

have been shown to downregulate T-cell activation and cytokine secretion upon binding to PD-1 (Freeman et al. (2000) J Exp Med 192:1027-34; Latchman et al. (2001) Nat Immunol 2:261-8; Carter et al. (2002) Eur J Immunol 32:634-43; Ohigashi et al. (2005) Clin Cancer Res 11:2947-53). The PD-1 pathway has been implicated in a number of autoimmune diseases (Francisco et al., (2010) Immunol Rev 236: 219-42).

[0005] There remains a need for therapeutics to treat autoimmune diseases, such as autoimmune diseases resulting from disruption of the PD-1 pathway.

SUMMARY

[0006] Provided herein is a multimeric binding molecule comprising two, five, or six bivalent binding units or variants or fragments thereof, where each binding unit comprises two IgA or IgM heavy chain constant regions or multimerizing fragments or variants thereof, each associated with a binding domain, where three to twelve of the binding domains are programmed cell death protein 1 (PD-1)-binding domains that specifically and agonistically bind to PD-1, where the binding molecule can activate PD-1-mediated signal transduction in a cell at a higher potency than an equivalent amount of a bivalent IgG antibody or fragment thereof comprising two of the same PD-1-binding domains, which also specifically binds to and agonizes PD-1. In some embodiment examples, the two, five, or six binding units are human, humanized, or chimeric immunoglobulin binding units. In one aspect, the present disclosure provides a multimeric binding molecule comprising five, six, or two bivalent binding units or variants or fragments thereof, wherein each binding unit comprises two IgM or IgA heavy chain constant regions or multimerizing fragments or variants thereof, each associated with a binding domain, wherein three to twelve of the binding domains are programmed cell death protein 1 (PD-1)-binding domains that specifically and agonistically bind to PD-1, wherein the binding molecule can activate PD-1-mediated signal transduction in a cell at a higher potency than an equivalent amount of a bivalent IgG antibody or fragment thereof comprising two of the same PD-1-binding domains, which also specifically binds to and agonizes PD-1.

[0007] In some embodiment examples, the three to twelve PD-1-binding domains comprise a heavy chain variable region (VH) and a light chain variable region (VL), where the VH and VL comprise six immunoglobulin complementarity determining regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, where the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the CDRs of an antibody comprising the VH and VL of

5 SEQ ID NO: 1 and SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4, SEQ ID NO: 5 and
SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10,
SEQ ID NO: 11 and SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14, SEQ ID NO:
25 and SEQ ID NO: 26, SEQ ID NO: 27 and SEQ ID NO: 28, SEQ ID NO: 29 and SEQ
ID NO: 30, SEQ ID NO: 31 and SEQ ID NO: 32, SEQ ID NO: 33 and SEQ ID NO: 34, or
SEQ ID NO: 49 and SEQ ID NO: 50, respectively, or the VH of any one of SEQ ID NO:
15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, or SEQ ID NO:
24 and the VL of any one of SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, or SEQ
ID NO: 22, or the CDRs of an antibody comprising the VH and VL of SEQ ID NO: 1 and

SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14, SEQ ID NO: 25 and SEQ ID NO: 26, SEQ ID NO: 27 and SEQ ID NO: 28, SEQ ID NO: 29 and SEQ ID NO: 30, SEQ ID NO: 31 and SEQ ID NO: 32, SEQ ID NO: 33 and SEQ ID NO: 34, or SEQ ID NO: 49 and SEQ ID NO: 50, respectively with one or two single amino acid substitutions in one or more of the HCDRs or LCDRs, or the VH of any one of SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, or SEQ ID NO: 24 and the VL of any one of SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, or SEQ ID NO: 22 with one or two single amino acid substitutions in one or more of the HCDRs or LCDRs.

[0008] In some embodiment examples, the three to twelve PD-1-binding domains comprise a heavy chain variable region (VH) and a light chain variable region (VL), where: (a) the VH and VL comprise six immunoglobulin complementarity determining regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, where the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the CDRs of an antibody comprising the VH and VL of SEQ ID NO: 1 and SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14, SEQ ID NO: 25 and SEQ ID NO: 26, SEQ ID NO: 27 and SEQ ID NO: 28, SEQ ID NO: 29 and SEQ ID NO: 30, SEQ ID NO: 31 and SEQ ID NO: 32, SEQ ID NO: 33 and SEQ ID NO: 34, or SEQ ID NO: 49 and SEQ ID NO: 50, respectively with zero, one, or two single amino acid substitutions in one or more of the HCDRs or LCDRs; (b) the VH and VL comprise six immunoglobulin complementarity determining regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, where the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the CDRs of an antibody comprising the VH of any one of SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, or SEQ ID NO: 24 and the VL of any one of SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, or SEQ ID NO: 22 with zero, one, or two single amino acid substitutions in one or more of the HCDRs or LCDRs; (c) the VH and VL comprise amino acid sequences at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 1 and SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14, SEQ ID NO: 25 and SEQ ID NO: 26,

SEQ ID NO: 27 and SEQ ID NO: 28, SEQ ID NO: 29 and SEQ ID NO: 30, SEQ ID NO: 31 and SEQ ID NO: 32, SEQ ID NO: 33 and SEQ ID NO: 34, or SEQ ID NO: 49 and SEQ ID NO: 50, respectively; or (d) the VH comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to of any one of SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, or SEQ ID NO: 24 and the VL comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to of any one of SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, or SEQ ID NO: 22.

[0009] In some embodimentexamples, the VH and VL comprise six immunoglobulin complementarity determining regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, where the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the CDRs of an antibody comprising the VH and VL of SEQ ID NO: 1 and SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4, SEQ ID NO: 13 and SEQ ID NO: 14, and SEQ ID NO: 25 and SEQ ID NO: 26, respectively, or the CDRs of an antibody comprising the VH and VL of SEQ ID NO: 1 and SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4, SEQ ID NO: 13 and SEQ ID NO: 14, and SEQ ID NO: 25 and SEQ ID NO: 26, respectively with one or two single amino acid substitutions in one or more of the HCDRs or LCDRs.

[0010] In some embodimentexamples, the three to twelve PD-1-binding domains of the binding molecule comprise an antibody VH and a VL, where the VH and VL comprise amino acid sequences at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 1 and SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14, SEQ ID NO: 25 and SEQ ID NO: 26, SEQ ID NO: 27 and SEQ ID NO: 28, SEQ ID NO: 29 and SEQ ID NO: 30, SEQ ID NO: 31 and SEQ ID NO: 32, SEQ ID NO: 33 and SEQ ID NO: 34, or SEQ ID NO: 49 and SEQ ID NO: 50, respectively, or the VH of any one of SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, or SEQ ID NO: 24 and the VL of any one of SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, or SEQ ID NO: 22. In some embodimentexamples, the three to twelve PD-1-binding domains of the binding molecule comprise an antibody VH and a VL, where the VH and VL comprise amino acid sequences at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 1 and SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4, SEQ ID NO: 13 and SEQ ID NO: 14, and SEQ ID NO: 25 and SEQ ID NO: 26, respectively.

[0011] In some embodimentexamples, the three to twelve PD-1-binding domains comprise antibody VH and VL regions comprising the amino acid sequences SEQ ID NO: 1 and SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14, SEQ ID NO: 25 and SEQ ID NO: 26, SEQ ID NO: 27 and SEQ ID NO: 28, SEQ ID NO: 29 and SEQ ID NO: 30, SEQ ID NO: 31 and SEQ ID NO: 32, SEQ ID NO: 33 and SEQ ID NO: 34, or SEQ ID NO: 49 and SEQ ID NO: 50, respectively, or the VH of any one of SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, or SEQ ID NO: 24 and the VL of any one of SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, or SEQ ID NO: 22. In some embodimentexamples, the three to twelve PD-1-binding domains comprise antibody VH and VL regions comprising the amino acid sequences SEQ ID NO: 1 and SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4, SEQ ID NO: 13 and SEQ ID NO: 14, or SEQ ID NO: 25 and SEQ ID NO: 26, respectively.

[0012] In some embodimentexamples, each binding unit comprises two heavy chains and two light chains, where the heavy chains and light chains comprise VH and VL amino acid sequences at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 1 and SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14, SEQ ID NO: 25 and SEQ ID NO: 26, SEQ ID NO: 27 and SEQ ID NO: 28, SEQ ID NO: 29 and SEQ ID NO: 30, SEQ ID NO: 31 and SEQ ID NO: 32, SEQ ID NO: 33 and SEQ ID NO: 34, or SEQ ID NO: 49 and SEQ ID NO: 50, respectively, or the VH of any one of SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, or SEQ ID NO: 24 and the VL of any one of SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, or SEQ ID NO: 22. In some embodimentexamples, each binding unit comprises two heavy chains and two light chains, where the heavy chains and light chains comprise VH and VL amino acid sequences at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 1 and SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4, SEQ ID NO: 13 and SEQ ID NO: 14, or SEQ ID NO: 25 and SEQ ID NO: 26, respectively.

[0013] In some embodimentexamples, the heavy chains and light chains comprise the VH and VL amino acid sequences SEQ ID NO: 1 and SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8, SEQ ID NO: 9

and SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14, SEQ ID NO: 25 and SEQ ID NO: 26, SEQ ID NO: 27 and SEQ ID NO: 28, SEQ ID NO: 29 and SEQ ID NO: 30, SEQ ID NO: 31 and SEQ ID NO: 32, SEQ ID NO: 33 and SEQ ID NO: 34, or SEQ ID NO: 49 and SEQ ID NO: 50, respectively, or the VH of any one of SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, or SEQ ID NO: 24 and the VL of any one of SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, or SEQ ID NO: 22. In some [embodimentexamples](#), the heavy chains and light chains comprise the VH and VL amino acid sequences SEQ ID NO: 1 and SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4, SEQ ID NO: 13 and SEQ ID NO: 14, or SEQ ID NO: 25 and SEQ ID NO: 26, respectively.

[0014] In some [embodimentexamples](#), the multimeric binding molecule is a dimeric binding molecule comprising two bivalent IgA or IgA-like binding units and a J chain or functional fragment or variant thereof, where each binding unit comprises two IgA heavy chain constant regions or multimerizing fragments or variants thereof, each comprising an IgA Cα3 domain and an IgA tailpiece domain. In some [embodimentexamples](#), each IgA heavy chain constant region or multimerizing fragment or variant thereof further comprises a Cα1 domain, a Cα2 domain, an IgA hinge region, or any combination thereof. In some [embodimentexamples](#), the IgA heavy chain constant regions or multimerizing fragments thereof are human IgA constant regions. In some [embodimentexamples](#), each binding unit comprises two IgA heavy chains each comprising a VH situated amino terminal to the IgA constant region or multimerizing fragment thereof, and two immunoglobulin light chains each comprising a VL situated amino terminal to an immunoglobulin light chain constant region.

[0015] In some [embodimentexamples](#), the multimeric binding molecule is a pentameric or a hexameric binding molecule comprising five or six bivalent IgM binding units, respectively, where each binding unit comprises two IgM heavy chain constant regions or multimerizing fragments thereof each associated with a PD-1-binding domain, where each IgM heavy chain constant region comprises an IgM Cμ4 and IgM tailpiece domain. In some [embodimentexamples](#), the IgM heavy chain constant regions or fragments or variants thereof each further comprise a Cμ1 domain, a Cμ2 domain, a Cμ3 domain, or any combination thereof. In some [embodimentexamples](#), the IgM heavy chain constant region is a human IgM constant region.

[0016] In some [embodimentexamples](#), each binding unit comprises two IgM heavy chains each comprising a VH situated amino terminal to the IgM constant region or fragment thereof,

and two immunoglobulin light chains each comprising a VL situated amino terminal to an immunoglobulin light chain constant region.

[0017] In some embodimentexamples, the multimeric binding molecule comprises SEQ ID NO: 35, SEQ ID NO: 36, or a multimerizing fragment thereof. In some embodimentexamples, the IgM constant region comprises a substitution relative to a wild-type human IgM constant region at position 310, 311, 313, and/or 315 of SEQ ID NO: 35 or SEQ ID NO: 36. In some embodimentexamples, the IgM constant region comprises two or more substitutions relative to a wild-type human IgM constant region at positions 46, 209, 272, or 440 of SEQ ID NO: 35 or SEQ ID NO: 36.

[0018] In some embodimentexamples, the multimeric binding molecule is pentameric, and further comprises a J-chain or functional fragment or variant thereof.

[0019] In some embodimentexamples, 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 that can affect serum half-life of the multimeric binding molecule; and where the multimeric binding molecule comprising the variant J-chain 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.

[0020] In some embodimentexamples, the J-chain or functional fragment thereof 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: 41). In some embodimentexamples, the amino acid corresponding to Y102 of SEQ ID NO: 41 is substituted with alanine (A), serine (S), or arginine (R). In some embodimentexamples, the amino acid corresponding to Y102 of SEQ ID NO: 41 is substituted with alanine (A).

[0021] In some embodimentexamples, the J-chain is a variant human J-chain and comprises the amino acid sequence SEQ ID NO: 42. In some embodimentexamples, the J-chain or functional fragment thereof comprises an amino acid substitution at the amino acid position corresponding to amino acid N49, amino acid S51, or both N49 and S51 of the mature human J-chain (SEQ ID NO: 41), where a single amino acid substitution corresponding to position S51 of SEQ ID NO: 41 is not a threonine (T) substitution. In some embodimentexamples, the position corresponding to N49 of SEQ ID NO: 41 is substituted with alanine (A), glycine (G), threonine (T), serine (S) or aspartic acid (D). In some embodimentexamples, the

position corresponding to N49 of SEQ ID NO: 41 or SEQ ID NO: 42 is substituted with alanine (A). In some [embodimentexamples](#), the position corresponding to S51 of SEQ ID NO: 41 or SEQ ID NO: 42 is substituted with alanine (A) or glycine (G). In some [embodimentexamples](#), the position corresponding to S51 of SEQ ID NO: 41 or SEQ ID NO: 42 is substituted with alanine (A).

[0022] In some [embodimentexamples](#), the J-chain or functional fragment or variant thereof further comprises a heterologous polypeptide, where the heterologous polypeptide is directly or indirectly fused to the J-chain or functional fragment or variant thereof.

[0023] In some [embodimentexamples](#), the heterologous polypeptide is fused to the J-chain or fragment thereof via a peptide linker. In some [embodimentexamples](#), the peptide linker comprises at least 5 amino acids, but no more than 25 amino acids. In some [embodimentexamples](#), the J-chain or functional fragment or variant thereof further comprises a heterologous polypeptide, where the heterologous polypeptide is fused to the J-chain or functional fragment or variant thereof via a peptide linker comprising at least 5 amino acids, but no more than 25 amino acids. In some [embodimentexamples](#), the peptide linker consists of GGGGS (SEQ ID NO: 43), GGGSGGGGS (SEQ ID NO: 44), GGGSGGGSGGGGS (SEQ ID NO: 45), GGGSGGGSGGGSGGGGS (SEQ ID NO: 46), or GGGSGGGSGGGSGGGSGGGGS (SEQ ID NO: 47). In some [embodimentexamples](#), the heterologous polypeptide is fused to the N-terminus of the J-chain or fragment or variant thereof, the C-terminus of the J-chain or fragment or variant thereof, or to both the N-terminus and C-terminus of the J-chain or fragment or variant thereof.

[0024] In some [embodimentexamples](#), the heterologous polypeptide can influence the absorption, distribution, metabolism and/or excretion (ADME) of the multimeric binding molecule.

[0025] In some [embodimentexamples](#), the heterologous polypeptide comprises an antigen binding domain. In some [embodimentexamples](#), the antigen binding domain of the heterologous polypeptide is an antibody or antigen-binding fragment thereof. In some [embodimentexamples](#), 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. In some [embodimentexamples](#), the antigen-binding fragment is a scFv fragment. In some [embodimentexamples](#), the antigen binding domain binds ICOS Ligand (ICOSLG), ICOS (CD278), Interleukin 6 (IL6), CD28, CD3, CD80, CD86, Tumor Necrosis Factor Alpha (TNFα), or Fibroblast Activation Protein (FAP).

[0026] In another aspect, aAlso provided herein is a composition comprising the multimeric binding molecule disclosed herein. In some embodimentexamples, the composition further comprises a pharmacologically acceptable excipient.

[0027] In another aspect, aAlso provided herein is a polynucleotide comprising a nucleic acid sequence that encodes a polypeptide subunit of the binding molecule disclosed herein. In some embodimentexamples, the polypeptide subunit comprises an IgM heavy chain constant region and at least an antibody VH portion of the PD-1-binding domain of the multimeric binding molecule.

[0028] In some embodimentexamples, the polypeptide subunit comprises a human IgM constant region or fragment thereof fused to the C-terminal end of a VH comprising: (a) HCDR1, HCDR2, and HCDR3 regions comprising the CDRs contained in the VH amino acid sequences SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, or SEQ ID NO: 49, or the CDRs contained in the VH amino acid sequences SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, or SEQ ID NO: 49 with one or two single amino acid substitutions in one or more of the HCDRs; or (b) an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, or SEQ ID NO: 49.

[0029] In some embodimentexamples, the polypeptide subunit comprises a light chain constant region and an antibody VL portion of the PD-1-binding domain of the multimeric binding molecule. In some embodimentexamples, the polypeptide subunit comprises a human kappa or lambda light chain constant region or fragment thereof fused to the C-terminal end of a VL comprising: (a) LCDR1, LCDR2, and LCDR3 regions comprising the CDRs contained in the VL amino acid sequences SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30,

SEQ ID NO: 32, SEQ ID NO: 34, or SEQ ID NO: 50, or the CDRs contained in the VL amino acid sequences SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, or SEQ ID NO: 50 with one or two single amino acid substitutions in one or more of the LCDRs; or (b) an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, or SEQ ID NO: 50.

[0030] In a further aspect, the present disclosure provides a composition comprising a polynucleotide comprising a nucleic acid sequence that encodes an IgM heavy chain constant region and at least an antibody VH portion of the PD-1-binding domain of the multimeric binding molecule as described herein, and a polynucleotide comprising a nucleic acid sequence that encodes a light chain constant region and an antibody VL portion of the PD-1-binding domain of the multimeric binding molecule as described herein, wherein the polynucleotides are on separate vectors or on a single vector. Also provided herein is a composition comprising a polynucleotide provided herein. In some ~~embodiment~~examples, the composition comprises a polynucleotide comprising a nucleic acid sequence that encodes an IgM heavy chain constant region and at least an antibody VH portion of the PD-1-binding domain of a multimeric binding molecule provided herein, and a polynucleotide comprising a nucleic acid sequence that encodes a light chain constant region and an antibody VL portion of the PD-1-binding domain of a multimeric binding molecule provided herein. In some ~~embodiment~~examples, the polynucleotides are on separate vectors. In some ~~embodiment~~examples, the polynucleotides are on a single vector. In some ~~embodiment~~examples, the composition further comprises a polynucleotide comprising a nucleic acid sequence encoding a J chain, or a functional fragment thereof, or a functional variant thereof.

[0031] Also provided herein is a vector or vectors disclosed herein.

[0032] In a further aspect, the present disclosure provides a host cell comprising the composition as described herein, wherein the host cell can express the binding molecule, or a subunit thereof. Also provided herein is a host cell comprising a polynucleotide provided herein, a composition provided herein, or a vector or vectors provided herein, where the host cell can express a binding molecule provided herein, or a subunit thereof.

[0033] In a further aspect, aAlso provided herein is a method of producing a binding molecule provided herein, comprising culturing a host cell provided herein, and recovering the binding molecule.

5 [0034] In a further aspect, the present disclosure provides a use of the multimeric binding molecule as described herein in the manufacture of a medicament for treating an autoimmune disorder, an inflammatory disorder, or a combination thereof, wherein the multimeric binding molecule exhibits greater potency than an equivalent amount of a monomeric or dimeric binding molecule binding to the same binding partner. Also
10 provided herein is a method for treating an autoimmune disorder, an inflammatory disorder, or a combination thereof in a subject in need of treatment comprising administering to the subject an effective amount of a multimeric binding molecule provided herein, where the multimeric binding molecule exhibits greater potency than an equivalent amount of a monomeric or dimeric binding molecule binding to the same binding partner. In some embodimentexamples, the subject is human.

[0035] In a further aspect, the present disclosure provides a use of the multimeric binding molecule as described herein in the manufacture of a medicament for preventing transplantation rejection, wherein the multimeric binding molecule exhibits greater potency than an equivalent amount of a monomeric or dimeric binding molecule binding to the same binding partner, and wherein the subject is a transplantation recipient. Also provided herein is a method for preventing transplantation rejection in a subject, comprising administering to the subject an effective amount of a multimeric binding molecule provided herein, where the multimeric binding molecule exhibits greater potency than an equivalent amount of a monomeric or dimeric binding molecule binding to the same binding partner, and where the subject is a transplantation recipient. In some embodimentexamples, the subject is human.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0036] FIG. 1 shows binding of anti-PD-1 IgG #1, anti-PD-1 IgM #1, anti-PD-1 IgG #2, and anti-PD-1 IgM #2 to human PD-1 in an ELISA assay.

[0037] FIG. 2 shows binding of anti-PD-1 IgG #1, anti-PD-1 IgM #1, anti-PD-1 IgG #2, and anti-PD-1 IgM #2 to human PD-1 in a cell-based assay.

[0038] FIGS. 3A-3B show activation of PD-1 signaling by anti-PD-1 IgG or IgM #1 (FIG. 3A) or anti-PD-1 IgG or IgM #2 (FIG. 3B).

[0039] FIGS. 4A-4B show activation of PD-1 signaling by anti-PD-1 IgG, IgG + cross-linker, or IgM #1 (FIG. 4A) or anti-PD-1 IgG, IgG + cross-linker, or IgM #2 (FIG. 4B).

[0040] FIGS. 5A-5D show activation of PD-1 signaling by anti-PD-1 IgG, IgG + cross-linker, or IgM #1 (FIG. 5A) or anti-PD-1 IgG, IgG + cross-linker, or IgM #2 (FIG. 5B) or anti-PD-1 IgG, IgG + cross-linker, or IgM #3 (FIG. 5C) or anti-PD-1 IgG, IgG + cross-linker, or IgM #4 (FIG. 5D).

[0041] FIGS. 6A-6D show activation of PD-1 signaling by anti-PD-1 IgG, IgG + cross-linker, pentameric IgM, or hexameric IgHM #1 (FIG. 6A) or anti-PD-1 IgG, IgG + cross-linker, pentameric IgM, or hexameric IgHM #2 (FIG. 6B) or anti-PD-1 IgG, IgG + cross-linker, pentameric IgM, or hexameric IgHM #3 (FIG. 6C) or anti-PD-1 IgG, IgG + cross-linker, pentameric IgM, or hexameric IgHM #5 (FIG. 6D).

DETAILED DESCRIPTION

Definitions

[0042] As used herein, the term "a" or "an" entity refers to one or more of that entity; for example, "a binding molecule," is understood to represent one or more binding molecules.

5 As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

WHAT IS CLAIMED IS:

1. A multimeric binding molecule comprising ~~two~~, five, ~~or six~~, or two bivalent binding units or variants or fragments thereof,
wherein each binding unit comprises two ~~IgA or IgM~~ or IgA heavy chain constant regions or multimerizing fragments or variants thereof, each associated with a binding domain,
wherein three to twelve of the binding domains are programmed cell death protein 1 (PD-1)-binding domains that specifically and agonistically bind to PD-1,
wherein the binding molecule can activate PD-1-mediated signal transduction in a cell at a higher potency than an equivalent amount of a bivalent IgG antibody or fragment thereof comprising two of the same PD-1-binding domains, which also specifically binds to and agonizes PD-1.
2. The multimeric binding molecule of claim 1, wherein the three to twelve PD-1-binding domains comprise a heavy chain variable region (VH) and a light chain variable region (VL), wherein:
 - (a) 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: 1 and SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14, SEQ ID NO: 25 and SEQ ID NO: 26, SEQ ID NO: 27 and SEQ ID NO: 28, SEQ ID NO: 29 and SEQ ID NO: 30, SEQ ID NO: 31 and SEQ ID NO: 32, SEQ ID NO: 33 and SEQ ID NO: 34, or SEQ ID NO: 49 and SEQ ID NO: 50, respectively with zero, one, or two single amino acid substitutions in one or more of the HCDRs or LCDRs;
 - (b) 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 of any one of SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, or SEQ ID NO: 24 and the VL

of any one of SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, or SEQ ID NO: 22 with zero, one, or two single amino acid substitutions in one or more of the HCDRs or LCDRs;

- (c) the VH and VL comprise amino acid sequences at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 1 and SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14, SEQ ID NO: 25 and SEQ ID NO: 26, SEQ ID NO: 27 and SEQ ID NO: 28, SEQ ID NO: 29 and SEQ ID NO: 30, SEQ ID NO: 31 and SEQ ID NO: 32, SEQ ID NO: 33 and SEQ ID NO: 34, or SEQ ID NO: 49 and SEQ ID NO: 50, respectively; or
- (d) the VH comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to of any one of SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, or SEQ ID NO: 24 and the VL comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to of any one of SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, or SEQ ID NO: 22.

3. The multimeric binding molecule of claim 2, 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: 1 and SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4, SEQ ID NO: 13 and SEQ ID NO: 14, and SEQ ID NO: 25 and SEQ ID NO: 26, respectively with zero, one, or two single amino acid substitutions in one or more of the HCDRs or LCDRs.

4. The multimeric binding molecule of claim 1, which is a dimeric binding molecule comprising two bivalent IgA or IgA-like binding units and a J chain or functional fragment or variant thereof, wherein each binding unit comprises two IgA heavy chain constant regions or multimerizing fragments or variants thereof, each comprising an IgA Cα3 domain and an IgA tailpiece domain.

5. The multimeric binding molecule of claim 4, wherein:
- (a) each IgA heavy chain constant region or multimerizing fragment or variant thereof further comprises a C α 1 domain, a C α 2 domain, an IgA hinge region, or any combination thereof;
 - (b) the IgA heavy chain constant regions or multimerizing fragments thereof are human IgA constant regions; and/or
 - (c) each binding unit comprises two IgA heavy chains each comprising a VH situated amino terminal to the IgA constant region or multimerizing fragment thereof, and two immunoglobulin light chains each comprising a VL situated amino terminal to an immunoglobulin light chain constant region.
6. The multimeric binding molecule of claim 1, which is a pentameric or a hexameric binding molecule comprising five or six bivalent IgM binding units, respectively, wherein each binding unit comprises two IgM heavy chain constant regions or multimerizing fragments thereof each associated with a PD-1-binding domain, wherein each IgM heavy chain constant region comprises an IgM C μ 4 and IgM tailpiece domain.
7. The multimeric binding molecule of claim 6, wherein:
- (a) the IgM heavy chain constant regions or 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;
 - (b) the IgM heavy chain constant region is a human IgM constant region;
 - (c) each binding unit comprises two IgM heavy chains each comprising a VH situated amino terminal to the IgM constant region or fragment thereof, and two immunoglobulin light chains each comprising a VL situated amino terminal to an immunoglobulin light chain constant region.
8. The multimeric binding molecule of claim 6, wherein the IgM constant region comprises:
- (a) SEQ ID NO: 35, SEQ ID NO: 36, or a multimerizing fragment thereof;

- (b) a substitution relative to a wild-type human IgM constant region at position 310, 311, 313, and/or 315 of SEQ ID NO: 35 or SEQ ID NO: 36; or
- (c) two or more substitutions relative to a wild-type human IgM constant region at positions 46, 209, 272, or 440 of SEQ ID NO: 35 or SEQ ID NO: 36.

9. The multimeric binding molecule of claim 6, which is pentameric, and further comprises a J-chain or functional fragment or variant thereof.

10. The multimeric 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 that can affect serum half-life of the multimeric binding molecule; and wherein the multimeric binding molecule comprising the variant J-chain 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 to be administered in the same way to the same animal species.

11. The multimeric binding molecule of claim 9, wherein the J-chain or functional fragment thereof comprises:

- (a) 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: 41);
- (b) 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: 41), wherein the amino acid corresponding to Y102 of SEQ ID NO: 41 is substituted with alanine (A);
- (c) 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: 41), wherein the J-chain is a variant human J-chain and comprises the amino acid sequence SEQ ID NO: 42; or
- (d) an amino acid substitution at the amino acid position corresponding to amino acid N49, amino acid S51, or both N49 and S51 of the mature human J-chain (SEQ ID

NO: 41), wherein a single amino acid substitution corresponding to position S51 of SEQ ID NO: 41 is not a threonine (T) substitution.

12. The multimeric binding molecule of claim 9, wherein the J-chain or functional fragment or variant thereof further comprises a heterologous polypeptide, wherein the heterologous polypeptide is fused to the J-chain or functional fragment or variant thereof via a peptide linker comprising at least 5 amino acids, but no more than 25 amino acids.

13. The multimeric binding molecule of claim 12, wherein the heterologous polypeptide is fused to the N-terminus of the J-chain or fragment or variant thereof, the C-terminus of the J-chain or fragment or variant thereof, or to both the N-terminus and C-terminus of the J-chain or fragment or variant thereof.

14. The multimeric binding molecule of claim 12, wherein the heterologous polypeptide comprises an antigen binding domain, and wherein:

- (a) the antigen binding domain of the heterologous polypeptide is an antibody or antigen-binding fragment thereof;
- (b) the antigen binding domain of the heterologous polypeptide is an antigen-binding fragment of an antibody, and 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;
- (c) the antigen binding domain of the heterologous polypeptide is an antigen-binding fragment of an antibody, and the antigen-binding fragment is a scFv fragment; and/or
- (d) the antigen binding domain of the heterologous polypeptide is an antigen-binding fragment of an antibody, and the antigen binding domain binds ICOS Ligand (ICOSLG), ICOS (CD278), Interleukin 6 (IL6), CD28, CD3, CD80, CD86, Tumor Necrosis Factor Alpha (TNF α), or Fibroblast Activation Protein (FAP).

15. A composition comprising the multimeric binding molecule of any one of claims 1 to 14.

16. A polynucleotide comprising a nucleic acid sequence that encodes a polypeptide subunit of the binding molecule of any one of claims 1 to 14.

17. The polynucleotide of claim 16, wherein the polypeptide subunit comprises an IgM heavy chain constant region and at least an antibody VH portion of the PD-1-binding domain of the multimeric binding molecule; or comprises a light chain constant region and an antibody VL portion of the PD-1-binding domain of the multimeric binding molecule.

18. A composition comprising a polynucleotide comprising a nucleic acid sequence that encodes an IgM heavy chain constant region and at least an antibody VH portion of the PD-1-binding domain of the multimeric binding molecule of any one of claims 1 to 14, and a polynucleotide comprising a nucleic acid sequence that encodes a light chain constant region and an antibody VL portion of the PD-1-binding domain of the multimeric binding molecule of any one of claims 1 to 14, wherein the polynucleotides are on separate vectors or on a single vector.

19. The composition of claim 18, further comprising a polynucleotide comprising a nucleic acid sequence encoding a J chain, or a functional fragment thereof, or a functional variant thereof.

20. The vector or the vectors of claim 19.

21. A host cell comprising the composition of claim 18, wherein the host cell can express the binding molecule, or a subunit thereof.

22. A method of producing the binding molecule, comprising culturing the host cell of claim 21, and recovering the binding molecule.

23. ~~A method for treating an autoimmune disorder, an inflammatory disorder, or a combination thereof in a subject in need of treatment comprising administering to the subject an effective amount of~~ Use of the multimeric binding molecule of any one of claims 1 to 14 in the manufacture of a medicament for treating an autoimmune disorder, an inflammatory disorder, or a combination thereof, wherein the multimeric binding molecule exhibits greater potency than an equivalent amount of a monomeric or dimeric binding molecule binding to the same binding partner.

24. ~~A method for preventing transplantation rejection in a subject, comprising administering to the subject an effective amount of~~ Use of the multimeric binding molecule of any one of claims 1 to 14 in the manufacture of a medicament for preventing transplantation rejection, wherein the multimeric binding molecule exhibits greater potency than an equivalent amount of a monomeric or dimeric binding molecule binding to the same binding partner, and wherein the subject is a transplantation recipient.

25. ~~The method of claim 23, wherein the subject is human.~~