

The assembly of IgM binding units into a pentameric structure is thought to involve the C μ 4 and tailpiece domains of the IgM constant region. *See, e.g., Braathen, R., et al., J. Biol. Chem. 277:42755-42762 (2002).*

[0008] Despite the advances made in the design of multimeric antibodies, there remains a need to be improve immunotherapy by engineering multimeric binding molecules.

SUMMARY

[0009] In one aspect, ~~t~~This disclosure provides a multimeric binding molecule that includes two or five bivalent binding units or multimerizing variants or fragments thereof and a modified J-chain, where each binding unit includes two IgA or IgM heavy chain constant regions or multimerizing variants or fragments thereof, each associated with an antigen-binding domain for a total of four or ten antigen-binding domains, where at least three of the antigen-binding domains of the binding molecule specifically bind to a target antigen. As provided, the modified J-chain includes (a) a J-chain or functional fragment or variant thereof ("J"), and (b) an immunostimulatory agent ("ISA"), where J and the ISA are associated as a fusion protein.

[0010] In certain embodimentexamples, the ISA includes a cytokine or receptor-binding fragment or variant thereof. For example, the cytokine or fragment or variant thereof includes IL-15 or IL-2, or a receptor-binding fragment or variant thereof. In certain embodimentexamples, the ISA includes (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 J 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.

[0011] In certain embodimentexamples, J is a wild-type human J-chain and includes the amino acid sequence SEQ ID NO: 2 or a functional fragment or variant thereof. In other embodimentexamples, J is a variant J-chain or fragment thereof including one or more single amino acid substitutions, deletions, or insertions relative to a wild-type J-chain that can affect serum half-life of the multimeric binding molecule; such that the multimeric binding molecule exhibits an increased serum half-life upon administration to an animal relative to a reference multimeric 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. For example, J can include an amino acid substitution at the amino acid position corresponding to amino acid Y102 of the mature wild-type human J-chain (SEQ ID NO: 2). The amino acid corresponding to Y102 of SEQ ID NO: 2 can be substituted with alanine (A), serine (S), or arginine (R), and in particular embodimentexamples Y102 can be substituted with alanine (A). In certain embodimentexamples J is a variant of the human J-chain and includes the amino acid sequence SEQ ID NO: 3 (“J*”) or amino acids 1-137 of SEQ ID NO: 86.

[0012] In some embodimentexamples, J is a variant J-chain or fragment thereof comprising one or more single amino acid substitutions, deletions, or insertions relative to a wild-type J-chain that reduces glycosylation of the J. In some embodimentexamples, the J comprises an amino acid substitution at the amino acid position corresponding to amino acid N49 of the mature wild-type human J-chain (SEQ ID NO: 2). In some embodimentexamples, the amino acid corresponding to Y102 of SEQ ID NO: 2 is substituted with aspartic acid (D).

[0013] In certain embodimentexamples, I includes the mature human IL-15 amino acid sequence of SEQ ID NO: 4 or a receptor-binding variant or fragment thereof. A receptor-binding variant can include at least one, but no more than ten, single amino acid insertions, deletions, or substitutions. In certain embodimentexamples, the single amino acid insertions, deletions, or substitutions reduce, but do not eliminate, the affinity of the IL-15 variant for its receptor. In certain embodimentexamples the variant I can include one, two, three, four, five, six, seven, or eight amino acid substitutions. In certain embodimentexamples, the amino acid substitutions can be at one or more of positions corresponding to N1, N4, D8, D30, D61, E64, N65, N72, or Q108 of SEQ ID NO: 4. For example, the amino acid substitutions can include one or more of substitutions N1D, N4D, D8N, D30N, D61N, E64Q, N65D, N72D, or Q108E, in SEQ ID NO: 4. In certain embodimentexamples, I includes SEQ ID NO: 4 except for: (a) a single amino acid substitution at a position selected from the group consisting of N1D, N4D, D8N, D30N, D61N, E64Q, N65D, N72D, and Q108E; (b) two amino acid substitutions at positions selected from the group consisting of N4D/N65D and N1D/N65D; or (c) three amino acid substitutions at positions D30N/E64Q/N65D. In certain embodimentexamples, I includes the amino acid sequence SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, or SEQ ID NO: 68.

[0014] In some embodimentexamples, the receptor-binding variant comprises at least one, but no more than ten, single amino acid insertions, deletions, or substitutions, and wherein the single amino acid insertions, deletions, or substitutions reduce the glycosylation of the IL-15 variant. In some embodimentexamples, I comprises one, two, three, four, five, six, seven, or eight amino acid substitutions. In some embodimentexamples, the amino acid substitutions are at one or more of positions corresponding to N71, S73, N79, or N112 of SEQ ID NO: 4. In some embodimentexamples, the amino acid substitutions comprise one or more of substitutions N71D, S73I, N79D, or N112D in SEQ ID NO: 4. In some embodimentexamples, I comprises SEQ ID NO: 4 except for one or more amino acid substitutions at a position selected from the group consisting of N71D, S73I, N79D, and N112D. In some embodimentexamples, I comprises the amino acid sequence of amino acids 246-361 of SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, or SEQ ID NO: 90.

[0015] In certain embodimentexamples, R, the sushi domain of the IL-15 receptor- α , includes the amino acid sequence SEQ ID NO: 5 or a variant or fragment thereof that is capable of associating with human IL-15. In other embodimentexamples, R consists essentially of or consists of the amino acid sequence SEQ ID NO: 5 or a variant thereof that is capable of associating with human IL-15.

[0016] In certain embodimentexamples, J and I are associated as a fusion protein. In certain embodimentexamples, J and R are associated as a fusion protein. In certain embodimentexamples, J, I, and R are associated as a fusion protein. According to these embodimentexamples, J, I, and/or R can be fused via linkers, which can be the same or different. In certain embodimentexamples, at least one linker includes, consists essentially of, or consists of the amino acid sequence GGGSGGGSGGGGS (SEQ ID NO: 78). In certain embodimentexamples, at least one linker includes, consists essentially of, or consists of the amino acid sequence GGSGGGSGGGSGGGSLQ (SEQ ID NO: 79). In certain embodimentexamples, J is J*, and the modified J-chain can be arranged from N-terminus to C-terminus as J*-R-I, J*-I-R, I-R-J*, R-I-J*, R-J*-I, I-J*-R, I-J*, or J*-I, where “-” is a linker. In certain embodimentexamples, the modified J-chain includes the amino acid sequence SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 77, SEQ ID NO: 85, SEQ ID NO: 86,

SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, or SEQ ID NO: 90. In a particular embodimentexample, the modified J-chain is arranged from N-terminus to C-terminus as J*-R-I, and can include the amino acid sequence SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 77, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, or SEQ ID NO: 90.

[0017] In other embodimentexamples, the ISA includes a variant of human IL-2, for example, "IL2v," that does not bind to the α -subunit of the IL-2 receptor. IL2v includes the amino acid sequence SEQ ID NO: 31, and one modified J-chain comprising IL2v includes the amino acid sequence SEQ ID NO: 32.

[0018] In certain embodimentexamples, the modified J-chain of a multimeric binding molecule as provided herein can further include an antigen-binding domain of an antibody fused thereto, in addition to the ISA. For example, the modified J-chain can include an antigen-binding domain binds to a target on an immune effector cell. In certain embodimentexamples, the immune effector cell is a CD8⁺ T cell, and the antigen binding domain can be a single-chain Fv (scFv) antibody fragment that specifically binds to CD3epsilon (CD3 ϵ). According to these embodimentexamples, the modified J-chain can include the amino acid sequence SEQ ID NO: 19.

[0019] In certain embodimentexamples, the multimeric binding molecule provided by this disclosure is pentameric and includes five binding units, where each binding unit includes two IgM heavy chain constant regions or multimerizing variants or fragments thereof. In other embodimentexamples, the multimeric binding molecule provided by this disclosure is dimeric and includes two binding units or multimerizing variants or fragments thereof, where each binding unit includes two IgA heavy chain constant regions or multimerizing variants or fragments thereof.

[0020] In certain embodimentexamples, the target antigen bound by the multimeric binding molecule provided by this disclosure is a tumor-associated antigen or a target that modulates a T cell response or NK cell response. For example, the target antigen can include a target that modulates a T cell response or an NK cell response. In certain embodimentexamples, the target is one that inhibits CD8⁺ T cell or NK cell activity and it is

desirable to inhibit such a target. For example, that target can be an inhibitory immune checkpoint protein, and the antigen-binding domains of the provided binding molecule antagonize the target, thereby stimulating CD8⁺ T cells or NK cells. For example, the inhibitory immune checkpoint protein includes a programmed cell death-1 protein (PD-1), a programmed cell death ligand-1 protein (PD-L1), a lymphocyte-activation gene 3 protein (LAG3), a T-cell immunoglobulin and mucin domain 3 protein (TIM3), a cytotoxic T-lymphocyte-associated protein 4 (CTLA4), a B- and T-lymphocyte attenuator protein (BTLA), a V-domain Ig suppressor of T-cell activation protein (VISTA), a T-cell immunoreceptor with Ig and ITIM Domains protein (TIGIT), a Killer-cell Immunoglobulin-like Receptor protein (KIR), a B7-H3 protein, a B7-H4 protein, or any combination thereof. In a particular [embodimentexample](#), the inhibitory immune checkpoint protein is PD-L1. According to this [embodimentexample](#), the antigen-binding domain can include a heavy chain variable region (VH) that includes the amino acid sequence SEQ ID NO: 33, SEQ ID NO: 91, SEQ ID NO: 92, or SEQ ID NO: 93 and a light chain variable region (VL) that includes the amino acid sequence SEQ ID NO: 34 or SEQ ID NO: 94.

[0021] In some [embodimentexamples](#), the inhibitory immune checkpoint protein comprises PD-L1, and the antigen-binding domain comprises a heavy chain variable region (VH) and a light chain variable region (VL) wherein the VH and VL comprise six immunoglobulin complementarity determining regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the CDRs of an antibody comprising the VH and VL of SEQ ID NO: 75 and SEQ ID NO: 76, SEQ ID NO: 96 and SEQ ID NO: 97, SEQ ID NO: 98 and SEQ ID NO: 99, SEQ ID NO: 100 and SEQ ID NO: 101, SEQ ID NO: 102 and SEQ ID NO: 103, SEQ ID NO: 104 and SEQ ID NO: 105, SEQ ID NO: 106 and SEQ ID NO: 107, SEQ ID NO: 108 and SEQ ID NO: 109, SEQ ID NO: 110 and SEQ ID NO: 111, SEQ ID NO: 112 and SEQ ID NO: 113, SEQ ID NO: 114 and SEQ ID NO: 115, SEQ ID NO: 116 and SEQ ID NO: 117, SEQ ID NO: 118 and SEQ ID NO: 119, SEQ ID NO: 120 and SEQ ID NO: 121, SEQ ID NO: 122 and SEQ ID NO: 123, SEQ ID NO: 124 and SEQ ID NO: 125, SEQ ID NO: 126 and SEQ ID NO: 127, SEQ ID NO: 128 and SEQ ID NO: 129, SEQ ID NO: 130 and SEQ ID NO: 131, SEQ ID NO: 132 and SEQ ID NO: 133, SEQ ID NO: 134 and SEQ ID NO: 135, SEQ ID NO: 136 and SEQ ID NO: 137, SEQ ID NO: 138 and SEQ ID NO: 139, SEQ ID NO: 140 and SEQ ID NO: 141, SEQ ID NO: 142 and SEQ ID NO: 143, SEQ ID NO: 144 and SEQ ID NO: 145, SEQ ID NO: 146 and SEQ ID NO: 147, SEQ ID NO: 148 and SEQ

ID NO: 149, SEQ ID NO: 150 and SEQ ID NO: 151, SEQ ID NO: 152 and SEQ ID NO: 153, SEQ ID NO: 154 and SEQ ID NO: 155, SEQ ID NO: 156 and SEQ ID NO: 157, SEQ ID NO: 158 and SEQ ID NO: 159, SEQ ID NO: 160 and SEQ ID NO: 161, SEQ ID NO: 162 and SEQ ID NO: 163, SEQ ID NO: 164 and SEQ ID NO: 165, SEQ ID NO: 166 and SEQ ID NO: 167, SEQ ID NO: 168 and SEQ ID NO: 169, SEQ ID NO: 170 and SEQ ID NO: 171, SEQ ID NO: 172 and SEQ ID NO: 173, SEQ ID NO: 174 and SEQ ID NO: 175, SEQ ID NO: 176 and SEQ ID NO: 177, SEQ ID NO: 178 and SEQ ID NO: 179, SEQ ID NO: 180 and SEQ ID NO: 181, SEQ ID NO: 182 and SEQ ID NO: 183, SEQ ID NO: 184 and SEQ ID NO: 185, SEQ ID NO: 186 and SEQ ID NO: 187, SEQ ID NO: 188 and SEQ ID NO: 189, SEQ ID NO: 190 and SEQ ID NO: 191, SEQ ID NO: 192 and SEQ ID NO: 193, SEQ ID NO: 194 and SEQ ID NO: 195, SEQ ID NO: 196 and SEQ ID NO: 197, SEQ ID NO: 198 and SEQ ID NO: 199, SEQ ID NO: 200 and SEQ ID NO: 201, SEQ ID NO: 202 and SEQ ID NO: 203, SEQ ID NO: 204 and SEQ ID NO: 205, SEQ ID NO: 206 and SEQ ID NO: 207, SEQ ID NO: 208 and SEQ ID NO: 209, SEQ ID NO: 210 and SEQ ID NO: 211, SEQ ID NO: 212 and SEQ ID NO: 213, SEQ ID NO: 214 and SEQ ID NO: 215, SEQ ID NO: 216 and SEQ ID NO: 217, SEQ ID NO: 218 and SEQ ID NO: 219, SEQ ID NO: 220 and SEQ ID NO: 221, or SEQ ID NO: 222 and SEQ ID NO: 223, respectively with zero, one, or two single amino acid substitutions in one or more of the HCDRs or LCDRs. In some embodimentexamples, the inhibitory immune checkpoint protein comprises PD-L1, and the antigen-binding domain comprises a heavy chain variable region (VH) and a light chain variable region (VL) wherein the VH and VL comprise six immunoglobulin complementarity determining regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the CDRs of an antibody comprising the VH and VL of SEQ ID NO: 134 and SEQ ID NO: 135, SEQ ID NO: 136 and SEQ ID NO: 137, SEQ ID NO: 138 and SEQ ID NO: 139, SEQ ID NO: 140 and SEQ ID NO: 141, SEQ ID NO: 142 and SEQ ID NO: 143, SEQ ID NO: 144 and SEQ ID NO: 145, SEQ ID NO: 146 and SEQ ID NO: 147, SEQ ID NO: 148 and SEQ ID NO: 149, SEQ ID NO: 166 and SEQ ID NO: 167, SEQ ID NO: 168 and SEQ ID NO: 169, SEQ ID NO: 170 and SEQ ID NO: 171, or SEQ ID NO: 186 and SEQ ID NO: 187, respectively with zero, one, or two single amino acid substitutions in one or more of the HCDRs or LCDRs, such as zero amino acid substitutions.

[0022] In some embodimentexamples, the inhibitory immune checkpoint protein comprises PD-L1, and the antigen-binding domain comprises a heavy chain variable region (VH) and a light

chain variable region (VL) wherein the VH and VL comprise amino acid sequences at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to the mature VH and VL amino acid sequences comprising SEQ ID NO: 75 and SEQ ID NO: 76, SEQ ID NO: 96 and SEQ ID NO: 97, SEQ ID NO: 98 and SEQ ID NO: 99, SEQ ID NO: 100 and SEQ ID NO: 101, SEQ ID NO: 102 and SEQ ID NO: 103, SEQ ID NO: 104 and SEQ ID NO: 105, SEQ ID NO: 106 and SEQ ID NO: 107, SEQ ID NO: 108 and SEQ ID NO: 109, SEQ ID NO: 110 and SEQ ID NO: 111, SEQ ID NO: 112 and SEQ ID NO: 113, SEQ ID NO: 114 and SEQ ID NO: 115, SEQ ID NO: 116 and SEQ ID NO: 117, SEQ ID NO: 118 and SEQ ID NO: 119, SEQ ID NO: 120 and SEQ ID NO: 121, SEQ ID NO: 122 and SEQ ID NO: 123, SEQ ID NO: 124 and SEQ ID NO: 125, SEQ ID NO: 126 and SEQ ID NO: 127, SEQ ID NO: 128 and SEQ ID NO: 129, SEQ ID NO: 130 and SEQ ID NO: 131, SEQ ID NO: 132 and SEQ ID NO: 133, SEQ ID NO: 134 and SEQ ID NO: 135, SEQ ID NO: 136 and SEQ ID NO: 137, SEQ ID NO: 138 and SEQ ID NO: 139, SEQ ID NO: 140 and SEQ ID NO: 141, SEQ ID NO: 142 and SEQ ID NO: 143, SEQ ID NO: 144 and SEQ ID NO: 145, SEQ ID NO: 146 and SEQ ID NO: 147, SEQ ID NO: 148 and SEQ ID NO: 149, SEQ ID NO: 150 and SEQ ID NO: 151, SEQ ID NO: 152 and SEQ ID NO: 153, SEQ ID NO: 154 and SEQ ID NO: 155, SEQ ID NO: 156 and SEQ ID NO: 157, SEQ ID NO: 158 and SEQ ID NO: 159, SEQ ID NO: 160 and SEQ ID NO: 161, SEQ ID NO: 162 and SEQ ID NO: 163, SEQ ID NO: 164 and SEQ ID NO: 165, SEQ ID NO: 166 and SEQ ID NO: 167, SEQ ID NO: 168 and SEQ ID NO: 169, SEQ ID NO: 170 and SEQ ID NO: 171, SEQ ID NO: 172 and SEQ ID NO: 173, SEQ ID NO: 174 and SEQ ID NO: 175, SEQ ID NO: 176 and SEQ ID NO: 177, SEQ ID NO: 178 and SEQ ID NO: 179, SEQ ID NO: 180 and SEQ ID NO: 181, SEQ ID NO: 182 and SEQ ID NO: 183, SEQ ID NO: 184 and SEQ ID NO: 185, SEQ ID NO: 186 and SEQ ID NO: 187, SEQ ID NO: 188 and SEQ ID NO: 189, SEQ ID NO: 190 and SEQ ID NO: 191, SEQ ID NO: 192 and SEQ ID NO: 193, SEQ ID NO: 194 and SEQ ID NO: 195, SEQ ID NO: 196 and SEQ ID NO: 197, SEQ ID NO: 198 and SEQ ID NO: 199, SEQ ID NO: 200 and SEQ ID NO: 201, SEQ ID NO: 202 and SEQ ID NO: 203, SEQ ID NO: 204 and SEQ ID NO: 205, SEQ ID NO: 206 and SEQ ID NO: 207, SEQ ID NO: 208 and SEQ ID NO: 209, SEQ ID NO: 210 and SEQ ID NO: 211, SEQ ID NO: 212 and SEQ ID NO: 213, SEQ ID NO: 214 and SEQ ID NO: 215, SEQ ID NO: 216 and SEQ ID NO: 217, SEQ ID NO: 218 and SEQ ID NO: 219, SEQ ID NO: 220 and SEQ ID NO: 221, or SEQ ID NO: 222 and SEQ ID NO: 223, respectively. In some embodimentexamples, the inhibitory immune checkpoint protein comprises PD-L1, and the

antigen-binding domain comprises a heavy chain variable region (VH) and a light chain variable region (VL) wherein the VH and VL comprise amino acid sequences at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to the mature VH and VL amino acid sequences comprising SEQ ID NO: 134 and SEQ ID NO: 135, SEQ ID NO: 136 and SEQ ID NO: 137, SEQ ID NO: 138 and SEQ ID NO: 139, SEQ ID NO: 140 and SEQ ID NO: 141, SEQ ID NO: 142 and SEQ ID NO: 143, SEQ ID NO: 144 and SEQ ID NO: 145, SEQ ID NO: 146 and SEQ ID NO: 147, SEQ ID NO: 148 and SEQ ID NO: 149, SEQ ID NO: 166 and SEQ ID NO: 167, SEQ ID NO: 168 and SEQ ID NO: 169, SEQ ID NO: 170 and SEQ ID NO: 171, or SEQ ID NO: 186 and SEQ ID NO: 187, respectively.

[0023] In other embodimentexamples, the target antigen bound by the multimeric binding molecule provided by this disclosure is a TNF receptor superfamily target. According to these embodimentexamples, the antigen-binding domains can agonize the target. For example, the target antigen can be GITR, OX40, or a combination thereof. In those embodimentexamples where the target antigen is GITR, the antigen-binding domain can include, for example, a heavy chain variable region (VH) and a light chain variable region (VL) including, respectively, the amino acid sequences SEQ ID NO: 35 and SEQ ID NO: 36, SEQ ID NO: 37 and SEQ ID NO: 38, SEQ ID NO: 39 and SEQ ID NO: 40, SEQ ID NO: 41 and SEQ ID NO: 42, or SEQ ID NO: 43 and SEQ ID NO: 44. In those embodimentexamples where the target antigen includes OX40, the antigen-binding domain can include, for example, a heavy chain variable region (VH) and a light chain variable region (VL) including, respectively, the amino acid sequences SEQ ID NO: 45 and SEQ ID NO: 46 or SEQ ID NO: 47 and SEQ ID NO: 48.

[0024] In other embodimentexamples, the target antigen bound by the multimeric binding molecule provided by this disclosure is a tumor-associated antigen. The tumor-associated antigen can include, for example, 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), CD33, or any combination thereof. In a particular aspectexample, the target antigen is CD20, and the antigen-

binding domain can include, for example, a heavy chain variable region (VH) and a light chain variable region (VL) including, respectively, the amino acid sequences SEQ ID NO: 49 and SEQ ID NO: 50.

[0025] In certain embodimentexamples at least four, at least five, at least six, at least seven, at least eight, at least nine or ten of the antigen-binding domains of the binding molecule specifically bind to the same target antigen.

[0026] In certain embodimentexamples, each binding unit of a multimeric binding molecule provided by the disclosure includes two IgA heavy chain constant regions or multimerizing fragments or variants thereof, each including an IgA C α 1 domain, an IgA hinge, an IgA C α 2 domain, IgA C α 3 domain, and an IgA tailpiece domain. In certain embodimentexamples, the IgA heavy chain constant regions include the amino acid sequence SEQ ID NO: 53, SEQ ID NO: 54, or any multimerizing variant or fragment thereof.

[0027] In certain embodimentexamples, each binding unit of a multimeric binding molecule provided by the disclosure includes two IgM heavy chain constant regions or multimerizing fragments or variants thereof, each including an IgM C μ 4 domain and an IgM tailpiece domain. In certain embodimentexamples, each IgM heavy chain constant region or multimerizing fragment or variant thereof further includes an IgM C μ 3 domain, an IgM C μ 2 domain, an IgM C μ 1 domain, or any combination thereof, situated N-terminal to the IgM C μ 4 and IgM tailpiece domains. In certain embodimentexamples, each IgM heavy chain constant region is a human IgM constant region or multimerizing variant or fragment thereof, including the amino acid sequence SEQ ID NO: 51, SEQ ID NO: 52, or a multimerizing variant or fragment thereof.

[0028] In certain embodimentexamples, each IgM heavy chain constant region is a variant of a human IgM constant region or multimerizing fragment thereof, which confers reduced CDC activity to the multimeric binding molecule relative to a multimeric binding molecule including IgM heavy chain constant regions including the amino acid sequence SEQ ID NO: 51, SEQ ID NO: 52. For example, each IgM heavy chain constant region can include a variant of the amino acid sequence SEQ ID NO: 51 or SEQ ID NO: 52, where the variant includes an amino acid substitution at position P311 of SEQ ID NO: 51 or SEQ ID NO: 52, an amino acid substitution at position P313 of SEQ ID NO: 51 or SEQ ID NO: 52, or

amino acid substitutions at positions P311 and P313 of SEQ ID NO: 51 or SEQ ID NO: 52.

[0029] In certain embodiment examples, each IgM heavy chain constant region is a variant of a human IgM constant region or multimerizing fragment thereof, which confers increased serum half-life to the multimeric binding molecule upon administration to a subject animal relative to a multimeric binding molecule including the reference IgM heavy chain constant regions, and is administered in the same way to the same animal species. For example, the variant IgM heavy chain constant regions can include amino acid substitutions at one or more amino acid positions corresponding to amino acid, E345A, S401A, E402A, or E403A of the wild-type human IgM constant region SEQ ID NO: 51 or SEQ ID NO: 52.

[0030] This disclosure also provides an isolated polynucleotide that includes a nucleic acid encoding a subunit polypeptide of the multimeric binding molecule provided by the disclosure, where the subunit polypeptide includes (a) an IgA or IgM heavy chain including an IgA or IgM heavy chain constant region or a multimerizing variant or fragment thereof associated with an antibody heavy chain variable region (VH), (b) an antibody light chain including an antibody light chain constant region associated with an antibody light chain variable region (VL), or (c) a modified J-chain including two or more of (i) a J-chain or functional fragment or variant thereof ("J"), (ii) an interleukin-15 (IL-15) protein or receptor-binding fragment or variant thereof ("I"), or (iii) an interleukin-15 receptor- α (IL-15R α) fragment including the sushi domain or a variant thereof capable of associating with I ("R"), where J and at least one of I and R are associated as a fusion protein, and where I and R can associate to function as an immune stimulatory complex, or (d) any combination thereof.

[0031] In another aspect, the present disclosure provides a composition comprising a first polynucleotide comprising a first nucleic acid encoding a first subunit polypeptide of the multimeric binding molecule as described herein, a second polynucleotide comprising a second nucleic acid encoding a second subunit polypeptide of the multimeric binding molecule as described herein, and a third polynucleotide comprising a third nucleic acid encoding a modified J-chain, wherein the first subunit polypeptide comprises an IgA or IgM heavy chain comprising an IgA or IgM heavy chain constant region or a multimerizing variant or fragment thereof associated with an antibody heavy chain

variable region (VH), wherein the second subunit polypeptide comprises an antibody light chain comprising an antibody light chain constant region associated with an antibody light chain variable region (VL), wherein the modified J-chain comprising two or more of (i) a J-chain or functional fragment or variant thereof ("J"), (ii) an interleukin-15 (IL-15) protein or receptor-binding fragment or variant thereof ("I"), or (iii) an interleukin-15 receptor- α (IL-15R α) fragment comprising the sushi domain or a variant thereof capable of associating with I ("R"), wherein J and at least one of I and R are associated as a fusion protein, and wherein I and R can associate to function as an immune stimulatory complex. In yet another aspect, the present disclosure provides an expression vector comprising a first polynucleotide comprising a first nucleic acid encoding a first subunit polypeptide of the multimeric binding molecule as described herein, a second polynucleotide comprising a second nucleic acid encoding a second subunit polypeptide of the multimeric binding molecule as described herein, and a third polynucleotide comprising a third nucleic acid encoding a modified J-chain, wherein the first subunit polypeptide comprises an IgA or IgM heavy chain comprising an IgA or IgM heavy chain constant region or a multimerizing variant or fragment thereof associated with an antibody heavy chain variable region (VH), wherein the second subunit polypeptide comprises an antibody light chain comprising an antibody light chain constant region associated with an antibody light chain variable region (VL), wherein the modified J-chain comprising two or more of (i) a J-chain or functional fragment or variant thereof ("J"), (ii) an interleukin-15 (IL-15) protein or receptor-binding fragment or variant thereof ("I"), or (iii) an interleukin-15 receptor- α (IL-15R α) fragment comprising the sushi domain or a variant thereof capable of associating with I ("R"), wherein J and at least one of I and R are associated as a fusion protein, and wherein I and R can associate to function as an immune stimulatory complex. In a further aspect, the present disclosure provides a host cell comprising the composition as described herein or the expression vector as described herein. Also provided is an expression vector that includes the polynucleotide and a host cell that includes the polynucleotide and/or the expression vector. In a further aspect, the disclosure also provides a method for producing the provided multimeric binding molecule that includes culturing the host cell and recovering the multimeric binding molecule.

[0032] In another aspect, the present disclosure provides a use of the multimeric binding molecule as described herein in the manufacture of a medicament for treating cancer. This

disclosure further provides a method for treating cancer, that includes administering to a subject in need of treatment an effective amount of the provided multimeric binding molecule.

WHAT IS CLAIMED IS:

1. A multimeric binding molecule comprising two or five bivalent binding units or multimerizing variants or fragments thereof and a modified J-chain, wherein each binding unit comprises two IgA or IgM heavy chain constant regions or multimerizing variants or fragments thereof, each associated with an antigen-binding domain for a total of four or ten antigen-binding domains,

wherein at least three of the antigen-binding domains of the binding molecule specifically bind to a target antigen, and

wherein the modified J-chain comprises (a) a J-chain or functional fragment or variant thereof ("J"), and (b) an immunostimulatory agent ("ISA"), wherein J and the ISA are associated as a fusion protein.

~~2. The multimeric binding molecule of claim 1, wherein the ISA comprises a cytokine or receptor-binding fragment or variant thereof;~~

~~3.2. The multimeric binding molecule of claim 2, and~~ wherein the cytokine or fragment or variant thereof comprises IL-15 or IL-2, or a receptor-binding fragment or variant thereof.

~~4.3. The multimeric binding molecule of any one of claims 1 to 3, 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 J 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.

~~4. The multimeric binding molecule of any one of claims 1 to 3, wherein:~~

~~5(a) J is a wild-type human J-chain and comprises the amino acid sequence SEQ ID NO: 2 or a functional fragment or variant thereof;~~

~~6. The multimeric binding molecule of any one of claims 1 to 5, wherein J is a variant J chain or fragment thereof comprising one or more single amino acid substitutions, deletions, or insertions relative to a wild-type J chain that can affect serum half-life of the multimeric binding molecule; and wherein the multimeric binding molecule exhibits an increased serum half-life upon administration to an animal relative to a reference multimeric 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.~~

~~7.(b) The multimeric binding molecule of claim 6, wherein the~~ J comprises an amino acid substitution at the amino acid position corresponding to amino acid Y102 of the mature wild-type human J-chain (SEQ ID NO: 2), wherein the amino acid corresponding to Y102 of SEQ ID NO: 2 is substituted with alanine (A);
or

~~8. The multimeric binding molecule of claim 7, wherein the amino acid corresponding to Y102 of SEQ ID NO: 2 is substituted with alanine (A), serine (S), or arginine (R).~~

~~9. The multimeric binding molecule of claim 8, wherein the amino acid corresponding to Y102 of SEQ ID NO: 2 is substituted with alanine (A).~~

~~10.(c) The multimeric binding molecule of claim 9, wherein~~ J is a variant human J-chain and comprises the amino acid sequence SEQ ID NO: 3 ("J*") or amino acids 1-137 of SEQ ID NO: 86.

~~11. The multimeric binding molecule of any one of claims 1 to 10, wherein J is a variant J chain or fragment thereof comprising one or more single amino acid substitutions, deletions, or insertions relative to a wild-type J chain that reduces glycosylation of the J-chain.~~

~~5. The multimeric binding molecule of claim 11, wherein the~~ J3 or claim 4, wherein:

~~12.(a) I comprises an amino acid substitution at the amino acid position corresponding to amino acid N49 of the mature wild-type human J-chain (IL-15 amino acid sequence of SEQ ID NO: 2).~~ 4 or a receptor-binding variant or fragment thereof;
or

~~13. The multimeric binding molecule of claim 12, wherein the amino acid corresponding to N49 of SEQ ID NO: 2 is substituted with aspartic acid (D).~~

~~14. The multimeric binding molecule of any one of claims 4 to 13, wherein I comprises the mature human IL-15 amino acid sequence of SEQ ID NO: 4 or a receptor-binding variant or fragment thereof.~~

~~15.(b) The multimeric binding molecule of claim 14,~~ wherein the receptor-binding variant comprises at least one, but no more than ten, single amino acid insertions, deletions, or substitutions, and wherein the single amino acid insertions, deletions, or substitutions reduce the affinity of the IL-15 variant for its receptor or reduce the glycosylation of the IL-15 variant.

~~16.6.~~ The multimeric binding molecule of claim ~~155~~, wherein ~~the single amino acid insertions, deletions, or substitutions reduce the affinity of the IL-15 variant for its receptor, wherein I and~~ comprises one, two, three, four, five, six, seven, or eight amino acid substitutions-, and wherein:

~~17.(a) The multimeric binding molecule of claim 16, wherein~~ the amino acid substitutions are at one or more of positions corresponding to N1, N4, D8, D30, D61, E64, N65, N72, or Q108 of SEQ ID NO: 4-;

~~18.(b) The multimeric binding molecule of claim 17, wherein~~ the amino acid substitutions comprise one or more of substitutions N1D, N4D, D8N, D30N, D61N, E64Q, N65D, N72D, or Q108E in SEQ ID NO: 4-;

~~19. The multimeric binding molecule of claim 18, wherein~~ I comprises SEQ ID NO: 4 except for:

~~(a)(c)~~ a single amino acid substitution at a position selected from the group consisting of N1D, N4D, D8N, D30N, D61N, E64Q, N65D, N72D, and Q108E;

~~(b)(d)~~ I comprises SEQ ID NO: 4 except for two amino acid substitutions at positions selected from the group consisting of N4D/N65D and N1D/N65D;-or

~~(c)(e)~~ I comprises SEQ ID NO: 4 except for three amino acid substitutions at positions D30N/E64Q/N65D-; or

~~20.(f) The multimeric binding molecule of claim 19, wherein~~ I comprises the amino acid sequence SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, or SEQ ID NO: 68.

~~21.7.~~ The multimeric binding molecule of claim ~~14, wherein the receptor-binding variant comprises at least one, but no more than ten, single amino acid insertions, deletions, or substitutions, and~~5, wherein the single amino acid insertions, deletions, or substitutions reduce the glycosylation of the IL-15 variant-, and wherein:

~~22. The multimeric binding molecule of claim 21, wherein~~ I comprises one, two, three, four, five, six, seven, or eight amino acid substitutions-

~~23.(a) The multimeric binding molecule of claim 22,~~ wherein the amino acid substitutions are at one or more of positions corresponding to N71, S73, N79, or N112 of SEQ ID NO: 4;~~;~~

~~24.(b) The multimeric binding molecule of claim 23,~~I comprises one, two, three, four, five, six, seven, or eight amino acid substitutions, wherein the amino acid substitutions comprise one or more of substitutions N71D, S73I, N79D, or N112D in SEQ ID NO: 4;~~;~~

~~25.(c) The multimeric binding molecule of claim 24,~~I comprises one, two, three, four, five, six, seven, or eight amino acid substitutions, wherein I comprises SEQ ID NO: 4 except for one or more amino acid substitutions at a position selected from the group consisting of N71D, S73I, N79D, and N112D~~;~~; or

~~26.(d) The multimeric binding molecule of claim 25, wherein~~ I comprises the amino acid sequence of amino acids 246-361 of SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, or SEQ ID NO: 90.

~~27.~~—The multimeric binding molecule of any one of claims ~~43 to 26~~ to 7, wherein R comprises the amino acid sequence SEQ ID NO: 5 or a variant or fragment thereof that is capable of associating with human IL-15~~;~~

~~28.8.~~ The multimeric binding molecule of any one of claims 4 to 20, wherein or R consists essentially of or consists of the amino acid sequence SEQ ID NO: 5 or a variant thereof that is capable of associating with human IL-15.

~~29.~~—The multimeric binding molecule of any one of claims ~~43~~ to 288, wherein J and I ~~are associated as a fusion protein.~~

~~30.~~—~~The multimeric binding molecule of any one of claims 4 to 28, wherein;~~ J and R ~~are associated as a fusion protein.~~

~~31.9.~~ The multimeric binding molecule of any one of claims 4 to 30, wherein J, I, and R are ; or J, I, and R are associated as a fusion protein.

~~32.~~—The multimeric binding molecule of claim ~~319~~, wherein J, I, and R are fused via linkers~~;~~

~~33.~~—~~The multimeric binding molecule of claim 32, wherein the~~ or wherein J, I, and R are fused via linkers ~~are the same or different.~~

~~34.10.~~ ~~The multimeric binding molecule of claim 32 or claim 33, wherein~~ and at least one linker comprises, consists essentially of, or consists of the amino acid sequence GGGGSGGGSGGGGS (SEQ ID NO: 78) or GGS GGGGSGGGSGGGGSLQ (SEQ ID NO: 79).

~~35.—The multimeric binding molecule of claim 32 or claim 33, wherein at least one linker comprises, consists essentially of, or consists of the amino acid sequence GGS GGGGSGGGSGGGGSLQ (SEQ ID NO: 79).~~

~~36.11.~~ The multimeric binding molecule of ~~any one of claims 6-9 to or 35~~ claim 10, wherein J is J*, and wherein the modified J-chain is arranged from N-terminus to C-terminus as J*-R-I, J*-I-R, I-R-J*, R-I-J*, R-J*-I, I-J*-R, I-J*, or J*-I, wherein “-” is a linker.

~~37.12.~~ The multimeric binding molecule of claim ~~36~~ 11, wherein the modified J-chain comprises the amino acid sequence SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 77, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, or SEQ ID NO: 90.

~~38.—The multimeric binding molecule of claim 36, wherein the modified J-chain is arranged from N-terminus to C-terminus as J*-R-I.~~

~~39.—The multimeric binding molecule of claim 38, wherein the modified J-chain comprises the amino acid sequence SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 77, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, or SEQ ID NO: 90.~~

~~40.—The multimeric binding molecule of any one of claims 1 to 3, wherein the ISA comprises a variant of human IL-2 (“IL2v”) that does not bind to the α -subunit of the IL-2 receptor.~~

~~41.—The multimeric binding molecule of claim 40, wherein IL2v comprises the amino acid sequence SEQ ID NO: 31.~~

~~42.—The multimeric binding molecule of claim 41, wherein the modified J-chain comprises the amino acid sequence SEQ ID NO: 32.~~

~~43.—The multimeric binding molecule of any one of claims 4 to 42, wherein the modified J-chain further comprises an antigen-binding domain of an antibody fused thereto.~~

~~44. The multimeric binding molecule of claim 43, wherein the antigen-binding domain binds to a target on an immune effector cell.~~

~~45. The multimeric binding molecule of claim 44, wherein the immune effector cell is a CD8⁺ T cell.~~

~~46. The multimeric binding molecule of claim 45, wherein the antigen-binding domain is a single-chain Fv (scFv) antibody fragment that specifically binds to CD3epsilon (CD3ε).~~

~~47. The multimeric binding molecule of claim 46, wherein the modified J-chain comprises SEQ ID NO: 19.~~

~~48.13.~~ The multimeric binding molecule of any one of claims 1 to ~~47~~12, which is pentameric and comprises five binding units, wherein each binding unit comprises two IgM heavy chain constant regions or multimerizing variants or fragments thereof.

14. The multimeric binding molecule of claim 13, wherein each IgM heavy chain constant region comprises an IgM Cμ4 domain and an IgM tailpiece domain or comprises an IgM Cμ4 domain and an IgM tailpiece domain and further comprises an IgM Cμ3 domain, an IgM Cμ2 domain, an IgM Cμ1 domain, or any combination thereof, situated N-terminal to the IgM Cμ4 and IgM tailpiece domains.

15. The multimeric binding molecule of claim 14, wherein each IgM heavy chain constant region is

(a) a human IgM constant region comprising the amino acid sequence SEQ ID NO: 51 or SEQ ID NO: 52;

(a)(b) a variant human IgM constant region, wherein the multimeric binding molecule has reduced CDC activity relative to a multimeric binding molecule comprising IgM heavy chain constant regions comprising the amino acid sequence SEQ ID NO: 51, SEQ ID NO: 52;

(c) a variant human IgM constant region with one or more single amino acid substitutions, deletions, or insertions relative to a reference IgM heavy chain constant region identical to the variant IgM heavy chain constant regions except for the one or more single amino acid substitutions, deletions, or insertions, and wherein the multimeric binding molecule exhibits increased serum half-life upon administration to a subject animal relative to a multimeric binding

molecule comprising the reference IgM heavy chain constant regions, and is administered in the same way to the same animal species.

16. The multimeric binding molecule of claim 15, wherein the multimeric binding molecule has reduced CDC activity relative to a multimeric binding molecule comprising IgM heavy chain constant regions comprising the amino acid sequence SEQ ID NO: 51, SEQ ID NO: 52, and wherein the variant human IgM constant region comprises an amino acid substitution at position K315 of SEQ ID NO: 51 or SEQ ID NO: 52, at position P311 of SEQ ID NO: 51 or SEQ ID NO: 52, an amino acid substitution at position P313 of SEQ ID NO: 51 or SEQ ID NO: 52, or amino acid substitutions at positions P311 and P313 of SEQ ID NO: 51 or SEQ ID NO: 52.

~~49. The multimeric binding molecule of any one of claims 1 to 47, which is dimeric and comprises two binding units or multimerizing variants or fragments thereof, wherein each binding unit comprises two IgA heavy chain constant regions or multimerizing variants or fragments thereof.~~

17. The multimeric binding molecule of any one of claims 1 to 49, wherein the target antigen comprises:

(a) a tumor-associated antigen;

~~50.~~ (b) a target that modulates a T cell response or NK cell response.

~~51. The multimeric binding molecule of claim 50, wherein the target antigen comprises a target that modulates a T cell response or an NK cell response.~~

~~52. The multimeric binding molecule of claim 51, wherein the target inhibits CD8+ T cell or NK cell activity.~~

~~53.~~ (c) The multimeric binding molecule of claim 52, wherein the target comprises an inhibitory immune checkpoint protein, and wherein the antigen-binding domains antagonize the target, thereby stimulating CD8+ T cells or NK cells;
or

~~54. The multimeric binding molecule of claim 53, wherein the inhibitory immune checkpoint protein comprises~~ a programmed cell death-1 protein (PD-1), a programmed cell death ligand-1 protein (PD-L1), a lymphocyte-activation gene 3 protein (LAG3), a T-cell immunoglobulin and mucin domain 3 protein (TIM3), a cytotoxic T-lymphocyte-associated protein 4 (CTLA4), a B- and T-lymphocyte attenuator protein (BTLA), a V-domain Ig

suppressor of T-cell activation protein (VISTA), a T-cell immunoreceptor with Ig and ITIM Domains protein (TIGIT), a Killer-cell Immunoglobulin-like Receptor protein (KIR), a B7-H3 protein, a B7-H4 protein, ~~or any combination thereof.~~

~~55.—The multimeric binding molecule of claim 54, wherein the inhibitory immune checkpoint protein comprises PD-L1, and wherein the antigen-binding domain comprises a heavy chain variable region (VH) comprising the amino acid sequence SEQ ID NO: 33, SEQ ID NO: 91, SEQ ID NO: 92, or SEQ ID NO: 93 and a light chain variable region (VL) comprising the amino acid sequence SEQ ID NO: 34 or SEQ ID NO: 94.~~

~~56.—The multimeric binding molecule of claim 54, wherein the inhibitory immune checkpoint protein comprises PD-L1, and wherein the antigen-binding domain comprises a heavy chain variable region (VH) and a light chain variable region (VL) wherein the VH and VL comprise six immunoglobulin complementarity determining regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the CDRs of an antibody comprising the VH and VL of SEQ ID NO: 75 and SEQ ID NO: 76, SEQ ID NO: 96 and SEQ ID NO: 97, SEQ ID NO: 98 and SEQ ID NO: 99, SEQ ID NO: 100 and SEQ ID NO: 101, SEQ ID NO: 102 and SEQ ID NO: 103, SEQ ID NO: 104 and SEQ ID NO: 105, SEQ ID NO: 106 and SEQ ID NO: 107, SEQ ID NO: 108 and SEQ ID NO: 109, SEQ ID NO: 110 and SEQ ID NO: 111, SEQ ID NO: 112 and SEQ ID NO: 113, SEQ ID NO: 114 and SEQ ID NO: 115, SEQ ID NO: 116 and SEQ ID NO: 117, SEQ ID NO: 118 and SEQ ID NO: 119, SEQ ID NO: 120 and SEQ ID NO: 121, SEQ ID NO: 122 and SEQ ID NO: 123, SEQ ID NO: 124 and SEQ ID NO: 125, SEQ ID NO: 126 and SEQ ID NO: 127, SEQ ID NO: 128 and SEQ ID NO: 129, SEQ ID NO: 130 and SEQ ID NO: 131, SEQ ID NO: 132 and SEQ ID NO: 133, SEQ ID NO: 134 and SEQ ID NO: 135, SEQ ID NO: 136 and SEQ ID NO: 137, SEQ ID NO: 138 and SEQ ID NO: 139, SEQ ID NO: 140 and SEQ ID NO: 141, SEQ ID NO: 142 and SEQ ID NO: 143, SEQ ID NO: 144 and SEQ ID NO: 145, SEQ ID NO: 146 and SEQ ID NO: 147, SEQ ID NO: 148 and SEQ ID NO: 149, SEQ ID NO: 150 and SEQ ID NO: 151, SEQ ID NO: 152 and SEQ ID NO: 153, SEQ ID NO: 154 and SEQ ID NO: 155, SEQ ID NO: 156 and SEQ ID NO: 157, SEQ ID NO: 158 and SEQ ID NO: 159, SEQ ID NO: 160 and SEQ ID NO: 161, SEQ ID NO: 162 and SEQ ID NO: 163, SEQ ID NO: 164 and SEQ ID NO: 165, SEQ ID NO: 166 and SEQ ID NO: 167, SEQ ID NO: 168 and SEQ ID NO: 169, SEQ ID NO: 170 and SEQ ID NO: 171, SEQ ID NO: 172 and SEQ ID NO: 173, SEQ ID NO: 174 and SEQ ID NO: 175, SEQ ID NO: 176 and SEQ ID NO: 177, SEQ ID NO: 178 and SEQ ID NO: 179, SEQ ID NO: 180 and SEQ ID NO: 181, SEQ ID NO: 182 and SEQ ID NO: 183, SEQ ID NO: 184~~

~~and SEQ ID NO: 185, SEQ ID NO: 186 and SEQ ID NO: 187, SEQ ID NO: 188 and SEQ ID NO: 189, SEQ ID NO: 190 and SEQ ID NO: 191, SEQ ID NO: 192 and SEQ ID NO: 193, SEQ ID NO: 194 and SEQ ID NO: 195, SEQ ID NO: 196 and SEQ ID NO: 197, SEQ ID NO: 198 and SEQ ID NO: 199, SEQ ID NO: 200 and SEQ ID NO: 201, SEQ ID NO: 202 and SEQ ID NO: 203, SEQ ID NO: 204 and SEQ ID NO: 205, SEQ ID NO: 206 and SEQ ID NO: 207, SEQ ID NO: 208 and SEQ ID NO: 209, SEQ ID NO: 210 and SEQ ID NO: 211, SEQ ID NO: 212 and SEQ ID NO: 213, SEQ ID NO: 214 and SEQ ID NO: 215, SEQ ID NO: 216 and SEQ ID NO: 217, SEQ ID NO: 218 and SEQ ID NO: 219, SEQ ID NO: 220 and SEQ ID NO: 221, or SEQ ID NO: 222 and SEQ ID NO: 223, respectively with zero, one, or two single amino acid substitutions in one or more of the HCDRs or LCDRs.~~

~~57. The multimeric binding molecule of claim 54 or claim 56, wherein the inhibitory immune checkpoint protein comprises PD-L1, and wherein the antigen-binding domain comprises a heavy chain variable region (VH) and a light chain variable region (VL) wherein the VH and VL comprise amino acid sequences at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to the mature VH and VL amino acid sequences comprising SEQ ID NO: 75 and SEQ ID NO: 76, SEQ ID NO: 96 and SEQ ID NO: 97, SEQ ID NO: 98 and SEQ ID NO: 99, SEQ ID NO: 100 and SEQ ID NO: 101, SEQ ID NO: 102 and SEQ ID NO: 103, SEQ ID NO: 104 and SEQ ID NO: 105, SEQ ID NO: 106 and SEQ ID NO: 107, SEQ ID NO: 108 and SEQ ID NO: 109, SEQ ID NO: 110 and SEQ ID NO: 111, SEQ ID NO: 112 and SEQ ID NO: 113, SEQ ID NO: 114 and SEQ ID NO: 115, SEQ ID NO: 116 and SEQ ID NO: 117, SEQ ID NO: 118 and SEQ ID NO: 119, SEQ ID NO: 120 and SEQ ID NO: 121, SEQ ID NO: 122 and SEQ ID NO: 123, SEQ ID NO: 124 and SEQ ID NO: 125, SEQ ID NO: 126 and SEQ ID NO: 127, SEQ ID NO: 128 and SEQ ID NO: 129, SEQ ID NO: 130 and SEQ ID NO: 131, SEQ ID NO: 132 and SEQ ID NO: 133, SEQ ID NO: 134 and SEQ ID NO: 135, SEQ ID NO: 136 and SEQ ID NO: 137, SEQ ID NO: 138 and SEQ ID NO: 139, SEQ ID NO: 140 and SEQ ID NO: 141, SEQ ID NO: 142 and SEQ ID NO: 143, SEQ ID NO: 144 and SEQ ID NO: 145, SEQ ID NO: 146 and SEQ ID NO: 147, SEQ ID NO: 148 and SEQ ID NO: 149, SEQ ID NO: 150 and SEQ ID NO: 151, SEQ ID NO: 152 and SEQ ID NO: 153, SEQ ID NO: 154 and SEQ ID NO: 155, SEQ ID NO: 156 and SEQ ID NO: 157, SEQ ID NO: 158 and SEQ ID NO: 159, SEQ ID NO: 160 and SEQ ID NO: 161, SEQ ID NO: 162 and SEQ ID NO: 163, SEQ ID NO: 164 and SEQ ID NO: 165, SEQ ID NO: 166 and SEQ ID NO: 167, SEQ ID NO: 168 and SEQ ID NO: 169, SEQ ID NO: 170 and SEQ ID NO: 171, SEQ ID NO: 172 and SEQ ID NO: 173, SEQ ID NO: 174 and SEQ ID NO: 175, SEQ ID NO: 176 and SEQ ID NO: 177, SEQ ID NO: 178 and SEQ ID~~

~~NO: 179, SEQ ID NO: 180 and SEQ ID NO: 181, SEQ ID NO: 182 and SEQ ID NO: 183, SEQ ID NO: 184 and SEQ ID NO: 185, SEQ ID NO: 186 and SEQ ID NO: 187, SEQ ID NO: 188 and SEQ ID NO: 189, SEQ ID NO: 190 and SEQ ID NO: 191, SEQ ID NO: 192 and SEQ ID NO: 193, SEQ ID NO: 194 and SEQ ID NO: 195, SEQ ID NO: 196 and SEQ ID NO: 197, SEQ ID NO: 198 and SEQ ID NO: 199, SEQ ID NO: 200 and SEQ ID NO: 201, SEQ ID NO: 202 and SEQ ID NO: 203, SEQ ID NO: 204 and SEQ ID NO: 205, SEQ ID NO: 206 and SEQ ID NO: 207, SEQ ID NO: 208 and SEQ ID NO: 209, SEQ ID NO: 210 and SEQ ID NO: 211, SEQ ID NO: 212 and SEQ ID NO: 213, SEQ ID NO: 214 and SEQ ID NO: 215, SEQ ID NO: 216 and SEQ ID NO: 217, SEQ ID NO: 218 and SEQ ID NO: 219, SEQ ID NO: 220 and SEQ ID NO: 221, or SEQ ID NO: 222 and SEQ ID NO: 223, respectively.~~

~~58.—The multimeric binding molecule of claim 50 or claim 51, wherein the target comprises a TNF receptor superfamily target, and wherein the antigen-binding domains can agonize the target.~~

~~59.—The multimeric binding molecule of claim 58, wherein the target antigen comprises GITR, OX40, or a combination thereof, and wherein the antigen-binding domains can agonize the target.~~

~~60.—The multimeric binding molecule of claim 59, wherein the target antigen comprises GITR, and wherein the antigen-binding domain comprises a heavy chain variable region (VH) and a light chain variable region (VL) comprising, respectively, the amino acid sequences SEQ ID NO: 35 and SEQ ID NO: 36, SEQ ID NO: 37 and SEQ ID NO: 38, SEQ ID NO: 39 and SEQ ID NO: 40, SEQ ID NO: 41 and SEQ ID NO: 42, or SEQ ID NO: 43 and SEQ ID NO: 44.~~

~~61.—The multimeric binding molecule of claim 59, wherein the target antigen comprises OX40, and wherein the antigen-binding domain comprises a heavy chain variable region (VH) and a light chain variable region (VL) comprising, respectively, the amino acid sequences SEQ ID NO: 45 and SEQ ID NO: 46 or SEQ ID NO: 47 and SEQ ID NO: 48.~~

~~62.—The multimeric binding molecule of any one of claims 1 to 49, wherein the target antigen comprises a tumor-associated antigen.~~

~~63.(d) The multimeric binding molecule of claim 62, wherein the tumor-associated antigen comprises~~GITR, OX40, 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, or any combination thereof.

~~64. The multimeric binding molecule of claim 63, wherein the target antigen comprises CD20, and wherein the antigen-binding domain comprises a heavy chain variable region (VH) and a light chain variable region (VL) comprising, respectively, the amino acid sequences SEQ ID NO: 49 and SEQ ID NO: 50.~~

18. The multimeric binding molecule of claim 17, wherein the target antigen comprises PD-L1, and wherein the antigen-binding domain comprises a heavy chain variable region (VH) comprising the amino acid sequence SEQ ID NO: 33, SEQ ID NO: 91, SEQ ID NO: 92, or SEQ ID NO: 93 and a light chain variable region (VL) comprising the amino acid sequence SEQ ID NO: 34 or SEQ ID NO: 94.

~~65.~~19. The multimeric binding molecule of any one of claims 1 to ~~64~~18, wherein at least four, at least five, at least six, at least seven, at least eight, at least nine or ten of the antigen-binding domains of the binding molecule specifically bind to the same target antigen.

~~66. The multimeric binding molecule of any one of claims 1 to 47 or 49 to 65, wherein each binding unit comprises two IgA heavy chain constant regions or multimerizing fragments or variants thereof, each comprising an IgA C α 1 domain, an IgA hinge, an IgA C α 2 domain, IgA C α 3 domain, and an IgA tailpiece domain.~~

~~67. The multimeric binding molecule of claim 66, wherein the IgA heavy chain constant regions comprise the amino acid sequence SEQ ID NO: 53, SEQ ID NO: 54, or any multimerizing variant or fragment thereof.~~

~~68. The multimeric binding molecule of any one of claims 1 to 46 or 48 to 65, wherein each binding unit comprises two IgM heavy chain constant regions or multimerizing fragments or variants thereof, each comprising an IgM C μ 4 domain and an IgM tailpiece domain.~~

~~69. The multimeric binding molecule of claim 68, wherein each IgM heavy chain constant region or multimerizing fragment or variant thereof further comprises an IgM C μ 3~~

domain, an IgM C μ 2 domain, an IgM C μ 1 domain, or any combination thereof, situated N-terminal to the IgM C μ 4 and IgM tailpiece domains.

70. — The multimeric binding molecule of claim 68 or claim 69, wherein each IgM heavy chain constant region is a human IgM constant region or multimerizing variant or fragment thereof, comprising the amino acid sequence SEQ ID NO: 51, SEQ ID NO: 52, or a multimerizing variant or fragment thereof.

71. — The multimeric binding molecule of claim 69 or 70, comprising a variant human IgM constant region, wherein the multimeric binding molecule has reduced CDC activity relative to a multimeric binding molecule comprising IgM heavy chain constant regions comprising the amino acid sequence SEQ ID NO: 51, SEQ ID NO: 52, or a multimerizing variant or fragment thereof.

72. — The multimeric binding molecule of claim 71, wherein each IgM heavy chain constant region comprises a variant of the amino acid sequence SEQ ID NO: 51 or SEQ ID NO: 52, wherein the variant comprises an amino acid substitution at position P311 of SEQ ID NO: 51 or SEQ ID NO: 52, an amino acid substitution at position P313 of SEQ ID NO: 51 or SEQ ID NO: 52, or amino acid substitutions at positions P311 and P313 of SEQ ID NO: 51 or SEQ ID NO: 52.

73. — The multimeric binding molecule of claim 69 or 70, wherein each IgM heavy chain constant region is a variant human IgM constant region with one or more single amino acid substitutions, deletions, or insertions relative to a reference IgM heavy chain constant region identical to the variant IgM heavy chain constant regions except for the one or more single amino acid substitutions, deletions, or insertions, and wherein the multimeric binding molecule exhibits increased serum half-life upon administration to a subject animal relative to a multimeric binding molecule comprising the reference IgM heavy chain constant regions, and is administered in the same way to the same animal species.

74. — The multimeric binding molecule of claim 73, wherein the variant IgM heavy chain constant regions comprise amino acid substitutions at one or more amino acid positions corresponding to amino acid, E345A, S401A, E402A, or E403A of the wild-type human IgM constant region SEQ ID NO: 51 or SEQ ID NO: 52.

20. A composition comprising a first polynucleotide comprising a first nucleic acid encoding a first subunit polypeptide of the multimeric binding molecule of any one of claims 1 to 19, a second polynucleotide comprising a second nucleic acid encoding a second subunit polypeptide of the multimeric binding molecule of any one of claims 1 to 19, and a third polynucleotide comprising a third nucleic acid encoding a modified

J-chain, wherein the first subunit polypeptide comprises an IgA or IgM heavy chain comprising an IgA or IgM heavy chain constant region or a multimerizing variant or fragment thereof associated with an antibody heavy chain variable region (VH), wherein the second subunit polypeptide comprises an antibody light chain comprising an antibody light chain constant region associated with an antibody light chain variable region (VL), wherein the modified J-chain comprising two or more of (i) a J-chain or functional fragment or variant thereof ("J"), (ii) an interleukin-15 (IL-15) protein or receptor-binding fragment or variant thereof ("I"), or (iii) an interleukin-15 receptor- α (IL-15R α) fragment comprising the sushi domain or a variant thereof capable of associating with I ("R"), wherein J and at least one of I and R are associated as a fusion protein, and wherein I and R can associate to function as an immune stimulatory complex.

~~75.21. An isolated polynucleotide comprising a nucleic acid encoding a subunit polypeptide of the multimeric binding molecule of any one of claims 1 to 74, wherein the subunit polypeptide comprises (a)~~An expression vector comprising a first polynucleotide comprising a first nucleic acid encoding a first subunit polypeptide of the multimeric binding molecule of any one of claims 1 to 19, a second polynucleotide comprising a second nucleic acid encoding a second subunit polypeptide of the multimeric binding molecule of any one of claims 1 to 19, and a third polynucleotide comprising a third nucleic acid encoding a modified J-chain, wherein the first subunit polypeptide comprises an IgA or IgM heavy chain comprising an IgA or IgM heavy chain constant region or a multimerizing variant or fragment thereof associated with an antibody heavy chain variable region (VH), ~~(b)~~wherein the second subunit polypeptide comprises an antibody light chain comprising an antibody light chain constant region associated with an antibody light chain variable region (VL), ~~or (c)~~wherein the modified J-chain comprising two or more of (i) a J-chain or functional fragment or variant thereof ("J"), (ii) an interleukin-15 (IL-15) protein or receptor-binding fragment or variant thereof ("I"), or (iii) an interleukin-15 receptor- α (IL-15R α) fragment comprising the sushi domain or a variant thereof capable of associating with I ("R"), wherein J and at least one of I and R are associated as a fusion protein, and wherein I and R can associate to function as an immune stimulatory complex,~~or (d) any combination thereof.~~

~~76.—The polynucleotide of claim 75, wherein the subunit polypeptide comprises an IgM heavy chain comprising an IgM constant region or multimerizing fragment or variant thereof.~~

~~77.—The polynucleotide of claim 76, wherein the IgM constant region or fragment or variant thereof comprises the amino acid sequence SEQ ID NO: 51 or SEQ ID NO: 52.~~

~~78.—The polynucleotide of claim 75, wherein the subunit polypeptide comprises the antibody light chain.~~

~~79.—The polynucleotide of claim 75, wherein the subunit polypeptide comprises the modified J chain.~~

~~80.—The polynucleotide of claim 79, wherein the subunit comprises the amino acid sequence SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, or SEQ ID NO: 32.~~

~~81.—The polynucleotide of any one of claims 75 to 80, which comprises two, three, or more nucleic acid sequences encoding two, three or more of the subunit polypeptides.~~

~~82.—An expression vector comprising the polynucleotide of any one of claims 75 to 81.~~

~~83.22.~~ A host cell comprising the polynucleotide composition of ~~any of claims~~claim 20 1 to ~~81~~ or the expression vector of claim ~~82~~21.

~~84.23.~~ A method for producing the multimeric binding molecule of any one of claims 1 to ~~74~~19, comprising culturing the host cell of claim ~~22~~83, and recovering the multimeric binding molecule.

~~85.24.~~ A method for treating cancer, comprising administering to a subject in need of ~~treatment~~Use of the multimeric binding molecule of any one of claims 1 to ~~74~~19 in the manufacture of a medicament for treating cancer.