 Mesylate	WO 1999/003854

TABLE 18

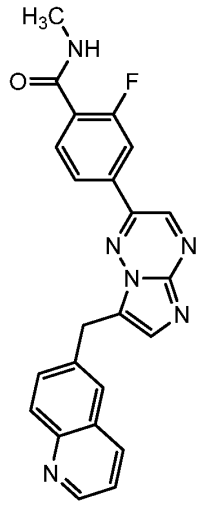
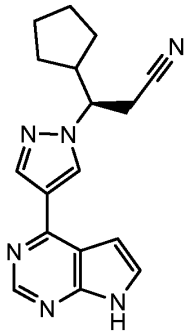
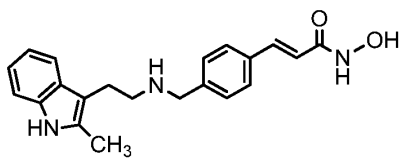
Compound Desig-nation	Generic Name Tradename	Compound Structure	Patents / Patent Application Publications
A12	Capmatinib	 Dihydrochloric salt	EP 2099447 US 7,767,675 US 8,420,645
A13	Ruxolitinib Phosphate JAKAFI®	 H_3PO_4	WO 2007/070514 EP 2474545 US 7,598,257 WO 2014/018632
A14	Panobinostat		WO 2014/072493 WO 2002/022577 EP 1870399

TABLE 18

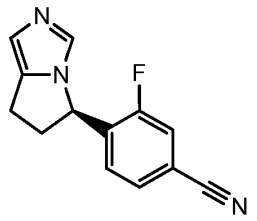
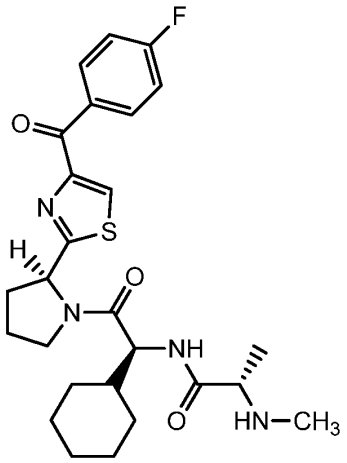
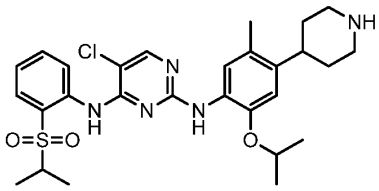
Compound Desig-nation	Generic Name Tradename	Compound Structure	Patents / Patent Application Publications
A15	Osilodrostat		WO 2007/024945
A16			WO 2008/016893 EP 2051990 US 8,546,336
A17	ceritinib ZYKADIA™		WO 2008/073687 US 8,039,479

TABLE 18

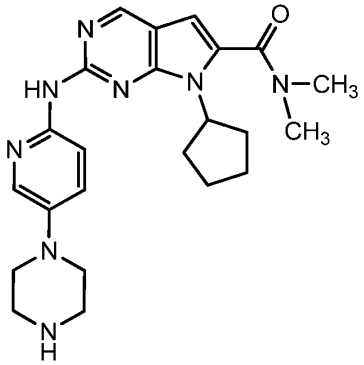
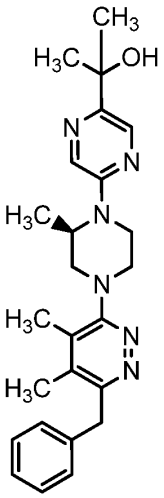
Compound Desig-nation	Generic Name Tradename	Compound Structure	Patents / Patent Application Publications
A18	Ribociclib KISQALI®		US 8,415,355 US 8,685,980
A19			WO 2010/007120
A20		Human monoclonal antibody to PRLR	US 7,867,493

TABLE 18

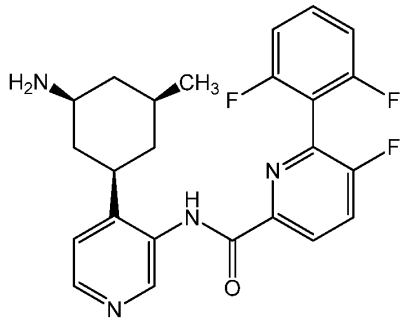
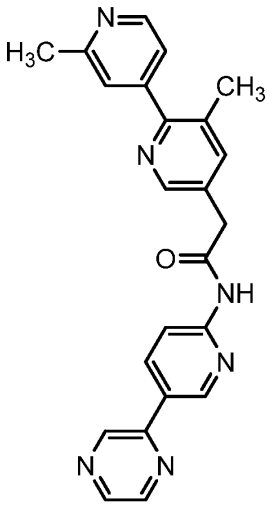
Compound Desig-nation	Generic Name Tradename	Compound Structure	Patents / Patent Application Publications
A21			WO 2010/026124 EP 2344474 US 2010/0056576 WO2008/106692
A22	WNT974		WO 2010/101849

TABLE 18

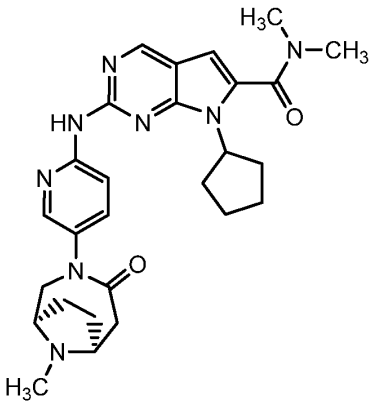
Compound Desig-nation	Generic Name Tradename	Compound Structure	Patents / Patent Application Publications
A23			WO 2011/101409
A24		Human monoclonal antibody to HER3, , *e.g.*, LJM716	WO 2012/022814 EP 2606070 US 8,735,551
A25		Antibody Drug Conjugate (ADC)	WO 2014/160160, *e.g.*, Ab: 12425 (*see* Table 1, paragraph [00191]) Linker: SMCC (*see* paragraph [00117] Payload: DM1 (*see* paragraph [00111] *See* also Claim 29
A26		Monoclonal antibody or Fab to M-CSF, *e.g.*, MCS110	WO 2004/045532

TABLE 18

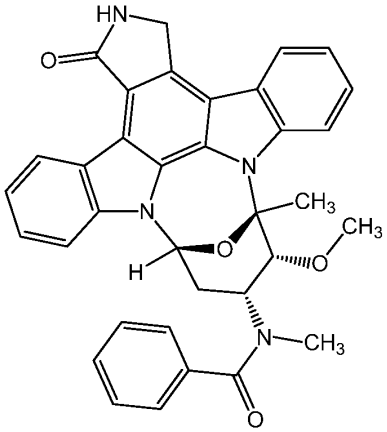
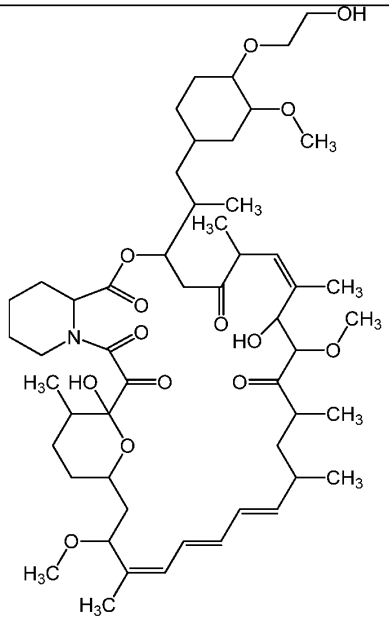
Compound Desig-nation	Generic Name Tradename	Compound Structure	Patents / Patent Application Publications
A27	Midostaurin		WO 2003/037347 EP 1441737 US 2012/252785
A28	Everolimus AFINITOR®		WO 2014/085318

TABLE 18

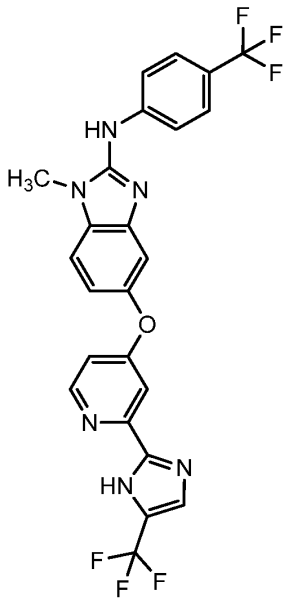
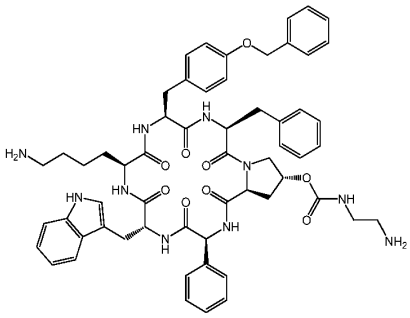
Compound Desig-nation	Generic Name Tradename	Compound Structure	Patents / Patent Application Publications
A29			WO 2007/030377 US 7,482,367
A30	Pasireotide diaspartate SIGNIFOR®		US 7,473,761

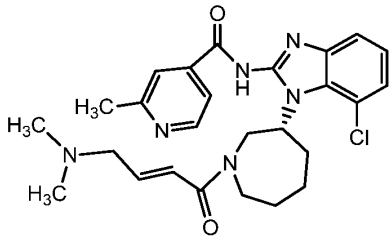
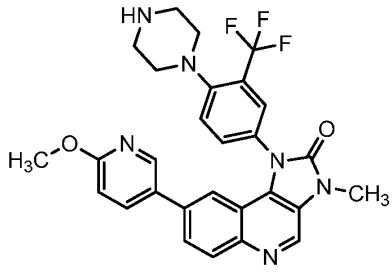
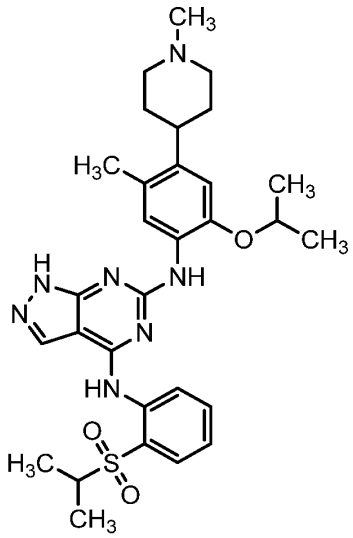
TABLE 18			
Compound Desig-nation	**Generic Name** **Tradename**	**Compound Structure**	**Patents / Patent Application Publications**
A31			WO 2013/184757
A32			WO 2006/122806
A33			WO 2008/073687 US 8,372,858

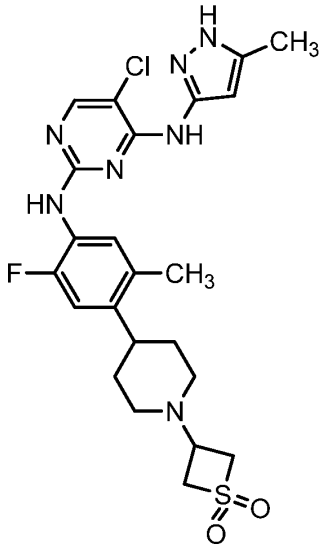
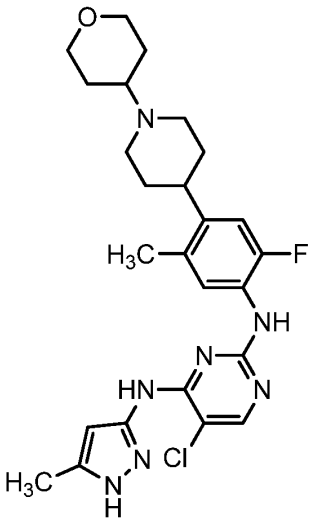
TABLE 18			
Compound Desig-nation	**Generic Name** **Tradename**	**Compound Structure**	**Patents / Patent Application Publications**
A34			WO 2010/002655 US 8,519,129
A35			WO 2010/002655 US 8,519,129

TABLE 18

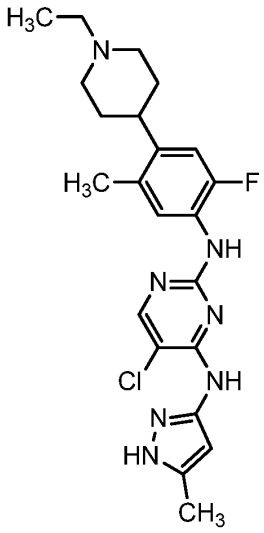
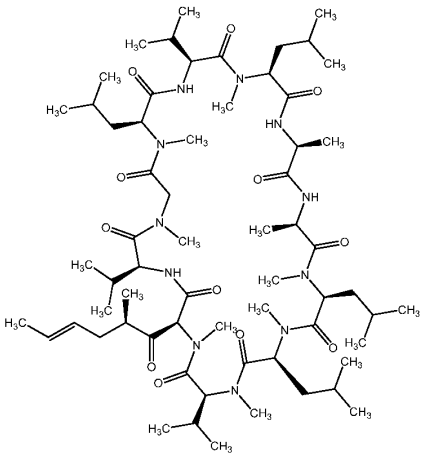
Compound Desig-nation	Generic Name Tradename	Compound Structure	Patents / Patent Application Publications
A36			WO 2010/002655
A37	Valspodar AMDRAY™		EP 296122

TABLE 18

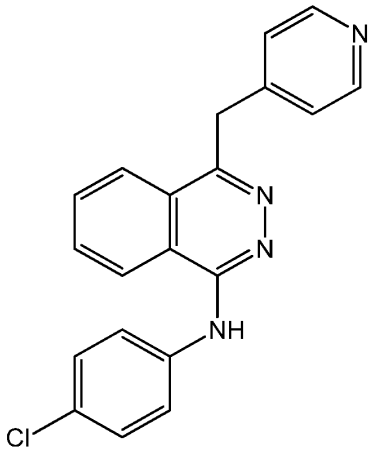
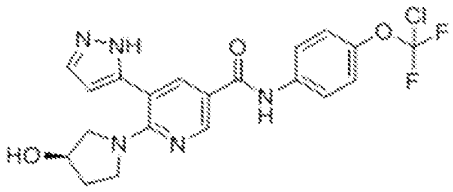
Compound Desig-nation	Generic Name Tradename	Compound Structure	Patents / Patent Application Publications
A38	Vatalanib succinate	 succinate	WO 98/35958
A39		IDH inhibitor, *e.g.*, IDH305	WO2014/141104
A40	Asciminib	BCR-ABL inhibitor 	WO2013/171639 WO2013/171640 WO2013/171641 WO2013/171642
A41		cRAF inhibitor	WO2014/151616
A42		ERK1/2 ATP competitive inhibitor	WO2015/066188

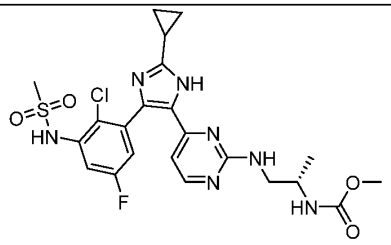
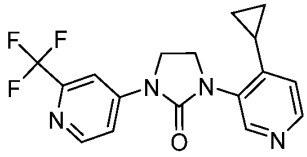
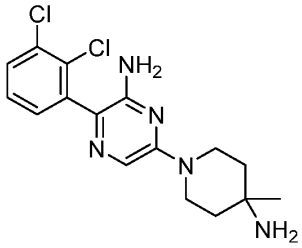
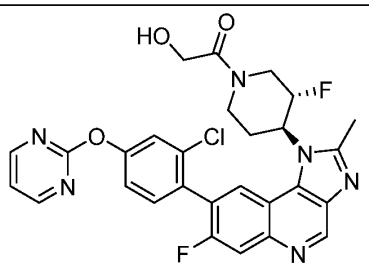
TABLE 18			
Compound Desig-nation	**Generic Name Tradename**	**Compound Structure**	**Patents / Patent Application Publications**
A43			WO2011/023773
A44			WO2012/149413
A45	SHP099		WO2015/107493
A46		SHP2 inhibitor of Formula I	WO2015/107495
A47			WO2015/022662

TABLE 18

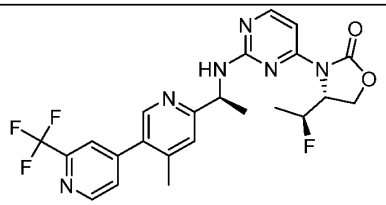
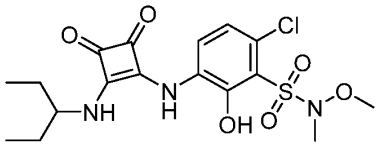
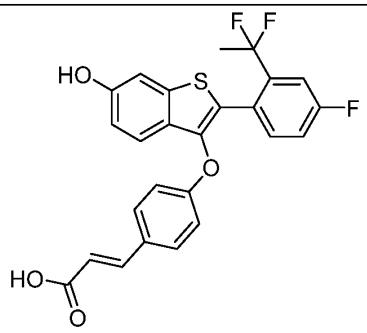
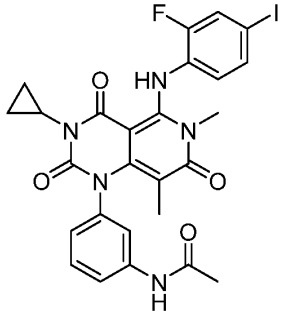
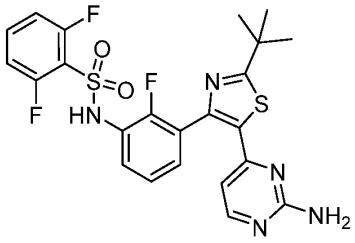
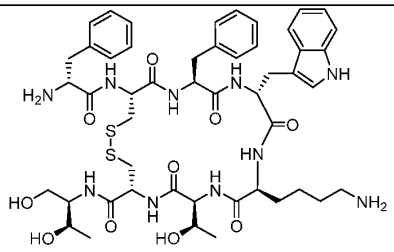
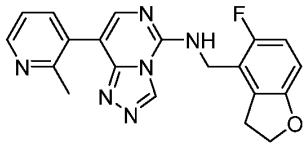
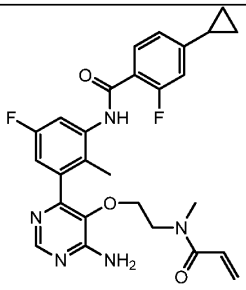
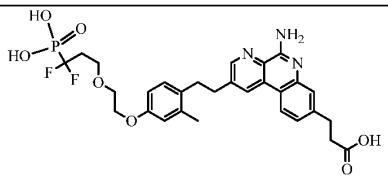
Compound Desig-nation	Generic Name Tradename	Compound Structure	Patents / Patent Application Publications
A48			WO2014/141104
A49		 or a choline salt thereof	WO2010/015613 WO2013030803 US 7,989,497,
A50		A2A receptor antagonist of Formula (I)	WO 2017/025918 WO2011/121418 US 8,796,284
A51			WO2014/130310
A52	trametinib		WO2005/121142 US 7,378,423

TABLE 18

Compound Desig-nation	Generic Name Tradename	Compound Structure	Patents / Patent Application Publications
A53	dabrafenib		WO 2009/137391 US 7,994,185
A54	octreotide		US 4,395,403 EP 0 029 579
A55			WO 2016/103155 US 9580437 EP 3237418
A56			US 9,512,084 WO/2015/079417
A57			WO2011/049677

[0836] In some embodiments, a CD19 binding molecule is administered in combination with one or more of NIZ985, a GITR agonist such as GWN323, PTK787, MBG453, mAb12425, CLR457, BGT226, BYL719, AMN107, ABL001, IDH305/LQS305, LJM716, MCS110, WNT974/LGK974, BLZ945, NIR178, QBM076, MBG453, CGS-20267, LHS534, LKG960,

LDM099/SHP099, TNO155, LCL161, MAP855/LQN716, RAD001, LEJ511, LDK378, LOU064, LSZ102, LEQ506, RAF265/CHIR265, canakinumab, gevokizumab, Anakinra, Rilonacept, CGS-20267, PSC833, GGP-57148B, CGM097, HDM201, LBH589, PKC412, LHC165, MAK683, INC280, INC424, LJE704, LAG525, and NIS793.

[0837] In some embodiments, the CD19 binding molecule is administered in combination with a standard treatment.

[0838] Standard treatment for multiple myeloma and associated diseases includes chemotherapy, stem cell transplant (autologous or allogeneic), radiation therapy, and other drug therapies. Frequently used anti-myeloma drugs include alkylating agents (*e.g.*, bendamustine, cyclophosphamide and melphalan), proteasome inhibitors (*e.g.*, bortezomib), corticosteroids (*e.g.*, dexamethasone and prednisone), and immunomodulators (*e.g.*, thalidomide and lenalidomide or Revlimid®), or any combination thereof. Biphosphonate drugs are also frequently administered in combination with the standard anti-MM treatments to prevent bone loss. Patients older than 65-70 years of age are unlikely candidates for stem cell transplant. In some cases, double-autologous stem cell transplants are options for patients less than 60 years of age with suboptimal response to the first transplant. The compositions and methods of the present disclosure can be administered in combination with any of the currently prescribed treatments for multiple myeloma.

[0839] Hodgkin's lymphoma is commonly treated with radiation therapy, chemotherapy, or hematopoietic stem cell transplantation. The most common therapy for non-Hodgkin's lymphoma is R-CHOP, which consists of four different chemotherapies (cyclophosphamide, doxorubicin, vincristine, and prenisolone) and rituximab (Rituxan®). Other therapies commonly used to treat NHL include other chemotherapeutic agents, radiation therapy, stem cell transplantation (autologous or allogeneic bone marrow transplantation), or biological therapy, such as immunotherapy. Other examples of biological therapeutic agents include, but are not limited to, rituximab (Rituxan®), tositumomab (Bexxar®), epratuzumab (LymphoCide®), and alemtuzumab (MabCampath®). The compositions and methods of the present disclosure can be administered in combination with any of the currently prescribed treatments for Hodgkin's lymphoma or non-Hodgkin's lymphoma.

[0840] Standard treatment for WM consists of chemotherapy, specifically with rituximab (Rituxan®). Other chemotherapeutic drugs can be used in combination, such as chlorambucil (Leukeran®), cyclophosphamide (Neosar®), fludarabine (Fludara®), cladribine (Leustatin®), vincristine, and/or thalidomide. Corticosteriods, such as prednisone, can also be administered in combination with the chemotherapy. Plasmapheresis, or plasma exchange, is commonly used throughout treatment of the patient to alleviate some symptoms by removing the

paraprotein from the blood. In some cases, stem cell transplantation is an option for some patients.

[0841] The CD19 binding molecules of the disclosure can be administered in combination with an agent which reduces or ameliorates a side effect associated with the administration of such binding molecules, incuding MBMs that bind to both CD19 and CD3. Side effects associated with the administration of MBMs that bind to both CD19 and CD3 can include, but are not limited to, cytokine release syndrome ("CRS") and hemophagocytic lymphohistiocytosis (HLH), also termed Macrophage Activation Syndrome (MAS). Symptoms of CRS can include high fevers, nausea, transient hypotension, hypoxia, and the like. CRS can include clinical constitutional signs and symptoms such as fever, fatigue, anorexia, myalgias, arthalgias, nausea, vomiting, and headache. CRS can include clinical skin signs and symptoms such as rash. CRS can include clinical gastrointestinal signs and symptoms such as nausea, vomiting and diarrhea. CRS can include clinical respiratory signs and symptoms such as tachypnea and hypoxemia. CRS can include clinical cardiovascular signs and symptoms such as tachycardia, widened pulse pressure, hypotension, increased cardiac output (early) and potentially diminished cardiac output (late). CRS can include clinical coagulation signs and symptoms such as elevated d-dimer, hypofibrinogenemia with or without bleeding. CRS can include clinical renal signs and symptoms such as azotemia. CRS can include clinical hepatic signs and symptoms such as transaminitis and hyperbilirubinemia. CRS can include clinical neurologic signs and symptoms such as headache, mental status changes, confusion, delirium, word finding difficulty or frank aphasia, hallucinations, tremor, dymetria, altered gait, and seizures.

[0842] Accordingly, the methods described herein can comprise administering a MBM that binds to both CD19 and CD3 to a subject and further administering one or more agents to manage elevated levels of a soluble factor resulting from treatment with the MBM. In one embodiment, the soluble factor elevated in the subject is one or more of IFN-γ, TNFα, IL-2 and IL-6. In an embodiment, the factor elevated in the subject is one or more of IL-1, GM-CSF, IL-10, IL-8, IL-5 and fraktalkine. Therefore, an agent administered to treat this side effect can be an agent that neutralizes one or more of these soluble factors. In one embodiment, the agent that neutralizes one or more of these soluble forms is an antibody or antigen binding fragment thereof. Examples of such agents include, but are not limited to a steroid (*e.g.*, corticosteroid), an inhibitor of TNFα, and inhibitor of IL-1R, and an inhibitor of IL-6. An example of a TNFα inhibitor is an anti-TNFα antibody molecule such as, infliximab, adalimumab, certolizumab pegol, and golimumab. Another example of a TNFα inhibitor is a fusion protein such as entanercept. Small molecule inhibitor of TNFα include, but are not limited to, xanthine derivatives (*e.g.* pentoxifylline) and bupropion. An example of an IL-6 inhibitor is an anti-IL-6

antibody molecule such as tocilizumab (toc), sarilumab, elsilimomab, CNTO 328, ALD518/BMS-945429, CNTO 136, CPSI-2364, CDP6038, VX30, ARGX-109, FE301, and FM101. In one embodiment, the anti-IL-6 antibody molecule is tocilizumab. An example of an IL-1R based inhibitor is anakinra.

[0843] In some embodiment, the subject is administered a corticosteroid, such as, *e.g.*, methylprednisolone, hydrocortisone, among others. In some embodiments, the subject is administered a corticosteroid, *e.g.*, methylprednisolone, hydrocortisone, in combination with Benadryl and Tylenol prior to the administration of a CD19 binding molecule, *e.g.*, a MBM that binds CD19 and CD3.

[0844] In some embodiments, the subject is administered a vasopressor, such as, *e.g.*, norepinephrine, dopamine, phenylephrine, epinephrine, vasopressin, or any combination thereof.

[0845] In an embodiment, the subject can be administered an antipyretic agent. In an embodiment, the subject can be administered an analgesic agent.

8. EXAMPLES

[0846] The examples below relate, in part, to the identification of novel CD19 binders, NEG218 and NEG258, that bind to human CD19 and are cross-reactive with cynomolgus (cyno) CD19, their incorporation into bispecific (BSP) and trispecific (TSP) binding molecules that engage CD3 and, in the case of the TSPs, CD2, as well as extensive characterization of the anti-tumor and immunostimulatory activities of the BSPs and TSPs.

[0847] In functional assays, the TSPs, particularly CD3hi TSP1, demonstrate enhanced tumor cell killing and T cell activation & proliferation as compared to the corresponding BSPs, While both CD3hi TSP1 and CD3med TSP1 demonstrate effective anti-tumor responses on established tumors in tumor-bearing mice, T-cell activation by CD3hi TSP1 is particularly effective at enriching T cells with a younger and more functional phenotype. Additionally, CD3hi TSP1 is particularly effective in activating CD28neg CD8-T cells, the exhausted/terminally differentiated cytotoxic T cells. Further, CD3hi TSP1-treated T cells better retain ability to kill target cells upon repeated challenges.

[0848] Altogether, this evidence presented herein indicates that the use of CD2 co-stimulation, particularly via a CD58 moiety, results in a CD19 binding molecule that can engage T cells in a manner that can achieve optimal T cell activation and prevent exhaustion, potentially resulting in a more effective and durable anti-tumor response.

[0849] The TSPs, particularly CD3hi TSP1, are optimized for a combination of factors, ranging from a novel CD19 binding domain that cross-reacts with cyno CD19, the inclusion of a CD2 binding moiety, the nature and affinity of the T-cell binding moieties (CD58 vs. an anti-CD2

antibody, the relatively “high” or "medium" affinity of the CD3 binding moiety), and the configuration of the binding moieties in the molecules (e.g., CD19 at the N-terminus), all of which individually confer advantageous properties that are expected to result in superior CD19 therapeutics.

8.1. Example 1: Production of anti-CD3-anti-CD19 IgG1 bispecific and trispecific binding molecules in knob-into-holes format

8.1.1. Example 1A: Initial BBM and TBM constructs

[0850] BBMs having a CD3 ABM and a CD19 ABM (shown schematically in FIG. 3A), and TBMs having a CD3 ABM, a CD19 ABM, and a CD2 ABM (shown schematically in FIG. 3B) were produced in a knob-into-hole (KIH) format. Each BBM and TBM of this Example comprises a first half antibody (shown schematically as the left half of each construct shown in FIGS. 3A-3B) and a second half antibody (shown schematically as the right half of each construct shown in FIGS. 3A-3B).

8.1.1.1. Materials and Methods

8.1.1.1.1. Plasmids encoding BBMs and TBMs

[0851] Plasmids for all constructs were synthesized and codon optimized for expression in mammalian cells.

[0852] For each bispecific construct, three plasmids were synthesized. A first plasmid encoding an anti-CD19 heavy chain was synthesized as a fusion comprising (in the N-terminal to C-terminal direction) (i) an anti-CD19 VH domain and (ii) a constant hIgG1 domain containing T366S, L368A, and Y407V mutations for a hole to facilitate heterodimerization as well as silencing mutations. A second plasmid encoding a light chain was synthesized as a fusion comprising (in the N-terminal to C-terminal direction) (i) an anti-CD19 VL domain and (ii) a constant human kappa sequence. The proteins encoded by the first and second plasmids form the first half antibody. A third plasmid encoding the second half antibody was synthesized as a fusion comprising (in the N-terminal to C-terminal direction) (i) an anti-CD3 single chain variable fragment (having the VH and VL domains of an anti-CD3 antibody designated as CD3hi (as defined in the following paragraph)), (ii) a linker, and (iii) a constant hIgG1 domain containing a T366W mutation for a knob to facilitate heterodimerization as well as silencing mutations.

[0853] For each trispecific construct, three plasmids were synthesized. A first plasmid encoding an anti-CD19 heavy chain was synthesized as a fusion comprising (in the N-terminal to C-terminal direction) (i) an anti-CD19 VH domain fused to a constant hIgG1 CH1 domain, (ii) a linker, (iii) an anti-CD3 scFv with VH and VL domains of an anti-CD3 antibody having high, medium, or low affinity to CD3 (in relative terms), and referred to herein as CD3hi, CD3med or CD3lo (from anti-CD3 antibodies having an affinity to CD3 of 16 nM, 30 nM, or 48 nm, respectively, as measured by Biacore), (iv) a second linker, and (v) an hIgG1 Fc domain

containing T366S, L368A, and Y407V mutations for a hole to facilitate heterodimerization as well as silencing mutations. It should be understood that with respect to the mentioned Biacore affinity values and relative terms in the construct names, these are used merely for identification purposes and are not intended to represent absolute affinity values. A second plasmid encoding a light chain was synthesized as a fusion comprising (in the N-terminal to C-terminal direction) (i) an anti-CD19 VL domain and (ii) a constant human kappa sequence. The proteins encoded by the first and second plasmids form the first half antibody. A third plasmid encoding the second half antibody was synthesized as a fusion comprising (in the N-terminal to C-terminal direction) (i) the IgV domain of CD58 (CD58-6) and (ii) a constant hIgG1 domain containing a T366W mutation for a knob to facilitate heterodimerization as well as silencing mutations.

[0854] Control constructs corresponding to the CD3hi TSP1 (which has a NEG258-based CD19 binding arm) and CD3hi TSP2 (which has a NEG218-based CD19 binding arm) trispecific constructs were produced in which the CD2 ABM was replaced with a Vhh against hen egg lysozyme (such control constructs having the names CD3hi TSP1L and CD3hi TSP2L, respectively).

[0855] Amino acid sequences for components of the constructs are shown in Table 19A-1 (without Fc sequences) and Table 19A-2 (with Fc sequences).

TABLE 19A-1
Amino acid sequences

Construct Name	Chain Description	Amino Acid Sequence	SEQ ID NO:
CD3hi TSP1	First Half Antibody Heavy Chain (Fc sequence not shown)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYWIQWVRQAPG QRLEWMGAVYPGDADTRYTQKFQGRVTLTADRSASTAYMELS SLRSEDTAVYYCGRDAGLEYYALDYWGQGTLVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKRVEPKSCGGGGSGGGGSEVQLVESGGGLVQPGGSLKLS CAASGFTFNTYAMNWVRQASGKGLEWVGRIRSKYNNYATYYA DSVKDRFTISRDDSKSTLYLQMNSLKTEDTAVYYCVRHGNFGN SYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGS QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKP GQAPRGLIGGTNKRAPWTPARFSGSLLGDKAALTLSGAQPED EAEYFCALWYSNLWVFGGGTKLTVLGGGGS	758
	First Half Antibody Light Chain	EIVMTQSPATLSVSPGERATLSCRASQDVGTAVAWYQQKPGQ APRLLIYWASTRHTGIPARFSGSGSGTEFTLTISSLQSEDFAVYF CQQYANFPLYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST YSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	759
	Second Half Antibody (Fc sequence not shown)	SQQIYGVVYGNVTFHVPSNVPLKEVLWKKQKDKVAELENSEFR AFSSFKNRVYLDTVSGSLTIYNLTSSDEDEYEMESPNITDTMKF FLYVLESGGGGS	760

TABLE 19A-1
Amino acid sequences

Construct Name	Chain Description	Amino Acid Sequence	SEQ ID NO:
CD3med TSP1	First Half Antibody Heavy Chain (Fc sequence not shown)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYWIQWVRQAPG QRLEWMGAVYPGDADTRYTQKFQGRVTLTADRSASTAYMELS SLRSEDTAVYYCGRDAGLEYYALDYWGQGTLVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKRVEPKSCGGGGSGGGGSEVQLVESGGGLVQPGGSLKLS CAASGFTFNTYAMNWVRQASGKGLEWVGRIRSKYNNYATYYA DSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVRHGNFGN SYVSWFAHWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGS QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTSSNYANWVQQKP GQAPRGLIGGTNKRAPWTPARFSGSLLGGKAALTLSGAQPED EAEYYCALWYSNLWVFGGGTKLTVLGGGGS	761
	First Half Antibody Light Chain	EIVMTQSPATLSVSPGERATLSCRASQDVGTAVAWYQQKPGQ APRLLIYWASTRHTGIPARFSGSGSGTEFTLTISSLQSEDFAVYF CQQYANFPLYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST YSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	759
	Second Half Antibody (Fc sequence not shown)	SQQIYGVVYGNVTFHVPSNVPLKEVLWKKQKDKVAELENSEFR AFSSFKNRVYLDTVSGSLTIYNLTSSDEDEYEMESPNITDTMKF FLYVLESGGGGS	760
CD3lo TSP1	First Half Antibody Heavy Chain (Fc sequence not shown)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYWIQWVRQAPG QRLEWMGAVYPGDADTRYTQKFQGRVTLTADRSASTAYMELS SLRSEDTAVYYCGRDAGLEYYALDYWGQGTLVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKRVEPKSCGGGGSGGGGSEVQLVESGGGLVQPGGSLKLS CAASGFTFNTYAMNWVRQASGKGLEWVGRIRSKYNNYATYYA DSVKDRFTISRDDSKSTAYLQMNSLKTEDTAVYYCVRHGNFGN SYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGS QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKP GQAPRGLIGGTNKRAPWTPARFSGSLLGDKAALTLSGAQPED EAEYFCALWYSNLWVFGGGTKLTVLGGGGS	762
	First Half Antibody Light Chain	EIVMTQSPATLSVSPGERATLSCRASQDVGTAVAWYQQKPGQ APRLLIYWASTRHTGIPARFSGSGSGTEFTLTISSLQSEDFAVYF CQQYANFPLYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST YSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	759
	Second Half Antibody (Fc sequence not shown)	SQQIYGVVYGNVTFHVPSNVPLKEVLWKKQKDKVAELENSEFR AFSSFKNRVYLDTVSGSLTIYNLTSSDEDEYEMESPNITDTMKF FLYVLESGGGGS	760
CD3hi TSP2	First Half Antibody Heavy Chain (Fc sequence not shown)	EVQLVQSGAEVKKPGESLKISCKASGYSFTNYWMNWVRQMP GKGLEWMGMIHPSDSEIRLNQKFQGQVTLSVDKSIGTAYMQW SSLKASDTAMYYCSRWYYLSSPMDYWGQGTTVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKRVEPKSCGGGGSGGGGSEVQLVESGGGLVQPGGSLKLS CAASGFTFNTYAMNWVRQASGKGLEWVGRIRSKYNNYATYYA DSVKDRFTISRDDSKSTLYLQMNSLKTEDTAVYYCVRHGNFGN SYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGS QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKP GQAPRGLIGGTNKRAPWTPARFSGSLLGDKAALTLSGAQPED EAEYFCALWYSNLWVFGGGTKLTVLGGGGS	763

TABLE 19A-1
Amino acid sequences

Construct Name	Chain Description	Amino Acid Sequence	SEQ ID NO:
	First Half Antibody Light Chain	EIVMTQSPATLSVSPGERATLSCRASQDVGTAVAWYQQKPGQ APRLLIYWASTRHTGIPARFSGSGSGTEFTLTISSLQSEDFAVYF CQQYSSYPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	764
	Second Half Antibody (Fc sequence not shown)	SQQIYGVVYGNVTFHVPSNVPLKEVLWKKQKDKVAELENSEFR AFSSFKNRVYLDTVSGSLTIYNLTSSDEDEYEMESPNITDTMKF FLYVLESGGGGS	760
CD3hi TSP1L	First Half Antibody Heavy Chain (Fc sequence not shown)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYWIQWVRQAPG QRLEWMGAVYPGDADTRYTQKFQGRVTLTADRSASTAYMELS SLRSEDTAVYYCGRDAGLEYYALDYWGQGTLVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKRVEPKSCGGGGSGGGGSEVQLVESGGGLVQPGGSLKLS CAASGFTFNTYAMNWVRQASGKGLEWVGRIRSKYNNYATYYA DSVKDRFTISRDDSKSTLYLQMNSLKTEDTAVYYCVRHGNFGN SYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGS QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKP GQAPRGLIGGTNKRAPWTPARFSGSLLGDKAALTLSGAQPED EAEYFCALWYSNLWVFGGGTKLTVLGGGGS	758
	First Half Antibody Light Chain	EIVMTQSPATLSVSPGERATLSCRASQDVGTAVAWYQQKPGQ APRLLIYWASTRHTGIPARFSGSGSGTEFTLTISSLQSEDFAVYF CQQYANFPLYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST YSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	759
	Second Half Antibody (Fc sequence not shown)	DVQLQASGGGSVQAGGSLRLSCAASGYTIGPYCMGWFRQAP GKEREGVAAINMGGGITYYADSVKGRFTISQDNAKNTVYLLMN SLEPEDTAIYYCAADSTIYASYYECGHGLSTGGYGYDSWGQGT QVTVSSGGGGS	765
CD3hi TSP2L	First Half Antibody Heavy Chain (Fc sequence not shown)	EVQLVQSGAEVKKPGESLKISCKASGYSFTNYWMNWVRQMP GKGLEWMGMIHPSDSEIRLNQKFQGQVTLSVDKSIGTAYMQW SSLKASDTAMYYCSRWYYLSSPMDYWGQGTTVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKRVEPKSCGGGGSGGGGSEVQLVESGGGLVQPGGSLKLS CAASGFTFNTYAMNWVRQASGKGLEWVGRIRSKYNNYATYYA DSVKDRFTISRDDSKSTLYLQMNSLKTEDTAVYYCVRHGNFGN SYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGS QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKP GQAPRGLIGGTNKRAPWTPARFSGSLLGDKAALTLSGAQPED EAEYFCALWYSNLWVFGGGTKLTVLGGGGS	763
	First Half Antibody Light Chain	EIVMTQSPATLSVSPGERATLSCRASQDVGTAVAWYQQKPGQ APRLLIYWASTRHTGIPARFSGSGSGTEFTLTISSLQSEDFAVYF CQQYSSYPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	764
	Second Half Antibody (Fc sequence not shown)	DVQLQASGGGSVQAGGSLRLSCAASGYTIGPYCMGWFRQAP GKEREGVAAINMGGGITYYADSVKGRFTISQDNAKNTVYLLMN SLEPEDTAIYYCAADSTIYASYYECGHGLSTGGYGYDSWGQGT QVTVSSGGGGS	765

TABLE 19A-1
Amino acid sequences

Construct Name	Chain Description	Amino Acid Sequence	SEQ ID NO:
CD3hi BSP1 - 2 arm	First Half Antibody Heavy Chain (Fc sequence not shown)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYWIQWVRQAPG QRLEWMGAVYPGDADTRYTQKFQGRVTLTADRSASTAYMELS SLRSEDTAVYYCGRDAGLEYYALDYWGQGTLVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKRVEPKSC	766
	First Half Antibody Light Chain	EIVMTQSPATLSVSPGERATLSCRASQDVGTAVAWYQQKPGQ APRLLIYWASTRHTGIPARFSGSGSGTEFTLTISSLQSEDFAVYF CQQYANFPLYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST YSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	759
	Second Half Antibody (Fc sequence not shown)	EVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAS GKGLEWVGRIRSKYNNYATYYADSVKDRFTISRDDSKSTLYLQ MNSLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS GGGGSGGGGSGGGGSGGGGSQAVVTQEPSLTVSPGGTVTLT CRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPWTPA RFSGSLLGDKAALTLSGAQPEDEAEYFCALWYSNLWVFGGGT KLTVLGGGGS	767
CD3hi BSP2 - 2 arm	First Half Antibody Heavy Chain (Fc sequence not shown)	EVQLVQSGAEVKKPGESLKISCKASGYSFTNYWMNWVRQMP GKGLEWMGMIHPSDSEIRLNQKFQGQVTLSVDKSIGTAYMQW SSLKASDTAMYYCSRWYYLSSPMDYWGQGTTVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKRVEPKSC	768
	First Half Antibody Light Chain	EIVMTQSPATLSVSPGERATLSCRASQDVGTAVAWYQQKPGQ APRLLIYWASTRHTGIPARFSGSGSGTEFTLTISSLQSEDFAVYF CQQYSSYPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	764
	Second Half Antibody (Fc sequence not shown)	EVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAS GKGLEWVGRIRSKYNNYATYYADSVKDRFTISRDDSKSTLYLQ MNSLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS GGGGSGGGGSGGGGSGGGGSQAVVTQEPSLTVSPGGTVTLT CRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPWTPA RFSGSLLGDKAALTLSGAQPEDEAEYFCALWYSNLWVFGGGT KLTVLGGGGS	767

[0856] Table 19A-2 below shows the full length amino acid sequences of the constructs shown in Table 19A-1, including Fc sequences.

TABLE 19A-2
Amino acid sequences

Construct Name	Chain Description	Amino Acid Sequence	SEQ ID NO:
CD3hi TSP1	First Half Antibody Heavy Chain (includes Fc sequence)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYWIQW VRQAPGQRLEWMGAVYPGDADTRYTQKFQGRVTLT ADRSASTAYMELSSLRSEDTAVYYCGRDAGLEYYAL DYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTA ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK RVEPKSCGGGGSGGGGSEVQLVESGGGLVQPGGSL KLSCAASGFTFNTYAMNWVRQASGKGLEWVGRIRSK YNNYATYYADSVKDRFTISRDDSKSTLYLQMNSLKTE DTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSG GGGSGGGGSGGGGSGGGGSQAVVTQEPSLTVSPG GTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIG GTNKRAPWTPARFSGSLLGDKAALTLSGAQPEDEAE YFCALWYSNLWVFGGGTKLTVLGGGGSDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVA VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR VVSVLTVLHQDWLNGKEYKCKVSNKALAAPIEKTISKA KGQPREPQVCTLPPSREEMTKNQVSLSCAVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	1077
	First Half Antibody Light Chain	EIVMTQSPATLSVSPGERATLSCRASQDVGTAVAWY QQKPGQAPRLLIYWASTRHTGIPARFSGSGSGTEFTL TISSLQSEDFAVYFCQQYANFPLYTFGQGTKLEIKRTV AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	759
	Second Half Antibody (includes Fc sequence)	SQQIYGVVYGNVTFHVPSNVPLKEVLWKKQKDKVAE LENSEFRAFSSFKNRVYLDTVSGSLTIYNLTSSDEDEY EMESPNITDTMKFFLYVLESGGGGSDKTHTCPPCPA PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVAVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVS VLTVLHQDWLNGKEYKCKVSNKALAAPIEKTISKAKG QPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD KSRWQQGNVFSCSVMHEALHNRYTQKSLSLSPGK	1078

TABLE 19A-2
Amino acid sequences

Construct Name	Chain Description	Amino Acid Sequence	SEQ ID NO:
CD3med TSP1	First Half Antibody Heavy Chain (includes Fc sequence)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYWIQW VRQAPGQRLEWMGAVYPGDADTRYTQKFQGRVTLT ADRSASTAYMELSSLRSEDTAVYYCGRDAGLEYYAL DYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTA ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK RVEPKSCGGGGSGGGGSEVQLVESGGGLVQPGGSL KLSCAASGFTFNTYAMNWVRQASGKGLEWVGRIRSK YNNYATYYADSVKDRFTISRDDSKNTAYLQMNSLKTE DTAVYYCVRHGNFGNSYVSWFAHWGQGTLVTVSSG GGGSGGGGSGGGGSGGGGSQAVVTQEPSLTVSPG GTVTLTCGSSTGAVTSSNYANWVQQKPGQAPRGLIG GTNKRAPWTPARFSGSLLGGKAALTLSGAQPEDEAE YYCALWYSNLWVFGGGTKLTVLGGGGSDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVA VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR VVSVLTVLHQDWLNGKEYKCKVSNKALAAPIEKTISKA KGQPREPQVCTLPPSREEMTKNQVSLSCAVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	1079
	First Half Antibody Light Chain	EIVMTQSPATLSVSPGERATLSCRASQDVGTAVAWY QQKPGQAPRLLIYWASTRHTGIPARFSGSGSGTEFTL TISSLQSEDFAVYFCQQYANFPLYTFGQGTKLEIKRTV AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	759
	Second Half Antibody (includes Fc sequence)	SQQIYGVVYGNVTFHVPSNVPLKEVLWKKQKDKVAE LENSEFRAFSSFKNRVYLDTVSGSLTIYNLTSSDEDEY EMESPNITDTMKFFLYVLESGGGGSDKTHTCPPCPA PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVAVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVS VLTVLHQDWLNGKEYKCKVSNKALAAPIEKTISKAKG QPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD KSRWQQGNVFSCSVMHEALHNRYTQKSLSLSPGK	1078

TABLE 19A-2
Amino acid sequences

Construct Name	Chain Description	Amino Acid Sequence	SEQ ID NO:
CD3lo TSP1	First Half Antibody Heavy Chain (includes Fc sequence)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYWIQW VRQAPGQRLEWMGAVYPGDADTRYTQKFQGRVTLT ADRSASTAYMELSSLRSEDTAVYYCGRDAGLEYYAL DYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTA ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK RVEPKSCGGGGSGGGGSEVQLVESGGGLVQPGGSL KLSCAASGFTFNTYAMNWVRQASGKGLEWVGRIRSK YNNYATYYADSVKDRFTISRDDSKSTAYLQMNSLKTE DTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSG GGGSGGGGSGGGGSGGGGSQAVVTQEPSLTVSPG GTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIG GTNKRAPWTPARFSGSLLGDKAALTLSGAQPEDEAE YFCALWYSNLWVFGGGTKLTVLGGGGSDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVA VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR VVSVLTVLHQDWLNGKEYKCKVSNKALAAPIEKTISKA KGQPREPQVCTLPPSREEMTKNQVSLSCAVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	1080
	First Half Antibody Light Chain	EIVMTQSPATLSVSPGERATLSCRASQDVGTAVAWY QQKPGQAPRLLIYWASTRHTGIPARFSGSGSGTEFTL TISSLQSEDFAVYFCQQYANFPLYTFGQGTKLEIKRTV AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	759
	Second Half Antibody (includes Fc sequence)	SQQIYGVVYGNVTFHVPSNVPLKEVLWKKQKDKVAE LENSEFRAFSSFKNRVYLDTVSGSLTIYNLTSSDEDEY EMESPNITDTMKFFLYVLESGGGGSDKTHTCPPCPA PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVAVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVS VLTVLHQDWLNGKEYKCKVSNKALAAPIEKTISKAKG QPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD KSRWQQGNVFSCSVMHEALHNRYTQKSLSLSPGK	1078

TABLE 19A-2
Amino acid sequences

Construct Name	Chain Description	Amino Acid Sequence	SEQ ID NO:
CD3hi TSP2	First Half Antibody Heavy Chain (includes Fc sequence)	EVQLVQSGAEVKKPGESLKISCKASGYSFTNYWMNW VRQMPGKGLEWMGMIHPSDSEIRLNQKFQGQVTLSV DKSIGTAYMQWSSLKASDTAMYYCSRWYYLSSPMD YWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAA LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKR VEPKSCGGGGSGGGGSEVQLVESGGGLVQPGGSLK LSCAASGFTFNTYAMNWVRQASGKGLEWVGRIRSKY NNYATYYADSVKDRFTISRDDSKSTLYLQMNSLKTED TAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGG GGSGGGGSGGGGSGGGGSQAVVTQEPSLTVSPGG TVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGG TNKRAPWTPARFSGSLLGDKAALTLSGAQPEDEAEY FCALWYSNLWVFGGGTKLTVLGGGGSDKTHTCPPC PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVAVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVV SVLTVLHQDWLNGKEYKCKVSNKALAAPIEKTISKAK GQPREPQVCTLPPSREEMTKNQVSLSCAVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	1081
	First Half Antibody Light Chain	EIVMTQSPATLSVSPGERATLSCRASQDVGTAVAWY QQKPGQAPRLLIYWASTRHTGIPARFSGSGSGTEFTL TISSLQSEDFAVYFCQQYSSYPYTFGQGTKLEIKRTVA APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKAD YEKHKVYACEVTHQGLSSPVTKSFNRGEC	764
	Second Half Antibody (includes Fc sequence)	SQQIYGVVYGNVTFHVPSNVPLKEVLWKKQKDKVAE LENSEFRAFSSFKNRVYLDTVSGSLTIYNLTSSDEDEY EMESPNITDTMKFFLYVLESGGGGSDKTHTCPPCPA PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVAVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVS VLTVLHQDWLNGKEYKCKVSNKALAAPIEKTISKAKG QPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD KSRWQQGNVFSCSVMHEALHNRYTQKSLSLSPGK	1078

TABLE 19A-2
Amino acid sequences

Construct Name	Chain Description	Amino Acid Sequence	SEQ ID NO:
CD3hi TSP1L	First Half Antibody Heavy Chain (includes Fc sequence)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYWIQW VRQAPGQRLEWMGAVYPGDADTRYTQKFQGRVTLT ADRSASTAYMELSSLRSEDTAVYYCGRDAGLEYYAL DYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTA ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK RVEPKSCGGGGSGGGGSEVQLVESGGGLVQPGGSL KLSCAASGFTFNTYAMNWVRQASGKGLEWVGRIRSK YNNYATYYADSVKDRFTISRDDSKSTLYLQMNSLKTE DTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSG GGGSGGGGSGGGGSGGGGSQAVVTQEPSLTVSPG GTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIG GTNKRAPWTPARFSGSLLGDKAALTLSGAQPEDEAE YFCALWYSNLWVFGGGTKLTVLGGGGSDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVA VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR VVSVLTVLHQDWLNGKEYKCKVSNKALAAPIEKTISKA KGQPREPQVCTLPPSREEMTKNQVSLSCAVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	1077
	First Half Antibody Light Chain	EIVMTQSPATLSVSPGERATLSCRASQDVGTAVAWY QQKPGQAPRLLIYWASTRHTGIPARFSGSGSGTEFTL TISSLQSEDFAVYFCQQYANFPLYTFGQGTKLEIKRTV AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	759
	Second Half Antibody (includes Fc sequence)	DVQLQASGGGSVQAGGSLRLSCAASGYTIGPYCMG WFRQAPGKEREGVAAINMGGGITYYADSVKGRFTIS QDNAKNTVYLLMNSLEPEDTAIYYCAADSTIYASYYEC GHGLSTGGYGYDSWGQGTQVTVSSGGGGSDKTHT CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYAS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALAAPIEKTI SKAKGQPREPQVYTLPPCREEMTKNQVSLWCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNRYTQKSLSLSP GK	1082

TABLE 19A-2
Amino acid sequences

Construct Name	Chain Description	Amino Acid Sequence	SEQ ID NO:
CD3hi TSP2L	First Half Antibody Heavy Chain (includes Fc sequence)	EVQLVQSGAEVKKPGESLKISCKASGYSFTNYWMNW VRQMPGKGLEWMGMIHPSDSEIRLNQKFQGQVTLSV DKSIGTAYMQWSSLKASDTAMYYCSRWYYLSSPMD YWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAA LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKR VEPKSCGGGGSGGGGSEVQLVESGGGLVQPGGSLK LSCAASGFTFNTYAMNWVRQASGKGLEWVGRIRSKY NNYATYYADSVKDRFTISRDDSKSTLYLQMNSLKTED TAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGG GGSGGGGSGGGGSGGGGSQAVVTQEPSLTVSPGG TVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGG TNKRAPWTPARFSGSLLGDKAALTLSGAQPEDEAEY FCALWYSNLWVFGGGTKLTVLGGGGSDKTHTCPPC PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVAVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVV SVLTVLHQDWLNGKEYKCKVSNKALAAPIEKTISKAK GQPREPQVCTLPPSREEMTKNQVSLSCAVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	1081
	First Half Antibody Light Chain	EIVMTQSPATLSVSPGERATLSCRASQDVGTAVAWY QQKPGQAPRLLIYWASTRHTGIPARFSGSGSGTEFTL TISSLQSEDFAVYFCQQYSSYPYTFGQGTKLEIKRTVA APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKAD YEKHKVYACEVTHQGLSSPVTKSFNRGEC	764
	Second Half Antibody (includes Fc sequence)	DVQLQASGGGSVQAGGSLRLSCAASGYTIGPYCMG WFRQAPGKEREGVAAINMGGGITYYADSVKGRFTIS QDNAKNTVYLLMNSLEPEDTAIYYCAADSTIYASYYEC GHGLSTGGYGYDSWGQGTQVTVSSGGGGSDKTHT CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYAS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALAAPIEKTI SKAKGQPREPQVYTLPPCREEMTKNQVSLWCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNRYTQKSLSLSP GK	1082

TABLE 19A-2
Amino acid sequences

Construct Name	Chain Description	Amino Acid Sequence	SEQ ID NO:
CD3hi BSP1 - 2 arm	First Half Antibody Heavy Chain (includes Fc sequence)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYWMQW VRQAPGQRLEWMGAVYPGDADTRYTQKFQGRVTLT ADRSASTAYMELSSLRSEDTAVYYCGRDAGLEYYAL DYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTA ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK RVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDT LMISRTPEVTCVVVAVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCK VSNKALAAPIEKTISKAKGQPREPQVCTLPPSREEMT KNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK	1083
	First Half Antibody Light Chain	EIVMTQSPATLSVSPGERATLSCRASQDVGTAVAWY QQKPGQAPRLLIYWASTRHTGIPARFSGSGSGTEFTL TISSLQSEDFAVYFCQQYANFPLYTFGQGTKLEIKRTV AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	759
	Second Half Antibody (includes Fc sequence)	EVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMN WVRQASGKGLEWVGRIRSKYNNYATYYADSVKDRFT ISRDDSKSTLYLQMNSLKTEDTAVYYCVRHGNFGNSY VSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSG GGGSQAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTS NYANWVQQKPGQAPRGLIGGTNKRAPWTPARFSGS LLGDKAALTLSGAQPEDEAEYFCALWYSNLWVFGGG TKLTVLGGGGSDKTHTCPPCPAPELLGGPSVFLFPPK PKDTLMISRTPEVTCVVVAVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKE YKCKVSNKALAAPIEKTISKAKGQPREPQVYTLPPCR EEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNRYTQKSLSLSPGK	1084
CD3hi BSP2 - 2 arm	First Half Antibody Heavy Chain (includes Fc sequence)	EVQLVQSGAEVKKPGESLKISCKASGYSFTNYWMNW VRQMPGKGLEWMGMIHPSDSEIRLNQKFQGQVTLSV DKSIGTAYMQWSSLKASDTAMYYCSRWYYLSSPMD YWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAA LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKR VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVAVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKV SNKALAAPIEKTISKAKGQPREPQVCTLPPSREEMTK NQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK	1085
	First Half Antibody Light Chain	EIVMTQSPATLSVSPGERATLSCRASQDVGTAVAWY QQKPGQAPRLLIYWASTRHTGIPARFSGSGSGTEFTL TISSLQSEDFAVYFCQQYSSYPYTFGQGTKLEIKRTVA	764

TABLE 19A-2 Amino acid sequences			
Construct Name	**Chain Description**	**Amino Acid Sequence**	**SEQ ID NO:**
		APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKAD YEKHKVYACEVTHQGLSSPVTKSFNRGEC	
	Second Half Antibody (includes Fc sequence)	EVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMN WVRQASGKGLEWVGRIRSKYNNYATYYADSVKDRFT ISRDDSKSTLYLQMNSLKTEDTAVYYCVRHGNFGNSY VSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSG GGGSQAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTS NYANWVQQKPGQAPRGLIGGTNKRAPWTPARFSGS LLGDKAALTLSGAQPEDEAEYFCALWYSNLWVFGGG TKLTVLGGGGSDKTHTCPPCPAPELLGGPSVFLFPPK PKDTLMISRTPEVTCVVVAVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKE YKCKVSNKALAAPIEKTISKAKGQPREPQVYTLPPCR EEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNRYTQKSLSLSPGK	1084

8.1.1.1.2. Expression and purification

[0857] BBMs and TBMs were expressed transiently by co-transfection of the respective chains in HEK293 cells. Briefly, transfection of the cells with the heavy and light chain plasmids was performed using PEI as transfection reagent with a final DNA:PEI ratio of 1:3. 1 mg of plasmid per liter of culture was used for transfection of cultures having 2.0 million cells/mL of serum media. After 5 days of expression, BBMs and TBMs were harvested by clarification of the media via centrifugation and filtration. Purification was performed via anti-CH1 affinity batch binding (CaptureSelect IgG-CH1 Affinity Matrix, Thermo-Fisher Scientific, Waltham, MA, USA) or Protein A (rProteinA Sepharose, Fast flow, GE Healthcare, Uppsala, Sweden) batch binding using 1ml resin/100 mL supernatant. The protein was allowed to bind for a minimum of 2 hours with gentle mixing, and the supernatant loaded onto a gravity filtration column. The resin was washed with 20-50 CV of PBS. BBMs and TBMs were eluted with 20 CV of 50 mM citrate, 90 mM NaCl pH 3.2. 50mM sucrose. The eluted BBM and TBM fractions were adjusted to pH 5.5 with 1 M sodium citrate 50mM sucrose. Preparative size exclusion chromatography was performed using Hi Load 16/60 Superdex 200 grade column (GE Healthcare Life Sciences, Uppsala, Sweden) as a final polishing step when aggregates were present. To confirm that the identity of the proteins of the BBMs and TBMs expressed matched the predicted masses for the primary amino acid sequences, proteins were analyzed by high-performance liquid chromatography coupled to mass spectrometry.

8.1.1.1.3. CD3 affinity measurements

[0858] The affinity of the CD3hi, CD3med, and CD3lo mAbs to CD3 were determined at 25 °C using a Biacore T200 system. Briefly, anti-hFc IgG1 was immobilized on a CM5 chip. After capturing CD3-Fc (1 µg/ml in HBS-EP+ buffer, flow rate of 50 µl/min, with a 30 second injection time) kinetic data was acquired by subsequent injections of 1:2 dilution series of the different antibodies in HBS-EP+ buffer.

[0859] Data were evaluated using the Biacore T200 evaluation software version 1.0. The raw data were double referenced, *i.e.* the response of the measuring flow cell was corrected for the response of the reference flow cell, and in a second step the response of a blank injection was subtracted. Finally, the sensorgrams were fitted by applying 1:1 binding model to calculate kinetic rate constants and dissociation equilibrium constants. R_{max} was set at local. Data were processed individually for each run.

8.1.2. Example 1B: Additional BBM and TBM constructs

[0860] A one-arm BBM having a CD3 ABM and a CD19 ABM (CD3hi BSP1, shown schematically in FIG. 3C) and a TBM corresponding to CD3hi TSP1 but with a lysozyme binding arm in place of the CD19 binding arm (CD3hi TSP1C) were produced. The amino acid sequences of the CD3hi BSP1 and CD3hi TSP1C constructs are shown in Table 19B.

TABLE 19B
Amino acid sequences

Construct Name	Chain Description	Amino Acid Sequence	SEQ ID NO:
CD3hi BSP1	First Half Antibody Heavy Chain (includes Fc sequence)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYWIQWVRQ APGQRLEWMGAVYPGDADTRYTQKFQGRVTLTADRSAST AYMELSSLRSEDTAVYYCGRDAGLEYYALDYWGQGTLVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKRVEPKSCGGGGSGGGGSEVQLV ESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQASGKG LEWVGRIRSKYNNYATYYADSVKDRFTISRDDSKSTLYLQM NSLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVS SGGGGSGGGGSGGGGSGGGGSQAVVTQEPSLTVSPGGT VTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRA PWTPARFSGSLLGDKAALTLSGAQPEDEAEYFCALWYSNL WVFGGGTKLTVLGGGGSDKTHTCPPCPAPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVVAVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKV SNKALAAPIEKTISKAKGQPREPQVCTLPPSREEMTKNQVS LSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF FLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK	1077
	First Half Antibody Light Chain	EIVMTQSPATLSVSPGERATLSCRASQDVGTAVAWYQQKP GQAPRLLIYWASTRHTGIPARFSGSGSGTEFTLTISSLQSE DFAVYFCQQYANFPLYTFGQGTKLEIKRTVAAPSVFIFPPS DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ	759

TABLE 19B
Amino acid sequences

Construct Name	Chain Description	Amino Acid Sequence	SEQ ID NO:
		ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGL SSPVTKSFNRGEC	
	Second Half Antibody (includes Fc sequence)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY RVVSVLTVLHQDWLNGKEYKCKVSNKALAAPIEKTISKAKG QPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNRYTQKSLSLSPGK	1102
CD3hi TSP1C	First Half Antibody Heavy Chain (includes Fc sequence)	QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWSWIR QSPGRGLEWLGRIYYRSKWYNDYAVSVKSRITINPDTSKN QFSLQLNSVTPEDTAVYYCARLDHRYHEDTVYPGMDVWG QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCGGGGSGG GGSEVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMNW VRQASGKGLEWVGRIRSKYNNYATYYADSVKDRFTISRDD SKSTLYLQMNSLKTEDTAVYYCVRHGNFGNSYVSWFAYW GQGTLVTVSSGGGGSGGGGSGGGGSGGGGSQAVVTQE PSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAP RGLIGGTNKRAPWTPARFSGSLLGDKAALTLSGAQPEDEA EYFCALWYSNLWVFGGGTKLTVLGGGGSDKTHTCPPCPA PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVAVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQD WLNGKEYKCKVSNKALAAPIEKTISKAKGQPREPQVCTLPP SREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK	1100
	First Half Antibody Light Chain	DIELTQPPSVSVAPGQTARISCSGDNLPAYTVTWYQQKPG QAPVLVIYDDSDRPSGIPERFSGSNSGNTATLTISGTQAED EADYYCASWDPSSGVVFGGGTKLTVLGQPKAAPSVTLFPP SSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVET TTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGST VEKTVAPTECS	1101
	Second Half Antibody (includes Fc sequence)	SQQIYGVVYGNVTFHVPSNVPLKEVLWKKQKDKVAELENS EFRAFSSFKNRVYLDTVSGSLTIYNLTSSDEDEYEMESPNIT DTMKFFLYVLESGGGGSDKTHTCPPCPAPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVVAVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKV SNKALAAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQVS LWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF FLYSKLTVDKSRWQQGNVFSCSVMHEALHNRYTQKSLSLS PGK	1078

[0861] Additionally, CD3hi TSP1, CD3med TSP1, CD3hi BSP1, and CD3hi TSP1C constructs were each produced in a second version having a second half antibody sequence varying from the second half antibody sequence for the construct set forth in Table 19A-2 (in the case of CD3med TSP1 and CD3hi TSP1) or Table 19B (in the case of CD3hi BSP1 and CD3hi TSP1C) by one amino acid in the Fc sequence. Specifically, the second half antibody sequences in Table 19A-2 and Table 19B have an arginine residue where the second versions have a histidine residue. The arginine residue was included in the constructs to facilitate purification via

Protein A binding. The versions of the constructs set forth in Table 19A-2 and Table 19B are referred to herein as "R variants" and the versions of the constructs set forth in Table 19C, below, are referred to herein as "H variants." It is believed that the functional activity of a construct's R variant does not differ significantly from the functional activity of its H variant. Nucleotide sequences encoding H variants of CD3hi TSP1, CD3med TSP1, CD3hi BSP1, and CD3hi TSP1C are shown in Table 19D.

TABLE 19C
Amino acid sequences (H variants)

Construct Name	Chain Description	Amino Acid Sequence	SEQ ID NO:
CD3hi TSP1 (H variant)	First Half Antibody Heavy Chain (includes Fc sequence)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYWIQWVRQ APGQRLEWMGAVYPGDADTRYTQKFQGRVTLTADRSAST AYMELSSLRSEDTAVYYCGRDAGLEYYALDYWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKRVEPKSCGGGGSGGGGSEVQL VESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQASGK GLEWVGRIRSKYNNYATYYADSVKDRFTISRDDSKSTLYLQ MNSLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTV SSGGGGSGGGGSGGGGSGGGGSQAVVTQEPSLTVSPG GTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTN KRAPWTPARFSGSLLGDKAALTLSGAQPEDEAEYFCALWY SNLWVFGGGTKLTVLGGGGSDKTHTCPPCPAPELLGGPS VFLFPPKPKDTLMISRTPEVTCVVVAVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEY KCKVSNKALAAPIEKTISKAKGQPREPQVCTLPPSREEMTK NQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS DGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	1077
	First Half Antibody Light Chain	EIVMTQSPATLSVSPGERATLSCRASQDVGTAVAWYQQKP GQAPRLLIYWASTRHTGIPARFSGSGSGTEFTLTISSLQSE DFAVYFCQQYANFPLYTFGQGTKLEIKRTVAAPSVFIFPPS DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG LSSPVTKSFNRGEC	759
	Second Half Antibody (includes Fc sequence)	SQQIYGVVYGNVTFHVPSNVPLKEVLWKKQKDKVAELENS EFRAFSSFKNRVYLDTVSGSLTIYNLTSSDEDEYEMESPNIT DTMKFFLYVLESGGGGSDKTHTCPPCPAPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVVAVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKV SNKALAAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQVS LWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK	1086

TABLE 19C
Amino acid sequences (H variants)

Construct Name	Chain Description	Amino Acid Sequence	SEQ ID NO:
CD3med TSP1 (H variant)	First Half Antibody Heavy Chain (includes Fc sequence)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYWIQWVRQ APGQRLEWMGAVYPGDADTRYTQKFQGRVTLTADRSAST AYMELSSLRSEDTAVYYCGRDAGLEYYALDYWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKRVEPKSCGGGGSGGGGSEVQL VESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQASGK GLEWVGRIRSKYNNYATYYADSVKDRFTISRDDSKNTAYL QMNSLKTEDTAVYYCVRHGNFGNSYVSWFAHWGQGTLV TVSSGGGGSGGGGSGGGGSGGGGSQAVVTQEPSLTVSP GGTVTLTCGSSTGAVTSSNYANWVQQKPGQAPRGLIGGT NKRAPWTPARFSGSLLGGKAALTLSGAQPEDEAEYYCAL WYSNLWVFGGGTKLTVLGGGGSDKTHTCPPCPAPELLGG PSVFLFPPKPKDTLMISRTPEVTCVVVAVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK EYKCKVSNKALAAPIEKTISKAKGQPREPQVCTLPPSREEM TKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK	1079
	First Half Antibody Light Chain	EIVMTQSPATLSVSPGERATLSCRASQDVGTAVAWYQQKP GQAPRLLIYWASTRHTGIPARFSGSGSGTEFTLTISSLQSE DFAVYFCQQYANFPLYTFGQGTKLEIKRTVAAPSVFIFPPS DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG LSSPVTKSFNRGEC	759
	Second Half Antibody (includes Fc sequence)	SQQIYGVVYGNVTFHVPSNVPLKEVLWKKQKDKVAELENS EFRAFSSFKNRVYLDTVSGSLTIYNLTSSDEDEYEMESPNIT DTMKFFLYVLESGGGGSDKTHTCPPCPAPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVVAVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKV SNKALAAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQVS LWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK	1086
CD3hi BSP1 (H variant)	First Half Antibody Heavy Chain (includes Fc sequence)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYWIQWVRQ APGQRLEWMGAVYPGDADTRYTQKFQGRVTLTADRSAST AYMELSSLRSEDTAVYYCGRDAGLEYYALDYWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKRVEPKSCGGGGSGGGGSEVQL VESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQASGK GLEWVGRIRSKYNNYATYYADSVKDRFTISRDDSKSTLYLQ MNSLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTV SSGGGGSGGGGSGGGGSGGGGSQAVVTQEPSLTVSPG GTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTN KRAPWTPARFSGSLLGDKAALTLSGAQPEDEAEYFCALWY SNLWVFGGGTKLTVLGGGGSDKTHTCPPCPAPELLGGPS VFLFPPKPKDTLMISRTPEVTCVVVAVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEY KCKVSNKALAAPIEKTISKAKGQPREPQVCTLPPSREEMTK NQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS DGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	1077

TABLE 19C
Amino acid sequences (H variants)

Construct Name	Chain Description	Amino Acid Sequence	SEQ ID NO:
	First Half Antibody Light Chain	EIVMTQSPATLSVSPGERATLSCRASQDVGTAVAWYQQKP GQAPRLLIYWASTRHTGIPARFSGSGSGTEFTLTISSLQSE DFAVYFCQQYANFPLYTFGQGTKLEIKRTVAAPSVFIFPPS DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG LSSPVTKSFNRGEC	759
	Second Half Antibody (includes Fc sequence)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY RVVSVLTVLHQDWLNGKEYKCKVSNKALAAPIEKTISKAKG QPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK	1099
CD3hi TSP1C (H variant)	First Half Antibody Heavy Chain (includes Fc sequence)	QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWSWIR QSPGRGLEWLGRIYYRSKWYNDYAVSVKSRITINPDTSKN QFSLQLNSVTPEDTAVYYCARLDHRYHEDTVYPGMDVWG QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCGGGGSGG GGSEVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMNW VRQASGKGLEWVGRIRSKYNNYATYYADSVKDRFTISRDD SKSTLYLQMNSLKTEDTAVYYCVRHGNFGNSYVSWFAYW GQGTLVTVSSGGGGSGGGGSGGGGSGGGGSQAVVTQE PSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAP RGLIGGTNKRAPWTPARFSGSLLGDKAALTLSGAQPEDEA EYFCALWYSNLWVFGGGTKLTVLGGGGSDKTHTCPPCPA PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVAVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALAAPIEKTISKAKGQPREPQVCTLP PSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGK	1100
	First Half Antibody Light Chain	DIELTQPPSVSVAPGQTARISCSGDNLPAYTVTWYQQKPG QAPVLVIYDDSDRPSGIPERFSGSNSGNTATLTISGTQAED EADYYCASWDPSSGVVFGGGTKLTVLGQPKAAPSVTLFPP SSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVET TTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGST VEKTVAPTECS	1101
	Second Half Antibody (includes Fc sequence)	SQQIYGVVYGNVTFHVPSNVPLKEVLWKKQKDKVAELENS EFRAFSSFKNRVYLDTVSGSLTIYNLTSSDEDEYEMESPNIT DTMKFFLYVLESGGGGSDKTHTCPPCPAPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVVAVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKV SNKALAAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQVS LWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK	1086

TABLE 19D
Nucleotide sequences (H variants)

Construct Name	Chain Description	Amino Acid Sequence	SEQ ID NO:
CD3hi TSP1 (H variant)	First Half Antibody Heavy Chain (includes signal peptide sequence)	ATGCCACTGCTGCTTCTACTGCCACTCCTGTGGGCAGGA GCACTGGCCCAAGTGCAACTGGTGCAGTCCGGTGCCGA AGTGAAGAAGCCCGGTGCCTCTGTGAAGGTGTCCTGCAA GGCGTCGGGATACACGTTCACCACTTACTGGATTCAGTG GGTCAGACAGGCCCCGGGACAGAGACTGGAGTGGATGG GAGCCGTGTACCCCGGAGATGCAGACACTCGCTACACCC AGAAGTTCCAGGGCCGCGTGACTTTGACCGCCGACAGAA GCGCCAGCACCGCCTACATGGAGCTTTCATCCCTCCGGA GCGAGGATACTGCCGTATACTATTGCGGAAGGGATGCCG GCCTGGAATACTATGCCCTCGACTACTGGGGACAGGGGA CCCTCGTGACTGTGTCCAGCGCGAGCACCAAGGGCCCC AGCGTGTTCCCGCTGGCCCCATCATCCAAGTCCACCTCG GGAGGGACTGCTGCGCTCGGTTGCCTTGTGAAGGACTAC TTCCCCGAGCCCGTGACTGTGTCGTGGAACAGCGGGGC TCTGACCAGCGGGGTTCACACCTTTCCCGCCGTGCTGCA GTCCTCGGGACTCTACAGCCTGTCCTCCGTGGTCACGGT CCCGTCGTCGTCGCTGGGGACCCAGACCTACATTTGCAA CGTGAACCACAAACCCTCCAACACAAAAGTGGACAAAAG GGTGGAACCTAAGTCCTGTGGAGGGGGTGGATCAGGCG GAGGAGGATCGGAAGTCCAGCTCGTCGAATCAGGGGGA GGGCTTGTGCAACCAGGAGGCTCCCTCAAGCTGTCTTGC GCAGCGTCCGGTTTCACTTTCAACACTTATGCGATGAATT GGGTCCGCCAAGCCAGTGGGAAGGGCCTGGAGTGGGTC GGACGGATCAGATCCAAGTACAACAACTACGCGACATAC TACGCCGACTCCGTGAAGGATCGCTTCACCATCAGCCGG GATGACTCCAAGAGCACCTTGTACCTCCAAATGAACAGC CTTAAGACCGAGGACACTGCGGTGTACTACTGCGTGAGA CACGGCAACTTCGGAAACTCCTACGTGTCCTGGTTCGCC TACTGGGGACAGGGCACCCTTGTCACTGTGTCAAGCGGA GGCGGTGGTTCGGGTGGCGGAGGTTCCGGAGGAGGAG GTTCGGGCGGTGGTGGATCACAGGCCGTCGTGACTCAG GAACCATCCCTGACTGTGTCCCCCGGTGGAACCGTGACC CTCACCTGTCGCTCCTCAACCGGAGCCGTGACCACCTCC AACTACGCTAATTGGGTGCAGCAGAAGCCAGGACAAGCC CCACGGGGACTGATTGGGGGCACCAACAAGAGGGCTCC TTGGACCCCAGCCCGCTTCTCGGGCTCCCTGTTGGGCGA CAAGGCCGCTCTGACCCTGTCCGGTGCACAGCCGGAGG ATGAAGCCGAATACTTCTGCGCGCTGTGGTACTCCAACC TCTGGGTGTTCGGCGGAGGGACCAAGCTGACTGTGTTG GGAGGAGGGGGGAGTGACAAGACTCACACGTGTCCGCC TTGCCCAGCACCCGAGCTACTGGGAGGACCGAGCGTGT TCCTGTTTCCCCCGAAGCCGAAGGATACCCTGATGATCT CCCGCACTCCTGAAGTGACTTGCGTGGTGGTGGCAGTGT CCCACGAGGACCCGGAAGTCAAGTTTAATTGGTACGTGG ATGGCGTGGAGGTGCACAACGCAAAGACCAAGCCTCGC GAGGAGCAGTACGCCAGCACCTACCGGGTGGTGTCCGT CCTGACGGTGCTGCACCAGGACTGGCTGAACGGGAAGG AGTACAAGTGCAAAGTGTCAAATAAGGCTTTGGCCGCCC CTATTGAGAAAACCATCTCAAAGGCCAAGGGCCAACCCA GGGAACCTCAAGTGTGCACCCTCCCACCTTCGCGAGAAG AGATGACCAAGAACCAGGTGTCCCTGTCCTGCGCCGTGA AGGGCTTCTACCCCTCCGATATCGCCGTGGAGTGGGAAT CTAACGGACAGCCGGAGAACAACTACAAGACCACTCCGC CGGTGCTGGACAGCGACGGCTCCTTCTTCCTCGTGTCGA	1089

TABLE 19D
Nucleotide sequences (H variants)

Construct Name	Chain Description	Amino Acid Sequence	SEQ ID NO:
		AACTGACCGTGGACAAGTCACGGTGGCAGCAGGGCAAT GTGTTCAGCTGCTCAGTCATGCATGAGGCCCTCCACAAC CACTACACTCAGAAGTCCCTGTCGCTTTCCCCCGGAAAA	
	First Half Antibody Light Chain (includes signal peptide sequence)	ATGTCGGTCCTGACCCAAGTGCTGGCCCTCCTTCTCCTG TGGCTGACCGGGACCAGATGCGAAATCGTCATGACTCAG AGCCCGGCAACCCTGTCCGTGAGCCCTGGAGAACGGGC CACTCTGAGCTGTCGGGCGTCACAGGACGTGGGAACTG CCGTGGCCTGGTATCAGCAGAAGCCGGGACAGGCTCCT AGGTTGCTCATCTACTGGGCGTCCACTCGCCACACCGGA ATCCCAGCCCGCTTCTCCGGCTCGGGTTCTGGCACCGAG TTCACCCTGACCATTTCCTCCCTCCAATCCGAGGATTTCG CCGTGTACTTCTGCCAACAATACGCCAACTTCCCCCTGTA CACATTTGGCCAGGGGACCAAGCTGGAGATTAAGCGTAC GGTGGCCGCTCCCAGCGTGTTCATCTTCCCCCCCAGCGA CGAGCAGCTGAAGAGCGGCACCGCCAGCGTGGTGTGCC TGCTGAACAACTTCTACCCCCGGGAGGCCAAGGTGCAGT GGAAGGTGGACAACGCCCTGCAGAGCGGCAACAGCCAG GAGAGCGTCACCGAGCAGGACAGCAAGGACTCCACCTA CAGCCTGAGCAGCACCCTGACCCTGAGCAAGGCCGACT ACGAGAAGCATAAGGTGTACGCCTGCGAGGTGACCCACC AGGGCCTGTCCAGCCCCGTGACCAAGAGCTTCAACAGG GGCGAGTGC	1090
	Second Half Antibody (includes signal peptide sequence)	ATGCCTCTGCTGCTCCTGCTGCCTCTGCTCTGGGCCGGA GCTTTGGCATCACAGCAAATCTACGGCGTGGTGTACGGC AACGTGACCTTCCATGTCCCCTCCAATGTGCCGCTGAAG GAAGTGCTCTGGAAGAAGCAGAAGGACAAGGTCGCGGA ACTGGAAAACTCCGAGTTTCGCGCCTTCTCCTCCTTCAAA AACCGGGTGTACCTGGACACCGTGTCCGGGAGCCTTACT ATCTACAACCTGACCTCCTCGGACGAGGATGAGTATGAG ATGGAGAGCCCAAACATTACCGACACCATGAAGTTCTTCC TCTACGTGCTGGAATCGGGTGGAGGCGGAAGCGATAAG ACTCACACGTGTCCACCTTGTCCCGCACCCGAACTCCTG GGGGGACCTTCCGTGTTTCTCTTCCCCCCTAAACCGAAG GACACCTTGATGATCTCCCGCACTCCTGAAGTGACCTGT GTGGTGGTGGCCGTGTCCCACGAGGACCCAGAAGTCAA GTTTAATTGGTACGTGGACGGAGTCGAGGTGCACAACGC GAAAACCAAACCGCGGGAGGAGCAGTACGCCTCCACCTA CCGGGTGGTGTCCGTCCTCACTGTGCTGCACCAGGACTG GCTCAACGGAAAGGAGTACAAGTGCAAAGTGTCCAACAA AGCCTTGGCGGCCCCAATCGAAAAGACGATCTCCAAGGC CAAGGGACAGCCGCGCGAACCTCAAGTCTACACCCTGCC TCCTTGCCGCGAGGAAATGACCAAGAACCAGGTGTCACT GTGGTGTCTGGTCAAGGGATTCTACCCTTCCGATATCGC AGTGGAGTGGGAAAGCAACGGCCAACCAGAGAACAACTA TAAGACCACACCCCCGGTGCTCGATTCCGACGGCTCATT CTTCCTGTACTCCAAGCTGACCGTGGACAAGTCACGGTG GCAGCAGGGGAACGTGTTCAGCTGCTCCGTGATGCATGA AGCCCTGCACAATCATTACACTCAGAAGTCCCTGTCGCT GAGCCCCGGAAAA	1091
CD3med TSP1 (H variant)	First Half Antibody Heavy Chain (includes signal	ATGCCACTGCTGCTTCTACTGCCACTCCTGTGGGCAGGA GCACTGGCCCAAGTGCAACTGGTGCAGTCCGGTGCCGA AGTGAAGAAGCCCGGTGCCTCTGTGAAGGTGTCCTGCAA GGCGTCGGGATACACGTTCACCACTTACTGGATTCAGTG GGTCAGACAGGCCCCGGGACAGAGACTGGAGTGGATGG	1092

TABLE 19D
Nucleotide sequences (H variants)

Construct Name	Chain Description	Amino Acid Sequence	SEQ ID NO:
	peptide sequence)	GAGCCGTGTACCCCGGAGATGCAGACACTCGCTACACCC AGAAGTTCCAGGGCCGCGTGACTTTGACCGCCGACAGAA GCGCCAGCACCGCCTACATGGAGCTTTCATCCCTCCGGA GCGAGGATACTGCCGTATACTATTGCGGAAGGGATGCCG GCCTGGAATACTATGCCCTCGACTACTGGGGACAGGGGA CCCTCGTGACTGTGTCCAGCGCGAGCACCAAGGGCCCG AGCGTGTTCCCATTGGCCCCGTCGTCAAAGTCCACCTCT GGCGGAACTGCGGCTCTGGGATGTCTCGTGAAGGACTA CTTTCCGGAACCCGTGACTGTGTCCTGGAACAGCGGCGC CCTCACTTCCGGCGTGCATACCTTCCCTGCCGTGCTGCA GTCCTCCGGCCTGTACAGCCTCAGCAGCGTCGTGACTGT GCCCTCCTCGTCCTTGGGCACCCAGACCTACATCTGCAA CGTCAACCACAAGCCCTCGAACACCAAAGTGGATAAGCG GGTGGAACCCAAGAGCTGTGGAGGGGGTGGCTCAGGAG GAGGGGGATCCGAAGTGCAGCTCGTGGAGTCCGGAGGA GGCCTGGTGCAGCCTGGGGGATCCCTCAAGCTTAGCTG CGCCGCATCAGGCTTCACCTTCAACACCTACGCCATGAA CTGGGTCCGCCAAGCATCCGGAAAGGGCCTGGAATGGG TCGGGAGAATCAGATCCAAGTACAACAACTACGCCACGT ACTACGCGGACTCCGTCAAGGACCGGTTCACTATTAGCC GGGATGACTCCAAGAATACCGCGTACCTTCAGATGAACT CGCTCAAAACCGAGGACACTGCCGTGTATTACTGCGTGC GGCACGGAAACTTCGGGAACAGTTACGTGTCCTGGTTCG CCCATTGGGGTCAAGGCACCCTGGTCACCGTGTCCTCGG GTGGTGGTGGCTCCGGTGGAGGAGGATCGGGGGGGTGG AGGATCTGGGGGAGGCGGATCACAGGCGGTCGTGACTC AGGAGCCCTCCCTGACCGTGTCGCCTGGTGGCACCGTG ACTCTGACTTGCGGAAGCTCAACAGGCGCCGTGACCTCC TCGAACTACGCCAACTGGGTGCAACAGAAGCCGGGACAA GCCCCTAGGGGACTGATCGGGGGGGACCAACAAGCGCGC TCCGTGGACTCCCGCGAGGTTCTCCGGAAGCCTCCTGG GAGGGAAGGCAGCCCTGACCCTGTCCGGAGCTCAGCCA GAAGATGAGGCCGAGTACTATTGCGCCCTGTGGTACTCG AATCTGTGGGTGTTTGGAGGCGGCACCAAGCTGACCGTC CTGGGTGGTGGCGGAAGCGACAAGACTCACACGTGTCC GCCTTGCCCAGCACCCGAGCTACTGGGAGGACCGAGCG TGTTCCTGTTTCCCCCGAAGCCGAAGGATACCCTGATGA TCTCCCGCACTCCTGAAGTGACTTGCGTGGTGGTGGCAG TGTCCCACGAGGACCCGGAAGTCAAGTTTAATTGGTACG TGGATGGCGTGGAGGTGCACAACGCAAAGACCAAGCCT CGCGAGGAGCAGTACGCCAGCACCTACCGGGTGGTGTC CGTCCTGACGGTGCTGCACCAGGACTGGCTGAACGGGA AGGAGTACAAGTGCAAAGTGTCAAATAAGGCTTTGGCCG CCCCTATTGAGAAAACCATCTCAAAGGCCAAGGGCCAAC CCAGGGAACCTCAAGTGTGCACCCTCCCACCTTCGCGAG AAGAGATGACCAAGAACCAGGTGTCCCTGTCCTGCGCCG TGAAGGGCTTCTACCCCTCCGATATCGCCGTGGAGTGGG AATCTAACGGACAGCCGGAGAACAACTACAAGACCACTC CGCCGGTGCTGGACAGCGACGGCTCCTTCTTCCTCGTGT CGAAACTGACCGTGGACAAGTCACGGTGGCAGCAGGGC AATGTGTTCAGCTGCTCAGTCATGCATGAGGCCCTCCAC AACCACTACACTCAGAAGTCCCTGTCGCTTTCCCCCGGA AAA	

TABLE 19D
Nucleotide sequences (H variants)

Construct Name	Chain Description	Amino Acid Sequence	SEQ ID NO:
	First Half Antibody Light Chain (includes signal peptide sequence)	GAAATCGTCATGACTCAGAGCCCGGCAACCCTGTCCGTG AGCCCTGGAGAACGGGCCACTCTGAGCTGTCGGGCGTC ACAGGACGTGGGAACTGCCGTGGCCTGGTATCAGCAGA AGCCGGGACAGGCTCCTAGGTTGCTCATCTACTGGGCGT CCACTCGCCACACCGGAATCCCAGCCCGCTTCTCCGGCT CGGGTTCTGGCACCGAGTTCACCCTGACCATTTCCTCCC TCCAATCCGAGGATTTCGCCGTGTACTTCTGCCAACAATA CGCCAACTTCCCCCTGTACACATTTGGCCAGGGGACCAA GCTGGAGATTAAGCGTACGGTGGCCGCTCCCAGCGTGTT CATCTTCCCCCCCAGCGACGAGCAGCTGAAGAGCGGCA CCGCCAGCGTGGTGTGCCTGCTGAACAACTTCTACCCCC GGGAGGCCAAGGTGCAGTGGAAGGTGGACAACGCCCTG CAGAGCGGCAACAGCCAGGAGAGCGTCACCGAGCAGGA CAGCAAGGACTCCACCTACAGCCTGAGCAGCACCCTGAC CCTGAGCAAGGCCGACTACGAGAAGCATAAGGTGTACGC CTGCGAGGTGACCCACCAGGGCCTGTCCAGCCCCGTGA CCAAGAGCTTCAACAGGGGCGAGTGC	1093
	Second Half Antibody (includes signal peptide sequence)	ATGCCTCTGCTGCTCCTGCTGCCTCTGCTCTGGGCCGGA GCTTTGGCATCACAGCAAATCTACGGCGTGGTGTACGGC AACGTGACCTTCCATGTCCCCTCCAATGTGCCGCTGAAG GAAGTGCTCTGGAAGAAGCAGAAGGACAAGGTCGCGGA ACTGGAAAACTCCGAGTTTCGCGCCTTCTCCTCCTTCAAA AACCGGGTGTACCTGGACACCGTGTCCGGGAGCCTTACT ATCTACAACCTGACCTCCTCGGACGAGGATGAGTATGAG ATGGAGAGCCCAAACATTACCGACACCATGAAGTTCTTCC TCTACGTGCTGGAATCGGGTGGAGGCGGAAGCGATAAG ACTCACACGTGTCCACCTTGTCCCGCACCCGAACTCCTG GGGGGACCTTCCGTGTTTCTCTTCCCCCCTAAACCGAAG GACACCTTGATGATCTCCCGCACTCCTGAAGTGACCTGT GTGGTGGTGGCCGTGTCCCACGAGGACCCAGAAGTCAA GTTTAATTGGTACGTGGACGGAGTCGAGGTGCACAACGC GAAAACCAAACCGCGGGAGGAGCAGTACGCCTCCACCTA CCGGGTGGTGTCCGTCCTCACTGTGCTGCACCAGGACTG GCTCAACGGAAAGGAGTACAAGTGCAAAGTGTCCAACAA AGCCTTGGCGGCCCCAATCGAAAAGACGATCTCCAAGGC CAAGGGACAGCCGCGCGAACCTCAAGTCTACACCCTGCC TCCTTGCCGCGAGGAAATGACCAAGAACCAGGTGTCACT GTGGTGTCTGGTCAAGGGATTCTACCCTTCCGATATCGC AGTGGAGTGGGAAAGCAACGGCCAACCAGAGAACAACTA TAAGACCACACCCCCGGTGCTCGATTCCGACGGCTCATT CTTCCTGTACTCCAAGCTGACCGTGGACAAGTCACGGTG GCAGCAGGGGAACGTGTTCAGCTGCTCCGTGATGCATGA AGCCCTGCACAATCATTACACTCAGAAGTCCCTGTCGCT GAGCCCCGGAAAA	1091
CD3hi BSP1 (H variant)	First Half Antibody Heavy Chain (includes signal peptide sequence)	ATGCCACTGCTGCTTCTACTGCCACTCCTGTGGGCAGGA GCACTGGCCCAAGTGCAACTGGTGCAGTCCGGTGCCGA AGTGAAGAAGCCCGGTGCCTCTGTGAAGGTGTCCTGCAA GGCGTCGGGATACACGTTCACCACTTACTGGATTCAGTG GGTCAGACAGGCCCCGGGACAGAGACTGGAGTGGATGG GAGCCGTGTACCCCGGAGATGCAGACACTCGCTACACCC AGAAGTTCCAGGGCCGCGTGACTTTGACCGCCGACAGAA GCGCCAGCACCGCCTACATGGAGCTTTCATCCCTCCGGA GCGAGGATACTGCCGTATACTATTGCGGAAGGGATGCCG GCCTGGAATACTATGCCCTCGACTACTGGGGACAGGGGA	1089

TABLE 19D
Nucleotide sequences (H variants)

Construct Name	Chain Description	Amino Acid Sequence	SEQ ID NO:
		CCCTCGTGACTGTGTCCAGCGCGAGCACCAAGGGCCCC AGCGTGTTCCCGCTGGCCCCATCATCCAAGTCCACCTCG GGAGGGACTGCTGCGCTCGGTTGCCTTGTGAAGGACTAC TTCCCCGAGCCCGTGACTGTGTCGTGGAACAGCGGGGC TCTGACCAGCGGGGTTCACACCTTTCCCGCCGTGCTGCA GTCCTCGGGACTCTACAGCCTGTCCTCCGTGGTCACGGT CCCGTCGTCGTCGCTGGGGACCCAGACCTACATTTGCAA CGTGAACCACAAACCCTCCAACACAAAAGTGGACAAAAG GGTGGAACCTAAGTCCTGTGGAGGGGGTGGATCAGGCG GAGGAGGATCGGAAGTCCAGCTCGTCGAATCAGGGGGA GGGCTTGTGCAACCAGGAGGCTCCCTCAAGCTGTCTTGC GCAGCGTCCGGTTTCACTTTCAACACTTATGCGATGAATT GGGTCCGCCAAGCCAGTGGGAAGGGCCTGGAGTGGGTC GGACGGATCAGATCCAAGTACAACAACTACGCGACATAC TACGCCGACTCCGTGAAGGATCGCTTCACCATCAGCCGG GATGACTCCAAGAGCACCTTGTACCTCCAAATGAACAGC CTTAAGACCGAGGACACTGCGGTGTACTACTGCGTGAGA CACGGCAACTTCGGAAACTCCTACGTGTCCTGGTTCGCC TACTGGGGACAGGGCACCCTTGTCACTGTGTCAAGCGGA GGCGGTGGTTCGGGTGGCGGAGGTTCCGGAGGAGGAG GTTCGGGCGGTGGTGGATCACAGGCCGTCGTGACTCAG GAACCATCCCTGACTGTGTCCCCCGGTGGAACCGTGACC CTCACCTGTCGCTCCTCAACCGGAGCCGTGACCACCTCC AACTACGCTAATTGGGTGCAGCAGAAGCCAGGACAAGCC CCACGGGGACTGATTGGGGGCACCAACAAGAGGGCTCC TTGGACCCCAGCCCGCTTCTCGGGCTCCCTGTTGGGCGA CAAGGCCGCTCTGACCCTGTCCGGTGCACAGCCGGAGG ATGAAGCCGAATACTTCTGCGCGCTGTGGTACTCCAACC TCTGGGTGTTCGGCGGAGGGACCAAGCTGACTGTGTTG GGAGGAGGGGGGAGTGACAAGACTCACACGTGTCCGCC TTGCCCAGCACCCGAGCTACTGGGAGGACCGAGCGTGT TCCTGTTTCCCCCGAAGCCGAAGGATACCCTGATGATCT CCCGCACTCCTGAAGTGACTTGCGTGGTGGTGGCAGTGT CCCACGAGGACCCGGAAGTCAAGTTTAATTGGTACGTGG ATGGCGTGGAGGTGCACAACGCAAAGACCAAGCCTCGC GAGGAGCAGTACGCCAGCACCTACCGGGTGGTGTCCGT CCTGACGGTGCTGCACCAGGACTGGCTGAACGGGAAGG AGTACAAGTGCAAAGTGTCAAATAAGGCTTTGGCCGCCC CTATTGAGAAAACCATCTCAAAGGCCAAGGGCCAACCCA GGGAACCTCAAGTGTGCACCCTCCCACCTTCGCGAGAAG AGATGACCAAGAACCAGGTGTCCCTGTCCTGCGCCGTGA AGGGCTTCTACCCCTCCGATATCGCCGTGGAGTGGGAAT CTAACGGACAGCCGGAGAACAACTACAAGACCACTCCGC CGGTGCTGGACAGCGACGGCTCCTTCTTCCTCGTGTCGA AACTGACCGTGGACAAGTCACGGTGGCAGCAGGGCAAT GTGTTCAGCTGCTCAGTCATGCATGAGGCCCTCCACAAC CACTACACTCAGAAGTCCCTGTCGCTTTCCCCCGGAAAA	
	First Half Antibody Light Chain (includes signal peptide sequence)	ATGTCGGTCCTGACCCAAGTGCTGGCCCTCCTTCTCCTG TGGCTGACCGGGACCAGATGCGAAATCGTCATGACTCAG AGCCCGGCAACCCTGTCCGTGAGCCCTGGAGAACGGGC CACTCTGAGCTGTCGGGCGTCACAGGACGTGGGAACTG CCGTGGCCTGGTATCAGCAGAAGCCGGGACAGGCTCCT AGGTTGCTCATCTACTGGGCGTCCACTCGCCACACCGGA ATCCCAGCCCGCTTCTCCGGCTCGGGTTCTGGCACCGAG	1090

TABLE 19D
Nucleotide sequences (H variants)

Construct Name	Chain Description	Amino Acid Sequence	SEQ ID NO:
		TTCACCCTGACCATTTCCTCCCTCCAATCCGAGGATTTCG CCGTGTACTTCTGCCAACAATACGCCAACTTCCCCCTGTA CACATTTGGCCAGGGGACCAAGCTGGAGATTAAGCGTAC GGTGGCCGCTCCCAGCGTGTTCATCTTCCCCCCCAGCGA CGAGCAGCTGAAGAGCGGCACCGCCAGCGTGGTGTGCC TGCTGAACAACTTCTACCCCCGGGAGGCCAAGGTGCAGT GGAAGGTGGACAACGCCCTGCAGAGCGGCAACAGCCAG GAGAGCGTCACCGAGCAGGACAGCAAGGACTCCACCTA CAGCCTGAGCAGCACCCTGACCCTGAGCAAGGCCGACT ACGAGAAGCATAAGGTGTACGCCTGCGAGGTGACCCACC AGGGCCTGTCCAGCCCCGTGACCAAGAGCTTCAACAGG GGCGAGTGC	
	Second Half Antibody (includes signal peptide sequence)	ATGCCTCTGCTGCTCCTGCTGCCTCTGCTCTGGGCCGGA GCTTTGGCAGATAAGACTCACACGTGTCCACCTTGTCCC GCACCCGAACTCCTGGGGGGACCTTCCGTGTTTCTCTTC CCCCCTAAACCGAAGGACACCTTGATGATCTCCCGCACT CCTGAAGTGACCTGTGTGGTGGTGGCCGTGTCCCACGA GGACCCAGAAGTCAAGTTTAATTGGTACGTGGACGGAGT CGAGGTGCACAACGCGAAAACCAAACCGCGGGAGGAGC AGTACGCCTCCACCTACCGGGTGGTGTCCGTCCTCACTG TGCTGCACCAGGACTGGCTCAACGGAAAGGAGTACAAGT GCAAAGTGTCCAACAAAGCCTTGGCGGCCCCAATCGAAA AGACGATCTCCAAGGCCAAGGGACAGCCGCGCGAACCT CAAGTCTACACCCTGCCTCCTTGCCGCGAGGAAATGACC AAGAACCAGGTGTCACTGTGGTGTCTGGTCAAGGGATTC TACCCTTCCGATATCGCAGTGGAGTGGGAAAGCAACGGC CAACCAGAGAACAACTATAAGACCACACCCCCGGTGCTC GATTCCGACGGCTCATTCTTCCTGTACTCCAAGCTGACC GTGGACAAGTCACGGTGGCAGCAGGGGAACGTGTTCAG CTGCTCCGTGATGCATGAAGCCCTGCACAATCATTACACT CAGAAGTCCCTGTCGCTGAGCCCCGGAAAA	1103
CD3hi TSP1C (H variant)	First Half Antibody Heavy Chain (includes signal peptide sequence)	ATGCCTCTGCTGCTCCTGTTGCCCCTGCTGTGGGCTGGA GCCTTGGCCCAAGTGCAGCTTCAGCAGTCGGGACCCGG ACTCGTGAAGCCGTCGCAGACGCTGTCCCTGACCTGTGC CATTAGCGGCGACTCCGTGAGCAGCAACAGCGCAGCCT GGTCCTGGATTCGGCAGTCACCTGGTCGGGGGCTTGAAT GGCTGGGACGGATCTACTACCGCTCGAAATGGTATAACG ACTACGCCGTGTCTGTGAAGTCCAGGATCACCATCAACC CGGACACCTCCAAGAATCAGTTCTCCCTCCAACTGAACTC AGTGACCCCAGAGGACACCGCCGTCTACTACTGCGCGA GACTGGATCACCGCTACCATGAAGATACCGTGTACCCGG GGATGGACGTCTGGGGCCAGGGTACTCTCGTCACTGTGT CCTCCGCGTCCACTAAGGGCCCCAGCGTGTTCCCGCTG GCCCCATCATCCAAGTCCACCTCGGGAGGGACTGCTGC GCTCGGTTGCCTTGTGAAGGACTACTTCCCCGAGCCCGT GACTGTGTCGTGGAACAGCGGGGCTCTGACCAGCGGGG TTCACACCTTTCCCGCCGTGCTGCAGTCCTCGGGACTCT ACAGCCTGTCCTCCGTGGTCACGGTCCCGTCGTCGTCGC TGGGGACCCAGACCTACATTTGCAACGTGAACCACAAAC CCTCCAACACAAAAGTGGACAAAAGGGTGGAACCTAAGT CCTGTGGAGGGGGTGGATCAGGCGGAGGAGGATCGGAA GTCCAGCTCGTCGAATCAGGGGGAGGGCTTGTGCAACC AGGAGGCTCCCTCAAGCTGTCTTGCGCAGCGTCCGGTTT CACTTTCAACACTTATGCGATGAATTGGGTCCGCCAAGCC	1104

TABLE 19D Nucleotide sequences (H variants)			
Construct Name	**Chain Description**	**Amino Acid Sequence**	**SEQ ID NO:**
		AGTGGGAAGGGCCTGGAGTGGGTCGGACGGATCAGATC CAAGTACAACAACTACGCGACATACTACGCCGACTCCGT GAAGGATCGCTTCACCATCAGCCGGGATGACTCCAAGAG CACCTTGTACCTCCAAATGAACAGCCTTAAGACCGAGGA CACTGCGGTGTACTACTGCGTGAGACACGGCAACTTCGG AAACTCCTACGTGTCCTGGTTCGCCTACTGGGGACAGGG CACCCTTGTCACTGTGTCAAGCGGAGGCGGTGGTTCGG GTGGCGGAGGTTCCGGAGGAGGAGGTTCGGGCGGTGGT GGATCACAGGCCGTCGTGACTCAGGAACCATCCCTGACT GTGTCCCCCGGTGGAACCGTGACCCTCACCTGTCGCTCC TCAACCGGAGCCGTGACCACCTCCAACTACGCTAATTGG GTGCAGCAGAAGCCAGGACAAGCCCCACGGGGACTGAT TGGGGGCACCAACAAGAGGGCTCCTTGGACCCCAGCCC GCTTCTCGGGCTCCCTGTTGGGCGACAAGGCCGCTCTGA CCCTGTCCGGTGCACAGCCGGAGGATGAAGCCGAATACT TCTGCGCGCTGTGGTACTCCAACCTCTGGGTGTTCGGCG GAGGGACCAAGCTGACTGTGTTGGGAGGAGGGGGGAGT GACAAGACTCACACGTGTCCGCCTTGCCCAGCACCCGAG CTACTGGGAGGACCGAGCGTGTTCCTGTTTCCCCCGAAG CCGAAGGATACCCTGATGATCTCCCGCACTCCTGAAGTG ACTTGCGTGGTGGTGGCAGTGTCCCACGAGGACCCGGA AGTCAAGTTTAATTGGTACGTGGATGGCGTGGAGGTGCA CAACGCAAAGACCAAGCCTCGCGAGGAGCAGTACGCCA GCACCTACCGGGTGGTGTCCGTCCTGACGGTGCTGCAC CAGGACTGGCTGAACGGGAAGGAGTACAAGTGCAAAGT GTCAAATAAGGCTTTGGCCGCCCCTATTGAGAAAACCATC TCAAAGGCCAAGGGCCAACCCAGGGAACCTCAAGTGTGC ACCCTCCCACCTTCGCGAGAAGAGATGACCAAGAACCAG GTGTCCCTGTCCTGCGCCGTGAAGGGCTTCTACCCCTCC GATATCGCCGTGGAGTGGGAATCTAACGGACAGCCGGA GAACAACTACAAGACCACTCCGCCGGTGCTGGACAGCGA CGGCTCCTTCTTCCTCGTGTCGAAACTGACCGTGGACAA GTCACGGTGGCAGCAGGGCAATGTGTTCAGCTGCTCAGT CATGCATGAGGCCCTCCACAACCACTACACTCAGAAGTC CCTGTCGCTTTCCCCCGGAAAA	
	First Half Antibody Light Chain (includes signal peptide sequence)	ATGTCCGTGCTGACCCAAGTCTTGGCGCTGCTGCTGCTG TGGCTCACTGGCACCCGCTGTGACATTGAACTGACCCAG CCGCCTTCAGTGTCCGTGGCACCCGGACAGACCGCGAG GATTAGCTGCTCCGGGGACAACCTCCCGGCCTACACTGT GACCTGGTATCAGCAGAAGCCCGGACAAGCCCCTGTGCT TGTCATCTACGACGACTCGGATCGGCCAAGCGGCATCCC CGAGAGATTCTCCGGCTCGAACAGCGGGAACACCGCCA CGCTCACTATCTCGGGAACCCAGGCCGAAGATGAGGCTG ACTACTACTGCGCCTCATGGGATCCGTCCTCCGGAGTGG TGTTCGGTGGCGGAACTAAGCTGACCGTGCTGGGTCAGC CTAAGGCGGCGCCCTCAGTGACCCTGTTCCCTCCGTCGT CTGAAGAACTCCAGGCCAACAAGGCCACCCTCGTGTGCC TGATTTCGGACTTCTACCCGGGAGCCGTCACTGTGGCCT GGAAGGCCGACAGCAGCCCAGTGAAGGCCGGCGTGGAA ACTACCACCCCGTCCAAGCAGTCCAACAATAAGTACGCA GCCAGCTCCTACCTGTCCCTGACCCCCGAACAATGGAAG TCACACAGATCCTACTCCTGTCAAGTCACCCACGAGGGC AGCACTGTCGAAAAGACCGTGGCACCGACTGAGTGCTCG	1105

TABLE 19D Nucleotide sequences (H variants)			
Construct Name	**Chain Description**	**Amino Acid Sequence**	**SEQ ID NO:**
	Second Half Antibody (includes signal peptide sequence)	ATGCCTCTGCTGCTCCTGCTGCCTCTGCTCTGGGCCGGA GCTTTGGCATCACAGCAAATCTACGGCGTGGTGTACGGC AACGTGACCTTCCATGTCCCCTCCAATGTGCCGCTGAAG GAAGTGCTCTGGAAGAAGCAGAAGGACAAGGTCGCGGA ACTGGAAAACTCCGAGTTTCGCGCCTTCTCCTCCTTCAAA AACCGGGTGTACCTGGACACCGTGTCCGGGAGCCTTACT ATCTACAACCTGACCTCCTCGGACGAGGATGAGTATGAG ATGGAGAGCCCAAACATTACCGACACCATGAAGTTCTTCC TCTACGTGCTGGAATCGGGTGGAGGCGGAAGCGATAAG ACTCACACGTGTCCACCTTGTCCCGCACCCGAACTCCTG GGGGGACCTTCCGTGTTTCTCTTCCCCCCTAAACCGAAG GACACCTTGATGATCTCCCGCACTCCTGAAGTGACCTGT GTGGTGGTGGCCGTGTCCCACGAGGACCCAGAAGTCAA GTTTAATTGGTACGTGGACGGAGTCGAGGTGCACAACGC GAAAACCAAACCGCGGGAGGAGCAGTACGCCTCCACCTA CCGGGTGGTGTCCGTCCTCACTGTGCTGCACCAGGACTG GCTCAACGGAAAGGAGTACAAGTGCAAAGTGTCCAACAA AGCCTTGGCGGCCCCAATCGAAAAGACGATCTCCAAGGC CAAGGGACAGCCGCGCGAACCTCAAGTCTACACCCTGCC TCCTTGCCGCGAGGAAATGACCAAGAACCAGGTGTCACT GTGGTGTCTGGTCAAGGGATTCTACCCTTCCGATATCGC AGTGGAGTGGGAAAGCAACGGCCAACCAGAGAACAACTA TAAGACCACACCCCCGGTGCTCGATTCCGACGGCTCATT CTTCCTGTACTCCAAGCTGACCGTGGACAAGTCACGGTG GCAGCAGGGGAACGTGTTCAGCTGCTCCGTGATGCATGA AGCCCTGCACAATCATTACACTCAGAAGTCCCTGTCGCT GAGCCCCGGAAAA	1091

8.2. Example 2: Ability of BBMs to elicit redirected T-cell cytotoxic activity (RTCC) against CD19+ target cells

8.2.1. Materials and Methods

[0862] A RTCC assay with the BBMs of Example 1A was performed to measure the ability of the BBMs to elicit RTCC against CD19+ Nalm6-luc and Karpas422-luc cells. Nalm-6 is a human B cell precursor leukemia cell line and Karpas422 is a human B-cell non-hodgkin lymphoma cell line. Briefly, Nalm6 and Karpas422 cells engineered to express the firefly luciferase reporter gene were cultured in RPMI1640 culture media with 10% fetal bovine serum (FBS). 10,000 target cells with serial diluted BBMs or gH isotype antibody control (αgH-CD3hi) were seeded on 384-well flat-bottom microtiter plate. Primary human T cells were isolated from cryopreserved peripheral blood mononuclear cells (PBMCs) and expanded using anti-CD3 and anti-CD28 dynabeads (Thermo fisher, catalog# 11131D) and subsequently cryopreserved. Expanded T cells were thawed and aliquoted to the plate to achieve an effector cell (*i.e.*, T cell) to target cell (*i.e.*, cancer cell) ratio (E:T ratio) of 3:1. Plates were incubated in a 37°C incubator with 5% CO2 overnight. Following the co-incubation, Bright Glo (Promega, catalog# E2620)

was added to all wells and the luminescence signal was subsequently measured on an Envision (Perkin Elmer). Target cells with Bright Glo served as maximal signal. The percent RTCC of target cells was calculated using the following formula: [100- (sample/maximal signal)*100%].

8.2.2. Results

[0863] Results are shown in FIGS. 4A-4B. BBMs based on both NEG258 and NEG218 mediated RTCC activity against Nalm6-luc and Karpas422-luc cells whereas gH isotype antibody (control) was not active, as expected.

8.3. Example 3: Ability of BBMs to elicit T-cell proliferation

8.3.1. Materials and Methods

[0864] The BBMs described in Example 1A, containing the variable regions of NEG258 and NEG218, were evaluated for their ability to induce T cell proliferation upon co-culture with CD19 expressing target cells. Briefly, Karpas422 and Nalm-6 target cells stably expressing firefly luciferase were irradiated on the day of the assay and plated at a density of 60,000 cells per well in a Costar 96 well plate (Corning, Cat # 3904) in T Cell Media (TCM) [RPMI-1640 (ThermoFisher Scientific, Cat # 11875-085), 10% FBS (Seradigm, Cat # 1500-500), 1% L-Glutamine (Thermo Fisher Scientific, Cat # 25830-081), 1% Non Essential Amino Acids (Thermo Fisher Scientific, Cat # 11140-050),1% Pen/Strep (Thermo Fisher Scientific, Cat # 15070063), 1% HEPES (Thermo Fisher Scientific, Cat # 15630080), Sodium Pyruvate (Thermo Fisher Scientific, Cat # 11360-070), 0.1% Beta-mercaptoethanol (Thermo Fisher Scientific, Cat # 21985-023)]. Peripheral blood mononuclear cells (PBMCs) previously isolated from Leukopak donors (Hemacare) and cryopreserved were thawed and Pan T cells were isolated by negative selection using the Pan T cell Isolation Kit, human [Miltenyi Biotec, Cat # 130-096-535] following the manufacturer's protocol. Isolated T cells were labelled with 5 µM Cell Trace Violet (CTV) (Thermo Fisher Scientific, Cat # C34557) following the manufacturer's protocol and 60,000 CTV labeled T cells were co-cultured with 60,000 target cells to achieve an E:T ratio of 1:1. A dilution series of the NEG258- and NEG218-based BBMs and control binding molecules (αgH-CD3hi) ranging from 16 pM-10,000 pM was added to cells and the plates were incubated in a 5% CO2, 37ºC incubator for 96 hrs. After incubation, the cells were harvested, treated with Human TruStain FcX (Fc Block) [Biolegend, Cat # 422302] following manufacturer instructions and then stained with Fixable Viability Dye eFlour 780 (ThermoFisher Scientific, Cat # 65-0865-14) by incubation at 4C for 30 mins. The cells were then washed twice using FACS Buffer and stained with PerCP-Cy5.5 conjugated anti-human CD3 mAb (Biolegend, Cat # 317336) by incubation at 4ºC for 30 mins. The samples were then run on BD LSR Fortessa and analyzed using FlowJo to determine % proliferated CD3+ T cells based on CD3 staining and dilution of Cell Trace Violet dye.

8.3.2. Results

[0865] Both NEG258- and NEG218-based BBMs induced proliferation of T cells upon co-culture with two different CD19 expressing target cell lines (FIGS. 5A-5B). The T cell proliferation effect was dose-dependent, and the NEG258-based BBM showed more potent activity than the NEG218-based BBM. The control antibody did not induce any T cell proliferation indicating that CD19 target-specific engagement was required for the proliferation of T cells.

8.4. Example 4: Ability of TBMs to elicit CD2 dependent T cell activation.

8.4.1. Materials & Methods

[0866] A Jurkat cell line (JNL, an immortalized human T-cell line) that stably expresses a luciferase reporter gene driven by the NFAT promoter was used to measure T cell activation. The level of CD2 expression in JNL cells was confirmed by flow cytometry (FIG. 6A). In order to generate CD2 knockout (KO) cells by CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), JNL cells were electroporated with a CD2 Cas9 ribonucleoprotein complex. $CD2^-$ cells were subsequently sorted to enrich for a uniform $CD2^-$ population (Fig. 6B). A JNL reporter assay with $CD2^+$ and $CD2^-$ JNL cells was then performed to measure bispecfic or trispecific construct-dependent T cell activation. In brief, 10,000 Nalm6 or Karpas422 cells with serial diluted BBMs or TBMs of Example 1A (*i.e.,* R variants) were seeded on 384-well flat-bottom microtiter plate. JNL cells were then added to the plate to achieve effector to target ratio of 3:1. Plates were incubated at a 37°C incubator with 5% CO_2 for overnight. Following the co-incubation, Bright Glo (Promega, catalog# E2620) was added to all wells and the luminescence signal was subsequently measured on an Envision (Perkin Elmer).

8.4.2. Results

[0867] Both BBMs and TBMs induced dose-dependent increase in luminescence when incubated with CD2 WT JNL cells, and the response level was higher with TBMs (FIGS. 6C-6F). When CD2-KO JNL cells were used as effector, decreased T cell activation was observed with TBMs as compared to corresponding BBMs, suggesting that the advantage of TBMs is dependent on CD2 expression on the T cells.

8.5. Example 5: Binding of NEG258- and NEG218-based TBMs to cyno B cells

8.5.1. Materials and Methods

[0868] Cynomolgus (cyno) PBMCs (iQ Biosciences #IQB-MnPB102) were depleted of CD3+ cells using MACS positive selection (Miltenyi #130-092-012). The remaining cell population was resuspended in a FACS buffer. 100,000 cells per well were plated in a V-bottom 96-well plate, and incubated on ice for one hour with TBMs of Example 1A (*i.e.,* R variants) at 1ug/mL. Following two washes with FACS buffer, the cells were incubated with Alexa-647 labeled anti-

human Fc secondary antibody (Jackson Immuno #109-605-098) and cyno cross reactive FITC mouse anti-human CD20 antibody (BD Pharmingen # 556632) for one hour on ice. Following two washes with FACS buffer, cells were resuspended in 100 µL of buffer and data was collected on a Beckman Coulter Cytoflex. Cells were analyzed using CytExper v2.3 and gated through CD20 positive population.

8.5.2. Results

[0869] Due to their proximal evolutionary relationship to humans, cynomolgus monkeys are the most appropriate preclinical model to analyze the therapeutic effect and potential toxicity of antibody therapeutics, and therefore it is useful for antibodies in clinical development to bind to cynomolgus homolog of their human target. As shown in FIGS. 7A-7B, both the NEG258- and NEG218 TBMs bind to cyno B cells, indicating that the CD19 binding arm recognizes cyno CD19.

8.6. Example 6: Ability of TBMs to induce T cell activation upon cyno B cells depletion in PBMCs

8.6.1. Materials & Methods

[0870] An *ex vivo* cyno B cell depletion assay was conducted to measure the ability of NEG258-based TBMs of Example 1 to lyse CD20 positive B cells in PBMCs (peripheral blood mononuclear cells). In brief, PBMCs were isolated from cynomolgus (cyno) monkey whole blood (BioIVT) using ficoll gradient centrifugation. Isolated PBMCs and serial diluted TBMs of Example 1A (*i.e.*, R variants) were seeded on 96-well flat-bottom microtiter plate. Plates were incubated in a 37°C incubator with 5% CO2 overnight. After 24h of incubation, samples were harvested and simultaneously stained for CD3 and CD20 to identify B and T cells within the PBMC population. To allow quantitative analysis of the cell population, 75,600 counting beads were added prior to the acquisition by flow cytometry. For each sample, 20,000 beads were acquired in order to determine the absolute numbers of B cells. The percent B cell depletion was determined by calculation of the ratio between the number of B cells and the number of beads. For detection of T cell activation, the cells were stained with anti-CD3, anti-CD69 and anti-CD25 (Biolegend and BD Biosciences).

8.6.2. Results

[0871] Both NEG258-based TBMs depleted cyno B cells (FIG. 8A) and induced activation of CD3+ T cells as evidence by upregulation of CD69 and CD25 expression (FIGS. 8C-8H). As expected, neither B cell depletion nor T cell activation occurred in the absence of added TBM. These results show both the ability of the NEG258-based TBMs induce activation of cyno T cells as well as the specificity of the activation.

8.7. Example 7: Re-directed T cell cytotoxicity by CD19 TBMs

[0872] NEG258- and NEG218 based TBMs of Example 1A (*i.e.,* R variants) (having CD3 ABMs with the VH and VL domains of an anti-CD3 antibody having an affinity to CD3 of 16 nM as measured by Biacore) were analyzed for their potential to induce T cell-mediated apoptosis in tumor target cells.

8.7.1. Materials and Methods

[0873] In one study, the TBMs were compared across multiple donor effector cells. Briefly, huCD19-expressing Nalm6 or Karpas422 target cells were engineered to overexpress firefly luciferase. Cells were harvested and resuspended in RPMI medium (Invitrogen # 11875-093) with 10% FBS. 2,500 target cells per well were plated in a flat-bottom 384-well plate. Human pan T effector cells were isolated via MACS negative selection (Miltenyi Biotec #130-096-535) from two donors from cryopreserved PBMC (Cellular Technologies Limited #CTL-UP1) then added to the plate to obtain a final E:T ratio of 3:1 or 5:1. Co-cultured cells were incubated with a serial dilution of all constructs and controls. For normalization, average maximum luminescence refers to target cells co-incubated with effector cells, but without any test construct. After an incubation of 24, 48, 72 or 96 hr at 37°C, 5% CO2, OneGlo luciferase substrate (Promega #E6120) was added to the plate. Luminescence was measured on an Envision plate reader after a 10 minute incubation. Percent specific lysis was calculated using the following equation: Specific lysis (%) = (1- (sample luminescence / average maximum luminescence)) * 100

8.7.2. Results

[0874] As shown in FIGS. 9A-9P, the TBMs show cytotoxic activity against both Nalm6 target cells (FIGS. 9A-9H) and Karpas422 cells (FIGS. 9I-P) at multiple time points, E:T ratios and effector T cell donors. The NEG258-based TBM appears to be more potent than the NEG218-based TBM.

8.8. Example 8: Re-directed T cell cytotoxicity by TBMs with different CD3 affinities

[0875] The NEG258-based TBMs of Example 1A (*i.e.,* R variants) with CD3 ABMs (comprising the VH and VL domains of anti-CD3 antibodies having affinities to CD3 of 16 nM, 30 nM and 48 nM as measured by Biacore) were analyzed for their potential to induce T cell-mediated apoptosis in tumor target cells.

8.8.1. Materials and Methods

[0876] In one study, the TBMs were compared across multiple donor effector cells. Briefly, huCD19-expressing Nalm6 and Karpas422 target cells were engineered to overexpress firefly luciferase. Cells were harvested and resuspended in RPMI medium (Invitrogen # 11875-093) with 10% FBS. 2,500 target cells per well were plated in a flat-bottom 384-well plate. Human

pan T effector cells were isolated via MACS negative selection (Miltenyi Biotec #130-096-535) from two donors from cryopreserved PBMCs (Cellular Technologies Limited #CTL-UP1), then added to the plate to obtain a final E:T ratio of 3:1 or 5:1. Co-cultured cells were incubated with serial dilutions of a TBM or control. For normalization, average maximum luminescence refers to target cells co-incubated with effector cells, but without any test construct. After an incubation of 24, 48, 72 or 96 hr at 37°C, 5% CO2, OneGlo luciferase substrate (Promega #E6120) was added to the plate. Luminescence was measured on an Envision plate reader after a 10 minute incubation. Percent specific lysis was calculated using the following equation: Specific lysis (%) = (1- (sample luminescence / average maximum luminescence)) * 100

8.8.2. Results

[0877] As shown in FIGS. 10A-10P, the TBMs show cytotoxic activity against both Nalm6 target cells (FIGS. 10A-10H) and Karpas422 (FIGS. 10I-10P) at multiple time points, E:T ratios and effector T cell donors.

8.9. Example 9: RTCC activity of the NEG258-based TBMs vs. BBMs and TBMs that do not bind to CD2

[0878] The NEG258- based TBMs of Example 1A (*i.e.,* R variants) containing either a CD2 binding arm or a control lysozyme binding arm were compared for their potential to induce T cell-mediated apoptosis in Nalm6 or Karpas422 target cells target cells. The study also included blinatumomab as a control. Blinatumomab is a bispecific T cell engager, or BiTE, that binds to both CD19 and CD3 but lacks an Fc domain (see, *e.g.*, U.S. Patent No. 10,191,034).

8.9.1. Materials and Methods

[0879] The purified TBMs were compared across multiple donor effector cells. Briefly, huCD19-expressing Nalm6 and Karpas422 target cells were engineered to overexpress firefly luciferase. Cells were harvested and resuspendend in RPMI medium (Invitrogen # 11875-093) with 10% FBS. 5,000 target cells per well were plated in a flat-bottom 384-well plate. Human pan T effector cells were isolated via negative selection (Stemcell Technologies #17951) from two donors from cryopreserved PBMCs that were separated from a leukopak (Hemacare #PB001F-1) by Ficoll density gradient centrifugation. Purified T cells were then added to the plate to obtain a final E:T ratio of 3:1, 1:1, 1:3 or 1:5. Co-cultured cells were incubated with serial dilutions of all constructs and controls. For normalization, average maximum luminescence refers to target cells co-incubated with effector cells, but without any test construct. After an incubation of 48, 72 or 96 hr at 37°C, 5% CO2, OneGlo luciferase substrate (Promega #E6120) was added to the plate. Luminescence was measured on an Envision plate reader after a 10 minute incubation. Percent specific lysis was calculated using the following equation: Specific lysis (%) = (1- (sample luminescence / average maximum luminescence)) * 100

8.9.2. Results

[0880] As shown in FIGS. 11A-11L, both types of TBMs show cytotoxic activity against both Nalm6 target cells (FIGS. 11A-11H) and Karpas422 cells (FIGS. 11I-11L). The TBM containing a CD2 binding arm demonstrated superior cytotoxic activity compared to the control TBM with a lysozyme binding arm and to blinatumomab, particularly at lower E:T ratios.

8.10. Example 10: Cytokine Release Assay

[0881] NEG258- and NEG218-based TBMs of Example 1A (*i.e.,* R variants) were analyzed for their ability to induce T cell-mediated de novo secretion of cytokines in the presence of tumor target cells.

8.10.1. Materials and Methods

[0882] Briefly, huCD19-expressing Nalm6 target cells were harvested and resuspended in RPMI medium with 10% FBS. 20,000 target cells per well were plated in a flat-bottom 96-well plate. Human pan T effector cells were isolated via MACS negative selection from cryopreserved PBMC then added to the plate to obtain a final E:T ratio of 5:1. Co-cultured cells were incubated with serial dilutions of all constructs and controls. After an incubation of 24 hr at 37°C, 5% CO2, the supernatants were harvested by centrifugation at 300 x g for 5 min for subsequent analysis.

[0883] A multiplexed ELISA was performed according to the manufacturer's instructions using a V-PLEX Proinflammatory Panel 1 Kit (MesoScale Discovery #K15049D).

8.10.2. Results

[0884] As shown in FIGS. 12A-12C, both NEG258- and NEG218-based TBMs induce significant cytokine secretion by T cells at all dose levels measured. These figures indicate that they can be effective at lower doses.

8.11. Example 11: Binding of NEG258- and NEG218-based TBMs to human and cyno CD19

8.11.1. Materials and Methods

[0885] The mouse cell line 300.19 was engineered to overexpress either human CD19 or cyno CD19. Cells were cultured in in RPMI medium (Invitrogen #11875-093) with 10% FBS and 2-mercaptoethanol. Cells were harvested and resuspended in FACS buffer (PBS containing 1% FBS). 50,000 cells per well were plated in a V-bottom 96-well plate. Each cell line was incubated with serial dilutions of TBMs of Example 1A (*i.e.,* R variants) for one hour on ice. Cells were centrifuged for 4 min at 400xg and washed with FACS buffer. This was repeated twice, and then the cells were incubated with Alexa-647 labeled anti-human Fc secondary antibody (Jackson Immuno #109-605-098) for 30 min on ice. The cells were washed twice,

then resuspended in 100 µL of FACS buffer. FACS data was collected on a Beckman Coulter Cytoflex and analysis was performed using CytExpert v2.3.

8.11.2. Results

[0886] As shown in FIGS. 13A-13B, the NEG258- and NEG218-based TBMs bind to cell lines engineered to overexpress both human and cyno CD19. NEG258 appears to bind equally to both human and cyno while NEG218 appears to have greater affinity for cyno CD19 than human CD19. Of the two, NEG258 appears to have greater affinity for both human CD19 and cyno CD19.

8.12. Example 12: Engineering CD58 for Improved Stability

8.12.1. Background

[0887] Human CD58 contains a signal peptide of 29 amino acids and two Ig-like domains. The most N-terminal Ig-like domain, referred to as domain 1, is of V-type, similar to a variable region of an antibody, and the second domain, named domain 2, is of C-type, is similar to a constant regions of an antibody. A schematic overview of the CD58 domain structure is shown in FIG. 14.

[0888] As illustrated in Examples 1-11, domain 1 of CD58, which interacts with CD2, can be used in lieu of an anti-CD2 antibody binding fragment in multispecific binding molecules. The use of a CD58 binding arm rather than an anti-CD2 binding arm reduces non-specific immune activation in the absence of target cells. However, CD58 exhibits lower stability than immunoglobulins.

[0889] In order to improve stability of human CD58 domain 1, the protein was engineered to include a pair of cysteine that form a disulfide bridge upon expression to stabilize the molecule.

[0890] Four different pairs of amino acids were engineered to be replaced by cysteines: (1) V45 and M105, (2) V45 and M114, (3) V54 and G88 and (4) W56 and L90.

8.12.2. Materials and Methods

8.12.2.1. Recombinant expression

[0891] To assess the binding and biophysical characteristics, the CD58 disulfide variants were transiently produced and purified from HEK293 cells along with the CD2 extracellular domain. All plasmids were codon optimized for mammalian expression. Human and cyno CD2 constructs were produced with a C-terminal Avi-Tag and a N terminal 8xhis tag (SEQ ID NO: 769) followed by a EVNLYFQS sequence (SEQ ID NO: 770) for cleavage of the histag after purification. CD2 constructs were site selectively biotinylated during expression via co-transfection of a plasmid encoding the BirA enzyme. CD58 was expressed with a C-terminal 8xhis tag (SEQ ID NO: 769). Transient expression and purification in HEK293F cells was performed with standard methodology. The sequences are shown in Table 20.

TABLE 20		
Protein Name	**AA Sequence**	**SEQ ID NO:**
Human CD2	SKEITNALETWGALGQDINLDIPSFQMSDDIDDIKWEKTS DKKKIAQFRKEKETFKEKDTYKLFKNGTLKIKHLKTDDQ DIYKVSIYDTKGKNVLEKIFDLKIQERVSKPKISWTCINTT LTCEVMNGTDPELNLYQDGKHLKLSQRVITHKWTTSLS AKFKCTAGNKVSKESSVEPVSCPEKGLDGGGGSGLNDI FEAQKIEWHE	771
Cyno CD2	SKEIRNALETWGALGQDIDLDIPSFQMSDDIDDIRWEKT SDKKKIAQFRKEKETFEEKDAYKLFKNGTLKIKHLKIHDQ DSYKVSIYDTKGKNVLEKTFDLKIQERVSEPKISWTCINT TLTCEVMNGTDPELNLYQDGKHVKLSQRVITHKWTTSL SAKFKCTAGNKVSKESRMETVSCPEKGLDGGGGSGLN DIFEAQKIEWHE	772
CD58 Full ECD	SQQIYGVVYGNVTFHVPSNVPLKEVLWKKQKDKVAELE NSEFRAFSSFKNRVYLDTVSGSLTIYNLTSSDEDEYEME SPNITDTMKFFLYVLESLPSPTLTCALTNGSIEVQCMIPE HYNSHRGLIMYSWDCPMEQCKRNSTSIYFKMENDLPQ KIQCTLSNPLFNTTSSIILTTCIPSSGHSRHRGGGGSHHH HHHHH	773
CD58_IgV	SQQIYGVVYGNVTFHVPSNVPLKEVLWKKQKDKVAELE NSEFRAFSSFKNRVYLDTVSGSLTIYNLTSSDEDEYEME SPNITDTMKFFLYVLESGGGGSHHHHHHHH	774
IgV V45C_M105C	SQQIYGVVYGNVTFHCPSNVPLKEVLWKKQKDKVAELE NSEFRAFSSFKNRVYLDTVSGSLTIYNLTSSDEDEYECE SPNITDTMKFFLYVLESGGGGSHHHHHHHH	775
IgV V54C_G88C	SQQIYGVVYGNVTFHVPSNVPLKECLWKKQKDKVAELE NSEFRAFSSFKNRVYLDTVSCSLTIYNLTSSDEDEYEME SPNITDTMKFFLYVLESGGGGSHHHHHHHH	776
IgV V45C_M114C	SQQIYGVVYGNVTFHCPSNVPLKEVLWKKQKDKVAELE NSEFRAFSSFKNRVYLDTVSGSLTIYNLTSSDEDEYEME SPNITDTCKFFLYVLESGGGGSHHHHHHHH	777

[0892] For expression, transfection was performed using PEI as transfection reagent. For small scale (<5L) transfections, cells were grown in shake flasks on an orbital shaker (100 rpm) in a humidified incubator (85%) at 8% CO2). Transfection was done with a ratio of 1 DNA: 3 PEI. 1mg/L culture of plasmid was used for transfection at 2.0 million cells/mL in Expi293 medium. After 5 days of expression, the culture was centrifuged and filtrated. Purification was performed via Nickel-NTA batch binding using 1ml resin/100 mL supernatant. The protein was allowed to bind for a minimum of 2 hours with gentle mixing, and the mixture was loaded onto a gravity filtration column. The resin was washed with 30 CV of PBS. Proteins were eluted with imidazole. The eluted protein was concentrated and finally purified via a preparative size exclusion chromatography (Hi Load 16/60 Superdex 75 grade column, GE Healthcare Life Sciences, Uppsala, Sweden). To confirm that the identity of the proteins expressed matched

the predicted masses for the primary amino acid sequences, proteins were analyzed by high-performance liquid chromatography coupled to mass spectrometry.

8.12.2.2. Stability

[0893] Disulfide stabilized variants were assessed for improved thermal stability using both differential scanning calorimetry (DSC) and differential scanning fluorimetry (DSF) using standard techniques. For DSF, 1-3 ug of each construct was add to 1x Sypro Orange (Thermo-Fisher) in 25ul total volume in 96-well PCR plate. Using a Bio-Rad CFX96 RT-PCR system equipped with C1000 Thermal Cycler, the temperature was increased from 25°C to 95°C at 0.5°C/minute and the fluorescence monitored. The manufacturer-supplied software was used to determine Tm.

[0894] For DSC, all samples were dialyzed into HEPES-buffered saline (HBS) and diluted to final concentration of 0.5 mg/mL. Tm and Tonset were determined using a MicroCal VP-Capillary DSC system (Malvern) by increasing temperature from 25°C to 100°C at 1°C/minute with a filtering period of 2 seconds and a mid-gain setting.

8.12.2.3. Binding affinity

[0895] To ensure the binding affinity remained uncompromised by the additional of the stabilizing disulfide variance, isothermal calorimetry (ITC) was performed on the resulting recombinant CD58 proteins to determine their apparent KD and binding stoicheometry (n) to recombinant human CD2.

[0896] Briefly, recombinant human CD2 and recombinant human CD58 variants were dialyzed into HEPES-buffered saline (HBS). CD2 was diluted to final concentration of 100 µM, CD58 variants were diluted to 10 µM. CD2 was titrated into 10 µM of CD58 variants via multiple injections and ΔH (kcal/mole) determined using a MicroCal VP-ITC isothermal titration calorimeter (Malvern). Titrations of CD2 into HBS were used as a reference and KD and n determined from the resulting data.

8.12.3. Results

[0897] Results for both DSF and DSC measurements for the constructs are shown in Table 21 below.

TABLE 21

	By Differential Scanning Fluorimetry (DSF)	By Differential Scanning Calorimetry (DSC)	
CD58 variant	**Tm (°C)**	**Tmonset (°C)**	**Tm (°C)**
CD58 Full ECD	59.5	48.8	65.0
CD58_IgV	48.5	46.3	60.9

TABLE 21			
	By Differential Scanning Fluorimetry (DSF)	By Differential Scanning Calorimetry (DSC)	
CD58 variant	Tm (°C)	Tmonset (°C)	Tm (°C)
IgV V45C_M105C	48.5	43.9	66.8
IgV V54C_G88C	76.5	66.7	80.9
IgV V45C_M114C	63.5	49.6	72.5

[0898] Results of the affinity studies are shown in Table 22 below. Addition of stabilizing disulfide had no detrimental impact on the affinity or the binding stoicheometry.

TABLE 22		
CD58 variant	KD (uM)	n
CD58 Full ECD	0.57 (±0.05)	0.92 (±0.01)
CD58_IgV	0.61 (±0.07)	0.96 (±0.01)
IgV V45C_M105C	0.88 (±0.06)	0.97 (±0.01)
IgV V54C_G88C	0.60 (±0.06)	0.83 (±.0.01)
IgV V45C_M114C	0.38 (±0.03)	0.88 (±.0.01)

8.13. Example 13: Production of anti-CD3-anti-CD19-CD58 IgG1 TBMs in knob-into-holes format

8.13.1. Materials and methods

[0899] Constructs were synthesized and codon optimized for expression in mammalian cells. For each trispecific construct, three plasmids were synthesized. A first plasmid encoding an anti-CD19 heavy chain was synthesized as a fusion comprising (in the N-terminal to C-terminal direction) (i) a VH domain fused to a constant hIgG1 CH1 domain, (ii) a linker, (iii) an anti-CD3 scFv, (iv) a second linker and (v) a hIgG1 Fc domain containing mutations for a hole to facilitate heterodimerization as well as silencing mutations. A second plasmid encoding a light chain was synthesized as a fusion comprising (in the N-terminal to C-terminal direction) an anti-CD19 VL domain and (ii) a constant human kappa sequence. A third plasmid encoding a second half antibody was synthesized as a fusion comprising (in the N-terminal to C-terminal direction) a CD58 disulfide stabilized variant fused to a constant hIgG1 domain containing mutations for a knob to facilitate heterodimerization as well as silencing mutations. The sequences are shown in Table 23.

TABLE 23			
Trispecific Name	Chain Description	Sequence	SEQ ID NO:
CD19_CTL119 _CD3_16nM-CD58_Full ECD Trispecific	First Half Antibody Heavy Chain	QVQLQESGPGLVKPSETLSLTCTVSGVSLPDYG VSWIRQPPGKGLEWIGVIWGSETTYYQSSLKSR VTISKDNSKNQVSLKLSSVTAADTAVYYCAKHY YYGGSYAMDYWGQGTLVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNS	778

TABLE 23

Trispecific Name	Chain Description	Sequence	SEQ ID NO:
	(Fc sequence not shown)	GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKRVEPKSCGGGGS GGGGSEVQLVESGGGLVQPGGSLKLSCAASGF TFNTYAMNWVRQASGKGLEWVGRIRSKYNNYA TYYADSVKDRFTISRDDSKSTLYLQMNSLKTED TAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVS SGGGGSGGGGSGGGGSGGGGSQAVVTQEPS LTVSPGGTVTLTCRSSTGAVTTSNYANWVQQK PGQAPRGLIGGTNKRAPWTPARFSGSLLGDKA ALTLSGAQPEDEAEYFCALWYSNLWVFGGGTK LTVLGGGGS	
	First Half Antibody Heavy Chain (includes Fc sequence)	QVQLQESGPGLVKPSETLSLTCTVSGVSLPDYG VSWIRQPPGKGLEWIGVIWGSETTYYQSSLKSR VTISKDNSKNQVSLKLSSVTAADTAVYYCAKHY YYGGSYAMDYWGQGTLVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKRVEPKSCGGGGS GGGGSEVQLVESGGGLVQPGGSLKLSCAASGF TFNTYAMNWVRQASGKGLEWVGRIRSKYNNYA TYYADSVKDRFTISRDDSKSTLYLQMNSLKTED TAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVS SGGGGSGGGGSGGGGSGGGGSQAVVTQEPS LTVSPGGTVTLTCRSSTGAVTTSNYANWVQQK PGQAPRGLIGGTNKRAPWTPARFSGSLLGDKA ALTLSGAQPEDEAEYFCALWYSNLWVFGGGTK LTVLGGGGSDKTHTCPPCPAPELLGGPSVFLFP PKPKDTLMISRTPEVTCVVVAVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALAAPIEKTISKAKGQP REPQVCTLPPSRDELTKNQVSLSCAVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLVSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPGK	1094
	Second Half Antibody (Fc sequence not shown)	FSQQIYGVVYGNVTFHVPSNVPLKEVLWKKQKD KVAELENSEFRAFSSFKNRVYLDTVSGSLTIYNL TSSDEDEYEMESPNITDTMKFFLYVLESLPSPTL TCALTNGSIEVQCMIPEHYNSHRGLIMYSWDCP MEQCKRNSTSIYFKMENDLPQKIQCTLSNPLFN TTSSIILTTCIPSSGHSRHRGGGS	779
	Second Half Antibody (includes Fc sequence)	FSQQIYGVVYGNVTFHVPSNVPLKEVLWKKQKD KVAELENSEFRAFSSFKNRVYLDTVSGSLTIYNL TSSDEDEYEMESPNITDTMKFFLYVLESLPSPTL TCALTNGSIEVQCMIPEHYNSHRGLIMYSWDCP MEQCKRNSTSIYFKMENDLPQKIQCTLSNPLFN TTSSIILTTCIPSSGHSRHRGGGSDKTHTCPPCP APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY ASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPCREEMTKNQ VSLWCLVKGFYPSDIAVEWESNGQPENNYKTT	1095

TABLE 23

Trispecific Name	Chain Description	Sequence	SEQ ID NO:
		PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNRYTQKSLSLSPGK	
	First Half Antibody Light Chain	EIVMTQSPATLSLSPGERATLSCRASQDISKYLN WYQQKPGQAPRLLIYHTSRLHSGIPARFSGSGS GTDYTLTISSLQPEDFAVYFCQQGNTLPYTFGQ GTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSGNSQESVTEQ DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ GLSSPVTKSFNRGEC	780
CD19_CTL119 _CD3_16nM-CD58_IgV Trispecific	First Half Antibody Heavy Chain (Fc sequence not shown)	QVQLQESGPGLVKPSETLSLTCTVSGVSLPDYG VSWIRQPPGKGLEWIGVIWGSETTYYQSSLKSR VTISKDNSKNQVSLKLSSVTAADTAVYYCAKHY YYGGSYAMDYWGQGTLVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKRVEPKSCGGGGS GGGGSEVQLVESGGGLVQPGGSLKLSCAASGF TFNTYAMNWVRQASGKGLEWVGRIRSKYNNYA TYYADSVKDRFTISRDDSKSTLYLQMNSLKTED TAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVS SGGGGSGGGGSGGGGSGGGGSQAVVTQEPS LTVSPGGTVTLTCRSSTGAVTTSNYANWVQQK PGQAPRGLIGGTNKRAPWTPARFSGSLLGDKA ALTLSGAQPEDEAEYFCALWYSNLWVFGGGTK LTVLGGGGS	778
	First Half Antibody Heavy Chain (includes Fc sequence)	QVQLQESGPGLVKPSETLSLTCTVSGVSLPDYG VSWIRQPPGKGLEWIGVIWGSETTYYQSSLKSR VTISKDNSKNQVSLKLSSVTAADTAVYYCAKHY YYGGSYAMDYWGQGTLVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKRVEPKSCGGGGS GGGGSEVQLVESGGGLVQPGGSLKLSCAASGF TFNTYAMNWVRQASGKGLEWVGRIRSKYNNYA TYYADSVKDRFTISRDDSKSTLYLQMNSLKTED TAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVS SGGGGSGGGGSGGGGSGGGGSQAVVTQEPS LTVSPGGTVTLTCRSSTGAVTTSNYANWVQQK PGQAPRGLIGGTNKRAPWTPARFSGSLLGDKA ALTLSGAQPEDEAEYFCALWYSNLWVFGGGTK LTVLGGGGSDKTHTCPPCPAPELLGGPSVFLFP PKPKDTLMISRTPEVTCVVVAVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALAAPIEKTISKAKGQP REPQVCTLPPSRDELTKNQVSLSCAVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLVSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPGK	1094

TABLE 23

Trispecific Name	Chain Description	Sequence	SEQ ID NO:
	Second Half Antibody (Fc sequence not shown)	SQQIYGVVYGNVTFHVPSNVPLKEVLWKKQKD KVAELENSEFRAFSSFKNRVYLDTVSGSLTIYNL TSSDEDEYEMESPNITDTMKFFLYVLESGGGGS	760
	Second Half Antibody (includes Fc sequence)	SQQIYGVVYGNVTFHVPSNVPLKEVLWKKQKD KVAELENSEFRAFSSFKNRVYLDTVSGSLTIYNL TSSDEDEYEMESPNITDTMKFFLYVLESGGGGS DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVAVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYASTYRVVSVLTVLHQDWLNGKE YKCKVSNKALAAPIEKTISKAKGQPREPQVYTLP PCREEMTKNQVSLWCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNRYTQKSLSLSPGK	1078
	First Half Antibody Light Chain	EIVMTQSPATLSLSPGERATLSCRASQDISKYLN WYQQKPGQAPRLLIYHTSRLHSGIPARFSGSGS GTDYTLTISSLQPEDFAVYFCQQGNTLPYTFGQ GTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSGNSQESVTEQ DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ GLSSPVTKSFNRGEC	780
CD19_CTL119_CD3_16nM-CD58_IgV_V45C_M105C Trispecific	First Half Antibody Heavy Chain (Fc sequence not shown)	QVQLQESGPGLVKPSETLSLTCTVSGVSLPDYG VSWIRQPPGKGLEWIGVIWGSETTYYQSSLKSR VTISKDNSKNQVSLKLSSVTAADTAVYYCAKHY YYGGSYAMDYWGQGTLVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKRVEPKSCGGGGS GGGGSEVQLVESGGGLVQPGGSLKLSCAASGF TFNTYAMNWVRQASGKGLEWVGRIRSKYNNYA TYYADSVKDRFTISRDDSKSTLYLQMNSLKTED TAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVS SGGGGSGGGGSGGGGSGGGGSQAVVTQEPS LTVSPGGTVTLTCRSSTGAVTTSNYANWVQQK PGQAPRGLIGGTNKRAPWTPARFSGSLLGDKA ALTLSGAQPEDEAEYFCALWYSNLWVFGGGTK LTVLGGGGS	778
	First Half Antibody Heavy Chain (includes Fc sequence)	QVQLQESGPGLVKPSETLSLTCTVSGVSLPDYG VSWIRQPPGKGLEWIGVIWGSETTYYQSSLKSR VTISKDNSKNQVSLKLSSVTAADTAVYYCAKHY YYGGSYAMDYWGQGTLVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKRVEPKSCGGGGS GGGGSEVQLVESGGGLVQPGGSLKLSCAASGF TFNTYAMNWVRQASGKGLEWVGRIRSKYNNYA TYYADSVKDRFTISRDDSKSTLYLQMNSLKTED	1094

TABLE 23

Trispecific Name	Chain Description	Sequence	SEQ ID NO:
		TAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVS SGGGGSGGGGSGGGGSGGGGSQAVVTQEPS LTVSPGGTVTLTCRSSTGAVTTSNYANWVQQK PGQAPRGLIGGTNKRAPWTPARFSGSLLGDKA ALTLSGAQPEDEAEYFCALWYSNLWVFGGGTK LTVLGGGGSDKTHTCPPCPAPELLGGPSVFLFP PKPKDTLMISRTPEVTCVVVAVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALAAPIEKTISKAKGQP REPQVCTLPPSRDELTKNQVSLSCAVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLVSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPGK	
	Second Half Antibody (Fc sequence not shown)	SQQIYGVVYGNVTFHCPSNVPLKEVLWKKQKD KVAELENSEFRAFSSFKNRVYLDTVSGSLTIYNL TSSDEDEYECESPNITDTMKFFLYVLESGS	781
	Second Half Antibody (includes Fc sequence)	SQQIYGVVYGNVTFHCPSNVPLKEVLWKKQKD KVAELENSEFRAFSSFKNRVYLDTVSGSLTIYNL TSSDEDEYECESPNITDTMKFFLYVLESGSDKT HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYASTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPCR EEMTKNQVSLWCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNRYTQKSLSLSPGK	1096
	First Half Antibody Light Chain	EIVMTQSPATLSLSPGERATLSCRASQDISKYLN WYQQKPGQAPRLLIYHTSRLHSGIPARFSGSGS GTDYTLTISSLQPEDFAVYFCQQGNTLPYTFGQ GTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSGNSQESVTEQ DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ GLSSPVTKSFNRGEC	780
CD19_CTL119 _CD3_16nM-CD58_ IgV V54C_G88C Trispecific	First Half Antibody Heavy Chain (Fc sequence not shown)	QVQLQESGPGLVKPSETLSLTCTVSGVSLPDYG VSWIRQPPGKGLEWIGVIWGSETTYYQSSLKSR VTISKDNSKNQVSLKLSSVTAADTAVYYCAKHY YYGGSYAMDYWGQGTLVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKRVEPKSCGGGGS GGGGSEVQLVESGGGLVQPGGSLKLSCAASGF TFNTYAMNWVRQASGKGLEWVGRIRSKYNNYA TYYADSVKDRFTISRDDSKSTLYLQMNSLKTED TAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVS SGGGGSGGGGSGGGGSGGGGSQAVVTQEPS LTVSPGGTVTLTCRSSTGAVTTSNYANWVQQK PGQAPRGLIGGTNKRAPWTPARFSGSLLGDKA	778

TABLE 23			
Trispecific Name	**Chain Description**	**Sequence**	**SEQ ID NO:**
		ALTLSGAQPEDEAEYFCALWYSNLWVFGGGTK LTVLGGGGS	
	First Half Antibody Heavy Chain (includes Fc sequence)	QVQLQESGPGLVKPSETLSLTCTVSGVSLPDYG VSWIRQPPGKGLEWIGVIWGSETTYYQSSLKSR VTISKDNSKNQVSLKLSSVTAADTAVYYCAKHY YYGGSYAMDYWGQGTLVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKRVEPKSCGGGGS GGGGSEVQLVESGGGLVQPGGSLKLSCAASGF TFNTYAMNWVRQASGKGLEWVGRIRSKYNNYA TYYADSVKDRFTISRDDSKSTLYLQMNSLKTED TAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVS SGGGGSGGGGSGGGGSGGGGSQAVVTQEPS LTVSPGGTVTLTCRSSTGAVTTSNYANWVQQK PGQAPRGLIGGTNKRAPWTPARFSGSLLGDKA ALTLSGAQPEDEAEYFCALWYSNLWVFGGGTK LTVLGGGGSDKTHTCPPCPAPELLGGPSVFLFP PKPKDTLMISRTPEVTCVVVAVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALAAPIEKTISKAKGQP REPQVCTLPPSRDELTKNQVSLSCAVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLVSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPGK	1094
	Second Half Antibody (Fc sequence not shown)	SQQIYGVVYGNVTFHVPSNVPLKECLWKKQKD KVAELENSEFRAFSSFKNRVYLDTVSCSLTIYNL TSSDEDEYEMESPNITDTMKFFLYVLESGS	782
	Second Half Antibody (includes Fc sequence)	SQQIYGVVYGNVTFHVPSNVPLKECLWKKQKD KVAELENSEFRAFSSFKNRVYLDTVSCSLTIYNL TSSDEDEYEMESPNITDTMKFFLYVLESGSDKT HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYASTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPCR EEMTKNQVSLWCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNRYTQKSLSLSPGK	1097
	First Half Antibody Light Chain	EIVMTQSPATLSLSPGERATLSCRASQDISKYLN WYQQKPGQAPRLLIYHTSRLHSGIPARFSGSGS GTDYTLTISSLQPEDFAVYFCQQGNTLPYTFGQ GTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSGNSQESVTEQ DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ GLSSPVTKSFNRGEC	780

TABLE 23

Trispecific Name	Chain Description	Sequence	SEQ ID NO:
CD19_CTL119 _CD3_16nM-CD58 _ IgV V45C_M114C Trispecific	First Half Antibody Heavy Chain (Fc sequence not shown)	QVQLQESGPGLVKPSETLSLTCTVSGVSLPDYG VSWIRQPPGKGLEWIGVIWGSETTYYQSSLKSR VTISKDNSKNQVSLKLSSVTAADTAVYYCAKHY YYGGSYAMDYWGQGTLVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKRVEPKSCGGGGS GGGGSEVQLVESGGGLVQPGGSLKLSCAASGF TFNTYAMNWVRQASGKGLEWVGRIRSKYNNYA TYYADSVKDRFTISRDDSKSTLYLQMNSLKTED TAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVS SGGGGSGGGGSGGGGSGGGGSQAVVTQEPS LTVSPGGTVTLTCRSSTGAVTTSNYANWVQQK PGQAPRGLIGGTNKRAPWTPARFSGSLLGDKA ALTLSGAQPEDEAEYFCALWYSNLWVFGGGTK LTVLGGGGS	778
	First Half Antibody Heavy Chain (includes Fc sequence)	QVQLQESGPGLVKPSETLSLTCTVSGVSLPDYG VSWIRQPPGKGLEWIGVIWGSETTYYQSSLKSR VTISKDNSKNQVSLKLSSVTAADTAVYYCAKHY YYGGSYAMDYWGQGTLVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKRVEPKSCGGGGS GGGGSEVQLVESGGGLVQPGGSLKLSCAASGF TFNTYAMNWVRQASGKGLEWVGRIRSKYNNYA TYYADSVKDRFTISRDDSKSTLYLQMNSLKTED TAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVS SGGGGSGGGGSGGGGSGGGGSQAVVTQEPS LTVSPGGTVTLTCRSSTGAVTTSNYANWVQQK PGQAPRGLIGGTNKRAPWTPARFSGSLLGDKA ALTLSGAQPEDEAEYFCALWYSNLWVFGGGTK LTVLGGGGSDKTHTCPPCPAPELLGGPSVFLFP PKPKDTLMISRTPEVTCVVVAVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALAAPIEKTISKAKGQP REPQVCTLPPSRDELTKNQVSLSCAVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLVSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPGK	1094
	Second Half Antibody (Fc sequence not shown)	SQQIYGVVYGNVTFHCPSNVPLKEVLWKKQKD KVAELENSEFRAFSSFKNRVYLDTVSGSLTIYNL TSSDEDEYEMESPNITDTCKFFLYVLESGS	783
	Second Half Antibody (includes Fc sequence)	SQQIYGVVYGNVTFHCPSNVPLKEVLWKKQKD KVAELENSEFRAFSSFKNRVYLDTVSGSLTIYNL TSSDEDEYEMESPNITDTCKFFLYVLESGSDKT HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYASTYRVVSVLTVLHQDWLNGKEYKC	1098

TABLE 23			
Trispecific Name	**Chain Description**	**Sequence**	**SEQ ID NO:**
		KVSNKALPAPIEKTISKAKGQPREPQVYTLPPCR EEMTKNQVSLWCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNRYTQKSLSLSPGK	
	First Half Antibody Light Chain	EIVMTQSPATLSLSPGERATLSCRASQDISKYLN WYQQKPGQAPRLLIYHTSRLHSGIPARFSGSGS GTDYTLTISSLQPEDFAVYFCQQGNTLPYTFGQ GTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSGNSQESVTEQ DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ GLSSPVTKSFNRGEC	780

[0900] Trispecific binding molecules were expressed transiently by co-transfection of the respective chains in HEK293 cells.

[0901] Briefly, transfection was performed using PEI as transfection reagent. For small scale (<5L) transfections, cells were grown in shake flasks on an orbital shaker (115 rpm) in a humidified incubator (85%) at 5% CO2). Plasmids were combined with PEI at a final ratio of 1 DNA: 3 PEI. 1mg/L culture of plasmid was used for transfection at 2.0million cells/mL serum media. After 5 days of expression, the TBMs were harvested by clarification of the media via centrifugation and filtration. Purification was performed via anti-CH1 affinity batch binding (CaptureSelect IgG-CH1 Affinity Matrix, Thermo-Fisher Scientific, Waltham, MA, USA) or Protein A (rProteinA Sepharose, Fast flow, GE Healthcare, Uppsala, Sweden) batch binding using 1ml resin/100 mL supernatant. The protein was allowed to bind for a minimum of 2 hours with gentle mixing, and the supernatant loaded onto a gravity filtration column. The resin was washed with 20-50 CV of PBS. TBMs were eluted with 20 CV of 50 mM citrate, 90 mM NaCl pH 3.2. 50mM sucrose The eluted TBMs were adjusted to pH 5.5 with 1 M sodium citrate 50mM sucrose. Preparative size exclusion chromatography was performed using Hi Load 16/60 Superdex 200 grade column (GE Healthcare Life Sciences, Uppsala, Sweden) as a final polishing step when aggregates were presente. To confirm that the identity of the proteins of the TBMs expressed matched the predicted masses for the primary amino acid sequences, proteins were analyzed by high-performance liquid chromatography coupled to mass spectrometry.

8.13.2. Results

[0902] As shown in Table 24 below, inclusion of stabilizing disulfide variants had no adverse impact on overall expression yields of increased aggregate content upon purification.

TABLE 24		
	Expression (mg/L)	**%HMWS**

CD19_CTL119_CD3_16nM-CD58_Full ECD Trispecific (Full ECD WT)	20	< 10%
CD19_CTL119_CD3_16nM-CD58_IgV Trispecific (IgV WT)	20	~10
CD19_CTL119_CD3_16nM-CD58_IgV_ V45C_M105C Trispecific (IgV V45C_M105C)	55	~10
CD19_CTL119_CD3_16nM-CD58_ IgV V54C_G88C Trispecific (IgV V54C_G88C)	65	~10
CD19_CTL119_CD3_16nM-CD58 _ IgV V45C_M114C Trispecific (IgV V45C_M114C)	63	~10

8.14. Example 14: Re-directed T cell cytotoxicity with TBMs containing CD58 variants

[0903] TBMs of Example 13 containing the variant CD58 domains were analyzed for their potential to induce T cell-mediated apoptosis in tumor target cells.

8.14.1. Materials and Methods

[0904] Briefly, huCD19-expressing Nalm6 target cells were engineered to overexpress firefly luciferase. Cells were harvested and resuspendend in RPMI medium (Invitrogen # 11875-093) with 10% FBS. 10,000 target cells per well were plated in a flat-bottom 96-well plate. Human pan T effector cells were isolated via MACS negative selection (Miltenyi Biotec #130-096-535) from two donors from cryopreserved PBMC (Cellular Technologies Limited #CTL-UP1) then added to the plate to obtain a final E:T ratio of 5:1. Co-cultured cells were incubated with a serial dilution of all constructs and controls. For normalization, average maximum luminescence refers to target cells co-incubated with effector cells, but without any test construct. After an incubation of either 24 or 48 hr at 37°C, 5% CO2, OneGlo luciferase substrate (Promega #E6120) was added to the plate. Luminescence was measured on an Envision plate reader after a 10 minute incubation. Percent specific lysis was calculated using the following equation: Specific lysis (%) = (1- (sample luminescence / average maximum luminescence)) * 100

8.14.2. Results

[0905] As shown in FIG. 15, the TBMs containing the variant CD58 domains show comparable cytotoxic activity to a TBM with wild type CD58.

8.15. Example 15: T-cell activation with TBMs containing CD58 variants

[0906] As an alternative to primary T cell activation, a Jurkat-NFAT reporter cell line was used to evaluate the functional activity of the TBMs of Example 13 containing the variant CD58 domains.

8.15.1. Materials and Methods

[0907] The Jurkat T cell line (E6-1) was transfected with a NFAT-luciferase reporter construct and a stable, clonal cell line Jurkat cells with NFAT-LUC reporter (JNL), was selected for further characterization based on strong induction of the NFAT reporter following PMA and ionomycin stimulation.

[0908] The Jurkat reporter cell line for was used for determination of non-specific activation of NFAT.

[0909] Purified TBMs were tested for their potential to induce NFAT activation in the absence of target cells.

[0910] Jurkat cells with NFAT-LUC reporter (JNL) were grown in RPMI-1640 media containing 2mM glutamine and 10% fetal bovine serum with puromycin at 0.5 ug/ml. 100,000 JNL cells per well were plated in a flat-bottom 96-well plate and were incubated with serial dilutions of the TBMs and controls. After an incubation of 6 hr at 37°C, 5% CO2, OneGlo luciferase substrate (Promega #E6120) was added to the plate. Luminescence was measured on an Envision plate reader after a 10 minute incubation.

8.15.2. Results

[0911] As shown in FIG. 16, the TBMs containing the variant CD58 domains show tumor-independent (*i.e.*, non-target cell specific) activitation levels comparable to or lower than TBMs containing wild type CD58.

8.16. Example 16: CD19 and CD58 expression on various cell lines

8.16.1. Materials and Methods

[0912] Cell surface expression of CD19 and CD58 was determined on OCI-LY-19 (a human B-cell non-Hodgkin lymphoma cell line), Karpas-422 (a human B-cell non-Hodgkin lymphoma cell line), Toledo (a human B-cell non-Hodgkin lymphoma cell ine), and Nalm-6 (B cell precursor leukemia cell line) cell lines by flow cytometry using APC labelled anti-CD19 (Biolegend, Cat # 302212) and APC-labelled anti-CD58 (Biolegend, Cat # 330918) and respective isotype control antibodies. The samples were run on BD LSR Fortessa and analyzed using FlowJo.

8.16.2. Results

[0913] The cell lines have different level of CD19 and CD58 expression (FIGS. 17A-H). The ranking for CD19 expression among the cell lines was OCI-LY-19 > Karpas 422 > Toledo = Nalm-6. The ranking for CD58 expression was OCI-LY-19 > Nalm-6 > Karpas = Toledo.

8.17. Example 17: RTCC and cytokine secretion activity of the NEG258-based TBMs vs. a one-arm BBM that does not bind to CD2 and a TBM that does not bind to CD19

[0914] CD3hi TSP1, CD3med TSP1, CD3 hi BSP1, and CD3hi TSP1C (H variants) were compared for their potential to induce T cell-mediated apoptosis in Karpas422 target cells.

8.17.1. Materials and Methods

[0915] An RTCC assay with huCD19-expressing Karpas422 target cells was performed according to the Materials and Methods described in Example 9, but with a final E:T ratio of 1:1 and a 96 hour incubation.

8.17.2. Results

[0916] As shown in FIGS. 18A-18B, CD3hi TSP1, CD3med TSP1, and CD3hi BSP1 show cytotoxic activity against Karpas422 target cells, with CD3hi TSP1 having the highest cytotoxic activity.

8.18. Example 18: Cytokine Release Assay

[0917] CD3hi TSP1, CD3med TSP1, CD3 hi BSP1, and CD3hi TSP1C (H variants) were analyzed for their ability to induce T cell-mediated de novo secretion of cytokines in the presence of Karpas422 cells.

8.18.1. Materials and Methods

[0918] A cytokine release assay was performed as in Example 10, but with Karpas422 cells at a final E:T ratio of 1:1 and an incubation of 48 hours.

8.18.2. Results

[0919] As shown in FIGS. 19A-19F, CD3hi TSP1, CD3med TSP1, and CD3hi BSP1 induced cytokine secretion by T cells, with CD3hi TSP1 inducing the highest levels of cytokine secretion, followed by CD3med TSP1, which was similar to CD3hi BSP1.

8.19. Example 19: TBM and BBM binding to T cells

[0920] Binding of CD3hi TSP1, CD3med TSP1, CD3hi BSP1, and CD3hi TSP1C (H variants) to T cells was evaluated using flow cytometry.

8.19.1. Materials and Methods

[0921] Peripheral blood mononuclear cells (PBMCs) previously isolated and cryopreserved from 2 Leukopak donors (Hemacare) were thawed and Pan T cells were isolated by negative selection using the Pan T cell Isolation Kit, human (Miltenyi Biotec, Cat # 130-096-535) following the manufacturer's protocol. T cells were resuspended in FACS Buffer and 100,000 cells were added to each well of a 96 well round bottom plate. A dilution series of CD3med TSP1, CD3hi TSP1, CD3hi BSP1, and CD3hi TSP1C ranging from 33 µg/ml – 0.005 µg/ml was added to cells and incubated on ice for 1 hour. Cells were washed twice, resuspended in 100 µl

of anti-human IgG secondary antibody and incubated on ice for another hour. After the incubation, cells were washed twice, resuspended in 100 µl of fixable viability dye and incubated on ice for 30 min. After washing twice again, cells were resuspended in 120 µl of FACS buffer. The cells were then run on BD LSR Fortessa and data was analyzed using FlowJo to determine the MFI of anti-human IgG secondary antibody, which was plotted against antibody concentration.

8.19.2. Results

[0922] All antibodies showed different degree of binding to T cells (FIG. 20). CD3hi TSP1 was the strongest binder followed by CD3med TSP1, with BSP1 being the weakest binder. Without being bound by theory, it is believed that the improved binding of the TBMs can be attributed to co-engagement of CD2 and CD3 arms, thereby increasing the binding avidity to T cells.

8.20. Example 20: TBM and BBM mediated T cell proliferation

[0923] CD3hi TSP1, CD3med TSP1, CD3hi BSP1, and CD3hi TSP1C (H variants), and blinatumomab were evaluated for their ability to induce T cells proliferation upon co-culture with CD19 expressing OCI-LY-19, Karpas422, and Toledo target cells.

8.20.1. Materials and Methods

[0924] Briefly, OCI-LY-19, Karpas422, and Toledo target cells stably expressing firefly luciferase were plated in a 96 well plate in T Cell Media (TCM) (RPMI-1640, ThermoFisher Scientific, Cat # 11875-085), 10% FBS (Seradigm, Cat # 1500-500), 1% L-Glutamine (Thermo Fisher Scientific, Cat # 25830-081), 1% Non Essential Amino Acids (Thermo Fisher Scientific, Cat # 11140-050),1% Pen/Strep (Thermo Fisher Scientific, Cat # 15070063), 1% HEPES (Thermo Fisher Scientific, Cat # 15630080), Sodium Pyruvate (Thermo Fisher Scientific, Cat # 11360-070), 0.1% Beta-mercaptoethanol (Thermo Fisher Scientific, Cat # 21985-023)]. PBMCs previously isolated and cryopreserved from 2 Leukopak donors were thawed and Pan T cells were isolated (as described earlier). Isolated T cells were labelled with 5 µM Cell Trace Violet (CTV) (Thermo Fisher Scientific, Cat # C34557) following the manufacturer's protocol and were co-cultured with target cells at an E:T ratio of 1:3. A dilution series of CD3med TSP1, CD3hi TSP1, CD3hi BSP1, CD3hi TSP1C, and blinatumomab ranging from 2.5 nM – 0.0006 nM was added to cells and the plates were incubated in a 5% CO2, 37ºC incubator for 96 hrs. After incubation, the cells were harvested, treated with Human TruStain FcX (Fc Block) (Biolegend, Cat # 422302) and stained with Fixable Viability Dye eFlour 780 (ThermoFisher Scientific, Cat # 65-0865-14), followed by staining with PerCP-Cy5.5 conjugated anti-human CD3 mAb (Biolegend, Cat # 317336). All staining steps were performed according to manufacturer's protocol. Flow analysis were performed using BD LSR Fortessa and FlowJo software to

determine % proliferated CD3+ T cells based on CD3 staining and dilution of Cell Trace Violet dye.

8.20.2. Results

[0925] All CD19 targeting antibodies induced proliferation of T cells upon co-culture with different CD19 expressing target cell lines (FIGS. 21A-21C). The T cell proliferation effect was dose-dependent, and CD3hi TSP1 showed more potent activity than CD3med TSP1 and CD3hi BSP1. The control antibody did not induce any T cell proliferation indicating that CD19 target-specific engagement was required for the proliferation of T cells. Blinatumomab mediated the most potent T cell proliferation in the presence of OCI-LY-19 and Toledo cells. In the presence of Karpas420, CD3hi TSP1 more effectively induced T cell proliferation as shown by the maximum percentage of proliferated T cells.

8.21. Example 21: RTCC activity of NEG258-based TBMs with different CD3 affinities vs. a BBM and blinatumomab

[0926] The NEG258-based TBMs containing CD3 binding arms with different affinities (CD3hi TSP1 and CD3med TSP1 (H variants)) and a BBM (CD3hi BSP1 (H variant)) were compared for their potential to induce T cell-mediated apoptosis in Karpas422 target cells. The study also included blinatumomab as a control.

8.21.1. Materials and Methods

[0927] An RTCC assay with huCD19-expressing Karpas422 target cells was performed according to the Materials and Methods described in Example 9, but with a final E:T ratio of 1:1 and a 96 hour incubation.

8.21.2. Results

[0928] As shown in FIGS. 22A-22B, both types of TBMs show cytotoxic activity against Karpas422 cells. The TBMs demonstrated superior cytotoxic activity compared to the BBM. CD3hi TSP1 showed similar or superior cytotoxic activity compared to blinatumomab.

8.22. Example 22: RTCC activity of NEG258-based TBMs with different CD3 affinities vs. a BBM and TBMs that do not bind to CD19 against multiple B cell lymphoma cell lines

[0929] CD3hi TSP1, CD3med TSP1, CD3hi BSP1, and CD3hi TSP1C (H variants) were compared for their potential to induce T cell-mediated apoptosis in Oci-Ly19, Toledo, Nalm6, Nalm6 KO and K562 target cells. Oci-Ly19, Toledo, Nalm6 cells express hCD19 antigen. Nalm6 KO and K562 target cells lacking hCD19 expression were used to assess target-independent killing. The study also included blinatumomab as a control.

8.22.1. Materials and Methods

[0930] Nalm6 KO was generated from Nalm6 parental cell line by using CRISPR-CAS9 technology and was confirmed to lack hCD19 expression. Oci-Ly19, Toledo, Nalm6, Nalm6 KO and K562 target cells were engineered to overexpress firefly luciferase. RTCC assays were performed with the different cells lines according to the Materials and Methods described in Example 9, but with a final E:T ratio of 1:1 and a 48 hour incubation.

8.22.2. Results

[0931] CD3hi TSP1 and CD3med TSP1 showed cytotoxic activity against Oci-Ly19, Toledo and Nalm6, but showed minimal activity against antigen-negative Nalm6 KO and K562 (FIGS. 23A-23J). The TBMs demonstrated superior cytotoxic activity compared to the BBM. CD3hi TSP1 showed comparable cytotoxic activity to blinatumomab.

8.23. Example 23: Cytokine Release Assay of the NEG258-based TBMs with different CD3 affinities vs. a BBM and TBMs that do not bind to CD19 against multiple B cell lymphoma cell lines

[0932] CD3hi TSP1, CD3med TSP1, CD3hi BSP1 and CD3hi TSP1C (H variants) were compared for their potential to induce T cell-mediated de novo secretion of cytokines in Oci-Ly19, Toledo, Nalm6, Nalm6 KO and K562 target cells. Oci-Ly19, Toledo, Nalm6 cells express hCD19 antigen. Nalm6 KO and K562 target cells that lack hCD19 expression were used to assess target-independent cytokine release. The study also included blinatumomab as a control.

8.23.1. Materials and Methods

[0933] Target cells were harvested and resuspended in RPMI medium (Invitrogen # 11875-093) with 10% FBS. 5,000 target cells per well were plated in a flat-bottom 384-well plate. Human pan T effector cells were isolated via negative selection (Stemcell Technologies #17951) from two donors from cryopreserved PBMCs that were separated from a leukopak (Hemacare #PB001F-1) by Ficoll density gradient centrifugation. Purified T cells were then added to the plate to obtain a final E:T ratio of 1:1. After an incubation of 48 hr at 37°C, 5% CO2, the supernatants were harvested for subsequent analysis. A multiplexed ELISA was performed according to the manufacturer's instructions using a human cytokine custom 3-plex 384 4-spot kit (MesoScale Discovery #N31IB-1).

8.23.2. Results

[0934] As shown in FIGS. 24A-24J, both NEG258-based TBMs induce significant cytokine secretion by T cells in a dose-dependent manner when incubated with Oci-Ly19, Toledo and Nalm6 cells. Minimal cytokine secretion was detected when incubating with antigen-negative Nalm6 KO and K562.

8.24. Example 24: Re-challenge RTCC assay with Karpas 422 & OCI-LY-19 cell lines

[0935] The effect of target cell re-challenge on the killing activity of CD3hi TSP1 (H variant), CD3med TSP1 (H variant), CD3hi BSP1 (H variant), and blinatumomab treated T cells was determined using a dose titration re-challenge RTCC assay.

8.24.1. Materials and Methods

[0936] OCI-LY-19 and Karpas422 target cells stably expressing firefly luciferase were plated in a Costar 6 well plate in T Cell Media (TCM). PBMCs previously isolated and cryopreserved from 2 Leukopak donors were thawed and Pan T cells were isolated (as described earlier). A co-culture of T cells and OCI-LY-19 or Karpas 422 cells at E:T ratio of 1:1 along with EC90 concentration (0.1nM for OCI-LY-19 and 0.5nM for Karpas 422) of CD3med TSP1, CD3hi TSP1, CD3hi BSP1, and blinatumomab was set-up. The plates were incubated for 4 days for OCI-LY-19 and 5 days for Karpas 422 cells. At the end of incubation, the killing of target cells was determined using the luminescence signal. The absolute T cell counts from each antibody treated condition was also determined. For the next round of rechallenge, it was ensured that the killing of target cells was equivalent across various antibody conditions. The T cell counts were normalized across different antibody conditions and another round of a single concentration rechallenge was set-up at E:T of 1:1 using the EC90 concentration with a 4 day incubation for both target cells. Additionally, a dose titration RTCC at a E:T of 1:1 and a concentration range of 2nM -0.000001 nM was set-up using T cells from the different antibody treated conditions with a 4 day incubation used for each cell line. The killing of target cells was determined using the luminescence signal to generate dose response curves. At the end of the challenge, the above process was repeated once more for Karpas 422 and twice more for OCI-LY-19 cells. The assay set-up is shown in FIG. 25A.

8.24.2. Results

[0937] As can be seen from FIGS. 25B-25H, CD3hi TSP1 was able to better retain killing ability upon repeated challenges with the target cells compared to CD3med TSP1, and CD3hi TSP1. CD3med TSP1 was the next best, with CD3hi BSP1 being the least active of all antibodies. CD3hi TSP1 demonstrated similar activity when compared to Blinatumomab in the first 2 or 3 rounds of re-challenges for Karpas422 and OCI-LY-19 cells, respectively. At the last re-challenge for OCI-LY-19, CD3hi TSP1 mediated more potent RTCC than blinatumomab (both in EC50 and maximum lysis), whereas for Karpas422, blinatumomab mediated higher maximum lysis than CD3hi TSP1 despite a similar EC50.

8.25. Example 25: Re-challenge T cell phenotyping with Karpas 422 & OCI-LY-19 cell lines

[0938] The effect of target cell re-challenge on the phenotype of CD3hi TSP1, CD3med TSP1, and CD3hi BSP1 (H variants) treated T cells was determined using a single concentration re-challenge assay.

8.25.1. Materials and Methods

[0939] OCI-LY-19 and Karpas422 target cells stably expressing firefly luciferase were plated in a Costar 6 well plate in T Cell Media (TCM). PBMCs previously isolated and cryopreserved from 2 Leukopak donors were thawed and Pan T cells were isolated (as described earlier). Co-cultures of T cells and OCI-LY-19 or Karpas 422 cells at E:T ratio of 1:1 was set-up and 1nM of CD3hi BSP1, CD3med TSP1, or CD3hi TSP1 was added. The plates were incubated for 4 days for OC-LY-19 and 5 days for Karpas 422 cells. At the end of incubation, the killing of target cells and absolute T cell counts from each antibody treated condition was determined. The T cell counts were normalized across different antibody conditions and two additional rounds of re-challenges were set-up the same way as the previous challenge with a 4 day incubation for both target cells, for a total of three challenges. After the third challenge, T cells from different antibody treated conditions were harvested on day 2 from the Karpas 422 co-cultures and on day 4 from OCI-LY-19 co-cultures and split into 2 fractions. One fraction was stained with blue fixable viability dye (ThermoFisher Scientific, Cat # L23105) prior to staining with a cocktail of anti-human CD3 (Biolegend, Cat # 317324), CD4 (Biolegend, Cat # 344608), CD8 (BD Biosciences, Cat # 563795), CD27 (Biolegend, Cat # 356412) & CD62L mAb (Biolegend, Cat # 304814). The second fraction was resuspended to 1e6/ml in TCM and stimulated with Cell Stimulation Cocktail (Tonbo Biosciences, Cat # TNB4975) for 4hrs at 37°C. Thereafter the cells were washed and sequentially stained with blue fixable viability dye (ThermoFisher Scientific, Cat # L23105), a cocktail of anti-human CD3 (Biolegend, Cat # 317324), CD4 (Biolegend, Cat # 344608), CD8 (BD Biosciences, Cat # 563795), followed by permeabilization using the FoxP3 transcription factor staining set (ThermoFisher Scientific, Cat # 00-5523-00) and final staining with anti-human IFNγ mAb (Biolegend, Cat # 400134) and IL-2 mAb (Biolegend, Cat # 400551) or respective isotype controls. All stainings were performed according to manufacturer's protocol. Flow analyses were performed using BD LSR Fortessa and FlowJo software.

8.25.2. Results

[0940] As shown in FIGS. 26A-26H (Karpas 422 model) and FIGS. 26I-26P (OCI-LY-19 model), CD3hi TSP1 better promoted enrichment of younger phenotype of T cells than CD3med TSP1 and CD3hi BSP1. CD3hi TSP1 was also able to induce better cytokine production from the T cells compared to the other CD19 binders tested.

8.26. Example 26: Ability of CD3hi TSP1 vs. CD3hi BSP1 to elicit T cell proliferation and cytokine production in presence of CD19+ target cells

[0941] CD3hi TSP1 and CD3hi BSP1 (R variants) were evaluated for their ability to induce T cell proliferation, cytokine production and changes in T cells' surface markers expression, upon co-culture with CD19-expressing Nalm6 target cells.

8.26.1. Materials and Methods

[0942] Nalm-6 target cells stably expressing firefly luciferase were irradiated at 50Gy on the day of the assay set up. Peripheral blood mononuclear cells (PBMCs) previously isolated from buffy coat donors (Bern Hospital) and cryopreserved were thawed and total T cells were isolated by negative selection using the human Pan T cell Isolation Kit (Miltenyi Biotec, Cat # 130-096-535) following the manufacturer's protocol. The positive fraction (called PBMCs-T cells depleted) was irradiated at 50Gy in order to be used as feeder for the co-culture. From the negative fraction, enriched in total T cells, $CD8^+$ T cells were isolated by an additional step of negative selection using EasySep™ Human CD8+ T Cell Enrichment Kit (Stem Cell, Cat# 19053). Untouched $CD8^+$ cells were then stained with an anti-CD28 antibody (Biolegend, Cat# 302922) and sorted with a FACSAria (BD) according to CD28 expression: $CD8^+CD28^+$ and $CD8^+CD28^-$. The purity of sorted cells was >95%.

[0943] After sorting, T cells were labelled with 2.5 µM of Carboxyfluorescein succinimidyl ester (CFSE, Thermo Scientific, Cat# C34554) following the manufacturer's protocol.

[0944] Each T cell subset (either $CD8^+CD28^+$ or $CD8^+CD28^-$) of CFSE-labelled T cells was co-cultured with Nalm6 target cells, seeding 50,000 T cells and 50,000 target cells to achieve an effector: target (E:T) ratio of 1:1. Cells were diluted and co-plated to obtain additional final E:T ratio of 1:3 or 1:6.

[0945] In the co-culture conditions where the presence of irradiated PBMCs-T cells depleted was required, 10,000 PBMCs were plated to obtain a 5:1 ratio, effector T cells: PBMCs.

[0946] The T cells-tumor cells co-culture was plated in a Costar 96 well plate (Corning, Cat # 3585) in T Cell Media [RPMI-1640 (ThermoFisher Scientific, Cat #21875-034); 10% FBS HyClone (GE healthcare, Cat # SH30070.03); 1% Non Essential Amino Acids (Thermo Fisher Scientific, Cat # 11140-050); 1% Pen/Strep (Thermo Fisher Scientific, Cat #15140122); 1% HEPES (Lonza, Cat # 17737E); Sodium Pyruvate (Thermo Fisher Scientific, Cat # 11360-070); 50µM Beta-mercaptoethanol (Thermo Fisher Scientific, Cat # 31350)].

[0947] CD3hi TSP1 andCD3hi BSP1, diluted in T cell Media, were added to the cells at different concentrations (1nM, 0.1nM and 0.01nM) and incubated in a 5% CO2, 37ºC incubator for 72 hrs. In order to be able to detect intracellular cytokines production, plates were incubated for the last 1.5 hrs of the co-culture with PMA (50 ng/ml; SIGMA, Cat# P1585)). Ionomycin

(500µg/ml; Calbiochem, Cat#407950); brefeldin (10µg/ml; Cell Signaling, Cat#9972) was also added for the last 1.5 hours of the incubation.

[0948] At the end of the 72hrs, cells were harvested and then stained with a viability die, Zombie Aqua (Biolegend, Cat #423102) by incubating at room temperature, for 10 mins. Cells were then washed twice using FACS Buffer and stained with antibodies against surface markers: anti-CD2 (Biolegend, Cat # 300214), anti-CD28 (Biolegend, Cat# 302922), anti-CCR7 (Biolegend, Cat# 353226), and anti-CD45RO (Biolegend, Cat# 304216). Intracellular IFN-g and granzyme B (GzB) were detected by treating T cells with BD cytofix cytoperm kit (BD, Cat# 555028) according to the manufacturer's instructions, and staining them with anti-IFNg (Biolegend, Cat# 502509) and anti-granzyme B antibodies (BD, Cat# 560213). Samples were washed with FACS buffer and acquired on a BD LSR Fortessa (BD). Analysis was performed with FLOWJO software (version 10.6.0; Tree Star Inc.).

8.26.2. Results

[0949] Both CD3hi TSP1 and CD3hi BSP1 induced proliferation of both $CD28^+$ and $CD28^-$ T cells upon co-culture with CD19 expressing target cell line Nalm6 (FIGS. 27A-27D). However, CD3hi TSP1 was more potent in inducing proliferation of both T cells subsets compared to CD3hi BSP1. The effect was observed at both concentrations tested and at the different E:T ratios used. The effect on T cell proliferation was observed both in presence or absence of irradiated PBMCs.

[0950] In presence of 1nM of CD3hi BSP1, no major differences were observed in terms of percentage of T cells producing IFN-g or granzyme B (GzB); however, in presence of CD3hi TSP1 there was a clear shift in the median fluorescence intensity (MFI) for both cytokines, indicating an increase in the expression of both IFNg and GzB, in particular among the $CD28^-$ T cells when co-cultured in presence of irradiated PBMCs (FIGS. 28A-28D). The effect of CD3hi TSP1 on cytokines producing-T cells is even more pronounced at 0.1nM: both in presence and absence of irradiated PBMCs there was a clear increase in GzB^+ T cells and $IFNg^+$ T cells both within CD28- and CD28+ T cell subsets, as MFI (FIGS. 28E-28H). Moreover, the proportion (%) of $CD28^-$ T cells $IFNg^+GzB^+$ was also more pronounced in presence of CD3hiTSP1 (FIGS. 28I-28L).

[0951] The combination of the expression profile of CD45RO and CCR7 define the distribution of the different T cell populations: naive ($CD45RO^-CCR7^+$), central memory (CM) ($CD45RO^+CCR7^+$), effector memory (EM) ($CD45RO^+CCR7^-$) and the terminally differentiated (TEMRA) ($CD45RO^-CCR7^-$). Changes in the T cell surface phenotype are shown in FIG. 29. There was no major effect of the CD3hi molecules on the $CD28^+$ cells that maintain the homogenous distribution of the different T cells populations, observed right after sorting, also

after 72hrs of co-culture. Conversely, there was an effect of CD3hi TSP1 on $CD28^-$ cells: while after sorting $CD28^-$ cells showed almost entirely a TEMRA phenotype, after 72 hrs treatment with CD3hiTSP1 $CD28^-$ cells re-aquired a central memory/effector memory phenotype with a concomitant decrease in the proportion of cells with a more terminally differentiated (TEMRA) profile.

8.27. Example 27: Ability of CD3hiTSP1 vs. CD3hi BSP1 molecules to elicit redirected T-cell cytotoxic activity (RTCC) against CD19+ target cells

[0952] An RTCC assay was set up with CD19+ Nalm6 cells, engineered to express the luciferase gene, and sorted CD8 T cells populations to measure the ability of CD3hi TSP1 and CD3hi BSP1 (R variants) to elicit cytotoxic activity of CD8 T cells subsets.

8.27.1. Materials and Methods

[0953] Peripheral blood mononuclear cells (PBMCs) previously isolated from buffy coat donors (Bern Hospital) and cryopreserved were thawed and total T cells were isolated by negative selection using the Pan T cell Isolation Kit, human (Miltenyi Biotec, Cat # 130-096-535) following the manufacturer's protocol. The positive fraction (called PBMCs-T cell depleted) was irradiated at 50Gy in order to be used as feeder in the co-culture.

[0954] From the negative fraction, enriched in total T cells, $CD8^+$T cells were then isolated by an additional step of negative selection using EasySep™ Human CD8+ T Cell Enrichment Kit (Stem Cell, Cat# 19053). Untouched $CD8^+$ cells were then stained with an anti-CD28 antibody (Biolegend, Cat# 302922) and sorted with a FACSAria (BD) according to the CD28 expression: $CD8^+CD28^+$ and $CD8^+CD28^-$. The purity of th sorted cells was >95%.

[0955] Each T cell subset (either $CD8^+CD28^+$ or $CD8^+CD28^-$) was then co-cultured in a 384-well flat-bottom microtiter plate (ThermoFisher Scientific, Cat #142761) with equivalent number of Nalm6 target cells to achieve an effector: target (E:T) ratio of 1:1 (3,000 T cells and 3,000 Target cells). The co-culture was set up in T Cell Media [RPMI-1640 (ThermoFisher Scientific, Cat #21875-034), 10% FBS HyClone (GE healthcare, Cat # SH30070.03), 1% Non Essential Amino Acids (Thermo Fisher Scientific, Cat # 11140-050),1% Pen/Strep (Thermo Fisher Scientific, Cat #15140122), 1% HEPES (Lonza, Cat # 17737E), Sodium Pyruvate (Thermo Fisher Scientific, Cat # 11360-070), 50µM Beta-mercaptoethanol (Thermo Fisher Scientific, Cat # 31350)]. Cells were diluted and co-plated to obtain additional final E:T ratio of 1:3 or 1:6. In the co-culture conditions where the presence of irradiated PBMCs-T cells depleted was required, 600 PBMCs were plated to obtain a 5:1 ratio, effector T cells: PBMCs.

[0956] CD3hi TSP1, CD3hi BSP1 and CD3hi TSP1C antibody control were added to the cells at different concentrations (1nM, 0.1nM and 0.01nM).

[0957] Plates were incubated in a 37°C incubator with 5% CO2 for 72 hrs. Following the co-incubation, One-Glo (Promega, catalog# E6110) was added to all wells and the luminescence signal was subsequently measured on an ELISA Reader 4.18 1 (Biotek, Synergy H1). Target cells with One-Glo served as maximal signal. The percent RTCC of target cells was calculated using the following formula: [100- (sample/maximal signal)*100%].

8.27.2. Results

[0958] Results are shown in FIGS. 30A-30D. Both CD3hi TSP1 and CD3hi BSP1 mediated RTCC activity against CD19+ Nalm6-luc target cells when compared to the control antibody CD3hi TSP1C. When 0.1nM or 1 nM of CD3hi TSP1 was used, an increase in the CD3hi TSP1-mediated RTCC was observed in the settings with $CD8^+CD28^-$ T cells (in presence of irradiated feeder) compared to the other treatments.

8.28. Example 28: Anti-tumor activity of CD3hi TSP1 and CD3med TSP1 in an adoptive transfer adaptation of the OCI-LY-19 diffuse large B-cell lymphoma tumor model in NSG mice

[0959] The anti-tumor activity of CD3hi TSP1 and CD3med TSP1 (H variants) were studied in an OCI-LY-19 diffuse large B-cell lymphoma (DLBCL) tumor model in NSG mice.

8.28.1. Materials and Methods

[0960] On Day 0, OCI-LY-19 cells were harvested and suspended in Hanks Balanced Salt Solution (HBSS) at a concentration of $500x10^6$ cells/mL. Female NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ mice (NSG mice) at ~6 weeks old (Jackson Laboratories, ME) were injected with $5x10^6$ OCI-LY-19 cells in 200µL subcutaneously on the right flank. Seven days following tumor inoculation, each mouse received an adoptive transfer (AdT) of $15X10^6$ of peripheral blood mononuclear cells (PBMCs) in 100µL via IV injection in the lateral tail vein. The PBMCs were previously isolated from a human leukopak, frozen and stored in Cryostor10 media in vapor phase liquid nitrogen tank until use. Immediately prior to AdT, PBMCs were thawed and suspended at a concentration of $100X10^6$cells/ml in Hanks Balanced Salt Solution (HBSS). When tumor burden (TB) reached an average of ~200 mm^3 volume measured via mechanical caliper, mice (n=8/group) were treated with a single IV administration of CD3hi TSP1 or CD3med TSP1 at dose levels 0.003 mg/kg, 0.01 mg/kg, 0.03 mg/kg, 0.1 mg/kg or 0.3 mg/kg. Anti-tumor activity of each antibody was compared to an untreated control group that received tumor implant and AdT but no treatment (tumor + AdT) (Table 25). The tumor only group was included to meter the allogeneic response observed with untreated control. All treatments were administered at 10 mL/kg according to individual mouse body weights. Anti-tumor activity was determined by percent change in tumor burden vs. change in untreated control (%ΔT/ΔC) or % regression.

[0961] Tumor burden and body weights were recorded twice weekly. Tumor burden was measured by bioluminescence signal intensity in p/s using a bioluminescence imaging system (IVIS200, Perkin Elmer). Anti-tumor activity was determined by %ΔT/ΔC using the formula: 100 X $\Delta TB_{treatment, time}/\Delta TB_{control\ group, time}$ if $\Delta TB \geq 0$; or % regression: (-1 X (100 X (TB_{final}-$TB_{initial}$/ $TB_{initial}$) if $\Delta TV < 0$. $TB_{initial}$ is the tumor burden on the day of treatment initiation. %ΔT/ΔC values <42% were considered to have anti-tumor activity. Percent body weight change was determined using the formula: 100 X (($BW_{time} - BW_{initial}$)/$BW_{initial}$). Statistical analysis using One-way ANOVA with Dunnett's multiple comparison test was performed using Graphpad Prism Software, Version. 7.03.

[0962] On day 25 following OCI-LY-19 implantation, all animals from the untreated control group were euthanized due to tumor burden.

8.28.2. Results

[0963] This study had minimal allogeneic response (FIGS. 31A-31B).

[0964] Antibody treatment with CD3hi TSP1 at 0.1 mg/kg and 0.3 mg/kg resulted in significant tumor regressions of 72.41% and 84.50%, respectively. Antibody treatment with CD3hi TSP1 at 0.03 mg/kg resulted in tumor regression of 13.74%. Antibody treatment with CD3hi TSP1 at 0.003 mg/kg exhibited significant anti-tumor activity at 1.38% ΔT/ΔC. Antibody treatment with CD3hi TSP1 at 0.003 mg/kg dose level was not active in this model (Table 25; FIG. 31A).

[0965] There was no antibody associated body weight loss with CD3hi TSP1. The body weight change observed with the treatment of CD3hi TSP1 was most likely due to the onset of graft-versus host disease (GvHD). Body weight loss is an endpoint parameter for both disease burden and onset of GvHD. At 35-42 days post-PBMC injection (28-35 days post-tumor implant), animals began to exhibit weight loss attributed to GvHD. Animals with high tumor burden also demonstrated disease-burden associated weight loss. Over the course of the study, body weights increased relative the initial measurement taken on the day of tumor implant (Table 25, FIG. 32A). However at the end of study, the body weight loss observed relative to the peak gain is indicative of GvHD and disease-burden induced weight loss.

[0966] Antibody treatment with CD3med TSP1 resulted in significant anti-tumor responses at 0.1 mg/kg (5.60% regression) and 0.3 mg/kg (36.33% regression). Treatment with CD3med TSP1 resulted in significant anti-tumor responses with %ΔT/ΔC values of 7.39% for the 0.03 mg/kg dose level. Antibody treatment with CD3med TSP1 at 0.003 and 0.01 mg/kg was not active in this model (Table 26; FIG. 31B).

[0967] There was no antibody associated body weight loss with CD3med TSP1. Body weight loss due to the onset of GvHD was not observed for this construct by the end of the study (Table 26, FIG. 32B).

TABLE 25

Test agent	Dose (mg/kg)	Schedule	Tumor Response			Host Response	
			ΔT/ΔC (%)	Regress-ion (%)	Δ Tumor burden from post-dose (mm^3) (Mean) Day 25	Δ Body weight from post-dose (%) (Mean ± SEM) Day 25	Survival (survivors/ total)
Tumor only	N/A	--	100	--	952.252		5/5
Tumor + AdT	N/A	--	100	--	822.216		8/8
CD3hi TSP1	0.003	Single dose/IV	24.20	--	198.9588	1.7±1.6	8/8
CD3hi TSP1	0.01	Single dose/IV	1.38	--	11.37875	-0.7 ± 1.8	8/8
CD3hi TSP1	0.03	Single dose/IV	--	13.74*	-29.7413	1.9± 2.0	8/8
CD3hi TSP1	0.1	Single dose/IV	--	72.41*	-155.988	1.2±2.3	8/8
CD3hi TSP1	0.3	Single dose/IV	--	84.50*	-187.737	-0.4±1.0	7/7**

*p < 0.05, Dunnett's multiple comparison test
**One animal was excluded from the study

TABLE 26

Test agent	Dose (mg/kg)	Schedule	Tumor Response			Host Response	
			ΔT/ΔC (%)	Regress-ion (%)	Δ Tumor burden from initial (mm^3) (Mean) Day 25	Δ Body weight from initial (%) (Mean ± SEM)	Survival (survivors/ total)
CD3med TSP1	0.003	Single dose/IV	45.36	--	372.94	4.2 ± 2.1	8/8
CD3med TSP1	0.01	Single dose/IV	57.13	--	469.6988	5.7 ± 2.7	8/8
CD3med TSP1	0.03	Single dose/IV	7.39	--	60.7375	2.6 ± 1.6	8/8
CD3med TSP1	0.1	Single dose/IV	--	5.60*	-12.1488	3.3 ± 2.0	8/8
CD3med TSP1	0.3	Single dose/IV	--	36.33*	-78.7388	3.54 ± 1.4	8/8

*p < 0.05, Dunnett's multiple comparison test

8.29. Example 29: Anti-tumor activity following multiple doses of CD3 TSP1, CD3hi BSP1 and CD3med TSP1 in the OCI-LY-19 in the adaptation of a DLBCL subcutaneous tumor model in huCD34+ NSG mice

[0968] The anti-tumor activity of CD3hi TSP1, CD3hi BSP1, and CD3med TSP1 (H variants) were studied in an OCI-LY-19 DLBCL subcutaneous tumor model in huCD34+ NSG mice.

8.29.1. Materials and Methods

[0969] The process of humanization of NGS mice used in this study is shown schematically in in FIG. 33. Briefly, female female NSG mice at ~6 weeks old (Jackson Laboratories, ME) underwent a preconditioning protocol to depopulate the bone marrow niche. This was accomplished by either chemical ablation or by X-ray irradiation to allow for reconstitution of a human immune system in each NSG mouse. Within twenty-four hours following preconditioning, 50,000 huCD34+ stem cells (huCD34+ SC) isolated from single umbilical cords (Lonza, StemCell) were introduced in 100µL via IV injection in the lateral tail vein. Each mouse received huCD34+ SC from a single donor. The huCD34+ SC were received frozen and stored in a -200°C liquid nitrogen tank until use. Immediately prior to inoculation, huCD34+ SC vials were removed from the liquid nitrogen tank, thawed in a bead-bath at 37°C and re-suspended in PBS at a final concentration of 500,000 cells/mL. For sixteen weeks post humanization, mice were monitored weekly for body weights and body condition. At week 16, mice were bled via the tail and human immune reconstitution (human engraftment) was ascertained by fluorescent activated cell sorting (FACS). Mice with ≥ 25% hCD45/total CD45 were considered stably engrafted and were eligible for study enrollment.

[0970] Following engraftment assessment, mice were implanted with tumor cells subcutaneously. On Day 0, OCI-LY-19 cells were harvested and suspended in Hanks Balanced Salt Solution (HBSS) at a concentration of $10x10^7$ cells/mL and then diluted 1:1 with matrigel to give a final concentration of $5x10^7$ cells/mL. Mice were implanted via subcutaneous (SQ) injection on the right flank with $5x10^6$ cells/mouse in 100µL volume. Fifteen days post implant (mean tumor volume ~ 250-300 mm^3 measured via calipers), mice were randomized on two parameters: donor and tumor volume. This ensured equal distribution of donors and comparable tumor volumes in each group. There were 3 treatment groups, n=8, and untreated control, n=5. Mice were treated weekly for 2-4 weeks via IV administration with CD3hi TSP1 (0.3mg/kg), CD3med TSP1 (1.0mg/kg), or CD3hi BSP1 (0.3mg/kg). Anti-tumor activity of each antibody was compared to an untreated $huCD34^+$ SC control group that received tumor implant (tumor + CD34+) (Table 27). All treatments were administered at 10 mL/kg according to individual mouse body weights. Anti-tumor activity was determined by percent change in tumor volume of treated vs. untreated control (%ΔT/ΔC) or % regression and durability of response was evaluated by monitoring % surviving animals over time. Animals whose TV, BW

or BCS (body condition score) that reached end point criteria by exceeding limits provisioned in the lab's animal use protocol (AUP) were euthanized.

[0971] Tumor burden (TV) and body weights were recorded twice weekly. Tumor burden was measured by calipers, capturing length and width, and the tumor volume was calculated using the formula $(w^2xL)/3.14$. Body weight was measured by scale. Both parameters were entered into an in-house system (INDIGO). Anti-tumor activity was determined by %ΔT/ΔC using the formula: $100 X \Delta TB_{treatment, time} / \Delta TB_{control group, time}$ if $\Delta TB \geq 0$; or % regression: $(-1 X (100 X (TB_{final} - TB_{initial} / TB_{initial})$ if $\Delta TV < 0$. $TB_{initial}$ is the tumor burden on the day of treatment initiation. %ΔT/ΔC values <42% were considered to have anti-tumor activity. Percent body weight change was determined using the formula: $100 X ((BW_{time} - BW_{initial})/BW_{initial})$. Statistical analysis using One-way ANOVA with Dunnett's multiple comparison test was performed using Graphpad Prism Software, Version. 7.03 (Day 24 post implant).

[0972] In addition, Time to endpoint was evaluated using KAPLAN-Meyer survival graph and analysis using Graphpad Prism Software, Version. 7.03, and was performed to compare differences in median time to endpoint (TTE). Mice which achieved tumor endpoint when tumor volume exceeded 1200 mm^3 and mice euthanized for reasons besides tumor volume related to disease progression, such as ulceration, metastasis, body weight loss or poor body condition were scored as dead ("1"). Animals euthanized for reasons other than tumor progression, such as adverse drug events, were censored ("0"). Log-Rank (Mantel-Cox) survival analysis was performed, and all pairwise multiple comparison procedures were performed using Holm-Sidak method with an overall significance level $P < 0.05$ (SigmaPlot 13.0). Graphical analysis of TTE was performed in Prism (GraphPad v7.03). Individual response criteria were also evaluated and scored as either Complete Response (CR), no detectable tumor at time of last measurement; Partial Response (PR), tumor volume less than baseline measurement at any time point followed by regrowth; or No Response (NR), tumor continues to increase over baseline measurement throughout the study. The last day of the study was captured at Day 39.

[0973] On day 24 following OCI-LY-19 implantation, all animals from the untreated control group were euthanized due to tumor burden. Statistical analysis was evaluated on Day 24.

8.29.2. Results

[0974] Treatment with all three antibodies showed significant differences in tumor activity compared to the tumor + $CD34^+$ control group. CD3hi TSP1 at 0.3 mg/kg resulted in significant tumor regressions of 47.4% whereas the CD3hi BSP1 did not achieve regressions (16.3% ΔT/ΔC). Treatment with CD3med TSP1 at 1.0mg/kg resulted in tumor regressions 64.5 % (Table 27, FIG. 34A).

[0975] There was treatment associated body weight loss with CD3hi TSP1, CD3med TSP1, and CD3hi BSP1 observed following the first dose only. The severity of body weight loss was impacted by donor as well, with different donors showing variable peak body weight loss. Without being bound by theory, the body weight change observed following the first dose is hypothesized to be target-mediated driven and exacerbated by the depletion of the inherent B cells. Body weight loss is an endpoint parameter for both disease burden and treatment induced responses. Animals with high tumor burden demonstrated disease-burden associated weight loss. Over the course of the study, body weights were observed to increase relative the initial measurement taken on the day of tumor implant, but decrease in response to the progressing disease burden (Table 27, FIG. 34B).

TABLE 27

Test agent	Dose (mg/kg)	Schedule	Tumor Response			Host Response	
			ΔT/ΔC (%)	**Regress-ion (%)**	**Δ Tumor burden from initial (mm^3) (Mean ± SEM) Day 24**	**Δ Body weight from initial (%) (Mean ± SEM) Day 24**	**Survival (survivors/ total)**
Tumor + CD34+	N/A	--	--	--	1733.60 ± 130	-5.12 ± 4.5	5/5
CD3hi TSP1	0.3	QWx3 dose/IV	--	47.4*	-175.93 ± 76.8	-13.51 ± 2.15	8/8
CD3hi BSP1	0.3	QWx3 dose/IV	16.25*		281.69 ± 292.5	-5.12 ± 1.7	8/8
CD3med TSP1	1.0	QWx3	--	64.3*	-215.32 ± 38.48	-11.30 ± 1.93	8/8

*p < 0.05, Dunnett's multiple comparisons test

8.30. Example 30: Anti-tumor activity in a single dose, dose range finding study comparing CD3hi TSP1 and CD3med TSP1 in a DLBCL subcutaneous tumor model in huCD34+ NSG mice

[0976] The anti-tumor activity of CD3hi TSP1, CD3hi BSP1, and CD3med TSP1 (H variants) were studied in an OCI-LY-19 DLBCL subcutaneous tumor model in huCD34+ NSG mice.

8.30.1. Materials and Methods

[0977] Female humanized CD34+ NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ mice (HuNSG mice) were purchased from Jackson Laboratories (Sacramento, CA). Mice were humanized using cord blood.

[0978] The engraftment levels of hCD45+ cells were determined prior to shipment and confirmed in-house prior to the start of the study. HuNSG mice that had over 25% hCD45+ cells in the peripheral blood were considered as engrafted and humanized. HuNSG derived from

different donors with different levels of engraftment were randomized into every treatment group in the study.

[0979] Following engraftment assessment, mice were implanted with tumor cells subcutaneously. On Day 0, OCI-LY-19 cells were harvested and suspended in Hanks Balanced Salt Solution (HBSS) at a concentration of $10x10^7$ cells/mL and then diluted 1:1 with matrigel to give a final concentration of $5x10^7$ cells/mL. Mice were implanted via subcutaneous (SQ) injection on the right flank with $5x10^6$ cells/mouse in 100µL volume. Nine days post implant, (mean tumor volume ~ 250-300 mm^3 measured via calipers) mice were randomized on two parameters: donor and tumor volume. This ensured equal distribution of donors and comparable tumor volumes in each group. There were 11 groups with n=8 treatment group and n=5 in the untreated control. Mice were administered a single dose via IV administration with CD3hi TSP1 or CD3med TSP1 across the following dose range 1.0 mg/kg, 0.3 mg/gk, 0.1 mg/kg and 0.01 mg/kg. Anti-tumor activity of each antibody was compared to an untreated huCD34+ SC control group that received tumor implant (tumor + CD34+) (Table 28). All treatments were administered at 10 mL/kg according to individual mouse body weights. Anti-tumor activity was determined by percent change in tumor volume of treated vs. untreated control (%ΔT/ΔC) or % regression and durability of response was evaluated by monitoring % surviving animals overtime. Animals whose TV, BW or BCS (body condition score) that reached end point criteria by exceeding limits provisioned in the lab's animal use protocol (AUP) were euthanized.

[0980] Tumor burden (TV) and body weights were recorded twice weekly. Tumor burden was measured by calipers, capturing length and width, and the tumor volume was calculated using the formula $(w^2xL)/3.14$. Body weight was measured by scale. Both parameters were entered into an in-house system (INDIGO). Anti-tumor activity was determined by %ΔT/ΔC using the formula: $100 \times \Delta TB_{treatment, time} / \Delta TB_{control\ group, time}$ if $\Delta TB \geq 0$; or % regression: $(-1 \times (100 \times (TB_{final} - TB_{initial} / TB_{initial})$ if $\Delta TV < 0$. $TB_{initial}$ is the tumor burden on the day of treatment initiation. (%ΔT/ΔC values <42% were considered to have anti-tumor activity). Percent body weight change was determined using the formula: $100 \times ((BW_{time} - BW_{initial})/BW_{initial})$. Statistical analysis using One-way ANOVA with Dunnett's multiple comparison test was performed using Graphpad Prism Software, Version. 7.03. In addition, durability of response was evaluated using KAPLAN-Meyer survival graph and analysis using Graphpad Prism Software, Version. 7.03.

[0981] On day 24 following OCI-LY-19 implantation, all animals from the untreated control group were euthanized due to tumor burden. Statistical analysis was evaluated on Day 24.

8.30.2. Results

[0982] There was a statistical significant difference in tumor activity observed with the 1.0 mg/kg, 0.3 mg/kg and 0.1 mg/kg doses of CD3hi TSP compared to the tumor + $CD34^+$ control group. CD3hi TSP1 at 1.0 mg/kg resulted in significant tumor regressions of 35.3%, whereas CD3hi TSP1 at 0.3 mg/kg and at 0.1 mg/kg showed robust significant anti-tumor activity with ΔT/ΔC values of 0.05% and 19.5%, respectively. The dose levels administered below 0.1 mg/kg did not achieve anti-tumor responses, with the 0.03 mg/kg dose of CD3hi TSP1 having a ΔT/ΔC value of 65.8% and the 0.01 mg/kg dose having a ΔT/ΔC value of 100% (Table 28, FIG. 35A).

[0983] Antibody treatments with CD3med TSP1 resulted in significant anti-tumor activity. CD3med TSP1 dosed at 1.0 mg/kg achieved significant tumor response with a ΔT/ΔC of 0.05%. The 0.3 mg/kg dose level of CD3med TSP1 did show anti-tumor activity (ΔT/ΔC: 26.9<42) but was not significant compared to the control. Doses lower that 0.3mg/kg did not show significant tumor activity with ΔT/ΔC values of 79.8%, 90.3%, and 100% for 0.1 mg/kg, 0.03 mg/kg and 0.01 mg/kg doses, respectively (Table 28, FIG. 35C).

[0984] There was treatment associated body weight loss observed across multiple dose levels following administration of CD3hi TSP1 and CD3med TSP1. The severity of body weight loss was a combination of both donor and dose level effects, with different donors showing variable peak body weight loss. Without being bound by theory, the body weight change observed following the dose is hypothesized to be target-mediated driven and exacerbated by the depletion of the inherent B cells. Body weight loss is an endpoint parameter for both disease burden and treatment induced responses. Animals with high tumor burden demonstrated disease-burden associated weight loss. Over the course of the study, body weights were observed to increase relative to the initial measurement taken on the day of tumor implant (Table 28, FIGS. 35B and 35D), but decrease in response to the progressing disease burden.

TABLE 28

Test agent	Dose (mg/kg)	Schedule	Tumor Response			Host Response	
			ΔT/ΔC (%)	**Regress-ion (%)**	**Δ Tumor burden from initial (mm^3) (Mean ± SEM) Day 24**	**Δ Body weight from initial (%) (Mean ± SEM) Day 24**	**Survival (survivors/ total) Day 24**
Untreated control	N/A	--	--	--	1057. ± 304.2	6.02 ± 1.72	5/5
CD3hi TSP1	1.0	Single dose/IV	---	35.3*	-91.6 ± 13.8	-1.96 ± 2.31	8/8
CD3hi TSP1	0.3	Single dose/IV	0.05*	--	-0.57 ± 95.0	-6.09 ± 2.34	8/8

TABLE 28

Test agent	Dose (mg/kg)	Schedule	Tumor Response			Host Response	
			ΔT/ΔC (%)	Regress-ion (%)	Δ Tumor burden from initial (mm^3) (Mean ± SEM) Day 24	Δ Body weight from initial (%) (Mean ± SEM) Day 24	Survival (survivors/ total) Day 24
CD3hi TSP1	0.1	Single dose/IV	19.5*	--	205.9 ± 170.6	-7.06 ± 3.02	8/8
CD3hi TSP1	0.03	Single dose/IV	65.8	--	695.0 ± 168.4	-3.32 ± 1.63	8/8
CD3hi-TSP1	0.01	Single dose/IV	100	--	1110.7 ± 201.7	4.54 ± 2.25	8/8
CD3med TSP1	1.0	Single dose/IV	0.05*	--	35.5 ± 57.6	3.1 ± 2.5	6/8
CD3med TSP1	0.3	Single dose/IV	26.9	--	284.0 ± 200.9	-3.0 ± 2.2	8/8
CD3med TSP1	0.1	Single dose/IV	79.8	--	843.2 ± 196.5	0.3 ± 2.5	8/8
CD3med TSP1	0.03	Single dose/IV	90.3	--	954.8 ± 180.1	4.8 ± 3.7	8/8
CD3med TSP1	0.01	Single dose/IV	100	--	1139.3 ± 155.7	12.7 ± 1.8	8/8

*$p < 0.05$, Dunnett's multiple comparison test

8.31. Example 31: Anti-tumor activity of CD3hi BSP1, CD3hi TSP1, and CD3med TSP1 in an adoptive transfer adaptation of the Daudi-Luc Burkitt's lymphoma subcutaneous tumor model in NSG mice

[0985] The anti-tumor activity of CD3hi BSP1, CD3hi TSP1, and CD3med TSP1 (H variants) were studied in an adoptive transfer adaptation of the Daudi-Luc Burkitt's lymphoma subcutaneous tumor model in NSG mice.

8.31.1. Materials and Methods

[0986] On Day 0, Daudi-Luc cells were harvested and suspended in a 1:1 mixture of Hanks Balanced Salt Solution (HBSS) and Matrigel at a concentration of $50x10^6$ cells/mL. Female NSG mice at ~6 weeks old (Jackson Laboratories, ME) were injected with $5x10^6$ Daudi-Luc cells in 100 µL subcutaneously (SQ) in the right flank. Three days following tumor inoculation, each mouse received an adoptive transfer (AdT) of $15x10^6$ of peripheral blood mononuclear cells (PBMCs) in 100µL via intravenous (IV) injection in the lateral tail vein. The PBMCs were previously isolated from a human leukopak, frozen and stored in Cryostor10 media in vapor phase liquid nitrogen tank until use. Immediately prior to AdT, PBMCs were thawed and suspended at a concentration of $150x10^6$cells/ml in Hanks Balanced Salt Solution (HBSS).

When tumor volume (TV) reached an average of ~250 cubic millimeters (mm^3) measured via caliper (Day 10 post-implant), mice (n=8/group) were treated with a single IV administration of CD3hi BSP1, CD3hi TSP1, or CD3med TSP1 at dose levels of 1.0 mg/kg, 0.3 mg/kg, or 0.1 mg/kg. Anti-tumor activity of each antibody was compared to an untreated control group that received tumor implant and AdT but no treatment (tumor + AdT) (Table 29). The tumor only group was included to meter the allogeneic response observed with untreated control. All treatments were administered at 10 mL/kg according to individual mouse body weights. Anti-tumor activity was determined by percent change in tumor volume vs. change in untreated control (%ΔT/ΔC) or % regression.

[0987] Tumor volume and body weights were recorded twice weekly. Tumor volume was measured by caliper. Anti-tumor activity was determined by %ΔT/ΔC using the formula: 100 X $\Delta TV_{treatment, time}/\Delta TV_{control\ group, time}$ if $\Delta TV \geq 0$; or % regression: (-1 X (100 X (TV_{final}-$TV_{initial}$/ $TV_{initial}$) if $\Delta TV < 0$. $TV_{initial}$ is the tumor volume on the day of treatment initiation. %ΔT/ΔC values <42% were considered to have anti-tumor activity. Percent body weight change was determined using the formula: 100 X (($BW_{time} - BW_{initial}$)/$BW_{initial}$). Statistical analysis using One-way ANOVA with Dunnett's multiple comparison test was performed using Graphpad Prism Software, Version. 7.03.

[0988] On day 36 following Daudi-Luc implantation, 25% of animals from the Tumor + AdT control group were euthanized due to tumor volume.

8.31.2. Results

[0989] This study had minimal allogeneic response (FIGS. 36A-36C).

[0990] Antibody treatment with CD3hi BSP1 at 1.0 mg/kg and 0.3 mg/kg resulted in significant tumor regressions of 85.21% and 73.26%, respectively. Antibody treatment with CD3hi BSP1 at 0.1 mg/kg exhibited significant anti-tumor activity (20.89% ΔT/ΔC value). Antibody treatment with CD3med TSP1 resulted in significant anti-tumor responses at all three dose levels: 1.0 mg/kg (90.86% regression), 0.3 mg/kg (85.13% regression), and 0.1 mg/kg (13.51% regression). Antibody treatment with CD3hi TSP1 resulted in significant tumor regressions at all three dose levels: 1.0 mg/kg (90.08% regression), 0.3 mg/kg (91.86% regression), and 0.1 mg/kg (87.52% regression).

[0991] There was no antibody associated body weight loss with any of the three constructs tested. Without being bound by theory, the body weight change observed at approximately Day 35 from baseline was most likely due to the onset of graft-versus host disease (GvHD). Body weight loss is an endpoint parameter for onset of GvHD. At 32--39 days post-PBMC injection (35-42 days post-tumor implant), animals began to exhibit weight loss attributed to GvHD. Over the course of the study, body weights increased relative the initial measurement taken on the

day of tumor implant (Table 29, FIGS. 37A-37C). However at the end of study, the body weight loss observed relative to the peak gain is indicative of GvHD-induced weight loss.

TABLE 29

Test agent	Dose (mg/kg)	Schedule	Tumor Response			Host Response	
			ΔT/ΔC (%)	Regress-ion (%)	Δ Tumor burden from initial (mm^3) (Mean) Day 36	Δ Body weight from initial (%) (Mean ± SEM) Day 36	Survival (survivors/ total)
Tumor only	N/A	-	119.42	--	1633.79	11.653±1.871	4/8
Tumor +Adt (Untreated Control)	N/A	-	100.00	--	1368.13	8.435±2.241	8/8
CD3hi BSP1	1.0	Single dose/IV	--	85.21	-124.67	0.296±3.360	8/8
CD3hi BSP1	0.3	Single dose/IV	--	73.26	-107.15	8.394±6.267	8/8
CD3hi BSP1	0.1	Single dose/IV	20.89	--	285.84	-0.359±2.569	8/8
CD3hi TSP1	1.0	Single dose/IV	--	90.08	-131.77	5.423±2.220	8/8
CD3hi TSP1	0.3	Single dose/IV	--	91.86	-134.39	2.254±2.975	8/8
CD3hi TSP1	0.1	Single dose/IV	--	87.52	-128.03	-0.506±4.777	8/8
CD3med TSP1	1.0	Single dose/IV	--	90.86	-132.92	3.839±1.597	8/8
CD3med TSP1	0.3	Single dose/IV	--	85.13	-124.57	3.214±1.737	8/8
CD3med TSP1	0.1	Single dose/IV	--	13.51	-19.77	0.165±2.561	8/8

8.32. Example 32: Design - Selection of Residue positions

[0992] Design strategies were tested to produce a set of antibodies with modified Fc regions that might exhibit desired properties such as diminished effector functions. Early studies defining key amino acid binding sites on IgG for Fc gamma receptors were performed by mutational analyses, and it was determined that the lower hinge, proximal CH2 region and glycosylation of N297 were critical (Shields et al., 2001). Mutations were introduced into the regions that interact with Fc gamma receptors with the goal to diminish residual binding to Fc gamma receptors. For this particular reason, it was necessary to test various combinations of

Fc positions and generate set of mutations without compromising antibody drug developability and immunogenicity risk. Several mutation sets were generated and compared to wildtype IgG1. In Examples 33- 36: LALAPA-IgG1 (L234A/L235A/P329A), LALAGA-IgG1 (L234A/L235A/G237A), LALAPG-IgG1 (L234A/L235A/P329G), DAPA-IgG1 (D265A/P329A), LALASKPA-IgG1 (L234A/L235A/S267K/P329A), DAPASK-IgG1 (D265A/P329A/S267K), GADAPA-IgG1 (G237A/D265A/P329A), GADAPASK-IgG1 (G237A/D265A/P329A/S267K) and DANAPA-IgG1 (D265A/N297A/P329A) were evaluated. Previously described DAPA and DANAPA silencing motifs were included for comparison. In Examples 37- : LALA (L234A/L235A), LALASKPA (L234A/L235A/S267K/P329A), GADAPASK (G237A/D265A/P329A/S267K) and DANAPA (D265A/N297A/P329A) mutation sets were evaluated.

8.33. Example 33: Expression and Purification of Fc modified CD3 antibodies

[0993] For the experiments described below antibodies against CD3 containing the indicated amino acid substitutions and expressed by the nucleotide sequences as indicated, were used as listed in Tables A and B. IgG1 molecules were expressed in HEK293 mammalian cells, and purified using protein A and size exclusion chromatography. In brief, heavy chain and light chain DNA of anti-CD3 WT IgG1 were synthesized at GeneArt (Regensburg, Germany) and cloned into a mammalian expression vector using restriction enzyme-ligation based cloning techniques. All variants described herein were then generated using PCR based mutagenesis. The resulting plasmids were co-transfected into HEK293T cells. For transient expression of antibodies, equal quantities of vector for each chain were co-transfected into suspension-adapted HEK293T cells using Polyethylenimine (PEI; Cat# 24765 Polysciences, Inc.). Typically, 100 ml of cells in suspension at a density of 1-2 Mio cells per ml was transfected with DNA containing 50 µg of expression vector encoding the heavy chain and 50 µg expression vectors encoding the light chain. The recombinant expression vectors were then introduced into the host cells and the construct produced by further culturing of the cells for a period of 7 days to allow for secretion into the culture medium (HEK, serum-fee medium) supplemented with 0.1% pluronic acid, 4mM glutamine, and 0.25 µg/ml antibiotic.

[0994] The produced construct was then purified from cell-free supernatant using immunoaffinity chromatography. MabSelect Sure resin (GE Healthcare Life Sciences), equilibrated with PBS buffer pH 7.4 was incubated with filtered conditioned media using liquid chromatography system (Aekta pure chromatography system, GE Healthcare Life Sciences). The resin was washed with PBS pH 7.4 before the constructs were eluted with elution buffer (50mM citrate, 90mM NaCl, pH 2.7). After capture, eluted proteins were pH neutralized using 1M TRIS pH 10.0 solution and polished using size exclusion chromatography technique

(HiPrep Superdex 200 16/60, GE Healthcare Life Sciences). Purified proteins were finally formulated in PBS buffer pH 7.4.

8.34. Example 34: Biophysical properties of Fc modified CD3 antibodies : SPR-Binding of modified antibodies to human Fc gamma receptors and human C1q

[0995] Surface plasmon resonance (SPR) experiments were performed to analyze the interaction of human activating receptors FcγR1A, FcγR3A (V158) and human C1q with IgG1 WT and antibody-Fc variants. Binding kinetics and their relative binding affinities were explored. The binding affinity is an important characteristic of an interaction between an antibody and an antigen. The equilibrium dissociation constant (KD) defines how strong the interaction is and therefore how much antibody-antigen complex is formed at equilibrium.

[0996] The knowledge of the antibody characteristics is not only essential during selection of the best therapeutic antibody candidate, but also important to understand the in vivo behavior and potentially predict cellular immune responses. The aim is to generate antibody variants with little or no binding to Fc gamma receptors to reduce or eliminate effector function aiming to improve the safety of monoclonal antibody therapeutics. Binding to human C1q was evaluated. All SPR buffers were prepared using deionized water. The samples were prepared in running buffer PBS pH 7.4 with 0.005% Tween-20. SPR measurements were measured on a Biacore T200 (GE-Healthcare Life Sciences) controlled by Biacore T200 control software version 2.0.1. Surface plasmon resonance was conducted using a Biacore T200 to assess binding affinity of antibody IgG1 WT and variants to human Fc receptors, including FcγR1A, and FcγR3A (V158) and human C1q.

[0997] The antibodies were covalently immobilized on a CM5 sensor chip whereas Fc gamma receptors or human C1q served as analytes in solution (Figure 38). For Fc gamma receptors binding evaluation (method 1), antibodies were diluted in 10 mM sodium acetate pH 4 and immobilized at a density of approximately 950 resonance units (RU`s) on the CM5 sensor chip applying a standard amine coupling procedure. Flow cell 1 was blank immobilized to serve as a reference. The kinetic binding data were collected by subsequent injections of 1:2 dilution series of the human Fc gamma receptors on all flow cells at a flow rate of 30 µl/min and at a temperature of 25°C. The Fc gamma receptors were diluted in the running buffer at concentrations ranging from 0.2 nM to 1000 nM (e.g. FcγR1A: 0.2 to 100 nM, FcγR3A V158: 1.95 to 1000 nM). The chip surface was regenerated using 20 mM glycine pH 2.0 solution after each measuring cycle. For human C1q binding evaluation (method 2), antibodies were diluted in 10 mM sodium acetate pH 4 and immobilized at a density of approximately 7000 resonance units (RU`s) on the CM5 sensor chip applying a standard amine coupling procedure. Flow cell 1 was blank immobilized to serve as a reference. The kinetic binding data were collected by

subsequent injections of 1:2 dilution series of the human C1q on all flow cells at a flow rate of 30 µl/min and at a temperature of 25°C. The human C1q was diluted in the running buffer at concentrations ranging from 0.49 nM to 250 nM. The chip surface was regenerated using 50 mM NaOH solution after each measuring cycle. Zero concentration samples (blank runs) were measured for both methods to allow double-referencing during data evaluation.

[0998] Data were evaluated using the Biacore T200 evaluation software. The raw data were double referenced, i.e. the response of the measuring flow cell was corrected for the response of the reference flow cell, and in a second step the response of a blank injection was subtracted. Then the sensorgrams were fitted by applying a 1:1 kinetic binding model to calculate dissociation equilibrium constants. In addition, the maximum response reached during the experiment was monitored. Maximum response describes the binding capacity of the surface in terms of the response at saturation. The maximum response values summarizing these interactions are given in Table 30. The SPR Biacore binding sensorgrams for each variant to each receptor were depicted in Figure 39, Concentration range: 0.2nM-100nM for FcgR1, 7.8 nM-4000nm for FcgR2A R131 d FcγR3A (V158 and F158). Figure 39A shows representative sensorgrams and response plots of WT and variants towards FcgammaR1A (Concentration range: 0.2nM-100nM for human FcγR1A). Figure 39B shows representative sensorgrams and response plots of WT and variants towards FcgammaR3A V158 (Concentration range: 1.95nM-1000nM for human FcγR3A V158). Figure 39C shows representative sensorgrams and response plots of WT and variants towards human C1q (Concentration range: 0.49nM-250nM for human C1q). All IgG1 antibody-Fc variants inhibit the binding to Fc gamma receptors compare to WT and little or no residual binding was measured. All IgG1 antibody-Fc variants inhibit the binding to human C1q compare to WT as low residual binding was measured.

Table 30
Maximum responses of WT-Fc and variants towards human FcgammaRs and C1q determined by surface plasmon resonance

Fc	SEQ ID NO	Maximum response at 100nM hFcgammaRIA (RU)	Maximum response at 1000nM hFcgammaRIIIA V158 (RU)	Maximum response at 250nM hC1q (RU)
WT		193	79	680
WT allotype		193	72	640
DANAPA		11	31	29
DAPA		63	15	85
LALAPA		26	19	270
LALAPG		18	33	245

Table 30 Maximum responses of WT-Fc and variants towards human FcgammaRs and C1q determined by surface plasmon resonance				
Fc	**SEQ ID NO**	**Maximum response at 100nM hFcgammaRIA (RU)**	**Maximum response at 1000nM hFcgammaRIIIA V158 (RU)**	**Maximum response at 250nM hC1q (RU)**
LALAGA		15	25	335
LALASKPA		9	20	95
DAPASK		13	15	86
GADAPA		16	9	203
GADAPASK		10	12	168

8.35. Example 35: Differential scanning calorimetry- melting temperature of modified antibodies

[0999] The thermal stability of engineered antibodies CH2 domains were compared using calorimetric measurements as shown in Table 31. Calorimetric measurements were carried out on a differential scanning micro calorimeter (Nano DSC, TA instruments). The cell volume was 0.5ml and the heating rate was 1°C/min. All proteins were used at a concentration of 1mg/ml in PBS (pH 7.4). The molar heat capacity of each protein was estimated by comparison with duplicate samples containing identical buffer from which the protein had been omitted. The partial molar heat capacities and melting curves were analyzed using standard procedure. Thermograms were baseline corrected and concentration normalized. The silent version LALASKPA (70°C) shows significantly better Tm compared to DANAPA (62°C).

TABLE 31 Melting temperatures of WT-Fc and variants	
Fc	**Melting temperature (Tm) of CH2 domain**
WT	**70**
WT allotype R214K	**70**
LALAPA	**70**
LALAGA	**70**
LALAPG	**70**
DAPA	**65**
LALASKPA	**70**
DAPASK	**65**
GADAPA	**65**
GADAPASK	**65**
DANAPA	**62**

Aggregation propensity post capture of IgG1 anti-CD3 antibody and Fc variants

[01000] Size exclusion chromatography measurements were performed to evaluate the aggregation propensity (% HMW) of IgG1 antibody and Fc modified derivatives. The produced and purified anti CD3 antibodies were applied to an analytical size exclusion chromatography column (SEC 200, GE Healthcare), equilibrated with PBS buffer pH 7.4. Results are summarized in Table 32.

Table 32
Higher molecular weight content (%) of anti-CD3 antibodies

Fc	(%) higher molecular weight
WT	**7.3**
WT allotype R214K	**7.7**
LALAPA	**1.8**
LALAGA	**2.9**
LALAPG	**4.2**
DAPA	**3.9**
LALASKPA	**4.9**
DAPASK	**3.6**
GADAPA	**8.0**
GADAPASK	**6.8**
DANAPA	**5.9**

8.36. Example 36: Anti-CD3 NFAT signalling assay

[01001] Jurkat reporter gene assay (RGA) for the nuclear factor of activated T-cells (NFAT) pathway was performed using Jurkat NFAT luciferized (JNL) cells and THP-1 cells (ATCC, TIB202). THP-1 cells express FcγRI, FcγRII, and FcγRIII. Cells were co-incubated for 6 hours at 37ºC, 5% CO2 at a 5:1 effector to tumor ratio with each sample at the various concentrations depicted. An equal volume of ONE-Glo™ reagent (Promega, E6110) was added to the culture volume. Plate was shaken for 2 minutes, then incubated for an additional 8 minutes protected from light. For JNL + THP + IFNg experiment, THP-1 cells were pre-treated with 100 u/mL IFNg for 48 hours at 37ºC, 5% CO2 before co-culture. IFNg stimulation increases FcγRI expression. Luciferase activity was quantitated on the EnVision plate reader (PerkinElmer). Data was analyzed and fit to a 5 parameter-logistic curve using GraphPad Prism.

[01002] In both treatments, WT showed the greatest NFAT activity. All silencing mutation sets overall showed significantly dampened NFAT activation. In the RGA, performed without

IFNg (Figure 40A), all silencing mutation sets showed comparable T-cell activation with the exception of DAPA. When the THP-1 cells were preincubated with IFNg (Figure 40B), the mutation sets showed lower activity, demonstrating strong Fc silencing, but some activity remaining in DAPA, LALAPA and GADAPA.

8.37. Example 37: Expression and Purification of modified antibodies

[01003] For the experiments described below antibodies were used as shown in Table 33. Designed molecules were expressed in HEK293 mammalian cells, and purified using protein A and size exclusion chromatography. In brief, heavy chains and light chain DNA were synthesized at GeneArt (Regensburg, Germany) and cloned into a mammalian expression vector using restriction enzyme-ligation based cloning techniques. The resulting plasmids were co-transfected into HEK293T cells. For transient expression of antibodies, equal quantities of vector for each chain were co-transfected into suspension-adapted HEK293T cells using Polyethylenimine (PEI; Cat# 24765 Polysciences, Inc.). Typically, 100 ml of cells in suspension at a density of 1-2 Mio cells per ml was transfected with DNA containing 33 µg of expression vector encoding the first heavy chain, 33 µg of expression vector encoding the second heavy chain and 33 µg expression vectors encoding the light chain. The recombinant expression vectors were then introduced into the host cells and the construct produced by further culturing of the cells for a period of 7 days to allow for secretion into the culture medium (HEK, serum-fee medium) supplemented with 0.1% pluronic acid, 4mM glutamine, and 0.25 µg/ml antibiotic.

[01004] The produced construct was then purified from cell-free supernatant using immunoaffinity chromatography. MabSelect Sure resin (GE Healthcare Life Sciences), equilibrated with PBS buffer pH 7.4 was incubated with filtered conditioned media using liquid chromatography system (Aekta pure chromatography system, GE Healthcare Life Sciences). The resin was washed with PBS pH 7.4 before the constructs were eluted with elution buffer (50mM citrate, 90mM NaCl, pH 2.7). After capture, eluted proteins were pH neutralized using 1M TRIS pH 10.0 solution and polished using size exclusion chromatography technique (HiPrep Superdex 200 16/60, GE Healthcare Life Sciences). Purified proteins were finally formulated in PBS buffer pH 7.4.

TABLE 33
Sequences of Antibodies and Fc variant

SEQ ID No	Description	Sequence
1110	Anti-CD19 light chain Amino acid sequence	eivmtqspatlsvspgeratlscrasqdvgtavawyqqkpgqaprlliywastr htgiparfsgsgsgteftltisslqsedfavyfcqqyanfplytfgqgtkleikrtvaa psvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteq dskdstyslsstltlskadyekhkvyacevthqglsspvtksfnrgec

TABLE 33
Sequences of Antibodies and Fc variant

SEQ ID No	Description	Sequence
1111	Anti-CD19 light chain Nucleic acid sequence	gagatcgtgatgacacagtctccagccacactgtccgtgtctccaggcgagag agctacactgagctgtagagccagccaggatgtgggaacagccgtggcctg gtatcagcagaaacctggacaagcccctcggctgctgatctactgggcctcta caagacacacaggcatccctgccagattttctggcagcggctctggcaccga gttcaccctgacaatctctagcctccagagcgaggacttcgccgtgtacttctgc cagcagtacgccaactttcccctgtacacctttggccagggcaccaagctgga aatcaagagaacagtggccgctccgagcgtgttcatctttccaccaagcgacg agcagctgaaaagcggcacagcctctgtcgtgtgcctgctgaacaacttctac cccagagaagccaaggtgcagtggaaggtggacaatgccctccagtccggc aatagccaagagagcgtgaccgagcaggacagcaaggatagcacataca gcctgagcagcacactgaccctgagcaaggccgactacgagaagcacaaa gtgtacgcctgcgaagtgacacaccagggcctgtctagccctgtgaccaaga gcttcaacagaggcgagtgc
1112	Anti-CD19-CD3 WT IgG1 Heavy Chain Amino acid sequence	qvqlvqsgaevkkpgasvkvsckasgytfttywiqwvrqapgqrlewmgav ypgdadtrytqkfqgrvtltadrsastaymelsslrsedtavyycgrdagleyyal dywgqgtlvtvssastkgpsvfplapsskstsggtaalgclvkdyfpepvtvsw nsgaltsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkpsntkvdkr vepkscggggsggggsevqlvesgglvqpggslklscaasgftfntyamn wvrqasgkglewvgrirskynnyatyyadsvkdrftisrddskstlylqmnslkt edtavyycvrhgnfgnsyvswfaywgqgtlvtvssggggsggggsggggsg gggsqavvtqepsltvspggtvtltcrsstgavttsnyanwvqqkpgqaprglig gtnkrapwtparfsgsllgdkaaltlsgaqpedeaeyfcalwysnlwvfgggtkl tvlggggsdkthtcppcpapellggpsvflfppkpkdtlmisrtpevtcvvvdvs hedpevkfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkey kckvsnkalpapiektiskakgqprepqvctlppsreemtknqvslscavkgfy psdiavewesngqpennykttppvldsdgsfflvskltvdksrwqqgnvfscs vmhealhnhytqkslslspgk
1113	Anti-CD19-CD3 WT IgG1 Heavy Chain	caggttcaactggttcagagcggagccgaagtgaagaaacctggggccagc gtgaaggtgtcctgtaaagccagcggctacacctttaccacctactggattcagt gggtccgacaggcccctggacagagacttgaatggatgggagctgtgtaccc cggcgacgccgataccagatacacccagaaattccagggcagagtgaccct

TABLE 33 Sequences of Antibodies and Fc variant		
SEQ ID No	Description	Sequence
	Nucleic acid sequence	gacagccgatagaagcgccagcacagcctacatggaactgagcagcctga gaagcgaggataccgccgtgtactactgtggcagagatgccggcctggaata ttacgccctggactattggggccagggcaccctggttacagtgtcctctgcctcta caaagggcccctccgtttttccactggctcctagcagcaagagcacatctggtg gaacagccgctctgggctgcctggtcaaggattactttcctgagcctgtgaccgt gtcctggaatagcggagcactgacaagcggcgtgcacacatttccagccgtg ctgcaaagcagcggcctgtactctctgtctagcgtggtcacagtgcctagctcta gcctgggcacccagacctacatctgcaacgtgaaccacaagcctagcaaca ccaaggtcgacaagagagtggaacccaagtcttgtggtggcggaggatctgg cggaggcggatctgaagttcagcttgttgaatctggcggcggactggtgcaac ctggcggatctctgaaactgtcttgtgccgcctccggcttcaccttcaatacctac gccatgaactgggttcgacaagcctccggcaaaggactggaatgggtcgga cggatcagaagcaagtacaacaactacgccacctactacgccgactccgtg aaggacagattcaccatcagccgggacgactccaagagcaccctgtacctcc agatgaactccctgaaaaccgaggacacagccgtctattattgcgtgcggcac ggcaacttcggcaacagctatgtgtcttggtttgcctactggggacagggaacc ctcgtgaccgtttcttcaggcggcggtggtagtggcggtggtggtagcggaggc ggtggatcaggtggcggcggttctcaagctgtggtcacacaagagcccagcct gacagtttctcctggcggaaccgtgacactgacctgtagatctagcaccggcg cagtgaccaccagcaattacgctaactgggtgcagcagaagcccggccaag ctcctagaggactgatcggaggcacaaacaagagagccccttggacaccag ccagattttctggctctctgctgggcgataaggccgctcttacactgtctggcgca cagcctgaagatgaggccgagtacttttgcgccctgtggtacagcaacctgtgg gtgttcggcggaggaacaaagctgacagttcttggaggcggcggaagcgac aagacccacacatgtcctccatgtcctgctccagaactgctcggcggaccctcc gtgtttctgttccctccaaagccaaaggacaccctgatgatcagcagaacccct gaagtgacctgcgtggtggtggatgtgtctcacgaggacccagaagtgaagtt caattggtacgtggacggcgtggaagtgcacaacgccaagaccaagcctag agaggaacagtacaacagcacctacagagtggtgtccgtgctgacagtgctg caccaggactggctgaacggcaaagagtacaagtgcaaggtgtccaacaa ggccctgcctgctcctatcgagaaaaccatcagcaaggccaagggccagcct agggaacctcaagtgtgcactctgcctccaagccgggaagagatgaccaag aatcaggtgtccctgagctgcgccgtgaagggcttttaccttccgatatcgccg

TABLE 33
Sequences of Antibodies and Fc variant

SEQ ID No	Description	Sequence
		tggaatgggagagcaatggccagccagagaacaactacaagaccacacct cctgtgctggacagcgacggctcattcttcctggtgtctaagctgaccgtggaca agagcagatggcagcagggcaacgtgttcagctgttctgtgatgcacgaggc cctgcacaaccactacacacagaagtccctgtctctgagccccggcaaa
1114	Anti-CD58 WT IgG1 Heavy Chain Amino acid sequence	sqqiygvvygnvtfhvpsnvplkevlwkkqkdkvaelensefrafssfknrvyl dtvsgsltiynltssdedeyemespnitdtmkfflyvlesggggsdkthtcppcp apellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgvev hnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektisk akgqprepqvytlppcreemtknqvslwclvkgfypsdiavewesngqpen nykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsls pgk
1115	Anti-CD58 WT IgG1 Heavy Chain Nucleic acid sequence	tctcagcagatctatggcgtggtgtacggcaacgtgaccttccacgtgccaagc aacgtgcccctgaaagaggtgctctggaagaaacagaaagacaaggtggc cgagctggaaaacagcgagttcagagccttcagcagcttcaagaaccgggt gtacctggataccgtgtccggcagcctgaccatctacaatctgaccagcagcg acgaggacgagtacgagatggaaagccccaacatcaccgacaccatgaag ttctttctgtacgtgctggaaagcggcggaggcggctctgataagacacacac atgtcctccatgtcctgctccagagctgctcggcggaccttctgttttcctgtttcca cctaagccaaaggacaccctgatgatcagcagaaccccctgaagtgacctgc gtggtggtggatgtgtctcacgaggaccccgaagtgaagttcaattggtacgtg gacggcgtggaagtgcacaacgccaagaccaagcctagagaggaacagt acaacagcacctacagagtggtgtccgtgctgaccgtgctgcaccaggattgg ctgaacggcaaagagtacaagtgcaaggtgtccaacaaggccctgcctgctc ctatcgagaaaaccatcagcaaggccaagggccagccaagagaacccca ggtttacaccctgcctccatgccgggaagagatgaccaagaatcaggtgtccc tgtggtgcctggtcaagggcttctacccttccgatatcgccgtggaatgggaga gcaatggccagcctgagaacaactacaagaccacacctcctgtgctggaca gcgacggctcattcttcctgtacagcaagctgacagtggacaagagcagatg gcagcagggcaacgtgttcagctgttctgtgatgcacgaggccctgcacaacc actacacccagaagtctctgtctctgagccccggcaaa
1116	Anti-CD19-CD3 DANAPA IgG1 Heavy Chain	qvqlvqsgaevkkpgasvkvsckasgytfttywiqwvrqapgqrlewmgav ypgdadtrytqkfqgrvtltadrsastaymelsslrsedtavyycgrdagleyyal dywgqgtlvtvssastkgpsvfplapsskstsggtaalgclvkdyfpepvtvsw

TABLE 33 Sequences of Antibodies and Fc variant		
SEQ ID No	Description	Sequence
	Amino acid sequence	nsgaltsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkpsntkvdkr vepkscggggsggggsevqlvesggglvqpggslklscaasgftfntyamn wvrqasgkglewvgrirskynnyatyyadsvkdrftisrddskstlylqmnslkt edtavyycvrhgnfgnsyvswfaywgqgtlvtvssggggsggggsggggsg gggsqavvtqepsltvspggtvtltcrsstgavttsnyanwvqqkpgqaprglig gtnkrapwtparfsgsllgdkaaltlsgaqpedeaeyfcalwysnlwvfgggtkl tvlggggsdkthtcppcpapellggpsvflfppkpkdtlmisrtpevtcvvvavs hedpevkfnwyvdgvevhnaktkpreeqyastyrvvsvltvlhqdwlngkey kckvsnkalaapiektiskakgqprepqvctlppsreemtknqvslscavkgfy psdiavewesngqpennykttppvldsdgsfflvskltvdksrwqqgnvfscs vmhealhnhytqkslslspgk
1117	Anti-CD19-CD3 DANAPA IgG1 Heavy Chain Nucleic acid sequence	caggttcaactggttcagagcggagccgaagtgaagaaacctggggccagc gtgaaggtgtcctgtaaagccagcggctacacctttaccacctactggattcagt gggtccgacaggcccctggacagagacttgaatggatgggagctgtgtaccc cggcgacgccgataccagatacacccagaaattccagggcagagtgaccct gacagccgatagaagcgccagcacagcctacatggaactgagcagcctga gaagcgaggataccgccgtgtactactgtggcagagatgccggcctggaata ttacgccctggactattggggccagggcaccctggttacagtgtcctctgcctcta caaagggcccctccgtttttccactggctcctagcagcaagagcacatctggtg gaacagccgctctgggctgcctggtcaaggattactttcctgagcctgtgaccgt gtcctggaatagcggagcactgacaagcggcgtgcacacatttccagccgtg ctgcaaagcagcggcctgtactctctgtctagcgtggtcacagtgcctagctcta gcctgggcacccagacctacatctgcaacgtgaaccacaagcctagcaaca ccaaggtcgacaagagagtggaacccaagtcttgtggtggcggaggatctgg cggaggcggatctgaagttcagcttgttgaatctggcggcggactggtgcaac ctggcggatctctgaaactgtcttgtgccgcctccggcttcaccttcaatacctac gccatgaactgggttcgacaagcctccggcaaaggactggaatgggtcgga cggatcagaagcaagtacaacaactacgccacctactacgccgactccgtg aaggacagattcaccatcagccgggacgactccaagagcaccctgtacctcc agatgaactccctgaaaaccgaggacacagccgtctattattgcgtgcggcac ggcaacttcggcaacagctatgtgtcttggtttgcctactggggacagggaacc ctcgtgaccgtttcttcaggcggcggtggtagtggcggtggtggtagcggaggc ggtggatcaggtggcggcggttctcaagctgtggtcacacaagagcccagcct

TABLE 33
Sequences of Antibodies and Fc variant

SEQ ID No	Description	Sequence
		gacagtttctcctggcggaaccgtgacactgacctgtagatctagcaccggcg cagtgaccaccagcaattacgctaactgggtgcagcagaagcccggccaag ctcctagaggactgatcggaggcacaaacaagagagccccttggacaccag ccagattttctggctctctgctgggcgataaggccgctcttacactgtctggcgca cagcctgaagatgaggccgagtacttttgcgccctgtggtacagcaacctgtgg gtgttcggcggaggaacaaagctgacagttcttggaggcggcggaagcgac aagacccacacatgtcctccatgtcctgctccagaactgctcggcggaccctcc gtgtttctgttccctccaaagccaaaggacaccctgatgatcagcagaacccct gaagtgacctgtgtggtggtggccgtgtctcacgaggacccagaagtgaagtt caattggtacgtggacggcgtggaagtgcacaacgccaagaccaagcctag agaggaacagtacgccagcacctacagagtggtgtctgtgctgacagtgctg caccaggactggctgaacggcaaagagtacaagtgcaaggtgtccaacaa ggccctggccgctcctatcgagaaaaccatctctaaggccaagggccagcct cgggaacctcaagtgtgcactcttccacctagccgggaagagatgaccaaga accaggtgtcactgagctgcgccgtgaagggcttctacccttctgatatcgccgt ggaatgggagagcaacggccagccagagaacaactacaagaccacacct cctgtgctggacagcgacggctcattcttcctggtgtctaagctgaccgtggaca agagcagatggcagcagggcaacgtgttcagctgttctgtgatgcacgaggc cctgcacaaccactacacacagaagtccctgtctctgagccccggcaaa
1118	Anti-CD58 DANAPA IgG1 Heavy Chain Amino acid sequence	sqqiygvvygnvtfhvpsnvplkevlwkkqkdkvaelensefrafssfknrvyl dtvsgsltiynltssdedeyemespnitdtmkfflyvlesggggsdkthtcppcp apellggpsvflfppkpkdtlmisrtpevtcvvvavshedpevkfnwyvdgvev hnaktkpreeqyastyrvvsvltvlhqdwlngkeykckvsnkalaapiektisk akgqprepqvytlppcreemtknqvslwclvkgfypsdiavewesngqpen nykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsls pgk
1119	Anti-CD58 DANAPA IgG1 Heavy Chain Nucleic acid sequence	tctcagcagatctatggcgtggtgtacggcaacgtgaccttccacgtgccaagc aacgtgcccctgaaagaggtgctctggaagaaacagaaagacaaggtggc cgagctggaaaacagcgagttcagagccttcagcagcttcaagaaccgggt gtacctggataccgtgtccggcagcctgaccatctacaatctgaccagcagcg acgaggacgagtacgagatggaaagccccaacatcaccgacaccatgaag ttctttctgtacgtgctggaaagcggcggaggcggctctgataagacacacac atgtcctccatgtcctgctccagagctgctcggcggaccttctgttttcctgtttcca

TABLE 33
Sequences of Antibodies and Fc variant

SEQ ID No	Description	Sequence
		cctaagccaaaggacaccctgatgatcagcagaaccctgaagtgacctgtg tggtggtggccgtgtctcacgaagatcccgaagtgaagttcaattggtacgtgg acggcgtggaagtgcacaacgccaagaccaagcctagagaggaacagta cgccagcacctatagagtggtgtccgtgctgacagtgctgcaccaggactggc tgaacggcaaagagtacaagtgcaaggtgtccaacaaggccctggccgctc ctatcgagaaaaccatctctaaggccaagggccagcctcgggaaccacagg tttacaccctgcctccatgccgggaagagatgaccaagaatcaggtgtccctgt ggtgcctggtcaagggcttctacccttccgatatcgccgtggaatgggagagca atggccagcctgagaacaactacaagaccacacctcctgtgctggacagcg acggctcattcttcctgtacagcaagctgaccgtggacaagagcagatggcag cagggcaacgtgttcagctgttctgtgatgcacgaggccctgcacaaccacta cacccagaagtctctgtctctgagccccggcaaa
1120	Anti-CD19-CD3 GADAPASK IgG1 Heavy Chain Amino acid sequence	qvqlvqsgaevkkpgasvkvsckasgytfttywiqwvrqapgqrlewmgav ypgdadtrytqkfqgrvtltadrsastaymelsslrsedtavyycgrdagleyyal dywgqgtlvtvssastkgpsvfplapsskstsggtaalgclvkdyfpepvtvsw nsgaltsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkpsntkvdkr vepkscggggsggggsevqlvesggglvqpggslklscaasgftfntyamn wvrqasgkglewvgrirskynnyatyyadsvkdrftisrddskstlylqmnslkt edtavyycvrhgnfgnsyvswfaywgqgtlvtvssggggsggggsggggsg gggsqavvtqepsltvspggtvtltcrsstgavttsnyanwvqqkpgqaprglig gtnkrapwtparfsgsllgdkaaltlsgaqpedeaeyfcalwysnlwvfgggtkl tvlggggsdkthtcppcpapellgapsvflfppkpkdtlmisrtpevtcvvvavk hedpevkfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkey kckvsnkalaapiektiskakgqprepqvctlppsreemtknqvslscavkgfy psdiavewesngqpennykttppvldsdgsfflvskltvdksrwqqgnvfscs vmhealhnhytqkslslspgk
1121	Anti-CD19-CD3 GADAPASK IgG1 Heavy Chain Nucleic acid sequence	caggttcaactggttcagagcggagccgaagtgaagaaacctggggccagc gtgaaggtgtcctgtaaagccagcggctacacctttaccacctactggattcagt gggtccgacaggcccctggacagagacttgaatggatgggagctgtgtaccc cggcgacgccgataccagatacacccagaaattccagggcagagtgaccct gacagccgatagaagcgccagcacagcctacatggaactgagcagcctga gaagcgaggataccgccgtgtactactgtggcagagatgccggcctggaata ttacgccctggactattggggccagggcaccctggttacagtgtcctctgcctcta

TABLE 33 Sequences of Antibodies and Fc variant		
SEQ ID No	Description	Sequence
		caaagggcccctccgtttttccactggctcctagcagcaagagcacatctggtg gaacagccgctctgggctgcctggtcaaggattactttcctgagcctgtgaccgt gtcctggaatagcggagcactgacaagcggcgtgcacacatttccagccgtg ctgcaaagcagcggcctgtactctctgtctagcgtggtcacagtgcctagctcta gcctgggcacccagacctacatctgcaacgtgaaccacaagcctagcaaca ccaaggtcgacaagagagtggaacccaagtcttgtggtggcggaggatctgg cggaggcggatctgaagttcagcttgttgaatctggcggcggactggtgcaac ctggcggatctctgaaactgtcttgtgccgcctccggcttcaccttcaatacctac gccatgaactgggttcgacaagcctccggcaaaggactggaatgggtcgga cggatcagaagcaagtacaacaactacgccacctactacgccgactccgtg aaggacagattcaccatcagccgggacgactccaagagcaccctgtacctcc agatgaactccctgaaaaccgaggacacagccgtctattattgcgtgcggcac ggcaacttcggcaacagctatgtgtcttggtttgcctactggggacagggaacc ctcgtgaccgtttcttcaggcggcggtggtagtggcggtggtggtagcggaggc ggtggatcaggtggcggcggttctcaagctgtggtcacacaagagcccagcct gacagtttctcctggcggaaccgtgacactgacctgtagatctagcaccggcg cagtgaccaccagcaattacgctaactgggtgcagcagaagcccggccaag ctcctagaggactgatcggaggcacaaacaagagagccccttggacaccag ccagattttctggctctctgctgggcgataaggccgctcttacactgtctggcgca cagcctgaagatgaggccgagtacttttgcgccctgtggtacagcaacctgtgg gtgttcggcggaggaacaaagctgacagttcttggaggcggcggaagcgac aagacccacacatgtcctccatgtcctgctccagaactgctgggagcccctag cgttttcctgtttcctccaaagccaaaggacaccctgatgatcagcagaacccct gaagtgacctgtgtggtggtggccgtgaagcacgaggacccagaagtgaagt tcaattggtacgtggacggcgtggaagtgcacaacgccaagaccaagccta gagaggaacagtacaacagcacctacagagtggtgtccgtgctgacagtgct gcaccaggactggctgaacggcaaagagtacaagtgcaaggtgtccaaca aggccctggccgctcctatcgagaaaaccatctctaaggccaagggccagcc tcgggaacctcaagtgtgcactcttccacctagccgggaagagatgaccaag aaccaggtgtcactgagctgcgccgtgaagggcttctacccttctgatatcgcc gtggaatgggagagcaacggccagccagagaacaactacaagaccacac ctcctgtgctggacagcgacggctcattcttcctggtgtctaagctgaccgtgga

TABLE 33
Sequences of Antibodies and Fc variant

SEQ ID No	Description	Sequence
		caagagcagatggcagcagggcaacgtgttcagctgttctgtgatgcacgag gccctgcacaaccactacacacagaagtccctgtctctgagccccggcaaa
1122	Anti-CD58 GADAPASK IgG1 Heavy Chain Amino acid sequence	sqqiygvvygnvtfhvpsnvplkevlwkkqkdkvaelensefrafssfknrvyl dtvsgsltiynltssdedeyemespnitdtmkfflyvlesggggsdkthtcppcp apellgapsvflfppkpkdtlmisrtpevtcvvvavkhedpevkfnwyvdgvev hnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalaapiektisk akgqprepqvytlppcreemtknqvslwclvkgfypsdiavewesngqpen nykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsls pgk
1123	Anti-CD58 GADAPASK IgG1 Heavy Chain Nucleic acid sequence	tctcagcagatctatggcgtggtgtacggcaacgtgaccttccacgtgccaagc aacgtgcccctgaaagaggtgctctggaagaaacagaaagacaaggtggc cgagctggaaaacagcgagttcagagccttcagcagcttcaagaaccgggt gtacctggataccgtgtccggcagcctgaccatctacaatctgaccagcagcg acgaggacgagtacgagatggaaagccccaacatcaccgacaccatgaag ttctttctgtacgtgctggaaagcggcggaggcggctctgataagacacacac atgtcctccatgtcctgctccagaactgctgggagcccctagcgttttcctgtttcct ccaaagccaaaggacaccctgatgatcagcagaaccccctgaagtgacctgt gtggtggtggccgtgaagcacgaagatcccgaagtgaagttcaattggtacgt ggacggcgtggaagtgcacaacgccaagaccaagcctagagaggaacag tacaacagcacctacagagtggtgtccgtgctgaccgtgctgcaccaggattg gctgaacggcaaagagtacaagtgcaaggtgtccaacaaggccctggccgc tcctatcgagaaaaccatctctaaggccaagggccagcctcgggaaccaca ggtttacaccctgcctccatgccgggaagagatgaccaagaatcaggtgtccc tgtggtgcctggtcaagggcttctacccttccgatatcgccgtggaatgggaga gcaatggccagcctgagaacaactacaagaccacacctcctgtgctggaca gcgacggctcattcttcctgtacagcaagctgacagtggacaagagcagatg gcagcagggcaacgtgttcagctgttctgtgatgcacgaggccctgcacaacc actacacccagaagtctctgtctctgagccccggcaaa
1124	Anti-CD19-CD3 LALA IgG1 Heavy Chain Amino acid sequence	qvqlvqsgaevkkpgasvkvsckasgytfttywiqwvrqapgqrlewmgav ypgdadtrytqkfqgrvtltadrsastaymelsslrsedtavyycgrdagleyyal dywgqgtlvtvssastkgpsvfplapsskstsggtaalgclvkdyfpepvtvsw nsgaltsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkpsntkvdkr vepkscggggsggggsevqlvesggglvqpggslklscaasgftfntyamn

TABLE 33 Sequences of Antibodies and Fc variant		
SEQ ID No	Description	Sequence
		wvrqasgkglewvgrirskynnyatyyadsvkdrftisrddskstlylqmnslkt edtavyycvrhgnfgnsyvswfaywgqgtlvtvssggggsggggsggggsg gggsqavvtqepsltvspggtvtltcrsstgavttsnyanwvqqkpgqaprglig gtnkrapwtparfsgsllgdkaaltlsgaqpedeaeyfcalwysnlwvfgggtkl tvlggggsdkthtcppcpapeaaggpsvflfppkpkdtlmisrtpevtcvvvdv shedpevkfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngke ykckvsnkalpapiektiskakgqprepqvctlppsreemtknqvslscavkgf ypsdiavewesngqpennykttppvldsdgsfflvskltvdksrwqqgnvfsc svmhealhnhytqkslslspgk
1125	Anti-CD19-CD3 LALA IgG1 Heavy Chain Nucleic acid sequence	caggttcaactggttcagagcggagccgaagtgaagaaacctggggccagc gtgaaggtgtcctgtaaagccagcggctacacctttaccacctactggattcagt gggtccgacaggcccctggacagagacttgaatggatgggagctgtgtaccc cggcgacgccgataccagatacacccagaaattccagggcagagtgaccct gacagccgatagaagcgccagcacagcctacatggaactgagcagcctga gaagcgaggataccgccgtgtactactgtggcagagatgccggcctggaata ttacgccctggactattggggccagggcaccctggttacagtgtcctctgcctcta caaagggcccctccgtttttccactggctcctagcagcaagagcacatctggtg gaacagccgctctgggctgcctggtcaaggattactttcctgagcctgtgaccgt gtcctggaatagcggagcactgacaagcggcgtgcacacatttccagccgtg ctgcaaagcagcggcctgtactctctgtctagcgtggtcacagtgcctagctcta gcctgggcacccagacctacatctgcaacgtgaaccacaagcctagcaaca ccaaggtcgacaagagagtggaacccaagtcttgtggtggcggaggatctgg cggaggcggatctgaagttcagcttgttgaatctggcggcggactggtgcaac ctggcggatctctgaaactgtcttgtgccgcctccggcttcaccttcaatacctac gccatgaactgggttcgacaagcctccggcaaaggactggaatgggtcgga cggatcagaagcaagtacaacaactacgccacctactacgccgactccgtg aaggacagattcaccatcagccgggacgactccaagagcaccctgtacctcc agatgaactccctgaaaaccgaggacacagccgtctattattgcgtgcggcac ggcaacttcggcaacagctatgtgtcttggtttgcctactggggacagggaacc ctcgtgaccgtttcttcaggcggcggtggtagtggcggtggtggtagcggaggc ggtggatcaggtggcggcggttctcaagctgtggtcacacaagagcccagcct gacagtttctcctggcggaaccgtgacactgacctgtagatctagcaccggcg cagtgaccaccagcaattacgctaactgggtgcagcagaagcccggccaag

TABLE 33
Sequences of Antibodies and Fc variant

SEQ ID No	Description	Sequence
		ctcctagaggactgatcggaggcacaaacaagagagccccttggacaccag ccagattttctggctctctgctgggcgataaggccgctcttacactgtctggcgca cagcctgaagatgaggccgagtacttttgcgccctgtggtacagcaacctgtgg gtgttcggcggaggaacaaagctgacagttcttggaggcggcggaagcgac aagacccacacatgtcctccatgtcctgctccagaagctgctggcggaccctc cgtgtttctgttccctccaaagccaaaggacaccctgatgatcagcagaacccc tgaagtgacctgcgtggtggtggatgtgtctcacgaggacccagaagtgaagtt caattggtacgtggacggcgtggaagtgcacaacgccaagaccaagcctag agaggaacagtacaacagcacctacagagtggtgtccgtgctgacagtgctg caccaggactggctgaacggcaaagagtacaagtgcaaggtgtccaacaa ggccctgcctgctcctatcgagaaaaccatcagcaaggccaagggccagcct agggaacctcaagtgtgcactctgcctccaagccgggaagagatgaccaag aatcaggtgtccctgagctgcgccgtgaagggcttttacccttccgatatcgccg tggaatgggagagcaatggccagccagagaacaactacaagaccacacct cctgtgctggacagcgacggctcattcttcctggtgtctaagctgaccgtggaca agagcagatggcagcagggcaacgtgttcagctgttctgtgatgcacgaggc cctgcacaaccactacacacagaagtccctgtctctgagccccggcaaa
1126	Anti-CD58 LALA IgG1 Heavy Chain Amino acid sequence	sqqiygvvygnvtfhvpsnvplkevlwkkqkdkvaelensefrafssfknrvyl dtvsgsltiynltssdedeyemespnitdtmkfflyvlesggggsdkthtcppcp apeaaggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgv evhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiekti skakgqprepqvytlppcreemtknqvslwclvkgfypsdiavewesngqp ennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqksl slspgk
1168	Anti-CD58 LALA IgG1 Heavy Chain Nucleic acid sequence	tctcagcagatctatggcgtggtgtacggcaacgtgaccttccacgtgccaagc aacgtgcccctgaaagaggtgctctggaagaaacagaaagacaaggtggc cgagctggaaaacagcgagttcagagccttcagcagcttcaagaaccgggt gtacctggataccgtgtccggcagcctgaccatctacaatctgaccagcagcg acgaggacgagtacgagatggaaagccccaacatcaccgacaccatgaag ttctttctgtacgtgctggaaagcggcggaggcggctctgataagacacacac atgtcctccatgtcctgctccagaagcagctggcggaccctctgttttcctgtttcc acctaagccaaaggacaccctgatgatcagcagaacccctgaagtgacctg cgtggtggtggatgtgtctcacgaggaccccgaagtgaagttcaattggtacgt

TABLE 33
Sequences of Antibodies and Fc variant

SEQ ID No	Description	Sequence
		ggacggcgtggaagtgcacaacgccaagaccaagcctagagaggaacag tacaacagcacctacagagtggtgtccgtgctgaccgtgctgcaccaggattg gctgaacggcaaagagtacaagtgcaaggtgtccaacaaggccctgcctgct cctatcgagaaaaccatcagcaaggccaagggccagccaagagaacccc aggtttacaccctgcctccatgccgggaagagatgaccaagaatcaggtgtcc ctgtggtgcctggtcaagggcttctacccttccgatatcgccgtggaatgggaga gcaatggccagcctgagaacaactacaagaccacacctcctgtgctggaca gcgacggctcattcttcctgtacagcaagctgacagtggacaagagcagatg gcagcagggcaacgtgttcagctgttctgtgatgcacgaggccctgcacaacc actacacccagaagtctctgtctctgagccccggcaaa
1127	Anti-CD19-CD3 LALASKPA IgG1 Heavy Chain Amino acid sequence	qvqlvqsgaevkkpgasvkvsckasgytfttywiqwvrqapgqrlewmgav ypgdadtrytqkfqgrvtltadrsastaymelsslrsedtavyycgrdagleyyal dywgqgtlvtvssastkgpsvfplapsskstsggtaalgclvkdyfpepvtvsw nsgaltsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkpsntkvdkr vepkscggggsggggsevqlvesggglvqpggslklscaasgftfntyamn wvrqasgkglewvgrirskynnyatyyadsvkdrftisrddskstlylqmnslkt edtavyycvrhgnfgnsyvswfaywgqgtlvtvssggggsggggsggggsg gggsqavvtqepsltvspggtvtltcrsstgavttsnyanwvqqkpgqaprglig gtnkrapwtparfsgsllgdkaaltlsgaqpedeaeyfcalwysnlwvfgggtkl tvlggggsdkthtcppcpapeaaggpsvflfppkpkdtlmisrtpevtcvvvdv khedpevkfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngke ykckvsnkalaapiektiskakgqprepqvctlppsreemtknqvslscavkgf ypsdiavewesngqpennykttppvldsdgsfflvskltvdksrwqqgnvfsc svmhealhnhytqkslslspgk
1128	Anti-CD19-CD3 LALASKPA IgG1 Heavy Chain Nucleic acid sequence	caggttcaactggttcagagcggagccgaagtgaagaaacctggggccagc gtgaaggtgtcctgtaaagccagcggctacacctttaccacctactggattcagt gggtccgacaggcccctggacagagacttgaatggatgggagctgtgtaccc cggcgacgccgataccagatacacccagaaattccagggcagagtgaccct gacagccgatagaagcgccagcacagcctacatggaactgagcagcctga gaagcgaggataccgccgtgtactactgtggcagagatgccggcctggaata ttacgccctggactattggggccagggcaccctggttacagtgtcctctgcctcta caaagggcccctccgtttttccactggctcctagcagcaagagcacatctggtg gaacagccgctctgggctgcctggtcaaggattactttcctgagcctgtgaccgt

TABLE 33
Sequences of Antibodies and Fc variant

SEQ ID No	Description	Sequence
		gtcctggaatagcggagcactgacaagcggcgtgcacacatttccagccgtg ctgcaaagcagcggcctgtactctctgtctagcgtggtcacagtgcctagctcta gcctgggcacccagacctacatctgcaacgtgaaccacaagcctagcaaca ccaaggtcgacaagagagtggaacccaagtcttgtggtggcggaggatctgg cggaggcggatctgaagttcagcttgttgaatctggcggcggactggtgcaac ctggcggatctctgaaactgtcttgtgccgcctccggcttcaccttcaatacctac gccatgaactgggttcgacaagcctccggcaaaggactggaatgggtcgga cggatcagaagcaagtacaacaactacgccacctactacgccgactccgtg aaggacagattcaccatcagccgggacgactccaagagcaccctgtacctcc agatgaactccctgaaaaccgaggacacagccgtctattattgcgtgcggcac ggcaacttcggcaacagctatgtgtcttggtttgcctactggggacagggaacc ctcgtgaccgtttcttcaggcggcggtggtagtggcggtggtggtagcggaggc ggtggatcaggtggcggcggttctcaagctgtggtcacacaagagcccagcct gacagtttctcctggcggaaccgtgacactgacctgtagatctagcaccggcg cagtgaccaccagcaattacgctaactgggtgcagcagaagcccggccaag ctcctagaggactgatcggaggcacaaacaagagagccccttggacaccag ccagattttctggctctctgctgggcgataaggccgctcttacactgtctggcgca cagcctgaagatgaggccgagtacttttgcgccctgtggtacagcaacctgtgg gtgttcggcggaggaacaaagctgacagttcttggaggcggcggaagcgac aagacccacacatgtcctccatgtcctgctccagaagctgctggcggaccctc cgtgtttctgttccctccaaagccaaaggacaccctgatgatcagcagaacccc tgaagtgacctgcgtggtggtggatgtgaagcacgaggacccagaagtgaa gttcaattggtacgtggacggcgtggaagtgcacaacgccaagaccaagcct agagaggaacagtacaacagcacctacagagtggtgtccgtgctgacagtg ctgcaccaggactggctgaacggcaaagagtacaagtgcaaggtgtccaac aaggccctggccgctcctatcgagaaaaccatctctaaggccaagggccag cctcgggaacctcaagtgtgcactcttccacctagccgggaagagatgacca agaaccaggtgtcactgagctgcgccgtgaagggcttctacccttctgatatcg ccgtggaatgggagagcaacggccagccagagaacaactacaagaccac acctcctgtgctggacagcgacggctcattcttcctggtgtctaagctgaccgtg gacaagagcagatggcagcagggcaacgtgttcagctgtagcgtgatgcac gaggccctgcacaaccactacacacagaagtccctgtctctgagccccggca aa

TABLE 33 Sequences of Antibodies and Fc variant		
SEQ ID No	**Description**	**Sequence**
1129	Anti-CD58 LALASKPA IgG1 Heavy Chain Amino acid sequence	sqqiygvvygnvtfhvpsnvplkevlwkkqkdkvaelensefrafssfknrvyl dtvsgsltiynltssdedeyemespnitdtmkfflyvlesggggsdkthtcppcp apeaaggpsvflfppkpkdtlmisrtpevtcvvvdvkhedpevkfnwyvdgv evhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalaapiekti skakgqprepqvytlppcreemtknqvslwclvkgfypsdiavewesngqp ennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqksl slspgk
1130	Anti-CD58 LALASKPA IgG1 Heavy Chain Nucleic acid sequence	tctcagcagatctatggcgtggtgtacggcaacgtgaccttccacgtgccaagc aacgtgcccctgaaagaggtgctctggaagaaacagaaagacaaggtggc cgagctggaaaacagcgagttcagagccttcagcagcttcaagaaccgggt gtacctggataccgtgtccggcagcctgaccatctacaatctgaccagcagcg acgaggacgagtacgagatggaaagccccaacatcaccgacaccatgaag ttctttctgtacgtgctggaaagcggcggaggcggctctgataagacacacac atgtcctccatgtcctgctccagaagcagctggcggaccctctgttttcctgtttcc acctaagccaaaggacaccctgatgatcagcagaaccctgaagtgacctg cgtggtggtggatgtgaagcacgaggaccccgaagtgaagttcaattggtacg tggacggcgtggaagtgcacaacgccaagaccaagcctagagaggaaca gtacaacagcacctacagagtggtgtccgtgctgaccgtgctgcaccaggatt ggctgaacggcaaagagtacaagtgcaaggtgtccaacaaggccctggcc gctcctatcgagaaaaccatctctaaggccaagggccagcctcgggaacca caggtttacaccctgcctccatgccgggaagagatgaccaagaatcaggtgtc cctgtggtgcctggtcaagggcttctacccttccgatatcgccgtggaatgggag agcaatggccagcctgagaacaactacaagaccacacctcctgtgctggac agcgacggctcattcttcctgtacagcaagctgacagtggacaagagcagat ggcagcagggcaacgtgttcagctgtagcgtgatgcacgaggccctgcacaa ccactacacccagaagtctctgtctctgagccccggcaaa

8.38. Example 38: Biophysical properties of modified antibodies: SPR- Binding of modified antibodies of Example 37 to human Fc gamma receptor 1A

[01005] Surface plasmon resonance (SPR) experiments were performed to analyze the interaction of human activating receptor FcγR1A towards WT and antibody-Fc variants. Binding kinetics and their relative binding affinities were explored. The binding affinity is an important characteristic of an interaction between an antibody and an antigen. The equilibrium

dissociation constant (K_D) defines how strong the interaction is and therefore how much antibody-antigen complex is formed at equilibrium. The knowledge of the antibody characteristics is not only essential during selection of the best therapeutic antibody candidate, but also important to understand the *in vivo* behavior and potentially predict cellular immune responses. The aim is to generate antibody variants with no or low binding to Fc gamma receptor to reduce or eliminate effector function in hope of improving the safety of monoclonal antibody therapeutics.

[01006] All SPR buffers were prepared using deionized water. The samples were prepared in running buffer PBS pH 7.4 with 0.005% Tween-20. SPR measurements were measured on a Biacore T200 (GE-Healthcare Life Sciences) controlled by Biacore T200 control software version 2.0.1.

[01007] Surface plasmon resonance was conducted using a Biacore T200 to assess binding affinity of antibody WT and variants to human FcγR1A.

[01008] The antibodies were covalently immobilized on a CM5 sensor chip whereas Fc gamma receptor 1A served as analyte in solution (Figure 38). Antibodies were diluted in 10 mM sodium acetate pH 4 and immobilized at a density of approximately 950 resonance units (RU) on the CM5 sensor chip applying a standard amine coupling procedure. Flow cell 1 was blank immobilized to serve as a reference. The kinetic binding data were collected by subsequent injections of 1:2 dilution series of the human Fc gamma receptor 1A on all flow cells at a flow rate of 30 µl/min and at a temperature of 25°C. The Fc gamma receptor was diluted in the running buffer at concentrations ranging from 0.2 nM to 25 nM. The chip surface was regenerated using 20 mM glycine pH 2.0 solution after each measuring cycle. Zero concentration samples (blank runs) were measured to allow double-referencing during data evaluation.

[01009] Data were evaluated using the Biacore T200 evaluation software. The raw data were double referenced, i.e. the response of the measuring flow cell was corrected for the response of the reference flow cell, and in a second step the response of a blank injection was subtracted. Then the sensorgrams were fitted by applying a 1:1 kinetic binding model to calculate dissociation equilibrium constants. In addition, the maximum response reached during the experiment was monitored. Maximum response describes the binding capacity of the surface in terms of the response at saturation.

[01010] The maximum response values summarizing these interactions are given in Table 34.

[01011] The SPR Biacore binding sensorgrams for each variant towards FcγR1A were depicted in Figure 41.

[01012] All variants inhibit the binding to Fc gamma receptors compare to WT as low residual binding was measured.

Table 34
Maximum responses of WT-Fc and variants towards human FcγRIA determined by surface plasmon resonance

Fc	Maximum response at 25nM hFcγRIA (RU)
WT	138
DANAPA	0
GADAPASK	0
LALA	44
LALASKPA	0

Differential scanning calorimetry- Melting temperature of modified antibodies

[01013] The thermal stability of engineered antibodies CH2 domains were compared using calorimetric measurements as shown in Table 35. Calorimetric measurements were carried out on a differential scanning micro calorimeter (Nano DSC, TA instruments). The cell volume was 1 ml and the heating rate was 1°C/min. All proteins were used at a concentration of 1mg/ml in PBS (pH 7.4). The molar heat capacity of each protein was estimated by comparison with duplicate samples containing identical buffer from which the protein had been omitted. The partial molar heat capacities and melting curves were analyzed using standard procedure. Thermograms were baseline corrected and concentration normalized. The silent version LALASKPA (68°C) shows significantly better Tm compared to DANAPA (57°C).

Table 35
Melting temperatures of WT-Fc and variants

Fc	Melting temperature (Tm) of CH2 domain
WT	**68**
DANAPA	**57**
GADAPASK	**59**
LALA	**68**
LALASKPA	**68**

Aggregation propensity post capture of Fc variants

[01014] Size exclusion chromatography measurements were performed to evaluate the aggregation propensity (% HMW) of WT and Fc modified derivatives. The produced and purified antibodies were applied to an analytical size exclusion chromatography column (SEC 200, GE Healthcare), equilibrated with PBS buffer pH 7.4. Results are summarized in Table 36.

TABLE 36 Higher molecular weight content (%) of antibodies	
Fc	**(%) higher molecular weight**
WT	**16.3**
DANAPA	**22.7**
GADAPASK	**16.1**
LALA	**18.1**
LALASKPA	**18.9**

8.39. Example 39: Anti-CD3 NFAT signalling assay

[01015] Jurkat reporter gene assay (RGA) for the nuclear factor of activated T-cells (NFAT) pathway was performed using Jurkat NFAT luciferized (JNL) cells and THP-1 cells (ATCC, TIB202). THP-1 cells express FcγRI, FcγRII, and FcγRIII. Cells were co-incubated for 6 hours at 37ºC, 5%CO2 at a 5:1 effector to tumor cell ratio with each sample at the various concentrations depicted. An equal volume of ONE-Glo™ reagent (Promega, E6120) was added to the culture volume. Plate was shaken for 2 minutes, then incubated for an additional 8 minutes protected from light. Luciferase activity was quantitated on the Biotek Synergy HT plate reader. Data were analyzed and fit to a 4 parameter-logistic curve using GraphPad Prism.

[01016] In summary, WT showed the greatest NFAT activity. All silencing mutation sets showed significantly dampened NFAT activation. In the RGA, performed without IFNg (Figure 42), all silencing mutation sets showed comparable T-cell activation with the exception of DANAPA.

8.40. Example 40: CD3hi TSP1 variant

[01017] A CD3hi TSP1 variant comprising knob-into-hole mutations in the opposite Fc regions as compared to CD3hi TSP1 was produced. The amino acid sequences of the variant are set forth in Table 37.

Table 37		
Chain Description	**Amino Acid Sequence**	**SEQ ID NO**
First Half Antibody Heavy Chain (includes Fc sequence)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYWIQWVRQAPGQRLE WMGAVYPGDADTRYTQKFQGRVTLTADRSASTAYMELSSLRSEDTA VYYCGRDAGLEYYALDYWGQGTLVTVSSASTKGPSVFPLAPSSKST SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCGGGGSGGG GSEVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQASGK GLEWVGRIRSKYNNYATYYADSVKDRFTISRDDSKSTLYLQMNSLKT EDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGG	1131

Table 37		
Chain Description	**Amino Acid Sequence**	**SEQ ID NO**
	SGGGGSGGGGSQAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYA NWVQQKPGQAPRGLIGGTNKRAPWTPARFSGSLLGDKAALTLSGAQ PEDEAEYFCALWYSNLWVFGGGTKLTVLGGGGSDKTHTCPPCPAPE LLGGPSVFLFPPKPKDTLMISRTPEVTCVVVAVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALAAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLVKGFY PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK	
First Half Antibody Light Chain	EIVMTQSPATLSVSPGERATLSCRASQDVGTAVAWYQQKPGQAPRL LIYWASTRHTGIPARFSGSGSGTEFTLTISSLQSEDFAVYFCQQYANF PLYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC	1132
Second Half Antibody (includes Fc sequence)	SQQIYGVVYGNVTFHVPSNVPLKEVLWKKQKDKVAELENSEFRAFSS FKNRVYLDTVSGSLTIYNLTSSDEDEYEMESPNITDTMKFFLYVLESG GGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVL HQDWLNGKEYKCKVSNKALAAPIEKTISKAKGQPREPQVCTLPPSRE EMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	1133

8.41. Example 41: CD3hi TSP1 variants

[01018] Additional Fc variants of CD3hi TSP1 are designed, and expressed and purified according to the methods described in Example 37. Amino acid sequences of the variants are set forth in Table 38.

TABLE 38 Exemplary TBM sequences		
SEQ ID No	**Description**	**Sequence**
1110	Anti-CD19 light chain Amino acid sequence	eivmtqspatlsvspgeratlscrasqdvgtavawyqqkpgqaprlliywastr htgiparfsgsgsgteftltisslqsedfavyfcqqyanfplytfgqgtkleikrtvaa psvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteq dskdstyslsstltlskadyekhkvyacevthqglsspvtksfnrgec
1134	Anti-CD19-CD3 LALAGA IgG1 Heavy Chain	qvqlvqsgaevkkpgasvkvsckasgytfttywiqwvrqapgqrlewmgav ypgdadtrytqkfqgrvtltadrsastaymelsslrsedtavyycgrdagleyyal dywgqgtlvtvssastkgpsvfplapsskstsggtaalgclvkdyfpepvtvsw

TABLE 38
Exemplary TBM sequences

SEQ ID No	Description	Sequence
	Amino acid sequence	nsgaltsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkpsntkvdkr vepkscggggsggggsevqlvesggglvqpggslklscaasgftfntyamn wvrqasgkglewvgrirskynnyatyyadsvkdrftisrddskstlylqmnslkt edtavyycvrhgnfgnsyvswfaywgqgtlvtvssggggsggggsggggsg gggsqavvtqepsltvspggtvtltcrsstgavttsnyanwvqqkpgqaprglig gtnkrapwtparfsgsllgdkaaltlsgaqpedeaeyfcalwysnlwvfgggtkl tvlggggsdkthtcppcpapeaagapsvflfppkpkdtlmisrtpevtcvvvdv shedpevkfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngke ykckvsnkalpapiektiskakgqprepqvctlppsreemtknqvslscavkgf ypsdiavewesngqpennykttppvldsdgsfflvskltvdksrwqqgnvfsc svmhealhnhytqkslslspgk
1135	Anti-CD58 LALAGA IgG1 Heavy Chain Amino acid sequence	sqqiygvvygnvtfhvpsnvplkevlwkkqkdkvaelensefrafssfknrvyl dtvsgsltiynltssdedeyemespnitdtmkfflyvlesggggsdkthtcppcp apeaagapsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgv evhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiekti skakgqprepqvytlppcreemtknqvslwclvkgfypsdiavewesngqp ennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqksl slspgk
1136	Anti-CD19-CD3 DAPASK IgG1 Heavy Chain Amino acid sequence	qvqlvqsgaevkkpgasvkvsckasgytfttywiqwvrqapgqrlewmgav ypgdadtrytqkfqgrvtltadrsastaymelsslrsedtavyycgrdagleyyal dywgqgtlvtvssastkgpsvfplapsskstsggtaalgclvkdyfpepvtvsw nsgaltsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkpsntkvdkr vepkscggggsggggsevqlvesggglvqpggslklscaasgftfntyamn wvrqasgkglewvgrirskynnyatyyadsvkdrftisrddskstlylqmnslkt edtavyycvrhgnfgnsyvswfaywgqgtlvtvssggggsggggsggggsg gggsqavvtqepsltvspggtvtltcrsstgavttsnyanwvqqkpgqaprglig gtnkrapwtparfsgsllgdkaaltlsgaqpedeaeyfcalwysnlwvfgggtkl tvlggggsdkthtcppcpapellggpsvflfppkpkdtlmisrtpevtcvvvavk hedpevkfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkey kckvsnkalaapiektiskakgqprepqvctlppsreemtknqvslscavkgfy psdiavewesngqpennykttppvldsdgsfflvskltvdksrwqqgnvfscs vmhealhnhytqkslslspgk

TABLE 38
Exemplary TBM sequences

SEQ ID No	Description	Sequence
1137	Anti-CD58 DAPASK IgG1 Heavy Chain Amino acid sequence	sqqiygvvygnvtfhvpsnvplkevlwkkqkdkvaelensefrafssfknrvyl dtvsgsltiynltssdedeyemespnitdtmkfflyvlesggggsdkthtcppcp apellggpsvflfppkpkdtlmisrtpevtcvvvavkhedpevkfnwyvdgvev hnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalaapiektisk akgqprepqvytlppcreemtknqvslwclvkgfypsdiavewesngqpen nykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsls pgk
1138	Anti-CD19-CD3 GADAPA IgG1 Heavy Chain Amino acid sequence	qvqlvqsgaevkkpgasvkvsckasgytfttywiqwvrqapgqrlewmgav ypgdadtrytqkfqgrvtltadrsastaymelsslrsedtavyycgrdagleyyal dywgqgtlvtvssastkgpsvfplapsskstsggtaalgclvkdyfpepvtvsw nsgaltsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkpsntkvdkr vepkscggggsggggsevqlvesgglvqpggslklscaasgftfntyamn wvrqasgkglewvgrirskynnyatyyadsvkdrftisrddskstlylqmnslkt edtavyycvrhgnfgnsyvswfaywgqgtlvtvssggggsggggsggggsg gggsqavvtqepsltvspggtvtltcrsstgavttsnyanwvqqkpgqaprglig gtnkrapwtparfsgsllgdkaaltlsgaqpedeaeyfcalwysnlwvfgggtkl tvlggggsdkthtcppcpapellgapsvflfppkpkdtlmisrtpevtcvvvavs hedpevkfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkey kckvsnkalaapiektiskakgqprepqvctlppsreemtknqvslscavkgfy psdiavewesngqpennykttppvldsdgsfflvskltvdksrwqqgnvfscs vmhealhnhytqkslslspgk
1139	Anti-CD58 GADAPA IgG1 Heavy Chain Amino acid sequence	sqqiygvvygnvtfhvpsnvplkevlwkkqkdkvaelensefrafssfknrvyl dtvsgsltiynltssdedeyemespnitdtmkfflyvlesggggsdkthtcppcp apellgapsvflfppkpkdtlmisrtpevtcvvvavshedpevkfnwyvdgvev hnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalaapiektisk akgqprepqvytlppcreemtknqvslwclvkgfypsdiavewesngqpen nykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsls pgk
1140	Anti-CD19-CD3 LALAPG IgG1 Heavy Chain Amino acid sequence	qvqlvqsgaevkkpgasvkvsckasgytfttywiqwvrqapgqrlewmgav ypgdadtrytqkfqgrvtltadrsastaymelsslrsedtavyycgrdagleyyal dywgqgtlvtvssastkgpsvfplapsskstsggtaalgclvkdyfpepvtvsw nsgaltsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkpsntkvdkr vepkscggggsggggsevqlvesgglvqpggslklscaasgftfntyamn

TABLE 38
Exemplary TBM sequences

SEQ ID No	Description	Sequence
		wvrqasgkglewvgrirskynnyatyyadsvkdrftisrddskstlylqmnslkt edtavyycvrhgnfgnsyvswfaywgqgtlvtvssggggsggggsggggsg gggsqavvtqepsltvspggtvtltcrsstgavttsnyanwvqqkpgqaprglig gtnkrapwtparfsgsllgdkaaltlsgaqpedeaeyfcalwysnlwvfgggtkl tvlggggsdkthtcppcpapeaaggpsvflfppkpkdtlmisrtpevtcvvvdv shedpevkfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngke ykckvsnkalgapiektiskakgqprepqvctlppsreemtknqvslscavkgf ypsdiavewesngqpennykttppvldsdgsfflvskltvdksrwqqgnvfsc svmhealhnhytqkslslspgk
1141	Anti-CD58 LALAPG IgG1 Heavy Chain Amino acid sequence	sqqiygvvygnvtfhvpsnvplkevlwkkqkdkvaelensefrafssfknrvyl dtvsgsltiynltssdedeyemespnitdtmkfflyvlesggggsdkthtcppcp apeaaggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgv evhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalgapiekti skakgqprepqvytlppcreemtknqvslwclvkgfypsdiavewesngqp ennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqksl slspgk
1142	Anti-CD19-CD3 LALAPA IgG1 Heavy Chain Amino acid sequence	qvqlvqsgaevkkpgasvkvsckasgytfttywiqwvrqapgqrlewmgav ypgdadtrytqkfqgrvtltadrsastaymelsslrsedtavyycgrdagleyyal dywgqgtlvtvssastkgpsvfplapsskstsggtaalgclvkdyfpepvtvsw nsgaltsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkpsntkvdkr vepkscggggsggggsevqlvesggglvqpggslklscaasgftfntyamn wvrqasgkglewvgrirskynnyatyyadsvkdrftisrddskstlylqmnslkt edtavyycvrhgnfgnsyvswfaywgqgtlvtvssggggsggggsggggsg gggsqavvtqepsltvspggtvtltcrsstgavttsnyanwvqqkpgqaprglig gtnkrapwtparfsgsllgdkaaltlsgaqpedeaeyfcalwysnlwvfgggtkl tvlggggsdkthtcppcpapeaaggpsvflfppkpkdtlmisrtpevtcvvvdv shedpevkfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngke ykckvsnkalaapiektiskakgqprepqvctlppsreemtknqvslscavkgf ypsdiavewesngqpennykttppvldsdgsfflvskltvdksrwqqgnvfsc svmhealhnhytqkslslspgk
1143	Anti-CD58 LALAPA IgG1 Heavy Chain	sqqiygvvygnvtfhvpsnvplkevlwkkqkdkvaelensefrafssfknrvyl dtvsgsltiynltssdedeyemespnitdtmkfflyvlesggggsdkthtcppcp apeaaggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgv

TABLE 38 Exemplary TBM sequences		
SEQ ID No	**Description**	**Sequence**
	Amino acid sequence	evhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalaapiektiskakgqprepqvytlppcreemtknqvslwclvkgfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk

9. SPECIFIC EMBODIMENTS, CITATION OF REFERENCES

[01019] While various specific embodiments have been illustrated and described, it will be appreciated that various changes can be made without departing from the spirit and scope of the disclosure(s).

[01020] The present disclosure is exemplified by the numbered embodiments set forth below.

1. A CD19 binding molecule that specifically binds to human CD19 and comprises

(a) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:14, SEQ ID NO:15, and SEQ ID NO:16; and

(b) a first variant human IgG1 Fc region and a second variant human IgG1 Fc region forming a Fc domain, wherein the first and second variant Fc regions comprise L234A, L235A, and G237A ("LALAGA") substitutions, L234A, L235A, S267K, and P329A ("LALASKPA") substitutions, D265A, P329A, and S267K ("DAPASK") substitutions, G237A, D265A, and P329A ("GADAPA") substitutions, G237A, D265A, P329A, and S267K ("GADAPASK") substitutions, L234A, L235A, and P329G ("LALAPG") substitutions, or L234A, L235A, and P329A ("LALAPA") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

2. A CD19 binding molecule that specifically binds to human CD19 and comprises

(a) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:17, SEQ ID NO:18, and SEQ ID NO:19; and

(b) a first variant human IgG1 Fc region and a second variant human IgG1 Fc region forming a Fc domain, wherein the first and second variant Fc regions comprise L234A, L235A, and G237A ("LALAGA") substitutions, L234A, L235A, S267K, and P329A

("LALASKPA") substitutions, D265A, P329A, and S267K ("DAPASK") substitutions, G237A, D265A, and P329A ("GADAPA") substitutions, G237A, D265A, P329A, and S267K ("GADAPASK") substitutions, L234A, L235A, and P329G ("LALAPG") substitutions, or L234A, L235A, and P329A ("LALAPA") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

3. A CD19 binding molecule that specifically binds to human CD19 and comprises

(a) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:20, SEQ ID NO:21, and SEQ ID NO:22; and

(b) a first variant human IgG1 Fc region and a second variant human IgG1 Fc region forming a Fc domain, wherein the first and second variant Fc regions comprise L234A, L235A, and G237A ("LALAGA") substitutions, L234A, L235A, S267K, and P329A ("LALASKPA") substitutions, D265A, P329A, and S267K ("DAPASK") substitutions, G237A, D265A, and P329A ("GADAPA") substitutions, G237A, D265A, P329A, and S267K ("GADAPASK") substitutions, L234A, L235A, and P329G ("LALAPG") substitutions, or L234A, L235A, and P329A ("LALAPA") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

4. A CD19 binding molecule that specifically binds to human CD19 and comprises

(a) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:23, SEQ ID NO:24, and SEQ ID NO:25; and

(b) a first human IgG1 variant Fc region and a second human IgG1 variant Fc region forming a Fc domain, wherein the first and second variant Fc regions comprise L234A, L235A, and G237A ("LALAGA") substitutions, L234A, L235A, S267K, and P329A ("LALASKPA") substitutions, D265A, P329A, and S267K ("DAPASK") substitutions, G237A, D265A, and P329A ("GADAPA") substitutions, G237A, D265A, P329A, and S267K ("GADAPASK") substitutions, L234A, L235A, and P329G ("LALAPG") substitutions, or L234A, L235A, and P329A ("LALAPA") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

5. The CD19 binding molecule of any one of embodiments 1 to 4, which comprises a VH having the amino acid sequence of SEQ ID NO:13.

6. The CD19 binding molecule of any one of embodiments 1 to 5, which comprises a VL having the amino acid sequence of SEQ ID NO:26.

7. A CD19 binding molecule that specifically binds to human CD19 and comprises

(a) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:27, SEQ ID NO:28, and SEQ ID NO:29, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:40, SEQ ID NO:41, and SEQ ID NO:42; and

(b) a first human IgG1 variant Fc region and a second human IgG1 variant Fc region forming a Fc domain, wherein the first and second variant Fc regions comprise L234A, L235A, and G237A ("LALAGA") substitutions, L234A, L235A, S267K, and P329A ("LALASKPA") substitutions, D265A, P329A, and S267K ("DAPASK") substitutions, G237A, D265A, and P329A ("GADAPA") substitutions, G237A, D265A, P329A, and S267K ("GADAPASK") substitutions, L234A, L235A, and P329G ("LALAPG") substitutions, or L234A, L235A, and P329A ("LALAPA") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

8. A CD19 binding molecule that specifically binds to human CD19 and comprises

(a) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:43, SEQ ID NO:44, and SEQ ID NO:45; and

(b) a first variant human IgG1 Fc region and a second variant human IgG1 Fc region forming a Fc domain, wherein the first and second variant Fc regions comprise L234A, L235A, and G237A ("LALAGA") substitutions, L234A, L235A, S267K, and P329A ("LALASKPA") substitutions, D265A, P329A, and S267K ("DAPASK") substitutions, G237A, D265A, and P329A ("GADAPA") substitutions, G237A, D265A, P329A, and S267K ("GADAPASK") substitutions, L234A, L235A, and P329G ("LALAPG") substitutions, or L234A, L235A, and P329A ("LALAPA") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

9. A CD19 binding molecule that specifically binds to human CD19 and comprises

(a) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:33, SEQ ID NO:34, and SEQ ID NO:35, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:46, SEQ ID NO:47, and SEQ ID NO:48; and

(b) a first variant human IgG1 Fc region and a second variant human IgG1 Fc region forming a Fc domain, wherein the first and second variant Fc regions comprise L234A, L235A, and G237A ("LALAGA") substitutions, L234A, L235A, S267K, and P329A ("LALASKPA") substitutions, D265A, P329A, and S267K ("DAPASK") substitutions, G237A, D265A, and P329A ("GADAPA") substitutions, G237A, D265A, P329A, and S267K ("GADAPASK") substitutions, L234A, L235A, and P329G ("LALAPG") substitutions, or L234A, L235A, and P329A ("LALAPA") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

10. A CD19 binding molecule that specifically binds to human CD19 and comprises

(a) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:36, SEQ ID NO:37, and SEQ ID NO:38, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:49, SEQ ID NO:50, and SEQ ID NO:51; and

(b) a first variant human IgG1 Fc region and a second variant human IgG1 Fc region forming a Fc domain, wherein the first and second variant Fc regions comprise L234A, L235A, and G237A ("LALAGA") substitutions, L234A, L235A, S267K, and P329A ("LALASKPA") substitutions, D265A, P329A, and S267K ("DAPASK") substitutions, G237A, D265A, and P329A ("GADAPA") substitutions, G237A, D265A, P329A, and S267K ("GADAPASK") substitutions, L234A, L235A, and P329G ("LALAPG") substitutions, or L234A, L235A, and P329A ("LALAPA") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

11. The CD19 binding molecule of any one of embodiments 7 to 10, which comprises a VH having the amino acid sequence of SEQ ID NO:39.

12. The CD19 binding molecule of any one of embodiments 7 to 11, which comprises a VL having the amino acid sequence of SEQ ID NO:52.

13. The CD19 binding molecule of any one of embodiments 1 to 12, which comprises an antibody, an antibody fragment, an scFv, a dsFv, a Fv, a Fab, an scFab, a (Fab')2, or a single domain antibody (SDAB).

14. The CD19 binding molecule of embodiment 13, which comprises an antibody or an antigen-binding domain thereof.

15. The CD19 binding molecule of embodiment 13, which comprises a scFv.

16. The CD19 binding molecule of any one of embodiments 1 to 12, which is a multispecific binding molecule (MBM) comprising:

(a) an antigen-binding module 1 (ABM1) that binds specifically to CD19; and

(b) an antigen-binding module 2 (ABM2) that binds specifically to a different target molecule, optionally wherein the target molecule is a component of a human T-cell receptor (TCR) complex.

17. The CD19 binding molecule of embodiment 16, wherein ABM1 is an antibody, an antibody fragment, an scFv, a dsFv, a Fv, a Fab, an scFab, a (Fab')2, a single domain antibody (SDAB), a VH or VL domain, or a camelid VHH domain.

18. The CD19 binding molecule of embodiment 17, wherein ABM1 is an scFv.

19. The CD19 binding molecule of embodiment 17, wherein ABM1 is a Fab.

20. The CD19 binding molecule of embodiment 17, wherein the Fab is a Fab heterodimer.

21. The CD19 binding molecule of embodiment 17, wherein ABM1 is an anti-CD19 antibody or an antigen-binding domain thereof.

22. The CD19 binding molecule of any one of embodiments 16 to 21, wherein ABM2 is a non-immunoglobulin scaffold based ABM.

23. The CD19 binding molecule of embodiment 22, wherein ABM2 is a Kunitz domain, an Adnexin, an Affibody, a DARPin, an Avimer, an Anticalin, a Lipocalin, a Centyrin, a Versabody, a Knottin, an Adnectin, a Pronectin, an Affitin/Nanofitin, an Affilin, an Atrimer/Tetranectin, a bicyclic peptide, a cys-knot, a Fn3 scaffold, an Obody, a Tn3, an Affimer, BD, an Adhiron, a Duocalin, an Alphabody, an Armadillo Repeat Protein, a Repebody, or a Fynomer

24. The CD19 binding molecule of any one of embodiments 16 to 21, wherein ABM2 is an immunoglobulin scaffold based ABM.

25. The CD19 binding molecule of embodiment 24, wherein ABM2 is an antibody, an antibody fragment, an scFv, a dsFv, a Fv, a Fab, an scFab, a (Fab')2, a single domain antibody (SDAB), a VH or VL domain, or a camelid VHH domain.

26. The CD19 binding molecule of embodiment 25, wherein ABM2 is an antibody or an antigen-binding domain thereof.

27. The CD19 binding molecule of embodiment 25, wherein ABM2 is an scFv.

28. The CD19 binding molecule of embodiment 25, wherein ABM2 is a Fab.

29. The CD19 binding molecule of embodiment 25, wherein ABM2 is a Fab heterodimer.

30. The CD19 binding molecule of any one of embodiments 16 to 29, in which ABM2 binds specifically to a component of a human T-cell receptor (TCR) complex.

31. The CD19 binding molecule of embodiment 30, wherein the component of the TCR complex is CD3.

32. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-1.

33. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-2.

34. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-3.

35. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-4.

36. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-5.

37. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-6.

38. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-7.

39. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-8.

40. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-9.

41. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-10.

42. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-11.

43. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-12.

44. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-13.

45. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-14.

46. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-15.

47. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-16.

48. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-17.

49. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-18.

50. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-19.

51. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-20.

52. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-21.

53. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-22.

54. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-23.

55. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-24.

56. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-25.

57. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-26.

58. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-27.

59. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-28.

60. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-29.

61. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-30.

62. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-31.

63. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-32.

64. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-33.

65. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-34.

66. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-35.

67. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-36.

68. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-37.

69. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-38.

70. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-39.

71. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-40.

72. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-41.

73. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-42.

74. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-43.

75. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-44.

76. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-45.

77. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-46.

78. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-47.

79. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-48.

80. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-49.

81. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-50.

82. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-51.

83. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-52.

84. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-53.

85. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-54.

86. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-55.

87. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-56.

88. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-57.

89. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-58.

90. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-59.

91. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-60.

92. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-61.

93. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-62.

94. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-63.

95. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-64.

96. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-65.

97. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-66.

98. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-67.

99. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-68.

100. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-69.

101. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-70.

102. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-71.

103. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-72.

104. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-73.

105. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-74.

106. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-75.

107. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-76.

108. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-77.

109. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-78.

110. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-79.

111. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-80.

112. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-81.

113. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-82.

114. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-83.

115. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-84.

116. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-85.

117. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-86.

118. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-87.

119. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-88.

120. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-89.

121. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-90.

122. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-91.

123. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-92.

124. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-93.

125. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-94.

126. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-95.

127. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-96.

128. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-97.

129. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-98.

130. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-99.

131. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-100.

132. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-101.

133. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-102.

134. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-103.

135. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-104.

136. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-105.

137. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-106.

138. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-107.

139. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-108.

140. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-109.

141. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-110.

142. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-111.

143. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-112.

144. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-113.

145. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-114.

146. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-115.

147. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-116.

148. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-117.

149. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-118.

150. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-119.

151. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-120.

152. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-121.

153. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-122.

154. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-123.

155. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-124.

156. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-125.

157. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-126.

158. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-127.

159. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-128.

160. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-129.

161. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the CDR sequences of CD3-130.

162. The CD19 binding molecule of any one of embodiments 32 to 161, wherein the CDRs are defined by Kabat numbering, as set forth in Table 12B.

163. The CD19 binding molecule of any one of embodiments 32 to 161, wherein the CDRs are defined by Chothia numbering, as set forth in Table 12C.

164. MBM of any one of embodiments 32 to 161, wherein the CDRs are defined by a combination of Kabat and Chothia numbering, as set forth in Table 12D.

165. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the heavy and light chain variable sequences of CD3-1, as set forth in Table 12A.

166. The of embodiment 31, wherein ABM2 comprises the heavy and light chain variable sequences of CD3-2, as set forth in Table 12A.

167. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the heavy and light chain variable sequences of CD3-3, as set forth in Table 12A.

168. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the heavy and light chain variable sequences of CD3-4, as set forth in Table 12A.

169. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the heavy and light chain variable sequences of CD3-5, as set forth in Table 12A.

170. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the heavy and light chain variable sequences of CD3-6, as set forth in Table 12A.

171. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the heavy and light chain variable sequences of CD3-7, as set forth in Table 12A.

172. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the heavy and light chain variable sequences of CD3-8, as set forth in Table 12A.

173. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the heavy and light chain variable sequences of CD3-9, as set forth in Table 12A.

174. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the heavy and light chain variable sequences of CD3-10, as set forth in Table 12A.

175. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the heavy and light chain variable sequences of CD3-11, as set forth in Table 12A.

176. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the heavy and light chain variable sequences of CD3-12, as set forth in Table 12A.

177. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the heavy and light chain variable sequences of CD3-13, as set forth in Table 12A.

178. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the heavy and light chain variable sequences of CD3-14, as set forth in Table 12A.

179. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the heavy and light chain variable sequences of CD3-15, as set forth in Table 12A.

180. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the heavy and light chain variable sequences of CD3-16, as set forth in Table 12A.

181. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the heavy and light chain variable sequences of CD3-17, as set forth in Table 12A.

182. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the heavy and light chain variable sequences of CD3-18, as set forth in Table 12A.

183. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the heavy and light chain variable sequences of CD3-19, as set forth in Table 12A.

184. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the heavy and light chain variable sequences of CD3-20, as set forth in Table 12A.

185. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the heavy and light chain variable sequences of CD3-21, as set forth in Table 12A.

186. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the heavy and light chain variable sequences of CD3-22, as set forth in Table 12A.

187. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the heavy and light chain variable sequences of CD3-23, as set forth in Table 12A.

188. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the heavy and light chain variable sequences of CD3-24, as set forth in Table 12A.

189. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the heavy and light chain variable sequences of CD3-25, as set forth in Table 12A.

190. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the heavy and light chain variable sequences of CD3-26, as set forth in Table 12A.

191. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the heavy and light chain variable sequences of CD3-27, as set forth in Table 12A.

192. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the heavy and light chain variable sequences of CD3-28, as set forth in Table 12A.

193. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the heavy and light chain variable sequences of CD3-129, as set forth in Table 12A.

194. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the heavy and light chain variable sequences of CD3-130, as set forth in Table 12A.

195. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the amino acid sequence of the scFv designated as CD3-12 in Table 12A.

196. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the amino acid sequence of the scFv designated as CD3-21 in Table 12A.

197. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the amino acid sequence of the scFv designated as CD3-22 in Table 12A.

198. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the amino acid sequence of the scFv designated as CD3-23 in Table 12A.

199. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the amino acid sequence of the scFv designated as CD3-24 in Table 12A.

200. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the amino acid sequence of the scFv designated as CD3-25 in Table 12A.

201. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the amino acid sequence of the scFv designated as CD3-26 in Table 12A.

202. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the amino acid sequence of the scFv designated as CD3-27 in Table 12A.

203. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the amino acid sequence of the scFv designated as CD3-28 in Table 12A.

204. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the amino acid sequence of the scFv designated as CD3-129 in Table 12A.

205. The CD19 binding molecule of embodiment 31, wherein ABM2 comprises the amino acid sequence of the scFv designated as CD3-130 in Table 12A.

206. The CD19 binding molecule of embodiment 30, wherein the component of the TCR complex is TCR-α, TCR-β, or a TCR-α/β dimer.

207. The CD19 binding molecule of embodiment 206, wherein the component of the TCR complex is TCR-α.

208. The CD19 binding molecule of embodiment 206, wherein the component of the TCR complex is TCR-β.

209. The CD19 binding molecule of embodiment 206, wherein the component of the TCR complex is a TCR-α/β dimer.

210. The CD19 binding molecule of embodiment 206, wherein ABM2 comprises the CDR sequences of BMA031.

211. The CD19 binding molecule of embodiment 210, wherein the CDR sequences are defined by Kabat numbering.

212. The CD19 binding molecule of embodiment 210, wherein the CDR sequences are defined by Chothia numbering.

213. The CD19 binding molecule of embodiment 210, wherein the CDR sequences are defined by a combination of Kabat and Chothia numbering.

214. The CD19 binding molecule of embodiment 210, wherein ABM2 comprises the heavy and light chain variable sequences of BMA031.

215. The CD19 binding molecule of embodiment 30, wherein the component of the TCR complex is TCR-γ, TCR-δ, or a TCR-γ/δ dimer.

216. The CD19 binding molecule of embodiment 215, wherein the component of the TCR complex is TCR-γ.

217. The CD19 binding molecule of embodiment 215, wherein the component of the TCR complex is TCR-δ.

218. The CD19 binding molecule of embodiment 215, wherein the component of the TCR complex is a TCR-γ/δ dimer.

219. The CD19 binding molecule of embodiment 215, wherein ABM2 comprises the CDR sequences of δTCS1.

220. The CD19 binding molecule of embodiment 219, wherein the CDR sequences are defined by Kabat numbering.

221. The CD19 binding molecule of embodiment 219, wherein the CDR sequences are defined by Chothia numbering.

222. The CD19 binding molecule of embodiment 219, wherein the CDR sequences are defined by a combination of Kabat and Chothia numbering.

223. The CD19 binding molecule of embodiment 219, wherein ABM2 comprises the heavy and light chain variable sequences of δTCS1.

224. The CD19 binding molecule of any one of embodiments 16 to 223, in which ABM1 is capable of binding CD19 at the same time as ABM2 is bound to its target molecule.

225. The CD19 binding molecule of any one of embodiments 16 to 224, which is a bispecific binding molecule (BBM).

226. The CD19 binding molecule of embodiment 225, which is bivalent.

227. The CD19 binding molecule of embodiment 226, wherein the CD19 binding molecule has any one of the configurations depicted in FIGS. 1B-1F.

228. The CD19 binding molecule of embodiment 227, wherein the CD19 binding molecule has the configuration depicted in FIG. 1B.

229. The CD19 binding molecule of embodiment 227, wherein the CD19 binding molecule has the configuration depicted in FIG. 1C.

230. The CD19 binding molecule of embodiment 227, wherein the CD19 binding molecule has the configuration depicted in FIG. 1D.

231. The CD19 binding molecule of embodiment 227, wherein the CD19 binding molecule has the configuration depicted in FIG. 1E.

232. The CD19 binding molecule of embodiment 227, wherein the CD19 binding molecule has the configuration depicted in FIG. 1F.

233. The CD19 binding molecule of any one of embodiments 227 to 232, which has the configuration referred to as B1 in Section 7.5.1.

234. The CD19 binding molecule of any one of embodiments 227 to 232, which has the configuration referred to as B2 in Section 7.5.1.

235. The CD19 binding molecule of embodiment 225, which is trivalent.

236. The CD19 binding molecule of embodiment 235, wherein the CD19 binding molecule has any one of the configurations depicted in FIGS. 1G-1Z.

237. The CD19 binding molecule of embodiment 236, wherein the CD19 binding molecule has the configuration depicted in FIG. 1G.

238. The CD19 binding molecule of embodiment 236, wherein the CD19 binding molecule has the configuration depicted in FIG. 1H.

239. The CD19 binding molecule of embodiment 236, wherein the CD19 binding molecule has the configuration depicted in FIG. 1I.

240. The CD19 binding molecule of embodiment 236, wherein the CD19 binding molecule has the configuration depicted in FIG. 1J.

241. The CD19 binding molecule of embodiment 236, wherein the CD19 binding molecule has the configuration depicted in FIG. 1K.

242. The CD19 binding molecule of embodiment 236, wherein the CD19 binding molecule has the configuration depicted in FIG. 1L.

243. The CD19 binding molecule of embodiment 236, wherein the CD19 binding molecule has the configuration depicted in FIG. 1M.

244. The CD19 binding molecule of embodiment 236, wherein the CD19 binding molecule has the configuration depicted in FIG. 1N.

245. The CD19 binding molecule of embodiment 236, wherein the CD19 binding molecule has the configuration depicted in FIG. 1O.

246. The CD19 binding molecule of embodiment 236, wherein the CD19 binding molecule has the configuration depicted in FIG. 1P.

247. The CD19 binding molecule of embodiment 236, wherein the CD19 binding molecule has the configuration depicted in FIG. 1Q.

248. The CD19 binding molecule of embodiment 236, wherein the CD19 binding molecule has the configuration depicted in FIG. 1R.

249. The CD19 binding molecule of embodiment 236, wherein the CD19 binding molecule has the configuration depicted in FIG. 1S.

250. The CD19 binding molecule of embodiment 236, wherein the CD19 binding molecule has the configuration depicted in FIG. 1T.

251. The CD19 binding molecule of embodiment 236, wherein the CD19 binding molecule has the configuration depicted in FIG. 1U.

252. The CD19 binding molecule of embodiment 236, wherein the CD19 binding molecule has the configuration depicted in FIG. 1V.

253. The CD19 binding molecule of embodiment 236, wherein the CD19 binding molecule has the configuration depicted in FIG. 1W.

254. The CD19 binding molecule of embodiment 236, wherein the CD19 binding molecule has the configuration depicted in FIG. 1X.

255. The CD19 binding molecule of embodiment 236, wherein the CD19 binding molecule has the configuration depicted in FIG. 1Y.

256. The CD19 binding molecule of embodiment 236, wherein the CD19 binding molecule has the configuration depicted in FIG. 1Z.

257. The CD19 binding molecule of any one of embodiments 235 to 256, which has the configuration referred to as T1 in Section 7.5.2.

258. The CD19 binding molecule of any one of embodiments 235 to 256, which has the configuration referred to as T2 in Section 7.5.2.

259. The CD19 binding molecule of any one of embodiments 235 to 256, which has the configuration referred to as T3 in Section 7.5.2.

260. The CD19 binding molecule of any one of embodiments 235 to 256, which has the configuration referred to as T4 in Section 7.5.2.

261. The CD19 binding molecule of any one of embodiments 235 to 256, which has the configuration referred to as T5 in Section 7.5.2.

262. The CD19 binding molecule of any one of embodiments 235 to 256, which has the configuration referred to as T6 in Section 7.5.2.

263. The CD19 binding molecule of embodiment 225, which is tetravalent.

264. The CD19 binding molecule of embodiment 263, wherein the CD19 binding molecule has any one of the configurations depicted in FIGS. 1AA-1AH.

265. The CD19 binding molecule of embodiment 264, wherein the CD19 binding molecule has the configuration depicted in FIG. 1AA.

266. The CD19 binding molecule of embodiment 264, wherein the CD19 binding molecule has the configuration depicted in FIG. 1AB.

267. The CD19 binding molecule of embodiment 264, wherein the CD19 binding molecule has the configuration depicted in FIG. 1AC.

268. The CD19 binding molecule of embodiment 264, wherein the CD19 binding molecule has the configuration depicted in FIG. 1AD.

269. The CD19 binding molecule of embodiment 264, wherein the CD19 binding molecule has the configuration depicted in FIG. 1AE.

270. The CD19 binding molecule of embodiment 264, wherein the CD19 binding molecule has the configuration depicted in FIG. 1AF.

271. The CD19 binding molecule of embodiment 264, wherein the CD19 binding molecule has the configuration depicted in FIG. 1AG.

272. The CD19 binding molecule of embodiment 264, wherein the CD19 binding molecule has the configuration depicted in FIG. 1AH.

273. The CD19 binding molecule of any one of embodiments 263 to 272, which has the configuration referred to as Tv1 in Section 7.5.3.

274. The CD19 binding molecule of any one of embodiments 263 to 272, which has the configuration referred to as Tv2 in Section 7.5.3.

275. The CD19 binding molecule of any one of embodiments 263 to 272, which has the configuration referred to as Tv3 in Section 7.5.3.

276. The CD19 binding molecule of any one of embodiments 263 to 272, which has the configuration referred to as Tv4 in Section 7.5.3.

277. The CD19 binding molecule of any one of embodiments 263 to 272, which has the configuration referred to as Tv5 in Section 7.5.3.

278. The CD19 binding molecule of any one of embodiments 263 to 272, which has the configuration referred to as Tv6 in Section 7.5.3.

279. The CD19 binding molecule of any one of embodiments 263 to 272, which has the configuration referred to as Tv7 in Section 7.5.3.

280. The CD19 binding molecule of any one of embodiments 263 to 272, which has the configuration referred to as Tv8 in Section 7.5.3.

281. The CD19 binding molecule of any one of embodiments 263 to 272, which has the configuration referred to as Tv9 in Section 7.5.3.

282. The CD19 binding molecule of any one of embodiments 263 to 272, which has the configuration referred to as Tv10 in Section 7.5.3.

283. The CD19 binding molecule of any one of embodiments 263 to 272, which has the configuration referred to as Tv11 in Section 7.5.3.

284. The CD19 binding molecule of any one of embodiments 263 to 272, which has the configuration referred to as Tv12 in Section 7.5.3.

285. The CD19 binding molecule of any one of embodiments 263 to 272, which has the configuration referred to as Tv13 in Section 7.5.3.

286. The CD19 binding molecule of any one of embodiments 263 to 272, which has the configuration referred to as Tv14 in Section 7.5.3.

287. The CD19 binding molecule of any one of embodiments 16 to 224, which is a trispecific binding molecule (TBM) comprising an antigen-binding module 3 (ABM3) that binds specifically to a target molecule other than CD19.

288. The CD19 binding molecule of embodiment 287, in which ABM2 binds specifically to a component of a human T-cell receptor (TCR) complex and ABM3 binds specifically to (i) human CD2 or (ii) a tumor associated antigen (TAA).

289. The CD19 binding molecule of embodiment 287 or embodiment 288, which is trivalent.

290. The CD19 binding molecule of embodiment 289, wherein the CD19 binding molecule has any one of the configurations depicted in FIGS. 2A-2P.

291. The CD19 binding molecule of embodiment 290, wherein the CD19 binding molecule has the configuration depicted in FIG. 2A.

292. The CD19 binding molecule of embodiment 290, wherein the CD19 binding molecule has the configuration depicted in FIG. 2B.

293. The CD19 binding molecule of embodiment 290, wherein the CD19 binding molecule has the configuration depicted in FIG. 2C.

294. The CD19 binding molecule of embodiment 290, wherein the CD19 binding molecule has the configuration depicted in FIG. 2D.

295. The CD19 binding molecule of embodiment 290, wherein the CD19 binding molecule has the configuration depicted in FIG. 2E.

296. The CD19 binding molecule of embodiment 290, wherein the CD19 binding molecule has the configuration depicted in FIG. 2F.

297. The CD19 binding molecule of embodiment 290, wherein the CD19 binding molecule has the configuration depicted in FIG. 2G.

298. The CD19 binding molecule of embodiment 290, wherein the CD19 binding molecule has the configuration depicted in FIG. 2H.

299. The CD19 binding molecule of embodiment 290, wherein the CD19 binding molecule has the configuration depicted in FIG. 2I.

300. The CD19 binding molecule of embodiment 290, wherein the CD19 binding molecule has the configuration depicted in FIG. 2J.

301. The CD19 binding molecule of embodiment 290, wherein the CD19 binding molecule has the configuration depicted in FIG. 2K.

302. The CD19 binding molecule of embodiment 290, wherein the CD19 binding molecule has the configuration depicted in FIG. 2L.

303. The CD19 binding molecule of embodiment 290, wherein the CD19 binding molecule has the configuration depicted in FIG. 2M.

304. The CD19 binding molecule of embodiment 290, wherein the CD19 binding molecule has the configuration depicted in FIG. 2N.

305. The CD19 binding molecule of embodiment 290, wherein the CD19 binding molecule has the configuration depicted in FIG. 2O.

306. The CD19 binding molecule of embodiment 290, wherein the CD19 binding molecule has the configuration depicted in FIG. 2P.

307. The CD19 binding molecule of any one of embodiments 290 to 306, which has the configuration referred to as T1 in Section 7.6.1.

308. CD19 binding molecule of any one of embodiments 290 to 306, which has the configuration referred to as T2 in Section 7.6.1.

309. CD19 binding molecule of any one of embodiments 290 to 306, which has the configuration referred to as T3 in Section 7.6.1.

310. CD19 binding molecule of any one of embodiments 290 to 306, which has the configuration referred to as T4 in Section 7.6.1.

311. CD19 binding molecule of any one of embodiments 290 to 306, which has the configuration referred to as T5 in Section 7.6.1.

312. CD19 binding molecule of any one of embodiments 290 to 306, which has the configuration referred to as T6 in Section 7.6.1.

313. The CD19 binding molecule of embodiment 287 or embodiment 288, which is tetravalent.

314. The CD19 binding molecule of embodiment 313, wherein the CD19 binding molecule has any one of the configurations depicted in FIGS. 2Q-2S.

315. The CD19 binding molecule of embodiment 314, wherein the CD19 binding molecule has the configuration depicted in FIG. 2Q.

316. The CD19 binding molecule of embodiment 314, wherein the CD19 binding molecule has the configuration depicted in FIG. 2R.

317. The CD19 binding molecule of embodiment 314, wherein the CD19 binding molecule has the configuration depicted in FIG. 2S.

318. The CD19 binding molecule of embodiment 287 or embodiment 288, which is pentavalent.

319. The CD19 binding molecule of embodiment 318, wherein the CD19 binding molecule has the configuration depicted in FIG. 2T.

320. The CD19 binding molecule of embodiment 287 or embodiment 288, which is hexavalent.

321. The CD19 binding molecule of embodiment 320, wherein the CD19 binding molecule has any one of the configurations depicted in FIGS. 2U-2V.

322. The CD19 binding molecule of embodiment 321, wherein the CD19 binding molecule has the configuration depicted in FIG. 2U.

323. The CD19 binding molecule of embodiment 321, wherein the CD19 binding molecule has the configuration depicted in FIG. 2V.

324. The CD19 binding molecule of any one of embodiments 287 to 323, in which ABM1 is capable of binding CD19 at the same time ABM3 is bound to its target molecule.

325. The CD19 binding molecule of any one of embodiments 287 to 324, in which ABM3 binds specifically to human CD2.

326. The CD19 binding molecule of embodiment 325, wherein ABM3 is a non-immunoglobulin scaffold based ABM.

327. The CD19 binding molecule of embodiment 326, wherein ABM3 is a Kunitz domain, an Adnexin, an Affibody, a DARPin, an Avimer, an Anticalin, a Lipocalin, a Centyrin, a Versabody, a Knottin, an Adnectin, a Pronectin, an Affitin/Nanofitin, an Affilin, an Atrimer/Tetranectin, a bicyclic peptide, a cys-knot, a Fn3 scaffold, an Obody, a Tn3, an Affimer, BD, an Adhiron, a Duocalin, an Alphabody, an Armadillo Repeat Protein, a Repebody, or a Fynomer.

328. The CD19 binding molecule of embodiment 326, wherein ABM3 comprises a receptor binding domain of a CD2 ligand.

329. The CD19-binding molecule of embodiment 325, wherein ABM3 is a CD58 moiety.

330. The CD19-binding molecule of embodiment 329, wherein the CD58 moiety comprises the amino acid sequence of CD58-1 as set forth in Table 15.

331. The CD19-binding molecule of embodiment 329, wherein the CD58 moiety comprises the amino acid sequence of CD58-2 as set forth in Table 15.

332. The CD19-binding molecule of embodiment 329, wherein the CD58 moiety comprises the amino acid sequence of CD58-3 as set forth in Table 15.

333. The CD19-binding molecule of embodiment 329, wherein the CD58 moiety comprises the amino acid sequence of CD58-4 as set forth in Table 15.

334. The CD19-binding molecule of embodiment 329, wherein the CD58 moiety comprises the amino acid sequence of CD58-5 as set forth in Table 15.

335. The CD19-binding molecule of embodiment 334, wherein the amino acid designated as B is a phenylalanine.

336. The CD19-binding molecule of embodiment 334, wherein the amino acid designated as B is a serine.

337. The CD19-binding molecule of any one of embodiments 334 to 336, wherein the amino acid designated as J is a valine.

338. The CD19-binding molecule of any one of embodiments 334 to 336, wherein the amino acid designated as J is a lysine.

339. The CD19-binding molecule of any one of embodiments 334 to 338, wherein the amino acid designated as O is a valine.

340. The CD19-binding molecule of any one of embodiments 334 to 338, wherein the amino acid designated as O is a glutamine.

341. The CD19-binding molecule of any one of embodiments 334 to 340, wherein the amino acid designated as U is a valine.

342. The CD19-binding molecule of any one of embodiments 334 to 340, wherein the amino acid designated as U is a lysine.

343. The CD19-binding molecule of any one of embodiments 334 to 342, wherein the amino acid designated as X is a threonine.

344. The CD19-binding molecule of any one of embodiments 334 to 342, wherein the amino acid designated as X is a serine.

345. The CD19-binding molecule of any one of embodiments 334 to 344, wherein the amino acid designated as Z is a leucine.

346. The CD19-binding molecule of any one of embodiments 334 to 344, wherein the amino acid designated as Z is a glycine.

347. The CD19-binding molecule of embodiment 329, wherein the CD58 moiety comprises the amino acid sequence of CD58-6 as set forth in Table 15.

348. The CD19-binding molecule of embodiment 329, wherein the CD58 moiety comprises the amino acid sequence of CD58-7 as set forth in Table 15.

349. The CD19-binding molecule of embodiment 348, wherein the amino acid designated as J is a valine.

350. The CD19-binding molecule of embodiment 348, wherein the amino acid designated as J is a lysine.

351. The CD19-binding molecule of any one of embodiments 348 to 350, wherein the amino acid designated as O is a valine.

352. The CD19-binding molecule of any one of embodiments 348 to 350, wherein the amino acid designated as O is a glutamine.

353. The CD19-binding molecule of embodiment 329, wherein the CD58 moiety comprises the amino acid sequence of CD58-8 as set forth in Table 15.

354. The CD19-binding molecule of embodiment 329, wherein the CD58 moiety comprises the amino acid sequence of CD58-9 as set forth in Table 15.

355. The CD19-binding molecule of embodiment 329, wherein the CD58 moiety comprises the amino acid sequence of CD58-10 as set forth in Table 15.

356. The CD19-binding molecule of embodiment 329, wherein the CD58 moiety comprises the amino acid sequence of CD58-11 as set forth in Table 15.

357. The CD19-binding molecule of embodiment 325, wherein ABM3 is a CD48 moiety.

358. The CD19 binding molecule of embodiment 357, wherein the CD48 moiety has at least 70% sequence identity to amino acids 27-220 of the amino acid sequence of Uniprot identifier P09326.

359. The CD19 binding molecule of embodiment 357, wherein the CD48 moiety has at least 80% sequence identity to amino acids 27-220 of the amino acid sequence ofUniprot identifier P09326.

360. The CD19 binding molecule of embodiment 357, wherein the CD48 moiety has at least 90% sequence identity to amino acids 27-220 of the amino acid sequence of Uniprot identifier P09326.

361. The CD19 binding molecule of embodiment 357, wherein the CD48 moiety has at least 95% sequence identity to amino acids 27-220 of the amino acid sequence of Uniprot identifier P09326.

362. The CD19 binding molecule of embodiment 357, wherein the CD48 moiety has at least 99% sequence identity to amino acids 27-220 of the amino acid sequence of Uniprot identifier P09326.

363. The CD19 binding molecule of embodiment 325, wherein ABM3 is an immunoglobulin scaffold based ABM.

364. The CD19 binding molecule of embodiment 363, wherein ABM3 is an antibody, an antibody fragment, an scFv, a dsFv, a Fv, a Fab, an scFab, a (Fab')2, a single domain antibody (SDAB), a VH or VL domain, or a camelid VHH domain.

365. The CD19-binding molecule of embodiment 363, wherein ABM3 is an anti-CD2 antibody or an antigen-binding domain thereof.

366. The CD19 binding molecule of embodiment 364, wherein ABM3 is an scFv.

367. The CD19 binding molecule of embodiment 364, wherein ABM3 is a Fab.

368. The CD19 binding molecule of embodiment 367, wherein ABM3 is a Fab heterodimer.

369. The CD19 binding molecule of any one of embodiments 363 to 368, wherein ABM3 comprises the CDR sequences of CD2-1.

370. The CD19 binding molecule of embodiment 369, wherein ABM3 comprises the heavy and light chain variable sequences of CD2-1.

371. The CD19 binding molecule of embodiment 369, wherein ABM3 comprises the heavy and light chain variable sequences of hu1CD2-1.

372. The CD19 binding molecule of embodiment 369, wherein ABM3 comprises the heavy and light chain variable sequences of hu2CD2-1.

373. The CD19 binding molecule of embodiment 369, wherein ABM3 comprises the CDR sequences of Medi 507.

374. The CD19 binding molecule of embodiment 373, wherein ABM3 comprises the heavy and light chain variable sequences of Medi 507.

375. The CD19 binding molecule of any one of embodiments 287 to 324, in which ABM3 binds specifically to a human TAA.

376. The CD19 binding molecule of embodiment 375, wherein ABM3 is a non-immunoglobulin scaffold based ABM.

377. The CD19 binding molecule of embodiment 376, wherein if TAA is a receptor, ABM3 comprises a receptor binding domain of a ligand of the receptor, and if TAA is a ligand, ABM3 comprises a ligand binding domain of a receptor of the ligand.

378. The CD19 binding molecule of embodiment 376, wherein ABM3 is a Kunitz domain, an Adnexin, an Affibody, a DARPin, an Avimer, an Anticalin, a Lipocalin, a Centyrin, a Versabody, a Knottin, an Adnectin, a Pronectin, an Affitin/Nanofitin, an Affilin, an Atrimer/Tetranectin, a bicyclic peptide, a cys-knot, a Fn3 scaffold, an Obody, a Tn3, an Affimer, BD, an Adhiron, a Duocalin, an Alphabody, an Armadillo Repeat Protein, a Repebody, or a Fynomer.

379. The CD19 binding molecule of embodiment 375, wherein ABM3 is an immunoglobulin scaffold based ABM.

380. The CD19 binding molecule of embodiment 379, wherein ABM3 is an antibody, an antibody fragment, an scFv, a dsFv, a Fv, a Fab, an scFab, a (Fab')2, a single domain antibody (SDAB), a VH or VL domain, or a camelid VHH domain.

381. The CD19 binding molecule of embodiment 380, wherein ABM3 is an antibody or an antigen-binding domain thereof.

382. The CD19 binding molecule of embodiment 380, wherein ABM3 is an scFv.

383. The CD19 binding molecule of embodiment 380, wherein ABM3 is a Fab.

384. The CD19 binding molecule of embodiment 383, wherein ABM3 is a Fab heterodimer.

385. The CD19 binding molecule of any one of embodiments 375 to 384, wherein the TAA is a TAA expressed on cancerous B cells that are B cell-derived plasma cells.

386. The CD19 binding molecule of any one of embodiments 375 to 385, wherein the TAA is a TAA expressed on cancerous B cells that are not plasma cells.

387. The CD19 binding molecule of any one of embodiments 375 to 386, wherein the TAA is selected from BCMA, CD20, CD22, CD123, CD33, CLL1, CD138, CS1, CD38, CD133, FLT3, CD52, TNFRSF13C, TNFRSF13B, CXCR4, PD-L1, LY9, CD200, FCGR2B, CD21, CD23, CD24, CD40L, CD72, CD79a, and CD79b.

388. The CD19 binding molecule of embodiment 387, wherein the TAA is BCMA.

389. The CD19 binding molecule of embodiment 387, wherein the TAA is CD20.

390. The CD19 binding molecule of embodiment 387, wherein the TAA is CD22.

391. The CD19 binding molecule of embodiment 387, wherein the TAA is CD123.

392. The CD19 binding molecule of embodiment 387, wherein the TAA is CD33.

393. The CD19 binding molecule of embodiment 387, wherein the TAA is CLL1.

394. The CD19 binding molecule of embodiment 387, wherein the TAA is CD138.

395. The CD19 binding molecule of embodiment 387, wherein the TAA is CS1.

396. The CD19 binding molecule of embodiment 387, wherein the TAA is CD38.

397. The CD19 binding molecule of embodiment 387, wherein the TAA is CD133.

398. The CD19 binding molecule of embodiment 387, wherein the TAA is FLT3.

399. The CD19 binding molecule of embodiment 387, wherein the TAA is CD52.

400. The CD19 binding molecule of embodiment 387, wherein the TAA is TNFRSF13C.

401. The CD19 binding molecule of embodiment 387, wherein the TAA is TNFRSF13B.

402. The CD19 binding molecule of embodiment 387, wherein the TAA is CXCR4.

403. The CD19 binding molecule of embodiment 387, wherein the TAA is PD-L1.

404. The CD19 binding molecule of embodiment 387, wherein the TAA is LY9.

405. The CD19 binding molecule of embodiment 387, wherein the TAA is CD200.

406. The CD19 binding molecule of embodiment 387, wherein the TAA is FCGR2B.

407. The CD19 binding molecule of embodiment 387, wherein the TAA is CD21.

408. The CD19 binding molecule of embodiment 387, wherein the TAA is CD23.

409. The CD19 binding molecule of embodiment 387, wherein ABM3 comprises a binding sequence set forth in Table 16 or Table 17.

410. The CD19 binding molecule of embodiment 409, which has the heavy and / or light chain variable regions of any one of BCMA-1 to BCMA-40 as set forth in Table 17A.

411. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-1, as set forth in Table 17A.

412. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-2, as set forth in Table 17A.

413. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-3, as set forth in Table 17A.

414. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-4, as set forth in Table 17A.

415. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-5, as set forth in Table 17A.

416. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-6, as set forth in Table 17A.

417. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-7, as set forth in Table 17A.

418. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-8, as set forth in Table 17A.

419. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-9, as set forth in Table 17A.

420. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-10, as set forth in Table 17A.

421. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-11, as set forth in Table 17A.

422. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-12, as set forth in Table 17A.

423. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-13, as set forth in Table 17A.

424. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-14, as set forth in Table 17A.

425. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-15, as set forth in Table 17A.

426. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-16, as set forth in Table 17A.

427. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-17, as set forth in Table 17A.

428. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-18, as set forth in Table 17A.

429. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-19, as set forth in Table 17A.

430. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-20, as set forth in Table 17A.

431. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-21, as set forth in Table 17A.

432. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-22, as set forth in Table 17A.

433. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-23, as set forth in Table 17A.

434. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-24, as set forth in Table 17A.

435. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-25, as set forth in Table 17A.

436. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-26, as set forth in Table 17A.

437. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-27, as set forth in Table 17A.

438. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-28, as set forth in Table 17A.

439. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-29, as set forth in Table 17A.

440. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-30, as set forth in Table 17A.

441. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-31, as set forth in Table 17A.

442. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-32, as set forth in Table 17A.

443. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-33, as set forth in Table 17A.

444. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-34, as set forth in Table 17A.

445. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-35, as set forth in Table 17A.

446. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-36, as set forth in Table 17A.

447. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-37, as set forth in Table 17A.

448. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-38, as set forth in Table 17A.

449. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-39, as set forth in Table 17A.

450. The CD19 binding molecule of embodiment 410, wherein ABM3 comprises the heavy and light chain variable sequences of BCMA-40, as set forth in Table 17A.

451. The CD19 binding molecule of embodiment 409, which has the heavy and / or light chain CDR sequences (as defined by Kabat) of any one of BCMA-1 to BCMA-40 as set forth in Tables 17B and 17E.

452. The CD19 binding molecule of embodiment 409, which has the heavy and / or light chain CDR sequences (as defined by Chothia) of any one of BCMA-1 to BCMA-40 as set forth in Tables 17C and 17F.

453. The CD19 binding molecule of embodiment 409, which has the heavy and / or light chain CDR sequences (as defined by a combination of Kabat and Chothia sequences) of any one of BCMA-1 to BCMA-40 as set forth in Tables 17D and 17G.

454. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-1.

455. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-2.

456. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-3.

457. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-4.

458. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-5.

459. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-6.

460. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-7.

461. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-8.

462. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-9.

463. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-10.

464. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-11.

465. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-12.

466. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-13.

467. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-14.

468. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-15.

469. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-16.

470. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-17.

471. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-18.

472. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-19.

473. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-20.

474. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-21.

475. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-22.

476. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-23.

477. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-24.

478. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-25.

479. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-26.

480. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-27.

481. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-28.

482. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-29.

483. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-30.

484. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-31.

485. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-32.

486. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-33.

487. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-34.

488. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-35.

489. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-36.

490. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-37.

491. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-38.

492. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-39.

493. The CD19 binding molecule of any one of embodiments 451 to 453, wherein ABM3 comprises the CDR sequences of BCMA-40.

494. The CD19 binding molecule of any one of embodiments 1 to 493, wherein the first variant Fc region and the second variant Fc region comprise L234A, L235A, and G237A ("LALAGA") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

495. The CD19 binding molecule of any one of embodiments 1 to 493, wherein the first variant Fc region and the second variant Fc region comprise L234A, L235A, S267K, and P329A ("LALASKPA") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

496. The CD19 binding molecule of any one of embodiments 1 to 493, wherein the first variant Fc region and the second variant Fc region comprise D265A, P329A, and S267K ("DAPASK") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

497. The CD19 binding molecule of any one of embodiments 1 to 493, wherein the first variant Fc region and the second variant Fc region comprise G237A, D265A, and P329A ("GADAPA") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

498. The CD19 binding molecule of any one of embodiments 1 to 493, wherein the first variant Fc region and the second variant Fc region comprise G237A, D265A, P329A, and S267K ("GADAPASK") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

499. The CD19 binding molecule of any one of embodiments 1 to 493, wherein the first variant Fc region and the second variant Fc region comprise L234A, L235A, and P329G ("LALAPG") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

500. The CD19 binding molecule of any one of embodiments 1 to 493, wherein the first variant Fc region and the second variant Fc region comprise L234A, L235A, and P329A ("LALAPA") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

501. The CD19 binding molecule of any one of embodiments 1 to 500, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 95% identity to FCV-1, FCV-2, FCV-3, FCV-4, FCV-5, FCV-6, or FCV-7.

502. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 95% identity to FCV-1.

503. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 96% identity to FCV-1.

504. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 97% identity to FCV-1.

505. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 98% identity to FCV-1.

506. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 99% identity to FCV-1.

507. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 95% identity to FCV-2.

508. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 96% identity to FCV-2.

509. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 97% identity to FCV-2.

510. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 98% identity to FCV-2.

511. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 99% identity to FCV-2.

512. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 95% identity to FCV-3.

513. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 96% identity to FCV-3.

514. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 97% identity to FCV-3.

515. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 98% identity to FCV-3.

516. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 99% identity to FCV-3.

517. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 95% identity to FCV-4.

518. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 96% identity to FCV-4.

519. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 97% identity to FCV-4.

520. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 98% identity to FCV-4.

521. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 99% identity to FCV-4.

522. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 95% identity to FCV-5.

523. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 96% identity to FCV-5.

524. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 97% identity to FCV-5.

525. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 98% identity to FCV-5.

526. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 99% identity to FCV-5.

527. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 95% identity to FCV-6.

528. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 96% identity to FCV-6.

529. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 97% identity to FCV-6.

530. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 98% identity to FCV-6.

531. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 99% identity to FCV-6.

532. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 95% identity to FCV-7.

533. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 96% identity to FCV-7.

534. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 97% identity to FCV-7.

535. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 98% identity to FCV-7.

536. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 99% identity to FCV-7.

537. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having 100% identity to FCV-1.

538. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having 100% identity to FCV-2.

539. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having 100% identity to FCV-3.

540. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having 100% identity to FCV-4.

541. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having 100% identity to FCV-5.

542. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having 100% identity to FCV-6.

543. The CD19 binding molecule of embodiment 501, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having 100% identity to FCV-7.

544. The CD19 binding molecule of any one of embodiments 1 to 543, wherein the first variant Fc region and the second variant Fc region together form an Fc heterodimer.

545. The CD19 binding molecule of embodiment 544, wherein the first and second variant Fc regions comprise the amino acid substitutions S364K/E357Q : L368D/K370S.

546. The CD19 binding molecule of embodiments 544, wherein the first and second variant Fc regions comprise the amino acid substitutions L368D/K370S : S364K.

547. The CD19 binding molecule of embodiment 544, wherein the first and second variant Fc regions comprise the amino acid substitutions L368E/K370S : S364K.

548. The CD19 binding molecule of embodiment 544, wherein the first and second variant Fc regions comprise the amino acid substitutions T411T/E360E/Q362E : D401K.

549. The CD19 binding molecule of embodiment 544, wherein the first and second variant Fc regions comprise the amino acid substitutions L368D 370S : S364 /E357L.

550. The CD19 binding molecule of embodiment 544, wherein the first and second variant Fc regions comprise the amino acid substitutions 370S : S364K/E357Q.

551. The CD19 binding molecule of embodiment 544, wherein the first and second variant Fc regions comprise the amino acid substitutions of any one of the steric variants listed in Figure 4 of WO 2014/110601 (reproduced in Table 4).

552. The CD19 binding molecule of embodiment 544, wherein the first and second variant Fc regions comprise the amino acid substitutions of any one of the variants listed in Figure 5 of WO 2014/110601 (reproduced in Table 4).

553. The CD19 binding molecule of embodiment 544, wherein the first and second variant Fc regions comprise the amino acid substitutions of any one of the variants listed in Figure 6 of WO 2014/110601 (reproduced in Table 4).

554. The CD19 binding molecule of any one of embodiments 1 to 553, wherein the first variant Fc region and/or the second variant Fc region further comprises an ablation variant modification selected from the ablation variant modifications set forth in Table 3.

555. The CD19 binding molecule of embodiment 554, wherein the ablation variant modification comprises G236R.

556. The CD19 binding molecule of embodiment 554, wherein the ablation variant modification comprises S239G.

557. The CD19 binding molecule of embodiment 554, wherein the ablation variant modification comprises S239K.

558. The CD19 binding molecule of embodiment 554, wherein the ablation variant modification comprises S239Q.

559. The CD19 binding molecule of embodiment 554, wherein the ablation variant modification comprises S239R.

560. The CD19 binding molecule of embodiment 554, wherein the ablation variant modification comprises V266D.

561. The CD19 binding molecule of embodiment 554, wherein the ablation variant modification comprises H268K.

562. The CD19 binding molecule of embodiment 554, wherein the ablation variant modification comprises E269R.

563. The CD19 binding molecule of embodiment 554, wherein the ablation variant modification comprises 299R.

564. The CD19 binding molecule of embodiment 554, wherein the ablation variant modification comprises 299K.

565. The CD19 binding molecule of embodiment 554, wherein the ablation variant modification comprises K322A.

566. The CD19 binding molecule of embodiment 554, wherein the ablation variant modification comprises A327G.

567. The CD19 binding molecule of embodiment 554, wherein the ablation variant modification comprises A327L.

568. The CD19 binding molecule of embodiment 554, wherein the ablation variant modification comprises A327N.

569. The CD19 binding molecule of embodiment 554, wherein the ablation variant modification comprises A327Q.

570. The CD19 binding molecule of embodiment 554, wherein the ablation variant modification comprises L328E.

571. The CD19 binding molecule of embodiment 554, wherein the ablation variant modification comprises L328R.

572. The CD19 binding molecule of embodiment 554, wherein the ablation variant modification comprises A330L.

573. The CD19 binding molecule of embodiment 554, wherein the ablation variant modification comprises A330S/P331S.

574. The CD19 binding molecule of embodiment 554, wherein the ablation variant modification comprises I332K.

575. The CD19 binding molecule of embodiment 554, wherein the ablation variant modification comprises I332R.

576. The CD19 binding molecule of embodiment 554, wherein the ablation variant modification comprises V266D/A327Q.

577. The CD19 binding molecule of embodiment 554, wherein the ablation variant modification comprises G236R/L328R.

578. The CD19 binding molecule of any one of embodiments 554 to 577, wherein both variant Fc regions comprise the ablation variant modification.

579. The CD19 binding molecule of any one of embodiments 1 to 578, wherein at least one of the Fc regions further comprises pI variant substitutions.

580. The CD19 binding molecule of embodiment 579 wherein the pI variant substitutions are selected from Table 4.

581. The CD19 binding molecule of embodiment 580, wherein the pI variant substitutions comprise the substitutions present in pI_ISO(-).

582. The CD19 binding molecule of embodiment 580, wherein the pI variant substitutions comprise the substitutions present in pI_(-)_isosteric_A.

583. The CD19 binding molecule of embodiment 580, wherein the pI variant substitutions comprise the substitutions present in pI_(-)_isosteric_B.

584. The CD19 binding molecule of embodiment 580, wherein the pI variant substitutions comprise the substitutions present in PI_ISO(+RR).

585. The CD19 binding molecule of embodiment 580, wherein the pI variant substitutions comprise the substitutions present in pI_ISO(+).

586. The CD19 binding molecule of embodiment 580, wherein the pI variant substitutions comprise the substitutions present in pI_(+)_isosteric_A.

587. The CD19 binding molecule of embodiment 580, wherein the pI variant substitutions comprise the substitutions present in pI_(+)_isosteric_B.

588. The CD19 binding molecule of embodiment 580, wherein the pI variant substitutions comprise the substitutions present in pI_(+)_isosteric_E269Q/E272Q.

589. The CD19 binding molecule of embodiment 580, wherein the pI variant substitutions comprise the substitutions present in pI_(+)_isosteric_E269Q/E283Q.

590. The CD19 binding molecule of embodiment 580, wherein the pI variant substitutions comprise the substitutions present in pI_(+)_isosteric_E2720/E283Q.

591. The CD19 binding molecule of embodiment 580, wherein the pI variant substitutions comprise the substitutions present in pI_(+)_isosteric_E269Q.

592. The CD19 binding molecule of any one of embodiments 1 to 591, wherein the first and/or second Fc region further comprises one or more amino acid substitution(s) selected from 434A, 434S, 428L, 308F, 259I, 428L/434S, 259I/308F, 436I/428L, 4361 or V/434S, 436V/428L, 252Y, 252Y/254T/256E, 259I/308F/428L, 236A, 239D, 239E, 332E, 332D, 239D/332E, 267D, 267E, 328F, 267E/328F, 236A/332E, 239D/332E/330Y, 239D, 332E/330L, 236R, 328R, 236R/328R, 236N/267E, 243L, 298A and 299T.

593. The CD19 binding molecule of any one of embodiments 1 to 591, wherein the first and/or second Fc region further comprises the amino acid substitution 434A, 434S or 434V.

594. The CD19 binding molecule of embodiment 593, wherein the first and/or second Fc region further comprises the amino acid substitution 428L.

595. The CD19 binding molecule of any one of embodiments 593 to 594, wherein the first and/or second Fc region further the amino acid substitution 308F.

596. The CD19 binding molecule of any one of embodiments 593 to 595, wherein the first and/or second Fc region further comprises the amino acid substitution 259I.

597. The CD19 binding molecule of any one of embodiments 593 to 596, wherein the first and/or second Fc region further comprises the amino acid substitution 436I.

598. The CD19 binding molecule of any one of embodiments 593 to 597, wherein the first and/or second Fc region further comprises the amino acid substitution 252Y.

599. The CD19 binding molecule of any one of embodiments 593 to 598, wherein the first and/or second Fc region further comprises the amino acid substitution 254T.

600. The CD19 binding molecule of any one of embodiments 593 to 599, wherein the first and/or second Fc region further comprises the amino acid substitution 256E.

601. The CD19 binding molecule of any one of embodiments 593 to 600, wherein the first and/or second Fc region further comprises the amino acid substitution 239D or 239E.

602. The CD19 binding molecule of any one of embodiments 593 to 601, wherein the first and/or second Fc region further comprises the amino acid substitution 332E or 332D.

603. The CD19 binding molecule of any one of embodiments 593 to 602, wherein the first and/or second Fc region further comprises the amino acid substitution 267D or 267E.

604. The CD19 binding molecule of any one of embodiments 593 to 603, wherein the first and/or second Fc region further comprises the amino acid substitution 330L.

605. The CD19 binding molecule of any one of embodiments 593 to 604, wherein the first and/or second Fc region further comprises the amino acid substitution 236R or 236N.

606. The CD19 binding molecule of any one of embodiments 593 to 605, wherein the first and/or second Fc region further comprises the amino acid substitution 328R.

607. The CD19 binding molecule of any one of embodiments 593 to 606, wherein the first and/or second Fc region further comprises the amino acid substitution 243L.

608. The CD19 binding molecule of any one of embodiments 593 to 607, wherein the first and/or second Fc region further comprises the amino acid substitution 298A.

609. The CD19 binding molecule of any one of embodiments 593 to 608, wherein the first and/or second Fc region further comprises the amino acid substitution 299T.

610. The CD19 binding molecule of any one of embodiments 1 to 609, wherein the first variant Fc region comprises the pI variant substitutions N208D/Q295E/N384D/Q418E/N421D (pI_(-)_isosteric_A).

611. The CD19 binding molecule of any one of embodiments 1 to 610, wherein the second variant Fc region comprises the pI variant substitutions N208D/Q295E/N384D/Q418E/N421D (pI_(-)_isosteric_A).

612. The CD19 binding molecule of embodiment 544, wherein the Fc heterodimer comprises any of the Fc modifications set forth in Table 4.

613. The CD19 binding molecule of embodiment 544, wherein the Fc heterodimer comprises knob-in-hole ("KIH") modifications.

614. The CD19 binding molecule of embodiment 613, wherein the first and second variant Fc regions comprise the amino acid substitutions T366W : T366S/L368A/Y407V.

615. The CD19 binding molecule of any one of 1 to 614, wherein the Fc domain has reduced or undetectable binding affinity to a Fc gamma receptor compared to a CD19 binding molecule comprising a wild-type human IgG1 Fc domain, optionally measured by surface plasmon resonance using a Biacore T200 instrument, wherein the Fc gamma receptor is selected from the group consisting of Fc gamma RIA and Fc gamma RIIIa V158 variant.

616. The CD19 binding molecule of any one of embodiments 1 to 615, wherein the CD19 binding molecule has reduced or undetectable effector function compared to a CD19 binding molecule comprising the wild-type human IgG1 Fc domain.

617. The CD19 binding molecule of any one of 1 to 616, wherein the binding molecule is capable of binding to one or more antigens without triggering detectable antibody-

dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), or complement dependent cytotoxicity (CDC).

618. The CD19 binding molecule of any one of embodiments 1 to 617, wherein the Fc domain has modified disulfide bond architecture.

619. The CD19 binding molecule of any one of embodiments 1 to 618, wherein the Fc domain has altered glycosylation patterns.

620. The CD19 binding molecule of any one of embodiments 1 to 619, wherein the Fc domain comprises a hinge region.

621. The CD19 binding molecule of embodiment 620, wherein the hinge region comprises any one of the hinge regions described in Section 7.4.2.

622. The CD19 binding molecule of embodiment 621, wherein the hinge region comprises the amino acid sequence of the hinge region designated H1.

623. The CD19 binding molecule of embodiment 621, wherein the hinge region comprises the amino acid sequence of the hinge region designated H2.

624. The CD19 binding molecule of embodiment 621, wherein the hinge region comprises the amino acid sequence of the hinge region designated H3.

625. The CD19 binding molecule of embodiment 621, wherein the hinge region comprises the amino acid sequence of the hinge region designated H4.

626. The CD19 binding molecule of embodiment 621, wherein the hinge region comprises the amino acid sequence of the hinge region designated H5.

627. The CD19 binding molecule of embodiment 621, wherein the hinge region comprises the amino acid sequence of the hinge region designated H6.

628. The CD19 binding molecule of embodiment 621, wherein the hinge region comprises the amino acid sequence of the hinge region designated H7.

629. The CD19 binding molecule of embodiment 621, wherein the hinge region comprises the amino acid sequence of the hinge region designated H8.

630. The CD19 binding molecule of embodiment 621, wherein the hinge region comprises the amino acid sequence of the hinge region designated H9.

631. The CD19 binding molecule of embodiment 621, wherein the hinge region comprises the amino acid sequence of the hinge region designated H10.

632. The CD19 binding molecule of embodiment 621, wherein the hinge region comprises the amino acid sequence of the hinge region designated H11.

633. The CD19 binding molecule of embodiment 621, wherein the hinge region comprises the amino acid sequence of the hinge region designated H12.

634. The CD19 binding molecule of embodiment 621, wherein the hinge region comprises the amino acid sequence of the hinge region designated H13.

635. The CD19 binding molecule of embodiment 621, wherein the hinge region comprises the amino acid sequence of the hinge region designated H14.

636. The CD19 binding molecule of embodiment 621, wherein the hinge region comprises the amino acid sequence of the hinge region designated H15.

637. The CD19 binding molecule of embodiment 621, wherein the hinge region comprises the amino acid sequence of the hinge region designated H16.

638. . The CD19 binding molecule of embodiment 621, wherein the hinge region comprises the amino acid sequence of the hinge region designated H17.

639. The CD19 binding molecule of embodiment 621, wherein the hinge region comprises the amino acid sequence of the hinge region designated H18.

640. The CD19 binding molecule of embodiment 621, wherein the hinge region comprises the amino acid sequence of the hinge region designated H19.

641. The CD19 binding molecule of embodiment 621, wherein the hinge region comprises the amino acid sequence of the hinge region designated H20.

642. The CD19 binding molecule of embodiment 621, wherein the hinge region comprises the amino acid sequence of the hinge region designated H21.

643. The CD19 binding molecule of any one of embodiments 16 to 642, which comprises at least one scFv domain.

644. The CD19 binding molecule of embodiment 643, wherein at least one scFv comprises a linker connecting the VH and VL domains.

645. The CD19 binding molecule of embodiment 644, wherein the linker is 5 to 25 amino acids in length.

646. The CD19 binding molecule of embodiment 645, wherein the linker is 12 to 20 amino acids in length.

647. The CD19 binding molecule of any one of embodiments 644 to 646, wherein the linker is a charged linker and/or a flexible linker.

648. The CD19 binding molecule of any one of embodiments 644 to 647, wherein the linker is selected from any one of linkers L1 through L54.

649. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L1.

650. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L2.

651. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L3.

652. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L4.

653. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L5.

654. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L6.

655. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L7.

656. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L8.

657. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L9.

658. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L10.

659. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L11.

660. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L12.

661. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L13.

662. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L14.

663. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L15.

664. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L16.

665. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L17.

666. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L18.

667. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L19.

668. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L20.

669. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L21.

670. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L22.

671. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L23.

672. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L24.

673. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L25.

674. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L26.

675. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L27.

676. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L28.

677. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L29.

678. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L30.

679. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L31.

680. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L32.

681. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L33.

682. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L34.

683. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L35.

684. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L36.

685. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L37.

686. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L38.

687. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L39.

688. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L40.

689. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L41.

690. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L42.

691. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L43.

692. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L44.

693. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L45.

694. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L46.

695. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L47.

696. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L48.

697. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L49.

698. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L50.

699. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L51.

700. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L52.

701. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L53.

702. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L54.

703. The CD19 binding molecule of any one of embodiments 16 to 702, which comprises at least one Fab domain.

704. The CD19 binding molecule of embodiment 703, wherein at least one Fab domain comprises any of the Fab heterodimerization modifications set forth in Table 2.

705. The CD19 binding molecule of embodiment 704, wherein at least one Fab domain comprises the Fab heterodimerization modifications designated as F1.

706. The CD19 binding molecule of embodiment 704, wherein at least one Fab domain comprises the Fab heterodimerization modifications designated as F2.

707. The CD19 binding molecule of embodiment 704, wherein at least one Fab domain comprises the Fab heterodimerization modifications designated as F3.

708. The CD19 binding molecule of embodiment 704, wherein at least one Fab domain comprises the Fab heterodimerization modifications designated as F4.

709. The CD19 binding molecule of embodiment 704, wherein at least one Fab domain comprises the Fab heterodimerization modifications designated as F5.

710. The CD19 binding molecule of embodiment 704, wherein at least one Fab domain comprises the Fab heterodimerization modifications designated as F6.

711. The CD19 binding molecule of embodiment 704, wherein at least one Fab domain comprises the Fab heterodimerization modifications designated as F7.

712. The CD19 binding molecule of any one of embodiments 16 to 711, which comprises at least two ABMs, an ABM and an ABM chain, or two ABM chains connected to one another via a linker.

713. The CD19 binding molecule of embodiment 712, wherein the linker is 5 to 25 amino acids in length.

714. The CD19 binding molecule of embodiment 713, wherein the linker is 12 to 20 amino acids in length.

715. The CD19 binding molecule of any one of embodiments 712 to 714, wherein the linker is a charged linker and/or a flexible linker.

716. The CD19 binding molecule of any one of embodiments 712 to 715, wherein the linker is selected from any one of linkers L1 through L54.

717. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L1.

718. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L2.

719. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L3.

720. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L4.

721. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L5.

722. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L6.

723. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L7.

724. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L8.

725. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L9.

726. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L10.

727. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L11.

728. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L12.

729. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L13.

730. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L14.

731. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L15.

732. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L16.

733. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L17.

734. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L18.

735. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L19.

736. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L20.

737. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L21.

738. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L22.

739. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L23.

740. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L24.

741. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L25.

742. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L26.

743. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L27.

744. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L28.

745. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L29.

746. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L30.

747. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L31.

748. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L32.

749. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L33.

750. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L34.

751. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L35.

752. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L36.

753. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L37.

754. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L38.

755. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L39.

756. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L40.

757. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L41.

758. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L42.

759. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L43.

760. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L44.

761. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L45.

762. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L46.

763. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L47.

764. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L48.

765. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L49.

766. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L50.

767. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L51.

768. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L52.

769. The CD19 binding molecule of embodiment 648, wherein the linker region comprises the amino acid sequence of the linker designated L53.

770. The CD19 binding molecule of embodiment 716, wherein the linker region comprises the amino acid sequence of the linker designated L54.

771. A CD19 binding molecule comprising:

(a) a first polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1120;

(b) a second polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1122; and

(c) a third polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1110.

772. A CD19 binding molecule comprising:

(a) a first polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1127;

(b) a second polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1129; and

(c) a third polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1110.

773. A CD19 binding molecule comprising:

(a) a first polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1124;

(b) a second polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1126; and

(c) a third polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1110.

774. A CD19 binding molecule comprising:

(a) a first polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1134;

(b) a second polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1135; and

(c) a third polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1110.

775. A CD19 binding molecule comprising:

(a) a first polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1136;

(b) a second polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1137; and

(c) a third polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1110.

776. A CD19 binding molecule comprising:

(a) a first polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1138;

(b) a second polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1139; and

(c) a third polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1110.

777. A CD19 binding molecule comprising:

(a) a first polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1140;

(b) a second polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1141; and

(c) a third polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1110.

778. A CD19 binding molecule comprising:

(a) a first polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1142;

(b) a second polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1143; and

(c) a third polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1110.

779. A CD19 binding molecule comprising:

(a) a first polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1077;

(b) a second polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:759; and

(c) a third polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1078 or SEQ ID NO:1086.

780. A CD19 binding molecule comprising:

(a) a first polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1079;

(b) a second polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:759; and

(c) a third polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1078 or SEQ ID NO:1086.

781. A CD19 binding molecule comprising:

(a) a first polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1077;

(b) a second polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:759; and

(c) a third polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1086.

782. A CD19 binding molecule comprising the sequences of a construct shown in Table 19-A1, 19A-2, 19-B, or 19C, preferably Table 19C.

783. A CD19 binding molecule comprising:

(a) a first polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1131;

(b) a second polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1132; and

(c) a third polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1133.

784. A conjugate comprising (a) the CD19 binding molecule of any one of embodiments 1 to 783, and (b) an agent.

785. The conjugate of embodiment 784, wherein the agent is a therapeutic agent, a diagnostic agent, a masking moiety, a cleavable moiety, a stabilizing agent, or any combination thereof.

786. The conjugate of embodiment 784, wherein the agent is any of the agents described in Section 7.12.

787. The conjugate of any one of embodiments 784 to 786, wherein the CD19-binding molecule is conjugated to a radionuclide.

788. The conjugate of any one of embodiments 784 to 786, wherein the CD19-binding molecule is conjugated to an alkylating agent.

789. The conjugate of any one of embodiments 784 to 786, wherein the CD19-binding molecule is conjugated to a topoisomerase inhibitor, which is optionally a topoisomerase I inhibitor or a topoisomerase II inhibitor.

790. The conjugate of any one of embodiments 784 to 786, wherein the CD19-binding molecule is conjugated to a DNA damaging agent.

791. The conjugate of any one of embodiments 784 to 786, wherein the CD19-binding molecule is conjugated to a DNA intercalating agent, optionally a groove binding agent such as a minor groove binding agent.

792. The conjugate of any one of embodiments 784 to 786, wherein the CD19-binding molecule is conjugated to a RNA/DNA antimetabolite.

793. The conjugate of any one of embodiments 784 to 786, wherein the CD19-binding molecule is conjugated to a kinase inhibitor.

794. The conjugate of any one of embodiments 784 to 786, wherein the CD19-binding molecule is conjugated to a protein synthesis inhibitor.

795. The conjugate of any one of embodiments 784 to 786, wherein the CD19-binding molecule is conjugated to a histone deacetylase (HDAC) inhibitor.

796. The conjugate of any one of embodiments 784 to 786, wherein the CD19-binding molecule is conjugated to a mitochondrial inhibitor, which is optionally an inhibitor of a phosphoryl transfer reaction in mitochondria.

797. The conjugate of any one of embodiments 784 to 786, wherein the CD19-binding molecule is conjugated to an antimitotic agent.

798. The conjugate of any one of embodiments 784 to 786, wherein the CD19-binding molecule is conjugated to a maytansinoid.

799. The conjugate of any one of embodiments 784 to 786, wherein the CD19-binding molecule is conjugated to a kinesin inhibitor.

800. The conjugate of any one of embodiments 784 to 786, wherein the CD19-binding molecule is conjugated to a kinesin-like protein KIF11 inhibitor.

801. The conjugate of any one of embodiments 784 to 786, wherein the CD19-binding molecule is conjugated to a V-ATPase (vacuolar-type H+ -ATPase) inhibitor.

802. The conjugate of any one of embodiments 784 to 786, wherein the CD19-binding molecule is conjugated to a pro-apoptotic agent.

803. The conjugate of any one of embodiments 784 to 786, wherein the CD19-binding molecule is conjugated to a Bcl2 (B-cell lymphoma 2) inhibitor.

804. The conjugate of any one of embodiments 784 to 786, wherein the CD19-binding molecule is conjugated to an MCL1 (myeloid cell leukemia 1) inhibitor.

805. The conjugate of any one of embodiments 784 to 786, wherein the CD19-binding molecule is conjugated to a HSP90 (heat shock protein 90) inhibitor.

806. The conjugate of any one of embodiments 784 to 786, wherein the CD19-binding molecule is conjugated to an IAP (inhibitor of apoptosis) inhibitor.

807. The conjugate of any one of embodiments 784 to 786, wherein the CD19-binding molecule is conjugated to an mTOR (mechanistic target of rapamycin) inhibitor.

808. The conjugate of any one of embodiments 784 to 786, wherein the CD19-binding molecule is conjugated to a microtubule stabilizer.

809. The conjugate of any one of embodiments 784 to 786, wherein the CD19-binding molecule is conjugated to a microtubule destabilizer.

810. The conjugate of any one of embodiments 784 to 786, wherein the CD19-binding molecule is conjugated to an auristatin.

811. The conjugate of any one of embodiments 784 to 786, wherein the CD19-binding molecule is conjugated to a dolastatin.

812. The conjugate of any one of embodiments 784 to 786, wherein the CD19-binding molecule is conjugated to a MetAP (methionine aminopeptidase).

813. The conjugate of any one of embodiments 784 to 786, wherein the CD19-binding molecule is conjugated to a CRM1 (chromosomal maintenance 1) inhibitor.

814. The conjugate of any one of embodiments 784 to 786, wherein the CD19-binding molecule is conjugated to a DPPIV (dipeptidyl peptidase IV) inhibitor.

815. The conjugate of any one of embodiments 784 to 786, wherein the CD19-binding molecule is conjugated to a proteasome inhibitor.

816. The conjugate of any one of embodiments 784 to 786, wherein the CD19-binding molecule is conjugated to a protein synthesis inhibitor.

817. The conjugate of any one of embodiments 784 to 786, wherein the CD19-binding molecule is conjugated to a CDK2 (cyclin-dependent kinase 2) inhibitor.

818. The conjugate of any one of embodiments 784 to 786, wherein the CD19-binding molecule is conjugated to a CDK9 (cyclin-dependent kinase 9) inhibitor.

819. The conjugate of any one of embodiments 784 to 786, wherein the CD19-binding molecule is conjugated to a RNA polymerase inhibitor.

820. The conjugate of any one of embodiments 784 to 786, wherein the CD19-binding molecule is conjugated to a DHFR (dihydrofolate reductase) inhibitor.

821. The conjugate of any one of embodiments 784 to 820, wherein the agent is attached to the CD19 binding molecule with a linker, which is optionally a cleavable linker or a non-cleavable linker.

822. The conjugate of any one of embodiments 784 to 821, which comprises an anti-CD19 binding molecule.

823. A pharmaceutical composition comprising (a) the CD19 binding molecule of any one of embodiments 1 to 783 or the conjugate of any one of embodiments 784 to 822 and (b) an excipient.

824. A method of treating a subject with a CD19-associated disease or disorder, comprising administering to the subject an effective amount of the CD19 binding molecule of any one of embodiments 1 to 783, the conjugate of any one of embodiments 784 to 822, or the pharmaceutical composition of embodiment 823.

825. The method of embodiment 824, wherein the CD19-associated disease or disorder is cancer.

826. The method of embodiment 824, wherein the disease or disorder is a plasma cell neoplasm.

827. The method of embodiment 824, wherein the disease or disorder is a B cell malignancy that expresses cell surface CD19.

828. The method of embodiment 824, wherein the disease or disorder is non-Hodgkin's lymphoma.

829. The method of embodiment 824, wherein the disease or disorder is diffuse large B-cell lymphoma (DLBCL).

830. The method of embodiment 829, wherein the DLBCL is relapsed or refractory DLBCL.

831. The method of embodiment 824, wherein the disease or disorder is Burkitt's lymphoma.

832. The method of embodiment 824, wherein the CD19-associated disease or disorder is a B cell malignancy.

833. The method of embodiment 832, wherein the B cell malignancy is a hematological cancer.

834. The method of embodiment 832, wherein the B cell malignancy is a malignant lymphoproliferative condition

835. The method of embodiment 832, wherein the B cell malignancy is a plasma cell dyscrasia.

836. The method of embodiment 832, wherein the B cell malignancy is an acute leukemia.

837. The method of embodiment 832, wherein the B cell malignancy is B cell acute lymphocytic leukemia (B-ALL).

838. The method of embodiment 832, wherein the B cell malignancy is relapsed and/or refractory B cell acute lymphocytic leukemia (B-ALL).

839. The method of embodiment 832, wherein the B cell malignancy is a non-Hodgkin's lymphoma (NHL).

840. The method of embodiment 832, wherein the B cell malignancy is a relapsed and/or refractory non-Hodgkin's lymphoma (NHL).

841. The method of embodiment 832, wherein the B cell malignancy is chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL).

842. The method of embodiment 832, wherein the B cell malignancy is relapsed and/or refractory chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL).

843. The method of embodiment 832, wherein the B cell malignancy is follicular lymphoma (FL), optionally wherein the FL is small cell FL or large cell FL.

844. The method of embodiment 832, wherein the B cell malignancy is relapsed and/or refractory follicular lymphoma (FL), optionally wherein the FL is small cell FL or large cell FL.

845. The method of embodiment 832, wherein the B cell malignancy is mantle cell lymphoma (MCL).

846. The method of embodiment 832, wherein the B cell malignancy is relapsed and/or refractory mantle cell lymphoma (MCL).

847. The method of embodiment 832, wherein the B cell malignancy is diffuse large B-cell lymphoma (DLBCL).

848. The method of embodiment 832, wherein the B cell malignancy is relapsed and/or refractory diffuse large B-cell lymphoma (DLBCL).

849. The method of embodiment 832, wherein the B cell malignancy is Burkitt lymphoma.

850. The method of embodiment 832, wherein the B cell malignancy is lymphoplasmacytic lymphoma (Waldenstrom macroglobulinemia).

851. The method of embodiment 832, wherein the B cell malignancy is MALT lymphoma (mucosa-associated lymphoid tissue lymphoma).

852. The method of embodiment 832, wherein the B cell malignancy is marginal zone lymphoma (MZL).

853. The method of embodiment 832, wherein the B cell malignancy is extranodal marginal zone lymphoma (EMZL).

854. The method of embodiment 832, wherein the B cell malignancy is nodal marginal zone B-cell lymphoma (NZML).

855. The method of embodiment 832, wherein the B cell malignancy is splenic marginal zone B-cell lymphoma (SMZL).

856. The method of any one of embodiments 839 to 855, wherein the subject has failed at least one prior line of standard of care therapy.

857. The method of embodiment 856, wherein the subject has failed up to five prior lines of standard of care therapies.

858. The method of embodiment 856 or embodiment 857, wherein the subject has failed one prior line of standard of care therapy.

859. The method of embodiment 856 or embodiment 857, wherein the subject has failed two prior lines of standard of care therapy.

860. The method of embodiment 856 or embodiment 857, wherein the subject has failed three prior lines of standard of care therapy.

861. The method of embodiment 856 or embodiment 857, wherein the subject has failed four prior lines of standard of care therapy.

862. The method of embodiment 856 or embodiment 857, wherein the subject has failed five prior lines of standard of care therapy.

863. The method of any one of embodiments 856 to 862, wherein the at least one prior line of standard of care therapies comprise an anti-CD20 therapy.

864. The method of embodiment 863, wherein the anti-CD20 therapy is rituximab.

865. The method of any one of embodiments 856 to 864, wherein the subject is intolerant to or ineligible for one or more other approved therapies.

866. The method of embodiment 865, wherein the one or more other approved therapies comprise an autologous stem cell transplant (ASCT).

867. The method of any one of embodiments 856 to 866, wherein the subject is a non-responder to a CAR composition.

868. The method of embodiment 867, wherein the CAR composition is an anti-CD19 CAR composition.

869. The method of embodiment 867 or embodiment 868, wherein the CAR composition comprises CTL019, tisagenlecleucel, axicabtagene ciloleucel, brexucabtagene autoleucel or lisocabtagene maraleucel.

870. The method of embodiment 832, wherein the B cell malignancy is a Hodgkin's lymphoma.

871. The method of embodiment 832, wherein the B cell malignancy is multiple myeloma.

872. The method of embodiment 832, wherein the B cell malignancy is hairy cell leukemia.

873. The method of embodiment 832, wherein the B cell malignancy is primary effusion lymphoma.

874. The method of embodiment 832, wherein the B cell malignancy is B cell prolymphocytic leukemia.

875. The method of embodiment 832, wherein the B cell malignancy is is plasmablastic lymphoma.

876. The method of embodiment 832, wherein the B cell malignancy is follicle center lymphoma.

877. The method of embodiment 832, wherein the B cell malignancy is precursor B-lymphoblastic leukemia.

878. The method of embodiment 832, wherein the B cell malignancy is high-grade B-cell lymphoma.

879. The method of embodiment 832, wherein the B cell malignancy is primary mediastinal large B-cell lymphoma.

880. The method of embodiment 824, wherein the CD19-associated disease or disorder is an autoimmune or inflammatory disorder.

881. The method of any of embodiments 824 to 880, further comprising administering at least one further agent to the subject.

882. The method of embodiment 881, wherein the at least one further agent comprises an immunomodulatory imide drug (IMiD).

883. The method of embodiment 882, wherein the immunomodulatory imide drug (IMiD) is lenalidomide, thalidomide, pomalidomide, or iberdomide.

884. The method of embodiment 883, wherein the immunomodulatory imide drug (IMiD) is lenalidomide.

885. A nucleic acid or plurality of nucleic acids encoding the CD19-binding molecule of any one of embodiments 1 to 783.

886. The nucleic acid or plurality of nucleic acids of embodiment 885 which is a DNA (are DNAs).

887. The nucleic acid or plurality of nucleic acids of embodiment 886 which are in the form of one or more vectors, optionally expression vectors.

888. The nucleic acid or plurality of nucleic acids of embodiment 885 which is a mRNA (are mRNAs).

889. A cell engineered to express the CD19 binding molecule of any one embodiments 1 to 783.

890. A cell transfected with one or more expression vectors comprising one or more nucleic acid sequences encoding the CD19 binding molecule of any one embodiments 1 to 783 under the control of one or more promoters.

891. The cell of embodiment 889 or embodiment 890, wherein expression of the CD19 binding molecule is under the control of an inducible promoter.

892. The cell of any one of embodiments 889 to 891, wherein the CD19 binding molecule is produced in secretable form.

893. A method of producing a CD19 binding molecule, comprising:

(a) culturing the cell of any one of embodiments 889 to 892 in conditions under which the CD19 binding molecule is expressed; and

(b) recovering the CD19-binding molecule from the cell culture.

10. INCORPORATION BY REFERENCE

[01021] All publications, patents, patent applications and other documents cited in this application are hereby incorporated by reference in their entireties for all purposes to the same extent as if each individual publication, patent, patent application or other document were individually indicated to be incorporated by reference for all purposes. In the event that there are any inconsistencies between the teachings of one or more of the references incorporated herein and the present disclosure, the teachings of the present specification are intended.

WHAT IS CLAIMED IS:

1. A CD19 binding molecule that specifically binds to human CD19 and comprises

(a) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:14, SEQ ID NO:15, and SEQ ID NO:16; and

(b) a first variant human IgG1 Fc region and a second variant human IgG1 Fc region forming a Fc domain, wherein the first and second variant Fc regions comprise L234A, L235A, and G237A ("LALAGA") substitutions, L234A, L235A, S267K, and P329A ("LALASKPA") substitutions, D265A, P329A, and S267K ("DAPASK") substitutions, G237A, D265A, and P329A ("GADAPA") substitutions, G237A, D265A, P329A, and S267K ("GADAPASK") substitutions, L234A, L235A, and P329G ("LALAPG") substitutions, or L234A, L235A, and P329A ("LALAPA") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

2. A CD19 binding molecule that specifically binds to human CD19 and comprises

(a) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:17, SEQ ID NO:18, and SEQ ID NO:19; and

(b) a first variant human IgG1 Fc region and a second variant human IgG1 Fc region forming a Fc domain, wherein the first and second variant Fc regions comprise L234A, L235A, and G237A ("LALAGA") substitutions, L234A, L235A, S267K, and P329A ("LALASKPA") substitutions, D265A, P329A, and S267K ("DAPASK") substitutions, G237A, D265A, and P329A ("GADAPA") substitutions, G237A, D265A, P329A, and S267K ("GADAPASK") substitutions, L234A, L235A, and P329G ("LALAPG") substitutions, or L234A, L235A, and P329A ("LALAPA") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

3. A CD19 binding molecule that specifically binds to human CD19 and comprises

(a) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:20, SEQ ID NO:21, and SEQ ID NO:22; and

(b) a first variant human IgG1 Fc region and a second variant human IgG1 Fc region forming a Fc domain, wherein the first and second variant Fc regions comprise L234A, L235A, and G237A ("LALAGA") substitutions, L234A, L235A, S267K, and P329A ("LALASKPA") substitutions, D265A, P329A, and S267K ("DAPASK") substitutions, G237A, D265A, and P329A ("GADAPA") substitutions, G237A, D265A, P329A, and S267K ("GADAPASK") substitutions, L234A, L235A, and P329G ("LALAPG") substitutions, or L234A, L235A, and P329A ("LALAPA") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

4. A CD19 binding molecule that specifically binds to human CD19 and comprises

(a) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:23, SEQ ID NO:24, and SEQ ID NO:25; and

(b) a first variant human IgG1 Fc region and a second variant human IgG1 Fc region forming a Fc domain, wherein the first and second variant Fc regions comprise L234A, L235A, and G237A ("LALAGA") substitutions, L234A, L235A, S267K, and P329A ("LALASKPA") substitutions, D265A, P329A, and S267K ("DAPASK") substitutions, G237A, D265A, and P329A ("GADAPA") substitutions, G237A, D265A, P329A, and S267K ("GADAPASK") substitutions, L234A, L235A, and P329G ("LALAPG") substitutions, or L234A, L235A, and P329A ("LALAPA") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

5. The CD19 binding molecule of any one of claims 1 to 4, which comprises a VH having the amino acid sequence of SEQ ID NO:13.

6. The CD19 binding molecule of any one of claims 1 to 5, which comprises a VL having the amino acid sequence of SEQ ID NO:26.

7. A CD19 binding molecule that specifically binds to human CD19 and comprises

(a) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:27, SEQ ID NO:28, and SEQ ID NO:29, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:40, SEQ ID NO:41, and SEQ ID NO:42; and

(b) a first variant human IgG1 Fc region and a second variant human IgG1 Fc region forming a Fc domain, wherein the first and second variant Fc regions comprise L234A, L235A, and G237A ("LALAGA") substitutions, L234A, L235A, S267K, and P329A

("LALASKPA") substitutions, D265A, P329A, and S267K ("DAPASK") substitutions, G237A, D265A, and P329A ("GADAPA") substitutions, G237A, D265A, P329A, and S267K ("GADAPASK") substitutions, L234A, L235A, and P329G ("LALAPG") substitutions, or L234A, L235A, and P329A ("LALAPA") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

8. A CD19 binding molecule that specifically binds to human CD19 and comprises

(a) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:43, SEQ ID NO:44, and SEQ ID NO:45; and

(b) a first variant human IgG1 Fc region and a second variant human IgG1 Fc region forming a Fc domain, wherein the first and second variant Fc regions comprise L234A, L235A, and G237A ("LALAGA") substitutions, L234A, L235A, S267K, and P329A ("LALASKPA") substitutions, D265A, P329A, and S267K ("DAPASK") substitutions, G237A, D265A, and P329A ("GADAPA") substitutions, G237A, D265A, P329A, and S267K ("GADAPASK") substitutions, L234A, L235A, and P329G ("LALAPG") substitutions, or L234A, L235A, and P329A ("LALAPA") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

9. A CD19 binding molecule that specifically binds to human CD19 and comprises

(a) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:33, SEQ ID NO:34, and SEQ ID NO:35, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:46, SEQ ID NO:47, and SEQ ID NO:48; and

(b) a first variant human IgG1 Fc region and a second variant human IgG1 Fc region forming a Fc domain, wherein the first and second variant Fc regions comprise L234A, L235A, and G237A ("LALAGA") substitutions, L234A, L235A, S267K, and P329A ("LALASKPA") substitutions, D265A, P329A, and S267K ("DAPASK") substitutions, G237A, D265A, and P329A ("GADAPA") substitutions, G237A, D265A, P329A, and S267K ("GADAPASK") substitutions, L234A, L235A, and P329G ("LALAPG") substitutions, or L234A, L235A, and P329A ("LALAPA") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

10. A CD19 binding molecule that specifically binds to human CD19 and comprises

(a) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:36, SEQ ID NO:37, and SEQ ID NO:38, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:49, SEQ ID NO:50, and SEQ ID NO:51; and

(b) a first variant human IgG1 Fc region and a second variant human IgG1 Fc region forming a Fc domain, wherein the first and second variant Fc regions comprise L234A, L235A, and G237A ("LALAGA") substitutions, L234A, L235A, S267K, and P329A ("LALASKPA") substitutions, D265A, P329A, and S267K ("DAPASK") substitutions, G237A, D265A, and P329A ("GADAPA") substitutions, G237A, D265A, P329A, and S267K ("GADAPASK") substitutions, L234A, L235A, and P329G ("LALAPG") substitutions, or L234A, L235A, and P329A ("LALAPA") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

11. The CD19 binding molecule of any one of claims 7 to 10, which comprises a VH having the amino acid sequence of SEQ ID NO:39.

12. The CD19 binding molecule of any one of claims 7 to 11, which comprises a VL having the amino acid sequence of SEQ ID NO:52.

13. The CD19 binding molecule of any one of claims 1 to 12, which is a multispecific binding molecule (MBM) comprising:

(a) an antigen-binding module 1 (ABM1) that binds specifically to CD19; and

(b) an antigen-binding module 2 (ABM2) that binds specifically to a different target molecule, optionally wherein the target molecule is a component of a human T-cell receptor (TCR) complex.

14. The CD19 binding molecule of claim 13, in which ABM2 binds specifically to a component of a human T-cell receptor (TCR) complex.

15. The CD19 binding molecule of claim 13, which is a bispecific binding molecule (BBM).

16. The CD19 binding molecule of claim 15, in which ABM2 binds specifically to a component of a human T-cell receptor (TCR) complex.

17. The CD19 binding molecule of claim 15 or claim 16, which is bivalent.

18. The CD19 binding molecule of claim 15 or claim 16, which is trivalent.

19. The CD19 binding molecule of claim 18, which has the configuration referred to as T1 in Section 7.5.2.

20. The CD19 binding molecule of claim 18, which has the configuration referred to as T2 in Section 7.5.2.

21. The CD19 binding molecule of claim 18, which has the configuration referred to as T3 in Section 7.5.2.

22. The CD19 binding molecule of claim 18, which has the configuration referred to as T4 in Section 7.5.2.

23. The CD19 binding molecule of claim 18, which has the configuration referred to as T5 in Section 7.5.2.

24. The CD19 binding molecule of claim 18, which has the configuration referred to as T6 in Section 7.5.2.

25. The CD19 binding molecule of claim 15 or claim 16, which is tetravalent.

26. The CD19 binding molecule of claim 25, which has the configuration referred to as Tv1 in Section 7.5.3.

27. The CD19 binding molecule of claim 25, which has the configuration referred to as Tv2 in Section 7.5.3.

28. The CD19 binding molecule of claim 25, which has the configuration referred to as Tv3 in Section 7.5.3.

29. The CD19 binding molecule of claim 25, which has the configuration referred to as Tv4 in Section 7.5.3.

30. The CD19 binding molecule of claim 25, which has the configuration referred to as Tv5 in Section 7.5.3.

31. The CD19 binding molecule of claim 25, which has the configuration referred to as Tv6 in Section 7.5.3.

32. The CD19 binding molecule of claim 25, which has the configuration referred to as Tv7 in Section 7.5.3.

33. The CD19 binding molecule of claim 25, which has the configuration referred to as Tv8 in Section 7.5.3.

34. The CD19 binding molecule of claim 25, which has the configuration referred to as Tv9 in Section 7.5.3.

35. The CD19 binding molecule of claim 25, which has the configuration referred to as Tv10 in Section 7.5.3.

36. The CD19 binding molecule of claim 25, which has the configuration referred to as Tv11 in Section 7.5.3.

37. The CD19 binding molecule of claim 25, which has the configuration referred to as Tv12 in Section 7.5.3.

38. The CD19 binding molecule of claim 25, which has the configuration referred to as Tv13 in Section 7.5.3.

39. The CD19 binding molecule of claim 25, which has the configuration referred to as Tv14 in Section 7.5.3.

40. The CD19 binding molecule of claim 13, which is a trispecific binding molecule (TBM) comprising an antigen-binding module 3 (ABM3) that binds specifically to a target molecule other than CD19.

41. The CD19 binding molecule of claim 40, in which ABM2 binds specifically to a component of a human T-cell receptor (TCR) complex and ABM3 binds specifically to (i) human CD2 or (ii) a tumor associated antigen (TAA).

42. The CD19 binding molecule of claim 40 or claim 41, which is trivalent.

43. The CD19 binding molecule of claim 42, which has the configuration referred to as T1 in Section 7.6.1.

44. The CD19 binding molecule of claim 42, which has the configuration referred to as T2 in Section 7.6.1.

45. The CD19 binding molecule of claim 42, which has the configuration referred to as T3 in Section 7.6.1.

46. The CD19 binding molecule of claim 42, which has the configuration referred to as T4 in Section 7.6.1.

47. The CD19 binding molecule of claim 42, which has the configuration referred to as T5 in Section 7.6.1.

48. The CD19 binding molecule of claim 42, which has the configuration referred to as T6 in Section 7.6.1.

49. The CD19 binding molecule of claim 40 or claim 41, which is tetravalent.

50. The CD19 binding molecule of claim 40 or claim 41, which is pentavalent.

51. The CD19 binding molecule of claim 40 or claim 41, which is hexavalent.

52. The CD19 binding molecule of any one of claims 13 to 51, in which ABM1 is capable of binding CD19 at the same time as ABM2 is bound to its target molecule.

53. The CD19 binding molecule of claim 52, in which ABM1 is capable of binding CD19 at the same time ABM3, if present, is bound to its target molecule.

54. The CD19 binding molecule of any one of claims 13 to 53, wherein ABM1 is an antibody, an antibody fragment, an scFv, a dsFv, a Fv, a Fab, an scFab, a (Fab')2, a single domain antibody (SDAB), a VH or VL domain, or a camelid VHH domain.

55. The CD19 binding molecule of claim 54, wherein ABM1 is an scFv.

56. The CD19 binding molecule of claim 54, wherein ABM1 is a Fab.

57. The CD19 binding molecule of claim 54, wherein the Fab is a Fab heterodimer.

58. The CD19 binding molecule of claim 54, wherein ABM1 is an anti-CD19 antibody or an antigen-binding domain thereof.

59. The CD19 binding molecule of any one of claims 13 to 58, wherein ABM2 is an antibody, an antibody fragment, an scFv, a dsFv, a Fv, a Fab, an scFab, a (Fab')2, a single domain antibody (SDAB), a VH or VL domain, or a camelid VHH domain.

60. The CD19 binding molecule of claim 59, wherein ABM2 is an scFv.

61. The CD19 binding molecule of claim 59, wherein ABM2 is a Fab.

62. The CD19 binding molecule of any one of claims 13 to 61, wherein the component of the TCR complex is CD3.

63. The CD19 binding molecule of claim 62, wherein ABM2 is an anti-CD3 antibody or an antigen-binding domain thereof.

64. The CD19 binding molecule of claim 63, wherein ABM2 comprises the CDR sequences of any of CD3-1 to CD3-130.

65. The CD19 binding molecule of any one of claims 13 to 61, wherein the component of the TCR complex is TCR-α, TCR-β, or a TCR-α/β dimer.

66. The CD19-binding molecule of claim 65, wherein ABM2 is an antibody or an antigen-binding domain thereof.

67. The CD19-binding molecule of claim 65, wherein ABM2 is a Fab.

68. The CD19 binding molecule of any one of claims 40 to 51 and claims 52 to 67 to the extent dependent from claims 40 to 51, in which ABM3 binds specifically to human CD2.

69. The CD19-binding molecule of claim 68, wherein ABM3 is a CD58 moiety.

70. The CD19-binding molecule of claim 69, wherein the CD58 moiety comprises the amino acid sequence of CD58-1 as set forth in Table 15.

71. The CD19-binding molecule of claim 69, wherein the CD58 moiety comprises the amino acid sequence of CD58-2 as set forth in Table 15.

72. The CD19-binding molecule of claim 69, wherein the CD58 moiety comprises the amino acid sequence of CD58-3 as set forth in Table 15.

73. The CD19-binding molecule of claim 69, wherein the CD58 moiety comprises the amino acid sequence of CD58-4 as set forth in Table 15.

74. The CD19-binding molecule of claim 69, wherein the CD58 moiety comprises the amino acid sequence of CD58-5 as set forth in Table 15.

75. The CD19-binding molecule of claim 69, wherein the CD58 moiety comprises the amino acid sequence of CD58-6 as set forth in Table 15.

76. The CD19-binding molecule of claim 69, wherein the CD58 moiety comprises the amino acid sequence of CD58-7 as set forth in Table 15.

77. The CD19-binding molecule of claim 69, wherein the CD58 moiety comprises the amino acid sequence of CD58-8 as set forth in Table 15.

78. The CD19-binding molecule of claim 69, wherein the CD58 moiety comprises the amino acid sequence of CD58-9 as set forth in Table 15.

79. The CD19-binding molecule of claim 69, wherein the CD58 moiety comprises the amino acid sequence of CD58-10 as set forth in Table 15.

80. The CD19-binding molecule of claim 69, wherein the CD58 moiety comprises the amino acid sequence of CD58-11 as set forth in Table 15.

81. The CD19-binding molecule of claim 69, wherein the CD58 moiety comprises the amino acid sequence of CD58-12 as set forth in Table 15.

82. The CD19-binding molecule of claim 68, wherein ABM3 is an anti-CD2 antibody or an antigen-binding domain thereof.

83. The CD19 binding molecule of any one of claims 40 to 51 and claims 52 to 67 to the extent dependent from claims 40 to 51, in which ABM3 binds specifically to a human TAA.

84. The CD19 binding molecule of claim 83, wherein the TAA is a TAA expressed on cancerous B cells that are B cell-derived plasma cells.

85. The CD19 binding molecule of claim 83 or claim 84, wherein the TAA is a TAA expressed on cancerous B cells that are not plasma cells.

86. The CD19 binding molecule of any one of claims 83 to 85, wherein the TAA is selected from BCMA, CD20, CD22, CD123, CD33, CLL1, CD138, CS1, CD38, CD133, FLT3, CD52, TNFRSF13C, TNFRSF13B, CXCR4, PD-L1, LY9, CD200, FCGR2B, CD21, CD23, CD24, CD40L, CD72, CD79a, and CD79b.

87. The CD19 binding molecule of claim 86, wherein ABM3 comprises a binding sequence set forth in Table 16 or Table 17.

88. The CD19 binding molecule of claim 87, which has the heavy and / or light chain variable regions of any one of BCMA-1 to BCMA-40 as set forth in Table 17A.

89. The CD19 binding molecule of claim 87, which has the heavy and / or light chain CDR sequences (as defined by Kabat) of any one of BCMA-1 to BCMA-40 as set forth in Tables 17B and 17E.

90. The CD19 binding molecule of claim 87, which has the heavy and / or light chain CDR sequences (as defined by Chothia) of any one of BCMA-1 to BCMA-40 as set forth in Tables 17C and 17F.

91. The CD19 binding molecule of claim 87, which has the heavy and / or light chain CDR sequences (as defined by a combination of Kabat and Chothia sequences) of any one of BCMA-1 to BCMA-40 as set forth in Tables 17D and 17G.

92. A CD19 binding molecule which is trispecific binding molecule (TBM) comprising:

(a) an antigen-binding module 1 (ABM1) that binds specifically to CD19 and comprises CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of

SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:17, SEQ ID NO:18, and SEQ ID NO:19;

(b) an antigen-binding module 2 (ABM2) that binds specifically to a component of a human T-cell receptor (TCR) complex;

(c) an antigen-binding module 3 (ABM3) that binds specifically to human CD2; and

(d) a first variant human IgG1 Fc region and a second variant human IgG1 Fc region forming a Fc domain, wherein the first and second variant Fc regions comprise L234A, L235A, and G237A ("LALAGA") substitutions, L234A, L235A, S267K, and P329A ("LALASKPA") substitutions, D265A, P329A, and S267K ("DAPASK") substitutions, G237A, D265A, and P329A ("GADAPA") substitutions, G237A, D265A, P329A, and S267K ("GADAPASK") substitutions, L234A, L235A, and P329G ("LALAPG") substitutions, or L234A, L235A, and P329A ("LALAPA") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

93. The CD19 binding molecule of claim 92 which is trivalent.

94. The CD19 binding molecule of claim 92 or claim 93, in which ABM1 is a Fab.

95. The CD19 binding molecule of any one of claims 92 to 94, in which the Fab comprises a VH having the amino acid sequence of SEQ ID NO:13 and a VL having the amino acid sequence of SEQ ID NO:26.

96. The CD19 binding molecule of any one of claims 92 to 95, wherein the component of the TCR complex is CD3.

97. The CD19 binding molecule of claim 96, wherein ABM2 is an anti-CD3 antibody or an antigen-binding domain thereof.

98. The CD19 binding molecule of claim 97, wherein ABM2 comprises the CDR sequences of CD3-21.

99. The CD19 binding molecule of claim 97 or claim 98, wherein ABM2 comprises the heavy and light chain variable sequences of CD3-21, as set forth in Table 12A.

100. The CD19 binding molecule of any one of claims 97 to 99, wherein the anti-CD3 antibody or antigen-binding domain thereof is in the form of a scFv.

101. The CD19 binding molecule of claim 100, wherein ABM2 comprises the amino acid sequence of the scFv designated as CD3-21 in Table 12A.

102. The CD19 binding molecule of any one of claims 92 to 101, wherein ABM3 is a CD58 moiety.

103. The CD19 binding molecule of any one of claims 92 to 102, wherein ABM3 comprises the amino acid sequence of CD58-6 as set forth in Table 15.

104. A CD19 binding molecule which is a trispecific binding molecule (TBM) comprising:

(a) an antigen-binding module 1 (ABM1) that binds specifically to CD19 and which is a Fab comprising: (i) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:17, SEQ ID NO:18, and SEQ ID NO:19; or (ii) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:43, SEQ ID NO:44, and SEQ ID NO:45;

(b) an antigen-binding module 2 (ABM2) that binds specifically to CD3 and which comprises the amino acid sequence of the scFv designated as CD3-21 in Table 12A;

(c) an antigen-binding module 3 (ABM3) that binds specifically to human CD2 and which comprises the amino acid sequence of CD58-6 as set forth in Table 15; and

(d) a first variant human IgG1 Fc region and a second variant human IgG1 Fc region forming a Fc domain, wherein the first and second variant Fc regions comprise L234A, L235A, and G237A ("LALAGA") substitutions, L234A, L235A, S267K, and P329A ("LALASKPA") substitutions, D265A, P329A, and S267K ("DAPASK") substitutions, G237A, D265A, and P329A ("GADAPA") substitutions, G237A, D265A, P329A, and S267K ("GADAPASK") substitutions, L234A, L235A, and P329G ("LALAPG") substitutions, or L234A, L235A, and P329A ("LALAPA") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

105. A CD19 binding molecule which is a trispecific binding molecule (TBM) comprising:

(a) an antigen-binding module 1 (ABM1) that binds specifically to CD19 and which is a Fab comprising: (i) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:17, SEQ ID NO:18, and SEQ ID NO:19; or (ii) CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:43, SEQ ID NO:44, and SEQ ID NO:45;

(b) an antigen-binding module 2 (ABM2) that binds specifically to CD3 and which comprises the amino acid sequence of the scFv designated as CD3-129 in Table 12A;

(c) an antigen-binding module 3 (ABM3) that binds specifically to human CD2 and which comprises the amino acid sequence of CD58-6 as set forth in Table 15; and

(d) a first variant human IgG1 Fc region and a second variant human IgG1 Fc region forming a Fc domain, wherein the first and second variant Fc regions comprise L234A, L235A, and G237A ("LALAGA") substitutions, L234A, L235A, S267K, and P329A ("LALASKPA") substitutions, D265A, P329A, and S267K ("DAPASK") substitutions, G237A, D265A, and P329A ("GADAPA") substitutions, G237A, D265A, P329A, and S267K ("GADAPASK") substitutions, L234A, L235A, and P329G ("LALAPG") substitutions, or L234A, L235A, and P329A ("LALAPA") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

106. The CD19 binding molecule of claim 104 or claim 105, wherein ABM1 comprises CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, and CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NO:17, SEQ ID NO:18, and SEQ ID NO:19.

107. The CD19 binding molecule of any one of claims 104 to 106, wherein ABM1 comprises a VH having the amino acid sequence of SEQ ID NO:13 and a VL having the amino acid sequence of SEQ ID NO:26.

108. The CD19 binding molecule of any one of claim 92 to 107, wherein the CD19 binding molecule has the configuration depicted in FIG. 2I and referred to as T2 in Section 7.6.1.

109. The CD19 binding molecule of any one of claims 1 to 108, wherein the first variant Fc region and the second variant Fc region comprise L234A, L235A, and G237A ("LALAGA") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

110. The CD19 binding molecule of any one of claims 1 to 108, wherein the first variant Fc region and the second variant Fc region comprise L234A, L235A, S267K, and P329A ("LALASKPA") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

111. The CD19 binding molecule of any one of claims 1 to 108, wherein the first variant Fc region and the second variant Fc region comprise D265A, P329A, and S267K ("DAPASK") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

112. The CD19 binding molecule of any one of claims 1 to 108, wherein the first variant Fc region and the second variant Fc region comprise G237A, D265A, and P329A ("GADAPA") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

113. The CD19 binding molecule of any one of claims 1 to 108, wherein the first variant Fc region and the second variant Fc region comprise G237A, D265A, P329A, and S267K ("GADAPASK") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

114. The CD19 binding molecule of any one of claims 1 to 108, wherein the first variant Fc region and the second variant Fc region comprise L234A, L235A, and P329G ("LALAPG") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

115. The CD19 binding molecule of any one of claims 1 to 108, wherein the first variant Fc region and the second variant Fc region comprise L234A, L235A, and P329A ("LALAPA") substitutions, wherein the amino acid residues are numbered according to the EU numbering system.

116. The CD19 binding molecule of any one of claims 1 to 108, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 95% identity to FCV-1, FCV-2, FCV-3, FCV-4, FCV-5, FCV-6, or FCV-7.

117. The CD19 binding molecule of claim 116, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to FCV-1.

118. The CD19 binding molecule of claim 116, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to FCV-2.

119. The CD19 binding molecule of claim 116, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to FCV-3.

120. The CD19 binding molecule of claim 116, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to FCV-4.

121. The CD19 binding molecule of claim 116, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to FCV-5.

122. The CD19 binding molecule of claim 116, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to FCV-6.

123. The CD19 binding molecule of claim 116, wherein the first variant Fc region and/or the second variant Fc region comprises a sequence having at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to FCV-7.

124. The CD19 binding molecule of any one of claims 1 to 123, wherein the first variant Fc region and the second variant Fc region together form an Fc heterodimer.

125. The CD19 binding molecule of claim 124, wherein the first and second variant Fc regions comprise the amino acid substitutions T366W : T366S/L368A/Y407V.

126. The CD19 binding molecule of any one of claims 124 to 125, the first and second variant Fc regions comprise the amino acid substitutions S354C : Y349C.

127. A CD19 binding molecule comprising:

(a) a first polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1120;

(b) a second polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1122; and

(c) a third polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1110.

128. A CD19 binding molecule comprising:

(a) a first polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1127;

(b) a second polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1129; and

(c) a third polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1110.

129. A CD19 binding molecule comprising:

(a) a first polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1134;

(b) a second polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1135; and

(c) a third polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1110.

130. A CD19 binding molecule comprising:

(a) a first polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1136;

(b) a second polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1137; and

(c) a third polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1110.

131. A CD19 binding molecule comprising:

(a) a first polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1138;

(b) a second polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1139; and

(c) a third polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1110.

132. A CD19 binding molecule comprising:

(a) a first polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1140;

(b) a second polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1141; and

(c) a third polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1110.

133. A CD19 binding molecule comprising:

(a) a first polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1142;

(b) a second polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1143; and

(c) a third polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1110.

134. A CD19 binding molecule comprising:

(a) a first polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1131;

(b) a second polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1132; and

(c) a third polypeptide whose amino acid sequence comprises the amino acid sequence of SEQ ID NO:1133.

135. A conjugate comprising (a) the CD19 binding molecule of any one of claims 1 to 134, and (b) an agent.

136. The conjugate of claim 135, wherein the agent is a therapeutic agent, a diagnostic agent, a masking moiety, a cleavable moiety, a stabilizing agent, or any combination thereof.

137. A pharmaceutical composition comprising (a) the CD19 binding molecule of any one of claims 1 to 134 or the conjugate of any one of claims 135 to 136 and (b) an excipient.

138. A nucleic acid or plurality of nucleic acids encoding the CD19-binding molecule of any one of claims 1 to 134.

139. A cell engineered to express the CD19 binding molecule of any one claims 1 to 134.

140. A method of producing a CD19 binding molecule, comprising:

(a) culturing the cell of claim 139 in conditions under which the CD19 binding molecule is expressed; and

(b) recovering the CD19-binding molecule from the cell culture.

141. A combination comprising the CD19 binding molecule of any one of claims 1 to 134 and at least one additional therapeutic agent.

142. The combination of claim 141, wherein the one or more additional therapeutic agents comprise an immunomodulatory imide drug (IMiD).

143. The combination of claim 142, wherein the immunomodulatory imide drug (IMiD) is lenalidomide, thalidomide, pomalidomide, or iberdomide.

144. The combination of claim 143, wherein the immunomodulatory imide drug (IMiD) is lenalidomide.

145. The CD19 binding molecule of any one of claims 1 to 134, the composition of claim 137 or combination of any one of claims 141 to 144 for use as a medicament.

146. The CD19 binding molecule of any one of claims 1 to 134, the composition of claim 137 or combination of any one of claims 141 to 144 for use in treating a CD19-associated disease or disorder.

147. Use of the antibody or antigen binding fragment thereof of any one of claims 1 to 134, the composition of claim 137 or combination of any one of claims 141 to 144 in the manufacture of a medicament to treat a CD19-associated disease or disorder.

148. A method of treating a subject with a CD19-associated disease or disorder, comprising administering to the subject an effective amount of the CD19 binding molecule of any one of claims 1 to 134, the composition of claim 137 or combination of any one of claims 141 to 144.

149. The CD19 binding molecule, combination or composition for use of claim 146, or use of claim 147 or method of claim 148, wherein the CD19-associated disease or disorder is cancer.

150. The CD19 binding molecule, combination or composition for use of claim 146, or use of claim 147 or method of claim 148, wherein the disease or disorder is a plasma cell neoplasm.

151. The CD19 binding molecule, combination or composition for use of claim 146, or use of claim 147 or method of claim 148, wherein the disease or disorder is a B cell malignancy that expresses cell surface CD19.

152. The CD19 binding molecule, combination or composition for use of claim 146, or use of claim 147 or method of claim 148, wherein the CD19-associated disease or disorder is non-Hodgkin's lymphoma.

153. The CD19 binding molecule, combination or composition for use of claim 146, or use of claim 147 or method of claim 148, wherein the CD19-associated disease or disorder is diffuse large B-cell lymphoma (DLBCL), optionally wherein the DLBCL is relapsed or refractory DLBCL.

154. The CD19 binding molecule, combination or composition for use of claim 146, or use of claim 147 or method of claim 148, wherein the CD19-associated disease or disorder is Burkitt's lymphoma.

155. The CD19 binding molecule, combination or composition for use of any one of claims 152 to 154, wherein the subject has failed at least one prior line of standard of care therapy.

156. The CD19 binding molecule, combination or composition for use of claim 155, wherein the subject has failed up to five prior lines of standard of care therapies.

157. The CD19 binding molecule, combination or composition for use of claim 155 or 156, wherein the subject has failed one prior line of standard of care therapy.

158. The CD19 binding molecule, combination or composition for use of claim 155 or 156,, wherein the subject has failed two prior lines of standard of care therapy.

159. The CD19 binding molecule, combination or composition for use of claim 155 or 156, wherein the subject has failed three prior lines of standard of care therapy.

160. The CD19 binding molecule, combination or composition for use of claim 155 or 156,, wherein the subject has failed four prior lines of standard of care therapy.

161. The CD19 binding molecule, combination or composition for use of claim 155 or 156, wherein the subject has failed five prior lines of standard of care therapy.

162. The CD19 binding molecule, combination or composition for use of any one of claims 155 to 161, wherein the at least one prior line of standard of care therapies comprise an anti-CD20 therapy.

163. The CD19 binding molecule, combination or composition for use of claim 162, wherein the anti-CD20 therapy is rituximab.

164. The CD19 binding molecule, combination or composition for use of any one of claims 155 to 163, wherein the subject is intolerant to or ineligible for one or more other approved therapies.

165. The CD19 binding molecule, combination or composition for use of claim 164, wherein the one or more other approved therapies comprise an autologous stem cell transplant (ASCT).

166. The CD19 binding molecule, combination or composition for use of any one of claims 155 to 165, wherein the subject is a non-responder to a CAR composition.

167. The CD19 binding molecule, combination or composition for use of claim 166, wherein the CAR composition is an anti-CD19 CAR composition.

168. The CD19 binding molecule, combination or composition for use of claim 166 or claim 167, wherein the CAR composition comprises CTL019, tisagenlecleucel, axicabtagene ciloleucel, brexucabtagene autoleucel or lisocabtagene maraleucel.