

fusions, however, a limited as an IgG fusion protein can only be expressed as a monomer or a dimer, limiting efficacy in some situations. Indeed, monomeric forms of the TNF receptor Fc fusion protein had greatly reduced TNF α inhibitory activity as compared to the dimer (Pepel, K., *et al*, *J. Exp. Med.* 174:1483-1489 (1991)).

[0005] Many reports have described polymerized therapeutic protein-Fc fusions through a variety of methods. In one report, IgG Fc regions were engineered to facilitate hexamerization of a malaria antigen-IgG fusion, but effector function of the IgG fusion portions were altered, and the molecules were not immunogenic when used to immunize animals, in contrast to the corresponding monomeric fusion proteins (Mekhaie, DN, *et al*, *Scientific Reports* 1, Doi: 10.1038/srep00124 (2011)). In another study, the human PD-L1 ectodomain was fused to wild-type human IgM constant region and expressed either with or without human J-chain and was tested in *in vitro* flow cytometry and plate-based immunoassays, but the ability of the constructs to induce signal transduction in PD-1-expressing cells was not tested (Ammann, JU., *et al*, *Eur. J. Immunol* 42:1354-1356 (2012)).

[0006] There remains a need for higher avidity Fc fusion therapeutics that maintain the stability and serum half-life characteristics of IgG Fc fusion proteins.

SUMMARY

[0007] This disclosure provides a multimeric binding molecule that includes two, five, or six bivalent binding units or variants or fragments thereof where each binding unit includes two IgA or IgM heavy chain constant regions or multimerizing fragments or variants thereof, each fused to a binding polypeptide or fragment thereof that specifically binds to a binding partner expressed on the surface of a cell, where the binding polypeptide is not an antibody or antigen-binding fragment of an antibody, and where binding of the binding polypeptide to the binding partner modulates signal transduction in the cell.

[0007a] In one aspect, the present disclosure provides a multimeric binding molecule comprising five or six bivalent binding units, wherein the multimeric binding molecule is an agonist of programmed cell death protein-1 (PD-1); wherein each binding unit comprises two IgM heavy chain constant regions or multimerizing fragments or variants thereof, each fused to a binding polypeptide or fragment thereof that specifically binds to a binding partner expressed on the surface of a cell, wherein the binding polypeptide is not

an antibody or antigen-binding fragment of an antibody, and wherein binding of the binding polypeptide to the binding partner modulates

signal transduction in the cell; wherein the binding polypeptide or fragment thereof comprises a receptor-binding fragment of human programmed cell death 1 ligand 1 (PD-L1) and the binding partner is PD-1; wherein the IgM heavy chain constant regions or multimerizing fragments or variants thereof each comprise: (a) SEQ ID NO: 3; (b) SEQ ID NO: 4; (c) SEQ ID NO: 6; or (d) SEQ ID NO: 7; and wherein the multimeric binding molecule can function as a PD-1 agonist at higher potency than an equivalent amount of a monomeric or dimeric PD-L1 ectodomain fused to an IgG Fc region.

[0007b] In certain examples at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven or twelve of the binding polypeptides bind to and modulate signal transduction of the same binding partner. Moreover, in certain examples the binding molecule can induce or inhibit signal transduction in the cell at a higher potency than an equivalent amount of a monovalent or divalent binding molecule with one or two binding polypeptides binding to the same binding partner.

[0008] This disclosure further provides a multimeric binding molecule that includes two, five, or six bivalent binding units or variants or fragments thereof where each binding unit includes two IgA or IgM heavy chain constant regions or multimerizing fragments or variants thereof, each fused to binding polypeptide, where at least three of the binding polypeptides include a receptor ectodomain that specifically binds to a binding partner that includes a ligand or receptor-binding fragment thereof, where the receptor ectodomain is not an antibody or antigen-binding fragment of an antibody, and where binding of the

domain containing receptor-5 (DR5), OX-40, CD40, 4-1BB, glucocorticoid-induced tumor necrosis factor receptor (GITR), or any combination thereof. As a further example, an immune checkpoint modulator receptor ectodomain can include, but is not limited to a ligand-binding fragment of PD-1, a ligand-binding fragment of CTLA4, a ligand-binding fragment of LAG3, a ligand-binding fragment of CD28, a ligand-binding fragment of immunoglobulin-like domain containing receptor 2 (ILDR2), a ligand-binding fragment of T-cell immunoglobulin mucin family member 3 (TIM-3), or any combination thereof. As a further example, a TGF β receptor can include, but is not limited to a ligand binding fragment of a TGF β R-1, a TGF β R-2, a TGF β R3, or any combination thereof.

[0018] In another aspect, the disclosure further provides an isolated polynucleotide comprising a nucleic acid sequence that encodes a subunit of a multimeric binding molecule as provided herein, wherein each subunit comprises the IgM heavy chain constant region fused to the binding polypeptide or fragment thereof. The disclosure further provides an isolated polynucleotide that includes a nucleic acid sequence that encodes a subunit of a multimeric binding molecule as provided herein, where each subunit includes an IgA or IgM heavy chain constant region or multimerizing fragment or variant thereof fused to a binding polypeptide or fragment thereof that specifically binds to a binding partner, or a receptor ectodomain that specifically binds to a ligand. Further disclosed in another aspect is a vector that includes the provided polynucleotide. In a further aspect, the present disclosure provides a host cell that includes the provided vector or polynucleotide. In certain examples, the provided host cell can further include an isolated polynucleotide that includes a nucleic acid sequence encoding a J-chain or functional fragment or variant thereof as provided by the disclosure.

[0018a] In a further aspect, the present disclosure provides the multimeric binding molecule as described herein for use in therapy, wherein the multimeric binding molecule exhibits greater potency than an equivalent amount of a monomeric or dimeric PD-L1 ectodomain fused to an IgG Fc region.

[0018b] In a further aspect, the present disclosure provides the 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 in a subject in need of treatment, wherein the multimeric binding molecule exhibits greater potency than an equivalent amount of a monomeric or dimeric PD-L1 ectodomain fused to an IgG Fc region.

[0018c] In a further aspect, the present disclosure provides the use of the multimeric binding molecule as described herein in the manufacture of a medicament for preventing transplantation rejection in a transplantation recipient, wherein the multimeric binding molecule exhibits greater potency than an equivalent amount of a monomeric or dimeric PD-L1 ectodomain fused to an IgG Fc region.

[0019] The disclosure further provides a method for treating an autoimmune disorder, an inflammatory disorder, or a combination thereof in a subject in need of treatment where the method includes administering to the subject an effective amount of a multimeric binding molecule as 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.

[0020] The disclosure further provides a method for preventing transplantation rejection in a transplantation recipient, where the method includes administering to the subject an effective amount of the multimeric binding molecule as 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.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0021] **FIGURE 1A** shows a prototype hexameric binding molecule that has six IgM-derived binding units each having two IgM-derived heavy chain fragments that include a C μ 2 domain, a C μ 3 domain, and a C μ 4-tp domain, where the IgM derived heavy chain fragments are fused to the C-terminus of a ligand or receptor binding polypeptide.

[0022] **FIGURE 1B** shows a prototype pentameric binding molecule that has five IgM-derived binding units each having two IgM-derived heavy chain fragments that include a C μ 2 domain, a C μ 3 domain, and a C μ 4-tp domain, where the IgM derived heavy chain fragments are fused to the C-terminus of binding polypeptide, and where the pentameric binding molecule further includes a modified J-chain bearing optional N- and C-terminal fusions of heterologous polypeptides, *e.g.*, scFv antibody binding domains.

[0023] **FIGURE 2** shows a prototype pentameric binding molecule has five IgM-derived binding units each having two IgM-derived heavy chain fragments that have a C μ 2 domain, a C μ 3 domain, and a C μ 4-tp domain, where the IgM derived heavy chain fragments are fused to the C-terminus of binding polypeptide, where the binding polypeptides are receptor ectodomains, and where the pentameric binding molecule further

includes a modified J-chain bearing optional N-and C-terminal fusions of heterologous polypeptides, *e.g.*, scFv antibody binding domains.

[0024] **FIGURE 3A-C** shows the structures of the PD-L1 binding molecules produced according to Example 1. **FIGURE 3A:** PD-L1-IgM; **FIGURE 3B:** PD-L1-H-IgM; **FIGURE 3C:** PD-L1-Fc.

[0025] **FIGURE 4** is a graph showing the ability of various PD-L1 binding molecules to stimulate PD-1 activity in reporter Jurkat T-cells.

WHAT IS CLAIMED IS:

1. A multimeric binding molecule comprising five or six bivalent binding units, wherein the multimeric binding molecule is an agonist of programmed cell death protein-1 (PD-1);

wherein each binding unit comprises two IgM heavy chain constant regions or multimerizing fragments or variants thereof, each fused to a binding polypeptide or fragment thereof that specifically binds to a binding partner expressed on the surface of a cell, wherein the binding polypeptide is not an antibody or antigen-binding fragment of an antibody, and wherein binding of the binding polypeptide to the binding partner modulates signal transduction in the cell;

wherein the binding polypeptide or fragment thereof comprises a receptor-binding fragment of human programmed cell death 1 ligand 1 (PD-L1) and the binding partner is PD-1;

wherein the IgM heavy chain constant regions or multimerizing fragments or variants thereof each comprise:

- (a) SEQ ID NO: 3;
- (b) SEQ ID NO: 4;
- (c) SEQ ID NO: 6; or
- (d) SEQ ID NO: 7; and

wherein the multimeric binding molecule can function as a PD-1 agonist at higher potency than an equivalent amount of a monomeric or dimeric PD-L1 ectodomain fused to an IgG Fc region.

2. The multimeric binding molecule of claim 1, wherein the receptor-binding fragment of PD-L1 comprises:
 - (a) amino acids 19-127 of SEQ ID NO: 8; or
 - (b) SEQ ID NO: 9.
3. The multimeric binding molecule of claim 1 or claim 2, which is pentameric, and further comprises a J-chain or functional fragment or variant thereof.
4. The multimeric binding molecule of claim 3, 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 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, and wherein the J-chain or functional fragment thereof comprises: an amino acid substitution at the amino acid position corresponding to amino acid Y102 of the wild-type human J-chain (SEQ ID NO: 15).

5. The multimeric binding molecule of claim 4, wherein:
 - (a) the amino acid corresponding to Y102 of SEQ ID NO: 15 is substituted with alanine (A), serine (S), or arginine (R);
 - (b) the amino acid corresponding to Y102 of SEQ ID NO: 15 is substituted with alanine (A); or
 - (c) the variant human J-chain comprises the amino acid sequence SEQ ID NO: 16.
6. The multimeric binding molecule of claim 4, wherein 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 human J-chain (SEQ ID NO: 15), wherein:
 - (a) the amino acid substitution corresponding to position S51 of SEQ ID NO: 15 is not a threonine (T) substitution;
 - (b) the position corresponding to N49 of SEQ ID NO: 15 is substituted with alanine (A), glycine (G), threonine (T), serine (S) or aspartic acid (D);
 - (c) the position corresponding to N49 of SEQ ID NO: 15 is substituted with alanine (A);
 - (d) the variant human J-chain comprises the amino acid sequence SEQ ID NO: 17;

- (e) the position corresponding to S51 of SEQ ID NO: 15 is substituted with alanine (A) or glycine (G);
 - (f) the position corresponding to S51 of SEQ ID NO: 15 is substituted with alanine (A); or
 - (g) the variant human J-chain comprises the amino acid sequence SEQ ID NO: 18.
7. The multimeric binding molecule of any one of claims 3 to 6, wherein the J-chain or functional fragment or variant thereof further comprises a heterologous polypeptide, wherein the heterologous polypeptide is directly fused to the J-chain or functional fragment or variant thereof or indirectly fused via a peptide linker comprising at least 5 amino acids, but no more than 25 amino acids to the J-chain or functional fragment or variant thereof, and 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.
8. The multimeric binding molecule of claim 7, wherein the heterologous polypeptide:
- (a) can influence the absorption, distribution, metabolism and/or excretion (ADME) of the multimeric binding molecule;
 - (b) comprises an antigen binding domain;
 - (c) is an antibody or antigen-binding fragment thereof;
 - (d) is an antigen-binding fragment of an antibody, and wherein the antigen-binding fragment comprises an Fab fragment, an Fab' fragment, an F(ab')₂ fragment, an Fd fragment, an Fv fragment, a single-chain Fv (scFv) fragment, a disulfide-linked Fv (sdFv) fragment, or any combination thereof; and/or
 - (e) is an antigen-binding fragment of an antibody, and wherein the antigen-binding fragment is a scFv fragment.
9. An isolated polynucleotide comprising a nucleic acid sequence that encodes a subunit of the multimeric binding molecule of any one of claims 1 to 8, wherein each subunit

comprises the IgM heavy chain constant region fused to the binding polypeptide or fragment thereof.

10. A vector comprising the polynucleotide of claim 9.
11. A host cell comprising the vector of claim 10.
12. The multimeric binding molecule of any one of claims 1 to 8 for use in therapy, wherein the multimeric binding molecule exhibits greater potency than an equivalent amount of a monomeric or dimeric PD-L1 ectodomain fused to an IgG Fc region.
13. Use of the multimeric binding molecule of any one of claims 1 to 8 in the manufacture of a medicament for treating an autoimmune disorder, an inflammatory disorder, or a combination thereof in a subject in need of treatment, wherein the multimeric binding molecule exhibits greater potency than an equivalent amount of a monomeric or dimeric PD-L1 ectodomain fused to an IgG Fc region.
14. Use of the multimeric binding molecule of any one of claims 1 to 8 in the manufacture of a medicament for preventing transplantation rejection in a transplantation recipient, wherein the multimeric binding molecule exhibits greater potency than an equivalent amount of a monomeric or dimeric PD-L1 ectodomain fused to an IgG Fc region.