CD20 BINDING MOLECULES AND USES THEREOF

Inventors: Bruce Keyt
Omar Duramad
Beatrice Wang
Ramesh Baliga
Fen Zhang

BACKGROUND

[0001] Since the advent of humanized antibodies, the therapeutic use of antibodies such as RITUXAN® (rituximab) has revolutionized the treatment of B-cell malignancies. Rituximab, a CD20 specific chimeric monoclonal antibody, is the first effective targeted therapy approved by the FDA for treatment of relapsed or refractory B-cell non-Hodgkin's lymphoma. This scientific achievement has not only changed the standard of practice for treatment of B-cell lymphoma, but has stimulated significant interest in next generation of CD20 mAbs. Many novel CD20 mAbs have entered the clinic, each with structural modifications to further improve efficacy. In this application, we describe a series of CD20 antibodies that can exhibit improved efficacy utilizing additional modes of action, as these CD20 antibodies are expressed in non-IgG formats, such as IgA and IgM.

[0002] CD20 is a 36 kDa non-glycosylated; tetra-spanning membrane protein (MS4A1 gene product) expressed exclusively on B-lymphocytes and more than 90% of B-lymphocytic lymphoma (see **FIG. 1**). CD20 is expressed at the late pre-B cell stage and is upregulated on most normal and malignant B lineage cells before it is down-regulated in terminally differentiated plasma cells. This B-lymphocyte surface molecule is involved with development and differentiation of B-cells into plasma cells.

[0003] Although rituximab exhibits significant anti-tumor activity in patients with B-cell non-Hodgkin's lymphoma, there is a need to improve the efficacy of antibody therapeutics for the treatment of B-cell neoplasms. Rituximab as single agent therapy results in a clinical response rate of 50%, and it is unclear why the remaining 50% of patients do not respond. In addition, a majority of responsive patients acquires resistance to further rituximab therapy. One mechanism of initial or acquired resistance is the down regulation or modulation of CD20 on the tumor cells (or clonal expansion of low expressing tumors). There is a clear unmet medical need for improved treatment for these refractory patients whose refractory tumors have minimizes CD20 expression. By increasing affinity and avidity with the multivalency of IgA or IgM, these newer agents aim to achieve improved response rates as compared to rituximab. Furthermore, altering

the effector functions by changing the antibody isotype may improve the potency and efficacy of CD20 antibodies.

[0004] Since the introduction of rituximab, much has been learned about potential mechanisms for the therapeutic efficacy of CD20 mAbs. Rituximab induces B-cell death primarily through complement-dependent lysis (CDC) and antibody dependent cellular toxicity (ADCC) effector mechanisms, and to a lesser degree via cellular apoptosis. CD20 antibodies are described as either Type I (such as rituximab/RITUXAN® or ofatumumab/ARZERRA®), which redistributes CD20 into detergent-resistant lipid rafts; and Type II such as tositumumab (B1) and obinutuzumab/GAZYVA®, which do not. Clustering by Type I antibodies promotes association with other cell surface proteins such as the B-cell receptor (BCR), and binding to C1q, resulting in potent complement-dependent cytotoxicity (CDC). IgM is highly potent at inducing complement dependent cytotoxicity, and as such IgM forms of CD20 antibodies can yield significantly greater efficacy. In contrast, Type II antibodies, such as tositumomab (B1), elicit antibody-dependent cellular cytotoxicity, but not complement-dependent cytotoxicity. Type II antibodies are very potent at directly triggering cell death via antibody-induced homotypic adhesion and lysosomal cell death. Oligomeric forms of antibodies, such as IgA and IgM exhibit increased multivalency and can display enhanced efficacy at inducing homotypic adhesion and cell death. Importantly, both type I and type II antibodies recruit FcR-expressing cells to mediate cellular effector functions such as antibody-dependent cellular cytotoxicity and antibody-dependent cellular phagocytosis.

[0005] Second-generation type I CD20 mAbs have been approved for human use. Ofatumumab is a fully human IgG1, type I CD20 mAb that exhibits antibody dependent cellular cytotoxicity (ADCC) but has stronger complement-dependent cytotoxicity (CDC) when compared to rituximab. As a type I antibody, ofatumumab is relatively ineffective in triggering cell death. Ofatumumab is FDA approved for treatment of chronic lymphocytic leukemia (CLL). Obinutuzumab/GA101 is a humanized IgG1 second-generation type II mAb which displays improved pro-apoptotic activity, enhanced ADCC but no complement-fixing activity. Obinutuzumab is FDA approved for treatment CLL. Another fully human IgG1κ CD20 antibody huMAb 1.5.3 (see U.S. Patent Publication No. 2007-0014720), was designed for enhance potency. Preclinical studies have shown that huMAb 1.5.3 has greater apoptosis as compared to rituximab, and exhibits both potent CDC as well as ADCC activity. HuMAb 1.5.3 appears to combine both type I and type II activities including effective cell killing through direct apoptosis induction, CDC and ADCC. IgM and IgA can contribute to further enhanced potency of CD20 antibodies with increased avidity which can mediate increased sensitivity and

cytotoxicity on low CD20 expressing tumor cells. Furthermore, the efficacy of type I and type II CD20 activities can be increased with IgA or IgM formats allowing the development of most potent anti-tumor antibodies utilizing optimally combined mechanisms of action, including the triggering direct apoptosis, enhanced CDC and ADCC.

SUMMARY

[0006] This disclosure provides a multimeric binding molecule that includes at least two bivalent binding units or variants or fragments thereof, where each binding unit includes at least two heavy chain constant regions or fragments thereof, each associated with an antigen-binding domain. At least one antigen binding domain of the provided binding molecule is a CD20 antigen binding domain that includes six immunoglobulin complementarity determining regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, where the HCDR1 includes the amino acid sequence of SEQ ID NO: 39 or SEQ ID NO: 39 with one or two single amino acid substitutions; the HCDR2 includes the amino acid sequence of SEQ ID NO: 40 or SEQ ID NO: 40 with one or two single amino acid substitutions; the HCDR3 includes the amino acid sequence of SEQ ID NO: 41, SEQ ID NO: 41 with one or two single amino acid substitutions; the LCDR1 includes the amino acid sequence of SEQ ID NO: 43, or SEQ ID NO: 43 with one or two single amino acid substitutions; the LCDR2 includes the amino acid sequence of SEQ ID NO: 44 or SEQ ID NO: 44 with one or two single amino acid substitutions; and the LCDR3 includes the amino acid sequence of SEQ ID NO: 45 or SEQ ID NO: 45 with one or two single amino acid substitutions.

[0007] The disclosure further provides a multimeric binding molecule that includes at least two bivalent binding units or variants or fragments thereof, where each binding unit includes at least two heavy chain constant regions or fragments thereof, each associated with an antigen-binding domain. At least one antigen binding domain of the provided binding molecule is a CD20 antigen binding domain that includes an antibody heavy chain variable region (VH) and an antibody light chain variable region (VL), where the VH includes an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 38, and the VL includes an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 42.

[0007a] In one aspect, there is provided a pentameric binding molecule comprising five bivalent binding units and a J-chain or functional fragment or variant thereof, wherein each binding unit comprises two IgM heavy chain constant regions, each associated with an antigen-binding domain, wherein at least one antigen binding domain of the binding molecule is a CD20 antigen binding domain comprising:

an antibody heavy chain variable region (VH) comprising immunoglobulin complementarity determining regions HCDR1, HCDR2, HCDR3, and an antibody light chain variable region (VL) comprising immunoglobulin complementarity determining regions LCDR1, LCDR2, and LCDR3, wherein the HCDR1 comprises the amino acid sequence of SEQ ID NO: 39; the HCDR2 comprises the amino acid sequence of SEQ ID NO: 40; the HCDR3 comprises the amino acid sequence of SEQ ID NO: 41; the LCDR1 comprises the amino acid sequence of SEQ ID NO: 43; the LCDR2 comprises the amino acid sequence of SEQ ID NO: 44; and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 45; wherein the binding molecule can direct complement-mediated killing of a CD-20-expressing cell at higher potency than an equivalent amount of a monospecific, bivalent reference IgG1 antibody that specifically binds to CD20; and wherein the monospecific, bivalent reference IgG1 antibody is 1.5.3, which comprises a VH having the amino acid sequence SEQ ID NO: 38 and a VL having the amino acid sequence SEQ ID NO: 42.

[0008] In certain examples a binding molecule provided by the disclosure is a dimeric binding molecule that includes two bivalent IgA binding units or fragments thereof and a J-chain or fragment or variant thereof, where each binding unit includes two IgA heavy chain constant

regions or fragments thereof each associated with an antigen-binding domain. A dimeric IgA binding molecule as provided herein can further include a secretory component, or fragment or variant thereof. The IgA heavy chain constant regions or fragments thereof can each include a Cα2 domain or a Cα3-tp domain and can, in certain examples, further include a Cα1 domain. In certain examples, the IgA heavy chain constant regions can be human IgA constant regions. In certain examples, each binding unit includes two IgA heavy chains each including a VH situated amino terminal to the IgA constant region or fragment thereof, and two immunoglobulin light chains each including a VL situated amino terminal to an immunoglobulin light chain constant region.

[0009] In certain examples a binding molecule provided by the disclosure is a is a pentameric or a hexameric binding molecule including five or six bivalent IgM binding units, respectively, where each binding unit includes two IgM heavy chain constant regions or fragments thereof each associated with an antigen-binding domain. The IgM heavy chain constant regions or fragments thereof can each include a Cμ3 domain and a Cμ4-tp domain and can, in certain examples further include a Cμ2 domain, a Cμ1 domain, or any combination thereof.

[0010] Where the binding molecule is pentameric, the binding molecule can further include a J-chain, or fragment thereof, or functional fragment thereof, or a functional variant thereof. In certain examples, the J-chain or fragment thereof includes the amino acid sequence SEQ ID NO: 49 or a functional fragment thereof. In certain examples, the J-chain or fragment thereof can further include a heterologous polypeptide. The heterologous polypeptide can be directly or indirectly fused to the J-chain or fragment thereof. In certain examples the heterologous polypeptide can be indirectly fused to the J-chain or fragment thereof via a peptide linker. In certain examples the peptide linker can include, e.g., at least 5 amino acids, but no more than 25 amino acids. In certain examples the peptide linker consists of GGGGSGGGGSGGGGS (SEQ ID NO: 67). The heterologous polypeptide can be fused to or near the N-terminus of the J-chain or fragment thereof, the C-terminus of the J-chain or fragment thereof, or to both the N-terminus and C-terminus of the J-chain or fragment thereof. In certain examples the heterologous polypeptide can include a binding domain, e.g., an antibody or antigen-binding fragment thereof. The antigen-binding fragment can be, for example, an Fab fragment, an Fab' fragment, an $F(ab')_2$ fragment, an Fd fragment, an Fv fragment, a single-chain Fv (scFv) fragment, a disulfide-linked Fv (sdFv) fragment, or any combination thereof. In certain examples the heterologous polypeptide can specifically bind to CD3ε. For example in certain examples the modified J-chain can include the amino acid sequence SEQ ID NO: 64 (V15J) or SEQ ID NO: 66 (J15V). Moreover in certain examples, these particular modified J-chains can further include a

signal peptide, where the modified J-chain then includes the amino acid sequence SEQ ID NO: 63 (V15J) or SEQ ID NO: 65 (J15V).

[0011] In certain examples, the IgM heavy chain constant regions can be human IgM heavy chain constant regions. In certain examples, each binding unit includes two IgM heavy chains each including a VH situated amino terminal to the IgM constant region or fragment thereof, and two immunoglobulin light chains each including a VL situated amino terminal to an immunoglobulin light chain constant region.

[0012] In certain examples, at least one binding unit of a multimeric binding molecule provided herein includes two of the CD20 antigen binding domains, which can be the same or different. In certain examples, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, or at least twelve copies of the same CD20 antigen binding domain.

[0013] In certain examples where the binding molecule is an IgM binding molecule, the two IgM heavy chains within at least one binding unit include the amino acid sequence SEQ ID NO: 56.

[0014] In certain examples where the binding molecule includes immunoglobulin light chains, the two light chain constant regions of a given binding unit can be human lambda constant regions or a human kappa constant regions. In certain examples the two light chain constant regions are identical and include the amino acid sequence SEQ ID NO: 58.

[0015] In certain examples, at least two, at least three, at least four, at least five, or at least six of the binding units of a multimeric binding molecule provided herein are identical.

[0016] In certain examples, the binding molecule as described above can direct complement-mediated, T-cell-mediated, or both complement-mediated and T-cell-mediated killing of a CD-20-expressing cell at higher potency than an equivalent amount of a monospecific, bivalent IgG1 antibody or fragment thereof that specifically binds to the same CD20 epitope as the one or more CD20 antigen binding domains of the binding molecule. In certain examples, the monospecific, bivalent IgG1 antibody is 1.5.3, which includes a VH having the amino acid sequence SEQ ID NO: 38 and a VL having the amino acid sequence SEQ ID NO: 42. In certain examples, the CD-20-expressing cell is a lymphoma cell line, for example, a Ramos cell line, a Raji cell line, a Daudi cell line, a Namalwa cell line, a Granta cell line, a Z138 cell line, a DoHH2 cell line, or a DB cell line. In certain examples, where the CD20-expressing cell is a Raji cell line, the binding molecule can direct complement-mediated killing with an IC_{50} at least four-fold, at least ten-fold, at least 50-fold, or at least 100-fold lower than the IC_{50} of an equivalent amount of the monospecific bivalent IgG1 antibody, as measured, *e.g.*, in μg/ml. In certain examples, where the CD20-expressing cell is a Ramos cell line, the binding molecule can

direct complement-mediated killing with an IC_{50} at least ten-fold, at least 20-fold, at least 30-fold, at least 40-fold, at least 50-fold, at least 60-fold, at least 70-fold, at least 80-fold, at least 90-fold, or at least 100-fold lower than the IC_{50} of an equivalent amount of the monospecific bivalent IgG1 antibody, as measured, *e.g.*, as molar equivalents. In certain examples, the CD-20-expressing cell is a malignant B cell in a subject with cancer, *e.g.*, a CD20-positive leukemia, lymphoma, or myeloma. In certain examples, the cancer is minimally responsive or non-responsive to rituximab therapy. In certain examples, the subject is human.

[0017] In another aspect, there is provided a composition comprising the binding molecule as described above, and a carrier.

[0018] The disclosure further provides a polynucleotide including a nucleic acid sequence that encodes a polypeptide subunit of a multimeric binding molecule as provided herein. In one aspect, there is provided a polynucleotide comprising a nucleic acid sequence that encodes a heavy chain polypeptide subunit of the binding molecule as provided herein, where the heavy chain polypeptide subunit comprises an IgM heavy chain constant region, and at least the antibody VH portion of the CD20 antigen binding domain. In certain examples, the heavy chain polypeptide subunit can include a human IgM constant region or fragment thereof fused to the C-terminal end of a VH that includes (a) an HCDR1, HCDR2, HCDR3, where the HCDR1 includes the amino acid sequence of SEQ ID NO: 39 or SEQ ID NO: 39 with one or two single amino acid substitutions; the HCDR2 includes the amino acid sequence of SEQ ID NO: 40 or SEQ ID NO: 40 with one or two single amino acid substitutions; the HCDR3 includes the amino acid sequence of SEQ ID NO: 41, SEQ ID NO: 41 with one or two single amino acid substitutions; or (b) an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 38. In certain examples the provided polynucleotide can encode the amino acid sequence SEQ ID NO: 56. In another aspect, there is provided a composition comprising the polynucleotide as described above.

[0019] The provided polynucleotide composition can further include, e.g., a nucleic acid sequence that encodes a light chain polypeptide subunit, where the light chain polypeptide subunit includes the antibody VL portion of the CD20 antigen binding domain. In certain examples the light chain polypeptide subunit can include a human antibody light chain constant region or fragment thereof fused to the C-terminal end of a VL that includes: (a) an LCDR1, LCDR2, and LCDR3, where the LCDR1 includes the amino acid sequence of SEQ ID NO: 43, or SEQ ID NO: 43 with one or two single amino acid substitutions; the LCDR2 includes the amino acid sequence of SEQ ID NO: 44 or SEQ ID NO: 44 with one or two single amino acid

substitutions; and the LCDR3 includes the amino acid sequence of SEQ ID NO: 45 or SEQ ID NO: 45 with one or two single amino acid substitutions; or (b) an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 42. In certain examples the nucleic acid sequence that encodes the light chain polypeptide subunit can encode the amino acid sequence SEQ ID NO: 58.

[0020] In certain examples of the provided polynucleotide composition, the nucleic acid sequence encoding the heavy chain polypeptide subunit and the nucleic acid sequence encoding the light chain polypeptide subunit can be on separate vectors, or they can be situated on a single vector.

[0021] The provided polynucleotide composition can further include, e.g., a nucleic acid sequence that encodes a J-chain, or functional fragment thereof, or a functional variant thereof. In certain examples the J-chain or fragment thereof can include the amino acid sequence SEQ ID NO: 49 or a functional fragment thereof. Moreover, the J-chain or fragment thereof can be a modified J-chain that further includes a heterologous polypeptide. The heterologous polypeptide can, in certain examples, be directly or indirectly fused to the J-chain or fragment thereof. In certain examples, the heterologous polypeptide can include an antibody or antigen-binding fragment thereof. In certain examples, the heterologous polypeptide can be, e.g., a scFv that can specifically bind to CD3ε. In certain examples the modified J-chain can include the amino acid sequence SEQ ID NO: 64 (V15J) or SEQ ID NO: 66 (J15V). In those examples where the modified J-chain further comprises a signal peptide, the modified J-chain can include the amino acid sequence SEQ ID NO: 63 (V15J) or SEQ ID NO: 65 (J15V). In certain examples of the provided polynucleotide composition, the nucleic acid sequence that encodes a J-chain, or functional fragment thereof, or a functional variant thereof can include SEQ ID NO: 68 or SEQ ID NO: 69.

[0022] In certain examples of the provided polynucleotide composition, the nucleic acid sequence encoding the heavy chain polypeptide subunit, the nucleic acid sequence encoding the light chain polypeptide subunit, and the nucleic acid sequence encoding the J-chain can be situated on a single vector, or they can be situated on two or three separate vectors. In one aspect, there is provided a vector or combination of vectors comprising the provided nucleic acid sequences. In another aspect, there is provided a host cell comprising the provided polynucleotide, the provided polynucleotide composition, or the provided vector or vectors, wherein the host cell can express the binding molecule as provided herein, or a subunit thereof. In yet another aspect, there is provided a method of producing the binding molecule as provided herein, comprising culturing the provided host cell, and recovering the binding molecule.

[0023] In certain examples, the disclosure provides a method for directing complement-mediated, T-cell-mediated, or both complement-mediated and T-cell-mediated killing of a CD20-expressing cell where the method includes contacting a CD20-expressing cell with a multimeric binding molecule as provided herein or a composition that includes the provided binding molecule. According to these examples, the binding molecule can direct complement-mediated, T-cell-mediated, or both complement-mediated and T-cell-mediated killing of a CD-20-expressing cell at higher potency than an equivalent amount of a monospecific, bivalent IgG1 antibody or fragment thereof that specifically binds to the same CD20 epitope as the CD20 antigen binding domain. In certain examples, the monospecific, bivalent IgG1 antibody is 1.5.3, which includes a VH having the amino acid sequence SEQ ID NO: 38 and a VL having the amino acid sequence SEQ ID NO: 42. In one aspect, there is provided an *in vitro* method for directing complement-mediated killing of a CD20-expressing cell comprising contacting a CD20-expressing cell with the binding molecule provided herein or the composition provided herein, wherein the binding molecule can direct complement-mediated killing of a CD-20-expressing cell at higher potency than an equivalent amount of a monospecific, bivalent IgG1 antibody or fragment thereof that specifically binds to CD20, wherein the monospecific, bivalent IgG1 antibody is 1.5.3, which comprises a VH having the amino acid sequence SEQ ID NO: 38 and a VL having the amino acid sequence SEQ ID NO: 42. In certain examples, the CD-20-expressing cell is a lymphoma cell line, *e.g.*, a Ramos cell line, a Raji cell line, a Daudi cell line, a Namalwa cell line, a Granta cell line, a Z138 cell line, a DoHH2 cell line, or a DB cell line. In certain examples, the CD20-expressing cell is a Raji cell line, and the binding molecule directs complement-mediated killing with an IC_{50} at least four-fold, at least ten-fold, at least 50-fold, or at least 100-fold lower than the IC_{50} of an equivalent amount of the monospecific bivalent IgG1 antibody, as measured, *e.g.*, in μg/ml. In certain examples, where the CD20-expressing cell is a Ramos cell line, the binding molecule can direct complement-mediated killing with an IC_{50} at least ten-fold, at least 20-fold, at least 30-fold, at least 40-fold, at least 50-fold, at least 60-fold, at least 70-fold, at least 80-fold, at least 90-fold, or at least 100-fold lower than the IC_{50} of an equivalent amount of the monospecific bivalent IgG1 antibody, as measured, *e.g.*, as molar equivalents. In certain examples, the CD-20-expressing cell is a malignant B cell in a subject with cancer, *e.g.*, a CD20-positive leukemia, lymphoma, or myeloma. In one aspect, there is provided use of the binding molecule provided herein or the composition provided herein in the preparation of a medicament for treating cancer in as subject in need thereof, wherein the cancer is a CD20-positive leukemia, lymphoma, or myeloma. In certain examples, the cancer is minimally responsive or non-responsive to rituximab therapy. In certain examples the subject is a human.

[0024] In other examples, the disclosure provides a method for directing complement-mediated, T-cell-mediated, or both complement-mediated and T-cell-mediated killing of a CD20-expressing cell, where the method includes contacting a CD20-expressing cell with a dimeric, pentameric, or hexameric binding molecule including two, five, or six bivalent binding units, respectively, where each binding unit includes two IgA or IgM heavy chain constant regions or fragments thereof and two antigen binding domains, where at least one antigen binding domain of the binding molecule is a CD20 antigen binding domain, and where the binding molecule can direct complement-mediated, T-cell-mediated, or both complement-mediated and T-cell-mediated killing of a CD-20-expressing cell at higher potency than an equivalent amount of a

monospecific, bivalent IgG1 antibody or fragment thereof that specifically binds to the same CD20 epitope as the CD20 antigen binding domain. Provided herein is use of a dimeric, pentameric, or hexameric binding molecule comprising two, five, or six bivalent binding units, respectively, wherein each binding unit comprises two IgA or IgM heavy chain constant regions or fragments thereof and two antigen binding domains, wherein at least one antigen binding domain of the binding molecule is a CD20 antigen binding domain in the preparation of a medicament for directing complement-mediated, T-cell-mediated, or both complement-mediated and T-cell-mediated killing of a CD20-expressing cell in a subject with cancer, wherein the binding molecule can direct complement-mediated killing of a CD-20-expressing cell at higher potency than an equivalent amount of a monospecific, bivalent IgG1 antibody or fragment thereof that specifically binds to the same CD20 epitope as the CD20 antigen binding domain, wherein the IgA or IgM heavy chain constant regions are optionally human IgA or IgM heavy chain constant regions.

[0025] According to these provided methods, the CD20 antigen binding domain can include six immunoglobulin complementarity determining regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, where the HCDR1 includes the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 2 with one or two single amino acid substitutions; the HCDR2 includes the amino acid sequence of SEQ ID NO: 3 or SEQ ID NO: 3 with one, two, three, four, or five single amino acid substitutions; the HCDR3 includes the amino acid sequence of SEQ ID NO: 4, SEQ ID NO: 4 with one, two, or three single amino acid substitutions, SEQ ID NO: 10, or SEQ ID NO: 31, the LCDR1 includes the amino acid sequence of SEQ ID NO: 6, or SEQ ID NO: 6 with one, two, or three single amino acid substitutions; the LCDR2 includes the amino acid sequence of SEQ ID NO: 7 or SEQ ID NO: 7 with one or two single amino acid substitutions; and the LCDR3 includes the amino acid sequence of SEQ ID NO: 8 or SEQ ID NO: 8 with one or two single amino acid substitutions.

[0026] According to these provided methods, the CD20 antigen binding domain can include a VH and a VL including, respectively: (a) an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 1 and an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 5; (b) an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 9 and an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 11; (c) an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 15 and an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 18; (d) an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 22 or SEQ ID NO: 23 and an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, or SEQ ID NO: 29; (e) an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 30 and an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 32; or (f) an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 35 and an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 37.

[0027] According to these provided methods, the IgA heavy chain constant regions or fragments thereof of the binding molecule can each include a Cα2 domain or a Cα3-tp domain,

and one or more IgA heavy chain constant regions or fragments thereof can further include a Cα1 domain. In certain examples, the IgA heavy chain constant region is a human IgA constant region. In certain examples a dimeric binding molecule according to these provided methods can further include a secretory component

[0028] According to these provided methods, the IgM heavy chain constant regions or fragments thereof of the binding molecule each include a Cμ3 domain and a Cμ4-tp domain, and in certain examples can further include a Cμ2 domain, a Cμ1 domain, or any combination thereof. In certain examples the IgM heavy chain constant region is a human IgM constant region.

[0029] According to these provided methods, where the binding molecule is dimeric or pentameric, the binding molecule can further include a J-chain, or functional fragment thereof, or a functional variant thereof. In certain examples, the J-chain or fragment thereof includes the amino acid sequence SEQ ID NO: 49 or a functional fragment thereof. In certain examples, the J-chain or fragment thereof can further include a heterologous polypeptide. The heterologous polypeptide can be directly or indirectly fused to the J-chain or fragment thereof. In certain examples the heterologous polypeptide can be indirectly fused to the J-chain or fragment thereof via a peptide linker. In certain examples the peptide linker can include, e.g., at least 5 amino acids, but no more than 25 amino acids. In certain examples the peptide linker consists of GGGGSGGGGSGGGGS (SEQ ID NO: 67). The heterologous polypeptide can be fused to or near the N-terminus of the J-chain or fragment thereof, the C-terminus of the J-chain or fragment thereof, or to both the N-terminus and C-terminus of the J-chain or fragment thereof. In certain examples the heterologous polypeptide can include a binding domain, e.g., an antibody or antigen-binding fragment thereof. The antigen-binding fragment can be, for example, an Fab fragment, an Fab' fragment, an $F(ab')_2$ fragment, an Fd fragment, an Fv fragment, a single-chain Fv (scFv) fragment, a disulfide-linked Fv (sdFv) fragment, or any combination thereof. In certain examples the heterologous polypeptide can specifically bind to CD3ε. For example in certain examples the modified J-chain can include the amino acid sequence SEQ ID NO: 64 (V15J) or SEQ ID NO: 66 (J15V). Moreover in certain examples, these particular modified J-chains can further include a signal peptide, where the modified J-chain then includes the amino acid sequence SEQ ID NO: 63 (V15J) or SEQ ID NO: 65 (J15V).

[0030] According to these provided methods, each binding unit of the binding molecule can include two IgA or IgM heavy chains each including a VH situated amino terminal to the IgA or IgM constant region or fragment thereof, and two immunoglobulin light chains each including a VL situated amino terminal to an immunoglobulin light chain constant region. In certain examples, at least one binding unit of the binding molecule includes two identical CD20

antigen binding domains, and where the two IgA or IgM heavy chains within the at least one binding unit are identical. In some examples, the two IgM heavy chains within at least one binding unit include the amino acid sequence SEQ ID NO: 52. In some examples, the two light chain constant regions are human lambda constant regions or human kappa constant regions that can be identical and include the amino acid sequence SEQ ID NO: 54.

[0031] According to these provided methods, the binding molecule can include at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, or at least twelve CD20 antigen binding domains that in certain examples can be identical. According to these provided methods at least two, at least three, at least four, at least five, or at least six of the binding units within the binding molecule can be identical.

[0032] According to these provided methods, the reference monospecific, bivalent IgG1 antibody can be rituximab, which includes a VH having the amino acid sequence SEQ ID NO: 1 and a VL having the amino acid sequence SEQ ID NO: 5.

[0033] According to these provided methods, the CD-20-expressing cell can be a lymphoma cell line, *e.g.*, a Ramos cell line, a Raji cell line, a Daudi cell line, a Namalwa cell line, a Granta cell line, a Z138 cell line, a DoHH2 cell line, or a DB cell line. In certain examples where the cell line is a Granta cell line, the binding molecule can direct complement-mediated killing of the cell line at about six times the potency of rituximab. In certain examples where the cell line is a Raji cell line or a Ramos cell line, and where the binding molecule can direct complement-mediated killing of the cell line at about three times the potency of rituximab.

[0034] According to these provided methods, the CD-20-expressing cell can be a malignant B cell in a subject with cancer, *e.g.*, a CD20-positive leukemia, lymphoma, or myeloma. In certain examples, the cancer is minimally responsive or non-responsive to rituximab therapy. In certain examples, the subject is human.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0035] **FIGURE 1**: The molecular diagram of CD20 molecule. CD20 is a tetraspanning-transmembrane protein that predominantly remains on the membrane of B cells without internalization upon antibody binding. The binding sites of CD20 monoclonal antibodies, rituximab and ofatumumab, are indicated. A linear diagram of CD20 is included to display the orientation of transmembrane (TM), extracellular domains (ECD) and cytoplasmic regions.

[0036] **FIGURE 2**: Schematic diagrams of IgG, IgM hexamer and IgM pentamer. IgG is displayed as 150 kDa protein with heavy and light chains indicated. IgM including a J-chain is

indicated as a pentamer of approximately 915 kDa. IgM without a J-chain is shown as a hexamer having a molecular weight of approximately 1080 kDa.

[0037] **FIGURE 3**: **Non-reducing SDS-PAGE of IgG and IgM**. IgG and IgM CD20 antibodies based on rituximab run on non-reducing, SDS denatured polyacrylamide gel electrophoresis. The first and last lanes are molecular weight standards. The murine and human IgG1 antibodies (second and third lanes, respectively) exhibit molecular weights of approximately 150 kDa. The IgM + J-chain version (a commercially available CD20 IgM antibody available from Invivogen) exhibits a molecular weight of about 1050 kDa (fourth lane). The anti-CDIM antibody IGM-55.5 (see PCT Publication No. WO 2013/120012) (fifth lane) is included as another IgM for comparison.

[0038] **FIGURE 4**: Assembly of Anti-CD20 IgM Oligomers. Non-reducing SDS-PAGE shows that anti-CD20 antibodies comprising the variable domains of rituximab and 1.5.3 can assemble as IgM. The rituximab IgM is shown in the first panel without J-chain (lane 2), and with J-chain (lane 3). The 1.5.3 IgM is shown in the second panel without J-chain (lane 2) and with J-chain (lane 3).

[0039] **FIGURE 5A:** ELISA results showing binding of 1.5.3 IgM and 1.5.3 IgG to CD20 at high antigen density (10 μg/ml).

[0040] **FIGURE 5B:** ELISA results showing binding of 1.5.3 IgM and 1.5.3 IgG to CD20 at low antigen density (0.3 μg/ml).

[0041] **FIGURE 6A-E** Anti-CD20 IgM is more potent than anti-CD20 IgG at complement dependent cytotoxicity. Human cultured leukemia or lymphoma cells were incubated with commercially-available anti-CD20 IgM or IgG plus 10% human complement. Cell viability was measured after 4 hours using a metabolic indicator dye (CCK8). In most of the lymphoma cell lines (Granta (**FIG. 6A**), Raji (**FIG. 6B**), and Ramos (**FIG. 6C**)), the IgM isotype of anti-CD20 was more potent than the corresponding IgG isotype, in the presence of complement. For comparison, the assay was also carried out in Namalwa cells (**FIG. 6E**), which exhibit minimal expression of CD20, and Nalm-6 cells (**FIG. 6D**), which are devoid of CD20 expression.

[0042] **FIGURE 7:** Anti-CD20 IgM is more potent than rituximab at complement dependent cytotoxicity in the Raji cell line as measured on a μg/ml basis.

[0043] **FIGURE 8A-B**: Anti-CD20 IgM is more effective than rituximab and 1.5.3 IgG at complement dependent cytotoxicity. Ramos cells were incubated with increasing concentrations of anti-CD20 IgM or IgG and 10% human complement. Cell viability was measured after 4 hours. **FIG. 8A** compares rituximab (IgG) and a rituximab-like IgM+J-chain.

FIG. 8B compares 1.5.3 (IgG), 1.5.3 IgM, and 1.5.3 IgM+J-chain. The tables in each panel show EC50 results in molar concentrations.

[0044] **FIGURE 9**: Complement-dependent cytotoxicity (CDC) activity of rituximab IgG1 (open circles), rituximab-derived anti-human CD20 IgM+J (closed circles), 1.5.3 IgG1 (open squares), and 1.5.3 anti- CD20 IgM+J (closed squares) on DOHH2 and Z138 cells.

[0045] **FIGURE 10**: Characterization of 1.5.3 IgM antibodies by gel electrophoresis and western blotting. Lane Key: 1: Marker; 2: 1.5.3 IgM+V15J; 3: 1.5.3 IgM+wtJ; 4: 1.5.3 IgM (hexamer); 5: Marker. **FIG. 10A** shows the hexamer and pentamer forms on a hybrid gel. **FIG. 10B** shows the antibodies resolved by non-reducing SDS-PAGE. **FIG. 10C** shows the antibodies resolved by reducing SDS-PAGE. The gel in **FIG. 10C** was transferred to a membrane and J-chain was detected by western blotting, shown in **FIG. 10D**.

[0046] **FIGURE 11**: 1.5.3 IgM + V15J (closed squares) elicits T-cell activation in a coculture of CD20+ RPMI8226 cells and engineered Jurkat T-cells to a greater extent than blinatumomab (closed circles) or 1.5.3 IgM + wt J (open squares).

[0047] **FIGURE 12**: T-cell activation by 1.5.3 IgM V15J as a function of CD20 expression using a series of tumor cell lines each expressing a different level of CD20 antigen (expressed as mean fluorescence intensity or MFI).

[0048] **FIGURE 13**: Tumor cell killing in human blood using the KILR™ detection assay. Key: diamonds: 1.5.3 IgM + V15J; squares: 1.5.3 IgM + wild type J; closed circles: 1.5.3 IgG; open circles Rituxan IgG; triangles: blinatumomab.

[0049] **FIGURE 14A-B**: T-cell directed B-cell killing *in vivo* in NSG mice engrafted with CD34+ cells to generate a human hematopoietic system. The mice were dosed with monospecific or bispecific 1.5.3 IgM and the number of human B-cells measured before and at 6 hours post dosing. **FIG. 14A** shows the results for the monospecific antibody with wild-type J-chain, and **FIG. 14B** shows the results for the bispecific with the V15J anti-CD3 J-chain.

[0050] **FIGURE 15**: Comparison of B-cell killing and recovery (at 10-days post dose) between 1.5.3+V15J (top row) and rituximab (bottom row).

DETAILED DESCRIPTION

Definitions

[0051] The term "a" or "an" entity refers to one or more of that entity; for example, "a binding molecule," is understood to represent one or more binding molecules. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

[0052] Furthermore, "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following embodiments: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0053] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, the Concise Dictionary of Biomedicine and Molecular Biology, Juo, Pei-Show, 2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 3rd ed., 1999, Academic Press; and the Oxford Dictionary Of Biochemistry And Molecular Biology, Revised, 2000, Oxford University Press, provide one of skill with a general dictionary of many of the terms used in this disclosure.

[0054] Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, amino acid sequences are written left to right in amino to carboxy orientation. The headings provided herein are not limitations of the various aspects or aspects of the disclosure, which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification in its entirety.

[0055] As used herein, the term “polypeptide” is intended to encompass a singular “polypeptide” as well as plural “polypeptides,” and refers to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as peptide bonds). The term "polypeptide" refers to any chain or chains of two or more amino acids, and does not refer to a specific length of the product. Thus, peptides, dipeptides, tripeptides, oligopeptides, “protein,” “amino acid chain,” or any other term used to refer to a chain or chains of two or more amino acids are included within the definition of "polypeptide," and the term “polypeptide” can be used instead of, or interchangeably with any of these terms. The term "polypeptide" is also intended to refer to the products of post-expression modifications of the polypeptide, including without limitation glycosylation, acetylation, phosphorylation, amidation, and derivatization by known protecting/blocking groups, proteolytic cleavage, or modification by non-naturally occurring amino acids. A polypeptide can be derived from a biological source or produced by recombinant technology, but is not necessarily translated from

a designated nucleic acid sequence. It can be generated in any manner, including by chemical synthesis.

[0056] A polypeptide as disclosed herein can be of a size of about 3 or more, 5 or more, 10 or more, 20 or more, 25 or more, 50 or more, 75 or more, 100 or more, 200 or more, 500 or more, 1,000 or more, or 2,000 or more amino acids. Polypeptides can have a defined three-dimensional structure, although they do not necessarily have such structure. Polypeptides with a defined three-dimensional structure are referred to as folded, and polypeptides which do not possess a defined three-dimensional structure, but rather can adopt a large number of different conformations, and are referred to as unfolded. As used herein, the term glycoprotein refers to a *protein* coupled to at least one carbohydrate moiety that is attached to the protein via an oxygen-containing or a nitrogen-containing side chain of an amino acid, *e.g.*, a serine or an asparagine.

[0057] By an "isolated" polypeptide or a fragment, variant, or derivative thereof is intended a polypeptide that is not in its natural milieu. No particular level of purification is required. For example, an isolated polypeptide can be removed from its native or natural environment. Recombinantly produced polypeptides and proteins expressed in host cells are considered isolated as disclosed herein, as are native or recombinant polypeptides which have been separated, fractionated, or partially or substantially purified by any suitable technique.

[0058] As used herein, the term “a non-naturally occurring polypeptide” or any grammatical variants thereof, is a conditional definition that explicitly excludes, but only excludes, those forms of the polypeptide that are, or could be, determined or interpreted by a judge or an administrative or judicial body, to be “naturally-occurring.”

[0059] Other polypeptides disclosed herein are fragments, derivatives, analogs, or variants of the foregoing polypeptides, and any combination thereof. The terms "fragment," "variant," "derivative" and "analog" as disclosed herein include any polypeptides which retain at least some of the properties of the corresponding native antibody or polypeptide, for example, specifically binding to an antigen. Fragments of polypeptides include, for example, proteolytic fragments, as well as deletion fragments, in addition to specific antibody fragments discussed elsewhere herein. Variants of, *e.g.*, a polypeptide include fragments as described above, and also polypeptides with altered amino acid sequences due to amino acid substitutions, deletions, or insertions. In certain aspects, variants can be non-naturally occurring. Non-naturally occurring variants can be produced using art-known mutagenesis techniques. Variant polypeptides can comprise conservative or non-conservative amino acid substitutions, deletions or additions. Derivatives are polypeptides that have been altered so as to exhibit additional

features not found on the original polypeptide. Examples include fusion proteins. Variant polypeptides can also be referred to herein as "polypeptide analogs." As used herein a "derivative" of a polypeptide can also refer to a subject polypeptide having one or more amino acids chemically derivatized by reaction of a functional side group. Also included as "derivatives" are those peptides that contain one or more derivatives of the twenty standard amino acids. For example, 4-hydroxyproline can be substituted for proline; 5-hydroxylysine can be substituted for lysine; 3-methylhistidine can be substituted for histidine; homoserine can be substituted for serine; and ornithine can be substituted for lysine.

[0060] A "conservative amino acid substitution" is one in which one amino acid is replaced with another amino acid having a similar side chain. Families of amino acids having similar side chains have been defined in the art, including basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). For example, substitution of a phenylalanine for a tyrosine is a conservative substitution. In certain embodiments, conservative substitutions in the sequences of the polypeptides and antibodies of the present disclosure do not abrogate the binding of the polypeptide or antibody containing the amino acid sequence, to the antigen to which the binding molecule binds. Methods of identifying nucleotide and amino acid conservative substitutions which do not eliminate antigen-binding are well-known in the art (see, *e.g.*, Brummell *et al.*, *Biochem.* 32: 1180-1 187 (1993); Kobayashi *et al.*, *Protein Eng.* 12(10):879-884 (1999); and Burks *et al.*, *Proc. Natl. Acad. Sci. USA* 94:.412-417 (1997)).

[0061] The term "polynucleotide" is intended to encompass a singular nucleic acid as well as plural nucleic acids, and refers to an isolated nucleic acid molecule or construct, *e.g.*, messenger RNA (mRNA), cDNA, or plasmid DNA (pDNA). A polynucleotide can comprise a conventional phosphodiester bond or a non-conventional bond (*e.g.*, an amide bond, such as found in peptide nucleic acids (PNA)). The terms "nucleic acid" or “nucleic acid sequence” refer to any one or more nucleic acid segments, *e.g.*, DNA or RNA fragments, present in a polynucleotide.

[0062] By an "isolated" nucleic acid or polynucleotide is intended any form of the nucleic acid or polynucleotide that is separated from its native environment. For example, gel-purified polynucleotide, or a recombinant polynucleotide encoding a polypeptide contained in a vector would be considered to be “isolated.” Also, a polynucleotide segment, *e.g.*, a PCR product,

which has been engineered to have restriction sites for cloning is considered to be "isolated." Further examples of an isolated polynucleotide include recombinant polynucleotides maintained in heterologous host cells or purified (partially or substantially) polynucleotides in a non-native solution such as a buffer or saline. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of polynucleotides, where the transcript is not one that would be found in nature. Isolated polynucleotides or nucleic acids further include such molecules produced synthetically. In addition, polynucleotide or a nucleic acid can be or can include a regulatory element such as a promoter, ribosome binding site, or a transcription terminator.

[0063] As used herein, the term "a non-naturally occurring polynucleotide" or any grammatical variants thereof, is a conditional definition that explicitly excludes, but only excludes, those forms of the nucleic acid or polynucleotide that are, or could be, determined or interpreted by a judge, or an administrative or judicial body, to be "naturally-occurring."

[0064] As used herein, a "coding region" is a portion of nucleic acid which consists of codons translated into amino acids. Although a "stop codon" (TAG, TGA, or TAA) is not translated into an amino acid, it can be considered to be part of a coding region, but any flanking sequences, for example promoters, ribosome binding sites, transcriptional terminators, introns, and the like, are not part of a coding region. Two or more coding regions can be present in a single polynucleotide construct, *e.g.*, on a single vector, or in separate polynucleotide constructs, *e.g.*, on separate (different) vectors. Furthermore, any vector can contain a single coding region, or can comprise two or more coding regions, *e.g.*, a single vector can separately encode an immunoglobulin heavy chain variable region and an immunoglobulin light chain variable region. In addition, a vector, polynucleotide, or nucleic acid can include heterologous coding regions, either fused or unfused to another coding region. Heterologous coding regions include without limitation, those encoding specialized elements or motifs, such as a secretory signal peptide or a heterologous functional domain.

[0065] In certain embodiments, the polynucleotide or nucleic acid is DNA. In the case of DNA, a polynucleotide comprising a nucleic acid which encodes a polypeptide normally can include a promoter and/or other transcription or translation control elements operably associated with one or more coding regions. An operable association is when a coding region for a gene product, *e.g.*, a polypeptide, is associated with one or more regulatory sequences in such a way as to place expression of the gene product under the influence or control of the regulatory sequence(s). Two DNA fragments (such as a polypeptide coding region and a promoter associated therewith) are "operably associated" if induction of promoter function results in the transcription of mRNA encoding the desired gene product and if the nature of the linkage

between the two DNA fragments does not interfere with the ability of the expression regulatory sequences to direct the expression of the gene product or interfere with the ability of the DNA template to be transcribed. Thus, a promoter region would be operably associated with a nucleic acid encoding a polypeptide if the promoter was capable of effecting transcription of that nucleic acid. The promoter can be a cell-specific promoter that directs substantial transcription of the DNA in predetermined cells. Other transcription control elements, besides a promoter, for example enhancers, operators, repressors, and transcription termination signals, can be operably associated with the polynucleotide to direct cell-specific transcription.

[0066] A variety of transcription control regions are known to those skilled in the art. These include, without limitation, transcription control regions which function in vertebrate cells, such as, but not limited to, promoter and enhancer segments from cytomegaloviruses (the immediate early promoter, in conjunction with intron-A), simian virus 40 (the early promoter), and retroviruses (such as Rous sarcoma virus). Other transcription control regions include those derived from vertebrate genes such as actin, heat shock protein, bovine growth hormone and rabbit ß-globin, as well as other sequences capable of controlling gene expression in eukaryotic cells. Additional suitable transcription control regions include tissue-specific promoters and enhancers as well as lymphokine-inducible promoters (*e.g.*, promoters inducible by interferons or interleukins).

[0067] Similarly, a variety of translation control elements are known to those of ordinary skill in the art. These include, but are not limited to ribosome binding sites, translation initiation and termination codons, and elements derived from picornaviruses (particularly an internal ribosome entry site, or IRES, also referred to as a CITE sequence).

[0068] In other embodiments, a polynucleotide can be RNA, for example, in the form of messenger RNA (mRNA), transfer RNA, or ribosomal RNA.

[0069] Polynucleotide and nucleic acid coding regions can be associated with additional coding regions which encode secretory or signal peptides, which direct the secretion of a polypeptide encoded by a polynucleotide as disclosed herein. According to the signal hypothesis, proteins secreted by mammalian cells have a signal peptide or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Those of ordinary skill in the art are aware that polypeptides secreted by vertebrate cells can have a signal peptide fused to the N-terminus of the polypeptide, which is cleaved from the complete or "full length" polypeptide to produce a secreted or "mature" form of the polypeptide. In certain embodiments, the native signal peptide, *e.g.*, an immunoglobulin heavy chain or light chain signal peptide is used, or a functional

derivative of that sequence that retains the ability to direct the secretion of the polypeptide that is operably associated with it. Alternatively, a heterologous mammalian signal peptide, or a functional derivative thereof, can be used. For example, the wild-type leader sequence can be substituted with the leader sequence of human tissue plasminogen activator (TPA) or mouse ß-glucuronidase.

[0070] As used herein, the term “CD20” refers to a membrane protein expressed on the surface of B lymphocytes. The CD20 protein is referred to in the literature by other names, *e.g.*, B-lymphocyte antigen CD20, B-lymphocyte cell-surface antigen B1, Bp35, CVID5, LEU-16, Membrane-spanning 4-domains subfamily A member 1, or MS4A2. The amino acid sequence for human CD20 (GenBank Accession No. NP_690605.1) is disclosed herein as SEQ ID NO: 50 (Table 2).

[0071] Disclosed herein are certain binding molecules, or antigen-binding fragments, variants, or derivatives thereof. Unless specifically referring to full-sized antibodies, the term "binding molecule" encompasses full-sized antibodies as well as antigen-binding subunits, fragments, variants, analogs, or derivatives of such antibodies, *e.g.*, engineered antibody molecules or fragments that bind antigen in a manner similar to antibody molecules, but which use a different scaffold.

[0072] As used herein, the term “binding molecule” refers in its broadest sense to a molecule that specifically binds to a target or molecular determinant, *e.g.*, an epitope or an antigenic determinant. As described further herein, a binding molecule can comprise one or more “antigen binding domains” described herein. A non-limiting example of a binding molecule is an antibody or fragment thereof that retains antigen-specific binding.

[0073] As used herein, the terms “binding domain” or “antigen binding domain” refer to a region of a binding molecule that is sufficient to specifically bind to an epitope. For example, an “Fv,” *e.g.*, a variable heavy chain and variable light chain of an antibody, either as two separate polypeptide subunits or as a single chain, is considered to be a “binding domain.” Other antigen binding domains include, without limitation, the variable heavy chain (VHH) of an antibody derived from a camelid species, or six immunoglobulin complementarity determining regions (CDRs) expressed in a fibronectin scaffold. A “binding molecule” as described herein can include one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve or more “antigen binding domains.”

[0074] The terms "antibody" and "immunoglobulin" can be used interchangeably herein. An antibody (or a fragment, variant, or derivative thereof as disclosed herein) includes at least the variable domain of a heavy chain (for camelid species) or at least the variable domains of a

heavy chain and a light chain. Basic immunoglobulin structures in vertebrate systems are relatively well understood. (*See*, *e.g.*, Harlow *et al.*, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 2nd ed. 1988). Unless otherwise stated, the term "antibody" encompasses anything ranging from a small antigen-binding fragment of an antibody to a full sized antibody, *e.g.*, an IgG antibody that includes two complete heavy chains and two complete light chains, an IgA antibody that includes four complete heavy chains and four complete light chains and can include a J-chain and/or a secretory component, or an IgM antibody that includes ten or twelve complete heavy chains and ten or twelve complete light chains and can include a J-chain.

[0075] As will be discussed in more detail below, the term "immunoglobulin" comprises various broad classes of polypeptides that can be distinguished biochemically. Those skilled in the art will appreciate that heavy chains are classified as gamma, mu, alpha, delta, or epsilon, (γ, μ, α, δ, ε) with some subclasses among them (*e.g.*, γ1-γ4 or α1-α2)). It is the nature of this chain that determines the "isotype" of the antibody as IgG, IgM, IgA IgG, or IgE, respectively. The immunoglobulin subclasses (subtypes) *e.g.*, IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, IgA_2, are well characterized and are known to confer functional specialization. Modified versions of each of these immunoglobulins are readily discernible to the skilled artisan in view of the instant disclosure and, accordingly, are within the scope of this disclosure.

[0076] Light chains are classified as either kappa or lambda (κ, λ). Each heavy chain class can be bound with either a kappa or lambda light chain. In general, the light and heavy chains are covalently bonded to each other, and the "tail" portions of the two heavy chains are bonded to each other by covalent disulfide linkages or non-covalent linkages when the immunoglobulins are expressed, *e.g.*, by hybridomas, B cells or genetically engineered host cells. In the heavy chain, the amino acid sequences run from an N-terminus at the forked ends of the Y configuration to the C-terminus at the bottom of each chain. The basic structure of certain antibodies, *e.g.*, IgG antibodies, includes two heavy chain subunits and two light chain subunits covalently connected via disulfide bonds to form a "Y" structure, also referred to herein as an "H2L2" structure, or a "binding unit."

[0077] The term "binding unit" is used herein to refer to the portion of a binding molecule, *e.g.*, an antibody or antigen-binding fragment thereof, which corresponds to a standard immunoglobulin structure, *e.g.*, two heavy chains or fragments thereof and two light chains or fragments thereof, or two heavy chains or fragments thereof derived, *e.g.*, from a camelid or condricthoid antibody. In certain aspects, *e.g.*, where the binding molecule is a bivalent IgG

antibody or antigen-binding fragment thereof, the terms "binding molecule" and "binding unit" are equivalent. In other aspects, *e.g.*, where the binding molecule is an IgA dimer, an IgM pentamer, or an IgM hexamer, the binding molecule comprises two or more "binding units." Two in the case of an IgA dimer, or five or six in the case of an IgM pentamer or hexamer, respectively. A binding unit need not include full-length antibody heavy and light chains, but will typically be bivalent, *i.e.*, will include two "antigen binding domains," as defined below. Certain binding molecules provided in this disclosure are dimeric, pentameric, or hexameric, and include two, five, or six bivalent binding units that include IgA or IgM constant regions or fragments thereof. As used herein, a binding molecule comprising two or more binding units, *e.g.*, two, five, or six binding units, can be referred to as "multimeric."

[0078] The term "native sequence J-chain" or "native J-chain" as used herein refers to J-chain of native sequence IgM or IgA antibodies of any animal species, including mature human J-chain, the amino acid sequence of which is presented as SEQ ID NO: 49.

[0079] The term "modified J-chain" is used herein to refer to variants of native sequence J-chain polypeptides comprising a heterologous moiety, *e.g.*, a heterologous polypeptide, *e.g.*, an extraneous binding domain introduced into the native sequence. The introduction can be achieved by any means, including direct or indirect fusion of the heterologous polypeptide or other moiety or by attachment through a peptide or chemical linker. The term "modified human J-chain" encompasses, without limitation, a native sequence human J-chain of the amino acid sequence of SEQ ID NO: 49 or functional fragment thereof modified by the introduction of a heterologous moiety, *e.g.*, a heterologous polypeptide, *e.g.*, an extraneous binding domain. In certain aspects the heterologous moiety does not interfere with efficient polymerization of IgM into a pentamer or IgA into a dimer and binding of such polymers to a target. Exemplary modified J-chains can be found, *e.g.*, in PCT Publication No. WO 2015/153912, which is incorporated herein by reference in its entirety.

[0080] The terms "valency," "bivalent," "multivalent" and grammatical equivalents, refer to the number of antigen binding domains in given binding molecule or binding unit. As such, the terms "bivalent", "tetravalent", and "hexavalent" in reference to a given binding molecule, *e.g.*, an IgM antibody or fragment thereof, denote the presence of two antigen binding domains, four antigen binding domains, and six antigen binding domains, respectively. In a typical IgM-derived binding molecule where each binding unit is bivalent, the binding molecule itself can have 10 or 12 valencies. In a typical IgA-derived binding molecule where each binding unit is bivalent, the binding molecule itself can have 4 valencies. A bivalent or multivalent binding molecule can be monospecific, *i.e.*, all of the antigen binding domains are the same, or can be

bispecific or multispecific, *e.g.*, where two or more antigen binding domains are different, *e.g.*, bind to different epitopes on the same antigen, or bind to entirely different antigens.

[0081] The term "epitope" includes any molecular determinant capable of specific binding to an antibody. In certain aspects, an epitope can include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl, or sulfonyl, and, in certain aspects, can have a three dimensional structural characteristics, and or specific charge characteristics. An epitope is a region of a target that is bound by an antibody.

[0082] "Multispecific binding molecules or antibodies" or "bispecific binding molecules or antibodies" refer to binding molecules, antibodies, or antigen-binding fragments thereof that have the ability to specifically bind to two or more different epitopes on the same or different target(s). "Monospecific" refers to the ability to bind only one epitope.

[0083] The term "target" is used in the broadest sense to include substances that can be bound by a binding molecule. A target can be, *e.g.*, a polypeptide, a nucleic acid, a carbohydrate, a lipid, or other molecule. Moreover, a "target" can, for example, be a cell, an organ, or an organism that comprises an epitope bound that can be bound by a binding molecule.

[0084] 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 variable light (VL) and variable heavy (VH) chain portions determine antigen recognition and specificity. Conversely, the constant domains of the light chain (CL) and the heavy chain (*e.g.*, CH1, CH2, CH3, or CH4) confer 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. The N-terminal portion is a variable region and at the C-terminal portion is a constant region; the CH3 (or CH4 in the case of IgM) and CL domains are at the carboxy-terminus of the heavy and light chain, respectively.

[0085] A "full length IgM antibody heavy chain" is a polypeptide that includes, in N-terminal to C-terminal direction, an antibody heavy chain variable domain (V_H), an antibody constant heavy chain constant domain 1 (CM1 or Cμ1), an antibody heavy chain constant domain 2 (CM2 or Cμ2), an antibody heavy chain constant domain 3 (CM3 or Cμ3), and an antibody heavy chain constant domain 4 (CM4 or Cμ4) that can include a tailpiece.

[0086] A "full length IgA antibody heavy chain" is a polypeptide that includes, in N-terminal to C-terminal direction, an antibody heavy chain variable domain (V_H), an antibody constant heavy chain constant domain 1 (CA1 or Cα1), an antibody heavy chain constant domain 2

(CA2 or Cα2), an antibody heavy chain constant domain 3 (CA3 or Cα3) that can include a tailpiece. The structure of monomeric and secretory IgA is described, *e.g.*, in Woof, JM and Russell, MW, *Mucosal Immunology 4*:590-597 (2011).

[0087] As indicated above, a variable region (*i.e.*, the "antigen binding domain") allows a binding molecule to selectively recognize and specifically bind epitopes on antigens. That is, the VL domain and VH domain (or just a VH domain for camelid or condricthoid antibodies (designated as VHH)), or subset of the complementarity determining regions (CDRs), of a binding molecule, *e.g.*, an antibody, can combine to form the antigen binding domain. More specifically, an antigen binding domain can be defined by three CDRs on each of the VH and VL chains (or 3 CDRs on a VHH). Certain antibodies form larger structures. For example, IgA can form a molecule that includes two H2L2 binding units, a J-chain, and a secretory component, covalently connected via disulfide bonds; and IgM can form a dimeric, pentameric, or hexameric molecule that includes two, five, or six H2L2 binding units and optionally a J-chain covalently connected via disulfide bonds.

[0088] The six "complementarity determining regions" or "CDRs" present in an antibody antigen binding domain are short, non-contiguous sequences of amino acids that are specifically positioned to form the antigen binding domain as the antibody assumes its three dimensional configuration in an aqueous environment. The remainder of the amino acids in the antigen binding domain, referred to as "framework" regions, show less inter-molecular variability. The framework regions largely adopt a β-sheet conformation and the CDRs form loops which connect, and in some cases form part of, the β-sheet structure. Thus, framework regions act to form a scaffold that provides for positioning the CDRs in correct orientation by inter-chain, non-covalent interactions. The antigen binding domain formed by the positioned CDRs defines a surface complementary to the epitope on the immunoreactive antigen. This complementary surface promotes the non-covalent binding of the antibody to its cognate epitope. The amino acids that make up the CDRs and the framework regions, respectively, can be readily identified for any given heavy or light chain variable region by one of ordinary skill in the art, since they have been defined in various different ways (*see*, "Sequences of Proteins of Immunological Interest," Kabat, E., *et al.*, U.S. Department of Health and Human Services, (1983); and Chothia and Lesk, *J. Mol. Biol.*, *196*:901-917 (1987), which are incorporated herein by reference in their entireties).

[0089] In the case where there are two or more definitions of a term which is used and/or accepted within the art, the definition of the term as used herein is intended to include all such meanings unless explicitly stated to the contrary. A specific example is the use of the term

"complementarity determining region" ("CDR") to describe the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. These particular regions have been described, for example, by Kabat *et al.*, U.S. Dept. of Health and Human Services, "Sequences of Proteins of Immunological Interest" (1983) and by Chothia *et al.*, *J. Mol. Biol.* 196:901-917 (1987), which are incorporated herein by reference. The Kabat and Chothia definitions include overlapping or subsets of amino acids when compared against each other. Nevertheless, application of either definition (or other definitions known to those of ordinary skill in the art) to refer to a CDR of an antibody or variant thereof is intended to be within the scope of the term as defined and used herein, unless otherwise indicated. The appropriate amino acids which encompass the CDRs as defined by each of the above cited references are set forth below in **Table 1** as a comparison. The exact amino acid numbers which encompass a particular CDR will vary depending on the sequence and size of the CDR. Those skilled in the art can routinely determine which amino acids comprise a particular CDR given the variable region amino acid sequence of the antibody.

Table 1 CDR Definitions*

	Kabat	**Chothia**
VH CDR1	31-35	26-32
VH CDR2	50-65	52-58
VH CDR3	95-102	95-102
VL CDR1	24-34	26-32
VL CDR2	50-56	50-52
VL CDR3	89-97	91-96

*Numbering of all CDR definitions in **Table 1** is according to the numbering conventions set forth by Kabat *et al.* (see below).

[0090] Immunoglobulin variable domains can also be analyzed, *e.g.*, using the IMGT information system (www://imgt.cines.fr/) (IMGT®/V-Quest) to identify variable region segments, including CDRs. *See, e.g.*, Brochet, X. *et al.*, *Nucl. Acids Res. 36*:W503-508 (2008).

[0091] Kabat *et al.* also defined a numbering system for variable domain sequences that is applicable to any antibody. One of ordinary skill in the art can unambiguously assign this system of "Kabat numbering" to any variable domain sequence, without reliance on any experimental data beyond the sequence itself. As used herein, "Kabat numbering" refers to the numbering system set forth by Kabat *et al.*, U.S. Dept. of Health and Human Services, "Sequence of Proteins of Immunological Interest" (1983). Unless use of the Kabat numbering

system is explicitly noted, however, consecutive numbering is used for amino acid sequences in this disclosure.

[0092] Binding molecules, *e.g.*, antibodies or antigen-binding fragments, variants, or derivatives thereof include, but are not limited to, polyclonal, monoclonal, human, humanized, or chimeric antibodies, single chain antibodies, epitope-binding fragments, *e.g.*, Fab, Fab' and $F(ab')_2$, Fd, Fvs, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv), fragments comprising either a VL or VH domain, fragments produced by a Fab expression library. ScFv molecules are known in the art and are described, *e.g.*, in US patent 5,892,019.

[0093] By "specifically binds," it is generally meant that a binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof binds to an epitope via its antigen binding domain, and that the binding entails some complementarity between the antigen binding domain and the epitope. According to this definition, a binding molecule is said to "specifically bind" to an epitope when it binds to that epitope, via its antigen binding domain more readily than it would bind to a random, unrelated epitope. The term "specificity" is used herein to qualify the relative affinity by which a certain binding molecule binds to a certain epitope. For example, binding molecule "A" can be deemed to have a higher specificity for a given epitope than binding molecule "B," or binding molecule "A" can be said to bind to epitope "C" with a higher specificity than it has for related epitope "D."

[0094] A binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof disclosed herein can be said to bind a target antigen with an off rate (k(off)) of less than or equal to 5×10^{-2} sec^{-1}, 10^{-2} sec^{-1}, 5×10^{-3} sec^{-1}, 10^{-3} sec^{-1}, 5×10^{-4} sec^{-1}, 10^{-4} sec^{-1}, 5×10^{-5} sec^{-1}, or 10^{-5} sec^{-1} 5×10^{-6} sec^{-1}, 10^{-6} sec^{-1}, 5×10^{-7} sec^{-1} or 10^{-7} sec^{-1}.

[0095] A binding molecule, *e.g.*, an antibody or antigen-binding fragment, variant, or derivative disclosed herein can be said to bind a target antigen with an on rate (k(on)) of greater than or equal to 10^3 M^{-1} sec^{-1}, 5×10^3 M^{-1} sec^{-1}, 10^4 M^{-1} sec^{-1}, 5×10^4 M^{-1} sec^{-1}, 10^5 M^{-1} sec^{-1}, 5×10^5 M^{-1} sec^{-1}, 10^6 M^{-1} sec^{-1}, or 5×10^6 M^{-1} sec^{-1} or 10^7 M^{-1} sec^{-1}.

[0096] A binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof is said to competitively inhibit binding of a reference antibody or antigen binding fragment to a given epitope if it preferentially binds to that epitope to the extent that it blocks, to some degree, binding of the reference antibody or antigen binding fragment to the epitope. Competitive inhibition can be determined by any method known in the art, for example, competition ELISA assays. A binding molecule can be said to competitively inhibit binding of the reference antibody or antigen binding fragment to a given epitope by at least 90%, at least 80%, at least 70%, at least 60%, or at least 50%.

[0097] As used herein, the term "affinity" refers to a measure of the strength of the binding of an individual epitope with one or more antigen binding domains, *e.g.*, of an immunoglobulin molecule. *See*, *e.g.*, Harlow *et al.*, Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) at pages 27-28. As used herein, the term "avidity" refers to the overall stability of the complex between a population of antigen binding domains and an antigen. *See*, *e.g.*, Harlow at pages 29-34. Avidity is related to both the affinity of individual antigen binding domains in the population with specific epitopes, and also the valencies of the immunoglobulins and the antigen. For example, the interaction between a bivalent monoclonal antibody and an antigen with a highly repeating epitope structure, such as a polymer, would be one of high avidity. An interaction between a between a bivalent monoclonal antibody with a receptor present at a high density on a cell surface would also be of high avidity.

[0098] Binding molecules or antigen-binding fragments, variants or derivatives thereof as disclosed herein can also be described or specified in terms of their cross-reactivity. As used herein, the term "cross-reactivity" refers to the ability of a binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof, specific for one antigen, to react with a second antigen; a measure of relatedness between two different antigenic substances. Thus, a binding molecule is cross reactive if it binds to an epitope other than the one that induced its formation. The cross reactive epitope generally contains many of the same complementary structural features as the inducing epitope, and in some cases, can actually fit better than the original.

[0099] A binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof can also be described or specified in terms of their binding affinity to an antigen. For example, a binding molecule can bind to an antigen with a dissociation constant or K_D no greater than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M.

[0100] Antibody fragments including single-chain antibodies or other antigen binding domains can exist alone or in combination with one or more of the following: hinge region, CH1, CH2, CH3, or CH4 domains, J-chain, or secretory component. Also included are antigen-binding fragments that can include any combination of variable region(s) with one or more of a hinge region, CH1, CH2, CH3, or CH4 domains, a J-chain, or a secretory component. Binding molecules, *e.g.*, antibodies, or antigen-binding fragments thereof can be from any animal origin including birds and mammals. The antibodies can be human, murine, donkey, rabbit, goat, guinea pig, camel, llama, horse, or chicken antibodies. In another embodiment, the variable region can be condricthoid in origin (*e.g.*, from sharks). As used herein, "human" antibodies

include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulins and can in some instances express endogenous immunoglobulins and some not, as described *infra* and, for example in, U.S. Pat. No. 5,939,598 by Kucherlapati *et al.*

[0101] As used herein, the term "heavy chain subunit" includes amino acid sequences derived from an immunoglobulin heavy chain, a binding molecule, *e.g.*, an antibody comprising a heavy chain subunit can include at least one of: a VH domain, a CH1 domain, a hinge (*e.g.*, upper, middle, and/or lower hinge region) domain, a CH2 domain, a CH3 domain, a CH4 domain, or a variant or fragment thereof. For example, a binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof can include, without limitation, in addition to a VH domain: a CH1 domain; a CH1 domain, a hinge, and a CH2 domain; a CH1 domain and a CH3 domain; a CH1 domain, a hinge, and a CH3 domain; or a CH1 domain, a hinge domain, a CH2 domain, and a CH3 domain. In certain aspects a binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof can include, in addition to a VH domain, a CH3 domain and a CH4 domain; or a CH3 domain, a CH4 domain, and a J-chain. Further, a binding molecule for use in the disclosure can lack certain constant region portions, *e.g.*, all or part of a CH2 domain. It will be understood by one of ordinary skill in the art that these domains (*e.g.*, the heavy chain subunit) can be modified such that they vary in amino acid sequence from the original immunoglobulin molecule.

[0102] As used herein, the term "light chain subunit" includes amino acid sequences derived from an immunoglobulin light chain. The light chain subunit includes at least a VL, and can further include a CL (*e.g.*, Cκ or Cλ) domain.

[0103] Binding molecules, *e.g.*, antibodies or antigen-binding fragments, variants, or derivatives thereof can be described or specified in terms of the epitope(s) or portion(s) of an antigen that they recognize or specifically bind. The portion of a target antigen that specifically interacts with the antigen binding domain of an antibody is an "epitope," or an "antigenic determinant." A target antigen can comprise a single epitope or at least two epitopes, and can include any number of epitopes, depending on the size, conformation, and type of antigen.

[0104] As used herein the term "disulfide bond" includes the covalent bond formed between two sulfur atoms. The amino acid cysteine comprises a thiol group that can form a disulfide bond or bridge with a second thiol group.

[0105] As used herein, the term "chimeric antibody" refers to an antibody in which the immunoreactive region or site is obtained or derived from a first species and the constant region

(which can be intact, partial or modified) is obtained from a second species. In some embodiments the target binding region or site will be from a non-human source (*e.g.* mouse or primate) and the constant region is human.

[0106] The term "multispecific antibody, *e.g.*, "bispecific antibody" refers to an antibody that has antigen binding domains for two or more different epitopes within a single antibody molecule. Other binding molecules in addition to the canonical antibody structure can be constructed with two binding specificities. Epitope binding by bispecific or multispecific antibodies can be simultaneous or sequential. Triomas and hybrid hybridomas are two examples of cell lines that can secrete bispecific antibodies. Bispecific antibodies can also be constructed by recombinant means. (Ströhlein and Heiss, *Future Oncol. 6*:1387-94 (2010); Mabry and Snavely, *IDrugs. 13*:543-9 (2010)). A bispecific antibody can also be a diabody.

[0107] As used herein, the term "engineered antibody" refers to an antibody in which the variable domain in either the heavy and light chain or both is altered by at least partial replacement of one or more amino acids in either the CDR or framework regions. In certain aspects entire CDRs from an antibody of known specificity can be grafted into the framework regions of a heterologous antibody. Although alternate CDRs can be derived from an antibody of the same class or even subclass as the antibody from which the framework regions are derived, CDRs can also be derived from an antibody of different class, *e.g.*, from an antibody from a different species. An engineered antibody in which one or more "donor" CDRs from a non-human antibody of known specificity are grafted into a human heavy or light chain framework region is referred to herein as a "humanized antibody." In certain aspects not all of the CDRs are replaced with the complete CDRs from the donor variable region and yet the antigen binding capacity of the donor can still be transferred to the recipient variable domains. Given the explanations set forth in, *e.g.*, U. S. Pat. Nos. 5,585,089, 5,693,761, 5,693,762, and 6,180,370, it will be well within the competence of those skilled in the art, either by carrying out routine experimentation or by trial and error testing to obtain a functional engineered or humanized antibody.

[0108] As used herein the term "engineered" includes manipulation of nucleic acid or polypeptide molecules by synthetic means (*e.g.* by recombinant techniques, *in vitro* peptide synthesis, by enzymatic or chemical coupling of peptides or some combination of these techniques).

[0109] As used herein, the terms "linked," "fused" or "fusion" or other grammatical equivalents can be used interchangeably. These terms refer to the joining together of two more elements or components, by whatever means including chemical conjugation or recombinant means. An

"in-frame fusion" refers to the joining of two or more polynucleotide open reading frames (ORFs) to form a continuous longer ORF, in a manner that maintains the translational reading frame of the original ORFs. Thus, a recombinant fusion protein is a single protein containing two or more segments that correspond to polypeptides encoded by the original ORFs (which segments are not normally so joined in nature.) Although the reading frame is thus made continuous throughout the fused segments, the segments can be physically or spatially separated by, for example, in-frame linker sequence. For example, polynucleotides encoding the CDRs of an immunoglobulin variable region can be fused, in-frame, but be separated by a polynucleotide encoding at least one immunoglobulin framework region or additional CDR regions, as long as the "fused" CDRs are co-translated as part of a continuous polypeptide.

[0110] In the context of polypeptides, a "linear sequence" or a "sequence" is an order of amino acids in a polypeptide in an amino to carboxyl terminal direction in which amino acids that neighbor each other in the sequence are contiguous in the primary structure of the polypeptide. A portion of a polypeptide that is “amino-terminal” or “N-terminal” to another portion of a polypeptide is that portion that comes earlier in the sequential polypeptide chain. Similarly a portion of a polypeptide that is “carboxy-terminal” or “C-terminal” to another portion of a polypeptide is that portion that comes later in the sequential polypeptide chain. For example in a typical antibody, the variable domain is “N-terminal” to the constant region, and the constant region is “C-terminal” to the variable domain.

[0111] The term “expression” as used herein refers to a process by which a gene produces a biochemical, for example, a polypeptide. The process includes any manifestation of the functional presence of the gene within the cell including, without limitation, gene knockdown as well as both transient expression and stable expression. It includes without limitation transcription of the gene into RNA, *e.g.*, messenger RNA (mRNA), and the translation of such mRNA into polypeptide(s). If the final desired product is a biochemical, expression includes the creation of that biochemical and any precursors. Expression of a gene produces a "gene product." As used herein, a gene product can be either a nucleic acid, *e.g.*, a messenger RNA produced by transcription of a gene, or a polypeptide that is translated from a transcript. Gene products described herein further include nucleic acids with post transcriptional modifications, *e.g.*, polyadenylation, or polypeptides with post translational modifications, *e.g.*, methylation, glycosylation, the addition of lipids, association with other protein subunits, proteolytic cleavage, and the like.

[0112] Terms such as "treating" or "treatment" or "to treat" or "alleviating" or "to alleviate" refer to therapeutic measures that cure, slow down, lessen symptoms of, and/or halt or slow the

progression of an existing diagnosed pathologic condition or disorder. Terms such as "prevent," "prevention," "avoid," "deterrence" and the like refer to prophylactic or preventative measures that prevent the development of an undiagnosed targeted pathologic condition or disorder. Thus, "those in need of treatment" can include those already with the disorder; those prone to have the disorder; and those in whom the disorder is to be prevented.

[0113] By "subject" or "individual" or "animal" or "patient" or "mammal," is meant any subject, particularly a mammalian subject, for whom diagnosis, prognosis, or therapy is desired. Mammalian subjects include humans, domestic animals, farm animals, and zoo, sports, or pet animals such as dogs, cats, guinea pigs, rabbits, rats, mice, horses, swine, cows, bears, and so on.

[0114] As used herein, phrases such as "a subject that would benefit from therapy" and "an animal in need of treatment" includes subjects, such as mammalian subjects, that would benefit from administration of a binding molecule such as an antibody, comprising one or more antigen binding domains. Such binding molecules, *e.g.*, antibodies, can be used, *e.g.*, for a diagnostic procedures and/or for treatment or prevention of a disease.

IgM Binding Molecules

[0115] IgM is the first immunoglobulin produced by B cells in response to stimulation by antigen, and is present at around 1.5 mg/ml in serum with a half-life of 5 days. IgM is a dimeric, pentameric, or hexameric molecule. An IgM binding unit includes two light and two heavy chains. While IgG contains three heavy chain constant domains (CH1, CH2 and CH3), the heavy (μ) chain of IgM additionally contains a fourth constant domain (CH4), that includes a C-terminal "tailpiece." The human IgM constant region typically comprises the amino acid sequence SEQ ID NO: 47. The human Cμ1 region ranges from about amino acid 5 to about amino acid 102 of SEQ ID NO: 47; the human Cμ2 region ranges from about amino acid 114 to about amino acid 205 of SEQ ID NO: 47, the human Cμ3 region ranges from about amino acid 224 to about amino acid 319 of SEQ ID NO: 47, the Cμ 4 region ranges from about amino acid 329 to about amino acid 430 of SEQ ID NO: 47, and the tailpiece ranges from about amino acid 431 to about amino acid 453 of SEQ ID NO: 47 (Table 2).

[0116] Five IgM binding units can form a complex with an additional small polypeptide chain (the J-chain) to form an IgM antibody. The human J-chain comprises the amino acid sequence SEQ ID NO: 49 (Table 2). A typical IgM pentamer is depicted in **FIG. 2**. Without the J-chain, IgM binding units typically assemble into a hexamer. A typical IgM hexamer is depicted in **FIG. 2**. While not wishing to be bound by theory, the assembly of IgM binding units into a

hexameric or pentameric binding molecule is thought to involve the Cμ3 and Cμ4 domains. Accordingly, a hexameric or pentameric binding molecule provided in this disclosure typically includes IgM constant regions that include at least the Cμ3 and Cμ4 domains. A comparison of IgG antibodies and IgM antibodies by non-reducing polyacrylamide gel electrophoresis is shown in **FIG. 3**.

[0117] An IgM heavy chain constant region can additionally include a Cμ2 domain or a fragment thereof, a Cμ1 domain or a fragment thereof, and/or other IgM heavy chain domains. In certain aspects, a binding molecule as provided herein can include a complete IgM heavy (μ) chain constant domain (*e.g.*, SEQ ID NO: 47), or a variant, derivative, or analog thereof.

Pentameric or Hexameric CD20 Binding Molecules

[0118] This disclosure provides a hexameric or pentameric binding molecule, *i.e.*, a binding molecule with five or six "binding units" as defined herein, that can specifically bind to CD20, *e.g.*, human CD20. A binding molecule as provided herein can possess improved binding characteristics or biological activity as compared to a binding molecule composed of a single binding unit, *e.g.*, a bivalent IgG antibody. In certain aspects, the disclosure provides a pentameric or hexameric binding molecule comprising five or six bivalent binding units, respectively, where each binding unit includes two IgM heavy chain constant regions or fragments thereof. In certain aspects, the two IgM heavy chain constant regions are human heavy chain constant regions.

[0119] Where the binding molecule provided herein is pentameric, the binding molecule can further comprise a J-chain, or functional fragment thereof, or variant thereof. In certain aspects, the J-chain is a modified J-chain comprising a heterologous moiety or one or more heterologous moieties, *e.g.*, a heterologous polypeptide sequence, *e.g.*, an extraneous binding domain introduced into the native sequence. In certain aspects the extraneous binding domain specifically binds to CD3, *e.g.*, CD3ε. In certain aspects the modified J-chain comprises V15J (SEQ ID NO: 64) or J15V (SEQ ID NO: 66).

[0120] An IgM heavy chain constant region can include one or more of a Cμ1 domain, a Cμ2 domain, a Cμ3 domain, and/or a Cμ4 domain, provided that the constant region can serve a desired function in the binding molecule, *e.g.*, associate with second IgM constant region to form an antigen binding domain, or associate with other binding units to form a hexamer or a pentamer. In certain aspects the two IgM heavy chain constant regions or fragments thereof within an individual binding unit each comprise a Cμ3 domain or fragment thereof, a Cμ4 domain or fragment thereof, a tailpiece (TP) or fragment thereof, or any combination of a Cμ3

domain a Cμ domain, and a TP or fragment thereof. In certain aspects the two IgM heavy chain constant regions or fragments thereof within an individual binding unit each further comprise a Cμ2 domain or fragment thereof, a Cμ1 domain or fragment thereof, or a Cμ1 domain or fragment thereof and a Cμ2 domain or fragment thereof.

[0121] In certain aspects each of the two IgM heavy chain constant regions in a given binding unit is associated with an antigen binding domain, for example an Fv portion of an antibody, *e.g.*, a VH and a VL of a human or murine antibody. In a binding molecule as provided herein at least one antigen binding domain of the binding molecule is an anti-CD20 antigen binding domain, *i.e.*, an antigen binding domain that can specifically bind to CD20, *e.g.*, human CD20.

IgA Binding Molecules

[0122] IgA plays a role in mucosal immunity, and comprises about 15% of total immunoglobulin produced. IgA is a monomeric or dimeric molecule. An IgA binding unit typically includes two light and two heavy chains. IgA contains three heavy chain constant domains (Cα1, Cα2 and Cα3), and includes a C-terminal "tailpiece." Human IgA has two subtypes, IgA1 and IgA2. The human IgA1 constant region typically comprises the amino acid sequence SEQ ID NO: 59. The human Cα1 region ranges from about amino acid 6 to about amino acid 98 of SEQ ID NO: 59; the human Cα2 region ranges from about amino acid 125 to about amino acid 220 of SEQ ID NO: 59, the human Cα3 region ranges from about amino acid 228 to about amino acid 330 of SEQ ID NO: 59, and the tailpiece ranges from about amino acid 331 to about amino acid 352 of SEQ ID NO: 59 (Table 2). The human IgA2 constant region typically comprises the amino acid sequence SEQ ID NO: 60. The human Cα1 region ranges from about amino acid 6 to about amino acid 98 of SEQ ID NO: 60 (Table 2); the human Cα2 region ranges from about amino acid 112 to about amino acid 207 of SEQ ID NO: 60, the human Cα3 region ranges from about amino acid 215 to about amino acid 317 of SEQ ID NO: 60, and the tailpiece ranges from about amino acid 318 to about amino acid 340 of SEQ ID NO: 60.

[0123] Two IgA binding units can form a complex with two additional polypeptide chains, the J-chain (SEQ ID NO: 49) and the secretory component (SEQ ID NO: 62) to form a secretory IgA (sIgA) antibody. While not wishing to be bound by theory, the assembly of IgA binding units into a dimeric sIgA binding molecule is thought to involve the Cα3 and tailpiece domains. Accordingly, a dimeric sIgA binding molecule provided in this disclosure typically includes IgA constant regions that include at least the Cα3 and tailpiece domains.

[0124] An IgA heavy chain constant region can additionally include a Cα2 domain or a fragment thereof, a Cα1 domain or a fragment thereof, and/or other IgA heavy chain domains. In certain aspects, a binding molecule as provided herein can include a complete IgA heavy (α) chain constant domain (*e.g.*, SEQ ID NO: 59 or SEQ ID NO: 60), or a variant, derivative, or analog thereof.

Dimeric CD20 Binding Molecules

[0125] This disclosure provides a dimeric binding molecule, *e.g.*, a binding molecule with two IgA "binding units" as defined herein, which can specifically bind to CD20, *e.g.*, human CD20. A dimeric binding molecule as provided herein can possess improved binding characteristics or biological activity as compared to a binding molecule composed of a single binding unit, *e.g.*, a bivalent IgG antibody. For example, an IgA binding molecule can reach mucosal sites providing greater tissue distribution for the binding molecules provided herein. In certain aspects, the disclosure provides a dimeric binding molecule comprising two bivalent binding units, where each binding unit includes two IgA heavy chain constant regions or fragments thereof. In certain aspects, the two IgA heavy chain constant regions are human heavy chain constant regions.

[0126] A dimeric IgA binding molecule as provided herein can further comprise a J-chain, or fragment thereof, or variant thereof. A dimeric IgA binding molecule as provided herein can further comprise a secretory component, or fragment thereof, or variant thereof. In certain aspects, the J-chain is a modified J-chain comprising a heterologous moiety or one or more heterologous moieties, *e.g.*, a heterologous polypeptide, *e.g.*, an extraneous binding domain introduced into the native sequence. In certain aspects the extraneous binding domain specifically binds to CD3, *e.g.*, CD3ε. In certain aspects the modified J-chain comprises V15J (SEQ ID NO: 64) or J15V (SEQ ID NO: 66).

[0127] An IgA heavy chain constant region can include one or more of a Cα1 domain, a Cα2 domain, and/or a Cα3 domain, provided that the constant region can serve a desired function in the binding molecule, *e.g.*, associate with a light chain constant region to facilitate formation of an antigen binding domain, or associate with another IgA binding unit to form a dimeric binding molecule. In certain aspects the two IgA heavy chain constant regions or fragments thereof within an individual binding unit each comprise a Cα3 domain or fragment thereof, a tailpiece (TP) or fragment thereof, or any combination of a Cα3 domain, a TP, or fragment thereof. In certain aspects the two IgA heavy chain constant regions or fragments thereof within an individual binding unit each further comprise a Cα2 domain or fragment thereof, a Cα1

domain or fragment thereof, or a Cα1 domain or fragment thereof and a Cα2 domain or fragment thereof.

[0128] In certain aspects each of the two IgA heavy chain constant regions in a given antigen binding domain is associated with an antigen binding domain, for example an Fv portion of an antibody, *e.g.*, a VH and a VL of a human or murine antibody. In a binding molecule as provided herein at least one antigen binding domain of the binding molecule can be a CD20 antigen binding domain, *e.g.*, a human CD20 antigen binding domain.

Modified J-Chains

[0129] In certain aspects CD20 binding molecules provided herein can be bispecific, incorporating a modified J-chain. As provided herein and in PCT Publication No. WO 2015/153912 , a modified J-chain can comprise a heterologous moiety, *e.g.*, a heterologous polypeptide, *e.g.*, an extraneous binding domain, which can include, for example, a polypeptide binding domain capable of specifically binding to a target. The binding domain can be, for example, an antibody or antigen-binding fragment thereof, an antibody-drug conjugate or antigen-binding fragment thereof, or an antibody-like molecule. A polypeptide binding domain can be introduced into a J-chain by appropriately selecting the location and type of addition (*e.g.* direct or indirect fusion, chemical tethering, etc.).

[0130] In certain aspects, the binding domain can be an antibody or an antigen-binding fragment of an antibody, including monospecific, bispecific, and multi-specific antibodies and antibody fragments. The antibody fragment can be, without limitation, a Fab fragment, a Fab' fragment, a $F(ab')_2$ fragment, an scFv, $(scFv)_2$ fragment, single-chain antibody molecules, minibodies, or multispecific antibodies formed from antibody fragments. In certain aspects, the antibody fragment is a scFv.

[0131] In other aspects, the binding domain can be an antibody-like molecule, for example, a human domain antibody (dAb), Dual-Affinity Re-Targeting (DART) molecule, a diabody, a di-diabody, dual-variable domain antibody, a Stacked Variable Domain antibody, a Small Modular Immuno Pharmaceutical (SMIP), a Surrobody, a strand-exchange engineered domain (SEED)-body, or TandAb.

[0132] The binding domain can be introduced into the native J-chain sequence at any location that allows the binding of the binding domain to its binding target without interfering with the binding of the recipient IgM or IgA molecule to its binding target or binding targets or the ability of the J-chain to effectively incorporated into an IgA dimer or an IgM pentamer. In certain aspects the binding domain can be inserted at or near the C-terminus, at or near the

mature N-terminus (*i.e.*, amino acid number 23 of SEQ ID NO: 49 following cleavage of the signal peptide) or at an internal location that, based on the three-dimensional structure of the J-chain is accessible. In certain aspects, the binding domain can be introduced into the native sequence J-chain without about 10 residues from the C-terminus or without about 10 amino acid residues from the mature N-terminus, of the human J-chain of SEQ ID NO: 49. In another aspect, the binding domain can be introduced into the native sequence human J-chain of SEQ ID NO: 49 in between cysteine residues 114 and 123 of SEQ ID NO: 49, or at an equivalent location of another native sequence J-chain. In a further aspect, the binding domain can be introduced into a native sequence J-chain, such as a J-chain of SEQ ID NO: 49, at or near a glycosylation site. In certain aspects, the binding domain can be introduced into the native sequence human J-chain of SEQ ID NO: 49 within about 10 amino acid residues from the C-terminus.

[0133] Introduction can be accomplished by direct or indirect fusion, *i.e.* by the combination of the J-chain and binding domain in one polypeptide chain by in-frame combination of their coding nucleotide sequences, with or without a peptide linker. The peptide linker (indirect fusion), if used, can be about 1 to 50, or about 1 to 40, or about 1 to 30, or about 1 to 20, or about 1 to 10, or about 10 to 20 amino acids in length, and can be present at one or both ends of the binding domain to be introduced into the J-chain sequence. In certain aspects, the peptide linker is about 10 to 20, or 10 to 15 amino acids long. In certain aspects the peptide linker is 15 amino acids long. In certain aspects the peptide linker is $(GGGGS)_3$ (SEQ ID NO: 67).

[0134] It is also possible to introduce more than one heterologous polypeptide, *e.g.*, more than one binding domain, into a J-chain.

[0135] The modified J-chain can be produced by well-known techniques of recombinant DNA technology, by expressing a nucleic acid encoding the modified J-chain in a suitable prokaryotic or eukaryotic host organism.

[0136] The modified J-chain can also be co-expressed with the heavy and light chains of the recipient IgM or IgA binding molecules as described elsewhere herein. The recipient binding molecule, prior to modified J-chain incorporation, can be monospecific, bispecific or multi-specific, *e.g.*, a monospecific, bispecific, or multispecific IgA or IgM antibody. Bispecific and multi-specific IgM and IgA binding molecules, including antibodies, are described, for example, in U.S. Application Serial Nos. 61/874,277 and 61/937,984, the entire contents of which are hereby expressly incorporated by reference.

[0137] In certain aspects, an anti-CD20 IgM or IgA binding molecule as described herein can include a modified J-chain with binding specificity for an immune effector cell, such as a T-

cell, NK-cell, a macrophage, or a neutrophil. In certain aspects the effector cell is a T-cell and the binding target is CD3 (discussed below). By activating and redirecting effector cells, *e.g.* effector T-cells, to CD20-expressing B cells, *e.g.*, malignant B cells, a bispecific anti-CD20 x anti-CD3 IgM or IgA binding molecule as provided herein can produce an enhanced immune response against the target, the response comprising, *e.g.*, complement-mediated cytotoxicity and/or antibody dependent cellular cytotoxicity (ADCC), thereby further increasing potency and efficacy. In certain aspects, a bispecific anti-CD20 x anti-CD3 IgM or IgA binding molecule as provided herein comprising a modified J-chain can be used for the treatment of B-cell related cancers.

[0138] In the case of T-cells, cluster of differentiation 3 (CD3) is a multimeric protein complex, known historically as the T3 complex, and is composed of four distinct polypeptide chains (ε, γ, δ, ζ) that assemble and function as three pairs of dimers (εγ, εδ, ζζ). The CD3 complex serves as a T-cell co-receptor that associates non-covalently with the T-cell receptor (TCR). Components of this CD3 complex, especially CD3ε, can be targets for a modified J-chain of a bispecific IgM or IgA binding molecule provided herein.

[0139] In certain aspects, a bispecific anti-CD20 x anti-CD3 IgM or IgA binding molecule binds to CD20 via the antibody binding domains, while the J-chain is modified to bind to CD3ε.

[0140] In certain aspects the anti- CD3ε binding domain of a modified J-chain provided herein is a scFv. The anti CD3ε scFv can be fused at or near the N-terminus of the J-chain, or at or near the C-terminus of the J-chain either directly or indirectly with a synthetic linker introduced in between the scFv and the J-chain sequences, *e.g.*, a $(GGGGS)_3$ linker (SEQ ID NO: 67). In certain aspects the scFv comprises the VH and VL regions of visilizumab (Nuvion). In certain aspects the modified J-chain comprises a scFv comprising the VH of visilizumab, a $(GGGGS)_3$ linker, and the VL of visilizumab.

[0141] In certain aspects the modified J-chain comprises a scFv of visilizumab fused to the N-terminus of the human J-chain through a 15-amino acid $(GGGGS)_3$ linker, a modified J-chain referred to herein as V15J. V15J can further include a signal peptide to facilitate transport and assembly into an IgM or IgA binding molecules. The mature V15J protein is presented as SEQ ID NO: 64, the precursor version, comprising a 19-amino acid-immunoglobulin heavy chain signal peptide is presented as SEQ ID NO: 63. In certain aspects the modified J-chain comprises a scFv of visilizumab fused to the C-terminus of the human J-chain through a 15-amino acid $(GGGGS)_3$ linker, a modified J-chain referred to herein as J15V. J15V can further include a signal peptide to facilitate transport and assembly into an IgM or IgA binding molecules. The mature J15V protein is presented as SEQ ID NO: 65, the precursor version,

comprising the 22-amino acid-human J-chain signal peptide is presented as SEQ ID NO: 66. In certain aspects, other signal peptides can be used. Selection and inclusion of suitable signal peptides to facilitate expression, secretion, and incorporation of a modified J-chain into an anti-CD20 IgM or IgA binding molecule as provided herein is well within the capabilities of a person of ordinary skill in the art.

CD20 Binding Domains

[0142] In certain aspects the CD20 antigen binding domain comprises six immunoglobulin complementarity determining regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein at least one, at least two, at least three, at least four, at least five, or at least six CDRs are related to the corresponding CDRs of 1.5.3 disclosed in U.S. Patent Publication No. 2007-0014720. The CD20 antigen binding domain can include an HCDR1 comprising the amino acid sequence of SEQ ID NO: 39 or SEQ ID NO: 39 with one, two, three, four, or five single amino acid substitutions, *e.g.*, one or two single amino acid substitutions. The CD20 antigen binding domain can include an HCDR2 comprising the amino acid sequence of SEQ ID NO: 40 or SEQ ID NO: 40 with one, two, three, four, or five single amino acid substitutions, *e.g.*, one or two single amino acid substitutions. The CD20 antigen binding domain can include an HCDR3 comprising the amino acid sequence of SEQ ID NO: 41, or SEQ ID NO: 41 with one, two, three, four, or five single amino acid substitutions, *e.g.*, one or two single amino acid substitutions. The CD20 antigen binding domain can include an LCDR1 comprising the amino acid sequence of SEQ ID NO: 43, or SEQ ID NO: 43 with one, two, three, four, or five single amino acid substitutions, *e.g.*, one or two single amino acid substitutions. The CD20 antigen binding domain can include an LCDR2 comprising the amino acid sequence of SEQ ID NO: 44 or SEQ ID NO: 44 with one, two, three, four, or five single amino acid substitutions, *e.g.*, one or two single amino acid substitutions. The CD20 antigen binding domain can include an LCDR3 comprising the amino acid sequence of SEQ ID NO: 45 or SEQ ID NO: 45 with one, two, three, four, or five single amino acid substitutions, *e.g.*, one or two single amino acid substitutions. The CD20 antigen binding domain can include any one, any two, any three, any four, any five or all six of the CDR amino acid sequences as described above. In certain aspects the CD20 antigen binding domain includes an HCDR1 comprising the amino acid sequence SEQ ID NO: 39, an HCDR2 comprising the amino acid sequence SEQ ID NO: 40, an HCDR3 comprising the amino acid sequence SEQ ID NO: 41, an LCDR1 comprising the amino acid sequence SEQ ID NO: 43, an LCDR2 comprising the amino acid sequence SEQ ID NO: 44, and an LCDR3 comprising the amino acid sequence SEQ ID NO: 45.

[0143] In certain aspects the CD20 antigen binding domain comprises an antibody heavy chain variable region (VH) and an antibody light chain variable region (VL), wherein the VH region, the VL region, or both the VH and VL regions are related to the corresponding VH and VL of 1.5.3 disclosed in U.S. Patent Publication No. 2007-0014720. In certain aspects the VH can comprise an amino acid sequence at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 38. In certain aspects the VL can comprise an amino acid sequence at least at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 42. In certain aspects the VH comprises the amino acid sequence SEQ ID NO: 38 and the VL comprises the amino acid sequence SEQ ID NO: 42.

[0144] While a variety of different dimeric, pentameric, or hexameric binding molecules can be contemplated by a person of ordinary skill in the art based on this disclosure, and as such are included in this disclosure, in certain aspects, a binding molecule as described above is provided in which each binding unit comprises two IgA or IgM heavy chains each comprising a VH situated amino terminal to the IgA or IgM constant region or fragment thereof, and two immunoglobulin light chains each comprising a VL situated amino terminal to an immunoglobulin light chain constant region.

[0145] Moreover in certain aspects, at least one binding unit of the binding molecule, or at least two, at least three, at least four, at least five, or at least six binding units of the binding molecule, comprises or comprise two of the CD20 antigen binding domains as described above. In certain aspects the two CD20 antigen binding domains in the binding unit of the binding molecule, or the two, three, four, five, or six binding units of the binding molecule, can be different from each other, or they can be identical.

[0146] In certain aspects, the two IgM heavy chains within the one, two, three, four, five, or six binding unit(s) of the binding molecule are identical. In certain aspects, two identical IgM heavy chains within at least one binding unit, or within at least two, at least three, at least four, at least five, or at least six binding units of the binding molecule comprise the amino acid sequence SEQ ID NO: 56.

[0147] In certain aspects, the two light chains within the one, two, three, four, five, or six binding unit(s) of the binding molecule are identical. In certain aspects, two identical light chains within at least one binding unit, or within at least two, at least three, at least four, at least five, or at least six binding units of the binding molecule are kappa light chains, *e.g.*, human kappa light chains, or lambda light chains, *e.g.*, human lambda light chains. In certain aspects, two identical light chains within at least one binding unit, or within at least two, at least three, at

least four, at least five, or at least six binding units of the binding molecule each comprise the amino acid sequence SEQ ID NO: 58.

[0148] In certain aspects at least one, at least two, at least three, at least four, at least five, or at least six binding units of a dimeric, pentameric, or hexameric binding molecule provided by this disclosures comprises or each comprise two identical IgM heavy chains each comprising the amino acid sequence SEQ ID NO: 56, and two identical light chains each comprising the amino acid sequence SEQ ID NO: 58. According to this aspect, the CD20 antigen binding domains in the one, two, three, four, five, or six binding unit(s) of the binding molecule, can be identical. Further according to this aspect, a dimeric, pentameric, or hexameric binding molecule as provided herein can comprise at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, or at least twelve copies of a CD20 antigen binding domain as described above. In certain aspects at least two, at least three, at least four, at least five, or at least six of the binding units can be identical and, in certain aspects the binding units can comprise identical antigen binding domains, *e.g.*, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, or at least twelve CD20 antigen binding domains can be identical. In certain aspects the identical CD20 antigen binding domain can comprise a VH with the amino acid sequence SEQ ID NO: 38, and a VL with the amino acid sequence SEQ ID NO: 42.

[0149] In certain aspects, a dimeric, pentameric, or hexameric CD20 binding molecule as provided herein can possess advantageous structural or functional properties compared to other binding molecules. For example, the dimeric, pentameric, or hexameric CD20 binding molecule can possess improved activity in a biological assay, either *in vitro* or *in vivo*, than a corresponding binding molecule, *e.g.*, an IgG1 1.5.3 as disclosed in U.S. Patent Publication No. 2007-0014720. Biological assays include, but are not limited to complement-dependent cellular cytotoxicity (CDC) and antibody dependent cellular cytotoxicity (ADCC).

[0150] In certain aspects a dimeric, pentameric, or hexameric binding molecule as provided herein can direct complement-mediated, T-cell-mediated, or both complement-mediated and T-cell-mediated killing of a CD-20-expressing cell, *e.g.*, a CD20-expressing B cell, at higher potency than an equivalent amount of a monospecific, bivalent IgG1 antibody or fragment thereof that specifically binds to the same CD20 epitope as the CD20 antigen binding domain, *e.g.*, an IgG1 version of 1.5.3 comprising human IgG1 and a VH with the amino acid sequence SEQ ID NO: 38 and a VL with the amino acid sequence SEQ ID NO: 42. In certain aspects a dimeric, pentameric, or hexameric binding molecule as provided herein can direct complement-

mediated, T-cell-mediated, or both complement-mediated and T-cell-mediated killing of a CD20-expressing cell, *e.g.*, a CD20-expressing B cell at higher potency than an equivalent amount of monospecific, bivalent CD20 monoclonal antibody or fragment thereof, where the antibody is, or comprises the same VH and VL regions as, *e.g.*, rituximab (Genentech), ofatumumab (Glaxo SmithKline), veltuzumab (Takeda), ocaratuzumab (Lilly), tositumumab (Glaxo SmithKline), or obinutumumab (Roche/Genentech).

[0151] By "potency" is meant the least amount of a given binding molecule necessary to achieve a given biological result, *e.g.*, killing of 50% of the cells in a given assay, *e.g.*, a CDC or ADCC assay (IC_{50}). Potency can be expressed as a curve in which % survival of cells is on the Y axis, and binding molecule concentration (in, *e.g.*, μg/ml or μM) is on the X axis.

[0152] In certain aspects CDC can be measured *in vitro*, and the CD20-expressing cell can be an immortalized cell line, *e.g.*, a B-cell lymphoma cell line, *e.g.*, a Ramos cell line, a Raji cell line, a Daudi cell line, a Namalwa cell line, a Granta cell line, a Z138 cell line, a DoHH2 cell line, or a DB cell line. Similar cell lines are known and are easily obtained by a person of ordinary skill in the art.

[0153] In certain aspects, CDC can be measured or demonstrated *in vitro* or *in vivo*, and the CD-20-expressing cell line is a malignant B cell obtained from, or in, a subject, *e.g.*, a human patient, with cancer, *e.g.*, a B-cell related lymphoma, leukemia, or myeloma. In certain aspects the cancer is minimally responsive or non-responsive to conventional therapy, *e.g.*, chemotherapy, or monoclonal antibody therapy with one or more of, *e.g.*, rituximab (Genentech), ofatumumab (Glaxo SmithKline), veltuzumab (Takeda), ocaratuzumab (Lilly), tositumumab (Glaxo SmithKline), or obinutumumab (Roche/Genentech). In certain aspects a dimeric, pentameric, or hexameric binding molecule comprising any one or more of the binding domains described herein, *e.g.*, in **Table 5**, is provided.

[0154] In certain aspects, ADCC can be measured *in vitro* through T-cell activation assays, *e.g.*, by co-culturing CD20-expressing B-cells and engineered CD3-expression T-cells in the presence of a bispecific anti-CD20 x anti-CD3 IgM binding molecule as provided herein, and measuring T-cell activation through cytokine release, target cell lysis, or other detection method. In certain aspects ADCC can be measured through T-cell directed B-cell killing. In certain aspects the CD20-expressing cell can be an immortalized cell line, *e.g.*, a B-cell lymphoma cell line, *e.g.*, a Ramos cell line, a Raji cell line, a Daudi cell line, a Namalwa cell line, a Granta cell line, a Z138 cell line, a DoHH2 cell line, or a DB cell line. Similar cell lines are known and are easily obtained by a person of ordinary skill in the art. In certain aspects the CD20-expressing cell line can be derived from a patient suffering from a B-cell related cancer.

[0155] In certain aspects, the totality of killing of CD20+ cells, *e.g.*, by CDC, ADCC, and other modes of killing, *e.g.*, apoptosis, can be tested *in vitro* in an assay using whole blood that includes both T-cells and complement.

[0156] In certain aspects, *e.g.*, where the binding molecule is a pentameric binding molecule comprising five identical binding units each comprising two identical CD20 binding domains with, *e.g.*, the VH and VL of 1.5.3, tested in a CDC assay using, *e.g.*, the CD20-expressing Raji cell line, the binding molecule can direct complement mediated killing with an IC_{50} at least one-fold, at least two-fold, at least three-fold, at least four-fold, at least five-fold, at least ten-fold, at least 20-fold, at least thirty-fold, at least forty-fold, at least 50-fold, at least 100-fold, at least 150-fold, at least 200-fold or more lower than the IC_{50} of an equivalent amount of the monospecific bivalent IgG1 antibody, *e.g.*, 1.5.3 or rituximab, as measured, *e.g.*, in μg/ml. In certain aspects, where the CD20-expressing cell is a Ramos cell line, the binding molecule can direct complement-mediated killing with an IC_{50} at least ten-fold, at least 20-fold, at least 30-fold, at least 40-fold, at least 50-fold, at least 60-fold, at least 70-fold, at least 80-fold, at least 90-fold, or at least 100-fold lower than the IC_{50} of an equivalent amount of the monospecific bivalent IgG1 antibody, as measured, *e.g.*, as molar equivalents.

[0157] In certain aspects, *e.g.*, a pentameric binding molecule comprising five identical binding units each comprising two identical binding domains with, *e.g.*, the VH and VL of 1.5.3, or the VH and VL of rituximab, plus a wild-type or modified J-chain as provided herein can exhibit increased potency in a CDC assay performed in cells exhibiting lower CD20 expression levels. For example, rituximab-derived anti-human CD20 IgM+J or 1.5.3 anti- CD20 IgM+J, tested in a CDC assay using the CD20-expressing DoHH2 (CD20 high expression) and Z138 (CD20 low expression) cell lines, can direct complement-mediated killing with an IC_{50} at least one-fold, at least two-fold, at least three-fold, at least four-fold, at least five-fold, at least ten-fold, at least 20-fold, at least thirty-fold, at least forty-fold, at least 50-fold, at least 100-fold, at least 150-fold, at least 200-fold or more lower than the IC_{50} of an equivalent amount of the monospecific bivalent IgG1 antibody equivalents, *e.g.*, rituximab (IgG1), or 1.5.3 (IgG1), as measured, *e.g.*, as molar equivalents. In certain aspects, rituximab-derived anti-human CD20 IgM+J and 1.5.3 anti- CD20 IgM+J, tested in a CDC assay using the Z138 (CD20 low expression) cell line, can direct complement-mediated killing of the cell line under conditions where 50% killing (EC_{50}) with the equivalent IgG molecules cannot be achieved even at a concentration of 100 nM.

[0158] In certain aspects, a bispecific pentameric binding molecule comprising five identical binding units each comprising two identical CD20 binding domains with, *e.g.*, the VH and VL of 1.5.3, or the VH and VL of rituximab, plus a modified J-chain capable of binding to human

CD3, *e.g.*, V15J or J15V as provided herein, can exhibit increased potency in a ADCC assay. For example, rituximab-derived anti-human CD20 IgM+ V15J or J15V, or 1.5.3 anti- CD20 IgM+ V15J or J15V, tested in a T-cell activation assay, *e.g.*, using the CD20-expressing DB cell line co-cultured with engineered Jurkat T-cells, can facilitate T-cell mediated killing with an IC_{50} at least one-fold, at least two-fold, at least three-fold, at least four-fold, at least five-fold, at least ten-fold, at least 20-fold, at least thirty-fold, at least forty-fold, at least 50-fold, at least 100-fold, at least 150-fold, at least 200-fold or more lower than the IC_{50} of an equivalent amount of a monovalent bispecific binding molecule that binds B-cells and T-cells, *e.g.*, a bispecific anti-CD19 (monovalent) x anti-CD3 (monovalent) molecule blinatumomab.

[0159] In certain aspects, a monospecific or bispecific pentameric binding molecule comprising five identical binding units each comprising two identical binding domains with, *e.g.*, the VH and VL of 1.5.3, or the VH and VL of rituximab, plus a wild-type J-chain or a modified J-chain capable of binding to human CD3, *e.g.*, V15J or J15V as provided herein, can exhibit increased potency in a whole-blood *in vitro* cytotoxicity assay. For example, 1.5.3 anti- CD20 IgM+ V15J or J15V, or 1.5.3 anti- CD20 IgM+J, tested in a KILR™ *in vitro* cytotoxicity assay using the CD20-expressing KILR™ ARH-77 cell line co-cultured with Hirudin anti-coagulated human blood can achieve killing of the KILR™ ARH-77 cell line with an IC_{50} at least one-fold, at least two-fold, at least three-fold, at least four-fold, at least five-fold, at least ten-fold, at least 20-fold, at least thirty-fold, at least forty-fold, at least 50-fold, at least 100-fold, at least 150-fold, at least 200-fold or more lower than the IC_{50} of an equivalent amount of a monospecific bivalent anti-CD20 binding molecule, *e.g.*, 1.5.3 IgG, or a monovalent bispecific binding molecule that binds B-cells and T-cells, *e.g.*, a bispecific anti-CD19 (monovalent) x anti-CD3 (monovalent) molecule blinatumomab.

[0160] In certain aspects, a monospecific or bispecific pentameric binding molecule comprising five identical binding units each comprising two identical binding domains with, *e.g.*, the VH and VL of 1.5.3, or the VH and VL of rituximab, plus a wild-type J-chain or a modified J-chain capable of binding to human CD3, *e.g.*, V15J or J15V as provided herein, can exhibit significant B-cell killing *in vivo*, for example in a humanized mouse model as described elsewhere herein.

Polynucleotides, Vectors, and Host Cells

[0161] The disclosure further provides a polynucleotide, *e.g.*, an isolated, recombinant, and/or non-naturally-occurring polynucleotide, comprising a nucleic acid sequence that encodes a polypeptide subunit of the dimeric, pentameric, or hexameric binding molecule as described

above. By "polypeptide subunit" is meant a portion of a binding molecule, binding unit, or antigen binding domain that can be independently translated. Examples include, without limitation, an antibody variable domain, *e.g.*, a VH or a VL, a single chain Fv, an antibody heavy chain, an antibody light chain, an antibody heavy chain constant region, an antibody light chain constant region, and/or any fragment thereof.

[0162] In certain aspect, the polypeptide subunit can comprise an IgA or IgM heavy chain constant region and at least the antibody VH portion of the CD20 antigen binding domain. In certain aspects the polynucleotide can encode a polypeptide subunit comprising a human IgA or IgM constant region or fragment thereof fused to the C-terminal end of a VH, where the VH comprises an HCDR1, HCDR2, and HCDR3, wherein the HCDR1 comprises the amino acid sequence of SEQ ID NO: 39 or SEQ ID NO: 39 with one, two, three, four, or five single amino acid substitutions, *e.g.*, one or two single amino acid substitutions; the HCDR2 comprises the amino acid sequence of SEQ ID NO: 40 or SEQ ID NO: 40 with one, two, three, four, or five single amino acid substitutions, *e.g.*, or two single amino acid substitutions; the HCDR3 comprises the amino acid sequence of SEQ ID NO: 41, SEQ ID NO: 41 with one, two, three, four, or five single amino acid substitutions, *e.g.*, one or two single amino acid substitutions; or the VH comprises an amino acid sequence at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 38. In certain aspects the polypeptide subunit comprises the amino acid sequence SEQ ID NO: 56.

[0163] In certain aspects, the polypeptide subunit can comprise an antibody VL portion of a CD20 antigen binding domain as described above. In certain aspects the polypeptide subunit can comprise a human antibody light chain constant region or fragment thereof fused to the C-terminal end of a VL, where the VL comprises an LCDR1, LCDR2, and LCDR3, wherein the LCDR1 comprises the amino acid sequence of SEQ ID NO: 43, or SEQ ID NO: 43 with one, two, three, four, or five single amino acid substitutions, *e.g.*, one or two single amino acid substitutions; the LCDR2 comprises the amino acid sequence of SEQ ID NO: 44 or SEQ ID NO: 44 with one, two, three, four, or five single amino acid substitutions, *e.g.*, one or two single amino acid substitutions; and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 45 or SEQ ID NO: 45 with one, two, three, four, or five single amino acid substitutions, *e.g.*, one or two single amino acid substitutions; or the VL comprises an amino acid sequence at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 42. In certain aspects the polypeptide subunit comprises the amino acid sequence SEQ ID NO: 58.

[0164] In certain aspects, the polypeptide subunit can comprise an antibody VH or an antibody VL portion of a CD20 antigen binding domain comprising any one or more of the VH or VL amino acid sequences described above and/or in **Table 5**.

[0165] The disclosure further provides a composition comprising two or more polynucleotides, where the two or more polynucleotides collectively can encode a dimeric, pentameric, or hexameric binding molecule as described above. In certain aspects the composition can include a polynucleotide encoding an IgA or IgM heavy chain or fragment thereof, e.g, a human IgA or IgM heavy chain as described above where the IgA or IgM heavy chain comprises at least the VH of a CD20 antigen binding domain, and a polynucleotide encoding a light chain or fragment thereof, *e.g.*, a human kappa or lambda light chain that comprises at least the VL of a CD20 antigen binding domain. A polynucleotide composition as provided can further include a polynucleotide encoding a J-chain, *e.g.*, a human J-chain, or a fragment thereof or a variant thereof. In certain aspects the polynucleotides making up a composition as provided herein can be situated on two or three separate vectors, *e.g.*, expression vectors. Such vectors are provided by the disclosure. In certain aspects two or more of the polynucleotides making up a composition as provided herein can be situated on a single vector, *e.g.*, an expression vector. Such a vector is provided by the disclosure.

[0166] The disclosure further provides a host cell, *e.g.*, a prokaryotic or eukaryotic host cell, comprising a polynucleotide or two or more polynucleotides encoding a dimeric, pentameric, or hexameric CD20 binding molecule as provided herein, or any subunit thereof, a polynucleotide composition as provided herein, or a vector or two, three, or more vectors that collectively encode a dimeric, pentameric, or hexameric CD20 binding molecule as provided herein, or any subunit thereof. In certain aspects a host cell provided by the disclosure can express a dimeric, pentameric, or hexameric CD20 binding molecule as provided by this disclosure, or a subunit thereof.

[0167] In a related aspect, the disclosure provides a method of producing a dimeric, pentameric, or hexameric CD20 binding molecule as provided by this disclosure, where the method comprises culturing a host cell as described above, and recovering the binding molecule.

Methods of Use

[0168] This disclosure provides improved methods for directing complement-mediated, T-cell-mediated, or both complement-mediated and T-cell-mediated killing of cells that express CD20, *e.g.*, B cells, *e.g.*, malignant or immortalized B cells, using a dimeric, pentameric, or hexameric IgA- or IgM-based CD20 binding molecule. The methods described below can utilize binding

molecules comprising CD20 antigen binding domains derived from any CD20 antibody, including without limitation 1.5.3 as disclosed in U.S. Patent Publication No. 2007-0014720, rituximab, ofatumumab, veltuzumab, ocaratuzumab, obinutumumab, or variants, derivatives, or analogs thereof, where the dimeric, pentameric, or hexameric CD20 binding molecule can provide improved complement-mediated, T-cell-mediated, or both complement-mediated and T-cell-mediated killing of CD20-expressing cells as compared to an equivalent IgG antibody, fragment, variant, derivative, or analog thereof, comprising the same antigen binding domain. In certain aspects the IgA-or IgM-based CD20 binding molecule further comprises a J-chain, either a wild-type J-chain or a modified J-chain capable of binding to human CD3, *e.g.*, V15J or J15V as provided herein. Based on this disclosure, construction of a dimeric, pentameric, or hexameric IgA- or IgM-based CD20 binding molecule comprising any CD20 antigen binding domain of interest is well within the capabilities of a person of ordinary skill in the art. The improved activity can, for example, allow a reduced dose to be used, or can result in more effective killing of cells that are resistant to killing by the original antibody. By "resistant" is meant any degree of reduced activity of a CD20 antibody, *e.g.*, rituximab, on the CD20-expressing cell. Use of an IgA-based binding molecule, can allow, for example, greater tissue distribution for a binding molecule provided herein.

[0169] In certain aspects, this disclosure provides a method for directing improved complement-mediated, T-cell-mediated, or both complement-mediated and T-cell-mediated killing of a CD20-expressing cell, where the method includes contacting a CD20-expressing cell with a dimeric, pentameric, or hexameric binding molecule as described herein, where the binding molecule can direct complement-mediated, T-cell-mediated, or both complement-mediated and T-cell-mediated killing of a CD-20-expressing cell, *e.g.*, a CD20-expressing B cell, at higher potency than an equivalent amount of a monospecific, bivalent IgG, *e.g.*, IgG1 antibody or fragment thereof that specifically binds to the same CD20 epitope as the CD20 antigen binding domain, *e.g.*, rituximab, which comprises a human IgG1 and a VH with the amino acid sequence SEQ ID NO: 1 and a VL with the amino acid sequence SEQ ID NO: 5. The dimeric or pentameric binding molecule can further include a wild-type J-chain or a modified J-chain capable of binding to human CD3, *e.g.*, V15J or J15V as provided herein. In certain aspects a dimeric, pentameric, or hexameric binding molecule as provided herein can direct complement-mediated, T-cell-mediated, or both complement-mediated and T-cell-mediated killing of a CD20-expressing cell, *e.g.*, a CD20-expressing B cell at higher potency than an equivalent amount of monospecific, bivalent CD20 monoclonal antibody or fragment

thereof, where the antibody is, or comprises the same VH and VL regions as, *e.g.*, ofatumumab, veltuzumab, ocaratuzumab, or obinutumumab.

[0170] This disclosure thus provides a method for directing complement-mediated, T-cell-mediated, or both complement-mediated and T-cell-mediated killing of a CD20-expressing cell, where the method includes: contacting a CD20-expressing cell with a dimeric, pentameric, or hexameric binding molecule comprising two, five, or six bivalent binding units, respectively, wherein each binding unit comprises two IgA or IgM heavy chain constant regions or fragments thereof and two antigen binding domains. Non-limiting examples of suitable binding molecules include 1.5.3-based dimeric, pentameric, or hexameric binding molecule provided by this disclosure, or other binding molecules as described elsewhere in this disclosure. The dimeric or pentameric binding molecule can further include a wild-type J-chain or a modified J-chain capable of binding to human CD3, *e.g.*, V15J or J15V as provided herein. According to the method at least one antigen binding domain of the binding molecule is a CD20 antigen binding domain. Moreover, according to the method the binding molecule can direct complement-mediated, T-cell-mediated, or both complement-mediated and T-cell-mediated killing of a CD-20-expressing cell at higher potency than an equivalent amount of a monospecific, bivalent IgG, *e.g.*, IgG1 antibody or fragment thereof that specifically binds to the same CD20 epitope as the CD20 antigen binding domain, *e.g.*, a bivalent IgG1 antibody comprising a CD20 antigen binding domain similar to, *e.g.*, identical to, a CD20 antigen binding domain of the dimeric, pentameric, or hexameric binding molecule provided by this disclosure.

[0171] For example, the disclosure provides a method for directing complement-mediated, T-cell-mediated, or both complement-mediated and T-cell-mediated killing of a CD20-expressing cell, where the method includes: contacting a CD20-expressing cell with a dimeric, pentameric, or hexameric binding molecule comprising at least one antigen binding domain related to 1.5.3, as described elsewhere herein, where the binding molecule can direct complement-mediated, T-cell-mediated, or both complement-mediated and T-cell-mediated killing of a CD-20-expressing cell at higher potency than an equivalent amount of a monospecific, bivalent IgG1 antibody or fragment thereof that specifically binds to the same CD20 epitope as 1.5.3-related CD20 antigen binding domain. The dimeric or pentameric binding molecule can further include a wild-type J-chain or a modified J-chain capable of binding to human CD3, *e.g.*, V15J or J15V as provided herein. In certain aspects the monospecific, bivalent IgG1 antibody is 1.5.3, and comprises a VH having the amino acid sequence SEQ ID NO: 38 and a VL having the amino acid sequence SEQ ID NO: 42.

[0172] In certain aspects, *e.g.*, where the binding molecule is a pentameric binding molecule comprising five identical binding units each comprising two identical binding domains with the VH and VL of 1.5.3, tested in a CDC assay using the CD20-expressing Raji cell line, the binding molecule can direct complement-mediated killing with an IC_{50} at least one-fold, at least two-fold, at least three-fold, at least four-fold, at least five-fold, at least ten-fold, at least 20-fold, at least thirty-fold, at least forty-fold, at least 50-fold, at least 100-fold, at least 150-fold, at least 200-fold or more lower than the IC_{50} of an equivalent amount of the monospecific bivalent IgG1 antibody, *e.g.*, 1.5.3, as measured, *e.g.*, in μg/ml or in molar equivalents.

[0173] In certain aspects the CD-20-expressing cell is an immortalized cell line, *e.g.* a B cell leukemia or lymphoma cell line. The cell line can be, without limitation, a Ramos cell line, a Raji cell line, a Daudi cell line, a Namalwa cell line, a Granta cell line, a Z138 cell line, a DoHH2 cell line, or a DB cell line. Other cell lines that can be useful in the methods provided herein can easily be identified by a person of ordinary skill in the art.

[0174] In certain aspects the cell line is a Granta cell line, and the dimeric, pentameric, or hexameric binding molecule can direct complement-mediated killing of the cell line at about six times the potency of rituximab as measured in μg/ml. In certain aspects the cell line is a Ramos cell line, and the dimeric, pentameric, or hexameric binding molecule can direct complement-mediated killing of the cell line at about 30-40 times the potency of rituximab as measured in molar equivalents.

[0175] In certain aspects the cell line is a Raji cell line or a Ramos cell line, and the dimeric, pentameric, or hexameric binding molecule can direct complement-mediated killing of the cell line at about three times the potency of rituximab.

[0176] In certain aspects the CD20-expressing cell is a malignant B cell in a subject, *e.g.*, a human patient, with cancer. The cancer can be, for example, a CD20-positive leukemia, lymphoma, or myeloma. In certain aspects the CD20-expressing cell line or malignant B cell is resistant, *e.g.*, minimally responsive or non-responsive to killing by a commercially-available CD20 monoclonal antibody, *e.g.*, the CD20-expressing cell line or malignant B cell in a subject with cancer is minimally responsive or non-responsive to rituximab therapy.

[0177] In another aspect the disclosure provides a method for directing complement-mediated, T-cell-mediated, or both complement-mediated and T-cell-mediated killing of a CD20-expressing cell, where the method includes: contacting a CD20-expressing cell with a dimeric, pentameric, or hexameric binding molecule comprising at least one antigen binding domain related to the CD20 mAb rituximab, or a fragment, variant, derivative, or analog thereof. The

dimeric or pentameric binding molecule can further include a wild-type J-chain or a modified J-chain capable of binding to human CD3, *e.g.*, V15J or J15V as provided herein.

[0178] In certain aspects the CD20 antigen binding domain of the dimeric, pentameric, or hexameric binding molecule comprises six immunoglobulin complementarity determining regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein at least one, at least two, at least three, at least four, at least five, or at least six CDRs are related to the corresponding CDRs of the CD20 mAb rituximab. The CD20 antigen binding domain can include an HCDR1 comprising the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 2 with one, two, three, four, or five single amino acid substitutions, *e.g.*one or two single amino acid substitutions. The CD20 antigen binding domain can include an HCDR2 comprising the amino acid sequence of SEQ ID NO: 3 or SEQ ID NO: 3 with one, two, three, four, or five single amino acid substitutions. For example, the HCDR2 can comprise the amino acid sequence SEQ ID NO: 16. The CD20 antigen binding domain can include an HCDR3 comprising the amino acid sequence of SEQ ID NO: 4, or SEQ ID NO: 4 with one, two, three, four, or five single amino acid substitutions, *e.g.*, one, two, or three single amino acid substitutions. For example, the HCDR3 can comprise the amino acid sequence SEQ ID NO: 17, SEQ ID NO: 31, or SEQ ID NO: 36. The CD20 antigen binding domain can, in other aspects, include an HCDR3 comprising the amino acid sequence SEQ ID NO: 10, or SEQ ID NO: 10 with one, two, three, four, or five single amino acid substitutions. The CD20 antigen binding domain can, in other aspects, include an HCDR3 comprising the amino acid sequence SEQ ID NO: 31, or SEQ ID NO: 31 with one, two, three, four, or five single amino acid substitutions. The CD20 antigen binding domain can include an LCDR1 comprising the amino acid sequence of SEQ ID NO: 6, or SEQ ID NO: 6 with one, two, three, four, or five single amino acid substitutions, *e.g.*, one, two, or three single amino acid substitutions. For example, the LCDR1 can comprise the amino acid sequence SEQ ID NO: 12, SEQ ID NO: 19, SEQ ID NO: 25, SEQ ID NO: 27, or SEQ ID NO: 33. The CD20 antigen binding domain can include an LCDR2 comprising the amino acid sequence of SEQ ID NO: 7 or SEQ ID NO: 7 with one, two, three, four, or five single amino acid substitutions, *e.g.*, one or two single amino acid substitutions. For example the LCDR2 can comprise the amino acid sequence SEQ ID NO: 13 or SEQ ID NO: 20. The CD20 antigen binding domain can include an LCDR3 comprising the amino acid sequence of SEQ ID NO: 8 or SEQ ID NO: 8 with one, two, three, four, or five single amino acid substitutions, *e.g.*, one or two single amino acid substitutions. For example, the LCDR3 can comprise the amino acid sequence SEQ ID NO: 14, SEQ ID NO: 21, or SEQ ID NO: 34.

[0179] In certain aspects the CD20 antigen binding domain of the dimeric, pentameric, or hexameric binding molecule comprises a VH and a VL, wherein the VH region, the VL region, or both the VH and the VL regions are related to the corresponding VH and VL of rituximab. In certain aspects the CD20 antigen binding domain can comprise a VH amino acid sequence at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 1 and a VL amino acid sequence at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 5.

[0180] In certain aspects the VH and VL can be derived from the CD20 mAb described in U.S. Patent No. 7,679,900. For example, the CD20 antigen binding domain can comprise a VH amino acid sequence at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 9 and a VL amino acid sequence at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 11.

[0181] In certain aspects the VH and VL can be derived from the CD20 mAb described in U.S. Patent No. 8,153,125. For example, the CD20 antigen binding domain can comprise a VH amino acid sequence at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 15 and a VL amino acid sequence at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 18.

[0182] In certain aspects the VH and VL can be derived from the CD20 mAb described in U.S. Patent No. 8,337,844. For example, the CD20 antigen binding domain can comprise a VH amino acid sequence at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 22 or SEQ ID NO: 23 and a VL amino acid sequence at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, or SEQ ID NO: 29.

[0183] In certain aspects the VH and VL can be derived from the CD20 mAb described in U.S. Patent No. 8,337,844. For example, the CD20 antigen binding domain can comprise a VH amino acid sequence at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 30 and a VL amino acid sequence at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 32.

[0184] In certain aspects the VH and VL can be derived from the CD20 mAb described in U.S. Patent No. 7,151,164. For example, the CD20 antigen binding domain can comprise a VH amino acid sequence at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 35 and a VL amino acid sequence at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 37.

[0185] A dimeric, pentameric, or hexameric binding molecule for use in the methods provided herein, is a binding molecule with two, five, or six "binding units" as defined herein, that can specifically bind to CD20, *e.g.*, human CD20. In certain aspects, a dimeric, pentameric, or hexameric binding molecule for use in the methods provided herein comprises two, five, or six bivalent binding units, respectively, where each binding unit includes two IgA or IgM heavy chain constant regions or fragments thereof. In certain aspects, the two IgA or IgM heavy chain constant regions are human heavy chain constant regions.

[0186] Where the binding molecule for use in the methods provided herein is pentameric, the binding molecule can further comprise a J-chain, or fragment thereof, or variant thereof. The J-chain can be a wild-type J-chain or a modified J-chain capable of binding to human CD3, *e.g.*, V15J or J15V as provided herein.

[0187] An IgM heavy chain constant region of a binding molecule for use in the methods provided herein can include one or more of a Cμ1 domain, a Cμ2 domain, a Cμ3 domain, and/or a Cμ4 domain, provided that the constant region can serve a desired function in the binding molecule, *e.g.*, associate with second IgM constant region to form an antigen binding domain, or associate with other binding units to form a hexamer or a pentamer. In certain aspects the two IgM heavy chain constant regions or fragments thereof within an individual binding unit each comprise a Cμ3 domain or fragment thereof, a Cμ4 domain or fragment thereof, a tailpiece (TP) or fragment thereof, or any combination of a Cμ3 domain a Cμ domain, and a TP or fragment thereof. In certain aspects the two IgM heavy chain constant regions or fragments thereof within an individual binding unit each further comprise a Cμ2 domain or fragment thereof, a Cμ1 domain or fragment thereof, or a Cμ1 domain or fragment thereof and a Cμ2 domain or fragment thereof.

[0188] While a variety of different dimeric, pentameric, or hexameric binding molecules for use in the methods provided herein can be contemplated by a person of ordinary skill in the art based on this disclosure, and as such are included in this disclosure, in certain aspects, a binding molecule for use in the methods provided herein is provided in which each binding unit comprises two IgA or IgM heavy chains each comprising a VH situated amino terminal to the

IgA or IgM constant region or fragment thereof, and two immunoglobulin light chains each comprising a VL situated amino terminal to an immunoglobulin light chain constant region.

[0189] Moreover in certain aspects, at least one binding unit of the binding molecule for use in the methods provided herein, or at least two, at least three, at least four, at least five, or at least six binding units of the binding molecule for use in the methods provided herein, comprises or comprise two of the CD20 antigen binding domains as described above. In certain aspects the two CD20 antigen binding domains in the one, two, three, four, five, or six binding unit(s) of the binding molecule for use in the methods provided herein can be different from each other, or they can be identical.

[0190] In certain aspects, the two IgM heavy chains within the one, two, three, four, five, or six binding unit(s) of the binding molecule for use in the methods provided herein are identical. In certain aspects, two identical IgM heavy chains within at least one binding unit, or within at least two, at least three, at least four, at least five, or at least six binding units of the binding molecule for use in the methods provided herein comprise the amino acid sequence SEQ ID NO: 52.

[0191] In certain aspects, the two light chains within the one, two, three, four, five, or six binding unit(s) of the binding molecule for use in the methods provided herein are identical. In certain aspects, two identical light chains within at least one binding unit, or within at least two, at least three, at least four, at least five, or at least six binding units of the binding molecule for use in the methods provided herein are kappa light chains, *e.g.*, human kappa light chains, or lambda light chains, *e.g.*, human lambda light chains. In certain aspects, two identical light chains within at least one binding unit, or within at least two, at least three, at least four, at least five, or at least six binding units of the binding molecule for use in the methods provided herein each comprise the amino acid sequence SEQ ID NO: 54.

[0192] In certain aspects at least one, at least two, at least three, at least four, at least five, or at least six binding units of a pentameric or hexameric binding molecule for use in the methods provided herein comprises or each comprise two identical IgM heavy chains each comprising the amino acid sequence SEQ ID NO: 52, and two identical light chains each comprising the amino acid sequence SEQ ID NO: 54. According to this aspect, the CD20 antigen binding domains in the one, two, three, four, five, or six binding unit(s) of the binding molecule can be identical. Further according to this aspect, a dimeric, pentameric, or hexameric binding molecule for use in the methods provided herein can comprise at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, or at least twelve copies of a CD20 antigen binding domain as described

above. In certain aspects at least two, at least three, at least four, at least five, or at least six of the binding units can be identical and, in certain aspects the binding units can comprise identical antigen binding domains, *e.g.*, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, or at least twelve CD20 antigen binding domains can be identical. In certain aspects the identical CD20 antigen binding domain can comprise a VH with the amino acid sequence SEQ ID NO: 1 and a VL with the amino acid sequence SEQ ID NO: 5.

[0193] Dimeric, pentameric, or hexameric CD20 binding molecules for use in the methods provided herein can possess advantageous structural or functional properties compared to other binding molecules. For example, a dimeric, pentameric, or hexameric CD20 binding molecule for use in the methods provided herein can possess improved activity in a biological assay, either *in vitro* or *in vivo*, than a corresponding binding molecule, *e.g.*, rituximab or a variant, analog, or derivative thereof. Biological assays include, but are not limited to complement-dependent cellular cytotoxicity (CDC) and/or antibody dependent cellular cytotoxicity (ADCC).

Pharmaceutical Compositions and Administration Methods

[0194] Methods of preparing and administering a dimeric, pentameric, or hexameric CD20 binding molecule as provided herein to a subject in need thereof are well known to or are readily determined by those skilled in the art in view of this disclosure. The route of administration of a CD20 binding molecule can be, for example, oral, parenteral, by inhalation or topical. The term parenteral as used herein includes, *e.g.*, intravenous, intraarterial, intraperitoneal, intramuscular, subcutaneous, rectal, or vaginal administration. While these forms of administration are contemplated as suitable forms, another example of a form for administration would be a solution for injection, in particular for intravenous or intraarterial injection or drip. A suitable pharmaceutical composition can comprise a buffer (*e.g.* acetate, phosphate or citrate buffer), a surfactant (*e.g.* polysorbate), optionally a stabilizer agent (*e.g.* human albumin).

[0195] As discussed herein, a dimeric, pentameric, or hexameric CD20 binding molecule as provided herein can be administered in a pharmaceutically effective amount for the *in vivo* treatment of diseases or disorders in which it's desirable to deplete B cells. In this regard, it will be appreciated that the disclosed binding molecules can be formulated so as to facilitate administration and promote stability of the active agent. Pharmaceutical compositions accordingly can comprise a pharmaceutically acceptable, non-toxic, sterile carrier such as physiological saline, non-toxic buffers, preservatives and the like. A pharmaceutically effective

amount of a dimeric, pentameric, or hexameric CD20 binding molecule as provided herein means an amount sufficient to achieve effective binding to a target and to achieve a therapeutic benefit. Suitable formulations are described in Remington's Pharmaceutical Sciences (Mack Publishing Co.) 16th ed. (1980).

[0196] Certain pharmaceutical compositions provided herein can be orally administered in an acceptable dosage form including, *e.g.*, capsules, tablets, aqueous suspensions or solutions. Certain pharmaceutical compositions also can be administered by nasal aerosol or inhalation. Such compositions can be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, and/or other conventional solubilizing or dispersing agents.

[0197] The amount of a dimeric, pentameric, or hexameric CD20 binding molecule that can be combined with carrier materials to produce a single dosage form will vary depending, *e.g.*, upon the subject treated and the particular mode of administration. The composition can be administered as a single dose, multiple doses or over an established period of time in an infusion. Dosage regimens also can be adjusted to provide the optimum desired response (*e.g.*, a therapeutic or prophylactic response).

[0198] In keeping with the scope of the present disclosure, a dimeric, pentameric, or hexameric CD20 binding molecule as provided herein can be administered to a subject in need of therapy in an amount sufficient to produce a therapeutic effect. A dimeric, pentameric, or hexameric CD20 binding molecule as provided herein can be administered to the subject in a conventional dosage form prepared by combining the antibody or antigen-binding fragment, variant, or derivative thereof of the disclosure with a conventional pharmaceutically acceptable carrier or diluent according to known techniques. The form and character of the pharmaceutically acceptable carrier or diluent can be dictated by the amount of active ingredient with which it is to be combined, the route of administration and other well-known variables.

[0199] By "therapeutically effective dose or amount" or "effective amount" is intended an amount of a dimeric, pentameric, or hexameric CD20 binding molecule, that when administered brings about a positive therapeutic response with respect to treatment of a patient with a disease or condition to be treated.

[0200] Therapeutically effective doses of the compositions disclosed herein, for treatment of diseases or disorders in which B cell depletion is desired, can vary depending upon many different factors, including means of administration, target site, physiological state of the patient, whether the patient is human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic. In certain aspects, the subject or patient is a human, but

non-human mammals including transgenic mammals can also be treated. Treatment dosages can be titrated using routine methods known to those of skill in the art to optimize safety and efficacy.

[0201] The amount of a dimeric, pentameric, or hexameric CD20 binding molecule to be administered is readily determined by one of ordinary skill in the art without undue experimentation given this disclosure. Factors influencing the mode of administration and the respective amount of a dimeric, pentameric, or hexameric CD20 binding molecule include, but are not limited to, the severity of the disease, the history of the disease, and the age, height, weight, health, and physical condition of the individual undergoing therapy. Similarly, the amount of a dimeric, pentameric, or hexameric CD20 binding molecule to be administered will be dependent upon the mode of administration and whether the subject will undergo a single dose or multiple doses of this agent.

[0202] This disclosure also provides for the use of a dimeric, pentameric, or hexameric CD20 binding molecule in the manufacture of a medicament for treating, preventing, or managing a disease or disorder in which B cell depletion is desirable, *e.g.*, B cell lymphoma, leukemia, or myeloma.

[0203] This disclosure employs, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, for example, Sambrook *et al.*, ed. (1989) Molecular Cloning A Laboratory Manual (2nd ed.; Cold Spring Harbor Laboratory Press); Sambrook *et al.*, ed. (1992) Molecular Cloning: A Laboratory Manual, (Cold Springs Harbor Laboratory, NY); D. N. Glover ed., (1985) DNA Cloning, Volumes I and II; Gait, ed. (1984) Oligonucleotide Synthesis; Mullis *et al.* U.S. Pat. No. 4,683,195; Hames and Higgins, eds. (1984) Nucleic Acid Hybridization; Hames and Higgins, eds. (1984) Transcription And Translation; Freshney (1987) Culture Of Animal Cells (Alan R. Liss, Inc.); Immobilized Cells And Enzymes (IRL Press) (1986); Perbal (1984) A Practical Guide To Molecular Cloning; the treatise, Methods In Enzymology (Academic Press, Inc., N.Y.); Miller and Calos eds. (1987) Gene Transfer Vectors For Mammalian Cells, (Cold Spring Harbor Laboratory); Wu *et al.*, eds., Methods In Enzymology, Vols. 154 and 155; Mayer and Walker, eds. (1987) Immunochemical Methods In Cell And Molecular Biology (Academic Press, London); Weir and Blackwell, eds., (1986) Handbook Of Experimental Immunology, Volumes I-IV; Manipulating the Mouse Embryo, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1986); and in Ausubel *et al.* (1989) Current Protocols in Molecular Biology (John Wiley and Sons, Baltimore, Md.).

[0204] General principles of antibody engineering are set forth in Borrebaeck, ed. (1995) Antibody Engineering (2nd ed.; Oxford Univ. Press). General principles of protein engineering are set forth in Rickwood *et al.*, eds. (1995) Protein Engineering, A Practical Approach (IRL Press at Oxford Univ. Press, Oxford, Eng.). General principles of antibodies and antibody-hapten binding are set forth in: Nisonoff (1984) Molecular Immunology (2nd ed.; Sinauer Associates, Sunderland, Mass.); and Steward (1984) Antibodies, Their Structure and Function (Chapman and Hall, New York, N.Y.). Additionally, standard methods in immunology known in the art and not specifically described can be followed as in Current Protocols in Immunology, John Wiley & Sons, New York; Stites *et al.*, eds. (1994) Basic and Clinical Immunology (8th ed; Appleton & Lange, Norwalk, Conn.) and Mishell and Shiigi (eds) (1980) Selected Methods in Cellular Immunology (W.H. Freeman and Co., NY).

[0205] Standard reference works setting forth general principles of immunology include Current Protocols in Immunology, John Wiley & Sons, New York; Klein (1982) J., Immunology: The Science of Self-Nonself Discrimination (John Wiley & Sons, NY); Kennett *et al.*, eds. (1980) Monoclonal Antibodies, Hybridoma: A New Dimension in Biological Analyses (Plenum Press, NY); Campbell (1984) "Monoclonal Antibody Technology" in Laboratory Techniques in Biochemistry and Molecular Biology, ed. Burden *et al.*, (Elsevier, Amsterdam); Goldsby *et al.*, eds. (2000) Kuby Immunology (4th ed.; H. Freeman & Co.); Roitt *et al.* (2001) Immunology (6th ed.; London: Mosby); Abbas *et al.* (2005) Cellular and Molecular Immunology (5th ed.; Elsevier Health Sciences Division); Kontermann and Dubel (2001) Antibody Engineering (Springer Verlag); Sambrook and Russell (2001) Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Press); Lewin (2003) Genes VIII (Prentice Hall, 2003); Harlow and Lane (1988) Antibodies: A Laboratory Manual (Cold Spring Harbor Press); Dieffenbach and Dveksler (2003) PCR Primer (Cold Spring Harbor Press).

[0206] All of the references cited above, as well as all references cited herein, are incorporated herein by reference in their entireties.

[0207] The following examples are offered by way of illustration and not by way of limitation.

Examples

Example 1: Generation of DNA constructs for expression of CD20-hIgM

[0208] Plasmid constructs that can express pentameric or hexameric IgM binding molecules that can specifically bind to CD20 were produced by the following method.

[0209] DNA fragments encoding the VH and VL regions of rituximab (SEQ ID NOs 1 and 5, respectively) or 1.5.3 (SEQ ID NOs 38 and 42, respectively) were synthesized by a commercial vendor (Genescript), with an EcoRV restriction site on the '5 end and an XbaI restriction site on the 3' end for subcloning into heavy chain and light chain expression vectors. The synthesized DNA constructs were re-suspended in Tris-EDTA buffer at 1 μg/ml. DNA samples (1 μg) were digested with EcoRV and XbaI, and the synthesized VH and VL were separated from the carrier plasmid DNA by electrophoresis. The digested DNA was ligated to pre-digested plasmid DNA (pFUSEss-CHIg-hM*03 for μ chain, pFUSE2ss-CLIg-hk for kappa chain, available from Invivogen) by standard molecular biology techniques. The ligated DNAs were transformed into competent bacteria and plated on LB plates with multiple selective antibiotics. Several bacterial colonies were picked and DNA preparations were made by standard molecular biology techniques. The constructs encoding the heavy chain and light chains were verified by sequencing. The DNA and amino acid sequences of the rituximab IgM heavy chain are presented as SEQ ID NO: 51 and SEQ ID NO: 52, respectively, and the DNA and amino acid sequences of the rituximab light chain are presented as SEQ ID NO: 53 and SEQ ID NO: 54, respectively. The DNA and amino acid sequences of 1.5.3 IgM heavy chain are presented as SEQ ID NO: 55 and SEQ ID NO: 56, respectively, and the DNA and amino acid sequences of 1.5.3 light chain are presented as SEQ ID NO: 57 and SEQ ID NO: 58, respectively. The amino acid sequence of the human J-chain is presented as SEQ ID NO: 49.

[0210] The plasmid constructs encoding the IgM heavy chains and light chains or the heavy chains, light chains, and J-chain were cotransfected into CHO cells, and cells that express the CD20 IgM antibody, either with our without J-chain, were selected, all according to standard methods.

[0211] Antibodies present in the cell supernatants were recovered using Capture Select IgM (Catalog 2890.05, BAC, Thermo Fisher) according to the manufacturer's protocol. Antibodies were evaluated on SDS PAGE under non-reducing conditions to show assembly of pentamers and hexamers. NuPage LDS Sample Buffer (Life Technologies) was added to samples before loading onto a NativePage Novex 3-12% bis-Tris Gel (Life Technologies Catalog #BN1003). Novex Tris-Acetate SDS Running Buffer (Life Technologies Catalog #LA0041) was used for gel electrophoresis. The gel was run until the dye front reached the bottom of the gel. After electrophoresis, the gel was stained with Colloidal Blue Stain (Life Technologies Catalog #LC6025). The results are shown in **FIG. 4**. Under non-reducing conditions, the pentameric 1.5.3 IgM (five H2L2 IgM units plus J-chain, **FIG. 4**, second panel, lane 3) and the pentameric rituximab IgM (first panel, lane 3) produced a protein band of approximately 1,000,000

molecular weight, and hexameric 1.5.3 IgM (six H2L2 IgM units, **FIG. 4**, second panel, lane 2) produced a protein band of approximately 1,180,000.

Example 2: Binding and Activity of CD20-hIgM

Detection of Binding via CD20 ELISA Assay

[0212] 96-well white polystyrene ELISA plates (Pierce 15042) were coated with 100 µL per well of 10 µg/mL or 0.3 µg/mL human CD20 with N-Fc fusion (AcroBiosystems, CD0-H526a) overnight at 4 °C. Plates were then washed with 0.05% PBS-Tween and blocked with 2% BSA-PBS. After blocking, 100 µL of serial dilutions of 1.5.3 IgM, 1.5.3 IgG, standards, and controls were added to the wells and incubated at room temperature for 2 hours. The plates were then washed and incubated with HRP conjugated mouse anti-human kappa (Southern Biotech, 9230-05. 1:6000 diluted in 2% BSA-PBS) for 30 min. After 10 final washes using 0.05% PBS-Tween, the plates were read out using SuperSignal chemiluminescent substrate (ThermoFisher, 37070). Luminescent data were collected on an EnVision plate reader (Perkin-Elmer) and analyzed with GraphPad Prism using a 4-parameter logistic model.

[0213] The results are shown in **FIG. 5**A and **FIG. 5B**, comparing IgM vs. IgG by molar concentrations. The anti-CD20 IgM antibody exhibited more effective binding, at both CD20 antigen densities, and especially at the low CD20 antigen concentration (**FIG. 5B**).

Complement Dependent Cytotoxicity – Colorimetric assay

[0214] Granta (DSMZ cat. #ACC 342), Raji (ATCC cat. #CCL-86), Ramos (ATCC cat. #CRL-1596), Nalm-6 (DSMZ cat. #ACC 128), and Namalwa (ATCC cat. #CRL -1432) cell lines were from ATCC and DSMZ. 50,000 cells of each cell line were seeded in a 96-well plate. Cells were treated with serially diluted commercially-available anti-human CD20 IgM (Invivogen cat. #hcd20-mab5) or anti-human CD20 IgG1 (Invivogen cat. #hcd20-mab1). Human serum complement (Quidel cat. #A113) was added to each well at a final concentration of 10%. The reaction mixtures were incubated at 37°C for 1 hr. Cell Counting Kit-SK reagent (CCK-SK) (Dojindo cat. #CK04-13) was added at 1/10 the total reaction volume and plate was incubated for an additional 3 hours at 37°C. Absorbance at 450 nm was measured on a spectrophotometer.

[0215] The results are shown in **FIG. 6A-E**. The IgM CD20 antibody was 6 times more potent at cell killing than IgG in Granta cells (**FIG. 6A**), three time more potent in Raji cells (**FIG. 6B**), and three times more potent in Ramos cells (**FIG. 6C**). Neither antibody was effective in

killing Nalm-6 cells (**FIG. 6D**) or Namalwa cells (**FIG. 6E**), which express no, or low levels of CD20, respectively.

Complement Dependent Cytotoxicity – Luminescent Assay

[0216] (a) The CD20-expressing Raji cell line (ATCC cat. #CCL-86) was used. 50,000 cells were seeded in a 96-well plate. Cells were treated with the following serially diluted antibodies: rituximab (IgG1), rituximab-derived anti-human CD20 IgM+J as produced in Example 1, or 1.5.3 anti-human CD20 IgM+J, produced as described in Example 1. Human serum complement (Quidel cat. #A113) was added to each well at a final concentration of 10%. The reaction mixtures were incubated at 37°C for 4 hours. Cell Titer Glo reagent (Promega cat. #G7572) was added at a volume equal to the volume of culture medium present in each well. The plate was shaken for 2 minutes, incubated for 10 minutes at room temperature, and luminescence was measured on a luminometer.

[0217] The results are shown in **FIG. 7** and **Table 2**. Anti-CD20 as an IgG (rituximab) achieved approximately half-maximal Raji cell killing with complement, whereas the IgM isotype (rituximab) achieved nearly maximal complement dependent cytotoxicity. Both IgM antibodies were more potent at complement dependent cytotoxicity than rituximab, and in this experiment, the 1.5.3-like IgM antibody was more potent than the anti-human IgM CD20 antibody carrying the rituximab VH and VL. The 1.5.3-like IgM antibody exhibited four-fold increased potency compared to that of the type 1 anti-CD20 (rituximab as IgM).

Table 2: CDC (IC_{50}) on Raji cells (μg/ml)

	IC_{50} (μg/ml)
Anti-CD20 IgG1 (rituximab)	>50
Anti-CD20 IgM	2.0
1.5.3 IgM + J	0.5

[0218] (b) The CDC assay as described above was repeated using the CD20-expressing Ramos cell line with the following serially diluted antibodies: rituximab (IgG1), rituximab-derived anti-human CD20 IgM+J as produced in Example 1, 1.5.3 (IgG1), 1.5.3 anti- CD20 IgM, or 1.5.3 anti- CD20 IgM+J, produced as described in Example 1.

[0219] The results are shown in **FIG. 8A** (rituximab and rituximab-like IgM) and **FIG. 8B** (1.5.3, 1.5.3 IgM+J, and huMAb-like IgM. In this experiment, the IgG and IgM versions were

compared on a molar equivalent basis. The IC_{50}s for the IgM versions were all about 30 to 40 times more effective at complement dependent cytotoxicity than the IgG versions.

[0220] (c) Next, the antibodies were then compared for CDC activity on different cell lines with decreasing CD20 expression levels. The assay was carried out with the following serially diluted antibodies: rituximab (IgG1), rituximab-derived anti-human CD20 IgM+J as produced in Example 1, 1.5.3 (IgG1), and 1.5.3 anti- CD20 IgM+J, produced as described in Example 1. The cells used were DoHH2 cells (DSMZ No. ACC 47), and Z138 cells (ATCC CRL-3001) were used in this assay. Z138 cells exhibit lower expression levels of CD20 than DoHH2 cells, as shown **Table 4** below. The target cells were washed and resuspended in CDC assay medium (RPMI 1640, 10% heat-inactivated FBS) at a density of 1.0 x 10^6 cells/mL and 10 µL/well was added to a Nunc 384-well tissue culture-treated white polystyrene plate. Serial 3-fold dilutions of test antibodies were prepared in assay medium, 10 µL/well was added to the assay plate, and the plate was incubated for 2 hr at 37 °C in a 5% CO_2 incubator to allow opsonization to occur. Normal human serum complement (Quidel) was frozen in aliquots, thawed once for use, diluted to 30% in assay medium, and 10 µL/well was added to the assay plate. The plate was incubated for 4 hr at 37 °C in a 5% CO_2 incubator. Cell Titer-Glo reagent (Promega) was thawed for use and 15 µL/well was added to the assay plate. The plate was gently mixed for 2 min on a plate shaker to lyse the cells and then for another 10 min at room temperature before measuring luminescence on an EnVision plate reader (Perkin-Elmer). After subtracting background signal, percent viability was plotted against antibody concentration and EC50 values were determined using GraphPad Prism.

[0221] The results are shown in **FIG. 9** and in **Table 3**. On both cell lines, the IgM versions of the antibodies exhibited greater CDC killing than the IgG versions. On the lower CD20-expressing Z-138 cells, the CDC activity of the IgM versions were more than 100-fold improved relative to the IgG versions.

Table 3: CDC Activity Depends on CD20 Antigen Expression Level

	EC_{50} (nM)	
	DOHH-2	Z-138
Anti-CD20 IgG1 (rituximab)	22	>100
1.5.3 IgG	4.6	>100
Anti-CD20 IgM+J	0.09	0.41

1.5.3 IgM+J	0.14	1.9

Example 3: Preparation of a Bispecific anti-CD20 IgM Comprising a Modified J-Chain Binding CD3

[0222] Rituxan-like anti-human CD20 IgM and 1.5.3 anti-CD20 IgM were produced as described in Example 1.

[0223] Two different J-chain variants were constructed with distinct fusion sites incorporating variable regions from the anti-CD3 antibody visilizumab (Nuvion). Shown below are the sequences for two J-chains with the scFv corresponding to visilizumab (V) (VH-$(GGGGS)_3$-VL, double-underlined) fused to the J-chain (italics) through a $(GGGGS)_3$ linker (SEQ ID NO: 67, underlined) containing 15 amino acids in two different orientations – V15J and J15V. Each sequence contains an N-terminal signal peptide that is shown without underlining or italics. In certain aspects, other signal peptide sequences can be substituted for the signal peptides shown here.

[0224] SEQ ID NO: 63: precursor modified J-chain sequence for V15J (DNA Sequence: SEQ ID NO: 68):

MGWSYIILFLVATATGVHSQVQLVQSGAEVKKPGASVKVSCK
ASGYTFISYTMHWVRQAPGQGLEWMGYINPRSGYTHYNQKL
KDKATLTADKSASTAYMELSSLRSEDTAVYYCARSAYYDYD
GFAYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLS
ASVGDRVTITCSASSSVSYMNWYQQKPGKAPKRLIYDTSKLAS
GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWSSNPPTFGG
GTKLEIKGGGGSGGGGSGGGGS*QEDERIVLVDNKCKCARITSRII*
RSSEDPNEDIVERNIRIIVPLNNRENISDPTSPLRTRFVYHLSDLCKK
CDPTEVELDNQIVTATQSNICDEDSATETCYTYDRNKCYTAVVPLV
YGGETKMVETALTPDACYPD

[0225] SEQ ID NO: 64: mature modified J-chain sequence for V15J:

QVQLVQSGAEVKKPGASVKVSCKASGYTFISYTMHWVRQAP
GQGLEWMGYINPRSGYTHYNQKLKDKATLTADKSASTAYME
LSSLRSEDTAVYYCARSAYYDYDGFAYWGQGTLVTVSSGGG
GSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCSASSSVSY
MNWYQQKPGKAPKRLIYDTSKLASGVPSRFSGSGSGTDFTLTI
SSLQPEDFATYYCQQWSSNPPTFGGGTKLEIKGGGGSGGGGSG
GGGS*QEDERIVLVDNKCKCARITSRIIRSSEDPNEDIVERNIRIIVPL*
NNRENISDPTSPLRTRFVYHLSDLCKKCDPTEVELDNQIVTATQSNI
CDEDSATETCYTYDRNKCYTAVVPLVYGGETKMVETALTPDACYP
D

[0226] SEQ ID NO: 65: precursor modified J-chain sequence for J15V (DNA sequence: SEQ ID NO: 69):

MKNHLLFWGVLAVFIKAVHVKA*QEDERIVLVDNKCKCARITSRI IRSSEDPNEDIVERNIRIIVPLNNRENISDPTSPLRTRFVYHLSDLCK KCDPTEVELDNQIVTATQSNICDEDSATETCYTYDRNKCYTAVVPL VYGGETKMVETALTPDACYPD*GGGGSGGGGSGGGGSQVQLVQ SGAEVKKPGASVKVSCKASGYTFISYTMHWVRQAPGQGLEW MGYINPRSGYTHYNQKLKDKATLTADKSASTAYMELSSLRSE DTAVYYCARSAYYDYDGFAYWGQGTLVTVSSGGGGSGGGGS GGGGSDIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQK PGKAPKRLIYDTSKLASGVPSRFSGSGSGTDFTLTISSLQPEDFA TYYCQQWSSNPPTFGGGTKLEIK

[0227] SEQ ID NO: 66: mature modified J-chain sequence for J15V:

*QEDERIVLVDNKCKCARITSRIIRSSEDPNEDIVERNIRIIVPLNNRE NISDPTSPLRTRFVYHLSDLCKKCDPTEVELDNQIVTATQSNICDE DSATETCYTYDRNKCYTAVVPLVYGGETKMVETALTPDACYPD*GG GGSGGGGSGGGGSQVQLVQSGAEVKKPGASVKVSCKASGYT FISYTMHWVRQAPGQGLEWMGYINPRSGYTHYNQKLKDKAT LTADKSASTAYMELSSLRSEDTAVYYCARSAYYDYDGFAYW GQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGD RVTITCSASSSVSYMNWYQQKPGKAPKRLIYDTSKLASGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQWSSNPPTFGGGTKLE IK

[0228] The mature constructs each have a molecular weight of about 45 kD and can bind to soluble epsilon chain of CD3 (Sino Biological), or T-cells (data not shown).

[0229] The DNA constructs corresponding to the anti-CD20 heavy and light chains as well as those corresponding to either the wild-type (wt) J-chain, V15J or J15V J-chain sequences were co-transfected into mammalian cells, *e.g.*, HEK293 or CHO cells, and proteins were expressed and purified according to standard methods. See, *e.g.*, PCT Publication No. WO 2015/153912, which is incorporated herein by reference in its entirety. The J-chains fused to the anti-CD3 scFv with the 15 amino acid linker were able to incorporate with the anti-CD20 heavy and light chains to produce a pentameric form of bi-specific IgM.

[0230] Agarose-Acrylamide Hybrid Gel. IgM Constructs were separated by non-reducing SDS-PAGE adapted from a previously described method (Chugai Seiyaki Kabushiki Kaisha, 2010, Pub. No.: US 2010/0172899 A1). Briefly, the hybrid gel was mixed with 40% Acrylamide / Bis-Acrylamide, 37.5:1 (Sigma-Aldrich) and Ultrapure Agarose (Invitrogen) to final concentrations of 3.6% and 0.5%, respectively, in 0.375 M Tris Buffer, pH 8.8 and 15% glycerol. The resulting mixture was heated to 50 °C and polymerization was initiated with the addition of 0.08% TEMED and 0.08% of ammonium persulfate. The resulting solution was poured between two plates and the acrylamide was allowed to polymerize at 37 °C for 1 hour and then left at room temperature for 30 min to ensure complete polymerization. Protein

samples were loaded into the resulting hybrid gel and the gel was run in Tris-Acetate SDS Running Buffer (Novex) for 800 Vh. The gel was then fixed in 40% methanol, 10% acetic for 10 minutes, stained using a Colloidal Blue Staining Kit (Novex) for at least 3 hours and subsequently de-stained in water.

[0231] Non-Reducing SDS-Native-PAGE. Protein samples were loaded into a NativePAGE 3-12% Bis-Tris gel (Novex). Tris-Acetate SDS Running Buffer (Novex) was added and the gel was run at 40V for 15 min and then at 90V for 2 hours. The gel was then fixed in 40% methanol, 10% acetic acid for 10 minutes, stained using a Colloidal Blue Staining Kit (Novex) for at least 3 hours and subsequently de-stained in water.

[0232] J-Chain Western Blot. An acrylamide gel run under reducing conditions was washed in a 20% ethanol solution for 10 minutes and then the protein was transferred to an iBlot PVDF membrane (Invitrogen) using the iBlot Dry Blotting System (Invitrogen) at 20V for 10 minutes. After transfer the PVDF membrane was blocked using 2% bovine serum albumin, 0.05% Tween 20 for at least 12 hours. A 1/500 dilution of Pierce J-chain antibody (ThermoFisher) was added to the membrane, incubated for 1 hour, and then a 1/5000 dilution of peroxidase-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch) was added and allowed to incubate in darkness for 30 minutes. Finally, Super Signal West Pico Chemiluminescent Substrate (ThermoFisher) was added to the blot and the resulting signal was visualized using the ChemiDoc-It HR410 Imaging System (UVP) or by exposing the blot to X-ray film.

[0233] As shown in **FIG 10A** and **FIG. 10B**, the 1.5.3 IgM+wt J and 1.5.3 IgM+V15J proteins are visible as faster migrating pentamers distinguishable on a hybrid gel from the slower migrating hexamer form not containing a J-chain. Integration of the J-chain is confirmed on the reducing gel (**FIG. 10C**) as well as western blot with antibodies to the J-chain (**FIG 10D**).

Example 4: T-Cell Activation Assay

[0234] To demonstrate that a bispecific anti-CD20/anti-CD3 antibody could activate T-cells upon binding to the CD20 target the following assay was performed. Engineered Jurkat T-cells (Promega CS176403) and RPMI8226 cells (ATCC CCL-155) were cultured in RPMI (Invitrogen) supplemented with 10% Fetal Bovine Serum (Invitrogen). Serial dilutions of purified 1.5.3 IgM + V15J, Blinatumomab (bispecific CD19 x CD3), and monospecific 1.5.3 IgM were incubated with 7500 RPMI8226 cells in 20 μL in a white 384 well assay plate for 2h at 37 °C with 5% CO_2. The engineered Jurkat cells (25000) were added to mixture to final volume of 40 μL. The mixture was incubated for 5h at 37 °C with 5% CO_2. The cell mixtures

were then mixed with 20 μL lysis buffer containing luciferin (Promega, Cell Titer Glo) to measure luciferase reporter activity. Light output was measured by EnVision plate reader. EC50 was determined by 4 parameter curve fit using Prism software.

[0235] The results are shown in **FIG. 11**. T-cell activation with the 1.5.3-V15J antibody was greater than that seen with Blinatumomab on the RPMI8226 cell line. The maximal level of T-cell activation showed good correlation with the level of CD20 expression on cell surface as shown in **FIG. 12** using a series of tumor cell lines each expressing a different level of CD20 antigen.

Example 5: T-cell Directed B-Cell Killing – LDH Release Assay

[0236] In order to demonstrate that bispecific CD20 x CD3 IgM binding molecules can kill target cells in the presence of CD8+ T-cell acute lymphoblastic leukemia (TALL) cells, we performed co-culture experiments. 6 x 10^3 cancerous B cells were co-cultured with 3 x 10^4 TALL cells (ATCC CRL-11386) in the presence of different concentrations of test compounds (Rituxan IgM + V15J and 1.5.3 IgM +V15J) in 45 μL total volume of RPMI 1640 media supplemented with 10% heat-inactivated FBS per well on a 384-well black tissue culture plate. After 24 hours of incubation at 37 °C in a 5% CO_2 incubator, 15 μL of CytoTox-ONE substrate reagent (Promega, G7891) was added to each well to measure the level of LDH released from dead cells. The plates were shaken briefly to mix the reagents, and then incubated at room temperature for 90 min before measuring fluorescence signal (485 nm for excitation and 615 nm for emission) on an EnVision plate reader (Perkin-Elmer). The data was then analyzed with GraphPad Prism to determine the EC_{50}. As shown **Table 4**, the EC_{50} of cell killing correlated with the expression level of CD20 antigen on cell surface using both 1.5.3 IgM V15J and Rituximab IgM V15J with EC50 on DB cell line as low as 0.4 ng/mL (0.4 pM).

Table 4: T-cell Directed B-Cell Killing

		EC_{50} (ng/mL)	
Cell lines	**MFI**	**Rituxan IgM + V15J**	**IgM 1.5.3 + V15J**
DB	3400	0.5	0.4
DOHH2	1300	13	12
Z-138	800	30	33

Example 6: *In vitro* cytotoxicity assay using KILR™ detection kit

[0237] In order to examine the ability of 1.5.3 IgM + V15J to kill CD20+ tumor cells in whole blood (*i.e.*, with the inclusion of T-cells and complement), the KILR™ *in vitro* cytotoxicity detection kit was used. The KILR™ ARH-77 cell line (CD20+) was purchased from DiscoverX (97-1001C017) as target cells. 5-10 x 10^3 KILR™ ARH-77 cells were co-cultured with either human CD8+ T-cells (Precision for Medicine), PBMC (AllCells or Precision for Medicine), or Hirudin anti-coagulated human blood (AllCells) in the presence of different concentrations of test compounds (1.5.3 IgM+V15J, 1.5.3 IgM+wtJ, 1.5.3 IgG, Rituximab IgG and blinatumomab) in 200 µL total volume of RPMI 1640 media supplemented with 10% heat-inactivated FBS (when human blood was used for co-culture, less media were used because the whole blood took up 20-50% of the volume) on a 96-well U-bottom non-tissue culture-treated polystyrene plate (Corning Falcon). After 4-48 hours of incubation at 37 °C in a 5% CO_2 incubator, the plates were centrifuged at 27 xg for 5 min. 50 µL of the supernatants were transferred to a 96-well flat-bottom white polystyrene plate (Greiner Bio-One) to be mixed with 25 µL of KILR detection working solution (KILR™ detection kit: DiscoverX 97-0001M) for 1 hour incubation at room temperature before measuring luminescence on an EnVision plate reader (Perkin-Elmer). The target cells were lysed with the lysis buffer supplied with the KILR™ detection kit to establish total lysis control. Percent killing was plotted against antibody concentration and EC50 values were determined using GraphPad Prism.

[0238] The results in the presence of normal human complement in whole blood (with hirudin) are shown in **FIG. 13**. The 1.5.3 IgM+wtJ (diamonds) and IgM+V15J (squares) antibodies both showed very potent killing at four hours. Rituxan IgG (open circles) and Blinatumomab (triangles) were at least 30 times less potent.

Example 7: *In vivo* efficacy study using humanized NOD/SCID Gamma knock-out (NSG) mouse models

[0239] CD34+ humanized NSG mouse studies were performed by In-Vivo Technologies, Inc. These mice are surrogates for human immuno-oncology studies, in that they possess develop multi-lineage human immune cells. The mice were purchased from the Jackson Laboratory, and dosed with test articles through tail vein injection. In addition to a vehicle control, the test articles included 1.5.3 IgM+V15J at 3 μg, 1 μg, and 0.3 μg per mouse and rituximab at 1 μg and 0.3 μg per mouse. Blood samples were collected at 6h, 24h, and 10 days post dose through facial vein. For the PBMC humanized NSG mouse studies, frozen PBMCs from AllCells were sent to the Jackson Laboratory for injection. Each NSG mouse was injected with 10 million PBMCs after 100 cGy whole body irradiation. Blood samples were collected before and after tail vein dosing of test articles (as above) via retro-orbital bleeding at designated time points. Blood samples from both the CD34+ and PBMC mouse studies were sent back to IGM Biosciences Inc. for lymphocyte analysis. Blood samples were stained for human CD56, CD3, CD19 and CD45 markers to identify different population of human lymphocytes. CountBright Absolute Counting Beads (LifeTechnologies, C36950) were used to quantify the absolute number of lymphocytes in the blood samples. The lymphocyte levels were plotted and analyzed using GraphPad Prism.

[0240] Results at 6 hours post-dose for 1.5.3 IgM with wild-type J and 1.5.3 IgM with V15J, normalized to pre-dose B cell levels, are shown in **FIG. 14A** and **FIG. 14B**, respectively. The bispecific 1.5.3 IgM x V15J antibody showed potent T-cell dependent B-cell killing in the engrafted NSG mice with as little as 3 μg per mouse.

[0241] A comparison of the results over the full assay period between 1.5.3 IgM x V15J and rituximab are shown in **FIG. 15**.

Table 5: Sequences in the Disclosure

SEQ ID NO	Short Name	Sequence

SEQ ID NO	Short Name	Sequence
1	Rituximab VH	QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDT SYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTT VTVSA
2	Rituximab HCDR1	SYNMH
3	Rituximab HCDR2	AIYPGNGDTSYNQKFKG
4	Rituximab HCDR3	STYYGGDWYFNV
5	Rituximab VL	QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVP VRFSGSGSGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKR
6	Rituximab LCDR1	RASSSVSYIH
7	Rituximab LCDR2	ATSNLAS
8	Rituximab LCDR3	QQWTSNPPT
9	900 VH	EVQLVESGGG LVQPGGSLRL SCAASGYTFT SYNMHWVRQA PGKGLEWVGA IYPGNGDTSY NQKFKGRFTI SVDKSKNTLY LQMNSLRAED TAVYYCARVV YYSNSYWYFD VWGQGTLVTV SSASTKGPSV FPLAPSSKST SGGTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVPSSSLGT
10	900HCDR3	VVYYSNSYWYFDV
11	900 VL	DIQMTQSPSS LSASVGDRVT ITCRASSSVS YMHWYQQKPG KAPKPLIYAP SNLASGVPSR FSGSGSGTDF TLTISSLQPE DFATYYCQQW SFNPPTFGQG TKVEIKRTVA APSVFIFPPS DEQLKSGTAS VVCLLNNFYP REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLTL SKADYEKHKV YACEVTHQGL
12	900LCDR1	RASSSVSYMH
13	900LCDR2	APSNLAS
14	900LCDR3	QQWSFNPPT
15	125 VH	EVQLVQSGAEVKKPGESLKISCKGSGRTFTSYNMHWVRQMPGKGLEWMGAIYPLTGDT SYNQKSKLQVTISADKSISTAYLQWSSLKASDTAMYYCARSTYVGGDWQFDVWGKGTT VTVSS
16	125HCDR2	aiypltgdtsynqkskl
17	125HCDR3	styvggdwqfdv
18	125 VL	EIVLTQSPGTLSLSPGERATLSCRASSSVPYIHWYQQKPGQAPRLLIYATSALASGIP DRFSGSGSGTDFTLTISRLEPEDFAVYYCQQWLSNPPTFGQGTKLEIK
19	125LCDR1	RASSSVPYIH
20	125LCDR2	ATSALAS
21	125LCDR3	QQWLSNPPT
22	844 VH #2	QVQLQQPGAELKKPGASVKVSCKASGYTFTSYNMHWVKQTPGRGLEWTGAIYPGNGDT SYNQKFKGKTTLTADKSSSTAYMELSSLRSEDTAVYYCARSTYYGGDWYFNVWGAGTT VTVSA
23	844 VH #3	QVQLQQPGAELKKPGASVKVSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDT SYNQKFKGKTTLTADKSSSTAYMELSSLRSEDTAVYYCARSTYYGGDWYFNVWGAGTT VTVSA
24	844 VL #5	QIVLSQSPAIITASPGEKVTMTCRASTSASYIHWFQQKPTSSPKPWIYATSNLASGVP SRFSGSGSGTTYSMTISSLEAEDAATYYCQQWTSNPPTFGGGTKLEIK
25	844 VL #5 LCDR1	RASTSASYIH
26	844 VL #6	QIVLSQSPAIITASPGEKVTMTCRASTSVSYIHWFQQKPTSSPKPWIYATSNLASGVP SRFSGSGSGTTYSMTISSLEAEDAATYYCQQWTSNPPTFGGGTKLEIK
27	844 VL #6, #7 LCDR1	RASTSVSYIH

SEQ ID NO	Short Name	Sequence
28	844 VL #7	QIVLSQSPAIITASPGEKVTMTCRASTSVSYIHWFQQKPGSSPKPWIYATSNLASGVP SRFSGSGSGTTYSMTISSLEAEDAATYYCQQWTSNPPTFGGGTKLEIK
27	null	
29	844 VL #8	QIVLSQSPAIITASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVP SRFSGSGSGTTYSMTISSLEAEDAATYYCQQWTSNPPTFGGGTKLEIK
30	844 VH #10	EVQLQQSGAELKKPGASVKVSCKASGYTFTSYNMHWVKQTPGQGLEWIGAIYPGNGDT SYNQKFKGKTTLTADKSSSTAYMELSSLRSEDTAVYYCARSNYYGSSYWFFDVWGTGT TVTVSS
31	844 VH #10 HCDR3	SNYYGSSYWFFDV
32	844 VL #12	DIVLTQSPAIITASPGEKVTMTCRASSSVNYMDWYQKKPGSSPKPWIYATSNLASGVP SRFSGSGSGTTYSMTISSLEAEDAATYYCQQWSFNPPTFGGGTKLEIK
33	844 VL #12 LCDR1	RASSSVNYMD
34	844 VL #12 LCDR3	QQWSFNPPT
35	164 VH	QVQLQQSGAEVKKPGSSVKVSCKASGYTFTSYNMHWVKQAPGQGLEWIGAIYPGNGDT SYNQKFKGKATLTADESTNTAYMELSSLRSEDTAFYYCARSTYYGGDWYFDVWGQGTT VTVSS
36	164 VH HCDR3	STYYGGDWYFDV
37	164 VL	MGWSCIILFLVATATGVHSDIQLTQSPSSLSASVGDRVTMTCRASSSVSYIHWFQQKP GKAPKPWIYATSNLASGVPVRFSGSGSGTDYTFTISSLQPEDIATYYCQQWTSNPPTF GGGTKLEIK
38	1.5.3 VH	EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIYPGDSDT RYSPSFQGQVTISADKSITTAYLQWSSLKASDTAMYYCARHPSYGSGSPNFDYWGQGT LVTVSS
39	1.5.3 HCDR1	GYSFTSYWIG
40	1.5.3 HCDR2	IIYPGDSDTRYSPSFQG
41	1.5.3 HCDR3	HPSYGSGSPNFDY
42	1.5.3 VL	DIVMTQTPLSSPVTLGQPASISCRSSQSLVYSDGNTYLSWLQQRPGQPPRLLIYKISN RFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCVQATQFPLTFGGGTKVEIK
43	1.5.3 LCDR1	RSSQSLV YSDGNTYLS
44	1.5.3 LCDR2	KISNRF S
45	1.5.3 LCDR3	VQATQFPLT
46	human IgM constant region DNA	GCCCCAACCCTTTTCCCCCTCGTCTCCTGTGAGAATTCCCCGTCGGATACGAGCAGCG TGGCCGTTGGCTGCCTCGCACAGGACTTCCTTCCCGACTCCATCACTTTCTCCTGGAA ATACAAGAACAACTCTGACATCAGCAGCACCCGGGGCTTCCCATCAGTCCTGAGAGGG GGCAAGCACGCAGCCACCTCACAGGTGCTGCTGCCTTCCAAGGACGTCATGCAGGGCA CAGACGAACACGTGGTGTGCAAAGTCCAGCACCCCAACGGCAACAAAGAAAAGAACGT GCCTCTTCCAGTGATTGCTGAGCTGCCTCCCAAAGTGAGCGTCTTCGTCCCACCCCGC GACGGCTTCTTCGGCAACCCCCGCAAGTCCAAGCTCATCTGCCAGGCCACGGGTTTCA GTCCCCGGCAGATTCAGGTGTCCTGGCTGCGCGAGGGGAAGCAGGTGGGGTCTGGCGT CACCACGGACCAGGTGCAGGCTGAGGCAAAGGAGTCTGGGACCACGACCTACAAGGTG ACCAGCACACTGACCATCAAAGAGAGCGACTGGCTCAGCCAGAGCATGTTCACCTGCC GCGTGGATCACAGGGGCCTGACCTTCCAGCAGAATGCGTCCTCCATGTGTGGCCCCGA TCAAGACACAGCCATCCGGGTCTTCTCCATCCCCCCATCCTTTGCCAGCATCTTCCTC ACCAAGTCCACCAAGTTGACCTGCCTGGTCACAGACCTGACCACCTATGACAGCGTGA CCATCTCCTGGACCCGCCAGAATGGCGAAGCTGTGAAAACCCACACCAACATCTCCGA GAGCCACCCCAATGCCACTTTCAGCGCCGTGGGTGAGGCCAGCATCTGCGAGGATGAC TGGAATTCCGGGGAGAGGTTCACGTGCACCGTGACCCACACAGACCTGCCCTCGCCAC TGAAGCAGACCATCTCCCGGCCCAAGGGGGTGGCCCTGCACAGGCCCGATGTCTACTT GCTGCCACCAGCCCGGGAGCAGCTGAACCTGCGGGAGTCGGCCACCATCACGTGCCTG GTGACGGGCTTCTCTCCCGCGGACGTCTTCGTGCAGTGGATGCAGAGGGGCAGCCCT TGTCCCCGGAGAAGTATGTGACCAGCGCCCCAATGCCTGAGCCCCAGGCCCCAGGCCG

SEQ ID NO	Short Name	Sequence
		GTACTTCGCCCACAGCATCCTGACCGTGTCCGAAGAGGAATGGAACACGGGGGAGACC TACACCTGCGTGGTGGCCCATGAGGCCCTGCCCAACAGGGTCACCGAGAGGACCGTGG ACAAGTCCACCGGTAAACCCACCCTGTACAACGTGTCCCTGGTCATGTCCGACACAGC TGGCACCTGCTAC
47	human IgM constant region AA	GSASAPTLFPLVSCENSPSDTSSVAVGCLAQDFLPDSITFSWKYKNNSDISSTRGFPS VLRGGKYAATSQVLLPSKDVMQGTDEHVVCKVQHPNGNKEKNVPLPVIAELPPKVSVF VPPRDGFFGNPRKSKLICQATGFSPRQIQVSWLREGKQVGSGVTTDQVQAEAKESGPT TYKVTSTLTIKESDWLSQSMFTCRVDHRGLTFQQNASSMCVPDQDTAIRVFAIPPSFA SIFLTKSTKLTCLVTDLTTYDSVTISWTRQNGEAVKTHTNISESHPNATFSAVGEASI CEDDWNSGERFTCTVTHTDLPSPLKQTISRPKGVALHRPDVYLLPPAREQLNLRESAT ITCLVTGFSPADVFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEEWN TGETYTCVVAHEALPNRVTERTVDKSTGKPTLYNVSLVMSDTAGTCY
48	J-chain DNA	ATGAAGAACCATTTGCTTTTCTGGGGAGTCCTGGCGGTTTTTATTAAGGCTGTTCATG TGAAAGCCCAAGAAGATGAAAGGATTGTTCTTGTTGACAACAAATGTAAGTGTGCCCG GATTACTTCCAGGATCATCCGTTCTTCCGAAGATCCTAATGAGGACATTGTGGAGAGA AACATCCGAATTATTGTTCCTCTGAACAACAGGGAGAATATCTCTGATCCCACCTCAC CATTGAGAACCAGATTTGTGTACCATTTGTCTGACCTCTGTAAAAAATGTGATCCTAC AGAAGTGGAGCTGGATAATCAGATAGTTACTGCTACCCAGAGCAATATCTGTGATGAA GACAGTGCTACAGAGACCTGCTACACTTATGACAGAAACAAGTGCTACACAGCTGTGG TCCCACTCGTATATGGTGGTGAGACCAAAATGGTGGAAACAGCCTTAACCCCAGATGC CTGCTATCCTGACTAA
49	J-chain AA	mknhllfwgvlavfikavhvkaqederivlvdnkckcaritsriirssedpnediver niiivplnnrenisdptsplrtrfvyhlsdlckkcdpteveldnqivtatqsnicded satetcytydrnkcytavvplvyggetkmvetaltpdacypd
50	human CD20 amino acid	MTTPRNSVNGTFPAEPMKGPIAMQSGPKPLFRRMSSLVGPTQSFFMRESKTLGAVQIM NGLFHIALGGLLMIPAGIYAPICVTVWYPLWGGIMYIISGSLLAATEKNSRKCLVKGK MIMNSLSLFAAISGMILSIMDILNIKISHFLKMESLNFIRAHTPYINIYNCEPANPSE KNSPSTQYCYSIQSLFLGILSVMLIFAFFQELVIAGIVENEWKRTCSRPKSNIVLLSA EEKKEQTIEIKEEVVGLTETSSQPKNEEDIEIIPIQEEEEEETETNFPEPPQDQESSP IENDSSP
51	Ritux-IgM heavy chain DNA	CAGGTTCAGCTGCAGCAGCCCGGAGCCGAGCTGGTCAAACCTGGCGCTAGTGTGAAAA TGTCATGCAAGGCATCCGGATACACATTCACTAGCTATAACATGCACTGGGTGAAGCA GACCCCCGGCAGGGGTCTGGAGTGGATCGGAGCTATCTACCCCGGCAACGGAGACACA TCTTATAATCAGAAGTTTAAAGGCAAGGCCACCCTGACAGCTGATAAGTCCAGCTCTA CCGCATACATGCAGCTGAGTTCACTGACAAGCGAGGACTCCGCCGTGTACTATTGCGC CCGGTCCACTTACTATGGCGGAGATTGGTATTTCAATGTGTGGGGAGCAGGCACCACA GTCACCGTCTCGAGCGGCAGTGCTAGCGCCCCAACCCTTTTCCCCCTCGTCTCCTGTG AGAATTCCCCGTCGGATACGAGCAGCGTGGCCGTTGGCTGCCTCGCACAGGACTTCCT TCCCGACTCCATCACTTTCTCCTGGAAATACAAGAACAACTCTGACATCAGCAGCACC CGGGGCTTCCCATCAGTCCTGAGAGGGGGCAAGTACGCAGCCACCTCACAGGTGCTGC TGCCTTCCAAGGACGTCATGCAGGGCACAGACGAACACGTGGTGTGCAAAGTCCAGCA CCCCAACGGCAACAAAGAAAAGAACGTGCCTCTTCCAGTGATTGCTGAGCTGCCTCCC AAAGTGAGCGTCTTCGTCCCACCCCGCGACGGCTTCTTCGGCAACCCCCGCAAGTCCA AGCTCATCTGCCAGGCCACGGGTTTCAGTCCCCGGCAGATTCAGGTGTCCTGGCTGCG CGAGGGGAAGCAGGTGGGGTCTGGCGTCACCACGGACCAGGTGCAGGCTGAGGCCAAA GAGTCTGGGCCCACGACCTACAAGGTGACCAGCACACTGACCATCAAAGAGAGCGACT GGCTCAGCCAGAGCATGTTCACCTGCCGCGTGGATCACAGGGGCCTGACCTTCCAGCA GAATGCGTCCTCCATGTGTGTCCCCGATCAAGACACAGCCATCCGGGTCTTCGCCATC CCCCCATCCTTTGCCAGCATCTTCCTCACCAAGTCCACCAAGTTGACCTGCCTGGTCA CAGACCTGACCACCTATGACAGCGTGACCATCTCCTGGACCCGCCAGAATGGCGAAGC TGTGAAAACCCACACCAACATCTCCGAGAGCCACCCCAATGCCACTTTCAGCGCCGTG GGTGAGGCCAGCATCTGCGAGGATGACTGGAATTCCGGGGAGAGGTTCACGTGCACCG TGACCCACACAGACCTGCCCTCGCCACTGAAGCAGACCATCTCCCGGCCCAAGGGGGT GGCCCTGCACAGGCCCGATGTCTACTTGCTGCCACCAGCCCGGGAGCAGCTGAACCTG CGGGAGTCGGCCACCATCACGTGCCTGGTGACGGGCTTCTCTCCCGCGGACGTCTTCG TGCAGTGGATGCAGAGGGGGCAGCCCTTGTCCCCGGAGAAGTATGTGACCAGCGCCCC AATGCCTGAGCCCCAGGCCCCAGGCCGGTACTTCGCCCACAGCATCCTGACCGTGTCC GAAGAGGAATGGAACACGGGGGAGACCTACACCTGCGTGGTGGCCCATGAGGCCCTGC

SEQ ID NO	Short Name	Sequence
		CCAACAGGGTCACCGAGAGGACCGTGGACAAGTCCACCGGTAAACCCACCCTGTACAA CGTGTCCCTGGTCATGTCCGACACAGCTGGCACCTGCTACTGA
52	Ritux-IgM heavy chain AA	QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDT SYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTT VTVSSGSASAPTLFPLVSCENSPSDTSSVAVGCLAQDFLPDSITFSWKYKNNSDISST RGFPSVLRGGKYAATSQVLLPSKDVMQGTDEHVVCKVQHPNGNKEKNVPLPVIAELPP KVSVFVPPRDGFFGNPRKSKLICQATGFSPRQIQVSWLREGKQVGSGVTTDQVQAEAK ESGPTTYKVTSTLTIKESDWLSQSMFTCRVDHRGLTFQQNASSMCVPDQDTAIRVFAI PPSFASIFLTKSTKLTCLVTDLTTYDSVTISWTRQNGEAVKTHTNISESHPNATFSAV GEASICEDDWNSGERFTCTVTHTDLPSPLKQTISRPKGVALHRPDVYLLPPAREQLNL RESATITCLVTGFSPADVFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVS EEEWNTGETYTCVVAHEALPNRVTERTVDKSTGKPTLYNVSLVMSDTAGTCY-
53	Ritux-light chain DNA	CAAATTGTGCTGTCTCAGAGTCCAGCTATCCTGAGCGCATCTCCCGGAGAGAAGGTGA CCATGACATGCAGAGCCTCCAGCTCTGTCTCCTACATCCACTGGTTCCAGCAGAAGCC CGGCTCCTCCCCAAAACCCTGGATCTACGCCACCTCTAACCTGGCTAGTGGTGTGCCT GTCAGGTTTAGTGGATCAGGGTCCGGCACCAGCTACTCTCTGACAATCAGCCGGGTGG AGGCTGAAGACGCCGCTACATACTATTGCCAGCAGTGGACTTCTAATCCCCCTACCTT CGGCGGAGGGACAAAGCTGGAGATCAAGCGTACGGTGGCTGCACCATCTGTCTTCATC TTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGA ATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATC GGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTC AGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCG AAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTG TTAG
54	Ritux-light chain AA	QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVP VRFSGSGSGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFI FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC-
55	1.5.3 -IgM heavy chain DNA	GAGGTGCAGCTGGTGCAGTCCGGCGCCGAGGTGAAGAAGCCCGGCGAGTCCCTGAAGA TCTCCTGCAAGGGCTCCGGCTACTCCTTCACCTCCTACTGGATCGGCTGGGTGAGGCA GATGCCCGGCAAGGGCCTGGAGTGGATGGGCATCATCTACCCCGGCGACTCCGACACC AGGTACTCCCCCTCCTTCCAGGGCCAGGTGACCATCTCCGCCGACAAGTCCATCACCA CCGCCTACCTGCAGTGGTCCTCCCTGAAGGCCTCCGACACCGCCATGTACTACTGCGC CAGGCACCCCTCCTACGGCTCCGGCTCCCCCAACTTCGACTACTGGGGCCAGGGCACC CTGGTGACCGTGTCCTCCGGCAGTGCTAGCGCCCCAACCCTTTTCCCCCTCGTCTCCT GTGAGAATTCCCCGTCGGATACGAGCAGCGTGGCCGTTGGCTGCCTCGCACAGGACTT CCTTCCCGACTCCATCACTTTCTCCTGGAAATACAAGAACAACTCTGACATCAGCAGC ACCCGGGGCTTCCCATCAGTCCTGAGAGGGGGCAAGTACGCAGCCACCTCACAGGTGC TGCTGCCTTCCAAGGACGTCATGCAGGGCACAGACGAACACGTGGTGTGCAAAGTCCA GCACCCCAACGGCAACAAAGAAAAGAACGTGCCTCTTCCAGTGATTGCTGAGCTGCCT CCCAAAGTGAGCGTCTTCGTCCCACCCCGCGACGGCTTCTTCGGCAACCCCCGCAAGT CCAAGCTCATCTGCCAGGCCACGGGTTTCAGTCCCCGGCAGATTCAGGTGTCCTGGCT GCGCGAGGGGAAGCAGGTGGGGTCTGGCGTCACCACGGACCAGGTGCAGGCTGAGGCC AAAGAGTCTGGGCCCACGACCTACAAGGTGACCAGCACACTGACCATCAAAGAGAGCG ACTGGCTCAGCCAGAGCATGTTCACCTGCCGCGTGGATCACAGGGGCCTGACCTTCCA GCAGAATGCGTCCTCCATGTGTGTCCCCGATCAAGACACAGCCATCCGGGTCTTCGCC ATCCCCCCATCCTTTGCCAGCATCTTCCTCACCAAGTCCACCAAGTTGACCTGCCTGG TCACAGACCTGACCACCTATGACAGCGTGACCATCTCCTGGACCCGCCAGAATGGCGA AGCTGTGAAAACCCACACCAACATCTCCGAGAGCCACCCCAATGCCACTTTCAGCGCC GTGGGTGAGGCCAGCATCTGCGAGGATGACTGGAATTCCGGGGAGAGGTTCACGTGCA CCGTGACCCACACAGACCTGCCCTCGCCACTGAAGCAGACCATCTCCCGGCCCAAGGG GGTGGCCCTGCACAGGCCCGATGTCTACTTGCTGCCACCAGCCCGGGAGCAGCTGAAC CTGCGGGAGTCGGCCACCATCACGTGCCTGGTGACGGGCTTCTCTCCCGCGGACGTCT TCGTGCAGTGGATGCAGAGGGGGCAGCCCTTGTCCCCGGAGAAGTATGTGACCAGCGC CCCAATGCCTGAGCCCCAGGCCCCAGGCCGGTACTTCGCCCACAGCATCCTGACCGTG TCCGAAGAGGAATGGAACACGGGGGAGACCTACACCTGCGTGGTGGCCCATGAGGCCC TGCCCAACAGGGTCACCGAGAGGACCGTGGACAAGTCCACCGGTAAACCCACCCTGTA CAACGTGTCCCTGGTCATGTCCGACACAGCTGGCACCTGCTACTGA

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56	1.5.3 -IgM heavy chain AA	EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIYPGDSDT RYSPSFQGQVTISADKSITTAYLQWSSLKASDTAMYYCARHPSYGSGSPNFDYWGQGT LVTVSSGSASAPTLFPLVSCENSPSDTSSVAVGCLAQDFLPDSITFSWKYKNNSDISS TRGFPSVLRGGKYAATSQVLLPSKDVMQGTDEHVVCKVQHPNGNKEKNVPLPVIAELP PKVSVFVPPRDGFFGNPRKSKLICQATGFSPRQIQVSWLREGKQVGSGVTTDQVQAEA KESGPTTYKVTSTLTIKESDWLSQSMFTCRVDHRGLTFQQNASSMCVPDQDTAIRVFA IPPSFASIFLTKSTKLTCLVTDLTTYDSVTISWTRQNGEAVKTHTNISESHPNATFSA VGEASICEDDWNSGERFTCTVTHTDLPSPLKQTISRPKGVALHRPDVYLLPPAREQLN LRESATITCLVTGFSPADVFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTV SEEEWNTGETYTCVVAHEALPNRVTERTVDKSTGKPTLYNVSLVMSDTAGTCY-
57	1.5.3 light chain DNA	GACATCGTGATGACCCAGACCCCCCTGTCCTCCCCCGTGACCCTGGGCCAGCCCGCCT CCATCTCCTGCAGGTCCTCCCAGTCCCTGGTGTACTCCGACGGCAACACCTACCTGTC CTGGCTGCAGCAGAGGCCCGGCCAGCCCCCCAGGCTGCTGATCTACAAGATCTCCAAC AGGTTCTCCGGCGTGCCCGACAGGTTCTCCGGCTCCGGCGCCGGCACCGACTTCACCC TGAAGATCTCCAGGGTGGAGGCCGAGGACGTGGGCGTGTACTACTGCGTGCAGGCCAC CCAGTTCCCCCTGACCTTCGGCGGCGGCACCAAGGTGGAGATCAAGCGTACGGTGGCT GCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCT CTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGT GGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAG GACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAAC ACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAG CTTCAACAGGGGAGAGTGTTAG
58	1.5.3 light chain AA	DIVMTQTPLSSPVTLGQPASISCRSSQSLVYSDGNTYLSWLQQRPGQPPRLLIYKISN RFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCVQATQFPLTFGGGTKVEIKRTVA APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC-
59	human IgA1 constant region aa P01876	ASPTSPKVFPLSLCSTQPDGNVVIACLVQGFFPQEPLSVTWSESGQGVTARNFPPSQD ASGDLYTTSSQLTLPATQCLAGKSVTCHVKHYTNPSQDVTVPCPVPSTPPTPSPSTPP TPSPSCCHPRLSLHRPALEDLLLGSEANLTCTLTGLRDASGVTFTWTPSSGKSAVQGP PERDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKSGNTFRPEVHL LPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKYLTWASRQEPSQGTT TFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPTHVNVSVVMAEV DGTCY
60	human IgA2 constant region aa P01877	ASPTSPKVFPLSLDSTPQDGNVVVACLVQGFFPQEPLSVTWSESGQNVTARNFPPSQD ASGDLYTTSSQLTLPATQCPDGKSVTCHVKHYTNPSQDVTVPCPVPPPPPCCHPRLSL HRPALEDLLLGSEANLTCTLTGLRDASGATFTWTPSSGKSAVQGPPERDLCGCYSVSS VLPGCAQPWNHGETFTCTAAHPELKTPLTANITKSGNTFRPEVHLLPPPSEELALNEL VTLTCLARGFSPKDVLVRWLQGSQELPREKYLTWASRQEPSQGTTTFAVTSILRVAAE DWKKGDTFSCMVGHEALPLAFTQKTIDRMAGKPTHVNVSVVMAEVDGTCY
61	Human Secretory Component Precursor	MLLFVLTCLLAVFPAISTKSPIFGPEEVNSVEGNSVSITCYYPPTSVNRHTRKYWCRQ GARGGCITLISSEGYVSSKYAGRANLTNFPENGTFVVNIAQLSQDDSGRYKCGLGINS RGLSFDVSLEVSQGPGLLNDTKVYTVDLGRTVTINCPFKTENAQKRKSLYKQIGLYPV LVIDSSGYVNPNYTGRIRLDIQGTGQLLFSVVINQLRLSDAGQYLCQAGDDSNSNKKN ADLQVLKPEPELVYEDLRGSVTFHCALGPEVANVAKFLCRQSSGENCDVVVNTLGKRA PAFEGRILLNPQDKDGSFSVVITGLRKEDAGRYLCGAHSDGQLQEGSPIQAWQLFVNE ESTIPRSPTVVKGVAGGSVAVLCPYNRKESKSIKYWCLWEGAQNGRCPLLVDSEGWVK AQYEGRLSLLEEPGNGTFTVILNQLTSRDAGFYWCLTNGDTLWRTTVEIKIIEGEPNL KVPGNVTAVLGETLKVPCHFPCKFSSYEKYWCKWNNTGCQALPSQDEGPSKAFVNCDE NSRLVSLTLNLVTRADEGWYWCGVKQGHFYGETAAVYVAVEERKAAGSRDVSLAKADA APDEKVLDSGFREIENKAIQDPRLFAEEKAVADTRDQADGSRASVDSGSSEEQGGSSR ALVSTLVPLGLVLAVGAVAVGVARARHRKNVDRVSIRSYRTDISMSDFENSREFGAND NMGASSITQETSLGGKEEFVATTESTTETKEPKKAKRSSKEEAEMAYKDFLLQSSTVA AEAQDGPQEA
62	human secretory component mature	KSPIFGPEEVNSVEGNSVSITCYYPPTSVNRHTRKYWCRQGARGGCITLISSEGYVSS KYAGRANLTNFPENGTFVVNIAQLSQDDSGRYKCGLGINSRGLSFDVSLEVSQGPGLL NDTKVYTVDLGRTVTINCPFKTENAQKRKSLYKQIGLYPVLVIDSSGYVNPNYTGRIR LDIQGTGQLLFSVVINQLRLSDAGQYLCQAGDDSNSNKKNADLQVLKPEPELVYEDLR GSVTFHCALGPEVANVAKFLCRQSSGENCDVVVNTLGKRAPAFEGRILLNPQDKDGSF

SEQ ID NO	Short Name	Sequence
		SVVITGLRKEDAGRYLCGAHSDGQLQEGSPIQAWQLFVNEESTIPRSPTVVKGVAGGS VAVLCPYNRKESKSIKYWCLWEGAQNGRCPLLVDSEGWVKAQYEGRLSLLEEPGNGTF TVILNQLTSRDAGFYWCLTNGDTLWRTTVEIKIIEGEPNLKVPGNVTAVLGETLKVPC HFPCKFSSYEKYWCKWNNTGCQALPSQDEGPSKAFVNCDENSRLVSLTLNLVTRADEG WYWCGVKQGHFYGETAAVYVAVEERKAAGSRDVSLAKADAAPDEKVLDSGFREIENKA IQDPR
63	precursor modified J-chain sequence for V15J	MGWSYIILFLVATATGVHSQVQLVQSGAEVKKPGASVKVSCKASGYTFISYTMHWVRQ APGQGLEWMGYINPRSGYTHYNQKLKDKATLTADKSASTAYMELSSLRSEDTAVYYCA RSAYYDYDGFAYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVT ITCSASSSVSYMNWYQQKPGKAPKRLIYDTSKLASGVPSRFSGSGSGTDFTLTISSLQ PEDFATYYCQQWSSNPPTFGGGTKLEIKGGGGSGGGGSGGGGSQEDERIVLVDNKCKC ARITSRIIRSSEDPNEDIVERNIRIIVPLNNRENISDPTSPLRTRFVYHLSDLCKKCD PTEVELDNQIVTATQSNICDEDSATETCYTYDRNKCYTAVVPLVYGGETKMVETALTP DACYPD
64	mature modified J-chain sequence for V15J	QVQLVQSGAEVKKPGASVKVSCKASGYTFISYTMHWVRQAPGQGLEWMGYINPRSGYT HYNQKLKDKATLTADKSASTAYMELSSLRSEDTAVYYCARSAYYDYDGFAYWGQGTLV TVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQKP GKAPKRLIYDTSKLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWSSNPPTF GGGTKLEIKGGGGSGGGGSGGGGSQEDERIVLVDNKCKCARITSRIIRSSEDPNEDIV ERNIRIIVPLNNRENISDPTSPLRTRFVYHLSDLCKKCDPTEVELDNQIVTATQSNIC DEDSATETCYTYDRNKCYTAVVPLVYGGETKMVETALTPDACYPD
65	Precursor modified J-chain sequence for J15V	MKNHLLFWGVLAVFIKAVHVKAQEDERIVLVDNKCKCARITSRIIRSSEDPNEDIVER NIRIIVPLNNRENISDPTSPLRTRFVYHLSDLCKKCDPTEVELDNQIVTATQSNICDE DSATETCYTYDRNKCYTAVVPLVYGGETKMVETALTPDACYPDGGGGSGGGGSGGGGS QVQLVQSGAEVKKPGASVKVSCKASGYTFISYTMHWVRQAPGQGLEWMGYINPRSGYT HYNQKLKDKATLTADKSASTAYMELSSLRSEDTAVYYCARSAYYDYDGFAYWGQGTLV TVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQKP GKAPKRLIYDTSKLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWSSNPPTF GGGTKLEIK
66	mature modified J-chain sequence for J15V	QEDERIVLVDNKCKCARITSRIIRSSEDPNEDIVERNIRIIVPLNNRENISDPTSPLR TRFVYHLSDLCKKCDPTEVELDNQIVTATQSNICDEDSATETCYTYDRNKCYTAVVPL VYGGETKMVETALTPDACYPDGGGGSGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGASVKVSC KASGYTFISYTMHWVRQAPGQGLEWMGYINPRSGYTHYNQKLKDKATLTADKSASTAY MELSSLRSEDTAVYYCARSAYYDYDGFAYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQ MTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQKPGKAPKRLIYDTSKLASGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQWSSNPPTFGGGTKLEIK
67	$(GGGGS)_3$ linker	GGGGSGGGGSGGGGS
68	J-chain DNA sequence for V15J	ATGGGCTGGTCCTACATCATCCTCTTCCTCGTGGCCACAGCCACAGGCGTCCATAGCC AGGTGCAGCTGGTGCAGTCCGGCGCCGAAGTGAAGAAGCCTGGCGCCAGCGTGAAGGT GAGCTGCAAGGCTTCCGGCTACACCTTCATCTCCTACACCATGCACTGGGTGAGGCAA GCTCCTGGCCAGGGCCTGGAGTGGATGGGATACATCAACCCTCGGTCCGGCTATACCC ACTACAATCAGAAGCTGAAGGACAAGGCCACCCTGACCGCTGACAAGTCCGCCTCCAC CGCTTACATGGAGCTGTCCTCCCTGAGGTCCGAGGACACCGCCGTGTACTACTGTGCC AGGTCCGCCTACTACGACTACGACGGATTCGCTTACTGGGGCCAGGGCACCCTGGTGA CAGTGAGCTCCGGAGGAGGAGGCAGCGGCGGCGGCGGCAGCGGCGGCGGCGGCAGCGA TATCCAGATGACCCAGAGCCCTTCCAGCCTGTCCGCTTCCGTGGGCGACAGGGTGACC ATCACCTGCAGCGCTTCCTCCTCCGTGTCCTACATGAACTGGTACCAGCAGAAGCCTG GCAAGGCCCCCAAGAGGCTGATCTACGACACCTCCAAGCTGGCCTCCGGAGTGCCTTC CAGGTTCAGCGGCTCCGGCTCCGGAACCGACTTCACCCTGACCATTAGCTCCCTGCAG CCCGAGGACTTCGCCACCTACTACTGCCAGCAGTGGTCCAGCAACCCTCCCACCTTCG GCGGCGGCACAAAGCTGGAGATCAAGGGAGGAGGAGGATCCGGTGGTGGTGGTTCTGG CGGAGGTGGATCCCAAGAAGATGAAAGGATTGTTCTTGTTGACAACAAATGTAAGTGT GCCCGGATTACTTCCAGGATCATCCGTTCTTCCGAAGATCCTAATGAGGACATTGTGG AGAGAAACATCCGAATTATTGTTCCTCTGAACAACAGGGAGAATATCTCTGATCCCAC CTCACCATTGAGAACCAGATTTGTGTACCATTTGTCTGACCTCTGTAAAAAATGTGAT CCTACAGAAGTGGAGCTGGATAATCAGATAGTTACTGCTACCCAGAGCAATATCTGTG ATGAAGACAGTGCTACAGAGACCTGCTACACTTATGACAGAAACAAGTGCTACACAGC TGTGGTCCCACTCGTATATGGTGGTGAGACCAAAATGGTGGAAACAGCCTTAACCCCA

SEQ ID NO	Short Name	Sequence
		GATGCCTGCTATCCTGACTGA
69	J-chain DNA sequence for J15V	ATGAAGAACCATTTGCTTTTCTGGGGAGTCCTGGCGGTTTTTATTAAGGCTGTTCATG TGAAAGCCCAAGAAGATGAAAGGATTGTTCTTGTTGACAACAAATGTAAGTGTGCCCG GATTACTTCCAGGATCATCCGTTCTTCCGAAGATCCTAATGAGGACATTGTGGAGAGA AACATCCGAATTATTGTTCCTCTGAACAACAGGGAGAATATCTCTGATCCCACCTCAC CATTGAGAACCAGATTTGTGTACCATTTGTCTGACCTCTGTAAAAAATGTGATCCTAC AGAAGTGGAGCTGGATAATCAGATAGTTACTGCTACCCAGAGCAATATCTGTGATGAA GACAGTGCTACAGAGACCTGCTACACTTATGACAGAAACAAGTGCTACACAGCTGTGG TCCCACTCGTATATGGTGGTGAGACCAAAATGGTGGAAACAGCCTTAACCCCAGATGC CTGCTATCCTGACGGAGGAGGAGGATCCGGTGGTGGTGGTTCTGGCGGAGGTGGATCC CAGGTGCAGCTGGTGCAGTCCGGCGCCGAAGTGAAGAAGCCTGGCGCCAGCGTGAAGG TGAGCTGCAAGGCTTCCGGCTACACCTTCATCTCCTACACCATGCACTGGGTGAGGCA AGCTCCTGGCCAGGGCCTGGAGTGGATGGGATACATCAACCCTCGGTCCGGCTATACC CACTACAATCAGAAGCTGAAGGACAAGGCCACCCTGACCGCTGACAAGTCCGCCTCCA CCGCTTACATGGAGCTGTCCTCCCTGAGGTCCGAGGACACCGCCGTGTACTACTGTGC CAGGTCCGCCTACTACGACTACGACGGATTCGCTTACTGGGGCCAGGGCACCCTGGTG ACAGTGAGCTCCGGAGGAGGAGGCAGCGGTGGTGGCGGAAGCGGTGGAGGTGGCAGCG ATATCCAGATGACCCAGAGCCCTTCCAGCCTGTCCGCTTCCGTGGGCGACAGGGTGAC CATCACCTGCAGCGCTTCCTCCTCCGTGTCCTACATGAACTGGTACCAGCAGAAGCCT GGCAAGGCCCCCAAGAGGCTGATCTACGACACCTCCAAGCTGGCCTCCGGAGTGCCTT CCAGGTTCAGCGGCTCCGGCTCCGGAACCGACTTCACCCTGACCATTAGCTCCCTGCA GCCCGAGGACTTCGCCACCTACTACTGCCAGCAGTGGTCCAGCAACCCTCCCACCTTC GGAGGCGGCACAAAGCTGGAGATCAAGTGA

[0242] The breadth and scope of the present disclosure should not be limited by any of the above-described exemplary embodiments, but should be defined only in accordance with the following claims and their equivalents.

WHAT IS CLAIMED IS:

1. A pentameric binding molecule comprising five bivalent binding units and a J-chain or functional fragment or variant thereof, wherein each binding unit comprises two IgM heavy chain constant regions, each associated with an antigen-binding domain, wherein at least one antigen binding domain of the binding molecule is a CD20 antigen binding domain comprising:

an antibody heavy chain variable region (VH) comprising immunoglobulin complementarity determining regions HCDR1, HCDR2, HCDR3, and an antibody light chain variable region (VL) comprising immunoglobulin complementarity determining regions LCDR1, LCDR2, and LCDR3, wherein the HCDR1 comprises the amino acid sequence of SEQ ID NO: 39; the HCDR2 comprises the amino acid sequence of SEQ ID NO: 40; the HCDR3 comprises the amino acid sequence of SEQ ID NO: 41; the LCDR1 comprises the amino acid sequence of SEQ ID NO: 43; the LCDR2 comprises the amino acid sequence of SEQ ID NO: 44; and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 45;

wherein the binding molecule can direct complement-mediated killing of a CD-20-expressing cell at higher potency than an equivalent amount of a monospecific, bivalent reference IgG1 antibody that specifically binds to CD20; and

wherein the monospecific, bivalent reference IgG1 antibody is 1.5.3, which comprises a VH having the amino acid sequence SEQ ID NO: 38 and a VL having the amino acid sequence SEQ ID NO: 42.

2. The binding molecule of claim 1, wherein the VH comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 38, and the VL comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 42.

3. The binding molecule of claim 1 or claim 2, wherein each binding unit comprises two IgM heavy chains each comprising a VH situated amino terminal to the IgM constant region, and two immunoglobulin light chains each comprising a VL situated amino terminal to an immunoglobulin light chain constant region.

4. The binding molecule of any one of claims 1 to 3, wherein the IgM heavy chain constant regions each comprise a Cμ1 domain, a Cμ2 domain, a Cμ3 domain and a Cμ4-tp domain.

5. The binding molecule of claim 4, wherein the IgM heavy chain constant regions are human IgM constant regions.

6. The binding molecule of claim 5, wherein the binding units are identical, wherein the IgM heavy chains in each binding unit comprise the amino acid sequence SEQ ID NO: 56, and wherein the light chains in each binding unit comprise the amino acid sequence SEQ ID NO: 58.

7. The binding molecule of any one of claims 1 to 6, wherein the J-chain or functional fragment or variant thereof comprises the amino acid sequence SEQ ID NO: 49 or a functional fragment or variant thereof.

8. The binding molecule of any one of claims 1 to 7, wherein the J-chain or functional fragment or variant thereof is a modified J-chain further comprising a heterologous polypeptide, wherein the heterologous polypeptide is directly or indirectly fused to the J-chain or functional fragment or variant thereof.

9. The binding molecule of claim 8, wherein the heterologous polypeptide is fused to the J-chain or functional fragment or variant thereof via a peptide linker.

10. The binding molecule of claim 8 or claim 9, wherein a heterologous polypeptide is fused to the N-terminus of the J-chain or functional fragment or variant thereof, the C-terminus of the J-chain or functional fragment or variant thereof, or to both the N-terminus and C-terminus of the J-chain or functional fragment or variant thereof.

11. The binding molecule of any one of claims 8 to 10, wherein the heterologous polypeptide comprises an antibody or antigen-binding fragment thereof.

12. The binding molecule of claim 11, wherein the antigen-binding fragment comprises an Fab fragment, an Fab' fragment, an $F(ab')_2$ fragment, an Fd fragment, an Fv fragment, a single-chain Fv (scFv) fragment, a disulfide-linked Fv (sdFv) fragment, or any combination thereof.

13. The binding molecule of claim 11 or claim 12, wherein the heterologous polypeptide comprises a scFv antibody fragment that can specifically bind to CD3ε.

14. The binding molecule of claim 13, wherein the modified J-chain comprises the amino acid sequence SEQ ID NO: 64 (V15J) or SEQ ID NO: 66 (J15V).

15. The binding molecule of any one of claims 1 to 14, wherein the CD20-expressing cell is a Raji cell line, and wherein the binding molecule can direct complement-mediated killing with an IC_{50} at least four-fold, at least ten-fold, at least 50-fold, or at least 100-fold lower than the IC_{50} of an equivalent amount of the monospecific bivalent IgG1 antibody, as measured in µg/ml, or wherein the CD20-expressing cell is a Ramos cell line, and wherein the binding molecule can direct complement-mediated killing with an IC_{50} at least ten-fold, at least 20-fold, at least 30-fold, at least 40-fold, at least 50-fold, at least 60-fold, at least 70-fold, at least 80-fold, at least 90-fold, or at least 100-fold lower than the IC_{50} of an equivalent amount of the monospecific bivalent IgG1 antibody, as measured as molar equivalents,

wherein the monospecific bivalent IgG1 antibody is 1.5.3, which comprises a VH having the amino acid sequence SEQ ID NO: 38 and a VL having the amino acid sequence SEQ ID NO: 42.

16. The binding molecule of any one of claims 1 to 14, wherein the CD-20-expressing cell is a malignant B cell isolated from a subject with cancer.

17. The binding molecule of claim 16, wherein the cancer is a CD20-positive leukemia, lymphoma, or myeloma.

18. The binding molecule of any one of claim 16 or claim 17, wherein the cancer is minimally responsive or non-responsive to rituximab therapy.

19. The binding molecule of any one of claims 16 to 18, wherein the subject is human.

20. A composition comprising the binding molecule of any one of claims 1 to 19, and a carrier.

21. A polynucleotide comprising a nucleic acid sequence that encodes a heavy chain polypeptide subunit of the binding molecule of any one of claims 1 to 19, wherein the heavy chain polypeptide subunit comprises an IgM heavy chain constant region, and at least the antibody VH portion of the CD20 antigen binding domain.

22. A composition comprising the polynucleotide of claim 21.

23. The composition of claim 22, further comprising a nucleic acid sequence that encodes a light chain polypeptide subunit of the binding molecule of any one claims 1 to 19.

24. The composition of claim 23, wherein the nucleic acid sequence encoding the heavy chain polypeptide subunit and the nucleic acid sequence encoding the light chain polypeptide subunit are on separate vectors.

25. The composition of claim 23, wherein the nucleic acid sequence encoding the heavy chain polypeptide subunit and the nucleic acid sequence encoding the light chain polypeptide subunit are on a single vector.

26. The composition of any one of claims 22 to 25, further comprising a nucleic acid sequence that encodes the J-chain, or functional fragment or variant thereof of the binding molecule of any one of claims 1 to 19, situated on a separate vector or on the same vector as the nucleic acid sequence encoding the heavy chain polypeptide subunit and/or the nucleic acid sequence encoding the light chain polypeptide subunit.

27. A vector or combination of vectors comprising the nucleic acid sequences of any one of claims 21 to 26.

28. A host cell comprising the polynucleotide of claim 21, the composition of any one of claims 22 to 26, or the vector or vectors of claim 27, wherein the host cell can express the binding molecule of any one of claims 1 to 19, or a subunit thereof.

29. A method of producing the binding molecule of any one of claims 1 to 19, comprising culturing the host cell of claim 28, and recovering the binding molecule.

30. An *in vitro* method for directing complement-mediated killing of a CD20-expressing cell comprising contacting a CD20-expressing cell with the binding molecule of any one of claims 1 to 19 or the composition of claim 20, wherein the binding molecule can direct complement-mediated killing of a CD-20-expressing cell at higher potency than an equivalent amount of a monospecific, bivalent IgG1 antibody or fragment thereof that specifically binds to CD20, wherein the monospecific, bivalent IgG1 antibody is 1.5.3, which comprises a VH having the amino acid sequence SEQ ID NO: 38 and a VL having the amino acid sequence SEQ ID NO: 42.

31. Use of the binding molecule of any one of claims 1 to 19 or the composition of claim 20 in the preparation of a medicament for treating cancer in a subject in need thereof, wherein the cancer is a CD20-positive leukemia, lymphoma, or myeloma.

32. The use of claim 31, wherein the cancer is minimally responsive or non-responsive to rituximab therapy.

33. The use of claim 31 or 32, wherein the subject is human.