

WO 2018/017888, WO 2018/017763, WO 2018/017889, WO 2018/017761, and WO 2019/169314, the contents of which are incorporated herein by reference in their entireties.

[0005] There remains a need for therapeutic monoclonal antibodies that can more selectively target tumor or other diseased cells that overexpress the target antigen, while avoiding interactions with healthy cells that express the antigen at reduced density.

SUMMARY

[0006] This disclosure provides a multimeric binding molecule that includes two, five, or six bivalent binding units, that collectively include four, ten, or twelve binding unit-associated antigen binding domains, respectively. As provided herein each binding unit includes two antibody heavy chains, each having an IgA, IgA-like, IgM, or IgM-like heavy chain constant region or multimerizing fragment or variant thereof and a binding unit-associated antigen-binding domain or subunit thereof, where at least three of the binding-unit-associated antigen-binding domains of the binding molecule specifically bind to the same predetermined target on the surface of a cell, and where the binding molecule preferentially binds to a cell expressing the predetermined target at a higher density relative to a cell expressing the predetermined target at a lower density.

[0006a] In one aspect, the present disclosure provides a multimeric binding molecule comprising two, five, or six bivalent binding units collectively comprising four, ten, or twelve binding unit-associated antigen binding domains, respectively; wherein each binding unit comprises two antibody heavy chains, each comprising an IgA, IgA-like, IgM, or IgM-like heavy chain constant region or multimerizing fragment or variant thereof and a binding unit-associated antigen-binding domain or subunit thereof, wherein at least three of the binding-unit-associated antigen-binding domains of the binding molecule specifically bind to the same predetermined target on the surface of a cell (target antigen), and wherein the binding molecule preferentially binds to a cell expressing the predetermined target at a higher density (high density cell) relative to a cell expressing the predetermined target at a lower density (low density cell).

[0007] In certain ~~embodiment~~examples, each antibody heavy chain includes a heavy chain variable region (VH) portion of a binding-unit-associated antigen-binding domain. In certain ~~embodiment~~examples, at least three binding unit-associated antigen-binding domains that bind to the same predetermined target on the surface of a cell each include a heavy chain variable region (VH) and light chain variable region (VL). In certain

embodimentexamples at least three antigen-binding domains each bind to the same epitope on the predetermined target. In certain embodimentexamples the at least three binding-unit-associated antigen-binding domains that bind to the same predetermined target on the surface of a cell are identical.

- 5 [0008] In certain embodimentexamples 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 twelve binding-unit-associated antigen-binding domains bind to the same predetermined target on the surface of a cell and are identical. In certain embodimentexamples, the provided binding molecule preferentially binds to a cell expressing the predetermined target at a higher density
- 10 relative to a reference bivalent IgG antibody having just two of the identical binding-unit-associated antigen-binding domains that specifically bind to the predetermined target on the surface of the cell. In certain embodimentexamples, the provided binding molecule can bind to a cell expressing the

target antigen at higher density, where the reference bivalent IgG antibody having just two of the identical binding-unit-associated antigen-binding domains that specifically bind to the predetermined target on the surface of the cell cannot bind to the cell expressing the target antigen at higher density. In certain [embodimentexamples](#) the provided binding molecule does not detectably bind to a cell expressing the predetermined target at lower density. In certain [embodimentexamples](#) the cell expressing the predetermined target at high density is a cancer cell and the cell expressing the predetermined target at low density is a normal, healthy cell.

[0009] In certain [embodimentexamples](#) the multimeric binding molecule is pentameric or hexameric, and includes five or six bivalent IgM or IgM-like binding units, respectively, where each binding unit includes two IgM or IgM-like heavy chain constant regions or multimerizing fragments or variants thereof each associated with a binding unit-associated antigen-binding domain or subunit thereof. In certain [embodimentexamples](#) the IgM or IgM-like heavy chain constant regions or multimerizing fragments or variants thereof each include a C μ 4 domain and an IgM tailpiece (tp) domain, and can further include a C μ 1 domain, a C μ 2 domain, a C μ 3 domain, or any combination thereof. In certain [embodimentexamples](#) the IgM or IgM-like heavy chain constant regions are human IgM constant regions or human-derived IgM-like constant regions. In certain [embodimentexamples](#), each binding unit includes two IgM or IgM-like heavy chains each including a VH situated amino terminal to the IgM or IgM-like constant region or multimerizing fragment or variant thereof, and two immunoglobulin light chains each including a VL situated amino terminal to an immunoglobulin light chain constant region. In those [embodimentexamples](#) where the multimeric binding molecule is pentameric, it can further include a J-chain, or functional fragment or variant thereof.

[0010] In certain [embodimentexamples](#) the multimeric binding molecule is dimeric and includes two bivalent IgA or IgA-like binding units and a J-chain or a functional fragment or variant thereof, where each binding unit includes two IgA or IgA-like heavy chain constant regions or multimerizing fragments or variants thereof each associated with a binding unit-associated antigen-binding domain or subunit thereof. In certain [embodimentexamples](#) the dimeric binding molecule can further include a secretory component, or functional fragment or variant thereof. In certain [embodimentexamples](#) the IgA or IgA-like heavy chain constant regions or multimerizing fragments or variants thereof each include a C α 3 domain and an IgA tailpiece (tp) domain and can further include a C α 1 domain, a C α 2 domain, an IgA hinge

region, or any combination thereof. In certain [embodimentexamples](#) the IgA or IgA-like heavy chain constant regions or multimerizing fragments or variants thereof are human IgA heavy chain constant regions or human-derived IgA-like heavy chain constant regions. In certain [embodimentexamples](#) each binding unit includes two IgA or IgA-like heavy chains each including a VH situated amino terminal to the IgA or IgA-like heavy chain constant region or multimerizing fragment or variant thereof, and two immunoglobulin light chains each including a VL situated amino terminal to an immunoglobulin light chain constant region.

[0011] Certain multimeric binding molecules provided herein include a J-chain or fragment or variant thereof. In certain [embodimentexamples](#) the J-chain or fragment or variant thereof is a human or human-derived J-chain that includes the amino acid sequence SEQ ID NO: 42 or a functional fragment or variant thereof. In certain [embodimentexamples](#) the J-chain or functional fragment or variant thereof is a variant J-chain including one or more single amino acid substitutions, deletions, or insertions relative to a wild-type J-chain, which can affect serum half-life of the binding molecule. According to these [embodimentexamples](#), a binding molecule that includes the variant J-chain exhibits an increased serum half-life upon administration to an animal relative to a reference binding molecule that is identical except for the one or more single amino acid substitutions, deletions, or insertions, and is administered in the same way to the same animal species. More specifically, in certain [embodimentexample](#)s the J-chain or functional fragment or variant thereof includes an amino acid substitution at the amino acid position corresponding to amino acid Y102 of the wild-type human J-chain (SEQ ID NO: 42). Y102 of SEQ ID NO: 42 can be substituted with alanine (A), serine (S), or arginine (R). More specifically the amino acid corresponding to Y102 of SEQ ID NO: 42 can be substituted with alanine (A). In certain specific [embodimentexamples](#) the variant J-chain is a variant human J-chain and includes the amino acid sequence SEQ ID NO: 43 (J*).

[0012] In certain [embodimentexamples](#), the J-chain or fragment or variant thereof of a provided multimeric binding molecule is a modified J-chain and further includes a heterologous moiety, where the heterologous moiety is chemically conjugated or fused to the J-chain or fragment or variant thereof. In certain [embodimentexample](#)s the heterologous moiety is a heterologous polypeptide. The heterologous polypeptide can be fused to the J-chain or fragment or variant thereof via a peptide linker. For example the peptide linker can consist of the amino acid sequence SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, or SEQ ID NO: 61. In certain [embodimentexample](#)s the heterologous polypeptide is fused

to the N-terminus of the J-chain or fragment or variant thereof, to the C-terminus of the J-chain or fragment or variant thereof, or heterologous polypeptides can be fused to both the N- and C-terminus of the J-chain or fragment or variant thereof. In certain embodimentexamples at least two heterologous polypeptides can be fused to the J-chain or fragment or variant thereof, and the at least two heterologous polypeptides can be the same or different. In certain embodimentexamples at least one heterologous polypeptide includes a J-chain-associated antigen-binding domain. In certain embodimentexample the J-chain-associated antigen-binding domain is an antibody or antigen-binding fragment thereof, e.g., 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 embodimentexamples the J-chain-associated antigen-binding domain is a scFv fragment. In certain embodimentexamples the J-chain-associated antigen-binding domain specifically binds to an immune effector cell, e.g., a T cell, e.g., CD8⁺ cytotoxic T cell, or an NK cell.

[0013] Where the immune effector cell is a T cell, the J-chain-associated antigen-binding domain can be, e.g., a scFv fragment that specifically binds to CD3 ϵ . In certain embodimentexamples the anti-CD3 ϵ scFv fragment can include a heavy chain variable region (VH) and a light chain variable region (VL), where the VH includes the VH complementarity-determining regions VHCDR1, VHCDR2, and VHCDR3 including the amino acid sequences SEQ ID NO: 49, SEQ ID NO: 50, and SEQ ID NO: 51, respectively, or SEQ ID NO: 49, SEQ ID NO: 50, and SEQ ID NO: 51 with one, two, or three amino acid substitutions in one or more of the VHCDRs, and where the VL includes the VL complementarity-determining regions VLCDR1, VLCDR2, and VLCDR3 including the amino acid sequences SEQ ID NO: 53, SEQ ID NO: 54, and SEQ ID NO: 55, respectively, or SEQ ID NO: 53, SEQ ID NO: 54, and SEQ ID NO: 55 with one, two, or three amino acid substitutions in one or more of the VLCDRs. For example the scFv fragment can include the VH amino acid sequence SEQ ID NO: 48 and the VL amino a heavy chain variable region (VH) and a light chain variable region (VL), where the VH and VL include the amino acid sequences SEQ ID NO: 44 and SEQ ID NO: 45, respectively. In certain specific embodimentexamples, the modified J-chain can include amino acids 20 to 412 of SEQ ID NO: 46 (V15J), amino acids 20 to 412 of SEQ ID NO: 47 (V15J*), or amino acids 20 to 420 of SEQ ID NO: 56 (SJ*). Where the immune effector cell is an NK cell, the J-chain-associated antigen-binding domain can be, e.g., a scFv fragment that specifically binds to CD16.

[0014] In certain embodimentexamples, a modified J-chain of a provided multimeric binding molecule can include an immune stimulatory agent (“ISA”) fused or chemically conjugated to the J-chain or fragment or variant thereof. In certain embodimentexamples ISA can include a cytokine or receptor-binding fragment or variant thereof. For example the IS can include (a) an interleukin-15 (IL-15) protein or receptor-binding fragment or variant thereof (“I”), and (b) an interleukin-15 receptor- α (IL-15R α) fragment including the sushi domain or a variant thereof capable of associating with I (“R”), where the J-chain or fragment or variant thereof and at least one of I and R are associated as a fusion protein, and where I and R can associate to function as the ISA. In certain embodimentexamples the ISA can be fused to the J-chain via a peptide linker.

[0015] In certain embodimentexamples, the predetermined target of the provided multimeric binding molecule is B-cell maturation antigen (BCMA), CD19, CD20, EGFR, HER2 (ErbB2), ErbB3, ErbB4, CTLA4, PD-1, PD-L1, VEGF, VEGFR1, VEGFR2, CD52, CD30, prostate-specific membrane antigen (PSMA), CD38, GD2, SLAMF7, platelet-derived growth factor receptor A (PDGFRA), CD22, FLT3 (CD135), CD123, MUC-16, carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM-1), mesothelin, tumor-associated calcium signal transducer 2 (Trop-2), glypican-3 (GPC-3), human blood group H type 1 trisaccharide (Globo-H), sialyl Tn antigen (STn antigen), or CD33. In certain embodimentexamples, at least three identical binding unit-associated antigen-binding domains that are specific for the predetermined target are reduced-affinity variants of an antigen-binding domain of an existing antibody known to bind to the predetermined target, where the antigen-binding domain of an existing antibody includes a heavy chain variable region (VH) and light chain variable region (VL). In certain embodimentexamples the existing antibody is alemtuzumab, atezolizumab, atezolizumab, avelumab, bevacizumab, blinatumomab, brentuximab, capromab, cetuximab, daratumumab, denosumab, dinutuximab, durvalumab, elotuzumab, gemtuzumab, ibritumomab, ipilimumab, inotuzumab, necitumumab, nivolumab, nivolumab, obinutuzumab, ocrelizumab, ofatumumab, olaratumab, omalizumab, panitumumab, pembrolizumab, pertuzumab, ramucirumab, ranibizumab, rituximab, trastuzumab, or tremelimumab. In certain embodimentexamples the provided binding molecule binds to the predetermined target with a binding affinity for the predetermined target at least 5-fold, at least 10-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, at least 100-fold, at least 500-fold, or at least 1000-fold lower

than the existing antibody. In certain embodimentexamples the VH and VL of the antigen-binding domain of the existing antibody include, respectively, the amino acid sequences SEQ ID NO: 7 and SEQ ID NO: 8, SEQ ID NO: 14 and SEQ ID NO: 15, SEQ ID NO: 16 and SEQ ID NO: 17, SEQ ID NO: 18 and SEQ ID NO: 19, SEQ ID NO: 20 and SEQ ID NO: 21, SEQ ID NO: 22 and SEQ ID NO: 23, SEQ ID NO: 24 and SEQ ID NO: 25, SEQ ID NO: 26 and SEQ ID NO: 27, SEQ ID NO: 28 and SEQ ID NO: 29, SEQ ID NO: 30 and SEQ ID NO: 31, SEQ ID NO: 32 and SEQ ID NO: 33, SEQ ID NO: 34 and SEQ ID NO: 35, SEQ ID NO: 36 and SEQ ID NO: 37, SEQ ID NO: 38 and SEQ ID NO: 39, or SEQ ID NO: 40 and SEQ ID NO: 41.

[0016] More specifically, the antigen-binding domain of the existing antibody specifically binds to CD20, and includes the VH amino acid sequence SEQ ID NO: 7 and the VL amino acid sequence SEQ ID NO: 8, and the reduced-affinity variant includes an amino acid substitution at position N93 in the VL, SEQ ID NO: 8. For example N93 of SEQ ID NO: 8 can include a N93D or N93E amino acid substitution. In certain specific embodimentexamples the at least three identical binding unit-associated antigen-binding domains specific for the predetermined target include the VH amino acid sequence SEQ ID NO: 7 and the VL amino acid sequence SEQ ID NO: 9 or SEQ ID NO: 10. In certain embodimentexamples, the provided binding molecule can direct complement-directed cytotoxicity (CDC) of B cells expressing CD20 at high density at an EC50 concentration at least 2-fold, at least 5-fold, at least 10-fold, at least 50-fold, at least 100-fold, at least 500-fold or at least 1000-fold lower than the EC50 concentration for B cells expressing CD20 at low density. In certain embodimentexamples the provided binding molecule can direct CDC of B cells expressing CD20 at high density, but not B cells expressing CD20 at low density. In certain embodimentexamples the provided anti-CD20 binding molecule is a pentameric IgM or IgM-like binding molecule or a dimeric IgA or IgA-like binding molecule, and further includes a modified J-chain including a scFv that specifically binds to CD3ε. For example, the modified J-chain can include amino acids 20 to 412 of SEQ ID NO: 46 (V15J), amino acids 20 to 412 of SEQ ID NO: 47 (V15J*), or amino acids 20 to 420 of SEQ ID NO: 56 (SJ*). In certain embodimentexamples the provided anti-CD20 binding molecule can direct T-cell directed cellular cytotoxicity (TDCC) or both TDCC and CDC of B cells expressing CD20 at high density at an EC50 concentration at least 2-fold, at least 5-fold, at least 10-fold, at least 50-fold, at least 100-fold, at least 500-fold or at least 1000-fold lower than the EC50 concentration for a B cell line or normal B cells, expressing CD20 at low density. In certain

embodimentexamples the provided anti-CD20 binding molecule can direct TDCC or both TDCC and CDC of B cells expressing CD20 at high density, but not B cells expressing CD20 at low density. In certain embodimentexamples the B cells expressing CD20 at both high and low density are lymphoma cell lines. For example, the lymphoma cell line expressing CD20 at high density is a Ramos cell line, a Raji cell line, a DoHH-2 cell line, a JeKo-1 cell line, a Z-138 cell line, a Daudi cell line, a Granta cell line, or a DoHH2 cell line, and the lymphoma cell line expressing CD20 at low density is a CA46 cell line, a Nalm-1 cell line, a Toledo cell line, a BJAB cell line, a Kasumi-2 cell line, an RPMI 8226 cell line, an HT cell line, an SU-DHL-8 cell line, a JM1 cell line, a Namalwa cell line, a Nalm-6 cell line, or a Z138 cell line. In certain embodimentexamples the provided anti-CD20 binding molecule can direct CDC of Ramos cells, where an equivalent amount of a monospecific bivalent IgG1 antibody including the same antigen-binding domains as the binding molecule shows no detectable complement-mediated killing of Ramos cells. In certain embodimentexamples the B cells expressing CD20 at high density are CD20-positive malignant B cells in a subject with cancer, and the B cells expressing CD20 at low density are normal B cells in the subject with cancer. In certain embodimentexamples the cancer is a CD20-positive leukemia, lymphoma, or myeloma. In certain embodimentexamples, the subject is human.

[0017] In another aspect, ~~T~~the disclosure further provides a composition that includes the binding molecule provided by the disclosure.

[0018] In yet another aspect, the disclosure provides an isolated polynucleotide comprising a nucleic acid molecule that encodes the binding molecule as described herein. The disclosure further provides an isolated polynucleotide that includes a nucleic acid molecule that encodes the provided binding molecule, an antigen-binding and multimerizing fragment of the provided binding molecule, or a subunit thereof. In certain embodimentexamples the subunit is an antibody heavy chain or multimerizing fragment or variant thereof, an antibody light chain or fragment thereof, a J-chain or fragment or variant thereof, or any combination thereof. In a further aspect, ~~t~~The disclosure further provides a vector that includes the provided polynucleotide, and a host cell that includes the provided polynucleotide or the provided vector. In ~~a—another aspectrelated embodiment,~~ the disclosure provides a method for producing the provided binding molecule, where the method includes culturing the provided host cell and recovering the binding molecule.

[0019] In addition, in another aspect, the disclosure provides an IgM or IgM-like antibody that includes five bivalent binding units and a modified J-chain, where each binding unit includes two human IgM or human-derived IgM-like heavy chain constant regions or multimerizing fragments or variants thereof, each associated with a binding unit-associated antigen-binding domain,

where each binding unit-associated antigen-binding domain of the antibody includes the VH amino acid sequence SEQ ID NO: 7 and the VL amino acid sequence SEQ ID NO: 9 or SEQ ID NO: 10, where the modified J-chain includes a J-chain-associated binding domain including a scFv that specifically binds to CD3ε, and where the IgM antibody can direct TDCC, CDC, or both TDCC and CDC of B cells expressing CD20 at high density at an EC50 concentration at least 2-fold, at least 5-fold, at least 10-fold, at least 50-fold, at least 100-fold, at least 500-fold or at least 1000-fold lower than the EC50 concentration for a B cell line or normal B cells, expressing CD20 at low density. In certain embodimentexamples, the IgM or IgM-like antibody can direct CDC, TDCC, or both TDCC and CDC of B cells expressing CD20 at high density, but not B cells expressing CD20 at low density. In certain embodimentexamples, the modified J-chain includes amino acids 20 to 412 of SEQ ID NO: 46 (V15J), amino acids 20 to 412 of SEQ ID NO: 47 (V15J*), or amino acids 20 to 420 of SEQ ID NO: 56 (SJ*). In certain aspectexamples, the high and low density CD20-expressing cells are lymphoma cell lines, for example, the cell expressing CD20 at high density can be a Ramos cell line, and the cell expressing CD20 at low density can be a CA46 cell line. In certain embodimentexamples the provided IgM or IgM-like antibody can direct complement-mediated killing of Ramos cells, where an equivalent amount of a monospecific bivalent IgG1 antibody including the same antigen-binding domains shows no detectable complement-mediated killing of Ramos cells. In certain embodimentexamples the cell expressing CD20 at high density is a malignant B cell in a subject with cancer, and where the cell expressing CD20 at low density is a normal B cell in the subject with cancer. The cancer can be, for example, a CD20-positive leukemia, lymphoma, or myeloma. In certain embodimentexamples, the subject is human.

[0020] In a further aspect, the disclosure provides use of an effective amount of the binding molecule as described herein, the composition as described herein, or the IgM or IgM-like antibody as described herein in the preparation of a medicament for treating cancer. The disclosure further provides a method for treating cancer in a subject, where the method includes administering an effective amount of the provided binding molecule, the provided composition, or the provided IgM or IgM-like antibody to a subject in need of treatment.

[0021] The disclosure further provides for the use of an effective amount of the provided binding molecule, the provided composition, or the provided IgM or IgM-like antibody in the preparation of a medicament for treating cancer.

[0022] The disclosure further provides the provided binding molecule, the provided composition, or the provided IgM or IgM-like antibody, for use in treating cancer.

WHAT IS CLAIMED IS:

1. A multimeric binding molecule comprising two, five, or six bivalent binding units collectively comprising four, ten, or twelve binding unit-associated antigen binding domains, respectively;

wherein each binding unit comprises two antibody heavy chains, each comprising an IgA, IgA-like, IgM, or IgM-like heavy chain constant region or multimerizing fragment or variant thereof and a binding unit-associated antigen-binding domain or subunit thereof,

wherein at least three of the binding-unit-associated antigen-binding domains of the binding molecule specifically bind to the same predetermined target on the surface of a cell, (target antigen), and

wherein the binding molecule preferentially binds to a cell expressing the predetermined target at a higher density (high density cell) relative to a cell expressing the predetermined target at a lower density- (low density cell).

~~2. The binding molecule of claim 1, wherein each antibody heavy chain comprises a heavy chain variable region (VH) portion of a binding-unit-associated antigen-binding domain-~~

~~3. The binding molecule of claim 1 or claim 2, and~~ wherein the at least three binding unit-associated antigen-binding domains that bind to the same ~~predetermined target on the surface of a cell~~ antigen each comprise a VH situated amino terminal to the IgA, IgA-like, IgM, or IgM-like heavy chain ~~variable~~ constant region ~~(VH)~~ and a light chain variable region (VL);

~~4. The binding molecule of any one of claims 1 to 3, situated amino terminal to an immunoglobulin light chain constant region, and~~ wherein the at least three antigen-binding domains each bind to the same epitope on the ~~predetermined target-~~

~~5. The binding molecule of claim 4, antigen, or~~ wherein the at least three ~~binding-unit-associated antigen-binding domains that bind to the same predetermined target on the surface of a cell are identical-~~

~~6.2. The binding molecule of claim 5, wherein,~~ 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 twelve binding-unit-associated antigen-binding domains that bind to the same ~~predetermined target on the surface of a cell and~~ antigen are identical.

~~7.3.~~ The binding molecule of claim ~~5 or claim 62~~, which preferentially binds to a cell-expressing the ~~predetermined target at a higher~~high density cell relative to the binding of a reference bivalent IgG antibody having to the high density cell, wherein the reference bivalent IgG antibody has just two of the identical binding-unit-associated antigen-binding domains that specifically bind to the ~~predetermined target on the surface of the cell~~antigen.

~~8.4.~~ The binding molecule of claim ~~73~~, which can bind to ~~a cell-expressing the target antigen at higher~~high density cell, wherein the reference bivalent IgG antibody ~~having just two of the identical binding-unit-associated antigen-binding domains that specifically bind to the predetermined target on the surface of the cell~~ cannot bind to the ~~cell-expressing the target antigen at higher~~high density cell.

~~9.5.~~ The binding molecule of any one of claims 1 to ~~84~~, which does not detectably bind to ~~a cell-expressing the predetermined target at lower~~low density cell.

~~10.6.~~ The binding molecule of any one of claims 1 to ~~95~~, wherein the ~~cell-expressing the predetermined target at~~ high density cell is a cancer cell and the ~~cell-expressing the predetermined target at~~ low density cell is a normal cell.

~~11.7.~~ The binding molecule of any one of claims 1 to ~~106~~, which is pentameric or hexameric, comprising five or six bivalent IgM or IgM-like binding units, respectively, wherein each binding unit comprises two IgM or IgM-like heavy chain constant regions or multimerizing fragments or variants thereof each associated with a binding unit-associated antigen-binding domain or subunit thereof.

~~12.8.~~ The binding molecule of claim ~~117~~, wherein the IgM or IgM-like heavy chain constant regions or multimerizing fragments or variants thereof each comprise ~~a C μ 4 domain and an IgM tailpiece (tp) domain~~human IgM constant regions or human-derived IgM-like constant regions and the human IgM constant regions or human-derived IgM-like constant regions comprise (a) a C μ 4 domain and an IgM tailpiece (tp) domain or (b) a C μ 4 domain and an IgM tailpiece (tp) domain and a C μ 1 domain, a C μ 2 domain, a C μ 3 domain, or any combination thereof.

~~13.~~ The binding molecule of claim ~~11 or claim 12~~, wherein the IgM or IgM-like heavy chain constant regions or multimerizing fragments or variants thereof each further comprise ~~a C μ 1 domain, a C μ 2 domain, a C μ 3 domain, or any combination thereof~~.

~~14. The binding molecule of any one of claims 11 to 13, wherein the IgM or IgM-like heavy chain constant regions comprise human IgM constant regions or human-derived IgM-like constant regions.~~

~~15. The binding molecule of any one of claims 11 to 14, wherein each binding unit comprises two IgM or IgM-like heavy chains each comprising a VH-situated amino terminal to the IgM or IgM-like constant region or multimerizing fragment or variant thereof, and two immunoglobulin light chains each comprising a VL-situated amino terminal to an immunoglobulin light chain constant region.~~

~~16.9.~~ The binding molecule of any one of claims ~~11-7 to 15~~ or claim 8, which is pentameric, and further comprises a J-chain, or functional fragment or variant thereof.

~~17.10.~~ The binding molecule of any one of claims 1 to ~~406~~, which is dimeric and comprises two bivalent IgA or IgA-like binding units and a J-chain or a functional fragment or variant thereof, wherein each binding unit comprises two IgA or IgA-like heavy chain constant regions or multimerizing fragments or variants thereof each associated with a binding unit-associated antigen-binding domain or subunit thereof.

~~18. The binding molecule of claim 17, further comprising a secretory component, functional fragment or variant thereof.~~

~~19. The binding molecule of claim 17 or claim 18, wherein the IgA or IgA-like heavy chain constant regions or multimerizing fragments or variants thereof each comprise a Cα3 domain and an IgA tailpiece (tp) domain.~~

~~20. The binding molecule of claim 19, wherein the IgA or IgA-like heavy chain constant regions or multimerizing fragments or variants thereof each further comprise a Cα1 domain, a Cα2 domain, an IgA hinge region, or any combination thereof.~~

~~21. The binding molecule of any one of claims 17 to 20, wherein the IgA or IgA-like heavy chain constant regions or multimerizing fragments or variants thereof are human IgA heavy chain constant regions or human-derived IgA-like heavy chain constant regions.~~

~~22. The binding molecule of any one of claims 17 to 21, wherein each binding unit comprises two IgA or IgA-like heavy chains each comprising a VH-situated amino terminal to the IgA or IgA-like heavy chain constant region or multimerizing fragment or variant thereof, and two immunoglobulin light chains each comprising a VL-situated amino terminal to an immunoglobulin light chain constant region.~~

~~23.11.~~ The binding molecule of ~~any one of claims 169 or claim 10 to 22~~, wherein the J-chain or fragment or variant thereof is a human J-chain comprising the amino acid sequence SEQ ID NO: 42 or a functional fragment or variant thereof.

~~24.12.~~ The binding molecule of ~~any one of claims claim 916 to 23~~, wherein the J-chain or functional fragment or variant thereof is a variant J-chain comprising one or more single amino acid substitutions, deletions, or insertions relative to a wild-type J-chain, which can affect serum half-life of the binding molecule; and wherein the binding molecule exhibits an increased serum half-life upon administration to an animal relative to a reference binding molecule that is identical except for the one or more single amino acid substitutions, deletions, or insertions, and is administered in the same way to the same animal species; and wherein the J-chain or functional fragment or variant thereof comprises:

~~25.(a) The binding molecule of claim 24, wherein the J chain or functional fragment or variant thereof comprises~~ an amino acid substitution at the amino acid position corresponding to amino acid Y102 of the wild-type human J-chain (SEQ ID NO: 42);

~~(b) The binding molecule of claim 25, wherein an alanine (A), a serine (S), or an arginine (R) substitution at the amino acid position corresponding to Y102 of SEQ ID NO: 42;~~

~~26.(c) an alanine (A) substitution at~~ the amino acid corresponding to Y102 of SEQ ID NO: 42 ~~is substituted with alanine (A), serine (S), or arginine (R); or~~

~~27. The binding molecule of claim 26, wherein the amino acid corresponding to Y102 of SEQ ID NO: 42 is substituted with alanine (A).~~

~~28.(d) The binding molecule of claim 27, wherein the J chain is a variant human J-chain and comprises~~ the amino acid sequence SEQ ID NO: 43 (J*).

~~29.13.~~ The binding molecule of any one of claims ~~916 to 2812~~, wherein the J-chain or fragment or variant thereof is a modified J-chain further comprising a heterologous moiety, wherein the heterologous moiety is chemically conjugated or fused to the J-chain or fragment or variant thereof.

~~30.14.~~ The binding molecule of claim ~~1329~~, wherein: the heterologous moiety is a heterologous polypeptide-, and the heterologous polypeptide:

~~31. The binding molecule of claim 30, wherein the heterologous polypeptide is fused to the J-chain or fragment or variant thereof via a peptide linker.~~

~~32. The binding molecule of claim 31, wherein the peptide linker consists of SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, or SEQ ID NO: 61.~~

~~33. The binding molecule of any one of claims 30 to 32, wherein the heterologous polypeptide is fused to the N-terminus of the J-chain or fragment or variant thereof, to the C-terminus of the J-chain or fragment or variant thereof, or wherein heterologous polypeptides are fused to both the N- and C-terminus of the J-chain or fragment or variant thereof.~~

~~34. The binding molecule of any one of claims 30 to 33, comprising at least two heterologous polypeptides fused to the J-chain or fragment or variant thereof, wherein the at least two heterologous polypeptides can be the same or different.~~

~~35.(a) The binding molecule of any one of claims 30 to 34, wherein at least one heterologous polypeptide comprises a J-chain-associated antigen-binding domain;~~

~~36.(b) The binding molecule of claim 35, wherein the J-chain-associated antigen-binding domain is comprises an antibody or antigen-binding fragment thereof;~~

~~37.(c) The binding molecule of claim 36, 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;~~

~~38.(d) The binding molecule of claim 37, wherein the J-chain-associated antigen-binding domain comprises a scFv fragment;~~

~~39. The binding molecule of any one of claims 35 to 38, wherein the J-chain-associated antigen-binding domain specifically binds to an immune effector cell.~~

~~40. The binding molecule of claim 39, wherein the immune effector cell is a T cell or an NK cell.~~

~~(e) The binding molecule of claim 39 or claim 40, wherein the immune effector cell is a T cell, and wherein the J-chain-associated antigen-binding domain~~

- ~~41. — comprises a scFv fragment that specifically binds to CD3ε.~~
- ~~42. — The binding molecule of claim 41, wherein the T cell is a CD8+ cytotoxic T cell.~~
- ~~43.(f) The binding molecule of claim 41 or claim 42, wherein the scFv fragment comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein the VH comprises the VH complementarity-determining regions VHCDR1, VHCDR2, and VHCDR3 comprising the amino acid sequences SEQ ID NO: 49, SEQ ID NO: 50, and SEQ ID NO: 51, respectively, or SEQ ID NO: 49, SEQ ID NO: 50, and SEQ ID NO: 51 with one, two, or three amino acid substitutions in one or more of the VHCDRs, and wherein the VL comprises the VL complementarity-determining regions VLCDR1, VLCDR2, and VLCDR3 comprising the amino acid sequences SEQ ID NO: 53, SEQ ID NO: 54, and SEQ ID NO: 55, respectively, or SEQ ID NO: 53, SEQ ID NO: 54, and SEQ ID NO: 55 with one, two, or three amino acid substitutions in one or more of the VLCDRs.~~
- ~~44. — The binding molecule of claim 43, wherein the scFv fragment comprises the VH amino acid sequence SEQ ID NO: 48 and the VL amino acid sequence SEQ ID NO: 52.~~
- ~~45. — The binding molecule of claim 41 or claim 42, wherein the scFv fragment comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein the VH and VL comprise the amino acid sequences SEQ ID NO: 44 and SEQ ID NO: 45, respectively.~~
- ~~46.(g) The binding molecule of claim 41 or claim 42, comprises a scFv fragment that specifically binds to CD3ε, and wherein the modified J-chain comprises amino acids 20 to 412 of SEQ ID NO: 46 (V15J), amino acids 20 to 412 of SEQ ID NO: 47 (V15J*), or amino acids 20 to 420 of SEQ ID NO: 56 (SJ*).~~
- ~~47. — The binding molecule of claim 40, wherein the immune effector cell is an NK cell, and wherein the scFv fragment specifically binds to CD16.~~
- ~~48.(h) The binding molecule of any one of claims 29 to 47, wherein the modified J-chain further comprises an immune stimulatory agent (“ISA”) fused or chemically conjugated to the J-chain or fragment or variant thereof.~~

~~49. The binding molecule of claim 48, wherein the ISA comprises a cytokine or receptor-binding fragment or variant thereof.~~

~~50.(i) The binding molecule of claim 49, wherein the ISA comprises~~comprises an ISA comprising (a) an interleukin-15 (IL-15) protein or receptor-binding fragment or variant thereof (“I”), and (b) an interleukin-15 receptor- α (IL-15R α) fragment comprising the sushi domain or a variant thereof capable of associating with I (“R”), wherein the J-chain or fragment or variant thereof and at least one of I and R are associated as a fusion protein, and wherein I and R can associate to function as the ISA.

~~51. The binding molecule of any one of claims 48 to 50, wherein the ISA is fused to the J-chain via a peptide linker.~~

~~52.15.~~ The binding molecule of any one of claims 1 to ~~54~~14, wherein the predetermined target is B-cell maturation antigen (BCMA), CD19, CD20, EGFR, HER2 (ErbB2), ErbB3, ErbB4, CTLA4, PD-1, PD-L1, VEGF, VEGFR1, VEGFR2, CD52, CD30, prostate-specific membrane antigen (PSMA), CD38, GD2, SLAMF7, platelet-derived growth factor receptor A (PDGFRA), CD22, FLT3 (CD135), CD123, MUC-16, carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM-1), mesothelin, tumor-associated calcium signal transducer 2 (Trop-2), glypican-3 (GPC-3), human blood group H type 1 trisaccharide (Globo-H), sialyl Tn antigen (STn antigen), or CD33.

~~53.16.~~ The binding molecule of any one of claims 1 to ~~155~~2, wherein the at least three identical binding unit-associated antigen-binding domains specific for the predetermined target are reduced-affinity variants of an antigen-binding domain of an existing antibody known to bind to the predetermined target, wherein the antigen-binding domain of an existing antibody comprises a heavy chain variable region (VH) and light chain variable region (VL).

~~17.~~ The binding molecule of claim ~~165~~3, wherein:-

~~54.(a)~~ the existing antibody is alemtuzumab, atezolizumab, atezolizumab, avelumab, bevacizumab, blinatumomab, brentuximab, capromab, cetuximab, daratumumab, denosumab, dinutuximab, durvalumab, elotuzumab, gemtuzumab, ibritumomab, ipilimumab—, inotuzumab, necitumumab, nivolumab, nivolumab, obinutuzumab, ocrelizumab, ofatumumab, olaratumab,

omalizumab, panitumumab, pembrolizumab, pertuzumab, ramucirumab, ranibizumab, rituximab, trastuzumab, or tremelimumab-;

~~55. The binding molecule of claim 53 or claim 54, which binds to the predetermined target with a binding affinity for the predetermined target at least 5-fold, at least 10-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, at least 100-fold, at least 500-fold, or at least 1000-fold lower than the existing antibody.~~

~~56.(b) The binding molecule of any one of claims 53 to 55, wherein the~~ VH and VL of the antigen-binding domain of the existing antibody comprise, respectively, the amino acid sequences SEQ ID NO: 7 and SEQ ID NO: 8, SEQ ID NO: 14 and SEQ ID NO: 15, SEQ ID NO: 16 and SEQ ID NO: 17, SEQ ID NO: 18 and SEQ ID NO: 19, SEQ ID NO: 20 and SEQ ID NO: 21, SEQ ID NO: 22 and SEQ ID NO: 23, SEQ ID NO: 24 and SEQ ID NO: 25, SEQ ID NO: 26 and SEQ ID NO: 27, SEQ ID NO: 28 and SEQ ID NO: 29, SEQ ID NO: 30 and SEQ ID NO: 31, SEQ ID NO: 32 and SEQ ID NO: 33, SEQ ID NO: 34 and SEQ ID NO: 35, SEQ ID NO: 36 and SEQ ID NO: 37, SEQ ID NO: 38 and SEQ ID NO: 39, or SEQ ID NO: 40 and SEQ ID NO: 41-;

~~57.(c) The binding molecule of claim 56, wherein~~ the antigen-binding domain of the existing antibody comprises the VH amino acid sequence SEQ ID NO: 7 and the VL amino acid sequence SEQ ID NO: 8, and wherein the reduced-affinity variant comprises an amino acid substitution at position N93 in the VL, SEQ ID NO: 8-;

~~58. The binding molecule of claim 57, wherein the amino acid substitution is N93D or N93E.~~

~~(d) The binding molecule of claim 58, wherein the antigen-binding domain of the existing antibody comprises the VH amino acid sequence SEQ ID NO: 7 and the VL amino acid sequence SEQ ID NO: 8, and wherein the reduced-affinity variant comprises a glutamate (E) or aspartate (D) substitution at position N93 in the VL, SEQ ID NO: 8; or~~

~~59.(e)~~ the at least three identical binding unit-associated antigen-binding domains specific for the predetermined target comprise the VH amino acid sequence SEQ ID NO: 7 and the VL amino acid sequence SEQ ID NO: 9 or SEQ ID NO: 10.

~~60.—18.~~ The binding molecule of ~~any one of claims~~claim 1657 to or claim 5917, which ~~can direct complement-directed cytotoxicity (CDC) of B cells expressing CD20 at high density at an EC50 concentration at least 2-fold, at least binds to the predetermined target with a binding affinity for the predetermined target at least 5-fold, at least 10-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, at least 100-fold, at least 500-fold or at least 1000-fold lower than the existing antibody EC50 concentration for B cells expressing CD20 at low density.~~

~~61.—The binding molecule of any one of claims 57 to 60, which can direct CDC of B cells expressing CD20 at high density, but not B cells expressing CD20 at low density.~~

~~62.—The binding molecule of any one of claims 57 to 61, which is a pentameric IgM or IgM-like binding molecule or a dimeric IgA or IgA-like binding molecule, and further comprises a modified J chain comprising a scFv that specifically binds to CD3e.~~

~~63.—The binding molecule of claim 62, wherein the modified J chain comprises amino acids 20 to 412 of SEQ ID NO: 46 (V15J), amino acids 20 to 412 of SEQ ID NO: 47 (V15J*), or amino acids 20 to 420 of SEQ ID NO: 56 (SJ*).~~

~~64.—The binding molecule of claim 62 or claim 63, which can direct T-cell directed cellular cytotoxicity (TDCC) or both TDCC and CDC of B cells expressing CD20 at high density at an EC50 concentration at least 2-fold, at least 5-fold, at least 10-fold, at least 50-fold, at least 100-fold, at least 500-fold or at least 1000-fold lower than the EC50 concentration for a B cell line or normal B cells, expressing CD20 at low density.~~

~~65.—The binding molecule of any one of claims 62 to 64, which can direct TDCC or both TDCC and CDC of B cells expressing CD20 at high density, but not B cells expressing CD20 at low density.~~

~~66.—The binding molecule of any one of claims 60 to 65, wherein the B cells expressing CD20 at both high and low density are lymphoma cell lines.~~

~~67. The binding molecule of claim 66, wherein the lymphoma cell line expressing CD20 at high density is a Ramos cell line, a Raji cell line, a DoHH-2 cell line, a JeKo-1 cell line, a Z-138 cell line, a Daudi cell line, a Granta cell line, or a DoHH-2 cell line, and the lymphoma cell line expressing CD20 at low density is a CA46 cell line, a Nalm-1 cell line, a Toledo cell line, a BJAB cell line, a Kasumi-2 cell line, an RPMI-8226 cell line, an HT cell line, an SU-DHL-8 cell line, a JM1 cell line, a Namalwa cell line, a Nalm-6 cell line, or a Z138 cell line.~~

~~68. The binding molecule of any one of claim 66 or claim 67, which can direct CDC of Ramos cells, wherein an equivalent amount of a monospecific bivalent IgG1 antibody comprising the same antigen-binding domains as the binding molecule shows no detectable complement-mediated killing of Ramos cells.~~

~~69. The binding molecule of any one of claims 60 to 65, wherein the B cells expressing CD20 at high density are CD20-positive malignant B cells in a subject with cancer, and the B cells expressing CD20 at low density are normal B cells in the subject with cancer.~~

~~70. The binding molecule of claim 69, wherein the cancer is a CD20-positive leukemia, lymphoma, or myeloma.~~

~~71.18. The binding molecule of claim 69 or claim 70, wherein the subject is human.~~

~~72.19. A composition comprising the binding molecule of any one of claims 1 to 71.18, and a carrier.~~

~~73.20. An isolated polynucleotide comprising a nucleic acid molecule that encodes the binding molecule of any one of claims 1 to 18.71, or an antigen-binding and multimerizing fragment thereof, or a subunit thereof.~~

~~74. The polynucleotide of claim 73, wherein the subunit is an antibody heavy chain or multimerizing fragment or variant thereof, an antibody light chain or fragment thereof, a J-chain or fragment or variant thereof, or any combination thereof.~~

~~75.21. A vector comprising the polynucleotide of claim 73 or claim 74.20.~~

~~76.22. A host cell comprising the vector of claim 75.21.~~

~~77.23. A method for producing the binding molecule of any one of claims 1 to 71.18, comprising culturing the host cell of claim 76.22, and recovering the binding molecule.~~

~~78.24.~~ An IgM or IgM-like antibody comprising five bivalent binding units and a modified J-chain,

wherein each binding unit comprises two human IgM or human-derived IgM-like heavy chain constant regions or multimerizing fragments or variants thereof, each associated with a binding unit-associated antigen-binding domain,

wherein each binding unit-associated antigen-binding domain of the antibody comprises the VH amino acid sequence SEQ ID NO: 7 and the VL amino acid sequence SEQ ID NO: 9 or SEQ ID NO: 10,

wherein the modified J-chain comprises a J-chain-associated binding domain comprising a scFv that specifically binds to CD3ε, and

wherein the IgM antibody can direct TDCC, CDC, or both TDCC and CDC of B cells expressing CD20 at high density at an EC50 concentration at least 2-fold, at least 5-fold, at least 10-fold, at least 50-fold, at least 100-fold, at least 500-fold or at least 1000-fold lower than the EC50 concentration for a B cell line or normal B cells, expressing CD20 at low density.

~~79.—The IgM or IgM-like antibody of claim 78, which can direct CDC, TDCC, or both TDCC and CDC of B cells expressing CD20 at high density, but not B cells expressing CD20 at low density.~~

~~80.—The IgM or IgM-like antibody of claim 78 or claim 79, wherein the modified J-chain comprises amino acids 20 to 412 of SEQ ID NO: 46 (V15J), amino acids 20 to 412 of SEQ ID NO: 47 (V15J*), or amino acids 20 to 420 of SEQ ID NO: 56 (SJ*).~~

~~81.—The IgM or IgM-like antibody of any one of claims 78 to 80, wherein the high and low density CD20-expressing cells are lymphoma cell lines.~~

~~82.—The IgM or IgM-like antibody of claim 81, wherein the cell expressing CD20 at high density is a Ramos cell line, and the cell expressing CD20 at low density is a CA46 cell line.~~

~~83.—The IgM or IgM-like antibody of any one of claims 78 to 82, which can direct complement-mediated killing of Ramos cells, wherein an equivalent amount of a monospecific bivalent IgG1 antibody comprising the same antigen-binding domains shows no detectable complement-mediated killing of Ramos cells.~~

~~84. The IgM or IgM-like antibody of any one of claims 78 to 80, wherein the cell expressing CD20 at high density is a malignant B cell in a subject with cancer, and wherein the cell expressing CD20 at low density is a normal B cell in the subject with cancer.~~

~~85. The IgM or IgM-like antibody of claim 84, wherein the cancer is a CD20-positive leukemia, lymphoma, or myeloma.~~

~~86. The IgM or IgM-like antibody of claim 84 or claim 85, wherein the subject is human.~~

~~87. A method for treating cancer in a subject, comprising administering an effective amount of the binding molecule of any one of claims 1 to 71, the composition of claim 72, or the IgM or IgM-like antibody of any one of claims 78 to 86 to a subject in need of treatment.~~

~~88. 25. Use of an effective amount of the binding molecule of any one of claims 1 to 7118, the composition of claim 1972, or the IgM or IgM-like antibody of ~~any one of claims claim 24~~78 to 86 in the preparation of a medicament for treating cancer.~~

~~89. The binding molecule of any one of claims 1 to 71, the composition of claim 72, or the IgM or IgM-like antibody of any one of claims 78 to 86, for use in treating cancer.~~