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 dimeric, pentameric, or hexameric binding molecule comprising at least two bivalent binding units, or variants or fragments thereof, wherein each binding unit comprises at least two heavy chain constant regions or fragments thereof, 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:

(a) six immunoglobulin complementarity determining regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein the HCDR1 comprises the amino acid sequence of SEQ ID NO: 39 or SEQ ID NO: 39 with 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 or two single amino acid substitutions; the HCDR3 comprises the amino acid sequence of SEQ ID NO: 41, SEQ ID NO: 41 with one or two single amino acid substitutions; the LCDR1 comprises the amino acid sequence of SEQ ID NO: 43, or SEQ ID NO: 43 with 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 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 or two single amino acid substitutions; or

(b) an antibody heavy chain variable region (VH) and an antibody light chain variable region (VL), 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.

[0008] In certain embodiments 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 embodiments, further include a Cα1 domain. In certain embodiments, the IgA heavy chain constant regions can be human IgA constant regions. In certain embodiments, 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 embodiments 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 embodiments 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 embodiments, the J-chain or fragment thereof includes the amino acid sequence SEQ ID NO: 49 or a functional fragment thereof. In certain embodiments, 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 embodiments the heterologous polypeptide can be indirectly fused to the J-chain or fragment thereof via a peptide linker. In certain embodiments the peptide linker can include, e.g., at least 5 amino acids, but no more than 25 amino acids. In certain embodiments 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 embodiments 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 embodiments the heterologous polypeptide can specifically bind to CD3ε. For example in certain embodiments the modified J-chain can include the amino acid sequence SEQ ID NO: 64 (V15J) or SEQ ID NO: 66 (J15V). Moreover in certain embodiments, 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 embodiments, the IgM heavy chain constant regions can be human IgM heavy chain constant regions. In certain embodiments, 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 embodiments, 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 embodiments, 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 embodiments 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 embodiments 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 embodiments the two light chain constant regions are identical and include the amino acid sequence SEQ ID NO: 58.

[0015] In certain embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, the cancer is minimally responsive or non-responsive to rituximab therapy. In certain embodiments, 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 provide 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 or fragment thereof or an IgA heavy chain constant region or fragment thereof, and at least the antibody VH portion of the CD20 antigen binding domain. In certain embodiments, 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 embodiments the provided polynucleotide can encode the amino acid sequence SEQ ID NO: 56. In another aspect, there is provide 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 embodiments 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 embodiments the nucleic acid sequence that encodes the light chain polypeptide subunit can encode the amino acid sequence SEQ ID NO: 58.

[0020] In certain embodiments 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 embodiments 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 embodiments, be directly or indirectly fused to the J-chain or fragment thereof. In certain embodiments, the heterologous polypeptide can include an antibody or antigen-binding fragment thereof. In certain embodiments, the heterologous polypeptide can be, e.g., a scFv that can specifically bind to CD3ε. In certain embodiments the modified J-chain can include the amino acid sequence SEQ ID NO: 64 (V15J) or SEQ ID NO: 66 (J15V). In those embodiments 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 embodiments 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 embodiments 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. 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 embodiments, 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 embodiments, 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 embodiments, 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, T-cell-mediated, or both complement-mediated and T-cell-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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 in the preparation of a medicament for treating cancer in as subject in need thereof. In certain embodiments, the cancer is minimally responsive or non-responsive to rituximab therapy. In certain embodiments the subject is a human.

[0024] In other embodiments, 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. In one aspect, there is provided 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 embodiments, the IgA heavy chain constant region is a human IgA constant region. In certain embodiments 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 embodiments can further include a Cμ2 domain, a Cμ1 domain, or any combination thereof. In certain embodiments 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 embodiments, the J-chain or fragment thereof includes the amino acid sequence SEQ ID NO: 49 or a functional fragment thereof. In certain embodiments, 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 embodiments the heterologous polypeptide can be indirectly fused to the J-chain or fragment thereof via a peptide linker. In certain embodiments the peptide linker can include, e.g., at least 5 amino acids, but no more than 25 amino acids. In certain embodiments 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 embodiments 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 embodiments the heterologous polypeptide can specifically bind to CD3ε. For example in certain embodiments the modified J-chain can include the amino acid sequence SEQ ID NO: 64 (V15J) or SEQ ID NO: 66 (J15V). Moreover in certain embodiments, 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 embodiments, 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 embodiments, the two IgM heavy chains within at least one binding unit include the amino acid sequence SEQ ID NO: 52. In some embodiments, 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 embodiments 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 embodiments 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 embodiments 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 embodiments, the cancer is minimally responsive or non-responsive to rituximab therapy. In certain embodiments, 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

WHAT IS CLAIMED IS:

1. A dimeric, pentameric, or hexameric binding molecule comprising at least two bivalent binding units, or variants or fragments thereof, wherein each binding unit comprises at least two heavy chain constant regions or fragments thereof, 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:

(a) six immunoglobulin complementarity determining regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein the HCDR1 comprises the amino acid sequence of SEQ ID NO: 39 or SEQ ID NO: 39 with 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 or two single amino acid substitutions; the HCDR3 comprises the amino acid sequence of SEQ ID NO: 41, SEQ ID NO: 41 with one or two single amino acid substitutions; the LCDR1 comprises the amino acid sequence of SEQ ID NO: 43, or SEQ ID NO: 43 with 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 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 or two single amino acid substitutions; or

(b) an antibody heavy chain variable region (VH) and an antibody light chain variable region (VL), 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.

2. The binding molecule of claim 1, which is a dimeric binding molecule comprising two bivalent IgA binding units or fragments thereof, a J-chain or fragment or variant thereof, and optionally a secretory component, or fragment or variant thereof, wherein each binding unit comprises two IgA heavy chain constant regions or fragments thereof each associated with an antigen-binding domain, wherein each binding unit comprises two IgA heavy chains each comprising a VH situated amino terminal to the IgA constant region or fragment thereof, and two immunoglobulin light chains each comprising a VL situated amino terminal to an immunoglobulin light chain constant region.

3. The binding molecule of claim 2, wherein the IgA heavy chain constant regions or fragments thereof each comprise a Cα1 domain, a Cα2 domain and a Cα3-tp domain, wherein the IgA heavy chain constant regions are optionally human IgA constant regions.

4. The binding molecule of claim 1 or claim 1(b), which is a pentameric or a hexameric binding molecule comprising five or six bivalent IgM binding units, respectively, wherein each binding unit comprises two IgM heavy chain constant regions or fragments thereof each associated with an antigen-binding domain, wherein each binding unit comprises two IgM heavy chains each comprising a VH situated amino terminal to the 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.

5. The binding molecule of claim 4, wherein the pentameric binding molecule further comprises a J-chain or functional fragment thereof, or a functional variant thereof.

6. The binding molecule of claim 4, wherein the IgM heavy chain constant regions or fragments thereof each comprise a Cμ1 domain, a Cμ2 domain, a Cμ3 domain and a Cμ4-tp domain, wherein the IgM heavy chain constant regions or fragments thereof are optionally human IgM constant regions or fragments thereof.

7. The binding molecule of claim 4 or claim 6, wherein J-chain or fragment thereof comprises the amino acid sequence SEQ ID NO: 49 or a functional fragment thereof.

8. The binding molecule of any one of claims 4 to 7, wherein the J-chain or fragment thereof is a modified J-chain further comprises a heterologous polypeptide, wherein the heterologous polypeptide is directly or indirectly fused to the J-chain or fragment thereof.

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

10. The binding molecule of 8 or claim 9, wherein a heterologous polypeptide is fused to 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.

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 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 4 to 14, comprising 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 CD20 antigen binding domain.

16. The binding molecule of any one of claims 1 to 15, which 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 comprising a VH having the amino acid sequence SEQ ID NO: 38 and a VL having the amino acid sequence SEQ ID NO: 42.

17. A composition comprising the binding molecule of any one of claims 1 to 16, and a carrier.

18. 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 16, wherein the heavy chain polypeptide subunit comprises an IgM heavy chain constant region or fragment thereof or an IgA heavy chain constant region or fragment thereof, and at least the antibody VH portion of the CD20 antigen binding domain.

19. A composition comprising the polynucleotide of claim 18.

20. The composition of claim 19, further comprising a nucleic acid sequence that encodes a heavy chain polypeptide subunit of the binding molecule of any one claims 1 to 16.

21. The composition of claim 20, 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.

22. The composition of claim 20, 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.

23. The composition of any one of claims 20 to 22, further comprising a nucleic acid sequence that encodes a J-chain, or functional fragment thereof, or a functional variant thereof, 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.

24. A vector or combination of vectors comprising the nucleic acid sequences of any one of claims 21 to 23.

25. A host cell comprising the polynucleotide of claim 18, the composition of any one of claims 19 to 23, or the vector or vectors of claim 24, wherein the host cell can express the binding molecule of any one of claims 1 to 16, or a subunit thereof.

26. A method of producing the binding molecule of any one of claims 1 to 16, comprising culturing the host cell of claim 25, and recovering the binding molecule.

27. An *in vitro* method for directing complement-mediated, T-cell-mediated, or both complement-mediated and T-cell-mediated killing of a CD20-expressing cell comprising contacting a CD20-expressing cell with the binding molecule of any one of claims 1 to 16 or the composition of claim 17, 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.

28. Use of the binding molecule of any one of claims 1 to 16 or the composition of claim 17 in the preparation of a medicament for treating cancer in a subject in need thereof.

29. The use of claim 28, wherein the cancer is a CD20-positive leukemia, lymphoma, or myeloma.

30. The use of claim 28 or claim 29, wherein the cancer is minimally responsive or non-responsive to rituximab therapy.

31. 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.

32. The use of claim 31, wherein the CD20 antigen binding domain comprises a VH and a VL comprising, 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.

33. The use of claim 31 or claim 32, wherein the binding molecule is a pentameric IgM antibody, and further comprises a J-chain, or functional fragment thereof, or a functional variant thereof.

34. The use of claim 33, wherein the J-chain or fragment thereof further comprises a heterologous polypeptide, wherein the heterologous polypeptide is directly or indirectly fused to the J-chain or fragment thereof.

35. The use of claim 34, wherein a heterologous polypeptide is fused to 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.

36. The use of claim 34 or claim 35, wherein the heterologous polypeptide comprises an antibody or antigen-binding fragment thereof.

37. The use of claim 36, wherein the antigen-binding fragment is a scFv fragment.

38. The use of claim 36 or claim 37, wherein the heterologous polypeptide can specifically bind to CD3ε.

39. The use of claim 38, wherein the modified J-chain comprises the amino acid sequence SEQ ID NO: 64 (V15J) or SEQ ID NO: 66 (J15V).

40. The use of any one of claims 31 to 39, wherein the binding molecule comprises 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, wherein the 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 are identical.

41. The use of any one of claims 31 to 40, wherein the monospecific, bivalent IgG1 antibody is rituximab, which comprises a VH having the amino acid sequence SEQ ID NO: 1 and a VL having the amino acid sequence SEQ ID NO: 5.

42. The use of any one of claims 31 to 41, wherein the cancer is a CD20-positive leukemia lymphoma, or myeloma.

43. The use of claim 42, wherein the cancer is minimally responsive or non-responsive to rituximab therapy.

44. The use of any one of claims 31 to 43, wherein the subject is human.