

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, and

wherein 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.

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, wherein the at least three binding unit-associated antigen-binding domains that bind to the same predetermined target on the surface of a cell each comprise a heavy chain variable region (VH) and light chain variable region (VL).

4. The binding molecule of any one of claims 1 to 3, 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, 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. 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 bind to the same predetermined target on the surface of a cell and are identical.

7. The binding molecule of claim 5 or claim 6, which 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.

8. The binding molecule of claim 7, which can bind to a cell expressing the target antigen at higher density, 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 density.

9. The binding molecule of any one of claims 1 to 8, which does not detectably bind to a cell expressing the predetermined target at lower density.

10. The binding molecule of any one of claims 1 to 9, wherein 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 cell.

11. The binding molecule of any one of claims 1 to 10, 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. The binding molecule of claim 11, 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.

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

17. The binding molecule of any one of claims 1 to 10, 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, or 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 C α 3 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 C α 1 domain, a C α 2 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. The binding molecule of any one of claims 16 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. The binding molecule of any one of claims 16 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.

25. 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).

26. The binding molecule of claim 25, wherein the amino acid corresponding to Y102 of SEQ ID NO: 42 is substituted with alanine (A), serine (S), or arginine (R).

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. 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. The binding molecule of any one of claims 16 to 28, 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. The binding molecule of claim 29, wherein the heterologous moiety is a 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. 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. The binding molecule of claim 35, wherein the J-chain-associated antigen-binding domain is an antibody or antigen-binding fragment thereof.

37. 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. 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.

41. 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 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. 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. The binding molecule of claim 41 or claim 42, 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. 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. The binding molecule of claim 49, wherein the ISA comprises (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. The binding molecule of any one of claims 1 to 51, 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. The binding molecule of any one of claims 5 to 52, 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).

54. The binding molecule of claim 53, wherein 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. The binding molecule of any one of claims 53 to 55, wherein 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. 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.

59. The binding molecule of claim 58, wherein 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. The binding molecule of any one of claims 57 to 59, 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 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.

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 CD3ε.

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 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.

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

72. A composition comprising the binding molecule of any one of claims 1 to 71, and a carrier.

73. An isolated polynucleotide comprising a nucleic acid molecule that encodes the binding molecule of any one of claims 1 to 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. A vector comprising the polynucleotide of claim 73 or claim 74.
76. A host cell comprising the vector of claim 75.
77. A method for producing the binding molecule of any one of claims 1 to 71, comprising culturing the host cell of claim 76, and recovering the binding molecule.
78. 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 CD3 ϵ , 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. Use of 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 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.