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; and 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.

[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

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, 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

[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 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. 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,

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; and

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.

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 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.

16. The binding molecule of any one of claims 1 to 15, 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.

17. The binding molecule of any one of claims 1 to 15, wherein the CD-20-expressing cell is a malignant B cell isolated from a subject with cancer.

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

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

20. The binding molecule of any one of claims 17 to 19, wherein the subject is human.

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

22. 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 20, 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.

23. A composition comprising the polynucleotide of claim 22.

24. The composition of claim 23, further comprising a nucleic acid sequence that encodes a light chain polypeptide subunit of the binding molecule of any one claims 1 to 20.

25. The composition of claim 24, 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.

26. The composition of claim 24, 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.

27. The composition of any one of claims 23 to 26, 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 20, 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.

28. A vector or combination of vectors comprising the nucleic acid sequences of any one of claims 22 to 27.

29. A host cell comprising the polynucleotide of claim 22, the composition of any one of claims 23 to 27, or the vector or vectors of claim 28, wherein the host cell can express the binding molecule of any one of claims 1 to 20, or a subunit thereof.

30. A method of producing the binding molecule of any one of claims 1 to 20, comprising culturing the host cell of claim 29, and recovering the binding molecule.

31. 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 20 or the composition of claim 21, 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.

32. Use of the binding molecule of any one of claims 1 to 20 or the composition of claim 21 in the preparation of a medicament for treating cancer in a subject in need thereof.

33. The use of claim 32, wherein the cancer is a CD20-positive leukemia, lymphoma, or myeloma.

34. The use of claim 32 or claim 33, wherein the cancer is minimally responsive or non-responsive to rituximab therapy.

35. The use of any one of claims 32 to 34, wherein the subject is human.