

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 examples, each antibody heavy chain includes a heavy chain variable region (VH) portion of a binding-unit-associated antigen-binding domain. In certain 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 examples at least three

antigen-binding domains each bind to the same epitope on the predetermined target. In certain examples 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.

[0008] In certain examples 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 examples, the provided binding molecule preferentially binds to a cell expressing the predetermined target at a higher density 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 examples, 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 examples the provided binding molecule does not detectably bind to a cell expressing the predetermined target at lower density. In certain examples 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 examples 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 examples 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 examples the IgM or IgM-like heavy chain constant regions are human IgM constant regions or human-derived IgM-like constant regions. In certain examples, 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 examples where the multimeric binding molecule is pentameric, it can further include a J-chain, or functional fragment or variant thereof.

[0010] In certain examples 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 examples the dimeric binding molecule can further include a secretory component, or functional fragment or variant thereof. In certain examples 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 examples 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 examples each binding unit includes two IgA or IgA-like heavy chains each including a
5 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 examples the J-chain or fragment or variant thereof is a human
10 or human-derived J-chain that includes the amino acid sequence SEQ ID NO: 42 or a functional fragment or variant thereof. In certain examples 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 examples, a binding molecule
15 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 examples the J-chain or functional fragment or variant thereof includes an amino acid substitution at the amino
20 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 examples the variant J-chain is a variant human J-chain and includes the amino acid sequence SEQ ID NO: 43 (J*).

[0012] In certain examples, 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
25 fragment or variant thereof. In certain examples 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
30 ID NO: 61. In certain examples 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 examples 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 examples at least one heterologous polypeptide includes a J-chain-associated antigen-binding domain. In certain examples 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 examples the J-chain-associated antigen-binding domain is a scFv fragment. In certain examples 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 examples 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 acid sequence SEQ ID NO: 49, where the VH and VL include the amino acid sequences SEQ ID NO: 44 and SEQ ID NO: 45, respectively. In certain specific examples, 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 examples, 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 examples 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 examples the ISA can be fused to the J-chain via a peptide linker.

[0015] In certain examples, 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 examples, 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 examples 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 examples 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 examples 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 examples 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 examples, 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 examples 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 examples 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 examples 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

examples 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 examples 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 examples 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 examples 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 examples the cancer is a CD20-positive leukemia, lymphoma, or myeloma. In certain examples, the subject is human.

[0017] In another aspect, 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 examples 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, 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 another aspect, 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 examples, 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 examples, 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 examples, 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 examples 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 examples 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 examples, 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, and wherein the at least three binding unit-associated antigen-binding domains that bind to the same target antigen each comprise a VH situated amino terminal to the IgA, IgA-like, IgM, or IgM-like heavy chain constant region and a light chain variable region (VL) 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 target antigen, or wherein the 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 twelve binding-unit-associated antigen-binding domains that bind to the same target antigen are identical.

3. The binding molecule of claim 2, which preferentially binds to the high density cell relative to the binding of a reference bivalent IgG antibody 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 target antigen.

4. The binding molecule of claim 3, which can bind to the high density cell, wherein the reference bivalent IgG antibody cannot bind to the high density cell.

5. The binding molecule of any one of claims 1 to 4, which does not detectably bind to the low density cell.

6. The binding molecule of any one of claims 1 to 5, wherein the high density cell is a cancer cell and the low density cell is a normal cell.

7. The binding molecule of any one of claims 1 to 6, 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.

8. The binding molecule of claim 7, wherein the IgM or IgM-like heavy chain constant regions or multimerizing fragments or variants thereof each comprise 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.

9. The binding molecule of claim 7 or claim 8, which is pentameric, and further comprises a J-chain, or functional fragment or variant thereof.

10. The binding molecule of any one of claims 1 to 6, 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.

11. The binding molecule of claim 9 or claim 10, 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.

12. The binding molecule of claim 9, 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:

- (a) 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) an alanine (A), a serine (S), or an arginine (R) substitution at the amino acid position corresponding to Y102 of SEQ ID NO: 42;
- (c) an alanine (A) substitution at the amino acid corresponding to Y102 of SEQ ID NO: 42; or
- (d) the amino acid sequence SEQ ID NO: 43 (J*).

13. The binding molecule of any one of claims 9 to 12, 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.

14. The binding molecule of claim 13, wherein: the heterologous moiety is a heterologous polypeptide, and the heterologous polypeptide:

- (a) comprises a J-chain-associated antigen-binding domain;
- (b) comprises an antibody or antigen-binding fragment thereof;
- (c) 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;
- (d) comprises a scFv fragment;
- (e) comprises a scFv fragment that specifically binds to CD3ε;
- (f) comprises a scFv fragment that specifically binds to CD3ε, 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;

- (g) comprises a scFv fragment that specifically binds to CD3 ϵ , 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*)
- (h) comprises an immune stimulatory agent ("ISA");
- (i) 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.

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

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

- (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;
- (b) 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;
- (c) 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;
- (d) 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
- (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.

18. The binding molecule of claim 16 or claim 17, 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.

19. A composition comprising the binding molecule of any one of claims 1 to 18, and a carrier.

20. An isolated polynucleotide comprising a nucleic acid molecule that encodes the binding molecule of any one of claims 1 to 18.

21. A vector comprising the polynucleotide of claim 20.

22. A host cell comprising the vector of claim 21.

23. A method for producing the binding molecule of any one of claims 1 to 18, comprising culturing the host cell of claim 22, and recovering the binding molecule.

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.

25. Use of an effective amount of the binding molecule of any one of claims 1 to 18, the composition of claim 19, or the IgM or IgM-like antibody of claim 24 in the preparation of a medicament for treating cancer.