

MULTIMERIC T-CELL MODULATORY POLYPEPTIDES AND METHODS OF USE THEREOF**CROSS-REFERENCE**

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 63/023,834, filed May 12, 2020, and U.S. Provisional Patent Application No. 63/041,451, filed June 19, 2020, which applications are incorporated herein by reference in their entirety.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING PROVIDED AS A TEXT FILE

[0002] A Sequence Listing is provided herewith as a text file, "CUEB-133WO_SEQ_LIST2_ST25.txt" created on May 7, 2021 and having a size of 800 KB. The contents of the text file are incorporated by reference herein in their entirety.

INTRODUCTION

[0003] An adaptive immune response involves the engagement of the T cell receptor (TCR), present on the surface of a T cell, with a small peptide antigen non-covalently presented on the surface of an antigen presenting cell (APC) by a major histocompatibility complex (MHC; also referred to in humans as a human leukocyte antigen (HLA) complex). This engagement represents the immune system's targeting mechanism and is a requisite molecular interaction for T cell modulation (activation or inhibition) and effector function. Following epitope-specific cell targeting, the targeted T cells are activated through engagement of costimulatory proteins found on the APC with counterpart costimulatory proteins the T cells. Both signals – epitope/TCR binding and engagement of APC costimulatory proteins with T cell costimulatory proteins – are required to drive T cell specificity and activation or inhibition. The TCR is specific for a given epitope; however, the costimulatory protein not epitope specific and instead is generally expressed on all T cells or on large T cell subsets.

SUMMARY

[0004] The present disclosure provides T-cell modulatory multimeric polypeptides (TMMPs) that comprise an immunomodulatory polypeptide and that comprise an epitope-presenting Wilms tumor peptide. A T-cell modulatory multimeric polypeptide is useful for modulating the activity of a T cell, and for modulating an immune response in an individual.

BRIEF DESCRIPTION OF THE DRAWINGS

[0001] **FIG. 1A-1F** are schematic depictions of various TMMPs of the present disclosure.

[0002] FIG. 2A-2F are schematic depictions of various disulfide-linked TMMPs of the present disclosure.

[0003] FIG. 3A-3E provides an amino acid sequence of WT-1 polypeptides. The sequences of FIGs. 3A-3E are set forth in SEQ ID NOs: 399-403, respectively.

[0004] FIG. 4A-4E provide amino acid sequences of exemplary polypeptide chains of TMMPs of the present disclosure. The sequences of the exemplary polypeptide chains of FIGs. 4A-4E are set forth in SEQ ID NOs: 405-409, respectively. The epitope sequences are set forth as follows: FIG. 4D: CMTWNQMNL (SEQ ID NO:266); FIG. 4E: CYTWNQMNL (SEQ ID NO:267).

[0005] FIG. 5A-5H provide amino acid sequences of immunoglobulin Fc polypeptides. The sequences of FIGs. 5A-5G are set forth in SEQ ID NOs: 410-421, respectively. The sequence depicted in FIG. 5H is set forth in SEQ ID NO:487.

[0006] FIG. 6 provides a multiple amino acid sequence alignment of beta-2 microglobulin (β 2M) precursors (i.e., including the leader sequence) from *Homo sapiens* (NP_004039.1; SEQ ID NO: 19), *Pan troglodytes* (NP_001009066.1; SEQ ID NO: 19), *Macaca mulatta* (NP_001040602.1; SEQ ID NO: 20), *Bos taurus* (NP_776318.1; SEQ ID NO: 21) and *Mus musculus* (NP_033865.2; SEQ ID NO: 22). Amino acids 1-20 are a signal peptide.

[0007] FIG. 7A-7C provide amino acid sequences of full-length human HLA heavy chains of alleles A*0101 (SEQ ID NO: 23), A*1101 (SEQ ID NO: 24), A*2402 (SEQ ID NO: 25), and A*3303 (SEQ ID NO: 26) (FIG. 7A); full-length human HLA heavy chain of allele B*0702 (SEQ ID NO: 27) (FIG. 7B); and a full-length human HLA-C heavy chain (SEQ ID NO: 28) (FIG. 7C).

[0008] FIG. 8 provides an alignment of eleven mature MHC class I heavy chain amino acid sequences without their leader sequences, transmembrane domains, and intracellular domains. Top to bottom: SEQ ID NOs: 41-51.

[0009] FIGs. 9A-9B provide an alignment of HLA-A heavy chain amino acid sequences (FIG. 9A) and a consensus sequence (FIG. 9B; SEQ ID NO: 29).

[0010] FIGs. 10A-10B provide an alignment of HLA-B heavy chain amino acid sequences (FIG. 10A; SEQ ID NOs: 207-213, respectively) and a consensus sequence (FIG. 10B; SEQ ID NO: 30).

[0011] FIGs. 11A-11B provide an alignment of HLA-C heavy chain amino acid sequences (FIG. 11A; SEQ ID NOs: 214-222, respectively) and a consensus sequence (FIG. 11B; SEQ ID NO: 31).

[0012] FIG. 12 provides a consensus amino acid sequence for each of HLA-E, -F, and -G heavy chains (SEQ ID NOs: 32-34, respectively). Variable amino acid (aa) positions are indicated as "X" residues sequentially numbered; the locations of amino acids 84, 139, and 236 are double underlined.

[0013] FIG. 13 provides an alignment of consensus amino acid sequences for HLA-A (SEQ ID NO: 29), -B (SEQ ID NO: 30), -C (SEQ ID NO: 31), -E (SEQ ID NO: 32), -F (SEQ ID NO: 33), and -G (SEQ ID NO: 34).

[0014] FIG. 14A-14J provide amino acid sequences of polypeptide chains of double disulfide-linked TMMP of the present disclosure. The sequences of the polypeptide chains of FIGs. 14A-14I are set forth in SEQ ID NOs: 422-430, respectively. The epitope sequences are set forth as follows: FIG. 14B: VLDFAPPGA (SEQ ID NO: 259); FIG. 14C: RMFPNAPYL (SEQ ID NO: 260); FIG. 14F: VLDFAPPGA (SEQ ID NO: 259); FIG. 14G: RMFPNAPYL (SEQ ID NO: 260); FIG. 14H: YMFPNAPYL (SEQ ID NO: 264); FIG. 14I: YMFPNAPYL (SEQ ID NO: 264) and 14J (SEQ ID NO: 486).

[0015] FIG. 15 depicts expression and stability data for a WT1(37-45) epitope-containing TMMP of the present disclosure.

[0016] FIG. 16 depicts expression and stability data for a WT1(126-134) epitope-containing TMMP of the present disclosure.

[0017] FIG. 17A-17D provide schematic depictions of double disulfide-linked TMMP of the present disclosure.

[0018] FIG. 18A-18C provide schematic depictions of examples of configurations of disulfide-linked TMMPs of the present disclosure.

[0019] FIG. 19 provide schematic depictions of examples of positions of immunomodulatory polypeptides in TMMPs of the present disclosure.

[0020] FIG. 20A-20R provide amino acid sequences of exemplary polypeptide chains of TMMPs of the present disclosure. The sequences of the exemplary polypeptide chains of FIGs. 20A-20R are set forth in SEQ ID NOs: 431-448, respectively. The epitope sequences are set forth as follows: FIG. 20H: CYTWNQMNL (SEQ ID NO: 262); FIG. 20I: CYTWNQMNL (SEQ ID NO: 262); FIG. 20J: CYTWNQMNL (SEQ ID NO: 262); FIG. 20K: CYTWNQMNL (SEQ ID NO: 262); FIG. 20L: CYTWNQMNL (SEQ ID NO: 262); FIG. 20M: NYMNLGATL (SEQ ID NO: 263); FIG. 20N: NYMNLGATL (SEQ ID NO: 263); FIG. 20O: NYMNLGATL (SEQ ID NO: 263); FIG. 20P: NYMNLGATL (SEQ ID NO: 263); FIG. 20Q: NYMNLGATL (SEQ ID NO: 263); FIG. 20R: NYMNLGATL (SEQ ID NO: 263).

[0021] FIG. 21 depicts the effect of TMMPs, containing WT1 peptide epitopes and HLA-A*02 heavy chains, on antigen-specific CD8+ T cell expansion.

[0022] FIG. 22 depicts the effect of TMMPs containing WT1 peptide epitopes on expansion of WT1-specific CD8+ T cells from total PBMCs over a course of an 8-day re-stimulation culture following a 10-day priming culture.

- [0023] **FIG. 23** depicts production of TNF- α and IFN- γ by WT1-specific CD8⁺ T cells expanded with WT1 37-45 containing TMMPs having either the G2C or R12C/G2C framework.
- [0024] **FIG. 24** depicts production of TNF- α and IFN- γ by WT1-specific CD8⁺ T cells expanded with WT1 126-134 containing TMMPs having the R12C/G2C framework.
- [0025] **FIG. 25** depicts the effect of disulfide bonds on IL-2-driven immune cell activation.
- [0026] **FIG. 26** depicts the effect of TMMP containing variant IL-2 as the immunomodulatory polypeptide on CGLL-2 proliferation, compared to proleukine.
- [0027] **FIG. 27** depicts binding of the “1715 + 2380” TMPP to various Fc receptors.
- [0028] **FIG. 28** depicts the effect of TMMPs, containing the WT1 peptide epitope 235-243 (M236Y) and HLA-A*24 heavy chains, on antigen-specific CD8⁺ T cell expansion.
- [0029] **FIG. 29** depicts the effect of TMMPs, containing the WT1 peptide epitope 239-247 (Q240Y) and HLA-A*24 heavy chains, on antigen-specific CD8⁺ T cell expansion.
- [0030] **FIG. 30** depicts induction of CD69 expression by TMMPs with IL-2 polypeptide engineered at position 1 or 3, peptide epitope WT1 235-243 (M236Y), HLA-A24 heavy chains, and G2C or R12C/G2C disulfide frameworks.
- [0031] **FIG. 31** depicts the effect of TMMPs (with IL-2 polypeptide engineered at position 1 or 3, peptide epitope WT1 239-247 (Q240Y), HLA-A24 heavy chains, and G2C or R12C/G2C disulfide frameworks) on CD69 expression, compared to proleukine and recombinant human IL-2 (rhIL-2).
- [0032] **FIG. 32** depicts the effect of TMMPs (with IL-2 polypeptide engineered at position 1 or 3, peptide epitope WT1 235-243 (M236Y), HLA-A24 heavy chains, and G2C or R12C/G2C disulfide frameworks) on CTLL-2 proliferation, compared to proleukine.
- [0033] **FIG. 33** depicts the effect of TMMPs (with IL-2 polypeptide engineered at position 1 or 3, peptide epitope WT1 239-247 (Q240Y), HLA-A24 heavy chains, and G2C or R12C/G2C disulfide frameworks) on CTLL-2 proliferation, compared to proleukine.
- [0034] **FIG. 34** depicts binding of the “3425 + 3529” TMPP to various Fc receptors.
- [0035] **FIG. 35A-35F** provide amino acid sequences of exemplary polypeptide chains of TMMPs of the present disclosure, in which the polypeptide chains comprise the WT-1 peptide SMTWNQMNL (SEQ ID NO:451).
- [0036] **FIG. 36A-36F** provide amino acid sequences of exemplary polypeptide chains of TMMPs of the present disclosure, in which the polypeptide chains comprise the WT-1 peptide GCMTWNQMNL (SEQ ID NO:452).
- [0037] **FIG. 37A-37F** provide amino acid sequences of exemplary polypeptide chains of TMMPs of the present disclosure, in which the polypeptide chains comprise the WT-1 peptide SYTWNQMNL (SEQ ID NO:453).

[0038] FIG. 38A-38F provide amino acid sequences of exemplary polypeptide chains of TMMPs of the present disclosure, in which the polypeptide chains comprise the WT-1 peptide GCYTWNQMNL (SEQ ID NO:454).

[0039] FIG. 39 depicts expansion of WT1₃₇₋₄₅-specific CD8⁺ T cells from unprimed PBMCs, in which the expansion was induced by a TMMP of the present disclosure ("CUE-102/A02 WT1₃₇₋₄₅ IST").

[0040] FIG. 40A-40B depict expansion of WT1₃₇₋₄₅-specific CD8⁺ T cells from WT1₃₇₋₄₅ peptide-primed PBMCs, in which the expansion was induced by a TMMP of the present disclosure ("CUE-102/A02 WT1₃₇₋₄₅ IST").

[0041] FIG. 41A-41B depict production of TNF- α , IL-6, and IFN- γ , and upregulation of CD69, induced by a TMMP of the present disclosure ("CUE-102/A02 WT1₃₇₋₄₅ IST") or by wild-type IL-2.

[0042] FIG. 42A-42B depict CTL activity, against peptide-presenting target cells, of WT1₃₇₋₄₅ peptide-specific CD8⁺ T cells expanded from peptide-primed PBMCs in the presence of a TMMP of the present disclosure ("CUE-102/A02 WT1₃₇₋₄₅ IST").

[0043] FIG. 43 depicts the effect of TMMPs, containing the WT1 peptide epitope WT1 235-243(C235S; M236Y) and HLA-A*24 heavy chains, on antigen-specific CD8⁺ T cell expansion.

[0044] FIG. 44 depicts the effect of TMMPs, containing the WT1 peptide epitope WT1 239-247(Q240Y) and HLA-A*24 heavy chains, on antigen-specific CD8⁺ T cell expansion.

[0045] FIG. 45 depicts the effect of TMMPs containing the WT1 peptide epitope 37-45 on antigen-specific CD8⁺ T cell expansion in naïve HLA-A2 (AAD) transgenic mice.

[0046] FIG. 46A-46D provide amino acid sequences of HLA-E heavy chains.

[0047] FIG. 47A-47D provide amino acid sequences of HLA-G heavy chains.

DEFINITIONS

[0048] The terms "polynucleotide" and "nucleic acid," used interchangeably herein, refer to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. Thus, this term includes, but is not limited to, single-, double-, or multi-stranded DNA or RNA, genomic DNA, cDNA, DNA-RNA hybrids, or a polymer comprising purine and pyrimidine bases or other natural, chemically or biochemically modified, non-natural, or derivatized nucleotide bases.

[0049] The terms "peptide," "polypeptide," and "protein" are used interchangeably herein, and refer to a polymeric form of amino acids of any length, which can include coded and non-coded amino acids, chemically or biochemically modified or derivatized amino acids, and polypeptides having modified peptide backbones. Furthermore, as used herein, a "polypeptide" refers to a protein that includes modifications, such as deletions, additions, and substitutions (generally conservative in nature as would be known to a person in the art) to the native sequence, as long as the protein maintains the

desired activity. These modifications can be deliberate, as through site-directed mutagenesis, or can be accidental, such as through mutations of hosts that produce the proteins, or errors due to polymerase chain reaction (PCR) amplification or other recombinant DNA methods. References herein to a specific residue or residue number in a known polypeptide are understood to refer to the amino acid at that position in the wild-type polypeptide. To the extent that the sequence of the wild-type polypeptide is altered, either by addition or deletion of one or more amino acids, one of ordinary skill will understand that a reference to the specific residue or residue number will be correspondingly altered so as to refer to the same specific amino acid in the altered polypeptide, which would be understood to reside at an altered position number. For example, if an MHC class I polypeptide is altered by the addition of one amino acid at the N-terminus, then a reference to position 84 or a specific residue at position 84, will be understood to indicate the amino acids that are at position 85 on the altered polypeptide. Likewise, a reference herein to substitution of a specific amino acid at a specific position, e.g., Y84, is understood to refer to a substitution of an amino acid for the amino acid at position 84 in the wild-type polypeptide. A Y84C substitution is thus understood to be a substitution of Cys residue for the Tyr residue that is present in the wild-type sequence. If, e.g., the wild-type polypeptide is altered to change the amino acid at position 84 from its wild-type amino acid to an alternate amino acid, then the substitution for the amino acid at position 84 will be understood to refer to the substitution for the alternate amino acid. If in such case the polypeptide is also altered by the addition or deletion of one or more amino acids, then the reference to the substitution will be understood to refer to the substitution for the alternate amino acid at the altered position number. A reference to a "non-naturally occurring Cys residue" in a polypeptide, e.g., an MHC class I polypeptide, means that the polypeptide comprises a Cys residue in a location where there is no Cys in the corresponding wild-type polypeptide. This can be accomplished through routine protein engineering in which a cysteine is substituted for the amino acid that occurs in the wild-type sequence.

[0050] A polynucleotide or polypeptide has a certain percent "sequence identity" to another polynucleotide or polypeptide, meaning that, when aligned, that percentage of bases or amino acids are the same, and in the same relative position, when comparing the two sequences. Sequence identity can be determined in a number of different ways. To determine sequence identity, sequences can be aligned using various convenient methods and computer programs (e.g., BLAST, T-COFFEE, MUSCLE, MAFFT, etc.), available over the world wide web at sites including ncbi.nlm.nih.gov/BLAST, ebi.ac.uk/Tools/msa/tcoffee/, ebi.ac.uk/Tools/msa/muscle/, mafft.cbrc.jp/alignment/software/. See, e.g., Altschul et al. (1990), J. Mol. Biol. 215:403-10. Unless otherwise stated, sequence identity is determined using the BLAST computer program.

[0051] The term "conservative amino acid substitution" refers to the interchangeability in proteins of amino acid residues having similar side chains. For example, a group of amino acids having

aliphatic side chains consists of glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains consists of serine and threonine; a group of amino acids having amide containing side chains consisting of asparagine and glutamine; a group of amino acids having aromatic side chains consists of phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains consists of lysine, arginine, and histidine; a group of amino acids having acidic side chains consists of glutamate and aspartate; and a group of amino acids having sulfur containing side chains consists of cysteine and methionine. Exemplary conservative amino acid substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine-glycine, and asparagine-glutamine.

[0052] The term “immunological synapse” or “immune synapse” as used herein generally refers to the natural interface between two interacting immune cells of an adaptive immune response including, e.g., the interface between an antigen-presenting cell (APC) or target cell and an effector cell, e.g., a lymphocyte, an effector T cell, a natural killer cell, and the like. An immunological synapse between an APC and a T cell is generally initiated by the interaction of a T cell antigen receptor and major histocompatibility complex molecules, e.g., as described in Bromley et al., *Annu Rev Immunol.* 2001;19:375-96; the disclosure of which is incorporated herein by reference in its entirety.

[0053] “T cell” includes all types of immune cells expressing CD3, including T-helper cells (CD4⁺ cells), cytotoxic T-cells (CD8⁺ cells), T-regulatory cells (Treg), and NK-T cells.

[0054] The term “immunomodulatory polypeptide” (also referred to as a “co-stimulatory polypeptide”), as used herein, includes a polypeptide on an antigen presenting cell (APC) (e.g., a dendritic cell, a B cell, and the like) that specifically binds a cognate co-immunomodulatory polypeptide on a T cell, thereby providing a signal which, in addition to the primary signal provided by, for instance, binding of a TCR/CD3 complex with a major histocompatibility complex (MHC) polypeptide loaded with peptide, mediates a T cell response, including, but not limited to, proliferation, activation, differentiation, and the like. An immunomodulatory polypeptide can include, but is not limited to, CD7, B7-1 (CD80), B7-2 (CD86), PD-L1, PD-L2, 4-1BBL, OX40L, Fas ligand (FasL), inducible costimulatory ligand (ICOS-L), intercellular adhesion molecule (ICAM), CD30L, CD40, CD70, CD83, HLA-G, MICA, MICB, HVEM, lymphotoxin beta receptor, 3/TR6, ILT3, ILT4, HVEM, an agonist or antibody that binds Toll ligand receptor and a ligand that specifically binds with B7-H3.

[0055] As noted above, an “immunomodulatory polypeptide” (also referred to herein as a “MOD”) specifically binds a cognate co-immunomodulatory polypeptide on a T cell.

[0056] An “immunomodulatory domain” (“MOD”) of a TMMP of the present disclosure binds a cognate co-immunomodulatory polypeptide, which may be present on a target T cell.

[0057] As used herein the term “in vivo” refers to any process or procedure occurring inside of the body.

[0058] As used herein, “in vitro” refers to any process or procedure occurring outside of the body.

[0059] “Heterologous,” as used herein, means a nucleotide or polypeptide that is not found in the native nucleic acid or protein, respectively.

[0060] “Recombinant,” as used herein, means that a particular nucleic acid (DNA or RNA) is the product of various combinations of cloning, restriction, polymerase chain reaction (PCR) and/or ligation steps resulting in a construct having a structural coding or non-coding sequence distinguishable from endogenous nucleic acids found in natural systems. DNA sequences encoding polypeptides can be assembled from cDNA fragments or from a series of synthetic oligonucleotides, to provide a synthetic nucleic acid which is capable of being expressed from a recombinant transcriptional unit contained in a cell or in a cell-free transcription and translation system.

[0061] The terms “recombinant expression vector,” or “DNA construct” are used interchangeably herein to refer to a DNA molecule comprising a vector and at least one insert. Recombinant expression vectors are usually generated for the purpose of expressing and/or propagating the insert(s), or for the construction of other recombinant nucleotide sequences. The insert(s) may or may not be operably linked to a promoter sequence and may or may not be operably linked to DNA regulatory sequences.

[0062] As used herein, the term “affinity” refers to the equilibrium constant for the reversible binding of two agents (e.g., an antibody and an antigen) and is expressed as a dissociation constant (K_D). As used herein, the term “avidity” refers to the resistance of a complex of two or more agents to dissociation after dilution. The terms “immunoreactive” and “preferentially binds” are used interchangeably herein with respect to antibodies and/or antigen-binding fragments.

[0063] The term “binding,” as used herein (e.g. with reference to binding of a TMMP to a polypeptide (e.g., a T-cell receptor) on a T cell), refers to a non-covalent interaction between two molecules. Non-covalent binding refers to a direct association between two molecules, due to, for example, electrostatic, hydrophobic, ionic, and/or hydrogen-bond interactions, including interactions such as salt bridges and water bridges. “Affinity” refers to the strength of non-covalent binding, increased binding affinity being correlated with a lower K_D . “Specific binding” generally refers to binding of a ligand to a moiety that is its designated binding site or receptor. “Non-specific binding” generally refers to binding of a ligand to a moiety other than its designated binding site or receptor. Covalent binding” or “covalent bond,” as used herein, refers to the formation of one or more covalent chemical binds between two different molecules.

[0064] The terms "treatment", "treating" and the like are used herein to generally mean obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. "Treatment" as used herein covers any treatment of a disease or symptom in a mammal, and includes: (a) preventing the disease or symptom from occurring in a subject which may be predisposed to acquiring the disease or symptom but has not yet been diagnosed as having it; (b) inhibiting the disease or symptom, i.e., arresting its development; and/or (c) relieving the disease, i.e., causing regression of the disease. The therapeutic agent may be administered before, during or after the onset of disease or injury. The treatment of ongoing disease, where the treatment stabilizes or reduces the undesirable clinical symptoms of the patient, is of particular interest. Such treatment is desirably performed prior to complete loss of function in the affected tissues. The subject therapy will desirably be administered during the symptomatic stage of the disease, and in some cases after the symptomatic stage of the disease.

[0065] The terms "individual," "subject," "host," and "patient," are used interchangeably herein and refer to any mammalian subject for whom diagnosis, treatment, or therapy is desired. Mammals include, e.g., humans, non-human primates, rodents (e.g., rats; mice), lagomorphs (e.g., rabbits), ungulates (e.g., cows, sheep, pigs, horses, goats, and the like), etc.

[0066] Unless indicated otherwise, the term "substantially" is intended to encompass both "wholly" and "largely but not wholly". For example, an Ig Fc that "substantially does not induce cell lysis" means an Ig Fc that induces no cell lysis at all or that largely does not induce cell lysis.

[0067] As used herein, the term "about" used in connection with an amount indicates that the amount can vary by 10% of the stated amount. For example, "about 100" means an amount of from 90-110. Where about is used in the context of a range, the "about" used in reference to the lower amount of the range means that the lower amount includes an amount that is 10% lower than the lower amount of the range, and "about" used in reference to the higher amount of the range means that the higher amount includes an amount 10% higher than the higher amount of the range. For example, from about 100 to about 1000 means that the range extends from 90 to 1100.

[0068] Before the present disclosure is further described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present disclosure will be limited only by the appended claims.

[0069] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the disclosure.

[0070] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present disclosure, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[0071] It must be noted that as used herein and in the appended claims, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to a “T-cell modulatory multimeric polypeptide” includes a plurality of such polypeptides and reference to “the immunomodulatory polypeptide” includes reference to one or more immunomodulatory polypeptides and equivalents thereof known to those skilled in the art, and so forth. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as “solely,” “only” and the like in connection with the recitation of claim elements, or use of a “negative” limitation.

[0072] It is appreciated that certain features of the disclosure, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments pertaining to the invention are specifically embraced by the present invention and are disclosed herein just as if each and every combination was individually and explicitly disclosed. In addition, all sub-combinations of the various embodiments and elements thereof are also specifically embraced by the present disclosure and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.

[0073] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present disclosure is not entitled to antedate such publication. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

DETAILED DESCRIPTION

[0074] The present disclosure provides T-cell modulatory multimeric polypeptides that comprise an immunomodulatory polypeptide (“MOD”) and that comprise an epitope-presenting Wilms tumor-1 (WT-1) peptide. A TMMP is useful for modulating the activity of a T cell, and for modulating an immune response in an individual.

T-CELL MODULATORY MULTIMERIC POLYPEPTIDES

[0075] The present disclosure provides a T-cell modulatory multimeric polypeptide (TMMP) comprising: a) a first polypeptide; and b) a second polypeptide, wherein the TMMP comprises an epitope; a first major histocompatibility complex (MHC) polypeptide; a second MHC polypeptide; one or more MODs; and optionally an immunoglobulin (Ig) Fc polypeptide or a non-Ig scaffold. The present disclosure provides a TMMP, wherein the TMMP is a heterodimer comprising: a) a first polypeptide comprising a first MHC polypeptide; and b) a second polypeptide comprising a second MHC polypeptide, wherein the first polypeptide or the second polypeptide comprises an epitope (e.g., a peptide that presents an epitope); wherein the first polypeptide and/or the second polypeptide comprises one or more MODs that can be the same or different; and optionally an Ig Fc polypeptide or a non-Ig scaffold. A TMMP of the present disclosure is also referred to herein as a “multimeric polypeptide of the present disclosure” or a “synTac.” In some cases, the peptide epitope present in a TMMP of the present disclosure is a WT-1 peptide.

[0076] The present disclosure provides a TMMP comprising a heterodimeric polypeptide comprising: a) a first polypeptide comprising: i) a peptide epitope; and ii) a first MHC polypeptide; b) a second polypeptide comprising a second MHC polypeptide; and c) at least one MOD, where the first and/or the second polypeptide comprises the at least one (i.e., one or more) MODs. Optionally, the first or the second polypeptide comprises an Ig Fc polypeptide or a non-Ig scaffold. At least one of the one or more MODs is a variant MOD that exhibits reduced affinity to a cognate co-immunomodulatory polypeptide (“co-MOD”) compared to the affinity of a corresponding wild-type MOD for the co-MOD. The epitope present in a TMMP binds to a T-cell receptor (TCR) on a T cell with an affinity of at least 100 μ M (e.g., at least 10 μ M, at least 1 μ M, at least 100 nM, at least 10 nM, or at least 1 nM). A TMMP binds to a first T cell with an affinity that is at least 25% higher than the affinity with which the TMMP binds a second T cell, where the first T cell expresses on its surface the cognate co-MOD and a TCR that binds the epitope with an affinity of at least 100 μ M, and where the second T cell expresses on its surface the cognate co-MOD but does not express on its surface a TCR that binds the epitope with an affinity of at least 100 μ M (e.g., at least 10 μ M, at least 1 μ M, at least 100 nM, at least 10 nM, or at least 1 nM). In some cases, the peptide epitope present in a TMMP is a WT-1 peptide.

[0077] The present disclosure provides a TMMP, wherein the TMMP is:

[0078] A) a heterodimer comprising: a) a first polypeptide comprising a first MHC polypeptide; and b) a second polypeptide comprising a second MHC polypeptide, wherein the first polypeptide or the second polypeptide comprises an epitope (e.g., a peptide that presents an epitope to a T cell); wherein the first polypeptide and/or the second polypeptide comprises one or more MODs that can be the same or different, and wherein at least one of the one or more MODs may be a wild-type MOD or a variant of a wild-type MOD, wherein the variant MOD comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid substitutions compared to the amino acid sequence of the corresponding wild-type MOD; and wherein the first polypeptide or the second polypeptide optionally comprises an Ig Fc polypeptide or a non-Ig scaffold; or

[0079] B) a heterodimer comprising: a) a first polypeptide comprising a first MHC polypeptide; and b) a second polypeptide comprising a second MHC polypeptide, wherein the first polypeptide or the second polypeptide comprises an epitope; wherein the first polypeptide and/or the second polypeptide comprises one or more MODs that can be the same or different,

[0080] wherein at least one of the one or more MODs is a variant of a wild-type MOD, wherein the variant MOD comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid substitutions compared to the amino acid sequence of the corresponding wild-type MOD,

[0081] wherein at least one of the one or more MODs is a variant MOD that exhibits reduced affinity to a cognate co-MOD compared to the affinity of a corresponding wild-type MOD for the cognate co-MOD, and wherein the epitope binds to a TCR on a T cell with an affinity of at least 10^{-7} M, such that: i) the TMMP polypeptide binds to a first T cell with an affinity that is at least 25% higher than the affinity with which the TMMP binds a second T cell, wherein the first T cell expresses on its surface the cognate co-MOD and a TCR that binds the epitope with an affinity of at least 10^{-7} M, and wherein the second T cell expresses on its surface the cognate co-MOD but does not express on its surface a TCR that binds the epitope with an affinity of at least 10^{-7} M; and/or ii) the ratio of the binding affinity of a control TMMP, wherein the control comprises a wild-type MOD, to a cognate co-MOD to the binding affinity of the TMMP comprising a variant of the wild-type MOD to the cognate co-MOD, when measured by bio-layer interferometry, is in a range of from 1.5:1 to 10^6 :1; and wherein the variant MOD comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid substitutions compared to the amino acid sequence of the corresponding wild-type MOD; and

[0082] wherein the first polypeptide or the second polypeptide optionally comprises an Ig Fc polypeptide or a non-Ig scaffold; or

[0083] C) a heterodimer comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) an epitope; ii) a first MHC polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a second MHC polypeptide; and ii) optionally an Ig Fc

polypeptide or a non-Ig scaffold, wherein the TMMP comprises one or more MODs that can be the same or different, wherein at least one of the one or more MOD is: A) at the C-terminus of the first polypeptide; B) at the N-terminus of the second polypeptide; C) at the C-terminus of the second polypeptide; or D) at the C-terminus of the first polypeptide and at the N-terminus of the second polypeptide, and wherein at least one of the one or more MODs may be a wild-type MOD or a variant of a wild-type MOD, wherein the variant MOD comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid substitutions compared to the amino acid sequence of the corresponding wild-type MOD; and

[0084] optionally wherein at least one of the one or more MODs is a variant MOD that exhibits reduced affinity to a cognate co-MOD compared to the affinity of a corresponding wild-type MOD for the cognate co-MOD, and wherein the epitope binds to a TCR on a T cell with an affinity of at least 10^{-7} M, such that: i) the TMMP binds to a first T cell with an affinity that is at least 25% higher than the affinity with which the TMMP binds a second T cell, wherein the first T cell expresses on its surface the cognate co-MOD and a TCR that binds the epitope with an affinity of at least 10^{-7} M, and wherein the second T cell expresses on its surface the cognate co-MOD but does not express on its surface a TCR that binds the epitope with an affinity of at least 10^{-7} M; and/or ii) the ratio of the binding affinity of a control TMMP, wherein the control comprises a wild-type MOD, to a cognate co-MOD to the binding affinity of the TMMP comprising a variant of the wild-type MOD to the cognate co-MOD, when measured by bio-layer interferometry, is in a range of from 1.5:1 to 10^6 :1; and wherein the variant MOD comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid substitutions compared to the amino acid sequence of the corresponding wild-type MOD. In some cases, the peptide epitope present in a TMMP is a WT-1 peptide.

[0085] The present disclosure provides a TMMP comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) an epitope; ii) a first MHC polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a second MHC polypeptide; and ii) optionally an Ig Fc polypeptide or a non-Ig scaffold. A TMMP comprises one or more MODs, wherein at least one of the one or more MODs is: A) at the C-terminus of the first polypeptide; B) at the N-terminus of the second polypeptide; C) at the C-terminus of the second polypeptide; or D) at the C-terminus of the first polypeptide and at the N-terminus of the second polypeptide. At least one of the one or more MODs is a variant MOD that exhibits reduced affinity to a cognate co-MOD compared to the affinity of a corresponding wild-type MOD for the cognate co-MOD. The epitope present in a TMMP binds to a T-cell receptor (TCR) on a T cell with an affinity of at least 100 μ M (e.g., at least 10 μ M, at least 1 μ M, at least 100 nM, at least 10 nM, or at least 1 nM). A TMMP binds to a first T cell with an affinity that is at least 25% higher than the affinity with which the TMMP binds a second T cell, where the first T cell expresses on its surface the cognate co-MOD and a TCR that binds the epitope with an affinity of at least

100 μ M, and where the second T cell expresses on its surface the cognate co-MOD but does not express on its surface a TCR that binds the epitope with an affinity of at least 100 μ M (e.g., at least 10 μ M, at least 1 μ M, at least 100 nM, at least 10 nM, or at least 1 nM).

[0086]

[0087] A MOD present in a TMMP binds to its cognate co-MOD with an affinity that it at least 10% less, at least 15% less, at least 20% less, at least 25% less, at least 30% less, at least 35% less, at least 40% less, at least 45% less, at least 50% less, at least 55% less, at least 60% less, at least 65% less, at least 70% less, at least 75% less, at least 80% less, at least 85% less, at least 90% less, at least 95% less, or more than 95% less, than the affinity of a corresponding wild-type MOD for the cognate co-MOD.

[0088] The combination of the reduced affinity of the MOD for its cognate co-MOD, and the affinity of the epitope for a TCR, provides for enhanced selectivity of a TMMP. For example, a TMMP of the present disclosure binds selectively to a first T cell that displays both: i) a TCR specific for the epitope present in the TMMP; and ii) a co-MOD that binds to the MOD present in the TMMP, compared to binding to a second T cell that displays: i) a TCR specific for an epitope other than the epitope present in the TMMP; and ii) a co-MOD that binds to the MOD present in the TMMP. For example, a TMMP of the present disclosure binds to the first T cell with an affinity that is at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 2-fold, at least 2.5-fold, at least 5-fold, at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, at least 100-fold, or more than 100-fold, higher than the affinity to which it binds the second T cell.

[0089] In some cases, a TMMP, when administered to an individual in need thereof, induces both an epitope-specific T cell response and an epitope non-specific T cell response. In other words, in some cases, a TMMP, when administered to an individual in need thereof, induces an epitope-specific T cell response by modulating the activity of a first T cell that displays both: i) a TCR specific for the epitope present in the TMMP; ii) a co-MOD that binds to the MOD present in the TMMP; and induces an epitope non-specific T cell response by modulating the activity of a second T cell that displays: i) a TCR specific for an epitope other than the epitope present in the TMMP; and ii) a co-MOD that binds to the MOD present in the TMMP. The ratio of the epitope-specific T cell response to the epitope-non-specific T cell response is at least 2:1, at least 5:1, at least 10:1, at least 15:1, at least 20:1, at least 25:1, at least 50:1, or at least 100:1. The ratio of the epitope-specific T cell response to the epitope-non-specific T cell response is from about 2:1 to about 5:1, from about 5:1 to about 10:1, from about 10:1 to about 15:1, from about 15:1 to about 20:1, from about 20:1 to about 25:1, from about 25:1 to about 50:1, or from about 50:1 to about 100:1, or more than 100:1. "Modulating the activity" of a T cell can include one or more of: i) activating a cytotoxic (e.g., CD8⁺) T cell; ii) inducing cytotoxic activity of a cytotoxic

(e.g., CD8⁺) T cell; iii) inducing production and release of a cytotoxin (e.g., a perforin; a granzyme; a granulysin) by a cytotoxic (e.g., CD8⁺) T cell; iv) inhibiting activity of an autoreactive T cell; and the like.

[0090] The combination of the reduced affinity of the MOD for its cognate co-MOD, and the affinity of the epitope for a TCR, provides for enhanced selectivity of a TMMP. Thus, for example, a TMMP binds with higher avidity to a first T cell that displays both: i) a TCR specific for the epitope present in the TMMP; and ii) a co-MOD that binds to the MOD present in the TMMP, compared to the avidity to which it binds to a second T cell that displays: i) a TCR specific for an epitope other than the epitope present in the TMMP; and ii) a co-MOD that binds to the MOD present in the TMMP.

[0091] Binding affinity between a MOC and its cognate co-MOD can be determined by bio-layer interferometry (BLI) using purified MOD and purified cognate co-MOD. Binding affinity between a TMMP and its cognate co-MOD can be determined by BLI using purified TMMP and the cognate co-MOD. BLI methods are well known to those skilled in the art. See, e.g., Lad et al. (2015) *J. Biomol. Screen.* 20(4):498-507; and Shah and Duncan (2014) *J. Vis. Exp.* 18:e51383.

[0092] A BLI assay can be carried out using an Octet RED 96 (Pal FortéBio) instrument, or a similar instrument, as follows. A TMMP (e.g., a TMMP of the present disclosure; a control TMMP (where a control TMMP comprises a wild-type immunomodulatory polypeptide)) is immobilized onto an insoluble support (a “biosensor”). The immobilized TMMP is the “target.” Immobilization can be effected by immobilizing a capture antibody onto the insoluble support, where the capture antibody immobilizes the TMMP. For example, immobilization can be effected by immobilizing anti-Fc (e.g., anti-human IgG Fc) antibodies onto the insoluble support, where the immobilized anti-Fc antibodies bind to and immobilize the TMMP (where the TMMP comprises an IgFc polypeptide). A co-immunomodulatory polypeptide is applied, at several different concentrations, to the immobilized TMMP, and the instrument’s response recorded. Assays are conducted in a liquid medium comprising 25mM HEPES pH 6.8, 5% poly(ethylene glycol) 6000, 50 mM KCl, 0.1% bovine serum albumin, and 0.02% Tween 20 nonionic detergent. Binding of the co-immunomodulatory polypeptide to the immobilized TMMP is conducted at 30°C. As a positive control for binding affinity, an anti-MHC Class I monoclonal antibody can be used. For example, anti-HLA Class I monoclonal antibody W6/32 (American Type Culture Collection No. HB-95; Parham et al. (1979) *J. Immunol.* 123:342), which has a K_D of 7 nM, can be used. A standard curve can be generated using serial dilutions of the anti-MHC Class I monoclonal antibody. The co-immunomodulatory polypeptide, or the anti-MHC Class I mAb, is the “analyte.” BLI analyzes the interference pattern of white light reflected from two surfaces: i) from the immobilized polypeptide (“target”); and ii) an internal reference layer. A change in the number of molecules (“analyte”; e.g., co-immunomodulatory polypeptide; anti-HLA antibody) bound to the biosensor tip causes a shift in the interference pattern; this shift in interference pattern can be measured

in real time. The two kinetic terms that describe the affinity of the target/analyte interaction are the association constant (k_a) and dissociation constant (k_d). The ratio of these two terms (k_d/k_a) gives rise to the affinity constant K_D .

[0093] The BLI assay is carried out in a multi-well plate. To run the assay, the plate layout is defined, the assay steps are defined, and biosensors are assigned in Octet Data Acquisition software. The biosensor assembly is hydrated. The hydrated biosensor assembly and the assay plate are equilibrated for 10 minutes on the Octet instrument. Once the data are acquired, the acquired data are loaded into the Octet Data Analysis software. The data are processed in the Processing window by specifying method for reference subtraction, y-axis alignment, inter-step correction, and Savitzky-Golay filtering. Data are analyzed in the Analysis window by specifying steps to analyze (Association and Dissociation), selecting curve fit model (1:1), fitting method (global), and window of interest (in seconds). The quality of fit is evaluated. K_D values for each data trace (analyte concentration) can be averaged if within a 3-fold range. K_D error values should be within one order of magnitude of the affinity constant values; R^2 values should be above 0.95. See, e.g., Abdiche et al. (2008) *J. Anal. Biochem.* 377:209.

[0094] Unless otherwise stated herein, the affinity of a TMMP of the present disclosure for a cognate co-immunomodulatory polypeptide, or the affinity of a control TMMP (where a control TMMP comprises a wild-type immunomodulatory polypeptide) for a cognate co-immunomodulatory polypeptide, is determined using BLI, as described above.

[0095] In some cases, the ratio of: i) the binding affinity of a control TMMP (where the control comprises a wild-type immunomodulatory polypeptide) to a cognate co-immunomodulatory polypeptide to ii) the binding affinity of a TMMP of the present disclosure comprising a variant of the wild-type immunomodulatory polypeptide to the cognate co-immunomodulatory polypeptide, when measured by BLI (as described above), is at least 1.5:1, at least 2:1, at least 5:1, at least 10:1, at least 15:1, at least 20:1, at least 25:1, at least 50:1, at least 100:1, at least 500:1, at least 10^2 :1, at least 5×10^2 :1, at least 10^3 :1, at least 5×10^3 :1, at least 10^4 :1, at least 10^5 :1, or at least 10^6 :1. In some cases, the ratio of: i) the binding affinity of a control TMMP (where the control comprises a wild-type immunomodulatory polypeptide) to a cognate co-immunomodulatory polypeptide to ii) the binding affinity of a TMMP of the present disclosure comprising a variant of the wild-type immunomodulatory polypeptide to the cognate co-immunomodulatory polypeptide, when measured by BLI, is in a range of from 1.5:1 to 10^6 :1, e.g., from 1.5:1 to 10:1, from 10:1 to 50:1, from 50:1 to 10^2 :1, from 10^2 :1 to 10^3 :1, from 10^3 :1 to 10^4 :1, from 10^4 :1 to 10^5 :1, or from 10^5 :1 to 10^6 :1.

[0096] As an example, where a control TMMP comprises a wild-type IL-2 polypeptide, and where a TMMP of the present disclosure comprises a variant IL-2 polypeptide (comprising from 1 to 10 amino acid substitutions relative to the amino acid sequence of the wild-type IL-2 polypeptide) as the immunomodulatory polypeptide, the ratio of: i) the binding affinity of the control TMMP to an IL-2

receptor (i.e., the cognate co-immunomodulatory polypeptide) to ii) the binding affinity of the TMMP of the present disclosure to the IL-2 receptor, when measured by BLI, is at least 1.5:1, at least 2:1, at least 5:1, at least 10:1, at least 15:1, at least 20:1, at least 25:1, at least 50:1, at least 100:1, at least 500:1, at least 10^2 :1, at least 5×10^2 :1, at least 10^3 :1, at least 5×10^3 :1, at least 10^4 :1, at least 10^5 :1, or at least 10^6 :1. In some cases, where a control TMMP comprises a wild-type IL-2 polypeptide, and where a TMMP of the present disclosure comprises a variant IL-2 polypeptide (comprising from 1 to 10 amino acid substitutions relative to the amino acid sequence of the wild-type IL-2 polypeptide) as the immunomodulatory polypeptide, the ratio of: i) the binding affinity of the control TMMP to an IL-2 receptor (i.e., the cognate co-immunomodulatory polypeptide) to ii) the binding affinity of the TMMP of the present disclosure to the IL-2 receptor, when measured by BLI, is in a range of from 1.5:1 to 10^6 :1, e.g., from 1.5:1 to 10:1, from 10:1 to 50:1, from 50:1 to 10^2 :1, from 10^2 :1 to 10^3 :1, from 10^3 :1 to 10^4 :1, from 10^4 :1 to 10^5 :1, or from 10^5 :1 to 10^6 :1.

[0097] Binding affinity of a TMMP of the present disclosure to a target T cell can be measured according to the procedure describes in published PCT application WO 2019/051091, published March 14, 2019. See [0063].

[0098] In some cases, when measured as described in the preceding paragraph, a TMMP of the present disclosure exhibits selective binding to target T-cell, compared to binding of the TMMP library member to a control T cell that comprises: i) the cognate co-immunomodulatory polypeptide that binds the parental wild-type immunomodulatory polypeptide; and ii) a T-cell receptor that binds to an epitope other than the epitope present in the TMMP library member.

Dimerized TMMPs

[0099] A TMMP of the present disclosure can be dimerized; i.e., the present disclosure provides a multimeric polypeptide comprising a dimer of a TMMP of the present disclosure. Thus, the present disclosure provides a TMMP comprising: A) a first heterodimer comprising: a) a first polypeptide comprising: i) a peptide epitope; and ii) a first MHC polypeptide; and b) a second polypeptide comprising: i) a second MHC polypeptide, wherein the first heterodimer comprises one or more MODs; and B) a second heterodimer comprising: a) a first polypeptide comprising: i) a peptide epitope; and ii) a first MHC polypeptide; and b) a second polypeptide comprising: i) a second MHC polypeptide, wherein the second heterodimer comprises one or more MODs, and wherein the first heterodimer and the second heterodimer are covalently linked to one another. In some cases, the two TMMPs are identical to one another in amino acid sequence. In some cases, the first heterodimer and the second heterodimer are covalently linked to one another via a C-terminal region of the second polypeptide of the first heterodimer and a C-terminal region of the second polypeptide of the second heterodimer. In some cases, first heterodimer and the second heterodimer are covalently linked to one another via the C-terminal amino acid of the second polypeptide of the first heterodimer and the C-terminal region of the second

polypeptide of the second heterodimer; for example, in some cases, the C-terminal amino acid of the second polypeptide of the first heterodimer and the C-terminal region of the second polypeptide of the second heterodimer are linked to one another, either directly or via a linker. The linker can be a peptide linker. The peptide linker can have a length of from 1 amino acid to 200 amino acids (e.g., from 1 amino acid (aa) to 5 aa, from 5 aa to 10 aa, from 10 aa to 25 aa, from 25 aa to 50 aa, from 50 aa to 100 aa, from 100 aa to 150 aa, or from 150 aa to 200 aa). In some cases, the peptide epitope of the first heterodimer and the peptide epitope of the second heterodimer comprise the same amino acid sequence. In some cases, the first MHC polypeptide of the first and the second heterodimer is an MHC Class I β 2-microglobulin, and wherein the second MHC polypeptide of the first and the second heterodimer is an MHC Class I heavy chain. In some cases, the MODs of the first heterodimer and the MODs of the second heterodimer comprise the same amino acid sequence. In some cases, the MOD(s) of the first heterodimer and the MOD(s) of the second heterodimer are variant MODs that comprise from 1 to 10 amino acid substitutions compared to a corresponding parental wild-type MOD, and wherein the from 1 to 10 amino acid substitutions result in reduced affinity binding of the variant immunomodulatory polypeptide to a cognate co-immunomodulatory polypeptide. In some cases, the immunomodulatory polypeptide of the first heterodimer and the immunomodulatory polypeptide of the second heterodimer are each independently selected from the group consisting of IL-2, 4-1BBL, PD-L1, CD80, CD86, ICOS-L, OX-40L, FasL, JAG1 (CD339), TGF β , CD70, and ICAM. Examples, of suitable MHC polypeptides, MODs, and peptide epitopes are described below.

MHC polypeptides

[00100] As noted above, a TMMP of the present disclosure includes MHC polypeptides. For the purposes of the instant disclosure, the term “major histocompatibility complex (MHC) polypeptides” is meant to include MHC polypeptides of various species, including human MHC (also referred to as human leukocyte antigen (HLA)) polypeptides, rodent (e.g., mouse, rat, etc.) MHC polypeptides, and MHC polypeptides of other mammalian species (e.g., lagomorphs, non-human primates, canines, felines, ungulates (e.g., equines, bovines, ovines, caprines, etc.), and the like. The term “MHC polypeptide” is meant to include Class I MHC polypeptides (e.g., β -2 microglobulin and MHC class I heavy chain).

[00101] In some cases, the first MHC polypeptide is an MHC Class I β 2M (β 2M) polypeptide, and the second MHC polypeptide is an MHC Class I heavy chain (H chain) (“MHC-H”). In other instances, the first MHC polypeptide is an MHC Class I heavy chain polypeptide; and the second MHC polypeptide is a β 2M polypeptide. In some cases, both the β 2M and MHC-H chain are of human origin; i.e., the MHC-H chain is an HLA heavy chain, or a variant thereof. Unless expressly stated otherwise, a TMMP of the present disclosure does not include membrane anchoring domains (transmembrane regions) of an MHC Class I heavy chain, or a part of MHC Class I heavy chain sufficient to anchor the resulting TMMP to a cell (e.g., eukaryotic cell such as a mammalian cell) in which it is expressed. In

some cases, the MHC Class I heavy chain present in a TMMP of the present disclosure does not include a signal peptide, a transmembrane domain, or an intracellular domain (cytoplasmic tail) associated with a native MHC Class I heavy chain. Thus, e.g., in some cases, the MHC Class I heavy chain present in a TMMP of the present disclosure includes only the $\alpha 1$, $\alpha 2$, and $\alpha 3$ domains of an MHC Class I heavy chain. In some cases, the MHC Class I heavy chain present in a TMMP has a length of from about 270 amino acids (aa) to about 290 aa. In some cases, the MHC Class I heavy chain present in a TMMP has a length of 270 aa, 271 aa, 272 aa, 273 aa, 274 aa, 275 aa, 276 aa, 277 aa, 278 aa, 279 aa, 280 aa, 281 aa, 282 aa, 283 aa, 284 aa, 285 aa, 286 aa, 287 aa, 288 aa, 289 aa, or 290 aa.

[00102] In some cases, an MHC polypeptide of a TMMP is a human MHC polypeptide, where human MHC polypeptides are also referred to as “human leukocyte antigen” (“HLA”) polypeptides. In some cases, an MHC polypeptide of a TMMP is a Class I HLA polypeptide, e.g., a $\beta 2$ -microglobulin polypeptide, or a Class I HLA heavy chain polypeptide. Class I HLA heavy chain polypeptides include HLA-A heavy chain polypeptides, HLA-B heavy chain polypeptides, HLA-C heavy chain polypeptides, HLA-E heavy chain polypeptides, HLA-F heavy chain polypeptides, and HLA-G heavy chain polypeptides.

MHC Class I heavy chains

[00103] In some cases, an MHC Class I heavy chain polypeptide present in a TMMP comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to all or part (e.g., 50, 75, 100, 150, 200, or 250 contiguous amino acids) of the amino acid sequence of any of the human HLA heavy chain polypeptides depicted in **FIGs. 7-13**. In some cases, the MHC Class I heavy chain has a length of 270 aa, 271 aa, 272 aa, 273 aa, 274 aa, 275 aa, 276 aa, 277 aa, 278 aa, 279 aa, 280 aa, 281 aa, 282 aa, 283 aa, 284 aa, 285 aa, 286 aa, 287 aa, 288 aa, 289 aa, or 290 aa. In some cases, an MHC Class I heavy chain polypeptide present in a TMMP comprises 1-30, 1-5, 5-10, 10-15, 15-20, 20-25 or 25-30 amino acid insertions, deletions, and/or substitutions (in addition to those locations indicated as being variable in the heavy chain consensus sequences) of any one of the amino acid sequences depicted in **FIGs 7-13**. In some cases, the MHC Class I heavy chain does not include transmembrane or cytoplasmic domains. As an example, a MHC Class I heavy chain polypeptide of a TMMP can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to amino acids 25-300 (lacking all, or substantially all, of the leader, transmembrane and cytoplasmic sequence) or amino acids 25-365 (lacking the leader) of a human HLA-A heavy chain polypeptides depicted in any one of **FIG. 7A, 7B, and 7C**.

[00104] FIGs. 7A, 7B and 7C provide amino acid sequences of human leukocyte antigen (HLA) Class I heavy chain polypeptides. Signal sequences, amino acids 1-24, are bolded and underlined. FIG. 7A entry: 3A.1 is the HLA-A heavy chain (HLA-A*01:01:01:01 or A*0101) (NCBI accession

NP_001229687.1), SEQ ID NO:23; entry 3A.2 is from HLA-A*1101 SEQ ID NO:24; entry 3A.3 is from HLA-A*2402 SEQ ID NO:25 and entry 3A.4 is from HLA-A*3303 SEQ ID NO:26. FIG. 7B provides the sequence HLA-B*07:02:01 (HLA-B*0702) NCBI GenBank Accession NP_005505.2 (see also GenBank Accession AUV50118.1.). FIG. 7C provides the sequence HLA-C*0701 (GenBank Accession NP_001229971.1) (HLA-C*07:01:01:01 or HLA-Cw*070101, HLA-Cw*07 see GenBank Accession CAO78194.1).

[00105] **FIG. 8** provides an alignment of eleven mature MHC class I heavy chain amino acid sequences without their leader sequences or transmembrane domains or intracellular domains. The aligned sequences are human HLA-A, HLA-B, and HLA-C, a mouse H2K protein sequence, three variants of HLA-A (var.1, var. 2C, and var.2CP), and 3 human HLA-A variants (HLA-A*1101; HLA-A*2402; and HLA-A*3303). Indicated in the alignment are the locations (84 and 139 of the mature proteins) where cysteine residues may be introduced (e.g., by substitution) for the formation of a disulfide bond to stabilize the MHC H chain - β 2M complex. Also shown in the alignment is position 236 (of the mature polypeptide), which may be substituted by a cysteine residue that can form an inter-chain disulfide bond with β 2M (e.g., at aa 12). An arrow appears above each of those locations and the residues are bolded. The seventh HLA-A sequence shown in the alignment (var. 2c), shows the sequence of variant 2 substituted with C residues at positions 84, 139 and 236. The boxes flanking residues 84, 139 and 236 show the groups of five amino acids on either sides of those six sets of five residues, denoted aac1 (for “amino acid cluster 1”), aac2 (for “amino acid cluster 2”), aac3 (for “amino acid cluster 3”), aac4 (for “amino acid cluster 4”), aac5 (for “amino acid cluster 5”), and aac6 (for “amino acid cluster 6”), that may be replaced by 1 to 5 amino acids selected independently from (i) any naturally occurring amino acid or (ii) any naturally occurring amino acid except proline or glycine.

[00106] With regard to **FIG. 8**, in some cases: i) aac1 (amino acid cluster 1) may be the amino acid sequence GTLRG (SEQ ID NO:287) or that sequence with one or two amino acids deleted or substituted with other naturally occurring amino acids (e.g., L replaced by I, V, A or F); ii) aac2 (amino acid cluster 2) may be the amino acid sequence YNQSE (SEQ ID NO:288) or that sequence with one or two amino acids deleted or substituted with other naturally occurring amino acids (e.g., N replaced by Q, Q replaced by N, and/or E replaced by D); iii) aac3 (amino acid cluster 3) may be the amino acid sequence TAADM (SEQ ID NO:289) or that sequence with one or two amino acids deleted or substituted with other naturally occurring amino acids (e.g., T replaced by S, A replaced by G, D replaced by E, and/or M replaced by L, V, or I); iv) aac4 (amino acid cluster 4) may be the amino acid sequence AQTTK (SEQ ID NO:290) or that sequence with one or two amino acids deleted or substituted with other naturally occurring amino acids (e.g., A replaced by G, Q replaced by N, or T replaced by S, and/or K replaced by R or Q); v) aac5 (amino acid cluster 5) may be the amino acid sequence VETRP (SEQ ID NO:291) or that sequence with one or two amino acids deleted or substituted with other

naturally occurring amino acids (*e.g.*, V replaced by I or L, E replaced by D, T replaced by S, and/or R replaced by K); and/or vi) aac6 (amino acid cluster 6) may be the amino acid sequence GDGTF (SEQ ID NO:292) or that sequence with one or two amino acids deleted or substituted with other naturally occurring amino acids (*e.g.*, D replaced by E, T replaced by S, or F replaced by L, W, or Y).

[00107] **FIGS. 9-11** provide alignments of mature HLA class I heavy chain amino acid sequences (without leader sequences or transmembrane domains or intracellular domains). The aligned amino acid sequences in **FIG. 9A** are HLA-A class I heavy chains of the following alleles: A*0101, A*0201, A*0301, A*1101, A*2301, A*2402, A*2407, A*3303, and A*3401. The aligned amino acid sequences in **FIG. 10A** are HLA-B class I heavy chains of the following alleles: B*0702, B*0801, B*1502, B*3802, B*4001, B*4601, and B*5301. The aligned amino acid sequences in **FIG. 11A** are HLA-C class I heavy chains of the following alleles: C*0102, C*0303, C*0304, C*0401, C*0602, C*0701, C*0801, and C*1502. Indicated in the alignments are the locations (84 and 139 of the mature proteins) where cysteine residues may be introduced (*e.g.*, by substitution) for the formation of a disulfide bond to stabilize the HLA H chain - β 2M complex. Also shown in the alignment is position 236 (of the mature polypeptide), which may be substituted by a cysteine residue that can form an inter-chain disulfide bond with β 2M (*e.g.*, at aa 12). The boxes flanking residues 84, 139 and 236 show the groups of five amino acids on either sides of those six sets of five residues, denoted aac1 (for “amino acid cluster 1”), aac2 (for “amino acid cluster 2”), aac3 (for “amino acid cluster 3”), aac4 (for “amino acid cluster 4”), aac5 (for “amino acid cluster 5”), and aac6 (for “amino acid cluster 6”), that may be replaced by 1 to 5 amino acids selected independently from (i) any naturally occurring amino acid or (ii) any naturally occurring amino acid except proline or glycine.

[00108] **FIGS. 9A, 10A, and 11A** provide alignments of the amino acid sequences of mature HLA-A, -B, and -C class I heavy chains, respectively. The sequences are provided for the extracellular portion of the mature protein (without leader sequences or transmembrane domains or intracellular domains). As described in **FIG. 8**, the positions of aa residues 84, 139, and 236 and their flanking residues (aac1 to aac6) that may be replaced by 1 to 5 amino acids selected independently from (i) any naturally occurring amino acid or (ii) any naturally occurring amino acid except proline or glycine are also shown. **FIG. 9B, 10B, and 11B** provide consensus amino acid sequences for the HLA-A, -B, and -C sequences, respectively, provide in **FIG. 9A, 10A, and 11A**. The consensus sequences show the variable amino acid positions as “X” residues sequentially numbered and the locations of amino acids 84, 139 and 236 double underlined.

[00109] With regard to **FIG. 9A**, in some cases: i) aac1 (amino acid cluster 1) may be the amino acid sequence GTLRG (SEQ ID NO:287) or that sequence with one or two amino acids deleted or substituted with other naturally occurring amino acids (*e.g.*, L replaced by I, V, A or F); ii) aac2 (amino acid cluster 2) may be the amino acid sequence YNQSE (SEQ ID NO:288) or that sequence with one or

two amino acids deleted or substituted with other naturally occurring amino acids (*e.g.*, N replaced by Q, Q replaced by N, and/or E replaced by D); iii) aac3 (amino acid cluster 3) may be the amino acid sequence TAADM (SEQ ID NO:289) or that sequence with one or two amino acids deleted or substituted with other naturally occurring amino acids (*e.g.*, T replaced by S, A replaced by G, D replaced by E, and/or M replaced by L, V, or I); iv) aac4 (amino acid cluster 4) may be the amino acid sequence AQTTK (SEQ ID NO:290) or that sequence with one or two amino acids deleted or substituted with other naturally occurring amino acids (*e.g.*, A replaced by G, Q replaced by N, or T replaced by S, and or K replaced by R or Q); v) aac5 (amino acid cluster 5) may be the amino acid sequence VETRP (SEQ ID NO:291) or that sequence with one or two amino acids deleted or substituted with other naturally occurring amino acids (*e.g.*, V replaced by I or L, E replaced by D, T replaced by S, and/or R replaced by K); and/or vi) aac6 (amino acid cluster 6) may be the amino acid sequence GDGTF (SEQ ID NO:292) or that sequence with one or two amino acids deleted or substituted with other naturally occurring amino acids (*e.g.*, D replaced by E, T replaced by S, or F replaced by L, W, or Y).

[00110] With regard to **FIG. 10A**, in some cases: i) aac1 (amino acid cluster 1) may be the amino acid sequence RNLRG (SEQ ID NO:293) or that sequence with one or two amino acids deleted or substituted with other naturally occurring amino acids (*e.g.*, N replaced by T or I; and/or L replaced by A; and/or the second R replaced by L; and/or the G replaced by R); ii) aac2 (amino acid cluster 2) may be the amino acid sequence YNQSE (SEQ ID NO:288) or that sequence with one or two amino acids deleted or substituted with other naturally occurring amino acids (*e.g.*, N replaced by Q, Q replaced by N, and/or E replaced by D); iii) aac3 (amino acid cluster 3) may be the amino acid sequence TAADT (SEQ ID NO:294) or that sequence with one or two amino acids deleted or substituted with other naturally occurring amino acids (*e.g.*, the first T replaced by S; and/or A replaced by G; and/or D replaced by E; and/or the second T replaced by S); iv) aac4 (amino acid cluster 4) may be the amino acid sequence AQITQ (SEQ ID NO:295) or that sequence with one or two amino acids deleted or substituted with other naturally occurring amino acids (*e.g.*, A replaced by G; and/or the first Q replaced by N; and/or I replaced by L or V; and/or the T replaced by S; and/or the second Q replaced by N); v) aac5 (amino acid cluster 5) may be the amino acid sequence VETRP (SEQ ID NO:291) or that sequence with one or two amino acids deleted or substituted with other naturally occurring amino acids (*e.g.*, V replaced by I or L, E replaced by D, T replaced by S, and/or R replaced by K); and/or vi) aac6 (amino acid cluster 6) may be the amino acid sequence GDRTF (SEQ ID NO:296) or that sequence with one or two amino acids deleted or substituted with other naturally occurring amino acids (*e.g.*, D replaced by E; and/or T replaced by S; and/or R replaced by K or H; and/or F replaced by L, W, or Y).

[00111] With regard to **FIG. 11A**, in some cases: i) aac1 (amino acid cluster 1) may be the amino acid sequence RNLRG (SEQ ID NO:293) or that sequence with one or two amino acids deleted or substituted with other naturally occurring amino acids (*e.g.*, N replaced by K; and/or L replaced by A or

I; and/or the second R replaced by H; and/or the G replaced by T or S); ii) aac2 (amino acid cluster 2) may be the amino acid sequence YNQSE (SEQ ID NO:288) or that sequence with one or two amino acids deleted or substituted with other naturally occurring amino acids (*e.g.*, N replaced by Q, Q replaced by N, and/or E replaced by D); iii) aac3 (amino acid cluster 3) may be the amino acid sequence TAADT (SEQ ID NO:294) or that sequence with one or two amino acids deleted or substituted with other naturally occurring amino acids (*e.g.*, the first T replaced by S; and/or A replaced by G; and/or D replaced by E; and/or the second T replaced by S); iv) aac4 (amino acid cluster 4) may be the amino acid sequence AQITQ (SEQ ID NO:295) or that sequence with one or two amino acids deleted or substituted with other naturally occurring amino acids (*e.g.*, A replaced by G; and/or the first Q replaced by N; and/or I replaced by L; and/or the second Q replaced by N or K); v) aac5 (amino acid cluster 5) may be the amino acid sequence VETRP (SEQ ID NO:291) or that sequence with one or two amino acids deleted or substituted with other naturally occurring amino acids (*e.g.*, V replaced by I or L, E replaced by D, T replaced by S, and/or R replaced by K or H); and/or vi) aac6 (amino acid cluster 6) may be the amino acid sequence GDGTF (SEQ ID NO:292) or that sequence with one or two amino acids deleted or substituted with other naturally occurring amino acids (*e.g.*, D replaced by E; and/or T replaced by S; and/or F replaced by L, W, or Y).

HLA-A

[00112] In some cases, a TMMP comprises an HLA-A heavy chain polypeptide. The HLA-A heavy chain peptide sequences, or portions thereof, that may be that may be incorporated into a TMMP of the present disclosure include, but are not limited to, the alleles: A*0101, A*0201, A*0301, A*1101, A*2301, A*2402, A*2407, A*3303, and A*3401, which are aligned without all, or substantially all, of the leader, transmembrane and cytoplasmic sequences in **FIG. 9A**. Any of those alleles may comprise a mutation at one or more of positions 84, 139 and/or 236 (as shown in **FIG. 9A**) selected from: a tyrosine to alanine at position 84 (Y84A); a tyrosine to cysteine at position 84 (Y84C); an alanine to cysteine at position 139 (A139C); and an alanine to cysteine substitution at position 236 (A236C). In addition, HLA-A sequence having at least 75% (*e.g.*, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%) or 100% amino acid sequence identity to all or part (*e.g.*, 50, 75, 100, 150, 200, or 250 contiguous amino acids) of the sequence of those HLA-A alleles may also be employed (*e.g.*, it may comprise 1-25, 1-5, 5-10, 10-15, 15-20, 20-25, or 25-30 amino acid insertions, deletions, and/or substitutions).

[00113] In some cases, a TMMP comprises an HLA-A heavy chain polypeptide comprising the following HLA-A consensus amino acid sequence:

[00114] GSHSMRYFX1TSVSRPGRGEPFIAGVGYVDDTQFVRFDSDAASQX2MEPRAPWIEQEGPEYWDX3X4TX5X6X7KX8SQX9X10RX11X12LX13X14X15X16X17YYNQSEX18GSHTX19QX20MX21GCDVGX22DX23RFLRGYX24QX25AYDGKDYIALX26EDLRSWTAADMAAQX27T

X287X29KWEX**X30X31X32**EAEQ**X33RX34**YL**X35GX36**CV**X37X38**LRRYLENGKETLQRTD**X39**PK
 THMTHH**X40X41**SDHEATLRCWAL**X42**FYPAEITLTWQRDGEDQTQDTELVETRP**A**GDGTFQK
 W**X43**VVVPSG**X44**EQRYTCHVQHEGLPKPLTLRW**X45** (SEQ ID NO:29), wherein X1 is F, Y, S,
 or T; X2 is K or R; X3 is Q, G, E, or R; X4 is N or E; X5 is R or G; X6 is N or K; X7 is M or V; X8 is H
 or Q; X9 is T or I; X10 is D or H; X11 is A, V, or E; X12 is N or D; X13 is G or R; X14 is T or I; X15 is
 L or A; X16 is R or L; X17 is G or R; X18 is A or D; X19 is I, L, or V; X20 is I, R or M; X21 is F or Y;
 X22 is S or P; X23 is W or G; X24 is R, H, or Q; X25 is D or Y; X26 is N or K; X27 is T or I; X28 is K
 or Q; X29 is R or H; X30 is A or T; X31 is A or V; X32 is H or R; X33 is R, L, Q, or W; X34 is V or A;
 X35 is D or E; X36 is R or T; X37 is D or E; X38 is W or G; X39 is P or A; X40 is P or A; X41 is V or I;
 X42 is S or G; X43 is A or S; X44 is Q or E; and X45 is P or L.

[00115] As one example, an MHC Class I heavy chain polypeptide of a TMMP can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following human HLA-A heavy chain amino acid sequence:

GSLSMRYFFTSVSRPGRGEPRFIAVGYYVDDTQFVRFSDAASQRMEPRAPWIEQEGPEYWDGET
 RKKVKAHSQTHRVDLGLTRGYYNQSEAGSHTVQRMYGCDVGSDWRFLRGYHQYAYDGKDYIA
 LKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRYLENGKETLQRTDAPK
 THMTHHAVSDHEATLRCWALSFPYAEITLTWQRDGEDQTQDTELVETRPAGDGTFQKWAAVV
 VPSGQEQRYTCHVQHEGLPKPLTLRWEP (SEQ ID NO:44).

[00116] In some cases, an HLA-A heavy chain polypeptide suitable for inclusion in a TMMP comprises the following amino acid sequence:

GSLSMRYFFTSVSRPGRGEPRFIAVGYYVDDTQFVRFSDAASQRMEPRAPWIEQEGPEYWDGET
 RKKVKAHSQTHRVDLGLTRGYYNQSEAGSHTVQRMYGCDVGSDWRFLRGYHQYAYDGKDYIA
 LKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRYLENGKETLQRTDAPK
 THMTHHAVSDHEATLRCWALSFPYAEITLTWQRDGEDQTQDTELVETRPAGDGTFQKWAAVV
 VPSGQEQRYTCHVQHEGLPKPLTLRWEP (SEQ ID NO:44). This HLA-A heavy chain polypeptide

is also referred to as “HLA-A*0201” or simply “HLA-A02.” In some cases, the C-terminal Pro is not included in a TMMP. For example, in some cases, an HLA-A02 polypeptide suitable for inclusion in a TMMP comprises the following amino acid sequence:

GSLSMRYFFTSVSRPGRGEPRFIAVGYYVDDTQFVRFSDAASQRMEPRAPWIEQEGPEYWDGET
 RKKVKAHSQTHRVDLGLTRGYYNQSEAGSHTVQRMYGCDVGSDWRFLRGYHQYAYDGKDYIA
 LKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRYLENGKETLQRTDAPK
 THMTHHAVSDHEATLRCWALSFPYAEITLTWQRDGEDQTQDTELVETRPAGDGTFQKWAAVV
 VPSGQEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:449).

HLA-A (Y84C; A236C)

[00117] In some cases, an HLA-A heavy chain polypeptide suitable for inclusion in a TMMP comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following human HLA-A heavy chain (Y84C; A236C) amino acid sequence:

GSISMRYFFTSVSRPGRGEPRFIAVGYYDDTQFVRFSDAASQRMEPRAPWIEQEGPEYWDGET
RKVKAHSQTHRVDLGTLRGCYNQSEAGSHTVQRMYGCDVGSDWRFLRGYHQYAYDGKDYIA
LKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRYLENGKETLQRTDAPK
THMTHHAVSDHEATLRCWALSFPYAEITLTWQRDGEDQTQDTELVETRPCGDGTFQKWAAVV
VPSGQEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:488), where amino acid 84 is a Cys and
where amino acid 236 is a Cys.

HLA-A (Y84A; A236C)

[00118] In some cases, the MHC Class I heavy chain polypeptide comprises Y84A and A236C substitutions. For example, in some cases, the MHC Class I heavy chain polypeptide comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following human HLA-A heavy chain (Y84A; A236C) amino acid sequence:

GSISMRYFFTSVSRPGRGEPRFIAVGYYDDTQFVRFSDAASQRMEPRAPWIEQEGPEYWDGET
RKVKAHSQTHRVDLGTLRGA^YNQSEAGSHTVQRMYGCDVGSDWRFLRGYHQYAYDGKDYIA
LKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRYLENGKETLQRTDAPK
THMTHHAVSDHEATLRCWALSFPYAEITLTWQRDGEDQTQDTELVETRPC^CGDGTFQKWAAVV
VPSGQEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:48), where amino acid 84 is Ala and amino
acid 236 is Cys. In some cases, the Cys-236 forms an interchain disulfide bond with Cys-12 of a variant
β2M polypeptide that comprises an R12C substitution.

[00119] In some cases, an HLA-A heavy chain polypeptide suitable for inclusion in a TMMP is an HLA-A02 (Y84A; A236C) polypeptide comprising the following amino acid sequence:

GSISMRYFFTSVSRPGRGEPRFIAVGYYDDTQFVRFSDAASQRMEPRAPWIEQEGPEYWDGET
RKVKAHSQTHRVDLGTLRGA^YNQSEAGSHTVQRMYGCDVGSDWRFLRGYHQYAYDGKDYIA
LKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRYLENGKETLQRTDAPK
THMTHHAVSDHEATLRCWALSFPYAEITLTWQRDGEDQTQDTELVETRPC^CGDGTFQKWAAVV
VPSGQEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:48).

[00120] In some cases, an HLA-A heavy chain polypeptide suitable for inclusion in a TMMP is an HLA-A02 (Y84A; A236C) polypeptide comprising the following amino acid sequence:

GSISMRYFFTSVSRPGRGEPRFIAVGYYDDTQFVRFSDAASQRMEPRAPWIEQEGPEYWDGET
RKVKAHSQTHRVDLGTLRGA^YNQSEAGSHTVQRMYGCDVGSDWRFLRGYHQYAYDGKDYIA
LKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRYLENGKETLQRTDAPK

THMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTELVETRPCGDGTFQKWA^{AVV}
VPSGQEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:46).

HLA-A (Y84C; A139C)

[00121] In some cases, the MHC Class I heavy chain polypeptide comprises Y84C and A139C substitutions. For example, in some cases, the MHC Class I heavy chain polypeptide comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following human HLA-A heavy chain (Y84C; A139C) amino acid sequence:

GSHSMRYFFTSVSRPGRGEPRFIAVG^YVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDGET
RKVK^AHSQTHRVDLGLTRGCYNQSEAGSHTVQRM^YGCDVGS^DWRFLRGYHQYAYDGKDYIA
LKEDLRSWTAADMCAQTTHKWEAAHVAEQLRAYLEGTCVEWLRRYLENGKETLQRTDAPK
THMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTELVETRPAGDGTFQKWA^{AVV}
VPSGQEQRYTCHVQHEGLPKPLTLRWE^P (SEQ ID NO:299), where amino acid 84 is Cys and amino acid 139 is Cys. In some cases, Cys-84 forms an intrachain disulfide bond with Cys-139.

HLA-A11 (HLA-A*1101)

[00122] As one non-limiting example, an MHC Class I heavy chain polypeptide of a TMMP can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following human HLA-A11 heavy chain amino acid sequence:

GSHSMRYFYTSVSRPGRGEPRFIAVG^YVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDQE
TRNVKAQSQTDRVDLGLTRGY^YNQSEDGSHTIQIMYGCDVGPDGRFLRGYRQDAYDGKDYIA
LNEDLRSWTAADMAAQITKRKWEAAHAAEQRAYLEGTCVEWLRRYLENGKETLQRTDPPKT
HMTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDGTFQKWA^{AVVV}
PSGEEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:300). Such an MHC Class I heavy chain may be prominent in Asian populations, including populations of individuals of Asian descent.

HLA-A11 (Y84A; A236C)

[00123] As one non-limiting example, in some cases, the MHC Class I heavy chain polypeptide is an HLA-A11 allele that comprises Y84A and A236C substitutions. For example, in some cases, the MHC Class I heavy chain polypeptide comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following human HLA-A A11 heavy chain (Y84A; A236C) amino acid sequence:

GSHSMRYFYTSVSRPGRGEPRFIAVG^YVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDQE
TRNVKAQSQTDRVDLGLTRGAYNQSEDGSHTIQIMYGCDVGPDGRFLRGYRQDAYDGKDYIA
LNEDLRSWTAADMAAQITKRKWEAAHAAEQRAYLEGTCVEWLRRYLENGKETLQRTDPPKT
HMTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPCGDGTFQKWA^{AVVV}

PSGEEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:301), where amino acid 84 is Ala and amino acid 236 is Cys. In some cases, the Cys-236 forms an interchain disulfide bond with Cys-12 of a variant β 2M polypeptide that comprises an R12C substitution.

HLA-A11 (Y84C; A236C)

[00124] In some cases, the MHC Class I heavy chain polypeptide present in a TMMP comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following human HLA-A A11 heavy chain (Y84C; A236C) amino acid sequence:

GSHSMRYFYTSVSRPGRGEPRFIAVGYYVDDTQFVRFSDAASQRMEPRAPWIEQEGPEYWDQE
TRNVKAQSQTDRVDLGLTRGCYNQSEDGSHTIQIMYGCDVGPDGRFLRGYRQDAYDGKDYYIA
LNEDLRSWTAADMAAQITKRKWEAAHAAEQRAYLEGTCVEWLRRYLENGKETLQRTDPPKT
HMTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPCGDGTFQKWAAVVV
PSGEEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:301), where amino acid 84 is Cys and amino acid 236 is Cys.

HLA-A24 (HLA-A*2402)

[00125] As one non-limiting example, an MHC Class I heavy chain polypeptide of a TMMP can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following human HLA-A24 (also referred to as HLA-A*2402) heavy chain amino acid sequence:

GSHSMRYFSTSVSRPGRGEPRFIAVGYYVDDTQFVRFSDAASQRMEPRAPWIEQEGPEYWDEET
GKVKAHSQTDRENLRALRYYNQSEAGSHTLQMMFGCDVGS DGRFLRGYHQYAYDGKDYYIAL
KEDLRSWTAADMAAQITKRKWEAAHVAEQRAYLEGTCVDGLRRYLENGKETLQRTDPPKT
HMTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDGTFQKWAAVVV
PSGEEQRYTCHVQHEGLPKPLTLRWEPSSQPTVPIVGHAGLVLLGAVITGAVVA VMWRRNSS
DRKGGSYSQAASSDSAQGS DVS LTACKV (SEQ ID NO:302). Such an MHC Class I heavy chain may be prominent in Asian populations, including populations of individuals of Asian descent. In some cases, amino acid 84 is an Ala. In some cases, amino acid 84 is a Cys. In some cases, amino acid 236 is a Cys. In some cases, amino acid 84 is an Ala and amino acid 236 is a Cys. In some cases, amino acid 84 is an Cys and amino acid 236 is a Cys.

[00126] In some cases, an MHC Class I heavy chain polypeptide of a TMMP can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following human HLA-A24 (also referred to as HLA-A*2402) heavy chain amino acid sequence:

GSHSMRYFSTSVSRPGRGEPRFIAVGYYVDDTQFVRFSDAASQRMEPRAPWIEQEGPEYWDEET
GKVKAHSQTDRENLRALRYYNQSEAGSHTLQMMFGCDVGS DGRFLRGYHQYAYDGKDYYIAL

KEDLRSWTAADMAAQITKRKWEAAHVAEQQRAYLEGTCVDGLRRYLENGKETLQRTDPPKT
 HMTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDGTFQKWAAVVV
 PSGEEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:455), where amino acid 84 is Tyr and amino
 acid 236 is Ala (amino acids 84 and 236 are bold and underlined); and where the MHC Class I heavy
 chain has a length of about 275 amino acids.

[00127] In some cases, an MHC Class I heavy chain polypeptide of a TMMP can comprise an
 amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least
 98%, at least 99%, or 100%, amino acid sequence identity to the following human HLA-A24 (also
 referred to as HLA-A*2402) heavy chain amino acid sequence:

GSHSMRYFSTSVSRPGRGEPRFIAVGYYDDTQFVRFSDAASQRMEPRAPWIEQEGPEYWDEET
 GKVKHAHSQTDRENLRALRAYNQSEAGSHTLQMMFGCDVGSDGRFLRGYHQYAYDGKDYIAL
 KEDLRSWTAADMAAQITKRKWEAAHVAEQQRAYLEGTCVDGLRRYLENGKETLQRTDPPKT
 HMTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDGTFQKWAAVVV
 PSGEEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:456), where amino acid 84 is Ala and amino
 acid 236 is Ala (amino acids 84 and 236 are bold and underlined); and where the MHC Class I heavy
 chain has a length of about 275 amino acids.

[00128] In some cases, an MHC Class I heavy chain polypeptide of a TMMP can comprise an
 amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least
 98%, at least 99%, or 100%, amino acid sequence identity to the following human HLA-A24 (also
 referred to as HLA-A*2402) heavy chain amino acid sequence:

GSHSMRYFSTSVSRPGRGEPRFIAVGYYDDTQFVRFSDAASQRMEPRAPWIEQEGPEYWDEET
 GKVKHAHSQTDRENLRALRYYNQSEAGSHTLQMMFGCDVGSDGRFLRGYHQYAYDGKDYIAL
 KEDLRSWTAADMAAQITKRKWEAAHVAEQQRAYLEGTCVDGLRRYLENGKETLQRTDPPKT
 HMTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPCGDGTFQKWAAVVV
 PSGEEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:457), where amino acid 84 is Tyr and amino
 acid 236 is Cys (amino acids 84 and 236 are bold and underlined); and where the MHC Class I heavy
 chain has a length of about 275 amino acids.

[00129] In some cases, an MHC Class I heavy chain polypeptide of a TMMP can comprise an
 amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least
 98%, at least 99%, or 100%, amino acid sequence identity to the following human HLA-A24 (also
 referred to as HLA-A*2402) heavy chain amino acid sequence:

GSHSMRYFSTSVSRPGRGEPRFIAVGYYDDTQFVRFSDAASQRMEPRAPWIEQEGPEYWDEET
 GKVKHAHSQTDRENLRALRAYNQSEAGSHTLQMMFGCDVGSDGRFLRGYHQYAYDGKDYIAL
 KEDLRSWTAADMAAQITKRKWEAAHVAEQQRAYLEGTCVDGLRRYLENGKETLQRTDPPKT
 HMTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPCGDGTFQKWAAVVV

PSGEEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:458), where amino acid 84 is Ala and amino acid 236 is Cys (amino acids 84 and 236 are bold and underlined); and where the MHC Class I heavy chain has a length of about 275 amino acids.

[00130] In some cases, an MHC Class I heavy chain polypeptide of a can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following human HLA-A24 (also referred to as HLA-A*2402) heavy chain amino acid sequence:

GSISMRYFSTSVSRPGRGEPRFIAVGYYVDDTQFVRFSDAASQRMEPRAPWIEQEGPEYWDEET
GKVKHAHSQTDRENLRALRCYNQSEAGSHTLQMMFGCDVGS DGRFLRGYHQYAYDGKDYIAL
KEDLRSWTAADMAAQITKRKWEAAHVAEQQRAYLEGTCVDGLRRYLENGKETLQRTDPPKT
HMTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTEL VETRPAGDGTFQKWA AVVV
PSGEEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:459), where amino acid 84 is Cys and amino acid 236 is Ala (amino acids 84 and 236 are bold and underlined); and where the MHC Class I heavy chain has a length of about 275 amino acids.

[00131] In some cases, an MHC Class I heavy chain polypeptide of a TMMP can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following human HLA-A24 (also referred to as HLA-A*2402) heavy chain amino acid sequence:

GSISMRYFSTSVSRPGRGEPRFIAVGYYVDDTQFVRFSDAASQRMEPRAPWIEQEGPEYWDEET
GKVKHAHSQTDRENLRALRCYNQSEAGSHTLQMMFGCDVGS DGRFLRGYHQYAYDGKDYIAL
KEDLRSWTAADMAAQITKRKWEAAHVAEQQRAYLEGTCVDGLRRYLENGKETLQRTDPPKT
HMTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTEL VETRPCGDGTFQKWA AVVV
PSGEEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:346), where amino acid 84 is Cys and amino acid 236 is Cys (amino acids 84 and 236 are bold and underlined); and where the MHC Class I heavy chain has a length of about 275 amino acids.

HLA-A33 (HLA-A*3303)

[00132] As one non-limiting example, an MHC Class I heavy chain polypeptide of a TMMP can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following human HLA-A33 heavy chain amino acid sequence:

GSISMRYFTTSVSRPGRGEPRFIAVGYYVDDTQFVRFSDAASQRMEPRAPWIEQEGPEYWDNRN
TRNVKAHSQIDRVLDLGLRGYYNQSEAGSHTIQMMYGC DVGSDGRFLRGYQQDAYDGKDYIA
LNEDLRSWTAADMAAQITQRKWEAARVAEQLRAYLEGTCVEWLRRYLENGKETLQRTDPPKT
HMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTEL VETRPAGDGTFQKWA SVVV
PSGQEQRYTCHVQHEGLPKPLTLRWEPSQPTPIVGIIAGLVLF GAVFAGAVVA AVRWRRKSSD

RKGGSYSQAASSDSAQGSMSLTACKV (SEQ ID NO:303). Such an MHC Class I heavy chain may be prominent in Asian populations, including populations of individuals of Asian descent. In some cases, amino acid 84 is an Ala. In some cases, amino acid 84 is a Cys. In some cases, amino acid 236 is a Cys. In some cases, amino acid 84 is an Ala and amino acid 236 is a Cys. In some cases, amino acid 84 is an Cys and amino acid 236 is a Cys.

HLA-B

[00133] In some cases, a TMMP comprises an HLA-B heavy chain polypeptide. The HLA-B heavy chain peptide sequences, or portions thereof, that may be that may be incorporated into a TMMP include, but are not limited to, the alleles: B*0702, B*0801, B*1502, B*3802, B*4001, B*4601, and B*5301, which are aligned without all, or substantially all, of the leader, transmembrane and cytoplasmic sequences in **FIG. 10A**. Any of those alleles may comprise a mutation at one or more of positions 84, 139 and/or 236 (as shown in **FIG. 10A**) selected from: a tyrosine to alanine at position 84 (Y84A); a tyrosine to cysteine at position 84 (Y84C); an alanine to cysteine at position 139 (A139C); and an alanine to cysteine substitution at position 236 (A236C). In addition, a HLA-B polypeptide comprising an amino acid sequence having at least 75% (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%) or 100% amino acid sequence identity to all or part (e.g., 50, 75, 100, 150, 200, or 250 contiguous amino acids) of the sequence of those HLA-B alleles may also be employed (e.g., it may comprise 1-25, 1-5, 5-10, 10-15, 15-20, 20-25, or 25-30 amino acid insertions, deletions, and/or substitutions).

[00134] In some cases, a TMMP comprises an HLA-B heavy chain polypeptide comprising the following HLA-B consensus amino acid sequence:

[00135] GSHSMRYF**X1**T**X2X3**SRPGRGEP**FX4**VGYVDDT**X5**FVRFDSDA**X6**SPR**X7X8**PRAPWIEQEGPEYWDR**X9**TQ**X10X11**K**TX12X13**TQ**X14YX15X16**NL**X17X18X19X20**YYNQSEAGSH**X21X22QX23**MYGCDLGPDGRLLRGHDQSAYDGKDIALNEDL**X24**SWTAADTAAQ**IX25**QRK**X26**EAAR**X27**AEQ**X28RX29**YLEG**X30**CVEWLRRYLENGK**X31X32LX33**RADPPKTHVTHHP**X34**SDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDR**TFQKWA**AVVVP**SGEEQR**YTCHVQHEGLPKPLTLRWEP (SEQ ID NO:30), wherein X1 is H, Y, or D; X2 is A or S; X3 is M or V; X4 is A, S, or T; X5 is Q or L; X6 is A or T; X7 is E, M K, or T; X8 is A or T; X9 is E or N; X10 is I or K; X11 is Y, F, S, or C; X12 is N or Q; X13 is A or T; X14 is D or Y; X15 is E or V; X16 is S or N; X17 is T, N, or I; X18 is A or L; X19 is L, or R; X20 is R or G; X21 is T or I; X22 is L or I; X23 is R or S; X24 is R or S; X25 is S or T; X26 is L or W; X27 is E OR V; X28 is R, D, L or W; X29 is A or T; X30 is L, E or T; X31 is E or D; X32 is K or T; X33 is E or Q; and X34 is I or V.

[00136] As an example, an MHC Class I heavy chain polypeptide of a TMMP can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following human HLA-B heavy chain

amino acid sequence:

GSHSMRYFYTSVSRPGRGEPFRFISVGYYVDDTQFVRFSDAASPREEPRAPWIEQEGPEYWDRNT
QIYKAQAQTDRESLRNLRGYYNQSEAGSHTLQSMYGCDVGPDRLLRGHDQYAYDGKDYIAL
NEDLRSWTAADTAAQITQRKWEAAREAEQRRAYLEGECVEWLRRYLENGKDKLERADPPKTH
VTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDRTFQKWA AVVVPS
GEEQRYTCHVQHEGLPKPLTLRWEP (SEQ ID NO:207).

HLA-B (Y84A; A236C)

[00137] As one non-limiting example, in some cases, the MHC Class I heavy chain polypeptide is an HLA-B polypeptide that comprises Y84A and A236C substitutions. For example, in some cases, the MHC Class I heavy chain polypeptide comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following human HLA-B heavy chain (Y84A; A236C) amino acid sequence:

GSHSMRYFYTSVSRPGRGEPFRFISVGYYVDDTQFVRFSDAASPREEPRAPWIEQEGPEYWDRNT
QIYKAQAQTDRESLRNLRGAYNQSEAGSHTLQSMYGCDVGPDRLLRGHDQYAYDGKDYIAL
NEDLRSWTAADTAAQITQRKWEAAREAEQRRAYLEGECVEWLRRYLENGKDKLERADPPKTH
VTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPCGDRTFQKWA AVVVPS
GEEQRYTCHVQHEGLPKPLTLRWEP (SEQ ID NO:305), where amino acid 84 is Ala and amino acid 236 is Cys. In some cases, the Cys-236 forms an interchain disulfide bond with Cys-12 of a variant β 2M polypeptide that comprises an R12C substitution.

HLA-B (Y84C; A139C)

[00138] In some cases, the MHC Class I heavy chain polypeptide comprises Y84C and A139C substitutions. For example, in some cases, the MHC Class I heavy chain polypeptide comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following human HLA-B heavy chain (Y84C; A139C) amino acid sequence:

GSHSMRYFYTSVSRPGRGEPFRFISVGYYVDDTQFVRFSDAASPREEPRAPWIEQEGPEYWDRNT
QIYKAQAQTDRESLRNLRGCYNQSEAGSHTLQSMYGCDVGPDRLLRGHDQYAYDGKDYIAL
NEDLRSWTAADTCAQITQRKWEAAREAEQRRAYLEGECVEWLRRYLENGKDKLERADPPKTH
VTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDRTFQKWA AVVVPS
GEEQRYTCHVQHEGLPKPLTLRWEP (SEQ ID NO:306), where amino acid 84 is Cys and amino acid 139 is Cys. In some cases, Cys-84 forms an intrachain disulfide bond with Cys-139.

HLA-B*0702

[00139] As an example, in some cases, a MHC Class I heavy chain polypeptide present in a TMMP comprises an amino acid sequence of HLA-B*0702 (SEQ ID NO:207) in **FIG. 10A**, or a

sequence having at least 75% (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%) or 100%, amino acid sequence identity to all or part (e.g., 50, 75, 100, 150, 200, or 250 contiguous amino acids) of that sequence (e.g., it may comprise 1-25, 1-5, 5-10, 10-15, 15-20, 20-25, or 25-30 amino acid insertions, deletions, and/or substitutions). In some cases, where the HLA-B heavy chain polypeptide of TMMP of the present disclosure has less than 100% identity to the sequence labeled HLA-B in **FIG. 8**, or labeled "B*0702 in **FIG. 10A**, it may comprise a mutation at one or more of positions 84, 139 and/or 236 selected from: a tyrosine to alanine substitution at position 84 (Y84A); a tyrosine to cysteine substitution at position 84 (Y84C); an alanine to cysteine at position 139 (A139C); and an alanine to cysteine substitution at position 236 (A236C). In some cases, the HLA-B heavy chain polypeptide of TMMP of the present disclosure comprises Y84A and A236C substitutions. In some cases, the HLA-B*0702 heavy chain polypeptide of TMMP of the present disclosure comprises Y84C and A139C substitutions. In some cases, the HLA-B heavy chain polypeptide of TMMP of the present disclosure comprises Y84C, A139C, and A236C substitutions.

HLA-C

[00140] In some cases, a TMMP comprises an HLA-C heavy chain polypeptide. The HLA-C heavy chain polypeptide, or portions thereof, that may be that may be incorporated into a TMMP of the present disclosure include, but are not limited to, the alleles: C*0102, C*0303, C*0304, C*0401, C*0602, C*0701, C*0801, and C*1502, which are aligned without all, or substantially all, of the leader, transmembrane and cytoplasmic sequences in **FIG. 11A**. Any of those alleles may comprise a mutation at one or more of positions 84, 139 and/or 236 (as shown in **FIG. 11A**) selected from: a tyrosine to alanine substitution at position 84 (Y84A); a tyrosine to cysteine substitution at position 84 (Y84C); an alanine to cysteine substitution at position 139 (A139C); and an alanine to cysteine substitution at position 236 (A236C). In addition, an HLA-C polypeptide comprising an amino acid sequence having at least 75% (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%) or 100% amino acid sequence identity to all or part (e.g., 50, 75, 100, 150, 200, or 250 contiguous amino acids) of the sequence of those HLA-C alleles may also be employed (e.g., it may comprise 1-25, 1-5, 5-10, 10-15, 15-20, 20-25, or 25-30 amino acid insertions, deletions, and/or substitutions).

[00141] In some cases, a TMMP comprises an HLA-C heavy chain polypeptide comprising the following HLA-C consensus amino acid sequence:

[00142] X1SHSMX2YFX3TAVSX4PGRGEPX5FIX6VGYVDDTQFVX7FDSDAASPRGEPX8PWVEQEGPEYWDRETQX9YKRQAQX10DRVX11LRX12LRGYYNQSEX13X14SHX15X16QX17MX18GCDX19GPDGRLRLRGX20X21QX22AYDGKDYLALNEDLRSWTAADTAQAQITQRKX23EARX24AEQX25RAYLEGX26CVEWLRRYLX27NGKX28TLQRAEX29PKTHVTHHPX30SDHEATLRCWALGFYPAEITLTWQX31DGEDQTQDELVETRPAGDGTFQKWAAYX32VPSPGX33EQRYTCHX34QHEGLX35EPLTLX36WX37P (SEQ ID NO:31), wherein X1 is C or G; X2 is R or K; X3 is

F, Y, S, or D; X4 is R or W; X5 is H or R; X6 is A or S; X7 is Q or R; X8 is A or E; X9 is N or K; X10 is T or A; X11 is S or N; X12 is N or K; X13 is A or D; X14 is G or R; X15 is T or I; X16 is L or I; X17 is W or R; X18 is C, Y, F, or S; X19 is L, or V; X20 is Y or H; X21 is D or N; X22 is Y, F, S, or L; X23 is L or W; X24 is E, A, or T; X25 is R, L, or W; X26 is L or T; X27 is E or K; X28 is E or K; X29 is H or P; X30 is R or V; X31 is W or R; X32 is V or M; X33 is E or Q; X34 is M or V; X35 is P or Q; X36 is R or S; and X37 is P or G.

[00143] As an example, an MHC Class I heavy chain polypeptide of a TMMP can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following human HLA-C heavy chain amino acid sequence:

CSHSMRYFDTA VSRPGRGEP RFISVGYVDDTQFVRFDSDAASPRGEP RAPWVEQEGPEYWDRE
TQNYKRQAQADRVSLRNLRGYYNQSEDGSHTLQRMYGCDLGP DGRLLRGYDQSAYDGKDYI
ALNEDLRSWTAADTAAQITQRKLEAARAAEQLRAYLEGTCVEWLRRYLENGKETLQRAEPPKT
HVTHHPLSDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTEL VETRPAGDGT FQKWAAVVV
PSGQEQRYTCHMQHEGLQEPLTSLWEP (SEQ ID NO:219).

HLA-C (Y84A; A236C)

[00144] As one non-limiting example, in some cases, the MHC Class I heavy chain polypeptide is an HLA-C polypeptide that comprises Y84A and A236C substitutions. For example, in some cases, the MHC Class I heavy chain polypeptide comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following human HLA-C heavy chain (Y84A; A236C) amino acid sequence:

CSHSMRYFDTA VSRPGRGEP RFISVGYVDDTQFVRFDSDAASPRGEP RAPWVEQEGPEYWDRE
TQNYKRQAQADRVSLRNLRGA^YNQSEDGSHTLQRMYGCDLGP DGRLLRGYDQSAYDGKDYI
ALNEDLRSWTAADTAAQITQRKLEAARAAEQLRAYLEGTCVEWLRRYLENGKETLQRAEPPKT
HVTHHPLSDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTEL VETRP^CGDGT FQKWAAVVV
PSGQEQRYTCHMQHEGLQEPLTSLWEP (SEQ ID NO:308), where amino acid 84 is Ala and amino acid 236 is Cys. In some cases, the Cys-236 forms an interchain disulfide bond with Cys-12 of a variant β 2M polypeptide that comprises an R12C substitution.

HLA-C (Y84C; A139C)

[00145] In some cases, the MHC Class I heavy chain polypeptide comprises Y84C and A139C substitutions. For example, in some cases, the MHC Class I heavy chain polypeptide comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following human HLA-C heavy chain (Y84C; A139C) amino acid sequence:

CSHSMRYFDTA VSRPGRGEP RFISVGYVDDTQFVRFDSDAASPRGEP RAPWVEQEGPEYWDRE

TQNYKRQAQADRVSLRNLRGCYNQSEDGSHTLQRMYGCDLGPDRLLRGYDQSAYDGKDYI
ALNEDLRSWTAADTCAQITQRKLEAARAAEQLRAYLEGTCVEWLRRYLENGKETLQRAEPPKT
HVTHHPLSDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDGTQKWA AVVV
PSGQEQRYTCHMQHEGLQEPLTSLWEP (SEQ ID NO:397), where amino acid 84 is Cys and amino
acid 139 is Cys. In some cases, Cys-84 forms an intrachain disulfide bond with Cys-139.

HLA-C*0701

[00146] In some cases, a MHC Class I heavy chain polypeptide of a TMMP comprises an amino acid sequence of HLA-C*0701 of **FIG. 11A** (labeled HLA-C in **FIG. 8**), or an amino acid sequence having at least 75% (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%) or 100% amino acid sequence identity to all or part (e.g., 50, 75, 100, 150, 200, or 250 contiguous amino acids) of that sequence (e.g., it may comprise 1-25, 1-5, 5-10, 10-15, 15-20, 20-25, or 25-30 amino acid insertions, deletions, and/or substitutions). In some cases, where the HLA-C heavy chain polypeptide of a TMMP has less than 100% identity to the sequence labeled HLA-C*0701 in **FIG. 11A**, it may comprise a mutation at one or more of positions 84, 139 and/or 236 selected from: a tyrosine to alanine substitution at position 84 (Y84A); a tyrosine to cysteine substitution at position 84 (Y84C); an alanine to cysteine at position 139 (A139C); and an alanine to cysteine substitution at position 236 (A236C). In some cases, the HLA-C heavy chain polypeptide of a TMMP comprises Y84A and A236C substitutions. In some cases, the HLA-C*0701 heavy chain polypeptide of a T-Cell-MMP or its epitope conjugate comprises Y84C and A139C substitutions. In some cases, the HLA-C heavy chain polypeptide of a TMMP of the present disclosure comprises Y84C, A139C, and A236C substitutions.

Non-classical HLA-E, -F, and -G MHC Class I heavy chains

[00147] In some cases, a TMMP comprises a non-classical MHC Class I heavy chain polypeptide. Among the non-classical HLA heavy chain polypeptides, or portions thereof, that may be that may be incorporated into a TMMP of the present disclosure include, but are not limited to, those of HLA-E, -F, and -G alleles. Amino acid sequences for HLA-E, -F, and -G heavy chain polypeptides, (and the HLA-A, B and C alleles) may be found on the world wide web hla.alleles.org/nomenclature/index.html, the European Bioinformatics Institute (www.ebi.ac.uk), which is part of the European Molecular Biology Laboratory (EMBL), and at the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov).

[00148] Non-limiting examples of suitable HLA-E alleles include, but are not limited to, HLA-E*0101 (HLA-E*01:01:01:01), HLA-E*01:03 (HLA-E*01:03:01:01), HLA-E*01:04, HLA-E*01:05, HLA-E*01:06, HLA-E*01:07, HLA-E*01:09, and HLA-E*01:10. Non-limiting examples of suitable HLA-F alleles include, but are not limited to, HLA-F*0101 (HLA-F*01:01:01:01), HLA-F*01:02, HLA-F*01:03 (HLA-F*01:03:01:01), HLA-F*01:04, HLA-F*01:05, and HLA-F*01:06. Non-limiting examples of suitable HLA-G alleles include, but are not limited to, HLA-G*0101 (HLA-G*01:01:01:01),

HLA-G*01:02, HLA-G*01:03(HLA-G*01:03:01:01), HLA-G*01:04 (HLA-G*01:04:01:01), HLA-G*01:06, HLA-G*01:07, HLA-G*01:08, HLA-G*01:09: HLA-G*01:10, HLA-G*01:10, HLA-G*01:11, HLA-G*01:12, HLA-G*01:14, HLA-G*01:15, HLA-G*01:16, HLA-G*01:17, HLA-G*01:18: HLA-G*01:19, HLA-G*01:20, and HLA-G*01:22. Consensus sequences for those HLA E, -F and -G alleles without all, or substantially all, of the leader, transmembrane and cytoplasmic sequences are provided in **FIG. 12**, and aligned with consensus sequences of the above-mentioned HLA-A, -B and -C alleles in **FIG. 13**.

[00149] Amino acid sequences of suitable HLA-E heavy chain polypeptides are provided in FIG. 46A-46D, where FIG. 46A provides the amino acid sequence of HLA-E*01:01 (wild-type); FIG. 46B provides the amino acid sequence of HLA-E*01:01 with Y84C and A2346C substitutions; FIG. 46C provides the amino acid sequence of HLA-E*01:03 (wild-type); and FIG. 46D provides the amino acid sequence of HLA-E*01:03 with Y84C and A2346C substitutions.

[00150] Amino acid sequences of suitable HLA-G heavy chain polypeptides are provided in FIG. 47A-47D, where FIG. 47A provides the amino acid sequence of HLA-G*01:01 (wild-type); FIG. 47B provides the amino acid sequence of HLA-G*01:01 with Y84C and A2346C substitutions; FIG. 47C provides the amino acid sequence of HLA-G*01:04 (wild-type); and FIG. 47D provides the amino acid sequence of HLA-G*01:04 with Y84C and A2346C substitutions.

[00151] **FIG. 12** provides a consensus sequence for each of HLA-E, -F, and -G with the variable aa positions indicated as “X” residues sequentially numbered and the locations of aas 84, 139 and 236 double underlined.

[00152] **FIG. 13** provides an alignment of the consensus amino acid sequences for HLA-A, -B, -C, -E, -F, and -G, which are given in FIGs. 9-13. Variable residues in each sequence are listed as “X” with the sequential numbering removed. As indicated in FIG. 8, the locations of aas 84, 139 and 236 are indicated with their flanking five-amino acid clusters that may be replaced by 1 to 5 amino acids selected independently from (i) any naturally occurring amino acid or (ii) any naturally occurring amino acid except proline or glycine are also shown.

[00153] Any of the above-mentioned HLA-E, -F, and/or -G alleles may comprise a substitution at one or more of positions 84, 139 and/or 236 as shown in **FIG. 13** for the consensus sequences. In some cases, the substitutions may be selected from a: position 84 tyrosine to alanine (Y84A) or cysteine (Y84C), or, in the case of HLA-F, an R84A or R84C substitution; a position 139 alanine to cysteine (A139C), or, in the case of HLA-F, a V139C; and an alanine to cysteine substitution at position 236 (A236C). In addition, an HLA-E, -F and/or -G sequence having at least 75% (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%) or 100% amino acid sequence identity to all or part (e.g., 50, 75, 100, 150, 200, or 250 contiguous amino acids) of any of the consensus sequences of

set forth in **FIG. 13** may also be employed (e.g., the sequences may comprise 1-25, 1-5, 5-10, 10-15, 15-20, 20-25, or 25-30 amino acid insertions, deletions, and/or substitutions in addition to changes at variable residues listed therein).

Mouse H2K

[00154] In some cases, a MHC Class I heavy chain polypeptide present in a TMMP comprises an amino acid sequence of mouse H2K (SEQ ID NO:45) (Mouse H2K in **FIG. 8**), or a sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% amino acid sequence identity to all or part (e.g., 50, 75, 100, 150, 200, or 250 contiguous amino acids) of that sequence (e.g., it may comprise 1-25, 1-5, 5-10, 10-15, 15-20, 20-25, or 25-30 amino acid insertions, deletions, and/or substitutions). In some cases, where the Mouse H2K heavy chain polypeptide of a TMMP has less than 100% identity to the sequence labeled Mouse H2K in **FIG. 8**, it may comprise a mutation at one or more of positions 84, 139 and/or 236 selected from: a tyrosine to alanine at position 84 (Y84A); a tyrosine to cysteine at position 84 (Y84C); an alanine to cysteine at position 139 (A139C); and an alanine to cysteine substitution at position 236 (A236C). In some cases, the MOUSE H2K heavy chain polypeptide of a TMMP comprises Y84A and A236C substitutions. In some cases, the Mouse H2K heavy chain polypeptide of a TMMP of the present disclosure comprises Y84C and A139C substitutions. In some cases, the MOUSE H2K heavy chain polypeptide of a TMMP of the present disclosure comprises Y84C, A139C and A236C substitutions.

Exemplary combinations

[00155] Table 1, below, presents various combinations of MHC Class I heavy chain sequence modifications that can be incorporated in a TMMP of the present disclosure.

TABLE 1

Entry	HLA Heavy Chain Sequence	Sequence Identity Range [‡]	Specific Substitutions at aa positions 84, 139 and/or 236
1	HLA-A Consensus (FIG. 9B)	75%-99.8%, 80%-99.8%, 85%-99.8%, 90%-99.8%, 95%-99.8%, 98%-99.8%, or 99%-99.8%; or 1-25, 1-5, 5-10, 10-15, 15-20, or 20-25 aa insertions, deletions, and/or substitutions (not counting variable residues)	None; Y84C; Y84A; A139C; A236C; (Y84A & A236C); (Y84C & A139C); or (Y84C, A139C & A236C)
2	A*0101, A*0201, A*0301, A*1101, A*2402, A*2301, A*2402, A*2407, A*3303, or A*3401	75%-99.8%, 80%-99.8%, 85%-99.8%, 90%-99.8%, 95%-99.8%, 98%-99.8%, or 99%-99.8%; or 1-25, 1-5, 5-10, 10-15, 15-20, or 20-25 aa insertions, deletions, and/or substitutions	None; Y84C; Y84A; A139C; A236C; (Y84A & A236C); (Y84C & A139C); or (Y84C, A139C & A236C)

Entry	HLA Heavy Chain Sequence	Sequence Identity Range*	Specific Substitutions at aa positions 84, 139 and/or 236
	(FIG. 9A)		
3	HLA-B Consensus (FIG. 10B)	75%-99.8%, 80%-99.8%, 85%-99.8%, 90%-99.8%, 95%-99.8%, 98%-99.8%, or 99%-99.8%; or 1-25, 1-5, 5-10, 10-15, 15-20, or 20-25 aa insertions, deletions, and/or substitutions (not counting variable residues)	None; Y84C; Y84A; A139C; A236C; (Y84A & A236C); (Y84C & A139C); or (Y84C, A139C & A236C)
4	B*0702, B*0801, B*1502, B*3802, B*4001, B*4601, or B*5301 (FIG. 10A)	75%-99.8%, 80%-99.8%, 85%-99.8%, 90%-99.8%, 95%-99.8%, 98%-99.8%, or 99%-99.8%; or 1-25, 1-5, 5-10, 10-15, 15-20, or 20-25 aa insertions, deletions, and/or substitutions	None; Y84C; Y84A; A139C; A236C; (Y84A & A236C); (Y84C & A139C); or (Y84C, A139C & A236C)
5	HLA-C Consensus (FIG. 11B)	75%-99.8%, 80%-99.8%, 85%-99.8%, 90%-99.8%, 95%-99.8%, 98%-99.8%, or 99%-99.8%; or 1-25, 1-5, 5-10, 10-15, 15-20, or 20-25 aa insertions, deletions, and/or substitutions (not counting variable residues)	None; Y84C; Y84A; A139C; A236C; (Y84A & A236C); (Y84C & A139C); or (Y84C, A139C & A236C)
6	C*0102, C*0303, C*0304, C*0401, C*0602, C*0701, C*0801, or C*1502 (FIG. 11A)	75%-99.8%, 80%-99.8%, 85%-99.8%, 90%-99.8%, 95%-99.8%, 98%-99.8%, or 99%-99.8%; or 1-25, 1-5, 5-10, 10-15, 15-20, or 20-25 aa insertions, deletions, and/or substitutions	None; Y84C; Y84A; A139C; A236C; (Y84A & A236C); (Y84C & A139C); or (Y84C, A139C & A236C)
7	HLA-E, F, or G Consensus (FIG. 12)	75%-99.8%, 80%-99.8%, 85%-99.8%, 90%-99.8%, 95%-99.8%, 98%-99.8%, or 99%-99.8%; or 1-25, 1-5, 5-10, 10-15, 15-20, or 20-25 aa insertions, deletions, and/or substitutions (not counting variable residues)	None; Y84C; Y84A; A139C; A236C; (Y84A & A236C); (Y84C & A139C); or (Y84C, A139C & A236C)
8	MOUSE H2K (FIG. 8)	75%-99.8%, 80%-99.8%, 85%-99.8%, 90%-99.8%, 95%-99.8%, 98%-99.8%, or 99%-99.8%; or 1-25, 1-5, 5-10, 10-15, 15-20, or 20-25 aa insertions, deletions, and/or substitutions	None; Y84C; Y84A; A139C; A236C; (Y84A & A236C); (Y84C & A139C); or (Y84C, A139C & A236C)

* The Sequence Identity Range is the permissible range in sequence identity of a MHC-H polypeptide sequence incorporated into a TMMP relative to the corresponding portion of the sequences listed in FIG. 8-13 not counting the variable residues in the consensus sequences.

Beta-2 microglobulin

[00156] A β 2-microglobulin (β 2M) polypeptide of a TMMP of the present disclosure can be a human β 2M polypeptide, a non-human primate β 2M polypeptide, a murine β 2M polypeptide, and the like. In some instances, a β 2M polypeptide comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a β 2M amino acid sequence depicted in FIG. 6. In some instances, a β 2M polypeptide comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to amino acids 21 to 119 of a β 2M amino acid sequence depicted in FIG. 6.

[00157] In some cases, a suitable β 2M polypeptide comprises the following amino acid sequence:

[00158] IQRTPKIQVY SCHPAENGKS NFLNCYVSGF HPSDIEVDLLKNGERIEKVE
HSDLSFSKDW SFYLLYYTEF TPTEKDEYAC RVNHVTLSP KIVKWDRDM (SEQ ID NO:311);
and the HLA Class I heavy chain polypeptide comprises the following amino acid sequence:

[00159] GSHSMRYFFTSVSRPGRGEPRFIAVG YVDDTQFVRFSDAASQRMEPRAPWIEQE
GPEYWDGETRKVKAHSQTHRVDL(aa1){C}(aa2)AGSHTVQRMYGCDVGS DWRFLRGYHQYAY
DGKDYLKEDLRSW(aa3){C}(aa4))HKWEAAHVAEQLRAYLEGTCVEWLRRYLENGKETLQR
TDAPKTHMTHHAVSDHEATLRCWALSFP AEITLTWQRDGEDQTQDTEL(aa5)(C)(aa6)QKWAA
VVVPSGQEQR YTCHVQHEGLPKPLTLR WEP (SEQ ID NO:309), where the cysteine residues
indicated as {C} form a disulfide bond between the α 1 and α 2-1 helices and the (C) residue forms a
disulfide bond with the β 2M polypeptide cysteine at position 12. In the sequence above, “aa1” is “amino
acid cluster 1”; “aa2” is “amino acid cluster 2”; “aa3” is “amino acid cluster 3”; “aa4” is “amino acid
cluster 4”; “aa5” is “amino acid cluster 5”; and “aa6” is “amino acid cluster 6”; see, e.g., FIG. 10. Each
occurrence of aa1, aa2, aa3, aa4, aa5, and aa6 is and independently selected to be 1-5 amino acid
residues, wherein the amino acid residues are i) selected independently from any naturally occurring
(e.g., encoded) amino acid or ii) any naturally occurring amino acid except proline or glycine.

[00160] In some cases, an MHC polypeptide comprises a single amino acid substitution relative to
a reference MHC polypeptide (where a reference MHC polypeptide can be a wild-type MHC
polypeptide), where the single amino acid substitution substitutes an amino acid with a cysteine (Cys)
residue. Such cysteine residues, when present in an MHC polypeptide of a first polypeptide of a TMMP
of the present disclosure, can form a disulfide bond with a cysteine residue present in a second
polypeptide chain of a TMMP.

[00161] In some cases, a first MHC polypeptide in a first polypeptide of a TMMP, and/or the
second MHC polypeptide in the second polypeptide of a TMMP, includes an amino acid substitution to
substitute an amino acid with a cysteine, where the substituted cysteine in the first MHC polypeptide

forms a disulfide bond with a cysteine in the second MHC polypeptide, where a cysteine in the first MHC polypeptide forms a disulfide bond with the substituted cysteine in the second MHC polypeptide, or where the substituted cysteine in the first MHC polypeptide forms a disulfide bond with the substituted cysteine in the second MHC polypeptide.

[00162] For example, in some cases, one of following pairs of residues in an HLA β 2-microglobulin and an HLA Class I heavy chain is substituted with cysteines (where residue numbers are those of the mature polypeptide): 1) β 2M residue 12, HLA Class I heavy chain residue 236; 2) β 2M residue 12, HLA Class I heavy chain residue 237; 3) β 2M residue 8, HLA Class I heavy chain residue 234; 4) β 2M residue 10, HLA Class I heavy chain residue 235; 5) β 2M residue 24, HLA Class I heavy chain residue 236; 6) β 2M residue 28, HLA Class I heavy chain residue 232; 7) β 2M residue 98, HLA Class I heavy chain residue 192; 8) β 2M residue 99, HLA Class I heavy chain residue 234; 9) β 2M residue 3, HLA Class I heavy chain residue 120; 10) β 2M residue 31, HLA Class I heavy chain residue 96; 11) β 2M residue 53, HLA Class I heavy chain residue 35; 12) β 2M residue 60, HLA Class I heavy chain residue 96; 13) β 2M residue 60, HLA Class I heavy chain residue 122; 14) β 2M residue 63, HLA Class I heavy chain residue 27; 15) β 2M residue Arg3, HLA Class I heavy chain residue Gly120; 16) β 2M residue His31, HLA Class I heavy chain residue Gln96; 17) β 2M residue Asp53, HLA Class I heavy chain residue Arg35; 18) β 2M residue Trp60, HLA Class I heavy chain residue Gln96; 19) β 2M residue Trp60, HLA Class I heavy chain residue Asp122; 20) β 2M residue Tyr63, HLA Class I heavy chain residue Tyr27; 21) β 2M residue Lys6, HLA Class I heavy chain residue Glu232; 22) β 2M residue Gln8, HLA Class I heavy chain residue Arg234; 23) β 2M residue Tyr10, HLA Class I heavy chain residue Pro235; 24) β 2M residue Ser11, HLA Class I heavy chain residue Gln242; 25) β 2M residue Asn24, HLA Class I heavy chain residue Ala236; 26) β 2M residue Ser28, HLA Class I heavy chain residue Glu232; 27) β 2M residue Asp98, HLA Class I heavy chain residue His192; and 28) β 2M residue Met99, HLA Class I heavy chain residue Arg234. The amino acid numbering of the MHC/HLA Class I heavy chain is in reference to the mature MHC/HLA Class I heavy chain, without a signal peptide. For example, in some cases, residue 236 of the mature HLA-A amino acid sequence is substituted with a Cys. In some cases, residue 236 of the mature HLA-B amino acid sequence is substituted with a Cys. In some cases, residue 236 of the mature HLA-C amino acid sequence is substituted with a Cys. In some cases, residue 32 (corresponding to Arg-12 of mature β 2M) of an amino acid sequence depicted in FIG. 6 is substituted with a Cys.

[00163] In some cases, a β 2M polypeptide comprises the amino acid sequence: IQRTPKIQVY SRHPAENGKS NFLNCYVSGF HPSDIEVDLLKNGERIEKVE HSDLSFSKDW SFYLLYYTEF TPTEKDEYAC RVNHVTL SQP KIVKWDRDM (SEQ ID NO:310). In some cases, a β 2M polypeptide comprises the amino acid sequence: IQRTPKIQVY SCHPAENGKS NFLNCYVSGF

HPSDIEVDLLKNGERIEKVE HSDLSFSKDW SFYLLYYTEF TPTEKDEYAC RVNHVTL SQP
KIVKWDRDM (SEQ ID NO:311).

[00164] In some cases, an HLA Class I heavy chain polypeptide comprises the HLA-A*2402 amino acid sequence:

GSHSMRYFSTSVSRPGRGEPRFIAVG YVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDEET
GKVKAHSQTDRENLRALRYYNQSEAGSHTLQMMFGCDVGSDGRFLRGYHQYAYDGKDYIAL
KEDLRSWTAADMAAQITKRKWEAAHVAEQRAYLEGTCVDGLRRYLENGKETLQRTDPPKT
HMTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDGTFQKWAAVVV
PSGEEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:455).

[00165] In some cases, an HLA Class I heavy chain polypeptide comprises the HLA-A*2402 amino acid sequence:

GSHSMRYFSTSVSRPGRGEPRFIAVG YVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDEET
GKVKAHSQTDRENLRALRAYNQSEAGSHTLQMMFGCDVGSDGRFLRGYHQYAYDGKDYIAL
KEDLRSWTAADMAAQITKRKWEAAHVAEQRAYLEGTCVDGLRRYLENGKETLQRTDPPKT
HMTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDGTFQKWAAVVV
PSGEEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:456).

[00166] In some cases, an HLA Class I heavy chain polypeptide comprises the amino acid sequence:

GSHSMRYFSTSVSRPGRGEPRFIAVG YVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDEET
GKVKAHSQTDRENLRALRCYNQSEAGSHTLQMMFGCDVGSDGRFLRGYHQYAYDGKDYIAL
KEDLRSWTAADMAAQITKRKWEAAHVAEQRAYLEGTCVDGLRRYLENGKETLQRTDPPKT
HMTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDGTFQKWAAVVV
PSGEEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:459).

[00167] In some cases, an HLA Class I heavy chain polypeptide comprises the amino acid sequence:

GSHSMRYFSTSVSRPGRGEPRFIAVG YVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDEET
GKVKAHSQTDRENLRALRYYNQSEAGSHTLQMMFGCDVGSDGRFLRGYHQYAYDGKDYIAL
KEDLRSWTAADMAAQITKRKWEAAHVAEQRAYLEGTCVDGLRRYLENGKETLQRTDPPKT
HMTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPCGDGTFQKWAAVVV
PSGEEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:457).

[00168] In some cases, an HLA Class I heavy chain polypeptide comprises the amino acid sequence:

GSHSMRYFSTSVSRPGRGEPRFIAVG YVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDEET
GKVKAHSQTDRENLRALRAYNQSEAGSHTLQMMFGCDVGSDGRFLRGYHQYAYDGKDYIAL

KEDLRSWTAADMAAQITKRKWEAAHVAEQRAYLEGTCVDGLRRYLENGKETLQRTDPPKT
HMTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPCGDGTFQKWAAVVV
PSGEEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:458).

[00169] In some cases, an HLA Class I heavy chain polypeptide comprises the amino acid sequence:

GSHSMRYFSTSVSRPGRGEPRFIAVGYVDDTQFVRFSDAASQRMEPRAPWIEQEGPEYWDEET
GKVKAHSQTDRENLRALRCYNQSEAGSHTLQMMFGCDVGSDGRFLRGYHQYAYDGKDYIAL
KEDLRSWTAADMAAQITKRKWEAAHVAEQRAYLEGTCVDGLRRYLENGKETLQRTDPPKT
HMTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPCGDGTFQKWAAVVV
PSGEEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:346).

[00170] In some cases, an HLA Class I heavy chain polypeptide comprises the amino acid sequence:

GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFSDAASQRMEPRAPWIEQEGPEYWDGET
RKVKHAHSQTHRVDLGTLRGYYNQSEAGSHTVQRMYGCDVGSDWRFLRGYHQYAYDGKDYIA
LKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRYLENGKETLQRTDAPK
THMTHHAVSDHEATLRCWALSFPYPAEITLTWQRDGEDQTQDTELVETRPAGDGTFQKWAAVV
VPSGQEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:44).

[00171] In some cases, an HLA Class I heavy chain polypeptide comprises the amino acid sequence:

GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFSDAASQRMEPRAPWIEQEGPEYWDGET
RKVKHAHSQTHRVDLGTLRGYYNQSEAGSHTVQRMYGCDVGSDWRFLRGYHQYAYDGKDYIA
LKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRYLENGKETLQRTDAPK
THMTHHAVSDHEATLRCWALSFPYPAEITLTWQRDGEDQTQDTELVETRPCGDGTFQKWAAVV
VPSGQEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:312).

[00172] In some cases, an HLA Class I heavy chain polypeptide comprises the amino acid sequence:

GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFSDAASQRMEPRAPWIEQEGPEYWDGET
RKVKHAHSQTHRVDLGTLRGAYNQSEAGSHTVQRMYGCDVGSDWRFLRGYHQYAYDGKDYIA
LKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRYLENGKETLQRTDAPK
THMTHHAVSDHEATLRCWALSFPYPAEITLTWQRDGEDQTQDTELVETRPCGDGTFQKWAAVV
VPSGQEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:46).

[00173] In some cases, the β 2M polypeptide comprises the following amino acid sequence:

[00174] IQRTPKIQVY SCHPAENGKS NFLNCYVSGF HPSDIEVDLLKNGERIEKVE HSDLSFSKDW SFYLLYYTEF TPTEKDEYAC RVNHVTLSP KIVKWDRDM (SEQ ID NO:311); and the HLA Class I heavy chain polypeptide of a TMMP comprises the following amino acid sequence:

[00175] GSHSMRYFFTSVSRPGRGEPRFIAVGYYDDTQFVRFSDAASQRMEPRAPWIEQE GPEYWDGETRKVKAHSQTHRVDLGLRGYYNQSEAGSHTVQRMYGCDVGS DWRFLRGYHQY AYDGKDYLKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRLYLENGKE TLQRTDAPKTHMTHHAVSDHEATLRWALSFPYAEITLTWQRDGEDQTQDTEL VETRPCGDGT FQKWA AVVVPSGQEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:312), where the Cys residues that are underlined and in bold form a disulfide bond with one another in the TMMP.

[00176] In some cases, the β 2M polypeptide comprises the following amino acid sequence:

[00177] IQRTPKIQVY SCHPAENGKS NFLNCYVSGF HPSDIEVDLLKNGERIEKVE HSDLSFSKDW SFYLLYYTEF TPTEKDEYAC RVNHVTLSP KIVKWDRDM (SEQ ID NO:311); and the HLA Class I heavy chain polypeptide of a TMMP comprises the following amino acid sequence:

[00178] GSHSMRYFSTSVSRPGRGEPRFIAVGYYDDTQFVRFSDAASQRMEPRAPWIEQE GPEYWDEETGKVKAHSQTDRENLRALRYYNQSEAGSHTLQMMFGCDVGS DGRFLRGYHQYA YDGKDYLKEDLRSWTAADMAAQITKRKWEAAHVAEQQRAYLEGTCVDGLRRYLENGKET LQRTDPPKTHMTHHPISDHEATLRWALGFYPAEITLTWQRDGEDQTQDTEL VETRPCGDGT FQKWA AVVVPSGEEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:457), where the Cys residue at amino acid 236 in the HLA Class I heavy chain polypeptide and the Cys at residue 12 of the β 2M polypeptide form a disulfide bond with one another in the TMMP.

[00179] In some cases, the β 2M polypeptide comprises the amino acid sequence:

IQRTPKIQVYSCHPAENGKS NFLNCYVSGFHPSDIEVDLLKNGERIEKVEHSDLSFSKDW SFYLL YYTEFTPTEKDEYACRVNHVTLSPKIVKWDRDM (SEQ ID NO:311).

[00180] In some cases, the first polypeptide and the second polypeptide of a TMMP are disulfide linked to one another through: i) a Cys residue present in a linker connecting the peptide epitope and a β 2M polypeptide in the first polypeptide chain; and ii) a Cys residue present in an MHC Class I heavy chain in the second polypeptide chain. In some cases, the Cys residue present in the MHC Class I heavy chain is a Cys introduced as a Y84C substitution. In some cases, the linker connecting the peptide epitope and the β 2M polypeptide in the first polypeptide chain is GCGGS(G4S)_n (SEQ ID NO:315), where n is 1, 2, 3, 4, 5, 6, 7, 8, or 9. For example, in some cases, the linker comprises the amino acid sequence GCGSGGGGSGGGGSGGGGS (SEQ ID NO:316). As another example, the linker comprises the amino acid sequence GCGSGGGGSGGGGS (SEQ ID NO:317). Examples of disulfide-linked first and second polypeptides of a TMMP are depicted schematically in FIG. 2A-2F.

Multiple disulfide bonded TMMPs

[00181] In some cases, the first polypeptide and the second polypeptide of a TMMP of the present disclosure are linked to one another by at least two disulfide bonds (i.e., two interchain disulfide bonds). Examples of such multiple disulfide-linked TMMP are depicted schematically in FIG. 17A and 17B and FIG. 18A-18C. In addition, where a TMMP comprises an IgFc polypeptide, a heterodimeric TMMP can be dimerized, such that disulfide bonds link the IgFc polypeptides in the two heterodimeric TMMPs. Such an arrangement is depicted schematically in FIG. 17C and 17D, where disulfide bonds are represented by dashed lines. Unless otherwise stated, the at least two disulfide bonds described in the multiple disulfide-linked TMMPs in this section are not referring to disulfide bonds linking IgFc polypeptides in dimerized TMMPs.

[00182] As noted above, in some cases, the first polypeptide and the second polypeptide of a TMMP are linked to one another by at least two disulfide bonds (i.e., two interchain disulfide bonds). For example, in some instances, the first polypeptide and the second polypeptide of a TMMP are linked to one another by 2 interchain disulfide bonds. As another example, in some instances, the first polypeptide and the second polypeptide of a TMMP are linked to one another by 3 interchain disulfide bonds. As another example, in some instances, the first polypeptide and the second polypeptide of a TMMP of the present disclosure are linked to one another by 4 interchain disulfide bonds.

[00183] In some cases where a peptide epitope in a first polypeptide of a TMMP is linked to a β 2M polypeptide by a linker comprising a Cys, at least one of the at least two disulfide bonds links a Cys in the linker to a Cys in an MHC Class I heavy chain in the second polypeptide. In some cases, where a peptide epitope in a first polypeptide of a TMMP is linked to an MHC Class I heavy chain polypeptide by a linker, at least one of the at least two disulfide bonds links a Cys in the linker to a Cys in a β 2M polypeptide present in the second polypeptide.

[00184] In some cases, a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) exhibits increased stability, compared to a control TMMP that includes only one of the at least two disulfide bonds. In some cases, a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) exhibits increased *in vitro* stability, compared to a control TMMP that includes only one of the at least two disulfide bonds. For example, in some cases, a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) exhibits at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 50%, at least 2-fold, at least 5-fold, or at least 10-fold, greater *in vitro* stability, compared to a control TMMP that includes only one of the at least two disulfide bonds.

[00185] Whether a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) exhibits increased *in vitro* stability, compared to a control TMMP that includes only one of the at least two disulfide bonds, can be determined by measuring the amount disulfide-linked heterodimeric TMMP

present in a sample over time and/or under a specified condition and/or during purification of the TMMP.

[00186] For example, in some cases, a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) exhibits at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 50%, at least 2-fold, at least 5-fold, or at least 10-fold, greater *in vitro* stability, compared to a control TMMP that includes only one of the at least two disulfide bonds, when the TMMP is stored at 37°C for a period of time (e.g., for a period of time of from about 1 week to about 2 weeks, from about 2 weeks to about 4 weeks, or from about 4 weeks to about 2 months). For example, in some cases, the amount of disulfide-linked heterodimeric TMMP remaining after storing a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) *in vitro* at 37°C for 28 days is at least at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 50%, at least 2-fold, at least 5-fold, or at least 10-fold, greater than the amount of disulfide-linked heterodimeric TMMP remaining after storing a control TMMP (a TMMP that includes only one of the at least two disulfide bonds present in the multiple disulfide-linked TMMP) *in vitro* at 37°C for 28 days.

[00187] As an example, a double disulfide-linked TMMP comprising polypeptides 1715 and 2380, as depicted in FIG. 14A and 14B, exhibits greater *in vitro* stability, compared to a TMMP comprising polypeptides 2405 and 2380, where polypeptide 2405 is depicted in FIG. 14D, where such TMMP comprises only one disulfide linkage, where the single disulfide linkage is formed between: i) the Cys of the G2C linker between the epitope and the β 2M; and ii) the Cys provided by a Y84C substitution in the MHC Class I heavy chain. As another example, a double disulfide-linked TMMP comprising polypeptides 1715 and 2380, as depicted in FIG. 14A and 14B, exhibits greater *in vitro* stability, compared to a TMMP comprising polypeptides 1380 and 2380, where polypeptide 1380 is depicted in FIG. 14E, where such TMMP comprises only one disulfide linkage, where the single disulfide linkage is formed between: i) the Cys provided by an R12C substitution in the β 2M polypeptide; and ii) the Cys provided by the A236C substitution in the MHC Class I heavy chain.

[00188] In some cases, a multiple disulfide-linked TMMP exhibits increased *in vivo* stability, compared to a control TMMP that includes only one of the at least two disulfide bonds. For example, in some cases, a multiple disulfide-linked TMMP exhibits at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 50%, at least 2-fold, at least 5-fold, or at least 10-fold, greater *in vivo* stability, compared to a control TMMP that includes only one of the at least two disulfide bonds.

[00189] In some cases, the presence of two disulfide bonds in a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) provides for increased production of disulfide-linked heterodimeric TMMP, compared to the amount of disulfide-linked heterodimeric TMMP produced when the TMMP is a control TMMP that includes only one of the at least two disulfide bonds. For example, a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) can be produced in a

mammalian cell in *in vitro* cell culture, where the mammalian cell is cultured in a liquid cell culture medium. The TMMP can be secreted into the cell culture medium. The cells can be lysed, generating a cell lysate, and the TMMP can be present in the cell lysate. The TMMP can be purified from the cell culture medium and/or the cell lysate. For example, where the TMMP comprises an IgG1 Fc polypeptide, the cell culture medium and/or the cell lysate can be contacted with immobilized protein A (e.g., the cell culture medium and/or the cell lysate can be applied to a protein A column, where protein A is immobilized onto beads). TMMP present in the cell culture medium and/or the cell lysate becomes bound to the immobilized protein A. After washing the column to remove unbound materials, the bound TMMP is eluted, generating a protein A eluate. The amount of disulfide-linked heterodimeric TMMP present in the protein A eluate is at least 0.5%, at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, or at least 10%, higher than the amount of disulfide-linked heterodimeric TMMP present in the protein A eluate when the TMMP is a control TMMP that includes only one of the at least two disulfide bonds present in the multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP). In some cases, the percent of the total TMMP protein in the eluate that is non-aggregated disulfide-linked heterodimeric TMMP is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99%. The protein A eluate can be subjected to size exclusion chromatography (SEC) and/or one or more other additional purification steps.

[00190] In some cases, a TMMP comprises at least one heterodimer comprising: a) a first polypeptide comprising: i) a WT1 peptide epitope, where the WT1 peptide has a length of at least 4 amino acids (e.g., from 4 amino acids to 25 amino acids; e.g., the WT1 peptide has a length of 4, 5, 6, 7, 8, 9, 10-15, 15-20, or 20-25 amino acids); and ii) first MHC polypeptide; b) a second polypeptide comprising a second MHC polypeptide, and c) at least one MOD, where the first and/or the second polypeptide comprises the MOD, and where the heterodimer comprises 2 disulfide bonds between the first polypeptide and the second polypeptide (i.e., the heterodimer comprises: i) a first disulfide bond linking the first polypeptide and the second polypeptide; and ii) a second disulfide bond linking the first polypeptide and the second polypeptide). Expressed another way, the first polypeptide comprises a first Cys residue that forms a disulfide bond (a first disulfide bond) with a first Cys residue in the second polypeptide; and the first polypeptide comprises a second Cys residue that forms a disulfide bond (a second disulfide bond) with a second Cys residue in the second polypeptide.

[00191] In some cases, a TMMP comprises: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a peptide epitope; ii) a peptide linker; and iii) a β 2M polypeptide; and b) a second polypeptide comprising an MHC Class I heavy chain polypeptide, where one or both of the first and the second polypeptides comprises at least one MOD, where the TMMP comprises: a) a first disulfide linkage between: i) a Cys present in the linker between the peptide epitope and the β 2M polypeptide; and ii) a first Cys introduced into the MHC Class I heavy chain polypeptide; and b) at least

a second disulfide linkage between the first polypeptide and the second polypeptide, where the at least a second disulfide linkage is between: i) a Cys in the first polypeptide that is C-terminal to the Cys present in the linker; and ii) a Cys in the second polypeptide that is C-terminal to the first Cys introduced into the MHC Class I heavy chain polypeptide.

[00192] In some cases, a first and a second disulfide bond-forming Cys residues in a first or a second polypeptide of a TMMP are from about 10 amino acids to about 200 amino acids apart from one another. For example, in some cases, a first and a second disulfide bond-forming Cys residues in a first or a second polypeptide of a TMMP are from about 10 amino acids (aa) to about 15 aa, from about 15 aa to about 20 aa, from about 20 aa to about 25 aa, from about 25 aa to about 30 aa, from about 30 aa to about 40 aa, from about 40 aa to about 50 aa, from about 50 aa to about 60 aa, from about 60 aa to about 70 aa, from about 70 aa to about 80 aa, from about 80 aa to about 90 aa, from about 90 aa to about 100 aa, from about 100 aa to about 110 aa, from about 110 aa to about 120 aa, from about 120 aa to about 130 aa, from about 130 aa to about 140 aa, from about 140 aa to about 150 aa, from about 150 aa to about 160 aa, from about 160 aa to about 170 aa, from about 170 aa to about 180 aa, from about 180 aa to about 190 aa, or from about 190 aa to about 200 aa.

[00193] As an example, in some cases, the first and second disulfide bond-forming Cys residues in the first polypeptide of a TMMP are from about 10 amino acids to about 80 amino acid residues apart from one another. For example, in some cases, the second disulfide bond-forming Cys residue in the first polypeptide is from about 10 amino acids to about 80 amino acids (e.g., from about 10 amino acids (aa) to about 15 aa, from about 15 aa to about 20 aa, from about 20 aa to about 25 aa, from about 25 aa to about 30 aa, from about 30 aa to about 40 aa, from about 40 aa to about 50 aa, from about 50 aa to about 60 aa, from about 60 aa to about 70 aa, or from about 70 aa to about 80 aa) C-terminal to the first disulfide bond-forming Cys residue in the first polypeptide. In some cases, the second disulfide bond-forming Cys residue in the first polypeptide is 10 aa, 11 aa, 12 aa, 13 aa, 14 aa, 15 aa, 16 aa, 17 aa, 18 aa, 19 aa, 20 aa, 21 aa, 22 aa, 23 aa, 24 aa, or 25 aa, C-terminal to the first disulfide bond-forming Cys residue in the first polypeptide. In some cases, the second disulfide bond-forming Cys residue in the first polypeptide is 15 aa C-terminal to the first disulfide bond-forming Cys residue in the first polypeptide. In some cases, the second disulfide bond-forming Cys residue in the first polypeptide is 20 aa C-terminal to the first disulfide bond-forming Cys residue in the first polypeptide. In some cases, the second disulfide bond-forming Cys residue in the first polypeptide is 25 aa C-terminal to the first disulfide bond-forming Cys residue in the first polypeptide.

[00194] In some cases, the first and second disulfide bond-forming Cys residues in the second polypeptide of a TMMP of the present disclosure are from about 140 amino acids to about 160 amino acids apart from one another. For example, in some cases, the second disulfide bond-forming Cys residue in the second polypeptide is from about 140 amino acids to about 160 amino acids C-terminal to the first

disulfide bond-forming Cys residue in the second polypeptide. In some cases, the second disulfide bond-forming Cys residue in the second polypeptide is 140 amino acids (aa), 141 aa, 142 aa, 143 aa, 144 aa, 145 aa, 146 aa, 147 aa, 148 aa, 149 aa, 150 aa, 151 aa, 152 aa, 153 aa, 154 aa, 155 aa, 156 aa, 157 aa, 158 aa, 159 aa, or 160 aa, C-terminal to the first disulfide bond-forming Cys residue in the second polypeptide.

[00195] A multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) can comprise: a) a first polypeptide comprising: i) a WT1 peptide (e.g., a WT1 peptide of from 4 amino acids to about 25 amino acids); and ii) a first MHC polypeptide, where the first polypeptide comprises a peptide linker between the WT1 peptide and the first MHC polypeptide, where the peptide linker comprises a Cys residue, and where the first MHC polypeptide is a β 2M polypeptide that comprises an amino acid substitution that introduces a Cys residue; b) and a second polypeptide comprising a second MHC polypeptide, where the second MHC polypeptide is a Class I heavy chain comprising a Y84C substitution and an A236C substitution, based on the amino acid numbering of HLA-A*0201 (depicted in FIG. 9A), or at corresponding positions in another Class I heavy chain allele, where the TMMP comprises a disulfide bond between the Cys residue in the peptide linker and the Cys residue at amino acid position 84 of the Class I heavy chain or corresponding position of another Class I heavy chain allele, and where the TMMP comprises a disulfide bond between the introduced Cys residue in the β 2M polypeptide and the Cys at amino acid position 236 of the Class I heavy chain or corresponding position of another Class I heavy chain allele; and c) at least one MOD, where the first and/or the second polypeptide comprises the at least one MOD. Examples are depicted schematically in FIG. 17A and FIG. 17B.

[00196] In some cases, the peptide linker comprises the amino acid sequence GCGGS (SEQ ID NO:318). In some cases, the peptide linker comprises the amino acid sequence GCGGS(GGGGS) n (SEQ ID NO:319), where n is an integer from 1 to 10. In some cases, the peptide linker comprises the amino acid sequence GCGGS(GGGGS) n (SEQ ID NO:398), where n is 1. In some cases, the peptide linker comprises the amino acid sequence GCGGS(GGGGS) n (SEQ ID NO:320), where n is 2. In some cases, the peptide linker comprises the amino acid sequence GCGGS(GGGGS) n (SEQ ID NO:321), where n is 3. In some cases, the peptide linker comprises the amino acid sequence GCGGS(GGGGS) n (SEQ ID NO:322), where n is 4. In some cases, the peptide linker comprises the amino acid sequence GCGGS(GGGGS) n (SEQ ID NO:323), where n is 5. In some cases, the peptide linker comprises the amino acid sequence GCGGS(GGGGS) n (SEQ ID NO:324), where n is 6. In some cases, the peptide linker comprises the amino acid sequence GCGGS(GGGGS) n (SEQ ID NO:325), where n is 7. In some cases, the peptide linker comprises the amino acid sequence GCGGS(GGGGS) n (SEQ ID NO:326), where n is 8. In some cases, the peptide linker comprises the amino acid sequence GCGGS(GGGGS) n

(SEQ ID NO: 327), where n is 9. In some cases, the peptide linker comprises the amino acid sequence GCGGS(GGGGS)_n (SEQ ID NO:328), where n is 10.

[00197] In some cases, the peptide linker comprises the amino acid sequence CGGGS (SEQ ID NO:329). In some cases, the peptide linker comprises the amino acid sequence CGGGS(GGGGS)_n (SEQ ID NO: 330), where n is an integer from 1 to 10. In some cases, the peptide linker comprises the amino acid sequence CGGGS(GGGGS)_n (SEQ ID NO:331), where n is 1. In some cases, the peptide linker comprises the amino acid sequence CGGGS(GGGGS)_n (SEQ ID NO:332), where n is 2. In some cases, the peptide linker comprises the amino acid sequence CGGGS(GGGGS)_n (SEQ ID NO:333), where n is 3. In some cases, the peptide linker comprises the amino acid sequence CGGGS(GGGGS)_n (SEQ ID NO:334), where n is 4. In some cases, the peptide linker comprises the amino acid sequence CGGGS(GGGGS)_n (SEQ ID NO:335), where n is 5. In some cases, the peptide linker comprises the amino acid sequence CGGGS(GGGGS)_n (SEQ ID NO:336), where n is 6. In some cases, the peptide linker comprises the amino acid sequence CGGGS(GGGGS)_n (SEQ ID NO:337), where n is 7. In some cases, the peptide linker comprises the amino acid sequence CGGGS(GGGGS)_n (SEQ ID NO:338), where n is 8. In some cases, the peptide linker comprises the amino acid sequence CGGGS(GGGGS)_n (SEQ ID NO:339), where n is 9. In some cases, the peptide linker comprises the amino acid sequence CGGGS(GGGGS)_n (SEQ ID NO:340), where n is 10.

[00198] The following are non-limiting examples of MHC Class I heavy chain comprising a Y84C substitution and an A236C substitution, based on the amino acid numbering of HLA-A*0201 (depicted in FIG. 9A), or at corresponding positions in another Class I heavy chain allele.

HLA-A

[00199] In some cases, a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) comprises: a) a first polypeptide comprising: i) a WT1 peptide (e.g., a WT1 peptide of from 4 amino acids to about 25 amino acids); and ii) a first MHC polypeptide, where the first polypeptide comprises a peptide linker between the WT1 peptide and the first MHC polypeptide, where the peptide linker comprises a Cys residue, and where the first MHC polypeptide is a β 2M polypeptide that comprises an amino acid substitution that introduces a Cys residue; and b) a second polypeptide comprising an HLA-A MHC Class I heavy chain comprising an amino acid sequence having at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following amino acid sequence:

GSHSMRYFFTSVSRPGRGEPRFIAVGYYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDGET
RKVKAHSQTHRVDLGTLRGCYNQSEAGSHTVQRMYGCDVGSDWRFLRGYHQYAYDGKDZIA
LKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRLYLENGKETLQRTDAPK
THMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTELVETRPCGDGTFQKWAADV
VPSGQEQRYSYTHVQHEGLPKPLTLRWEP (SEQ ID NO:342), where amino acid 84 is a Cys and

amino acid 236 is a Cys; and c) at least one MOD, where the first and/or the second polypeptide comprises the at least one MOD. In some cases, the peptide linker comprises the amino acid sequence GCGGS (SEQ ID NO:318). In some cases, the peptide linker comprises the amino acid sequence GCGGS(GGGGS)_n (SEQ ID NO:319), where *n* is an integer from 1 to 10. In some cases, the β 2M polypeptide comprises an R12C substitution. For example, the β 2M polypeptide can comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following amino acid sequence:

IQRTPKIQVYSCHPAENGKSNFLNCYVSGFHPSDIEVDLLKNGERIEKVEHSDLSFSKDWSFYLL
YYTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDM (SEQ ID NO:311), where amino acid 12 is a Cys. The at least one MOD can be a polypeptide that exerts an activating/stimulating effect on the target T cell or a suppressing/inhibitory effect on the target T cell. For example, the at least one MOD can be a cytokine (e.g., an IL2 polypeptide, an IL7 polypeptide, an IL12 polypeptide, an IL15 polypeptide, an IL17 polypeptide, an IL21 polypeptide, an IL27 polypeptide, an IL-23 polypeptide, a TGF β polypeptide, and the like; and including all family members, e.g., IL17A, IL-17B, IL-17C, IL-17D, IL-17E, IL-17F, IL-17E), a 4-1BBL polypeptide, an ICOS-L polypeptide, an OX-40L polypeptide, a CD80 polypeptide, a CD86 polypeptide, (CD80 and CD86 are also known as B7-1 and B7-2, respectively), a CD40 polypeptide, a CD70 polypeptide, a JAG1 (CD339) polypeptide, an ICAM (CD540 polypeptide, a PD-L1 polypeptide, a FasL polypeptide, a PD-L2 polypeptide, a PD-1H (VISTA) polypeptide, an ICOS-L (CD275) polypeptide, a GITRL polypeptide, an HVEM polypeptide, a CXCL10 polypeptide, a CXCL9 polypeptide, a CXCL11 polypeptide, a CXCL13 polypeptide, and a CX3CL1 polypeptide, a Galectin-9 polypeptide, a CD83 polypeptide, a CD30L polypeptide, a HLA-G polypeptide, a MICA polypeptide, a MICB polypeptide, a HVEM (CD270) polypeptide, a lymphotoxin beta receptor polypeptide, a 3/TR6 polypeptide, an ILT3 polypeptide, an ILT4 polypeptide, a CXCL10 polypeptide, a CXCL9 polypeptide, a CXCL11 polypeptide, a CXCL13 polypeptide, or a CX3CL1 polypeptide. These MODs may be the wild type polypeptide or a variant of a wild type polypeptide. In some cases, the MOD is an activating (“stimulatory”) immunomodulatory polypeptide; e.g., the MOD may produce an activating/stimulating effect on a T cell. Examples of activating MODs include, e.g., CD80, CD86, 4-1BBL, OX40L, CD70, ICOS-L, CD40, ICAM (CD54), IL2, IL7, IL12, IL15, IL17, IL21, IL27, IL23, GITRL, TGF β , and lymphotoxin beta receptor. In some cases, the MOD is an inhibitory (“suppressing”) MOD; e.g., the MOD may produce a suppressing/inhibitory effect on a T cell. Examples of inhibitory MOD include, e.g., PD-1H, PD-L1, PD-L2, TGF β , FasL, HVEM, Galectin-9, ILT3, and ILT4. TGF β polypeptides may produce either an activating/stimulating effect or a suppressing/inhibitory effect, depending on the context.

[00200] In some cases, a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) comprises: a) a first polypeptide comprising: i) a WT1 peptide (e.g., a WT1 peptide of from 4 amino

acids to about 25 amino acids); and ii) a first MHC polypeptide, where the first polypeptide comprises a peptide linker between the WT1 peptide and the first MHC polypeptide, where the peptide linker comprises a Cys residue, and where the first MHC polypeptide is a β 2M polypeptide that comprises an amino acid substitution that introduces a Cys residue; and b) a second polypeptide comprising an HLA-A MHC Class I heavy chain comprising an amino acid sequence having at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following amino acid sequence:

GSLSMRYFSTSVSRPGRGEPRFIAVGYYVDDTQFVRFSDAASQRMEPRAPWIEQEGPEYWDEET
GKVKAHSTQDRENLRALRCYNQSEAGSHTLQMMFGCDVGSDGRFLRGYHQYAYDGKDYIAL
KEDLRSWTAADMAAQITKRKWEAAHVAEQRAYLEGTCVDGLRRYLENGKETLQRTDPPKT
HMTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPCGDGTFQKWAADV

PSGEEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:346), where amino acid 84 is a Cys and amino acid 236 is a Cys; and c) at least one MOD, where the first and/or the second polypeptide comprises the at least one immunomodulatory polypeptide. In some cases, the peptide linker comprises the amino acid sequence GCGGS (SEQ ID NO:318). In some cases, the peptide linker comprises the amino acid sequence GCGGS(GGGGS)_n (SEQ ID NO:319), where n is an integer from 1 to 10. In some cases, the β 2M polypeptide comprises an R12C substitution. For example, the β 2M polypeptide can comprise an amino acid sequence having at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following amino acid sequence:

IQRTPKIQVYSCHPAENGKSNFLNCYVSGFHPSDIEVDLLKNGERIEKVEHSDLSFSKDWSFYLL
YYTEFTPTEKDEYACRVNHVTLSPKIVKWDRDM (SEQ ID NO:311), where amino acid 12 is a

Cys. The at least one MOD can be a polypeptide that exerts an activating/stimulating effect on the target T cell or a suppressing/inhibitory effect on the target T cell. For example, the at least one MOD can be a cytokine (e.g., an IL2 polypeptide, an IL7 polypeptide, an IL12 polypeptide, an IL15 polypeptide, an IL17 polypeptide, an IL21 polypeptide, an IL27 polypeptide, an IL-23 polypeptide, a TGF β polypeptide, and the like; and including all family members, e.g., IL17A, IL-17B, IL-17C, IL-17D, IL-17E, IL-17F, IL-17E), a 4-1BBL polypeptide, an ICOS-L polypeptide, an OX-40L polypeptide, a CD80 polypeptide, a CD86 polypeptide, (CD80 and CD86 are also known as B7-1 and B7-2, respectively), a CD40 polypeptide, a CD70 polypeptide, a JAG1 (CD339) polypeptide, an ICAM (CD540) polypeptide, a PD-L1 polypeptide, a FasL polypeptide, a PD-L2 polypeptide, a PD-1H (VISTA) polypeptide, an ICOS-L (CD275) polypeptide, a GITRL polypeptide, an HVEM polypeptide, a CXCL10 polypeptide, a CXCL9 polypeptide, a CXCL11 polypeptide, a CXCL13 polypeptide, and a CX3CL1 polypeptide, a Galectin-9 polypeptide, a CD83 polypeptide, a CD30L polypeptide, a HLA-G polypeptide, a MICA polypeptide, a MICB polypeptide, a HVEM (CD270) polypeptide, a lymphotoxin beta receptor polypeptide, a 3/TR6 polypeptide, an ILT3 polypeptide, an ILT4 polypeptide, a CXCL10 polypeptide, a CXCL9 polypeptide,

a CXCL11 polypeptide, a CXCL13 polypeptide, and a CX3CL1 polypeptide. These MODs may be the wild type polypeptide or a variant of wild type polypeptide. Of these, the following MODs may produce an activating/stimulating effect: CD80, CD86, 4-1BBL, OX40L, CD70, ICOS-L, CD40, ICAM (CD54), IL2, IL7, IL12, IL15, IL17, IL21, IL27, IL23, GITRL, TGF β , lymphotoxin beta receptor, 3/TR6, ILT3, ILT4, CXCL10, CXCL9, CXCL11, CXCL13 and CX3CL1. Of these, the following MODs may produce a suppressing/inhibitory effect: PD-1H, PD-L1, PD-L2, TGF β , FasL, HVEM, Galectin-9, ILT3, ILT4. TGF β polypeptides may produce either an activating/stimulating effect or a suppressing/inhibitory effect, depending on the context. In some cases, the at least one MOD is a reduced affinity variant, as described elsewhere herein. In some cases, the first or the second polypeptide comprises an Ig Fc polypeptide.

[00201] In some cases, the at least one MOD is a reduced affinity variant, as described elsewhere herein. In some cases, the first or the second polypeptide comprises an Ig Fc polypeptide.

[00202] In some cases, a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) comprises an HLA-A Class I heavy chain polypeptide. In some cases, the HLA-A heavy chain polypeptide present in a multiple disulfide-linked TMMP of the present disclosure (e.g., a double disulfide-linked TMMP) comprises an amino acid sequence having at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the HLA-A*0101, HLA-A*0201, HLA-A*0202, HLA-A*1101, HLA-A*2301, HLA-A*2402, HLA-A*2407, HLA-A*3303, or HLA-A*3401 amino acid sequence depicted in FIG. 9A, where the HLA-A heavy chain polypeptide comprises Y84C and A236C substitutions.

HLA-A*0101 (Y84C; A236C)

[00203] In some cases, the HLA-A heavy chain polypeptide present in a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) comprises an amino acid sequence having at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following HLA-A*0101 (Y84C; A236C) amino acid sequence:

[00204] GSHSMRYFFTSVSRPGRGEPRFIAVGYYDDTQFVRFSDAASQKMEPRAPWIEQE
GPEYWDQETRNMKAHSQTDRLNLTGRLGCYNQSEDGSHTIQIMYGCDVGPDRFLRGYRQDA
YDGKDYIALNEDLRSWTAADMAAQITKRKWEAVHAAEQRRVYLEGRCVDGLRRYLENGKET
LQRDPPKTHMTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPCGDGTF
QKWA AVVPSGEEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:343), where amino acid 84 is a Cys and amino acid 236 is a Cys.

HLA-A*0201 (Y84C; A236C)

[00205] In some cases, the HLA-A heavy chain polypeptide present in a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) comprises an amino acid sequence having at least 95%,

at least 98%, at least 99%, or 100%, amino acid sequence identity to the following HLA-A*0201 (Y84C; A236C) amino acid sequence:

GSHSMRYFFTSVSRPGRGEPRFIAVGYYVDDTQFVRFSDAASQRMEPRAPWIEQEGPEYWDGET
RKVKHAHSQTHRVDLGTLRGCYNQSEAGSHTVQRMYGCDVGSDWRFLRGYHQYAYDGKDYIA
LKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRYLENGKETLQRTDAPK
THMTHHAVSDHEATLRCWALSFPYPAEITLTWQRDGEDQTQDTELVETRPGDGTQKWAHAVV
VPSGQEQRYSCHVQHEGLPKPLTLRWE (SEQ ID NO:342), where amino acid 84 is a Cys and
amino acid 236 is a Cys.

HLA-A*0202 (Y84C; A236C)

[00206] In some cases, the HLA-A heavy chain polypeptide present in a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) comprises an amino acid sequence having at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following HLA-A*0202 (Y84C; A236C) amino acid sequence:

GSHSMRYFFTSVSRPGRGEPRFIAVGYYVDDTQFVRFSDAASQRMEPRAPWIEQEGPEYWDGET
RKVKHAHSQTHRVDLGTLRGCYNQSEAGSHTVQRMYGCDVGSDWRFLRGYHQYAYDGKDYIA
LKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRYLENGKETLQRTDAPK
THMTHHAVSDHEATLRCWALSFPYPAEITLTWQRDGEDQTQDTELVETRPGDGTQKWAHAVV
VPSGQEQRYSCHVQHEGLPKPLTLRWE (SEQ ID NO:341), where amino acid 84 is a Cys and
amino acid 236 is a Cys.

HLA-A*1101 (Y84C; A236C)

[00207] In some cases, the HLA-A heavy chain polypeptide present in a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) comprises an amino acid sequence having at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following HLA-A*1101 (Y84C; A236C) amino acid sequence:

GSHSMRYFYTSVSRPGRGEPRFIAVGYYVDDTQFVRFSDAASQRMEPRAPWIEQEGPEYWDQE
TRNVKAQSQTDRVDLGTLRGCYNQSEDSHTIQIMYGCDVGPDRFLRGYRQDAYDGKDYIA
LNEDLRSWTAADMAAQITKRKWEAAHAAEQRAYLEGRCVEWLRRYLENGKETLQRTDPPK
THMTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPGDGTQKWAHAVV
VPSGEEQRYSCHVQHEGLPKPLTLRWE (SEQ ID NO:344), where amino acid 84 is a Cys and amino
acid 236 is a Cys.

HLA-A*2301 (Y84C; A236C)

[00208] In some cases, the HLA-A heavy chain polypeptide present in a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) comprises an amino acid sequence having at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following HLA-A*2301 (Y84C; A236C) amino acid sequence:

GSHSMRYFSTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDEET
GKVKAHSQTDRENLRALRCYNQSEAGSHTLQMMFGCDVGSDGRFLRGYHGYAYDGKDYIAL
KEDLRSWTAADMAAQITQRKWEAAARVAEQLRAYLEGTCVDGLRRYLENGKETLQRTDPPKTH
MTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPCGDGTFQKWA AVV VPS
GEEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:345), where amino acid 84 is a Cys and amino
acid 236 is a Cys.

HLA-A*2402 (Y84C; A236C)

[00209] In some cases, the HLA-A heavy chain polypeptide present in a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) comprises an amino acid sequence having at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following HLA-A*2402 (Y84C; A236C) amino acid sequence:

GSHSMRYFSTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDEET
GKVKAHSQTDRENLRALRCYNQSEAGSHTLQMMFGCDVGSDGRFLRGYHGYAYDGKDYIAL
KEDLRSWTAADMAAQITKRKWEAAHVAEQRAYLEGTCVDGLRRYLENGKETLQRTDPPKT
HMTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPCGDGTFQKWA AVV V
PSGEEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:346), where amino acid 84 is a Cys and amino
acid 236 is a Cys.

HLA-A*2407 (Y84C; A236C)

[00210] In some cases, the HLA-A heavy chain polypeptide present in a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) comprises an amino acid sequence having at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following HLA-A*2407 (Y84C; A236C) amino acid sequence:

GSHSMRYFSTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDEET
GKVKASQTDRENLRALRCYNQSEAGSHTLQMMFGCDVGSDGRFLRGYHGYAYDGKDYIAL
KEDLRSWTAADMAAQITKRKWEAAHVAEQRAYLEGTCVDGLRRYLENGKETLQRTDPPKT
HMTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPCGDGTFQKWA AVV V
PSGEEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:347), where amino acid 84 is a Cys and amino
acid 236 is a Cys.

HLA-A*3303 (Y84C; A236C)

[00211] In some cases, the HLA-A heavy chain polypeptide present in a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) comprises an amino acid sequence having at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following HLA-A*3303 (Y84C; A236C) amino acid sequence:

[00212] GSHSMRYFTTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQE
GPEYWDRNTRNVKAHSQIDRVDLGTLRGCYNQSEAGSHTIQMMYGCDVGSDGRFLRGYQQD

AYDGKDYIALNEDLRSWTAADMAAQITQRKWEAARVAEQLRAYLEGTCVEWLRRYLENGKETLQRTDPPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTELVETRPGDGT FQKWASVVVPSGQEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:348), where amino acid 84 is a Cys and amino acid 236 is a Cys.

HLA-A*3401 (Y84C; A236C)

[00213] In some cases, the HLA-A heavy chain polypeptide present in a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) comprises an amino acid sequence having at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following HLA-A*3401 (Y84C; A236C) amino acid sequence:

GSHSMRYFYTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDRN TRKVKAQSQTDRVDLGLRGYCYNQSEDSHTIQRMYGCDVGPDRFLRGYQQDAYDGKDYIA LNEDLRSWTAADMAAQITQRKWETAHEAEQWRAYLEGTCVEWLRRYLENGKETLQRTDAPK THMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTELVETRPGDGT FQKWASVV VPSGQEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:349), where amino acid 84 is a Cys and amino acid 236 is a Cys.

HLA-B

In some cases, a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) comprises: a) a first polypeptide comprising: i) a WT1 peptide (e.g., a WT1 peptide of from 4 amino acids to about 25 amino acids); and ii) a first MHC polypeptide, where the first polypeptide comprises a peptide linker between the WT1 peptide and the first MHC polypeptide, where the peptide linker comprises a Cys residue, and where the first MHC polypeptide is a β 2M polypeptide that comprises an amino acid substitution that introduces a Cys residue; and b) a second polypeptide comprising an HLA-B MHC Class I heavy chain comprising an amino acid sequence having at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following amino acid sequence:

GSHSMRYFYTSVSRPGRGEPRFISVGYVDDTQFVRFDSDAASPREPRAPWIEQEGPEYWDRNT QIYKAQAQTDRESLRNLRGCYNQSEAGSHTLQSMYGCDVGPDRLLRGHDQYAYDGKDYIAL NEDLRSWTAADTAAQITQRKWEAAREAEQRRAYLEGECVEWLRRYLENGKDKLERADPPKTH VTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPGDRTFQKWA AVVVPS GEEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:350), where amino acid 84 is a Cys and amino acid 236 is a Cys; and c) at least one MOD, where the first and/or the second polypeptide comprises the at least one MOD. In some cases, the peptide linker comprises the amino acid sequence GCGGS (SEQ ID NO:318). In some cases, the peptide linker comprises the amino acid sequence GCGGS(GGGGS)_n (SEQ ID NO:319), where n is an integer from 1 to 10. In some cases, the β 2M polypeptide comprises an R12C substitution. For example, the β 2M polypeptide can comprises an amino acid sequence having at

least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following amino acid sequence:

IQRTPKIQVYSCHPAENGKSNFLNCYVSGFHPSDIEVDLLKNGERIEKVEHSDLSFSKDWSFYLL
YYTEFTPTEKDEYACRVNHVTLSPKIVKWDRDM (SEQ ID NO:311), where amino acid 12 is a Cys. The at least one MOD can be a polypeptide that exerts an activating/stimulating effect on the target T cell or a suppressing/inhibitory effect on the target T cell. For example, the at least one MOD can be a cytokine (e.g., an IL2 polypeptide, an IL7 polypeptide, an IL12 polypeptide, an IL15 polypeptide, an IL17 polypeptide, an IL21 polypeptide, an IL27 polypeptide, an IL-23 polypeptide, a TGF β polypeptide, and the like; and including all family members, e.g., IL17A, IL-17B, IL-17C, IL-17D, IL-17E, IL-17F, IL-17E), a 4-1BBL polypeptide, an ICOS-L polypeptide, an OX-40L polypeptide, a CD80 polypeptide, a CD86 polypeptide, (CD80 and CD86 are also known as B7-1 and B7-2, respectively), a CD40 polypeptide, a CD70 polypeptide, a JAG1 (CD339) polypeptide, an ICAM (CD54) polypeptide, a PD-L1 polypeptide, a FasL polypeptide, a PD-L2 polypeptide, a PD-1H (VISTA) polypeptide, an ICOS-L (CD275) polypeptide, a GITRL polypeptide, an HVEM polypeptide, a CXCL10 polypeptide, a CXCL9 polypeptide, a CXCL11 polypeptide, a CXCL13 polypeptide, and a CX3CL1 polypeptide, a Galectin-9 polypeptide, a CD83 polypeptide, a CD30L polypeptide, a HLA-G polypeptide, a MICA polypeptide, a MICB polypeptide, a HVEM (CD270) polypeptide, a lymphotoxin beta receptor polypeptide, a 3/TR6 polypeptide, an ILT3 polypeptide, an ILT4 polypeptide, a CXCL10 polypeptide, a CXCL9 polypeptide, a CXCL11 polypeptide, a CXCL13 polypeptide, or a CX3CL1 polypeptide. These MODs may be the wild type polypeptide or a variant of a wild type polypeptide. In some cases, the MOD is an activating (“stimulatory”) immunomodulatory polypeptide; e.g., the MOD may produce an activating/stimulating effect on a T cell. Examples of activating MODs include, e.g., CD80, CD86, 4-1BBL, OX40L, CD70, ICOS-L, CD40, ICAM (CD54), IL2, IL7, IL12, IL15, IL17, IL21, IL27, IL23, GITRL, TGF β , and lymphotoxin beta receptor. In some cases, the immunomodulatory polypeptide is an inhibitory (“suppressing”) MOD; e.g., MOD may produce a suppressing/inhibitory effect on a T cell. Examples of inhibitory MODs include, e.g., PD-1H, PD-L1, PD-L2, TGF β , FasL, HVEM, Galectin-9, ILT3, and ILT4. TGF β polypeptides may produce either an activating/stimulating effect or a suppressing/inhibitory effect, depending on the context.

[00214] . In some cases, the at least one MOD is a reduced affinity variant, as described elsewhere herein. In some cases, the first or the second polypeptide comprises an Ig Fc polypeptide.

[00215] In some cases, a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) comprises an HLA-B Class I heavy chain polypeptide. In some cases, the HLA-B heavy chain polypeptide present in a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) comprises an amino acid sequence having at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the HLA-B*0702, HLA-B*0801, HLA-B*1502, HLA-B*3802, HLA-B*4001,

HLA-B*4601, or HLA-B*5301 amino acid sequence depicted in FIG. 10A, where the HLA-B heavy chain polypeptide comprises Y84C and A236C substitutions.

HLA-B*0702 (Y84C; A236C)

[00216] In some cases, the HLA-B heavy chain polypeptide present in a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) comprises an amino acid sequence having at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following HLA-B*0702 (Y84C; A236C) amino acid sequence:

[00217] GSHSMRYFYTSVSRPGRGEPFISVG YVDDTQFVRFDSDAASPREEPRAPWIEQE
GPEYWDRNTQIYKAQAQTDRESLRNLRGCYNQSEAGSHTLQSMYGCDVGPDRLLRGHDQYA
YDGKDYIALNEDLRSWTAADTAAQITQRKWEAAREAEQRRAYLEGECEVWLRRLRYLENGKDKL
ERADPPKTHVTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTEL VETRPCGDRTFQK
WAAVVVPSGEEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:350), where amino acid 84 is a Cys
and amino acid 236 is a Cys.

HLA-B*0801 (Y84C; A236C)

[00218] In some cases, the HLA-B heavy chain polypeptide present in a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) comprises an amino acid sequence having at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following HLA-B*0801 (Y84C; A236C) amino acid sequence:

[00219] GSHSMRYFDTAMSRPGRGEPFISVG YVDDTQFVRFDSDAASPREEPRAPWIEQE
GPEYWDRNTQIFKTNTQTDRESLRNLRGCYNQSEAGSHTLQSMYGCDVGPDRLLRGHNQYA
YDGKDYIALNEDLRSWTAADTAAQITQRKWEAARVAEQDRAYLEGTCVEWLRRLRYLENGKDTL
ERADPPKTHVTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTEL VETRPCGDRTFQK
WAAVVVPSGEEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:351), where amino acid 84 is a Cys
and amino acid 236 is a Cys.

HLA-B*1502 (Y84C; A236C)

[00220] In some cases, the HLA-B heavy chain polypeptide present in a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) comprises an amino acid sequence having at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following HLA-B*1502 (Y84C; A236C) amino acid sequence:

[00221] GSHSMRYFYTAMSRPGRGEPFIAVG YVDDTQFVRFDSDAASPRMAPRAPWIEQE
EGPEYWDRNTQISKTNQTYRESLRNLRGCYNQSEAGSHIIQRMYGCDVGPDRLLRGYDQSA
YDGKDYIALNEDLSSWTAADTAAQITQRKWEAAREAEQLRAYLEGLCVEWLRRLRYLENGKETL
QRADPPKTHVTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTEL VETRPCGDRTFQ

KWAAVVVPSGEEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:352), where amino acid 84 is a Cys and amino acid 236 is a Cys.

HLA-B*3802 (Y84C; A236C)

[00222] In some cases, the HLA-B heavy chain polypeptide present in a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) comprises an amino acid sequence having at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following HLA-B*3802 (Y84C; A236C) amino acid sequence:

[00223] GSHSMRYFYTSVSRPGRGEPFISVG YVDDTQFVRFDS DAASPREEPRAPWIEQE
GPEYWDRTNTQICKTNTQTYRENLR TALRCYNQSEAGSHTLQRM YGCDVGP DGRLLRGHNQFA
YDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRTYLEGTCVEWLRRLYLENGKETL
QRADPPKTHVTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTEL VETRPCGDRTFQ
KWAAVVVPSGEEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:353), where amino acid 84 is a Cys and amino acid 236 is a Cys.

HLA-B*4001 (Y84C; A236C)

[00224] In some cases, the HLA-B heavy chain polypeptide present in a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) comprises an amino acid sequence having at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following HLA-B*4001 (Y84C; A236C) amino acid sequence:

[00225] GSHSMRYFHTAMSRPGRGEPFITVGYVDDTLFVRFDS DATSPRKEPRAPWIEQE
GPEYWDRETQISKNTNTQTYRESLRNLRG CYNQSEAGSHTLQRM YGCDVGP DGRLLRGHNQYA
YDGKDYIALNEDLRSWTAADTAAQISQRKLEAARVAEQLRAYLEGECEWLRRLYLENGKDKL
ERADPPKTHVTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTEL VETRPCGDRTFQK
WAAVVVPSGEEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:354) where amino acid 84 is a Cys and amino acid 236 is a Cys.

HLA-B*4601 (Y84C; A236C)

[00226] In some cases, the HLA-B heavy chain polypeptide present in a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) comprises an amino acid sequence having at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following HLA-B*4601 (Y84C; A236C) amino acid sequence:

[00227] GSHSMRYFYTAMSRPGRGEPFIAVGYVDDTQFVRFDS DAASPRMAPRAPWIEQE
EGPEYWDRETQKYKRQAQTDRVSLRNLRG CYNQSEAGSHTLQRM YGCDVGP DGRLLRGHDQ
SAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAAREAEQWRAYLEGLCWEWLRRLYLENGKE
TLQRADPPKTHVTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTEL VETRPCGDRTF

QKWAADVVPSPGEEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:355) where amino acid 84 is a Cys and amino acid 236 is a Cys.

HLA-B*5301 (Y84C; A236C)

[00228] In some cases, the HLA-B heavy chain polypeptide present in a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) comprises an amino acid sequence having at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following HLA-B*5301 (Y84C; A236C) amino acid sequence:

[00229] GSHSMRYFYTAMSRPGRGEPFIAVGYVDDTQFVRFDSDAASPRTEPRAPWIEQE
GPEYWDRTNTQIFKTNTQTYRENLRIALRCYNQSEAGSHIIQRMYGCDLGPDRLLRGHDQSAY
DGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGLCWEWLRRYLENGKETLQ
RADPPKTHVTHHPVSDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPCGDRFTQK
WAAVVVPSPGEEQRYTCHVQHEGLPKPLTLRWE (SEQ ID NO:356) where amino acid 84 is a Cys and amino acid 236 is a Cys.

HLA-C

In some cases, a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) comprises: a) a first polypeptide comprising: i) a WT-1 peptide (e.g., 1 WT-1 peptide of from 4 amino acids to about 25 amino acids); and ii) a first MHC polypeptide, where the first polypeptide comprises a peptide linker between the WT-1 peptide and the first MHC polypeptide, where the peptide linker comprises a Cys residue, and where the first MHC polypeptide is a β 2M polypeptide that comprises an amino acid substitution that introduces a Cys residue; and b) a second polypeptide comprising an HLA-C MHC Class I heavy chain comprising an amino acid sequence having at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following amino acid sequence:

CSHSMRYFDTA VSRPGRGEPFISVGYVDDTQFVRFDSDAASPRGEPAPWVEQEGPEYWDRE
TQNYKRQAQADRVSLRNLRGCYNQSEDSHTLQRMYGCDLGPDRLLRGYDQSAYDGKDYI
ALNEDLRSWTAADTAAQITQRKLEAARAAEQLRAYLEGTCWEWLRRYLENGKETLQRAEPPKT
HVTHHPLSDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPCGDTGTFQKWAADVVP
PSGQEQRYTCHMQHEGLQEPLTSLWEP (SEQ ID NO:357), where amino acid 84 is a Cys and amino acid 236 is a Cys; and c) at least one MOD, where the first and/or the second polypeptide comprises the at least one MOD. In some cases, the peptide linker comprises the amino acid sequence GCGGS (SEQ ID NO:318). In some cases, the peptide linker comprises the amino acid sequence GCGGS(GGGGS)_n (SEQ ID NO:319), where n is an integer from 1 to 10. In some cases, the β 2M polypeptide comprises an R12C substitution. For example, the β 2M polypeptide can comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following amino acid sequence:

IQRTPKIQVYSCHPAENGKSNFLNCYVSGFHPSDIEVDLLKNGERIEKVEHSDLSFSKDWSFYLL
 YYTEFTPTEKDEYACRVNHVTLSPKIVKWDRDM (SEQ ID NO:311), where amino acid 12 is a
 Cys. The at least one MOD can be a polypeptide that exerts an activating/stimulating effect on the target
 T cell or a suppressing/inhibitory effect on the target T cell. For example, the at least one MOD can be a
 cytokine (e.g., an IL2 polypeptide, an IL7 polypeptide, an IL12 polypeptide, an IL15 polypeptide, an
 IL17 polypeptide, an IL21 polypeptide, an IL27 polypeptide, an IL-23 polypeptide, a TGF β polypeptide,
 and the like; and including all family members, e.g., IL17A, IL-17B, IL-17C, IL-17D, IL-17E, IL-17F,
 IL-17E), a 4-1BBL polypeptide, an ICOS-L polypeptide, an OX-40L polypeptide, a CD80 polypeptide, a
 CD86 polypeptide, (CD80 and CD86 are also known as B7-1 and B7-2, respectively), a CD40
 polypeptide, a CD70 polypeptide, a JAG1 (CD339) polypeptide, an ICAM (CD54) polypeptide, a PD-
 L1 polypeptide, a FasL polypeptide, a PD-L2 polypeptide, a PD-1H (VISTA) polypeptide, an ICOS-L
 (CD275) polypeptide, a GITRL polypeptide, an HVEM polypeptide, a CXCL10 polypeptide, a CXCL9
 polypeptide, a CXCL11 polypeptide, a CXCL13 polypeptide, and a CX3CL1 polypeptide, a Galectin-9
 polypeptide, a CD83 polypeptide, a CD30L polypeptide, a HLA-G polypeptide, a MICA polypeptide, a
 MICB polypeptide, a HVEM (CD270) polypeptide, a lymphotoxin beta receptor polypeptide, a 3/TR6
 polypeptide, an ILT3 polypeptide, an ILT4 polypeptide, a CXCL10 polypeptide, a CXCL9 polypeptide,
 a CXCL11 polypeptide, a CXCL13 polypeptide, or a CX3CL1 polypeptide. These MODs may be the
 wild type polypeptide or a variant of a wild type polypeptide. In some cases, the immunomodulatory
 polypeptide is an activating (“stimulatory”) MOD; e.g., the MOD may produce an activating/stimulating
 effect on a T cell. Examples of activating immunomodulatory polypeptides include, e.g., CD80, CD86,
 4-1BBL, OX40L, CD70, ICOS-L, CD40, ICAM (CD54), IL2, IL7, IL12, IL15, IL17, IL21, IL27, IL23,
 GITRL, TGF β , and lymphotoxin beta receptor. In some cases, the MOD is an inhibitory (“suppressing”)
 MOD; e.g., the MOD may produce a suppressing/inhibitory effect on a T cell. Examples of inhibitory
 MODs include, e.g., PD-1H, PD-L1, PD-L2, TGF β , FasL, HVEM, Galectin-9, ILT3, and ILT4. TGF β
 polypeptides may produce either an activating/stimulating effect or a suppressing/inhibitory effect,
 depending on the context.

[00230] In some cases, the at least one MOD is a reduced affinity variant, as described elsewhere
 herein. In some cases, the first or the second polypeptide comprises an Ig Fc polypeptide.

[00231] In some cases, a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP)
 comprises an HLA-C Class I heavy chain polypeptide. In some cases, the HLA-C heavy chain
 polypeptide present in a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP)
 comprises an amino acid sequence having at least 95%, at least 98%, or at least 99%, amino acid
 sequence identity to the HLA-C*0102, HLA-C*0303, HLA-C*0304, HLA-C*0401, HLA-C*0602,
 HLA-C*0701, HLA-C*0702, HLA-C*0801, or HLA-C*1502 amino acid sequence depicted in FIG.
 11A, where the HLA-C heavy chain polypeptide comprises Y84C and A236C substitutions.

HLA-C*01:02 (Y84C; A236C)

[00232] In some cases, the HLA-C heavy chain polypeptide present in a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) comprises an amino acid sequence having at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following HLA-C*01:02 (Y84C; A236C) amino acid sequence:

[00233] CSHSMKYFFTSVSRPGRGEPFISVGYVDDTQFVRFDSDAASPRGEPRAPWVEQE
GPEYWDRETQKYKRQAQTDRVSLRNLRG CYNQSEAGSHTLQWMCGCDLGPDRLLRGYDQY
AYDGKDYLALNEDLRSWTAADTAAQITQRKWEAAREAEQRRAYLEGTCVEWLRRYLENGKET
LQRAEHPKTHVTHHPVSDHEATLRCWALGFYPAEITLTWQWDGEDQTQDTELVETRPCGDGTF
QKWAAMVPSGEEQRYTCHVQHEGLPEPLTLRWEP (SEQ ID NO:358), where amino acid 84 is a
Cys and amino acid 236 is a Cys.

HLA-C*03:03 (Y84C; A236C)

[00234] In some cases, the HLA-C heavy chain polypeptide present in a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) comprises an amino acid sequence having at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following HLA-C*03:03 (Y84C; A236C) amino acid sequence:

[00235] GSHSMRYFYTAVSRPGRGEPHFIAVGYVDDTQFVRFDSDAASPRGEPRAPWVEQE
EGPEYWDRETQKYKRQAQTDRVSLRNLRG CYNQSEARSHIIQRMYGCDVGPDRLLRGYDQY
AYDGKDYLALNEDLRSWTAADTAAQITQRKWEAAREAEQLRAYLEGLCVEWLRRYLKNGKET
LQRAEHPKTHVTHHPVSDHEATLRCWALGFYPAEITLTWQWDGEDQTQDTELVETRPCGDGTF
QKWAAMVVPSGEEQRYTCHVQHEGLPEPLTLRWEP (SEQ ID NO:359), where amino acid 84 is a
Cys and amino acid 236 is a Cys.

HLA-C*03:04 (Y84C; A236C)

[00236] In some cases, the HLA-C heavy chain polypeptide present in a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) comprises an amino acid sequence having at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following HLA-C*03:04 (Y84C; A236C) amino acid sequence:

[00237] GSHSMRYFYTAVSRPGRGEPHFIAVGYVDDTQFVRFDSDAASPRGEPRAPWVEQE
EGPEYWDRETQKYKRQAQTDRVSLRNLRG CYNQSEAGSHIIQRMYGCDVGPDRLLRGYDQY
AYDGKDYLALNEDLRSWTAADTAAQITQRKWEAAREAEQLRAYLEGLCVEWLRRYLKNGKET
LQRAEHPKTHVTHHPVSDHEATLRCWALGFYPAEITLTWQWDGEDQTQDTELVETRPCGDGTF
QKWAAMVVPSGEEQRYTCHVQHEGLPEPLTLRWEP (SEQ ID NO:360), where amino acid 84 is a
Cys and amino acid 236 is a Cys.

HLA-C*0401 (Y84C; A236C)

[00238] In some cases, the HLA-C heavy chain polypeptide present in a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) comprises an amino acid sequence having at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following HLA-C*04:01 (Y84C; A236C) amino acid sequence:

[00239] GSHSMRYFSTSVSWPGRGEPRIAVGYVDDTQFVRFSDAASPRGEPREPWVEQ
EGPEYWDRETQKYKRQAQADRVNLRKLRGCYNQSEDGSHTLQRMFGCDLGPDRLLRGYNQ
FAYDGGDYIALNEDLRSWTAADTAAQITQRKWEAAREAEQRRAYLEGTCVEWLRRLYLENGKE
TLQRAEHPKTHVTHHPVSDHEATLRCWALGFYPAEITLTWQWDGEDQTQDTELVETRPCGDGT
FQKWAADVVPVGEEQRYTCHVQHEGLPEPLTLRWKP (SEQ ID NO:361), where amino acid 84 is a Cys and amino acid 236 is a Cys.

HLA-C*0602 (Y84C; A236C)

[00240] In some cases, the HLA-C heavy chain polypeptide present in a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) comprises an amino acid sequence having at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following HLA-C*06:02 (Y84C; A236C) amino acid sequence:

[00241] CSHSMRYFDTAVSRPGRGEPFISVG YVDDTQFVRFSDAASPRGEPRAPWVEQ
EGPEYWDRETQKYKRQAQADRVNLRKLRGCYNQSEDGSHTLQWMYGCDLGPDRLLRGYD
QSAYDGGDYIALNEDLRSWTAADTAAQITQRKWEAAREAEQWRAYLEGTCVEWLRRLYLENG
KETLQRAEHPKTHVTHHPVSDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPCGD
GTFQKWAADVVPVGEEQRYTCHVQHEGLPEPLTLRWEP (SEQ ID NO:362), where amino acid 84 is a Cys and amino acid 236 is a Cys.

HLA-C*0701 (Y84C; A236C)

[00242] In some cases, the HLA-C heavy chain polypeptide present in a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) comprises an amino acid sequence having at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following HLA-C*07:01 (Y84C; A236C) amino acid sequence:

[00243] CSHSMRYFDTAVSRPGRGEPFISVG YVDDTQFVRFSDAASPRGEPRAPWVEQ
EGPEYWDRETQNYKRQAQADRVSLRNLRGCYNQSEDGSHTLQRMYGCDLGPDRLLRGYDQ
SAYDGGDYIALNEDLRSWTAADTAAQITQRKLEAARAAEQRAYLEGTCVEWLRRLYLENGKE
TLQRAEPPKTHVTHHPLSDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPCGDGT
FQKWAADVVPVGEEQRYTCHMQHEGLQEPLTLWEP (SEQ ID NO:357), where amino acid 84 is a Cys and amino acid 236 is a Cys.

HLA-C*0702 (Y84C; A236C)

[00244] In some cases, the HLA-C heavy chain polypeptide present in a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) comprises an amino acid sequence having at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following HLA-C*07:02 (Y84C; A236C) amino acid sequence:

[00245] CSHSMRYFDTA VSRPGRGEP RFISVGYVDDTQFVRFDSDAASPRGEP RAPWVEQ
EGPEYWDRETQKYKRQAQADRVSLRNLRGCYNQSE DGSHTLQRMSGCDLGP DGRLLRGYDQS
AYDGKD YIALNEDLRSWTAADTAAQITQRKLEAARAAEQLRAYLEGTCVEWLRRYLENGKET
LQRAEPPKTHVTHHPLSDHEATLRCWALGFYP AEITLTWQRDGEDQTQDTELVETRPCGDGTF
QKWA AVVVPSGQEQR YTCHMQHEGLQEPLT LSWEP (SEQ ID NO:404), where amino acid 84 is a Cys and amino acid 236 is a Cys.

HLA-C*0801 (Y84C; A236C)

[00246] In some cases, the HLA-C heavy chain polypeptide present in a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) comprises an amino acid sequence having at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following HLA-C*08:01 (Y84C; A236C) amino acid sequence:

[00247] CSHSMRYFYTA VSRPGRGEP RFIAVGYVDDTQFVQFSDAASPRGEP RAPWVEQ
EGPEYWDRETQKYKRQAQTDRVSLRNLRGCYNQSE AGSHTLQRMYGCDLGP DGRLLRGYNQ
FAYDGKD YIALNEDLRSWTAADTAAQITQRKWEAARTAEQLRAYLEGTCVEWLRRYLENGKK
TLQRAEHPKTHVTHHPVSDHEATLRCWALGFYP AEITLTWQRDGEDQTQDTELVETRPCGDGT
FQKWA AVVVPSGEEQR YTCHVQHEGLPEPL TLRWGP (SEQ ID NO:363), where amino acid 84 is a Cys and amino acid 236 is a Cys.

HLA-C*1502 (Y84C; A236C)

[00248] In some cases, the HLA-C heavy chain polypeptide present in a multiple disulfide-linked TMMP (e.g., a double disulfide-linked TMMP) comprises an amino acid sequence having at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following HLA-C*15:02 (Y84C; A236C) amino acid sequence:

[00249] CSHSMRYFYTA VSRPGRGEP HFIAVGYVDDTQFVRFDSDAASPRGEP RAPWVEQ
EGPEYWDRETQNYKRQAQTDRVNLRLKLRGCYNQSE AGSHIIQRMYGCDLGP DGRLLRGHDQL
AYDGKD YIALNEDLRSWTAADTAAQITQRKWEAAREAEQLRAYLEGTCVEWLRRYLENGKET
LQRAEHPKTHVTHHPVSDHEATLRCWALGFYP AEITLTWQRDGEDQTQDTELVETRPCGDGTF
QKWA AVVVPSGEEQR YTCHVQHEGLPEPL TLRWEP (SEQ ID NO:364), where amino acid 84 is a Cys and amino acid 236 is a Cys.

Scaffold polypeptides

[00250] A TMMP can comprise an Fc polypeptide, or can comprise another suitable scaffold polypeptide.

[00251] Suitable scaffold polypeptides include antibody-based scaffold polypeptides and non-antibody-based scaffolds. Non-antibody-based scaffolds include, e.g., albumin, an XTEN (extended recombinant) polypeptide, transferrin, an Fc receptor polypeptide, an elastin-like polypeptide (see, e.g., Hassounch et al. (2012) *Methods Enzymol.* 502:215; e.g., a polypeptide comprising a pentapeptide repeat unit of (Val-Pro-Gly-X-Gly; SEQ ID NO:59), where X is any amino acid other than proline), an albumin-binding polypeptide, a silk-like polypeptide (see, e.g., Valluzzi et al. (2002) *Philos Trans R Soc Lond B Biol Sci.* 357:165), a silk-elastin-like polypeptide (SELP; see, e.g., Megeed et al. (2002) *Adv Drug Deliv Rev.* 54:1075), and the like. Suitable XTEN polypeptides include, e.g., those disclosed in WO 2009/023270, WO 2010/091122, WO 2007/103515, US 2010/0189682, and US 2009/0092582; see also Schellenberger et al. (2009) *Nat Biotechnol.* 27:1186). Suitable albumin polypeptides include, e.g., human serum albumin.

[00252] Suitable scaffold polypeptides will in some cases be a half-life extending polypeptides. Thus, in some cases, a suitable scaffold polypeptide increases the *in vivo* half-life (e.g., the serum half-life) of the TMMP, compared to a control TMMP lacking the scaffold polypeptide. For example, in some cases, a scaffold polypeptide increases the *in vivo* half-life (e.g., the serum half-life) of the TMMP, compared to a control TMMP lacking the scaffold polypeptide, by at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 50%, at least about 2-fold, at least about 2.5-fold, at least about 5-fold, at least about 10-fold, at least about 25-fold, at least about 50-fold, at least about 100-fold, or more than 100-fold. As an example, in some cases, an Fc polypeptide increases the *in vivo* half-life (e.g., the serum half-life) of the TMMP, compared to a control TMMP lacking the Fc polypeptide, by at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 50%, at least about 2-fold, at least about 2.5-fold, at least about 5-fold, at least about 10-fold, at least about 25-fold, at least about 50-fold, at least about 100-fold, or more than 100-fold.

Fc polypeptides

[00253] In some cases, the first and/or the second polypeptide chain of a TMMP of the present disclosure comprises an Fc polypeptide. The Fc polypeptide of a TMMP can be a human IgG1 Fc, a human IgG2 Fc, a human IgG3 Fc, a human IgG4 Fc, etc. In some cases, the Fc polypeptide comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to an amino acid sequence of an Fc region depicted in FIG. 5A-5G or 5H. In some cases, the Fc region comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least

about 99%, or 100%, amino acid sequence identity to the human IgG1 Fc polypeptide depicted in FIG. 5A. In some cases, the Fc region comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to the human IgG1 Fc polypeptide depicted in FIG. 5A; and comprises a substitution of N77; e.g., the Fc polypeptide comprises an N77A substitution. In some cases, the Fc polypeptide comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to the human IgG2 Fc polypeptide depicted in FIG. 5A; e.g., the Fc polypeptide comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to amino acids 99-325 of the human IgG2 Fc polypeptide depicted in FIG. 5A. In some cases, the Fc polypeptide comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to the human IgG3 Fc polypeptide depicted in FIG. 5A; e.g., the Fc polypeptide comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to amino acids 19-246 of the human IgG3 Fc polypeptide depicted in FIG. 5A. In some cases, the Fc polypeptide comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to the human IgM Fc polypeptide depicted in FIG. 5B; e.g., the Fc polypeptide comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to amino acids 1-276 of the human IgM Fc polypeptide depicted in FIG. 5B. In some cases, the Fc polypeptide comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to the human IgA Fc polypeptide depicted in FIG. 5C; e.g., the Fc polypeptide comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to amino acids 1-234 of the human IgA Fc polypeptide depicted in FIG. 5C.

[00254] In some cases, the Fc polypeptide comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to the human IgG4 Fc

polypeptide depicted in FIG. 5C. In some cases, the Fc polypeptide comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to amino acids 100 to 327 of the human IgG4 Fc polypeptide depicted in FIG. 5C.

[00255] In some cases, the IgG4 Fc polypeptide comprises the following amino acid sequence:
PPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNA
KTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTL
PPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSRLTVDKS
RWQEGNVFSCSVMEALHNHYTQKSLSLSPG (SEQ ID NO:365).

[00256] In some cases, the Fc polypeptide present in a TMMP comprises the amino acid sequence depicted in FIG. 5A (human IgG1 Fc). In some cases, the Fc polypeptide present in a TMMP comprises the amino acid sequence depicted in FIG. 5A (human IgG1 Fc), except for a substitution of N297 (N77 of the amino acid sequence depicted in FIG. 5A) with an amino acid other than asparagine. In some cases, the Fc polypeptide present in a TMMP comprises the amino acid sequence depicted in FIG. 5C (human IgG1 Fc comprising an N297A substitution, which is N77 of the amino acid sequence depicted in FIG. 5A). In some cases, the Fc polypeptide present in a TMMP comprises the amino acid sequence depicted in FIG. 5A (human IgG1 Fc), except for a substitution of L234 (L14 of the amino acid sequence depicted in FIG. 5A) with an amino acid other than leucine. In some cases, the Fc polypeptide present in a TMMP comprises the amino acid sequence depicted in FIG. 5A (human IgG1 Fc), except for a substitution of L235 (L15 of the amino acid sequence depicted in FIG. 5A) with an amino acid other than leucine. In some cases, the IgG1 Fc polypeptide comprises the C-terminal Lys depicted in FIG. 5A. In other cases, the IgG1 Fc polypeptide does not include the C-terminal Lys depicted in FIG. 5A.

[00257] In some cases, the Fc polypeptide present in a TMMP comprises the amino acid sequence depicted in FIG. 5E. In some cases, the Fc polypeptide comprises the amino acid sequence depicted in FIG. 5E, but without the C-terminal Lys. In some cases, the Fc polypeptide present in a TMMP comprises the amino acid sequence depicted in FIG. 5F. In some cases, the Fc polypeptide comprises the amino acid sequence depicted in FIG. 5F, but without the C-terminal Lys. In some cases, the Fc polypeptide present in a TMMP comprises the amino acid sequence depicted in FIG. 5G (human IgG1 Fc comprising an L234A substitution and an L235A substitution, corresponding to positions 14 and 15 of the amino acid sequence depicted in FIG. 5G). In some cases, the Fc polypeptide comprises the amino acid sequence depicted in FIG. 5G, but without the C-terminal Lys. In some cases, the Fc polypeptide present in a TMMP comprises the amino acid sequence depicted in FIG. 5A (human IgG1 Fc), except for a substitution of P331 (P111 of the amino acid sequence depicted in FIG. 5A) with an amino acid other than proline; in some cases, the substitution is a P331S substitution. In some cases, the Fc polypeptide present in a TMMP comprises the amino acid sequence depicted in FIG. 5A (human IgG1 Fc), except for

substitutions at L234 and L235 (L14 and L15 of the amino acid sequence depicted in FIG. 5A) with amino acids other than leucine. In some cases, the Fc polypeptide present in a TMMP comprises the amino acid sequence depicted in FIG. 5A (human IgG1 Fc), except for substitutions at L234 and L235 (L14 and L15 of the amino acid sequence depicted in FIG. 5A) with amino acids other than leucine, and a substitution of P331 (P111 of the amino acid sequence depicted in FIG. 5A) with an amino acid other than proline. In some cases, the Fc polypeptide present in a TMMP comprises the amino acid sequence depicted in FIG. 5E (human IgG1 Fc comprising L234F, L235E, and P331S substitutions (corresponding to amino acid positions 14, 15, and 111 of the amino acid sequence depicted in FIG. 5E)). In some cases, the Fc polypeptide present in a TMMP is an IgG1 Fc polypeptide that comprises L234A and L235A substitutions (substitutions of L14 and L15 of the amino acid sequence depicted in FIG. 5A with Ala), as depicted in FIG. 5G.

[00258] In some cases, the Fc polypeptide present in a TMMP has the following amino acid sequence:

DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLT
VDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:489); and has a length of 226 amino acids.

Linkers

[00259] A TMMP of the present disclosure can include one or more linkers, where the one or more linkers are between one or more of: i) an MHC Class I polypeptide and an Ig Fc polypeptide, where such a linker is referred to herein as “L1”; ii) a MOD and an MHC Class I polypeptide, where such a linker is referred to herein as “L2”; iii) a first MOD and a second MOD, where such a linker is referred to herein as “L3”; iv) a peptide antigen (“epitope”) and an MHC Class I polypeptide; v) an MHC Class I polypeptide and a dimerization polypeptide (e.g., a first or a second member of a dimerizing pair); and vi) a dimerization polypeptide (e.g., a first or a second member of a dimerizing pair) and an Ig Fc polypeptide.

[00260] As used herein, the phrase “a peptide linker between any two of the components of a TMMP” refers to a peptide linker between any two adjacent polypeptides within the TMMP. For example, as used herein, the phrase “a peptide linker between any two of the components of a TMMP” refers to a peptide linker between one or more of: i) a peptide and a β 2M polypeptide; ii) a β 2M polypeptide and an MHC class I heavy chain polypeptide; iii) an MHC class I heavy chain polypeptide and an Ig Fc polypeptide; iv) an MHC class I heavy chain polypeptide and a MOD; v) an Ig Fc polypeptide and a MOD; and vi) a first MOD and a second MOD.

[00261] Suitable linkers (also referred to as “spacers”) can be readily selected and can be of any of a number of suitable lengths, such as from 1 amino acid to 25 amino acids, from 3 amino acids to 20 amino acids, from 2 amino acids to 15 amino acids, from 3 amino acids to 12 amino acids, including 4 amino acids to 10 amino acids, 5 amino acids to 9 amino acids, 6 amino acids to 8 amino acids, or 7 amino acids to 8 amino acids. A suitable linker can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 amino acids in length. In some cases, a linker has a length of from 25 amino acids to 50 amino acids, e.g., from 25 to 30, from 30 to 35, from 35 to 40, from 40 to 45, or from 45 to 50 amino acids in length.

[00262] Exemplary linkers include glycine polymers (G)_n, glycine-serine polymers (including, for example, (GS)_n, (GSGGS)_n (SEQ ID NO:366) and (GGGS)_n (SEQ ID NO:367), where n is an integer of at least one), glycine-alanine polymers, alanine-serine polymers, and other flexible linkers known in the art. Glycine and glycine-serine polymers can be used; both Gly and Ser are relatively unstructured, and therefore can serve as a neutral tether between components. Glycine polymers can be used; glycine accesses significantly more phi-psi space than even alanine, and is much less restricted than residues with longer side chains (see Scheraga, *Rev. Computational Chem.* 11173-142 (1992)). Exemplary linkers can comprise amino acid sequences including, but not limited to, GGSG (SEQ ID NO:368), GSGGG (SEQ ID NO:369), GSGSG (SEQ ID NO:370), GSGGG (SEQ ID NO:371), GGGSG (SEQ ID NO:372), GSSSG (SEQ ID NO:373), and the like. Exemplary linkers can include, e.g., Gly(Ser₄)_n (SEQ ID NO:374), where n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In some cases, a linker comprises the amino acid sequence (GSSSS)_n (SEQ ID NO:375), where n is 4. In some cases, a linker comprises the amino acid sequence (GSSSS)_n (SEQ ID NO:376), where n is 5. In some cases, a linker comprises the amino acid sequence (GGGGS)_n (SEQ ID NO:377), where n is 1. In some cases, a linker comprises the amino acid sequence (GGGGS)_n (SEQ ID NO:378), where n is 2. In some cases, a linker comprises the amino acid sequence (GGGGS)_n (SEQ ID NO:379), where n is 3. In some cases, a linker comprises the amino acid sequence (GGGGS)_n (SEQ ID NO:380), where n is 4. In some cases, a linker comprises the amino acid sequence (GGGGS)_n (SEQ ID NO:381), where n is 5. In some cases, a linker comprises the amino acid sequence (GGGGS)_n (SEQ ID NO:382), where n is 6. In some cases, a linker comprises the amino acid sequence (GGGGS)_n (SEQ ID NO:383), where n is 7. In some cases, a linker comprises the amino acid sequence (GGGGS)_n (SEQ ID NO:384), where n is 8. In some cases, a linker comprises the amino acid sequence (GGGGS)_n (SEQ ID NO:385), where n is 9. In some cases, a linker comprises the amino acid sequence (GGGGS)_n (SEQ ID NO:386), where n is 10. In some cases, a linker comprises the amino acid sequence AAAGG (SEQ ID NO:283).

[00263] In some cases, a linker polypeptide, present in a first polypeptide of a TMMP, includes a cysteine residue that can form a disulfide bond with a cysteine residue present in a second polypeptide of a TMMP. In some cases, for example, a suitable linker comprises the amino acid sequence

GCGGSGGGGSGGGGS (SEQ ID NO:317). As another example, a suitable linker can comprise the amino acid sequence GCGGS(G4S)_n (SEQ ID NO:315), where n is 1, 2, 3, 4, 5, 6, 7, 8, or 9. For example, in some cases, the linker comprises the amino acid sequence GCGGSGGGGSGGGGSGGGGS (SEQ ID NO:316). As another example, the linker comprises the amino acid sequence GCGGSGGGGSGGGGS (SEQ ID NO:317).

Epitopes

[00264] In some cases, an epitope (a peptide presenting one or more epitopes) present in a TMMP is a WT-1 peptide, e.g., a WT-1 peptide that, together with MHC, presents an epitope to a TCR. Amino acid sequences of WT-1 isoforms are presented in FIG. 3A-3E. A WT-1 peptide that presents one or more epitopes is referred to herein as a “WT-1 peptide” or a “WT-1 epitope.” In some cases, a WT-1 epitope present in a TMMP of the present disclosure can be a peptide of from 4 to 25 contiguous amino acids (e.g., 4 amino acids (aa), 5 aa, 6 aa, 7 aa, 8 aa, 9 aa, 10-15 aa, 15-20 aa, or 20-25 aa) of an amino acid sequence having at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the WT-1 amino acid sequence depicted in any one of FIG. 3A-3E. In some cases, a WT-1 epitope present in a TMMP of the present disclosure can be a peptide of from 4 to 25 contiguous amino acids (e.g., 4 amino acids (aa), 5 aa, 6 aa, 7 aa, 8 aa, 9 aa, 10-15 aa, 15-20 aa, or 20-25 aa) of an amino acid sequence having at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the WT-1 amino acid sequence depicted in FIG. 3A. In some cases, a WT-1 epitope present in a TMMP can be a peptide of from 4 to 25 contiguous amino acids (e.g., 4 amino acids (aa), 5 aa, 6 aa, 7 aa, 8 aa, 9 aa, 10-15 aa, 15-20 aa, or 20-25 aa) of an amino acid sequence having at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the WT-1 amino acid sequence depicted in FIG. 3B. In some cases, a WT-1 epitope present in a TMMP of the present disclosure can be a peptide of from 4 to 25 contiguous amino acids (e.g., 4 amino acids (aa), 5 aa, 6 aa, 7 aa, 8 aa, 9 aa, 10-15 aa, 15-20 aa, or 20-25 aa) of an amino acid sequence having at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the WT-1 amino acid sequence depicted in FIG. 3C. In some cases, a WT-1 epitope present in a TMMP can be a peptide of from 4 to 25 contiguous amino acids (e.g., 4 amino acids (aa), 5 aa, 6 aa, 7 aa, 8 aa, 9 aa, 10-15 aa, 15-20 aa, or 20-25 aa) of an amino acid sequence having at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the WT-1 amino acid sequence depicted in FIG. 3D. In some cases, a WT-1 epitope present in a TMMP of the present disclosure can be a peptide of from 4 to 25 contiguous amino acids (e.g., 4 amino acids (aa), 5 aa, 6 aa, 7 aa, 8 aa, 9 aa, 10-15 aa, 15-20 aa, or 20-25 aa) of an amino acid sequence having at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the WT-1 amino acid sequence depicted in FIG. 3E. In some cases, a WT-1 epitope present in a TMMP is 6 amino acids in length. In some cases, a WT-1 epitope present in a TMMP is 7 amino acids in length. In some cases, a WT-1 epitope present in a TMMP is 8 amino acids in

length. In some cases, a WT-1 epitope present in a TMMP is 9 amino acids in length. In some cases, a WT-1 epitope present in a TMMP is 10 amino acids in length. In some cases, a WT-1 epitope present in a TMMP is 11 amino acids in length. In some cases, a WT-1 epitope present in a TMMP is from 6 amino acids to 25 amino acids in length. In some cases, a WT-1 epitope present in a TMMP is from 6 amino acids to 20 amino acids in length. In some cases, a WT-1 epitope present in a TMMP is from 7 amino acids to 25 amino acids in length. In some cases, a WT-1 epitope present in a TMMP is from 7 amino acids to 20 amino acids in length. In some cases, a WT-1 epitope present in a TMMP is at least 4 amino acids in length, at least 6 amino acids in length, or at least 7 amino acids in length.

[00265] An epitope present in a TMMP can have a length of from about 4 amino acids to about 25 amino acids, e.g., the epitope can have a length of from 4 amino acids (aa) to 10 aa, from 10 aa to 15 aa, from 15 aa to 20 aa, or from 20 aa to 25 aa. For example, an epitope present in a TMMP can have a length of 4 amino acids (aa), 5 aa, 6 aa, 7 aa, 8 aa, 9 aa, 10 aa, 11 aa, 12 aa, 13 aa, 14 aa, 15 aa, 16 aa, 17 aa, 18 aa, 19 aa, 20 aa, 21 aa, 22 aa, 23 aa, 24 aa, or 25 aa. In some cases, an epitope present in a TMMP has a length of from 5 amino acids to 10 amino acids, e.g., 5 aa, 6 aa, 7 aa, 8 aa, 9 aa, or 10 aa.

[00266] A WT-1 epitope present in a TMMP is a peptide specifically bound by a T-cell, i.e., the epitope is specifically bound by a WT-1 epitope-specific T cell. An epitope-specific T cell binds an epitope having a reference amino acid sequence, but does not substantially bind an epitope that differs from the reference amino acid sequence. For example, an epitope-specific T cell binds an epitope having a reference amino acid sequence, and binds an epitope that differs from the reference amino acid sequence, if at all, with an affinity that is less than 10^{-6} M, less than 10^{-5} M, or less than 10^{-4} M. An epitope-specific T cell can bind an epitope for which it is specific with an affinity of at least 10^{-7} M, at least 10^{-8} M, at least 10^{-9} M, or at least 10^{-10} M.

[00267] Examples of WT-1 peptides suitable for inclusion in a TMMP include, but are not limited to, CMTWNQMNLGATLKG (SEQ ID NO:223), WNQMNLGATLKGVAA (SEQ ID NO:224), CMTWNYMNLGATLKG (SEQ ID NO:225), WNYMNLGATLKGVAA (SEQ ID NO:226), MTWNQMNLGATLKG (SEQ ID NO:227), TWNQMNLGATLKGVA (SEQ ID NO:228), CMTWNLNMNLGATLKG (SEQ ID NO:229), MTWNLNMNLGATLKG (SEQ ID NO:230), TWNLNMNLGATLKGVA (SEQ ID NO:231), WNLNMNLGATLKGVAA (SEQ ID NO:232), MNLGATLKG (SEQ ID NO:233), MTWNYMNLGATLKG (SEQ ID NO:234), TWNYMNLGATLKGVA (SEQ ID NO:235), CMTWNQMNLGATLKGVA (SEQ ID NO:236), CMTWNLNMNLGATLKGVA (SEQ ID NO:237), CMTWNYMNLGATLKGVA (SEQ ID NO:238), GYLRNPTAC (SEQ ID NO:239), GALRNPTAL (SEQ ID NO:240), YALRNPTAC (SEQ ID NO:241), GLLRNPTAC (SEQ ID NO:242), RYRPHPGAL (SEQ ID NO:243), YQRPHPGAL (SEQ ID NO:244), RLRPHPGAL (SEQ ID NO:245), RIRPHPGAL (SEQ ID NO:246), QFPNHSFKHEDPMGQ (SEQ ID NO:247), HSFKHEDPY (SEQ ID NO:248), QFPNHSFKHEDPM (SEQ ID NO:249),

QFPNHSFKHEDPY (SEQ ID NO:250), KRPFMCAYPGCNK (SEQ ID NO:251), KRPFMCAYPGCYK (SEQ ID NO:252), FMCAYPGCY (SEQ ID NO:253), FMCAYPGCK (SEQ ID NO:254), KRPFMCAYPGCNKRY (SEQ ID NO:255), SEKRPFMCAYPGCNK (SEQ ID NO:256), KRPFMCAYPGCYKRY (SEQ ID NO:257), NLMNLGATL (SEQ ID NO:258), VLDFAPPGA (SEQ ID NO:259); RMFPNAPYL (SEQ ID NO:260); CMTWNQMN (SEQ ID NO:261); CYTWNQMNL (SEQ ID NO:262); NYMNLGATL (SEQ ID NO:263); YMFPNAPYL (SEQ ID NO:264); SLGEQQYSV (SEQ ID NO:265); CMTWNQMNL (SEQ ID NO:266); and NQMNLGATL (SEQ ID NO:267). In some cases, the WT-1 peptide present in a TMMP is CMTWNQMN (SEQ ID NO:261). In some cases, the WT-1 peptide present in a TMMP is CYTWNQMNL (SEQ ID NO:262).

[00268] In some cases, the WT-1 peptide present in a TMMP presents an HLA-A*2402-restricted epitope. WT-1 peptides that present an HLA-A*2402-restricted epitope include, e.g., CMTWNQMN (SEQ ID NO:261); NYMNLGATL (SEQ ID NO:263) (WT-1 239-247; Q240Y); CYTWNQMNL (SEQ ID NO:262) (WT-1 235-243); CMTWNQMNL (SEQ ID NO:266) (WT-1 235-243); NQMNLGATL (SEQ ID NO:267) (WT-1 239-247); and NLMNLGATL (SEQ ID NO:258) (WT-1 239-247; Q240L).

[00269] In some cases, the WT-1 peptide present in a TMMP presents an HLA-A*0201-restricted epitope. WT-1 peptides that present an HLA-A*0201-restricted epitope include, e.g., VLDFAPPGA (SEQ ID NO:259) (WT-1 37-45); RMFPNAPYL (SEQ ID NO:260) (WT-1 126-134); YMFPNAPYL (SEQ ID NO:264) (WT-1 126-134; R126Y); SLGEQQYSV (SEQ ID NO:265) (WT-1 187-195); and NLMNLGATL (SEQ ID NO:258) (WT-1 239-247; Q240L).

[00270] In some cases, a WT-1 peptide present in a TMMP presents an HLA-A*2402-restricted epitope and does not have an N-terminal Cys. For example, where a WT-1 peptide comprises an N-terminal Cys, the N-terminal Cys can be replaced by a Ser. As another example, where a WT-1 peptide comprises an N-terminal Cys, a Gly can be added to the N-terminus. For example, a WT-1 peptide present in a TMMP can comprise the amino acid sequence $X_1X_2X_3$ TWNQMNL (SEQ ID NO:460) or X_2X_3 TWNQMNL (SEQ ID NO:461), where each of X_1 , X_2 , and X_3 is independently any amino acid, with the proviso that the N-terminal amino acid is not a Cys, and where the WT-1 peptide epitope has a length from 9 to 25 amino acids. In some of these embodiments, the WT-1 peptide has a length of 9 amino acids or 10 amino acids. Examples of WT-1 peptides suitable for inclusion in a TMMP include, but are not limited to, SMTWNQMNL (SEQ ID NO:451), GCMTWNQMNL (SEQ ID NO:452), SYTWNQMNL (SEQ ID NO:453), or GCYTWNQMNL (SEQ ID NO:454). In some cases, a WT-1 peptide present in a TMMP of the present disclosure has the amino acid sequence SMTWNQMNL (SEQ ID NO:451); and has a length of 9 amino acids. In some cases, a WT-1 peptide present in a TMMP has the amino acid sequence GCMTWNQMNL (SEQ ID NO:452); and has a length of 10 amino acids. In some cases, a WT-1 peptide present in a TMMP has the amino acid sequence SYTWNQMNL (SEQ ID

NO:453); and has a length of 9 amino acids. In some cases, a WT-1 peptide present in a TMMP has the amino acid sequence GCYTWNQMNL (SEQ ID NO:454); and has a length of 10 amino acids.

HLA/peptide binding assays

[00271] Whether a given peptide (e.g., WT-1 peptide) binds a class I HLA (comprising an HLA heavy chain and a β 2M polypeptide), and, when bound to the HLA complex, can effectively present an epitope to a TCR, can be determined using any of a number of well-known methods. Assays include binding assays and T-cell activation assays.

Cell-based binding assay

[00272] As one example, a cell-based peptide-induced stabilization assay can be used to determine peptide-HLA class I binding. In this assay, a peptide of interest is allowed to bind to a TAP-deficient cell, i.e., a cell that has defective transporter associated with antigen processing (TAP) machinery, and consequently, few surface class I molecules. Such cells include, e.g., the human T2 cell line (T2 (174 x CEM.T2; American Type Culture Collection (ATCC) No. CRL-1992). Henderson et al. (1992) *Science* 255:1264. Without efficient TAP-mediated transport of cytosolic peptides into the endoplasmic reticulum, assembled class I complexes are structurally unstable, and retained only transiently at the cell surface. However, when T2 cells are incubated with an exogenous peptide capable of binding class I, surface peptide-HLA class I complexes are stabilized and can be detected by flow cytometry with, e.g., a pan anti-class I monoclonal antibody. The stabilization and resultant increased life-span of peptide-HLA complexes on the cell surface by the addition of a peptide validates their identity. Analysis can be carried out using flow cytometry, e.g., where the pan-HLA class I antibody comprises a fluorescent label. Binding of the peptide to various allelic forms of HLA H chains can be tested by genetically modifying the T2 cells to express an allelic HLA H chain of interest.

[00273] The following is a non-limiting example of use of a T2 assay to assess peptide binding to HLA A*0201. T2 cells are washed in cell culture medium, and concentrated to 10^6 cells/ml. Peptides of interest are prepared in cell culture medium and serially diluted providing concentrations of 200 μ M, 100 μ M, 20 μ M and 2 μ M. The cells are mixed 1:1 with each peptide dilution to give a final volume of 200 μ L and final peptide concentrations of 100 μ M, 50 μ M, 10 μ M and 1 μ M. A HLA A*0201 binding peptide, GILGFVFTL (SEQ ID NO: 395), and a non-HLA A*0201-restricted peptide, HPVG EADYF (SEQ ID NO: 396) (HLA-B*3501), are included as positive and negative controls, respectively. The cell/peptide mixtures are kept at 37°C 5% CO₂ for ten minutes; then incubated at room temperature overnight. Cells are then incubated for 2 hours at 37°C and stained with a fluorescently-labeled anti-human HLA antibody. The cells are washed twice with phosphate-buffered saline and analyzed using flow cytometry. The average mean fluorescence intensity (MFI) of the anti-HLA antibody staining is used to measure the strength of binding.

Biochemical binding assay

[00274] HLA polypeptides (HLA heavy chain polypeptide complexed with β 2M polypeptide) can be tested for binding to a peptide of interest in a cell-free *in vitro* assay system. For example, a labeled reference peptide (e.g., fluorescently labeled) is allowed to bind to HLA polypeptides (HLA heavy chain polypeptide complexed with β 2M polypeptide), to form an HLA-reference peptide complex. The ability of a test peptide of interest to displace the labeled reference peptide from the HLA-reference peptide complex is tested. The relative binding affinity is calculated as the amount of test peptide needed to displace the bound reference peptide. See, e.g., van der Burg et al. (1995) *Human Immunol.* 44:189.

[00275] As another example, a peptide of interest can be incubated with an HLA molecule (HLA heavy chain complexed with a β 2M polypeptide), and the stabilization of the HLA/peptide complex can be measured in an immunoassay format. The ability of a peptide of interest to stabilize an HLA molecule is compared to that of a control peptide presenting a known T-cell epitope. Detection of stabilization is based on the presence or absence of the native conformation of the HLA/peptide complex, detected using an anti-HLA antibody. See, e.g., Westrop et al. (2009) *J. Immunol. Methods* 341:76; Steinitz et al. (2012) *Blood* 119:4073; and U.S. Patent No. 9,205,144.

T-cell activation assays

[00276] Whether a given peptide binds a class I HLA (comprising an HLA heavy chain and a β 2M polypeptide), and, when bound to the HLA complex, can effectively present an epitope to a TCR, can be determined by assessing T-cell response to the peptide-HLA complex. T-cell responses that can be measured include, e.g., interferon-gamma (IFN γ) production, cytotoxic activity, and the like.

ELISPOT assay

[00277] Suitable assays include, e.g., an enzyme linked immunospot (ELISPOT) assay. In this assay, production of IFN γ by CD8⁺ T cells is measured following with an antigen-presenting cell (APC) that presents a peptide of interest complexed with HLA class I. Antibody to IFN γ is immobilized on wells of a multi-well plate. APCs are added to the wells, and incubated for a period of time with a peptide of interest, such that the peptide binds HLA class I on the surface of the APCs. CD8⁺ T cells specific for the peptide are added to the wells, and the plate is incubated for about 24 hours. The wells are then washed, and any IFN γ bound to the immobilized anti-IFN γ antibody is detected using a detectably labeled anti-IFN γ antibody. A colorimetric assay can be used. For example, the detectably labeled anti-IFN γ antibody can be a biotin-labeled anti-IFN γ antibody, which can be detected using, e.g., streptavidin conjugated to alkaline phosphatase. A BCIP/NBT (5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium) solution is added, to develop the assay. The presence of IFN γ -secreting T cells is identified by colored spots. Negative controls include APCs not contacted with the peptide. APCs expressing various HLA H chain alleles can be used to determine whether a peptide of interest effectively binds to a HLA class I molecule comprising a particular HLA H chain.

Cytotoxicity assays

[00278] Whether a given peptide binds to a particular HLA class I H chain and, when bound to a HLA class I complex comprising the H chain, can effectively present an epitope to a TCR, can also be determined using a cytotoxicity assay. A cytotoxicity assay involves incubation of a target cell with a cytotoxic CD8⁺ T cell. The target cell displays on its surface a peptide/HLA class I complex comprising a peptide of interest and an HLA class I molecule comprising an HLA H chain to be tested. The target cells can be radioactively labeled, e.g., with ⁵¹Cr. Whether the target cell effectively presents an epitope to a TCR on the cytotoxic CD8⁺ T cell, thereby inducing cytotoxic activity by the CD8⁺ T cell toward the target cell, is determined by measuring release of ⁵¹Cr from the lysed target cell. Specific cytotoxicity can be calculated as the amount of cytotoxic activity in the presence of the peptide minus the amount of cytotoxic activity in the absence of the peptide.

Detection of Antigen-specific T cells with peptide-HLA tetramers

[00279] As another example, multimers (e.g., tetramers) of peptide-HLA complexes are generated with fluorescent or heavy metal tags. The multimers can then be used to identify and quantify specific T cells via flow cytometry (FACS) or mass cytometry (CyTOF). Detection of epitope-specific T cells provides direct evidence that the peptide-bound HLA molecule is capable of binding to a specific TCR on a subset of antigen-specific T cells. See, e.g., Klenerman et al. (2002) *Nature Reviews Immunol.* 2:263.

Immunomodulatory polypeptides (“MODs”)

[00280] In some cases, a MOD present in a TMMP is a wild-type (“wt”) MOD. As discussed above, in other cases, a MOD present in a TMMP is a variant of a wt MOD that has reduced affinity for a co-MOD compared to the affinity of a corresponding wild-type MOD for the co-MOD. Suitable MODs that exhibit reduced affinity for a co-MOD can have from 1 amino acid (aa) to 20 aa differences from a wild-type MOD. For example, in some cases, a variant MOD present in a TMMP differs in amino acid sequence by 1 aa, 2 aa, 3 aa, 4 aa, 5 aa, 6 aa, 7 aa, 8 aa, 9 aa, or 10 aa, from a corresponding wild-type MOD. As another example, in some cases, a variant MOD present in a TMMP differs in amino acid sequence by 11 aa, 12 aa, 13 aa, 14 aa, 15 aa, 16 aa, 17 aa, 18 aa, 19 aa, or 20 aa, from a corresponding wild-type MOD.

[00281] As discussed above, a MOD may comprise a variant of a wt immunomodulatory polypeptide that may exhibit reduced binding to its co-MOD, including e.g., reduced binding to one or more chains or domains of the co-MOD. For example, a variant MOD present in a TMMP may bind its co-MOD with an affinity that it at least 10% less, at least 15% less, at least 20% less, at least 25% less, at least 30% less, at least 35% less, at least 40% less, at least 45% less, at least 50% less, at least 55% less, at least 60% less, at least 65% less, at least 70% less, at least 75% less, at least 80% less, at least 85% less, at least 90% less, at least 95% less, or more than 95% less, than the affinity of a corresponding wild-

type MOD for the co-MOD. Exemplary pairs of immunomodulatory polypeptide and cognate co-immunomodulatory polypeptide include, but are not limited to:

- [00282]** a) 4-1BBL (immunomodulatory polypeptide) and 4-1BB (cognate co-immunomodulatory polypeptide);
- [00283]** b) PD-L1 (immunomodulatory polypeptide) and PD1 (cognate co-immunomodulatory polypeptide);
- [00284]** c) IL-2 (immunomodulatory polypeptide) and IL-2 receptor (cognate co-immunomodulatory polypeptide);
- [00285]** d) CD80 (immunomodulatory polypeptide) and CD86 (cognate co-immunomodulatory polypeptide);
- [00286]** e) CD86 (immunomodulatory polypeptide) and CD28 (cognate co-immunomodulatory polypeptide);
- [00287]** f) OX40L (CD252) (immunomodulatory polypeptide) and OX40 (CD134) (cognate co-immunomodulatory polypeptide);
- [00288]** g) Fas ligand (immunomodulatory polypeptide) and Fas (cognate co-immunomodulatory polypeptide);
- [00289]** h) ICOS-L (immunomodulatory polypeptide) and ICOS (cognate co-immunomodulatory polypeptide);
- [00290]** i) ICAM (immunomodulatory polypeptide) and LFA-1 (cognate co-immunomodulatory polypeptide);
- [00291]** j) CD30L (immunomodulatory polypeptide) and CD30 (cognate co-immunomodulatory polypeptide);
- [00292]** k) CD40 (immunomodulatory polypeptide) and CD40L (cognate co-immunomodulatory polypeptide);
- [00293]** l) CD83 (immunomodulatory polypeptide) and CD83L (cognate co-immunomodulatory polypeptide);
- [00294]** m) HVEM (CD270) (immunomodulatory polypeptide) and CD160 (cognate co-immunomodulatory polypeptide);
- [00295]** n) JAG1 (CD339) (immunomodulatory polypeptide) and Notch (cognate co-immunomodulatory polypeptide);
- [00296]** o) JAG1 (immunomodulatory polypeptide) and CD46 (cognate co-immunomodulatory polypeptide);
- [00297]** p) CD80 (immunomodulatory polypeptide) and CTLA4 (cognate co-immunomodulatory polypeptide);

[00298] q) CD86 (immunomodulatory polypeptide) and CTLA4 (cognate co-immunomodulatory polypeptide); and

[00299] r) CD70 (immunomodulatory polypeptide) and CD27 (cognate co-immunomodulatory polypeptide).

[00300] As depicted schematically in FIG. 19, a MOD (i.e., one or more MODs) can be present in a TMMP at any of a variety of positions. FIG. 19 depicts the position of two copies of a variant IL-2 polypeptide; however, the MOD can be any of a variety of MODs, as described herein. As depicted in FIG. 19, a MOD can be: 1) N-terminal to the MHC class I heavy chain; 2) C-terminal to the MHC class I heavy chain and N-terminal to the Ig Fc polypeptide; in other words, between the MHC class I heavy chain and the Ig Fc polypeptide; 3) C-terminal to the Ig Fc polypeptide; 4) N-terminal to the peptide epitope; or 5) C-terminal to the β 2M polypeptide.

PD-L1 – wild-type and variants

[00301] A MOD present in a TMMP can be a wild-type PD-L1 polypeptide or a variant PD-L1 polypeptide.

[00302] In some cases, a MOD present in a TMMP is a PD-L1 polypeptide. In some cases, a PD-L1 polypeptide of a TMP comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a PD-L1 amino acid sequence set forth in SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3. PD-L1 variant MODs are described in published PCT application WO 2019/051091, published March 14, 2019. See [00157]-[00169].

[00303] In some cases, a variant PD-L1 polypeptide exhibits reduced binding affinity to PD-1 (e.g., a PD-1 polypeptide comprising the amino acid sequence set forth in SEQ ID NO:3), compared to the binding affinity of a PD-L1 polypeptide comprising the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2. For example, in some cases, a variant PD-L1 polypeptide of the present disclosure binds PD-1 (e.g., a PD-1 polypeptide comprising the amino acid sequence set forth in SEQ ID NO:3) with a binding affinity that is at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50% less, at least 55% less, at least 60% less, at least 65% less, at least 70% less, at least 75% less, at least 80% less, at least 85% less, at least 90% less, at least 95% less, or more than 95% less, than the binding affinity of a PD-L1 polypeptide comprising the amino acid sequence set forth in SEQ ID NO:1, SEQ ID NO: 2 or SEQ ID NO:3.

CD80 – wild-type and variants

[00304] A MOD present in a TMMP can be a wild-type CD80 polypeptide or a variant CD80 polypeptide. CD80 variant MODs are described in published PCT application WO 2019/051091, published March 14, 2019. See [00170]-[00196].

[00305] In some cases, a MOD present in a TMMP is a CD80 polypeptide. In some cases, a CD80 polypeptide of a TMMP comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7.

[00306] In some cases, a variant CD80 polypeptide exhibits reduced binding affinity to CD28, compared to the binding affinity of a CD80 polypeptide comprising the amino acid sequence set forth in SEQ ID NO:4 for CD28. For example, in some cases, a variant CD80 polypeptide binds CD28 with a binding affinity that is at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50% less, at least 55% less, at least 60% less, at least 65% less, at least 70% less, at least 75% less, at least 80% less, at least 85% less, at least 90% less, at least 95% less, or more than 95% less, than the binding affinity of a CD80 polypeptide comprising the amino acid sequence set forth in SEQ ID NO:4 for CD28 (e.g., a CD28 polypeptide comprising the amino acid sequence set forth in one of SEQ ID NO:5, 6, or 7).

CD86 – wild-type and variants

[00307] A MOD present in a TMMP can be a wild-type CD86 polypeptide or a variant CD86 polypeptide. CD80 variant MODs are described in published PCT application WO 2019/051091, published March 14, 2019. See [00197]-[00228].

[00308] In some cases, a MOD present in a TMMP is a CD86 polypeptide. In some cases, a CD86 polypeptide of a TMMP comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a CD86 amino acid sequence set forth in SEQ ID NO:8 or SEQ ID NO:9.

4-1BBL – wild-type and variants

[00309] A MOD present in a TMMP can be a wild-type 4-1BBL polypeptide or a variant 4-1BBL polypeptide. 4-1BBL variant MODs are described in published PCT application WO 2019/051091, published March 14, 2019. See [00229]-[00324].

[00310] In some cases, a MOD present in a TMMP is a 4-1BBL polypeptide. In some cases, a 4-1BBL polypeptide of a TMMP comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a 4-1BBL amino acid sequence set forth in SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, or SEQ ID NO:14.

[00311] In some cases, a variant 4-1BBL polypeptide exhibits reduced binding affinity to 4-1BB, compared to the binding affinity of a 4-1BBL polypeptide comprising the amino acid sequence set forth in one of SEQ ID NOs:10-13. For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure binds 4-1BB with a binding affinity that is at least 10% less, at least 15% less, at least 20%

less, at least 25%, at least 30% less, at least 35% less, at least 40% less, at least 45% less, at least 50% less, at least 55% less, at least 60% less, at least 65% less, at least 70% less, at least 75% less, at least 80% less, at least 85% less, at least 90% less, at least 95% less, or more than 95% less, than the binding affinity of a 4-1BBL polypeptide comprising the amino acid sequence set forth in one of SEQ ID NOs:10-13 for a 4-1BB polypeptide (e.g., a 4-1BB polypeptide comprising the amino acid sequence set forth in SEQ ID NO:14), when assayed under the same conditions.

IL-2 variants

[00312] In some cases, a variant MOD present in a TMMP of the present disclosure is a variant IL-2 polypeptide. Wild-type IL-2 binds to IL-2 receptor (IL-2R), i.e., a heterotrimeric polypeptide comprising IL-2R α , IL-2R β , and IL-2R γ .

[00313] A wild-type IL-2 amino acid sequence can be as follows: APTSSSTKKT
QLQL**EH**LLLD LQMILNGINN YKNPKLTRML **TFKF**YMPKKA TELKHLQCLEEELKPLEEVL
NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNRWITFC**Q**SIIS TLT
(SEQ ID NO:15).

[00314] Wild-type IL2 binds to an IL2 receptor (IL2R) on the surface of a cell. An IL2 receptor is in some cases a heterotrimeric polypeptide comprising an alpha chain (IL-2R α ; also referred to as CD25), a beta chain (IL-2R β ; also referred to as CD122; and a gamma chain (IL-2R γ ; also referred to as CD132). Amino acid sequences of human IL-2R α , IL2R β , and IL-2R γ can be as follows.

[00315] Human IL-2R α : ELCDDDPPE IPHATFKAMA YKEGTMLNCE CKRGFRRIKS
GSLYMLCTGN SSHSSWDNQC QCTSSATRNT TKQVTPQPEE QKERKTTEMQ SPMQPVDQAS
LPGHCREPPP WENEATERIY HFVVGQMVYY QCVQGYRALH RGPAESVCKM THGKTRWTQP
QLICTGEMET SQFPGEKPQ ASPEGRPESE TSCLVTTTDF QIQTEMAATM ETSIFTTEYQ
VAVAGCVFLL ISVLLLSGLT WQRRQRKSRR TI (SEQ ID NO:16).

[00316] Human IL-2R β : VNG TSQFTCFYNS RANISCVWSQ DGALQDTSCQ
VHAWPDRRRW NQTCELLPVS QASWACNLIL GAPDSQKLTT VDIVTLRVLC REGVRWRVMA
IQDFKPFENL RLMAISLQV VHVETHRCNI SWEISQASHY FERHLEFEAR TLSPGHTWEE
APLLTLKQKQ EWICLETLP DTQYEFQVRV KPLQGEFTTW SPWSQPLAFR TKPAALGKDT
IPWLGHLLVG LSGAFGFIL VYLLINCRNT GPWLKKVLKC NTPDPSKFFS QLSSEHGSDV
QKWLSPPFPS SSFSPGGLAP EISPLEVLER DKVTQLLLQQ DKVPEPASLS SNHSLTSCFT
NQGYFFFHLP DALEIEACQV YFTYDPYSEE DPDEGVAGAP TGSSPQLQP LSGEDDAYCT
FPSRDDLLLF SPSLLGGPSP PSTAPGGSGA GEERMPPSLQ ERVPRDWDPO PLGPPTPGVP
DLVDFQPPPE LVLREAGEEV PDAGPREGVS FPWSRPPGQG EFRALNARLP LNTDAYLSLQ
ELQGQDPHTL V (SEQ ID NO:17).

[00317] Human IL-2R γ : LNTTILTP NGNEDTTADF FLTTMPTDSL SVSTLPLPEV QCFVFNVEYM NCTWNSSSEP QPTNLTLLHYW YKNSDNDKVQ KCSHYLFSEE ITSGCQLQKK EIHLYQTFVV QLQDPREPRR QATQMLKLQN LVIPWAPENL TLHKLSESQL ELNWNNRFLN HCLEHLVQYR TDWDHSWTEQ SVDYRHKFSL PSVDGQKRYT FRVRSRFNPL CGSAQHWSEW SHPIHWGSNT SKENPFLFAL EAVVISVGSM GLIISLLCVY FWLERTMPRI PTLKNLEDLV TEYHGNFSAW SGVSKGLAES LQPDYSERLC LVSEIPPKGG ALGEGPGASP CNQHSPYWAP PCYTLKPET (SEQ ID NO:18).

[00318] In some cases, where a TMMP comprises a variant IL-2 polypeptide, a “cognate co-MOD” is an IL-2R comprising polypeptides comprising the amino acid sequences of SEQ ID NO:16, 17, and 18.

[00319] In some cases, a variant IL-2 polypeptide exhibits reduced binding affinity to IL-2R, compared to the binding affinity of a IL-2 polypeptide comprising the amino acid sequence set forth in SEQ ID NO:15. For example, in some cases, a variant IL-2 polypeptide binds IL-2R with a binding affinity that is at least 10% less, at least 15% less, at least 20% less, at least 25%, at least 30% less, at least 35% less, at least 40% less, at least 45% less, at least 50% less, at least 55% less, at least 60% less, at least 65% less, at least 70% less, at least 75% less, at least 80% less, at least 85% less, at least 90% less, at least 95% less, or more than 95% less, than the binding affinity of an IL-2 polypeptide comprising the amino acid sequence set forth in SEQ ID NO:15 for an IL-2R (e.g., an IL-2R comprising polypeptides comprising the amino acid sequence set forth in SEQ ID NOs:16-18), when assayed under the same conditions.

[00320] In some cases, a variant IL-2 polypeptide has a binding affinity to IL-2R that is from 100 nM to 100 μ M. As another example, in some cases, a variant IL-2 polypeptide has a binding affinity for IL-2R (e.g., an IL-2R comprising polypeptides comprising the amino acid sequence set forth in SEQ ID NOs:16-18) that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μ M, to about 1 μ M to about 5 μ M, from about 5 μ M to about 10 μ M, from about 10 μ M to about 15 μ M, from about 15 μ M to about 20 μ M, from about 20 μ M to about 25 μ M, from about 25 μ M to about 50 μ M, from about 50 μ M to about 75 μ M, or from about 75 μ M to about 100 μ M.

[00321] In some cases, a variant IL-2 polypeptide has a single amino acid substitution compared to the IL-2 amino acid sequence set forth in SEQ ID NO:15. In some cases, a variant IL-2 polypeptide has from 2 to 10 amino acid substitutions compared to the IL-2 amino acid sequence set forth in SEQ ID

NO:15. In some cases, a variant IL-2 polypeptide has 2 amino acid substitutions compared to the IL-2 amino acid sequence set forth in SEQ ID NO:15. In some cases, a variant IL-2 polypeptide has 3 amino acid substitutions compared to the IL-2 amino acid sequence set forth in SEQ ID NO:15. In some cases, a variant IL-2 polypeptide has 4 amino acid substitutions compared to the IL-2 amino acid sequence set forth in SEQ ID NO:15. In some cases, a variant IL-2 polypeptide has 5 amino acid substitutions compared to the IL-2 amino acid sequence set forth in SEQ ID NO:15. In some cases, a variant IL-2 polypeptide has 6 amino acid substitutions compared to the IL-2 amino acid sequence set forth in SEQ ID NO:15. In some cases, a variant IL-2 polypeptide has 7 amino acid substitutions compared to the IL-2 amino acid sequence set forth in SEQ ID NO:15. In some cases, a variant IL-2 polypeptide has 8 amino acid substitutions compared to the IL-2 amino acid sequence set forth in SEQ ID NO:15. In some cases, a variant IL-2 polypeptide has 9 amino acid substitutions compared to the IL-2 amino acid sequence set forth in SEQ ID NO:15. In some cases, a variant IL-2 polypeptide has 10 amino acid substitutions compared to the IL-2 amino acid sequence set forth in SEQ ID NO:15.

[00322] Suitable IL-2 variants include a polypeptide that comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to any one of the following amino acid sequences:

[00323] APTSSSTKKT QLQLEHLLLD LQMILNGINN YKNPKLTRML TXKFYMPKKA
TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFCQSIIS TLT (SEQ ID NO:181), where X is any amino acid other than Phe. In some cases, X is Ala. In some cases, X is Met. In some cases, X is Pro. In some cases, X is Ser. In some cases, X is Thr. In some cases, X is Trp. In some cases, X is Tyr. In some cases, X is Val. In some cases, X is His;

[00324] APTSSSTKKT QLQLEHLLLDX LQMILNGINN YKNPKLTRML TFKFYMPKKA
TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFCQSIIS TLT (SEQ ID NO:182), where X is any amino acid other than Asp. In some cases, X is Ala;

[00325] APTSSSTKKT QLQLXHLLLD LQMILNGINN YKNPKLTRML TFKFYMPKKA
TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFCQSIIS TLT (SEQ ID NO:183), where X is any amino acid other than Glu. In some cases, X is Ala.

[00326] APTSSSTKKT QLQLEXLLED LQMILNGINN YKNPKLTRML TFKFYMPKKA
TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFCQSIIS TLT (SEQ ID NO:184), where X is any amino acid other than His. In

some cases, X is Ala. In some cases, X is Thr. In some cases, X is Asn. In some cases, X is Cys. In some cases, X is Gln. In some cases, X is Met. In some cases, X is Val. In some cases, X is Trp;

[00327] APTSSSTKKT QLQLEXLLLD LQMILNGINN YKNPKLTRML TFKFYMPKKA
TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFCQSIIS TLT (SEQ ID NO:185), where X is any amino acid other than His. In some cases, X is Ala. In some cases, X is Arg. In some cases, X is Asn. In some cases, X is Asp. In some cases, X is Cys. In some cases, X is Glu. In some cases, X is Gln. In some cases, X is Gly. In some cases, X is Ile. In some cases, X is Lys. In some cases, X is Leu. In some cases, X is Met. In some cases, X is Phe. In some cases, X is Pro. In some cases, X is Ser. In some cases, X is Thr. In some cases, X is Tyr. In some cases, X is Trp. In some cases, X is Val;

[00328] APTSSSTKKT QLQLEHLLLD LQMILNGINN YKNPKLTRML TFKFXMPKKA
TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFCQSIIS TLT (SEQ ID NO:186), where X is any amino acid other than Tyr. In some cases, X is Ala;

[00329] APTSSSTKKT QLQLEHLLLD LQMILNGINN YKNPKLTRML TFKFYMPKKA
TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFCXSIIS TLT (SEQ ID NO:187), where X is any amino acid other than Gln. In some cases, X is Ala;

[00330] APTSSSTKKT QLQLEX₁LLLD LQMILNGINN YKNPKLTRML TX₂KFYMPKKA
TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFCQSIIS TLT (SEQ ID NO:188), where X₁ is any amino acid other than His, and where X₂ is any amino acid other than Phe. In some cases, X₁ is Ala. In some cases, X₂ is Ala. In some cases, X₁ is Ala; and X₂ is Ala. In some cases, X₁ is Thr; and X₂ is Ala;

[00331] APTSSSTKKT QLQLEHLLX₁ LQMILNGINN YKNPKLTRML TX₂KFYMPKKA
TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFCQSIIS TLT (SEQ ID NO:189), where X₁ is any amino acid other than Asp; and where X₂ is any amino acid other than Phe. In some cases, X₁ is Ala. In some cases, X₂ is Ala. In some cases, X₁ is Ala; and X₂ is Ala;

[00332] APTSSSTKKT QLQLX₁HLLX₂ LQMILNGINN YKNPKLTRML TX₃KFYMPKKA
TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFCQSIIS TLT (SEQ ID NO:190), where X₁ is any amino acid other than Glu; where X₂ is any amino acid other than Asp; and where X₃ is any amino acid other than Phe. In some cases, X₁ is Ala. In some cases, X₂ is Ala. In some cases, X₃ is Ala. In some cases, X₁ is Ala; X₂ is Ala; and X₃ is Ala;

[00333] APTSSSTKKT QLQLEX₁LLLX₂ LQMILNGINN YKNPKLTRML TX₃KFYMPKKA
TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFCQSIIS TLT (SEQ ID NO:191), where X₁ is any amino acid other than His;
where X₂ is any amino acid other than Asp; and where X₃ is any amino acid other than Phe. In some
cases, X₁ is Ala. In some cases, X₂ is Ala. In some cases, X₃ is Ala. In some cases, X₁ is Ala; X₂ is Ala;
and X₃ is Ala;

[00334] APTSSSTKKT QLQLEHLLLX₁ LQMILNGINN YKNPKLTRML TX₂KFYMPKKA
TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFCX₃SIIS TLT (SEQ ID NO:192), where X₁ is any amino acid other than Asp;
where X₂ is any amino acid other than Phe; and where X₃ is any amino acid other than Gln. In some
cases, X₁ is Ala. In some cases, X₂ is Ala. In some cases, X₃ is Ala. In some cases, X₁ is Ala; X₂ is Ala;
and X₃ is Ala;

[00335] APTSSSTKKT QLQLEHLLLX₁ LQMILNGINN YKNPKLTRML TX₂KFX₃MPKKA
TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFCQSIIS TLT (SEQ ID NO:193), where X₁ is any amino acid other than Asp;
where X₂ is any amino acid other than Phe; and where X₃ is any amino acid other than Tyr. In some
cases, X₁ is Ala. In some cases, X₂ is Ala. In some cases, X₃ is Ala. In some cases, X₁ is Ala; X₂ is Ala;
and X₃ is Ala;

[00336] APTSSSTKKT QLQLEX₁LLLX₂ LQMILNGINN YKNPKLTRML TX₃KFX₄MPKKA
TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFCQSIIS TLT (SEQ ID NO:194), where X₁ is any amino acid other than His;
where X₂ is any amino acid other than Asp; where X₃ is any amino acid other than Phe; and where X₄ is
any amino acid other than Tyr. In some cases, X₁ is Ala. In some cases, X₂ is Ala. In some cases, X₃ is
Ala. In some cases, X₄ is Ala. In some cases, X₁ is Ala; X₂ is Ala; X₃ is Ala; and X₄ is Ala;

[00337] APTSSSTKKT QLQLEHLLLX₁ LQMILNGINN YKNPKLTRML TX₂KFX₃MPKKA
TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFCX₄SIIS TLT (SEQ ID NO:195), where X₁ is any amino acid other than Asp;
where X₂ is any amino acid other than Phe; where X₃ is any amino acid other than Tyr; and where X₄ is
any amino acid other than Gln. In some cases, X₁ is Ala. In some cases, X₂ is Ala. In some cases, X₃ is
Ala. In some cases, X₄ is Ala. In some cases, X₁ is Ala; X₂ is Ala; X₃ is Ala; and X₄ is Ala;

[00338] APTSSSTKKT QLQLEX₁LLLX₂ LQMILNGINN YKNPKLTRML TX₃KFX₄MPKKA
TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFCX₅SIIS TLT (SEQ ID NO:196), where X₁ is any amino acid other than His;
where X₂ is any amino acid other than Asp; where X₃ is any amino acid other than Phe; where X₄ is any

amino acid other than Tyr; and where X₅ is any amino acid other than Gln. In some cases, X₁ is Ala. In some cases, X₂ is Ala. In some cases, X₃ is Ala. In some cases, X₄ is Ala. In some cases, X₅ is Ala. In some cases, X₁ is Ala; X₂ is Ala; X₃ is Ala; X₄ is Ala; X₅ is Ala; and

[00339] APTSSSTKKT QLQLE**X₁**LLLD LQMILNGINN YKNPKLTRML T**X₂**KFYMPKKA
TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFC**X₃**SIIS TLT (SEQ ID NO:197), where X₁ is any amino acid other than His;
where X₂ is any amino acid other than Phe; and where X₃ is any amino acid other than Gln. In some
cases, X₁ is Ala. In some cases, X₂ is Ala. In some cases, X₃ is Ala. In some cases, X₁ is Ala; X₂ is Ala;
and X₃ is Ala.

[00340] In some cases, a suitable variant IL-2 polypeptide comprises an amino acid sequence
having at least 90%, at least 95%, at least 98%, at least 99%, or 100% amino acid sequence identity to
the amino acid sequence: APTSSSTKKT QLQLEALLLD LQMILNGINN YKNPKLTRML
TAKFYMPKKA TELKHLQCLEEELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE
TTFMCEYADE TATIVEFLNRWITFCQSIIS TLT (SEQ ID NO:490), i.e., the variant IL-2 polypeptide
has the amino acid sequence of wild-type IL-2 but with H16A and F42A substitutions (shown in bold).
Alternatively, the foregoing sequence, but with substitutions other than Ala at H16 and/or F42 may be
employed, e.g., H16T may be employed instead of H16A. In some cases, a variant IL-2 polypeptide
present in a TMP comprises the amino acid sequence: APTSSSTKKT QLQLEALLLD LQMILNGINN
YKNPKLTRML **TAKFYMPKKA** TELKHLQCLEEELKPLEEVL NLAQSKNFHL RPRDLISNIN
VIVLELKGSE TTFMCEYADE TATIVEFLNRWITFCQSIIS TLT (SEQ ID NO:490). In some cases, a
variant IL-2 polypeptide present in a TMMP comprises the amino acid sequence: APTSSSTKKT
QLQLETLLLD LQMILNGINN YKNPKLTRML **TAKFYMPKKA** TELKHLQCLEEELKPLEEVL
NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNRWITFCQSIIS TLT
(SEQ ID NO:491). In some cases, a M comprises two copies of such a variant IL-2 polypeptide.

Additional polypeptides

[00341] A polypeptide chain of a TMMP of the present disclosure can include one or more
polypeptides in addition to those described above. Suitable additional polypeptides include epitope tags
and affinity domains. The one or more additional polypeptide can be included at the N-terminus of a
polypeptide chain of a TMMP, at the C-terminus of a polypeptide chain of a TMMP, or internally within
a polypeptide chain of a TMMP.

Epitope tag

[00342] Suitable epitope tags include, but are not limited to, hemagglutinin (HA; e.g.,
YPYDVPDYA (SEQ ID NO:271); FLAG (e.g., DYKDDDDK (SEQ ID NO:272); c-myc (e.g.,
EQKLISEEDL; SEQ ID NO:273), and the like.

Affinity domain

[00343] Affinity domains include peptide sequences that can interact with a binding partner, e.g., such as one immobilized on a solid support, useful for identification or purification. DNA sequences encoding multiple consecutive single amino acids, such as histidine, when fused to the expressed protein, may be used for one-step purification of the recombinant protein by high affinity binding to a resin column, such as nickel sepharose. Exemplary affinity domains are provided in published PCT application WO 2019/051091, published March 14, 2019. See [00355].

Drug conjugates

[00344] A polypeptide chain of a TMMP of the present disclosure can comprise a small molecule drug linked (e.g., covalently attached) to the polypeptide chain. For example, where a TMMP of the present disclosure comprises an Fc polypeptide, the Fc polypeptide can comprise a covalently linked small molecule drug. In some cases, the small molecule drug is a cancer chemotherapeutic agent, e.g., a cytotoxic agent. Disclosures of such drug conjugates and suitable chemotherapeutic agents are provided in published PCT application WO 2019/051091, published March 14, 2019. See [00356]-[00363].

Exemplary TMMPs

[00345] A TMMP of the present disclosure comprises at least one heterodimer comprising: a) a first polypeptide comprising: i) a WT-1 peptide epitope; and ii) first MHC polypeptide; b) a second polypeptide comprising a second MHC polypeptide, and c) at least one MOD, where the first and/or the second polypeptide comprises the MOD. Thus, in some cases, a TMMP comprises at least one heterodimer comprising: a) a first polypeptide comprising: i) a WT-1 peptide epitope; ii) first MHC polypeptide; and iii) at least one MOD; and b) a second polypeptide comprising a second MHC polypeptide. In other instances, a TMMP comprises at least one heterodimer comprising: a) a first polypeptide comprising: i) a WT-1 peptide epitope; and ii) first MHC polypeptide; and b) a second polypeptide comprising: i) a second MHC polypeptide; and ii) at least one MOD. In some cases, a TMMP comprises at least one heterodimer comprising: a) a first polypeptide comprising: i) a WT-1 peptide epitope; ii) first MHC polypeptide; and iii) at least one MOD ; and b) a second polypeptide comprising: i) a second MHC polypeptide; and ii) at least one MOD . In some cases, the at least one MOD is a wild-type immunomodulatory polypeptide. In other cases, the at least one MOD is a variant MOD that exhibits reduced affinity for a co-immunomodulatory polypeptide, compared to the affinity of a corresponding wild-type MOD for the co-immunomodulatory polypeptide. In some cases, a TMMP comprises two MODs, where the two MODs have the same amino acid sequence.

[00346] In some cases, a TMMP comprises: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a WT-1 peptide epitope; ii) a first MHC polypeptide; and iii) at least one MOD; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a second MHC polypeptide; and ii) an Ig Fc polypeptide. In some cases, the first MHC polypeptide is a β 2M

polypeptide; and the second MHC polypeptide is an HLA heavy chain polypeptide. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide with a Y84C substitution. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide with a Y84C substitution and an Ala at position 236. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide with a Y84A substitution. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide with a Y84A substitution and an A236C substitution. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide with a Y84C substitution and an A236C substitution. In some cases, the β 2M polypeptide comprises an Arg at position 12 (R12). In some cases, the β 2M polypeptide comprises an R12C substitution. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide with an A236C substitution. In some cases, the first polypeptide comprises, in order from N-terminus to C-terminus: i) a WT-1 peptide epitope; ii) a first MHC polypeptide; and iii) two MODs, where the two MODs have the same amino acid sequence. In some cases, the Ig Fc polypeptide is a human IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide comprising L234A and L235A substitutions. In some cases, the first and the second polypeptides are disulfide linked to one another. In some cases, the MOD is a variant IL-2 polypeptide comprising H16A and F42A substitutions. In some cases, the MOD is a variant IL-2 polypeptide comprising H16T and F42A substitutions. In some cases, a peptide linker is between one or more of: i) the second MHC polypeptide and the Ig Fc polypeptide; ii) the epitope and the first MHC polypeptide; iii) the first MHC polypeptide and the MOD; and (where the TMMP comprises two MODs on the first polypeptide chain) iv) between the two MODs. In some cases, the peptide linker comprises the amino acid sequence AAAGG (SEQ ID NO: 283). In some cases, the peptide linker comprises the amino acid sequence (GGGGS)_n (SEQ ID NO: 284), where n is an integer from 1 to 10 (e.g., where n is 2, 3, or 4). In some cases, the peptide linker comprises the amino acid sequence GCGGS(GGGGS)_n (SEQ ID NO:319), where n is an integer from 1 to 9 (e.g., where n is 2, 3, or 4). In some cases, the WT-1 peptide epitope is CMTWNQMNL (SEQ ID NO: 261). In some cases, the WT-1 peptide epitope is CYTWNQMNL (SEQ ID NO: 262). In some cases, the WT-1 peptide epitope is SMTWNQMNL (SEQ ID NO:451). In some cases, the WT-1 peptide epitope is GCMTWNQMNL (SEQ ID NO:452). In some cases, the WT-1 peptide epitope is SYTWNQMNL (SEQ ID NO:453). In some cases, the WT-1 peptide epitope is GCYTWNQMNL (SEQ ID NO:454).

[00347] In some cases, a TMMP comprises: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a WT-1 peptide epitope; and ii) a first MHC polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) at least one MOD; ii) a second MHC polypeptide; and iii) an Ig Fc polypeptide. In some cases, the first MHC polypeptide is a β 2M polypeptide; and the second MHC polypeptide is an HLA heavy chain polypeptide. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide. In some cases, the HLA heavy chain

polypeptide is an HLA-A24 polypeptide with an A236C substitution. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide with a Y84C substitution. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide with a Y84C substitution and an Ala at position 236. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide with a Y84A substitution. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide with a Y84A substitution and an A236C substitution. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide with a Y84C substitution and an A236C substitution. In some cases, the β 2M polypeptide comprises an Arg at position 12 (R12). In some cases, the β 2M polypeptide comprises an R12C substitution. In some cases, the second polypeptide comprises, in order from N-terminus to C-terminus: i) two MODs, where the two MODs have the same amino acid sequence; ii) a second MHC polypeptide; and iii) an Ig Fc polypeptide. In some cases, the Ig Fc polypeptide is a human IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide comprising L234A and L235A substitutions. In some cases, the first and the second polypeptides are disulfide linked to one another. In some cases, the MOD is a variant IL-2 polypeptide comprising H16A and F42A substitutions. In some cases, the MOD is a variant IL-2 polypeptide comprising H16T and F42A substitutions. In some cases, a peptide linker is between one or more of: i) the second MHC polypeptide and the Ig Fc polypeptide; ii) the epitope and the first MHC polypeptide; iii) the first MHC polypeptide and the MOD; and (where the TMMP comprises two MODs on the second polypeptide chain) iv) between the two MODs. In some cases, the peptide linker comprises the amino acid sequence AAAGG (SEQ ID NO: 283). In some cases, the peptide linker comprises the amino acid sequence (GGGGS)_n (SEQ ID NO: 284), where n is an integer from 1 to 10 (e.g., where n is 2, 3, or 4). In some cases, the peptide linker comprises the amino acid sequence GCGGS(GGGGS)_n (SEQ ID NO:319), where n is an integer from 1 to 9 (e.g., where n is 2, 3, or 4). In some cases, the WT-1 peptide epitope is CMTWNQMN (SEQ ID NO: 261). In some cases, the WT-1 peptide epitope is CYTWNQMNL (SEQ ID NO: 262). In some cases, the WT-1 peptide epitope is SMTWNQMNL (SEQ ID NO:451). In some cases, the WT-1 peptide epitope is GCMTWNQMNL (SEQ ID NO:452). In some cases, the WT-1 peptide epitope is SYTWNQMNL (SEQ ID NO:453). In some cases, the WT-1 peptide epitope is GCYTWNQMNL (SEQ ID NO:454).

[00348] In some cases, a TMMP comprises: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a WT-1 peptide epitope; and ii) a first MHC polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a second MHC polypeptide; ii) an Ig Fc polypeptide; and iii) at least one MOD. In some cases, the first MHC polypeptide is a β 2M polypeptide; and the second MHC polypeptide is an HLA heavy chain polypeptide. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide with an A236C substitution. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide with a Y84C substitution. In some cases, the HLA heavy

chain polypeptide is an HLA-A24 polypeptide with a Y84C substitution and an Ala at position 236. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide with a Y84A substitution. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide with a Y84A substitution and an A236C substitution. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide with a Y84C substitution and an A236C substitution. In some cases, the β 2M polypeptide comprises an Arg at position 12 (R12). In some cases, the β 2M polypeptide comprises an R12C substitution. In some cases, the second polypeptide comprises, in order from N-terminus to C-terminus: i) a second MHC polypeptide; ii) an Ig Fc polypeptide; and iii) two MODs, where the two MODs have the same amino acid sequence. In some cases, the Ig Fc polypeptide is a human IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide comprising L234A and L235A substitutions. In some cases, the first and the second polypeptides are disulfide linked to one another. In some cases, the MOD is a variant IL-2 polypeptide comprising H16A and F42A substitutions. In some cases, the MOD is a variant IL-2 polypeptide comprising H16T and F42A substitutions. In some cases, a peptide linker is between one or more of: i) the second MHC polypeptide and the Ig Fc polypeptide; ii) the epitope and the first MHC polypeptide; iii) the Ig Fc polypeptide and the MOD; and (where the TMMP comprises two MODs on the second polypeptide chain) iv) between the two immunomodulatory polypeptides. In some cases, the peptide linker comprises the amino acid sequence AAAGG (SEQ ID NO: 283). In some cases, the peptide linker comprises the amino acid sequence (GGGGS)_n (SEQ ID NO: 284), where n is an integer from 1 to 10 (e.g., where n is 2, 3, or 4). In some cases, the peptide linker comprises the amino acid sequence GCGGS(GGGGS)_n (SEQ ID NO:319), where n is an integer from 1 to 9 (e.g., where n is 2, 3, or 4). In some cases, the WT-1 peptide epitope is CMTWNQMN (SEQ ID NO: 261). In some cases, the WT-1 peptide epitope is CYTWNQMNL (SEQ ID NO: 262). In some cases, the WT-1 peptide epitope is SMTWNQMNL (SEQ ID NO:451). In some cases, the WT-1 peptide epitope is GCMTWNQMNL (SEQ ID NO:452). In some cases, the WT-1 peptide epitope is SYTWNQMNL (SEQ ID NO:453). In some cases, the WT-1 peptide epitope is GCYTWNQMNL (SEQ ID NO:454).

[00349] In some cases, a TMMP comprises: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a WT-1 peptide epitope; and ii) a first MHC polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) at least one MOD; ii) a second MHC polypeptide; and iii) an Ig Fc polypeptide. In some cases, the first MHC polypeptide is a β 2M polypeptide; and the second MHC polypeptide is an HLA heavy chain polypeptide. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide with an A236C substitution. In some cases, the Ig Fc polypeptide is a human IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide comprising L234A and L235A substitutions. In some cases, the first and the second polypeptides are disulfide linked to one another. In some cases, the MOD is a variant IL-2 polypeptide

comprising H16A and F42A substitutions. In some cases, the MOD is a variant IL-2 polypeptide comprising H16T and F42A substitutions. In some cases, the WT-1 peptide epitope is CMTWNQMN (SEQ ID NO: 261). In some cases, the WT-1 peptide epitope is CYTWNQMNL (SEQ ID NO: 262).

[00350] In some cases, a TMMP comprises: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) at least one MOD; ii) a WT-1 peptide epitope; and iii) a first MHC polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a second MHC polypeptide; and ii) an Ig Fc polypeptide. In some cases, the first MHC polypeptide is a β 2M polypeptide; and the second MHC polypeptide is an HLA heavy chain polypeptide. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide with an A236C substitution. In some cases, the first polypeptide comprises, in order from N-terminus to C-terminus: i) two MODs, where the two MODs have the same amino acid sequence; ii) a WT-1 peptide epitope; and iii) a first MHC polypeptide. In some cases, the Ig Fc polypeptide is a human IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide comprising L234A and L235A substitutions. In some cases, the first and the second polypeptides are disulfide linked to one another. In some cases, the MOD is a variant IL-2 polypeptide comprising H16A and F42A substitutions. In some cases, the MOD is a variant IL-2 polypeptide comprising H16T and F42A substitutions. In some cases, a peptide linker is between one or more of: i) the second MHC polypeptide and the Ig Fc polypeptide; ii) the epitope and the first MHC polypeptide; iii) the MOD and the epitope; and (where the TMMP comprises two MODs on the first polypeptide chain) iv) between the two MODs. In some cases, the peptide linker comprises the amino acid sequence AAAGG (SEQ ID NO: 283). In some cases, the peptide linker comprises the amino acid sequence (GGGGS)_n (SEQ ID NO: 284), where n is an integer from 1 to 10 (e.g., where n is 2, 3, or 4). In some cases, the WT-1 peptide epitope is CMTWNQMN (SEQ ID NO: 261). In some cases, the WT-1 peptide epitope is CYTWNQMNL (SEQ ID NO: 262).

[00351] In some cases, a TMMP comprises: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a WT-1 peptide epitope; and ii) a first MHC polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a second MHC polypeptide; ii) at least one MOD; and iii) an Ig Fc polypeptide. In some cases, the first MHC polypeptide is a β 2M polypeptide; and the second MHC polypeptide is an HLA heavy chain polypeptide. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide with an A236C substitution. In some cases, the second polypeptide comprises, in order from N-terminus to C-terminus: i) a second MHC polypeptide; ii) two MODs, where the two MODs have the same amino acid sequence; and iii) an Ig Fc polypeptide. In some cases, the Ig Fc polypeptide is a human IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide comprising L234A and L235A substitutions. In some cases, the first and the second

polypeptides are disulfide linked to one another. In some cases, the MOD is a variant IL-2 polypeptide comprising H16A and F42A substitutions. In some cases, the MOD is a variant IL-2 polypeptide comprising H16T and F42A substitutions. In some cases, a peptide linker is between one or more of: i) the second MHC polypeptide and the MOD; ii) the MOD and the Ig Fc polypeptide; iii) the epitope and the first MHC polypeptide; iii) the first MHC polypeptide and the MOD; and (where the TMMP comprises two MODs on the second polypeptide chain) iv) between the two MODs. In some cases, the peptide linker comprises the amino acid sequence AAAGG (SEQ ID NO: 283). In some cases, the peptide linker comprises the amino acid sequence (GGGGGS)_n (SEQ ID NO: 284), where n is an integer from 1 to 10 (e.g., where n is 2, 3, or 4). In some cases, the WT-1 peptide epitope is CMTWNQMN (SEQ ID NO: 261). In some cases, the WT-1 peptide epitope is CYTWNQMNL (SEQ ID NO: 262).

[00352] In some cases, a TMMP comprises: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) at least one MOD; ii) a WT-1 peptide epitope; and iii) a first MHC polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a second MHC polypeptide; and ii) an Ig Fc polypeptide. In some cases, the first MHC polypeptide is a β 2M polypeptide; and the second MHC polypeptide is an HLA heavy chain polypeptide. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide with an A236C substitution. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide with a Y84C substitution. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide with a Y84C substitution and an Ala at position 236. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide with a Y84A substitution. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide with a Y84A substitution and an A236C substitution. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide with a Y84C substitution and an A236C substitution. In some cases, the β 2M polypeptide comprises an Arg at position 12 (R12). In some cases, the β 2M polypeptide comprises an R12C substitution. In some cases, the first polypeptide comprises, in order from N-terminus to C-terminus: i) two MODs, where the two MODs have the same amino acid sequence; ii) a WT-1 peptide epitope; and iii) a first MHC polypeptide. In some cases, the Ig Fc polypeptide is a human IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide comprising L234A and L235A substitutions. In some cases, the first and the second polypeptides are disulfide linked to one another. In some cases, the MOD is a variant IL-2 polypeptide comprising H16A and F42A substitutions. In some cases, the MOD is a variant IL-2 polypeptide comprising H16T and F42A substitutions. In some cases, a peptide linker is between one or more of: i) the second MHC polypeptide and the Ig Fc polypeptide; ii) the epitope and the first MHC polypeptide; iii) the MOD and the epitope; and iv) (where the TMMP comprises two immunomodulatory polypeptides on the first polypeptide chain) the two MODs. In some cases, the peptide linker comprises the amino acid sequence AAAGG (SEQ ID NO:283). In some cases, the

peptide linker comprises the amino acid sequence (GGGGS)_n (SEQ ID NO: 284), where *n* is an integer from 1 to 10 (e.g., where *n* is 2, 3, or 4). In some cases, the peptide linker comprises the amino acid sequence GCGGS(GGGGS)_n (SEQ ID NO:319), where *n* is an integer from 1 to 9 (e.g., where *n* is 2, 3, or 4). In some cases, the WT-1 peptide epitope is CMTWNQMN (SEQ ID NO: 261). In some cases, the WT-1 peptide epitope is CYTWNQMNL (SEQ ID NO: 262). In some cases, the WT-1 peptide epitope is SMTWNQMNL (SEQ ID NO:451). In some cases, the WT-1 peptide epitope is GCMTWNQMNL (SEQ ID NO:452). In some cases, the WT-1 peptide epitope is SYTWNQMNL (SEQ ID NO:453). In some cases, the WT-1 peptide epitope is GCYTWNQMNL (SEQ ID NO:454).

[00353] In some cases, a TMMP comprises: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a WT-1 peptide epitope; and ii) a first MHC polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a second MHC polypeptide; ii) at least one MOD; and iii) an Ig Fc polypeptide. In some cases, the first MHC polypeptide is a β 2M polypeptide; and the second MHC polypeptide is an HLA heavy chain polypeptide. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide with an A236C substitution. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide with a Y84C substitution. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide with a Y84C substitution and an Ala at position 236. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide with a Y84A substitution. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide with a Y84A substitution and an A236C substitution. In some cases, the HLA heavy chain polypeptide is an HLA-A24 polypeptide with a Y84C substitution and an A236C substitution. In some cases, the β 2M polypeptide comprises an Arg at position 12 (R12). In some cases, the β 2M polypeptide comprises an R12C substitution. In some cases, the second polypeptide comprises, in order from N-terminus to C-terminus: i) a second MHC polypeptide; ii) two MODs, where the two MODs have the same amino acid sequence; and iii) an Ig Fc polypeptide. In some cases, the Ig Fc polypeptide is a human IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide comprising L234A and L235A substitutions. In some cases, the first and the second polypeptides are disulfide linked to one another. In some cases, the MOD is a variant IL-2 polypeptide comprising H16A and F42A substitutions. In some cases, the MOD is a variant IL-2 polypeptide comprising H16T and F42A substitutions. In some cases, a peptide linker is between one or more of: i) the second MHC polypeptide and the MOD; ii) the MOD and the Ig Fc polypeptide; iii) the epitope and the first MHC polypeptide; iii) the first MHC polypeptide and the MOD; and iv) (where the TMMP comprises two MODs on the second polypeptide chain) the two MODs. In some cases, the peptide linker comprises the amino acid sequence AAAGG (SEQ ID NO:283). In some cases, the peptide linker comprises the amino acid sequence (GGGGS)_n (SEQ ID NO:284), where *n* is an integer from 1 to 10 (e.g., where *n* is 2, 3, or 4). In some cases, the peptide linker comprises the amino

acid sequence GCGGS(GGGGS)_n (SEQ ID NO:319), where *n* is an integer from 1 to 9 (e.g., where *n* is 2, 3, or 4). In some cases, the WT-1 peptide epitope is CMTWNQMN (SEQ ID NO:261). In some cases, the WT-1 peptide epitope is CYTWNQMNL (SEQ ID NO:262). In some cases, the WT-1 peptide epitope is SMTWNQMNL (SEQ ID NO:451). In some cases, the WT-1 peptide epitope is GCMTWNQMNL (SEQ ID NO:452). In some cases, the WT-1 peptide epitope is SYTWNQMNL (SEQ ID NO:453). In some cases, the WT-1 peptide epitope is GCYTWNQMNL (SEQ ID NO:454).

[00354] As noted above, and as depicted schematically in FIG. 19, an MOD (i.e., one or more MODs) can be present in a TMMP of the present disclosure at any of a variety of positions. FIG. 19 depicts the position of two copies of a variant IL-2 polypeptide; however, the MOD can be any of a variety of MODs, as described herein. As depicted in FIG. 19, a MOD can be: 1) N-terminal to the MHC class I heavy chain (position 1); 2) C-terminal to the MHC class I heavy chain and N-terminal to the Ig Fc polypeptide; in other words, between the MHC class I heavy chain and the Ig Fc polypeptide (position 2); 3) C-terminal to the Ig Fc polypeptide (position 3); 4) N-terminal to the peptide epitope (position 4); or 5) C-terminal to the β 2M polypeptide (position 5). “Position 1” refers to a position of the MOD on the same polypeptide chain as the class I MHC heavy chain and N-terminal to the class I MHC heavy chain; e.g., where the TMMP comprises: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a peptide epitope (e.g., a WT-1 peptide); and ii) a β 2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) one or more MODs; and ii) a class I MHC heavy chain polypeptide. “Position 2” refers to a position of the MOD on the same polypeptide chain as the class I MHC heavy chain and C-terminal to the class I MHC heavy chain, but not at the C-terminus of the polypeptide chain; e.g., where the TMMP comprises: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a peptide epitope (e.g., a WT-1 peptide); and ii) a β 2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a class I MHC heavy chain polypeptide; ii) one or more MODs; and iii) an Ig Fc polypeptide. “Position 3” refers to a position of the MOD on the same polypeptide chain as the class I MHC heavy chain and at the C-terminus of the polypeptide chain; e.g., where the TMMP comprises: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a peptide epitope (e.g., a WT-1 peptide); and ii) a β 2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a class I MHC heavy chain polypeptide; ii) an Ig Fc polypeptide; and iii) one or more MODs. “Position 4” refers to a position of the MOD on the same polypeptide chain as the β 2M polypeptide and N-terminal to the peptide epitope and the β 2M polypeptide; e.g., where the TMMP comprises: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) one or more MODs; ii) a peptide epitope (e.g., a WT-1 peptide); and iii) a β 2M polypeptide; and b) a second polypeptide comprising a class I MHC heavy chain polypeptide (e.g., a second polypeptide comprising, in order from N-terminus to C-terminus: i) a class I MHC heavy chain polypeptide; and ii) an Ig Fc polypeptide. “Position 5” refers to a position

of the MOD on the same polypeptide chain as the β 2M polypeptide and C-terminal to the β 2M polypeptide (e.g., at the C-terminus of the polypeptide chain); e.g., where the TMMP comprises: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a peptide epitope (e.g., a WT-1 peptide); ii) a β 2M polypeptide; and iii) one or more MODs; and b) a second polypeptide comprising a class I MHC heavy chain polypeptide (e.g., a second polypeptide comprising, in order from N-terminus to C-terminus: i) a class I MHC heavy chain polypeptide; and ii) an Ig Fc polypeptide.

[00355] Furthermore, as discussed above and as depicted schematically in FIG. 18A-18C, the first polypeptide chain and the second polypeptide chain of a TMMP can be linked by one or more disulfide bonds. For example, a TMMP can comprise: a) a first polypeptide chain comprising an β 2M polypeptide having an R12C substitution; and b) a second polypeptide chain comprising a class I MHC heavy chain polypeptide having an A236C substitution; such that a disulfide bond forms between the Cys at position 12 of the β 2M polypeptide in the first polypeptide chain and the Cys at position 236 of the class I MHC heavy chain polypeptide in the second polypeptide chain. As another example, a TMMP can comprise: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a peptide epitope; ii) a peptide linker comprising a GCGGS(G4S)_n (SEQ ID NO:315) sequence, where n is 1, 2, or 3; and iii) a β 2M polypeptide; and b) a second polypeptide comprising a class I MHC heavy chain polypeptide having a Y84C substitution, such that a disulfide bond forms between the Cys in the peptide linker in the first polypeptide chain and the Cys at position 84 of the class I MHC heavy chain polypeptide in the second polypeptide chain. In other examples, a TMMP can comprise: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a peptide epitope; ii) a peptide linker comprising a GCGGS(G4S)_n (SEQ ID NO:315) sequence, where n is 1, 2, or 3; and iii) a β 2M polypeptide having an R12C substitution; and b) a second polypeptide comprising a class I MHC heavy chain polypeptide having a Y84C substitution and an A236C substitution; such that: i) a first disulfide bond forms between the Cys in the peptide linker in the first polypeptide chain and the Cys at position 84 of the class I MHC heavy chain polypeptide in the second polypeptide chain; and ii) a second disulfide bond forms between the Cys at position 12 of the β 2M polypeptide in the first polypeptide chain and the Cys at position 236 of the class I MHC heavy chain polypeptide in the second polypeptide chain. For simplicity, the first disulfide bond is referred to as “G2C/Y84C”; and the second disulfide bond is referred to as “R12C/A236C.” A TMMP can include: a) a G2C/Y84C disulfide bond and not an R12C/A236C disulfide bond; b) an R12C/A236C disulfide bond and not a G2C/Y84C disulfide bond; or c) a G2C/Y84C disulfide bond and an R12C/A236C disulfide bond.

[00356] A TMMP can include: a) a G2C/Y84C disulfide bond and not an R12C/A236C disulfide bond; and b) at least one immunomodulatory polypeptide at position 1. A TMMP can include: a) a G2C/Y84C disulfide bond and not an R12C/A236C disulfide bond; and b) at least one MOD at position 2. A TMMP of the present disclosure can include: a) a G2C/Y84C disulfide bond and not an

R12C/A236C disulfide bond; and b) at least one MOD at position 3. A TMMP can include: a) a G2C/Y84C disulfide bond and not an R12C/A236C disulfide bond; and b) at least one MOD at position 4. A TMMP can include: a) a G2C/Y84C disulfide bond and not an R12C/A236C disulfide bond; and b) at least one MOD at position 5.

[00357] A TMMP can include: a) an R12C/A236C disulfide bond and not a G2C/Y84C disulfide bond; and at least one MOD at position 1. A TMMP can include: a) an R12C/A236C disulfide bond and not a G2C/Y84C disulfide bond; and at least one MOD at position 2. A TMMP can include: a) an R12C/A236C disulfide bond and not a G2C/Y84C disulfide bond; and at least one MOD at position 3. A TMMP of the present disclosure can include: a) an R12C/A236C disulfide bond and not a G2C/Y84C disulfide bond; and at least one immunomodulatory polypeptide at position 4. A TMMP can include: a) an R12C/A236C disulfide bond and not a G2C/Y84C disulfide bond; and at least one MOD at position 5.

[00358] A TMMP can include: a) a G2C/Y84C disulfide bond and an R12C/A236C disulfide bond; and b) and at least one MOD at position 1. A TMMP can include: a) a G2C/Y84C disulfide bond and an R12C/A236C disulfide bond; and b) and at least one MOD at position 2. A TMMP can include: a) a G2C/Y84C disulfide bond and an R12C/A236C disulfide bond; and b) and at least one MOD at position 3. A TMMP of the present disclosure can include: a) a G2C/Y84C disulfide bond and an R12C/A236C disulfide bond; and b) and at least one MOD at position 4. A TMMP can include: a) a G2C/Y84C disulfide bond and an R12C/A236C disulfide bond; and b) and at least one MOD at position 5.

[00359] Non-limiting examples of amino acid sequences of first and second polypeptide chains of a TMMP of the present disclosure are provided in FIGs. 4A-4K and FIGs. 20A-20R.

[00360] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence designated "2752" as depicted in FIG. 4D; and b) a second polypeptide chain comprising the amino acid sequence designated "3159" as depicted in FIG. 4C.

[00361] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence designated "2753" as depicted in FIG. 4E; and b) a second polypeptide chain comprising the amino acid sequence designated "3159" as depicted in FIG. 4C. Such a TMMP comprises: a) a MOD at position 3 as depicted in FIG. 19; and b) an R12C/A236C disulfide bond (but not a G2C/Y84C disulfide bond).

[00362] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence designated "2752" as depicted in FIG. 4D; and b) a second polypeptide chain comprising the amino acid sequence designated "2750" as depicted in FIG. 4B.

[00363] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence designated "2753" as depicted in FIG. 4E; and b) a second polypeptide chain comprising

the amino acid sequence designated “2750” as depicted in FIG. 4B. Such a TMMP comprises: a) a MOD at position 1 as depicted in FIG. 19; and b) an R12C/A236C disulfide bond (but not a G2C/Y84C disulfide bond).

[00364] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence designated “2752” as depicted in FIG. 4D; and b) a second polypeptide chain comprising the amino acid sequence designated “3158” as depicted in FIG. 4A.

[00365] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence designated “2753” as depicted in FIG. 4E; and b) a second polypeptide chain comprising the amino acid sequence designated “3158” as depicted in FIG. 4A.

[00366] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence designated “2380” as depicted in FIG. 14B; and b) a second polypeptide chain comprising the amino acid sequence designated “1715” as depicted in FIG. 14A.

[00367] In some cases, a TMMP comprises: a) a first polypeptide chain comprising, in order from N-terminus to C-terminus: i) a WT-1 peptide of the sequence VLDFAPPGA (SEQ ID NO:259); ii) a linker having the amino acid sequence GCGSGGGGSGGGGS (SEQ ID NO:317); and iii) a β 2M polypeptide comprising a Cys at position 12 (e.g., a β 2M having the amino acid sequence set forth in SEQ ID NO:311); and b) a second polypeptide chain comprising, in order from N-terminus to C-terminus: i) a variant IL-2 polypeptide comprising H16A and F42A substitutions (i.e., comprising Ala at positions 16 and 42, e.g., the amino acid sequence set forth in SEQ ID NO:188, where X₁ is Ala and where X₂ is Ala); ii) a (GGGS)₄ linker; iii) a variant IL-2 polypeptide comprising H16A and F42A substitutions (i.e., comprising Ala at positions 16 and 42, e.g., the amino acid sequence set forth in SEQ ID NO:188, where X₁ is Ala and where X₂ is Ala); iv) a (GGGS)₄ linker; v) an HLA A0202 heavy chain comprising Cys at positions 84 and 236 (e.g., an HLA heavy chain polypeptide comprising the amino acid sequence set forth in SEQ ID NO:341); vi) an AAAGG linker; and vii) an Ig Fc polypeptide. In some cases, the Ig Fc polypeptide comprises an amino acid sequence having at least at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to an amino acid sequence of an Fc region depicted in FIG. 5A-5G or 5H. In some cases, the Ig Fc polypeptide is a variant Ig Fc polypeptide comprising one or more sequence variations relative to the wild-type polypeptide, where the ability of the Ig Fc polypeptide to induce cell lysis through complement-dependent cytotoxicity (CDC) and/or antibody-dependent cellular cytotoxicity (ADCC) is reduced or substantially eliminated. In some cases, the Ig Fc polypeptide is a variant human IgG1 Fc polypeptide comprising comprises an L234A and/or L235A substitutions (L14 and L15 in the amino acid sequence depicted in FIG. 5H. In some cases, the Ig Fc polypeptide comprises the amino acid sequence depicted in FIG. 5H and set forth in SEQ ID NO:487.

[00368] In some cases, a TMMP comprises: a) a first polypeptide chain comprising, in order from N-terminus to C-terminus: i) a WT-1 peptide of the sequence VLDFAPPGA (SEQ ID NO:259); ii) a linker having the amino acid sequence GCGSGGGSGGGGS (SEQ ID NO:317); and iii) a β 2M polypeptide comprising a Cys at position 12 (e.g., a β 2M having the amino acid sequence set forth in SEQ ID NO:311); and b) a second polypeptide chain comprising, in order from N-terminus to C-terminus: i) a variant IL-2 polypeptide comprising H16A and F42A substitutions (i.e., comprising Ala at positions 16 and 42, e.g., the amino acid sequence set forth in SEQ ID NO:188, where X₁ is Ala and where X₂ is Ala); ii) a (GGGGS)₄ linker; iii) a variant IL-2 polypeptide comprising H16A and F42A substitutions (i.e., comprising Ala at positions 16 and 42, e.g., the amino acid sequence set forth in SEQ ID NO:188, where X₁ is Ala and where X₂ is Ala); iv) a (GGGGS)₄ linker; v) an HLA A0202 heavy chain comprising Cys at positions 84 and 236 (e.g., an HLA heavy chain polypeptide comprising the amino acid sequence set forth in SEQ ID NO:341); vi) an AAAGG linker; and vii) an Ig Fc polypeptide comprising Ala at positions 14 and 15, and lacking a C-terminal Lys (e.g., an Ig Fc polypeptide comprising the amino acid sequence depicted in FIG. 5H and set forth in SEQ ID NO:487). For example, in some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence designated “2380” as depicted in FIG. 14B; and b) a second polypeptide chain comprising the amino acid sequence designated “1715 without C-terminal Lys” as depicted in FIG. 14J. The construct depicted in FIG. 14J (“1715 without C-terminal Lys”) is also referred to herein as “1715 Δ ”.

[00369] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence designated “2381” as depicted in FIG. 14B; and b) a second polypeptide chain comprising the amino acid sequence designated “1715” as depicted in FIG. 14A or 1715 Δ as depicted in FIG. 14J and as set forth in SEQ ID NO:486.

[00370] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence designated “2380” as depicted in FIG. 14B; and b) a second polypeptide chain comprising the amino acid sequence designated “2405” as depicted in FIG. 14D.

[00371] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence designated “2381” as depicted in FIG. 14B; and b) a second polypeptide chain comprising the amino acid sequence designated “2405” as depicted in FIG. 14D. In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence designated “2762” as depicted in FIG. 14F; and b) a second polypeptide chain comprising the amino acid sequence designated “2405” as depicted in FIG. 14D.

[00372] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence designated “2380” as depicted in FIG. 14B; and b) a second polypeptide chain comprising the amino acid sequence designated “1380” as depicted in FIG. 14E.

[00373] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence designated “2381” as depicted in FIG. 14B; and b) a second polypeptide chain comprising the amino acid sequence designated “1380” as depicted in FIG. 14E.

[00374] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence designated “3592” as depicted in FIG. 20A; and b) a second polypeptide chain comprising the amino acid sequence designated “3188” as depicted in FIG. 20H. Such a TMMP comprises: a) a MOD at position 1 as depicted in FIG. 19; and b) both a G2C/Y84C disulfide bond and an R12C/A236C disulfide bond.

[00375] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence designated “3425” as depicted in FIG. 20B; and b) a second polypeptide chain comprising the amino acid sequence designated “3188” as depicted in FIG. 20H. Such a TMMP comprises: a) a MOD at position 3 as depicted in FIG. 19; and b) both a G2C/Y84C disulfide bond and an R12C/A236C disulfide bond.

[00376] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence designated “3196” as depicted in FIG. 20C; and b) a second polypeptide chain comprising the amino acid sequence designated “3604” as depicted in FIG. 20I. Such a TMMP comprises: a) a MOD at position 5 as depicted in FIG. 19; and b) both a G2C/Y84C disulfide bond and an R12C/A236C disulfide bond.

[00377] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence designated “2764” as depicted in FIG. 20D; and b) a second polypeptide chain comprising the amino acid sequence designated “3603” as depicted in FIG. 20J. Such a TMMP comprises: a) a MOD at position 5 as depicted in FIG. 19; and b) an R12C/A236C disulfide bond (but not a G2C/Y84C disulfide bond).

[00378] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence designated “3593” as depicted in FIG. 20E; and b) a second polypeptide chain comprising the amino acid sequence designated “3192” as depicted in FIG. 20K. Such a TMMP comprises: a) a MOD at position 1 as depicted in FIG. 19; and b) a G2C/Y84C disulfide bond (but not an R12C/A236C disulfide bond).

[00379] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence designated “3426” as depicted in FIG. 20F; and b) a second polypeptide chain comprising the amino acid sequence designated “3192” as depicted in FIG. 20K. Such a TMMP comprises: a) a MOD at position 3 as depicted in FIG. 19; and b) a G2C/Y84C disulfide bond (but not an R12C/A236C disulfide bond).

[00380] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence designated “3197” as depicted in FIG. 20G; and b) a second polypeptide chain comprising the amino acid sequence designated “3605” as depicted in FIG. 20L. Such a TMMP comprises: a) a MOD at position 5 as depicted in FIG. 19; and b) a G2C/Y84C disulfide bond (but not an R12C/A236C disulfide bond).

[00381] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence designated “3592” as depicted in FIG. 20A; and b) a second polypeptide chain comprising the amino acid sequence designated “3529” as depicted in FIG. 20M. Such a TMMP comprises: a) a MOD at position 1 as depicted in FIG. 19; and b) both a G2C/Y84C disulfide bond and an R12C/A236C disulfide bond.

[00382] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence designated “3425” as depicted in FIG. 20B; and b) a second polypeptide chain comprising the amino acid sequence designated “3529” as depicted in FIG. 20M. Such a TMMP comprises: a) a MOD at position 3 as depicted in FIG. 19; and b) both a G2C/Y84C disulfide bond and an R12C/A236C disulfide bond; and also comprises a WT1 239-247 (Q240Y) epitope.

[00383] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence designated “3196” as depicted in FIG. 20C; and b) a second polypeptide chain comprising the amino acid sequence designated “3709” as depicted in FIG. 20N. Such a TMMP comprises: a) a MOD at position 3 as depicted in FIG. 19; and b) both a G2C/Y84C disulfide bond and an R12C/A236C disulfide bond.

[00384] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence designated “2750” as depicted in FIG. 4B; and b) a second polypeptide chain comprising the amino acid sequence designated “3528” as depicted in FIG. 20O. Such a TMMP comprises: a) a MOD at position 1 as depicted in FIG. 19; and b) an R12C/A236C disulfide bond (but not a G2C/Y84C disulfide bond).

[00385] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence designated “3159” as depicted in FIG. 4C; and b) a second polypeptide chain comprising the amino acid sequence designated “3528” as depicted in FIG. 20O. Such a TMMP comprises: a) a MOD at position 3 as depicted in FIG. 19; and b) an R12C/A236C disulfide bond (but not a G2C/Y84C disulfide bond).

[00386] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence designated “2764” as depicted in FIG. 20D; and b) a second polypeptide chain comprising the amino acid sequence designated “3708” as depicted in FIG. 20P. Such a TMMP comprises: a) a

MOD at position 5 as depicted in FIG. 19; and b) an R12C/A236C disulfide bond (but not a G2C/Y84C disulfide bond).

[00387] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence designated “3593” as depicted in FIG. 20E; and b) a second polypeptide chain comprising the amino acid sequence designated “3530” as depicted in FIG. 20Q. Such a TMMP comprises: a) a MOD at position 1 as depicted in FIG. 19; and b) a G2C/Y84C disulfide bond (but not an R12C/A236C disulfide bond).

[00388] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence designated “3426” as depicted in FIG. 20F; and b) a second polypeptide chain comprising the amino acid sequence designated “3530” as depicted in FIG. 20Q. Such a TMMP comprises: a) a MOD at position 3 as depicted in FIG. 19; and b) a G2C/Y84C disulfide bond (but not an R12C/A236C disulfide bond).

[00389] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence designated “3197” as depicted in FIG. 20G; and b) a second polypeptide chain comprising the amino acid sequence designated “3710” as depicted in FIG. 20R. Such a TMMP comprises: a) a MOD at position 5 as depicted in FIG. 19; and b) a G2C/Y84C disulfide bond (but not an R12C/A236C disulfide bond).

[00390] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence designated “3426” as depicted in FIG. 20F; and b) a second polypeptide chain comprising the amino acid sequence designated “3529” as depicted in FIG. 20M. Such a TMMP comprises: a) a MOD at position 3 as depicted in FIG. 19; and b) a G2C/Y84C disulfide bond (but not an R12C/A236C disulfide bond), and also includes a WT1 239-247 (Q240Y) epitope.

[00391] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence designated “3425” as depicted in FIG. 20B; and b) a second polypeptide chain comprising the amino acid sequence designated “3528” as depicted in FIG. 20O. Such a TMMP comprises: a) a MOD at position 3 as depicted in FIG. 19; and b) an R12C/A236C disulfide bond (but not a G2C/Y84C disulfide bond) and also includes a WT1 239-247 (Q240Y) epitope.

[00392] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence designated “3425” as depicted in FIG. 20B; and b) a second polypeptide chain comprising the amino acid sequence designated “3530” as depicted in FIG. 20Q. Such a TMMP comprises: a) a MOD at position 3 as depicted in FIG. 19; and b) a G2C/Y84C disulfide bond (but not an R12C/A236C disulfide bond), and also includes a WT1 239-247 (Q240Y) epitope.

[00393] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence designated “3159” as depicted in FIG. 4C; and b) a second polypeptide chain comprising

the amino acid sequence designated “3188” as depicted in FIG. 20H. Such a TMMP comprises: a) a MOD at position 3 as depicted in FIG. 19; and b) an R12C/A236C disulfide bond (but not a G2C/Y84C disulfide bond), and also includes a WT1 235-243 (M236Y) epitope.

Exemplary TMMPs with epitope SMTWNQMNL (WT1 (235-243; C235S))

[00394] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 35A; and b) a second polypeptide chain comprising the amino acid sequence depicted in FIG. 4B. Such a TMMP comprises: a) a MOD at position 1 as depicted in FIG. 19; and b) an R12C/A236C disulfide bond (but not a G2C/Y84C disulfide bond).

[00395] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 35A; and b) a second polypeptide chain comprising the amino acid sequence depicted in FIG. 4C. Such a TMMP comprises: a) a MOD at position 3 as depicted in FIG. 19; and b) an R12C/A236C disulfide bond (but not a G2C/Y84C disulfide bond).

[00396] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 35B; and b) a second polypeptide chain comprising the amino acid sequence depicted in FIG. 20A. Such a TMMP comprises: a) a MOD at position 1 as depicted in FIG. 19; and b) both a G2C/Y84C disulfide bond and an R12C/A236C disulfide bond.

[00397] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 35B; and b) a second polypeptide chain comprising the amino acid sequence depicted in FIG. 20B. Such a TMMP comprises: a) a MOD at position 3 as depicted in FIG. 19; and b) both a G2C/Y84C disulfide bond and an R12C/A236C disulfide bond.

[00398] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 35C; and b) a second polypeptide chain comprising the amino acid sequence depicted in FIG. 20E. Such a TMMP comprises: a) a MOD at position 1 as depicted in FIG. 19; and b) a G2C/Y84C disulfide bond (but not an R12C/A236C disulfide bond).

[00399] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 35C; and b) a second polypeptide chain comprising the amino acid sequence depicted in FIG. 20F. Such a TMMP comprises: a) a MOD at position 3 as depicted in FIG. 19; and b) a G2C/Y84C disulfide bond (but not an R12C/A236C disulfide bond).

[00400] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 35D; and b) a second polypeptide chain comprising the amino acid sequence depicted in FIG. 20G. Such a TMMP comprises: a) a MOD at position 5 as depicted in FIG. 19; and b) a G2C/Y84C disulfide bond (but not an R12C/A236C disulfide bond).

[00401] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 35E; and b) a second polypeptide chain comprising the amino acid

sequence depicted in FIG. 20C. Such a TMMP comprises: a) a MOD at position 5 as depicted in FIG. 19; and b) both a G2C/Y84C disulfide bond and an R12C/A236C disulfide bond.

[00402] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 35F; and b) a second polypeptide chain comprising the amino acid sequence depicted in FIG. 20D. Such a TMMP comprises: a) a MOD at position 5 as depicted in FIG. 19; and b) an R12C/A236C disulfide bond (but not a G2C/Y84C disulfide bond).

Exemplary TMMPs with epitope GCMTWNQMNL (WT1 (235-243; G-1))

[00403] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 36A; and b) a second polypeptide chain comprising the amino acid sequence depicted in FIG. 4B. Such a TMMP comprises: a) a MOD at position 1 as depicted in FIG. 19; and b) an R12C/A236C disulfide bond (but not a G2C/Y84C disulfide bond).

[00404] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 36A; and b) a second polypeptide chain comprising the amino acid sequence depicted in FIG. 4C. Such a TMMP comprises: a) a MOD at position 3 as depicted in FIG. 19; and b) an R12C/A236C disulfide bond (but not a G2C/Y84C disulfide bond).

[00405] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 36B; and b) a second polypeptide chain comprising the amino acid sequence depicted in FIG. 20A. Such a TMMP comprises: a) a MOD at position 1 as depicted in FIG. 19; and b) both a G2C/Y84C disulfide bond and an R12C/A236C disulfide bond.

[00406] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 36B; and b) a second polypeptide chain comprising the amino acid sequence depicted in FIG. 20B. Such a TMMP comprises: a) a MOD at position 3 as depicted in FIG. 19; and b) both a G2C/Y84C disulfide bond and an R12C/A236C disulfide bond.

[00407] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 36C; and b) a second polypeptide chain comprising the amino acid sequence depicted in FIG. 20E. Such a TMMP comprises: a) a MOD at position 1 as depicted in FIG. 19; and b) a G2C/Y84C disulfide bond (but not an R12C/A236C disulfide bond).

[00408] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 36C; and b) a second polypeptide chain comprising the amino acid sequence depicted in FIG. 20F. Such a TMMP comprises: a) a MOD at position 3 as depicted in FIG. 19; and b) a G2C/Y84C disulfide bond (but not an R12C/A236C disulfide bond).

[00409] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 36D; and b) a second polypeptide chain comprising the amino acid sequence depicted in FIG. 20G. Such a TMMP comprises: a) an immunomodulatory polypeptide at

position 5 as depicted in FIG. 19; and b) a G2C/Y84C disulfide bond (but not an R12C/A236C disulfide bond).

[00410] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 36E; and b) a second polypeptide chain comprising the amino acid sequence depicted in FIG. 20C. Such a TMMP comprises: a) a MOD at position 5 as depicted in FIG. 19; and b) both a G2C/Y84C disulfide bond and an R12C/A236C disulfide bond.

[00411] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 36F; and b) a second polypeptide chain comprising the amino acid sequence depicted in FIG. 20D. Such a TMMP comprises: a) a MOD at position 5 as depicted in FIG. 19; and b) an R12C/A236C disulfide bond (but not a G2C/Y84C disulfide bond).

Exemplary TMMPs with epitope SYTWNQMNL (WT1 (235-243; C235S; M236Y))

[00412] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 37A; and b) a second polypeptide chain comprising the amino acid sequence depicted in FIG. 4B. Such a TMMP comprises: a) a MOD at position 1 as depicted in FIG. 19; and b) an R12C/A236C disulfide bond (but not a G2C/Y84C disulfide bond).

[00413] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 37A; and b) a second polypeptide chain comprising the amino acid sequence depicted in FIG. 4C. Such a TMMP comprises: a) a MOD at position 3 as depicted in FIG. 19; and b) an R12C/A236C disulfide bond (but not a G2C/Y84C disulfide bond).

[00414] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 37B; and b) a second polypeptide chain comprising the amino acid sequence depicted in FIG. 20A. Such a TMMP comprises: a) a MOD at position 1 as depicted in FIG. 19; and b) both a G2C/Y84C disulfide bond and an R12C/A236C disulfide bond.

[00415] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 37B; and b) a second polypeptide chain comprising the amino acid sequence depicted in FIG. 20B. Such a TMMP comprises: a) a MOD at position 3 as depicted in FIG. 19; and b) both a G2C/Y84C disulfide bond and an R12C/A236C disulfide bond.

[00416] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 37C; and b) a second polypeptide chain comprising the amino acid sequence depicted in FIG. 20E. Such a TMMP comprises: a) a MOD at position 1 as depicted in FIG. 19; and b) a G2C/Y84C disulfide bond (but not an R12C/A236C disulfide bond).

[00417] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 37C; and b) a second polypeptide chain comprising the amino acid

sequence depicted in FIG. 20F. Such a TMMP comprises: a) a MOD at position 3 as depicted in FIG. 19; and b) a G2C/Y84C disulfide bond (but not an R12C/A236C disulfide bond).

[00418] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 37D; and b) a second polypeptide chain comprising the amino acid sequence depicted in FIG. 20G. Such a TMMP comprises: a) a MOD at position 5 as depicted in FIG. 19; and b) a G2C/Y84C disulfide bond (but not an R12C/A236C disulfide bond).

[00419] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 37E; and b) a second polypeptide chain comprising the amino acid sequence depicted in FIG. 20C. Such a TMMP comprises: a) a MOD at position 5 as depicted in FIG. 19; and b) both a G2C/Y84C disulfide bond and an R12C/A236C disulfide bond.

[00420] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 37F; and b) a second polypeptide chain comprising the amino acid sequence depicted in FIG. 20D. Such a TMMP comprises: a) a MOD at position 5 as depicted in FIG. 19; and b) an R12C/A236C disulfide bond (but not a G2C/Y84C disulfide bond).

Exemplary TMMPs with epitope GCYTWNQMNL (WT1 (235-243; G-1; M236Y))

[00421] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 38A; and b) a second polypeptide chain comprising the amino acid sequence depicted in FIG. 4B. Such a TMMP comprises: a) a MOD at position 1 as depicted in FIG. 19; and b) an R12C/A236C disulfide bond (but not a G2C/Y84C disulfide bond).

[00422] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 38A; and b) a second polypeptide chain comprising the amino acid sequence depicted in FIG. 4C. Such a TMMP comprises: a) a MOD at position 3 as depicted in FIG. 19; and b) an R12C/A236C disulfide bond (but not a G2C/Y84C disulfide bond).

[00423] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 38B; and b) a second polypeptide chain comprising the amino acid sequence depicted in FIG. 20A. Such a TMMP comprises: a) a MOD at position 1 as depicted in FIG. 19; and b) both a G2C/Y84C disulfide bond and an R12C/A236C disulfide bond.

[00424] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 38B; and b) a second polypeptide chain comprising the amino acid sequence depicted in FIG. 20B. Such a TMMP comprises: a) a MOD at position 3 as depicted in FIG. 19; and b) both a G2C/Y84C disulfide bond and an R12C/A236C disulfide bond.

[00425] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 38C; and b) a second polypeptide chain comprising the amino acid

sequence depicted in FIG. 20E. Such a TMMP comprises: a) a MOD at position 1 as depicted in FIG. 19; and b) a G2C/Y84C disulfide bond (but not an R12C/A236C disulfide bond).

[00426] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 38C; and b) a second polypeptide chain comprising the amino acid sequence depicted in FIG. 20F. Such a TMMP comprises: a) a MOD at position 3 as depicted in FIG. 19; and b) a G2C/Y84C disulfide bond (but not an R12C/A236C disulfide bond).

[00427] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 38D; and b) a second polypeptide chain comprising the amino acid sequence depicted in FIG. 20G. Such a TMMP comprises: a) a MOD at position 5 as depicted in FIG. 19; and b) a G2C/Y84C disulfide bond (but not an R12C/A236C disulfide bond).

[00428] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 38E; and b) a second polypeptide chain comprising the amino acid sequence depicted in FIG. 20C. Such a TMMP comprises: a) a MOD at position 5 as depicted in FIG. 19; and b) both a G2C/Y84C disulfide bond and an R12C/A236C disulfide bond.

[00429] In some cases, a TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence depicted in FIG. 38F; and b) a second polypeptide chain comprising the amino acid sequence depicted in FIG. 20D. Such a TMMP comprises: a) a MOD at position 5 as depicted in FIG. 19; and b) an R12C/A236C disulfide bond (but not a G2C/Y84C disulfide bond).

METHODS OF GENERATING A MULTIMERIC T-CELL MODULATORY POLYPEPTIDE

[00430] Methods of obtaining a TMMP comprising one or more variant MODs that exhibit lower affinity for a cognate co-MOD compared to the affinity of the corresponding parental wild-type immunomodulatory polypeptide for the co-immunomodulatory polypeptide are provided in accordance with published PCT application WO 2019/051091, published March 14, 2019. See [00364]-[00387].

NUCLEIC ACIDS

[00431] The present disclosure provides a nucleic acid comprising a nucleotide sequence encoding a TMMP of the present disclosure.

[00432] In some cases, the individual polypeptide chains of a TMMP are encoded in separate nucleic acids. In some cases, all polypeptide chains of a TMMP are encoded in a single nucleic acid. In some cases, a first nucleic acid comprises a nucleotide sequence encoding a first polypeptide of a TMMP; and a second nucleic acid comprises a nucleotide sequence encoding a second polypeptide of a TMMP. In some cases, a single nucleic acid comprises a nucleotide sequence encoding a first polypeptide of a TMMP and a second polypeptide of a TMMP.

Separate nucleic acids encoding individual polypeptide chains of a multimeric polypeptide

[00433] The present disclosure provides nucleic acids comprising nucleotide sequences encoding a TMMP. As noted above, in some cases, the individual polypeptide chains of a TMMP are encoded in separate nucleic acids. In some cases, nucleotide sequences encoding the separate polypeptide chains of a TMMP are operably linked to transcriptional control elements, e.g., promoters, such as promoters that are functional in a eukaryotic cell, where the promoter can be a constitutive promoter or an inducible promoter.

[00434] The present disclosure provides a first nucleic acid and a second nucleic acid, where the first nucleic acid comprises a nucleotide sequence encoding a first polypeptide of a TMMP, where the first polypeptide comprises, in order from N-terminus to C-terminus: a) an epitope (e.g., a T-cell epitope); b) a first MHC polypeptide; and c) a MOD (e.g., a wild-type MOD or a reduced-affinity variant MOD, as described above); and where the second nucleic acid comprises a nucleotide sequence encoding a second polypeptide of a TMMP, where the second polypeptide comprises, in order from N-terminus to C-terminus: a) a second MHC polypeptide; and b) an Ig Fc polypeptide. Suitable T-cell epitopes, MHC polypeptides, MODs, and Ig Fc polypeptides, are described above. In some cases, the nucleotide sequences encoding the first and the second polypeptides are operably linked to transcriptional control elements. In some cases, the transcriptional control element is a promoter that is functional in a eukaryotic cell. In some cases, the nucleic acids are present in separate expression vectors.

[00435] The present disclosure provides a first nucleic acid and a second nucleic acid, where the first nucleic acid comprises a nucleotide sequence encoding a first polypeptide of a TMMP, where the first polypeptide comprises, in order from N-terminus to C-terminus: a) an epitope (e.g., a T-cell epitope); and b) a first MHC polypeptide; and where the second nucleic acid comprises a nucleotide sequence encoding a second polypeptide of a TMMP, where the second polypeptide comprises, in order from N-terminus to C-terminus: a) a MOD (e.g., a wild-type MOD or a reduced-affinity variant MOD, as described above); b) a second MHC polypeptide; and c) an Ig Fc polypeptide. Suitable T-cell epitopes, MHC polypeptides, MODs, and Ig Fc polypeptides, are described above. In some cases, the nucleotide sequences encoding the first and the second polypeptides are operably linked to transcriptional control elements. In some cases, the transcriptional control element is a promoter that is functional in a eukaryotic cell. In some cases, the nucleic acids are present in separate expression vectors.

Nucleic acid encoding two or more polypeptides present in a multimeric polypeptide

[00436] The present disclosure provides a nucleic acid comprising nucleotide sequences encoding at least the first polypeptide and the second polypeptide of a TMMP. In some cases, where a TMMP of the present disclosure includes a first, second, and third polypeptide, the nucleic acid includes a nucleotide sequence encoding the first, second, and third polypeptides. In some cases, the nucleotide sequences encoding the first polypeptide and the second polypeptide of a TMMP includes a

proteolytically cleavable linker interposed between the nucleotide sequence encoding the first polypeptide and the nucleotide sequence encoding the second polypeptide. In some cases, the nucleotide sequences encoding the first polypeptide and the second polypeptide of a TMMP includes an internal ribosome entry site (IRES) interposed between the nucleotide sequence encoding the first polypeptide and the nucleotide sequence encoding the second polypeptide. In some cases, the nucleotide sequences encoding the first polypeptide and the second polypeptide of a TMMP includes a ribosome skipping signal (or *cis*-acting hydrolase element, CHYSEL (SEQ ID NO: 394)) interposed between the nucleotide sequence encoding the first polypeptide and the nucleotide sequence encoding the second polypeptide. Examples of nucleic acids are described below, where a proteolytically cleavable linker is provided between nucleotide sequences encoding the first polypeptide and the second polypeptide of a TMMP; in any of these embodiments, an IRES or a ribosome skipping signal can be used in place of the nucleotide sequence encoding the proteolytically cleavable linker.

[00437] In some cases, a first nucleic acid (e.g., a recombinant expression vector, an mRNA, a viral RNA, etc.) comprises a nucleotide sequence encoding a first polypeptide chain of a TMMP of the present disclosure; and a second nucleic acid (e.g., a recombinant expression vector, an mRNA, a viral RNA, etc.) comprises a nucleotide sequence encoding a second polypeptide chain of a TMMP of the present disclosure. In some cases, the nucleotide sequence encoding the first polypeptide, and the second nucleotide sequence encoding the second polypeptide, are each operably linked to transcriptional control elements, e.g., promoters, such as promoters that are functional in a eukaryotic cell, where the promoter can be a constitutive promoter or an inducible promoter.

[00438] The present disclosure provides a nucleic acid comprising a nucleotide sequence encoding a recombinant polypeptide, where the recombinant polypeptide comprises, in order from N-terminus to C-terminus: a) an epitope (e.g., a T-cell epitope); b) a first MHC polypeptide; c) a MOD (e.g., a wild-type MOD or a reduced-affinity variant MOD, as described above); d) a proteolytically cleavable linker; e) a second MHC polypeptide; and f) Ig Fc polypeptide. The present disclosure provides a nucleic acid comprising a nucleotide sequence encoding a recombinant polypeptide, where the recombinant polypeptide comprises, in order from N-terminus to C-terminus: a) a first leader peptide; b) the epitope; c) the first MHC polypeptide; d) the MOD (e.g., a reduced-affinity variant as described above); e) the proteolytically cleavable linker; f) a second leader peptide; g) the second MHC polypeptide; and h) the Ig Fc polypeptide. The present disclosure provides a nucleic acid comprising a nucleotide sequence encoding a recombinant polypeptide, where the recombinant polypeptide comprises, in order from N-terminus to C-terminus: a) an epitope; b) a first MHC polypeptide; c) a proteolytically cleavable linker; d) a MOD (e.g., a reduced-affinity variant as described above); e) a second MHC polypeptide; and f) an Ig Fc polypeptide. In some cases, the first leader peptide and the second leader peptide are a β 2-M leader peptide. In some cases, the nucleotide sequence is operably linked to a

transcriptional control element. In some cases, the transcriptional control element is a promoter that is functional in a eukaryotic cell.

[00439] Suitable MHC polypeptides are described above. In some cases, the first MHC polypeptide is a β 2-microglobulin polypeptide; and wherein the second MHC polypeptide is an MHC class I heavy chain polypeptide. In some cases, the β 2-microglobulin polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to a β 2M amino acid sequence depicted in FIG. 6. In some cases, the MHC class I heavy chain polypeptide is an HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, HLA-G, HLA-K, or HLA-L heavy chain. In some cases, the MHC class I heavy chain polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to the amino acid sequence depicted in any one of FIG. 3A-3C.

[00440] Suitable Fc polypeptides are described above. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide, an IgG2 Fc polypeptide, an IgG3 Fc polypeptide, an IgG4 Fc polypeptide, an IgA Fc polypeptide, or an IgM Fc polypeptide. In some cases, the Ig Fc polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to an amino acid sequence depicted in Figures 5A-5H.

[00441] Suitable MODs are described above.

[00442] Suitable proteolytically cleavable linkers are described above. In some cases, the proteolytically cleavable linker comprises an amino acid sequence selected from: a) LEVLFQGP (SEQ ID NO:388); b) ENLYTQS (SEQ ID NO:389); c) DDDDK (SEQ ID NO:390); d) LVPR (SEQ ID NO:391); and e) GSGATNFSLLKQAGDVEENPGP (SEQ ID NO:392).

[00443] In some cases, a linker between the epitope and the first MHC polypeptide comprises a first Cys residue, and the second MHC polypeptide comprises an amino acid substitution to provide a second Cys residue, such that the first and the second Cys residues provide for a disulfide linkage between the linker and the second MHC polypeptide. In some cases, first MHC polypeptide comprises an amino acid substitution to provide a first Cys residue, and the second MHC polypeptide comprises an amino acid substitution to provide a second Cys residue, such that the first Cys residue and the second Cys residue provide for a disulfide linkage between the first MHC polypeptide and the second MHC polypeptide.

[00444] The present disclosure provides a nucleic acid comprising a nucleotide sequence encoding a TMMP, where the TMMP comprises: a) a first polypeptide chain comprising, in order from N-terminus to C-terminus: i) a WT-1 peptide of the sequence VLDFAPPGA (SEQ ID NO:259); ii) a linker having the amino acid sequence GCGSGGGSGGGGS (SEQ ID NO:317); and iii) a β 2M polypeptide comprising a Cys at position 12 (e.g., a β 2M having the amino acid sequence set forth in SEQ ID NO:311); and b) a second polypeptide chain comprising, in order from N-terminus to C-

terminus: i) a variant IL-2 polypeptide comprising H16A and F42A substitutions (i.e., comprising Ala at positions 16 and 42, e.g., the amino acid sequence set forth in SEQ ID NO:188, where X₁ is Ala and where X₂ is Ala); ii) a (GGGGS)₄ linker; iii) a variant IL-2 polypeptide comprising H16A and F42A substitutions (i.e., comprising Ala at positions 16 and 42, e.g., the amino acid sequence set forth in SEQ ID NO:188, where X₁ is Ala and where X₂ is Ala); iv) a (GGGGS)₄ linker; v) an HLA A0202 heavy chain comprising Cys at positions 84 and 236 (e.g., an HLA heavy chain polypeptide comprising the amino acid sequence set forth in SEQ ID NO:341); vi) an AAAGG linker; and vii) an Ig Fc polypeptide. In some cases, the Ig Fc polypeptide is a variant Ig Fc polypeptide comprising one or more sequence variations relative to the wild type polypeptide, where the ability of the Ig Fc polypeptide to induce cell lysis through complement-dependent cytotoxicity (CDC) and/or antibody-dependent cellular cytotoxicity (ADCC) is reduced or substantially eliminated. In some cases, the Ig Fc polypeptide is a variant human IgG1 Fc polypeptide comprising comprises an L234A and/or L235A substitutions (L14 and L15 in the amino acid sequence depicted in FIG. 5H). In some cases, the Ig Fc polypeptide comprises the amino acid sequence depicted in FIG. 5H. In some cases, the first polypeptide and the second polypeptide are encoded on separate nucleic acids; e.g., the present disclosure provides a first nucleic acid comprising a nucleotide sequence encoding the first polypeptide and a second nucleic acid comprising a nucleotide sequence encoding the second polypeptide. In other cases, a single nucleic acid comprises nucleotide sequences encoding the first polypeptide and the second polypeptide; e.g., the present disclosure provides a nucleic acid comprising a first nucleotide sequence encoding the first polypeptide and a second nucleotide sequence encoding the second polypeptide. In some cases, the nucleic acid(s) is/are in expression vector(s).

[00445] The present disclosure provides a nucleic acid comprising a nucleotide sequence encoding a TMMP, where the TMMP comprises: a) a first polypeptide chain comprising, in order from N-terminus to C-terminus: i) a WT-1 peptide of the sequence VLDFAPPGA (SEQ ID NO:259); ii) a linker having the amino acid sequence GCGSGGGSGGGGS (SEQ ID NO:317); and iii) a β 2M polypeptide comprising a Cys at position 12 (e.g., a β 2M having the amino acid sequence set forth in SEQ ID NO:311); and b) a second polypeptide chain comprising, in order from N-terminus to C-terminus: i) a variant IL-2 polypeptide comprising H16A and F42A substitutions (i.e., comprising Ala at positions 16 and 42, e.g., the amino acid sequence set forth in SEQ ID NO:188, where X₁ is Ala and where X₂ is Ala); ii) a (GGGGS)₄ linker; iii) a variant IL-2 polypeptide comprising H16A and F42A substitutions (i.e., comprising Ala at positions 16 and 42, e.g., the amino acid sequence set forth in SEQ ID NO:188, where X₁ is Ala and where X₂ is Ala); iv) a (GGGGS)₄ linker; v) an HLA A0202 heavy chain comprising Cys at positions 84 and 236 (e.g., an HLA heavy chain polypeptide comprising the amino acid sequence set forth in SEQ ID NO:341); vi) an AAAGG linker; and vii) an Ig Fc polypeptide comprising Ala at positions 14 and 15, and lacking a C-terminal Lys (e.g., an Ig Fc polypeptide

comprising the amino acid sequence depicted in FIG. 5H and set forth in SEQ ID NO:487). In some cases, the first polypeptide and the second polypeptide are encoded on separate nucleic acids; e.g., the present disclosure provides a first nucleic acid comprising a nucleotide sequence encoding the first polypeptide and a second nucleic acid comprising a nucleotide sequence encoding the second polypeptide. In other cases, a single nucleic acid comprises nucleotide sequences encoding the first polypeptide and the second polypeptide; e.g., the present disclosure provides a nucleic acid comprising a first nucleotide sequence encoding the first polypeptide and a second nucleotide sequence encoding the second polypeptide. In some cases, the nucleic acid(s) is/are in expression vector(s).

[00446] The present disclosure provides a nucleic acid comprising a nucleotide sequence encoding a TMMP, where the TMMP comprises: a) a first polypeptide chain comprising the amino acid sequence designated "2380" as depicted in FIG. 14B and as set forth in SEQ ID NO:423; and b) a second polypeptide chain comprising the amino acid sequence 1715Δ as depicted in FIG. 14J and as set forth in SEQ ID NO:486. In some cases, the present disclosure provides: i) a first nucleic acid comprising a nucleotide sequence encoding the 2380 polypeptide as depicted in FIG. 14B and as set forth in SEQ ID NO:423; and ii) a second nucleic acid comprising a nucleotide sequence encoding the 1715Δ polypeptide as depicted in FIG. 14J and as set forth in SEQ ID NO:486. In some cases, the first nucleic acid is in a first expression vector and the second nucleic acid is in a second expression vector. In some cases, the present disclosure provides a nucleic acid comprising: i) a first nucleotide sequence encoding the 2380 polypeptide as depicted in FIG. 14B and as set forth in SEQ ID NO:423; and ii) a second nucleotide sequence encoding the 1715Δ polypeptide as depicted in FIG. 14J and as set forth in SEQ ID NO:486. In some cases, the nucleic acid is in an expression vector. Suitable expression vectors are described below.

Recombinant expression vectors

[00447] The present disclosure provides recombinant expression vectors comprising nucleic acids of the present disclosure. In some cases, the recombinant expression vector is a non-viral vector. In some cases, the recombinant expression vector is a viral construct, e.g., a recombinant adeno-associated virus construct (see, e.g., U.S. Patent No. 7,078,387), a recombinant adenoviral construct, a recombinant lentiviral construct, a recombinant retroviral construct, a non-integrating viral vector, etc.

[00448] Suitable expression vectors include, but are not limited to, viral vectors (e.g. viral vectors based on vaccinia virus; poliovirus; adenovirus (see, e.g., Li et al., Invest Ophthalmol Vis Sci 35:2543 2549, 1994; Borrás et al., Gene Ther 6:515 524, 1999; Li and Davidson, PNAS 92:7700 7704, 1995; Sakamoto et al., Hum Gene Ther 5:1088 1097, 1999; WO 94/12649, WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 and WO 95/00655); adeno-associated virus (see, e.g., Ali et al., Hum Gene Ther 9:81 86, 1998; Flannery et al., PNAS 94:6916 6921, 1997; Bennett et al., Invest Ophthalmol Vis Sci 38:2857 2863, 1997; Jomary et al., Gene Ther 4:683 690, 1997; Rolling et al., Hum Gene Ther 10:641 648, 1999; Ali et al., Hum Mol Genet 5:591 594, 1996; Srivastava in WO 93/09239, Samulski et al., J.

Vir. (1989) 63:3822-3828; Mendelson et al., Virol. (1988) 166:154-165; and Flotte et al., PNAS (1993) 90:10613-10617); SV40; herpes simplex virus; human immunodeficiency virus (see, e.g., Miyoshi et al., PNAS 94:10319 23, 1997; Takahashi et al., J Virol 73:7812 7816, 1999); a retroviral vector (e.g., Murine Leukemia Virus, spleen necrosis virus, and vectors derived from retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, a lentivirus, human immunodeficiency virus, myeloproliferative sarcoma virus, and mammary tumor virus); and the like.

[00449] Numerous suitable expression vectors are known to those of skill in the art, and many are commercially available. The following vectors are provided by way of example; for eukaryotic host cells: pXT1, pSG5 (Stratagene), pSVK3, pBPV, pMSG, and pSVLSV40 (Pharmacia). However, any other vector may be used so long as it is compatible with the host cell.

[00450] Depending on the host/vector system utilized, any of a number of suitable transcription and translation control elements, including constitutive and inducible promoters, transcription enhancer elements, transcription terminators, etc. may be used in the expression vector (see e.g., Bitter et al. (1987) *Methods in Enzymology*, 153:516-544).

[00451] In some cases, a nucleotide sequence encoding a DNA-targeting RNA and/or a site-directed modifying polypeptide is operably linked to a control element, e.g., a transcriptional control element, such as a promoter. The transcriptional control element may be functional in either a eukaryotic cell, e.g., a mammalian cell; or a prokaryotic cell (e.g., bacterial or archaeal cell). In some cases, a nucleotide sequence encoding a DNA-targeting RNA and/or a site-directed modifying polypeptide is operably linked to multiple control elements that allow expression of the nucleotide sequence encoding a DNA-targeting RNA and/or a site-directed modifying polypeptide in both prokaryotic and eukaryotic cells.

[00452] Non-limiting examples of suitable eukaryotic promoters (promoters functional in a eukaryotic cell) include those from cytomegalovirus (CMV) immediate early, herpes simplex virus (HSV) thymidine kinase, early and late SV40, long terminal repeats (LTRs) from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art. The expression vector may also contain a ribosome binding site for translation initiation and a transcription terminator. The expression vector may also include appropriate sequences for amplifying expression.

GENETICALLY MODIFIED HOST CELLS

[00453] The present disclosure provides a genetically modified host cell, where the host cell is genetically modified with a nucleic acid of the present disclosure.

[00454] Suitable host cells include eukaryotic cells, such as yeast cells, insect cells, and mammalian cells. In some cases, the host cell is a cell of a mammalian cell line. Suitable mammalian cell

lines include human cell lines, non-human primate cell lines, rodent (e.g., mouse, rat) cell lines, and the like. Suitable mammalian cell lines include, but are not limited to, HeLa cells (e.g., American Type Culture Collection (ATCC) No. CCL-2), CHO cells (e.g., ATCC Nos. CRL9618, CCL61, CRL9096), 293 cells (e.g., ATCC No. CRL-1573), Vero cells, NIH 3T3 cells (e.g., ATCC No. CRL-1658), Huh-7 cells, BHK cells (e.g., ATCC No. CCL10), PC12 cells (ATCC No. CRL1721), COS cells, COS-7 cells (ATCC No. CRL1651), RAT1 cells, mouse L cells (ATCC No. CCL1.3), human embryonic kidney (HEK) cells (ATCC No. CRL1573), HLHepG2 cells, and the like.

[00455] In some cases, the host cell is a mammalian cell that has been genetically modified such that it does not synthesize endogenous MHC β 2-M.

[00456] In some cases, the host cell is a mammalian cell that has been genetically modified such that it does not synthesize endogenous MHC Class I heavy chain. In some cases, the host cell is a mammalian cell that has been genetically modified such that it does not synthesize endogenous MHC β 2-M and such that it does not synthesize endogenous MHC Class I heavy chain.

COMPOSITIONS

[00457] The present disclosure provides compositions, including pharmaceutical compositions, comprising a TMMP (synTac) of the present disclosure. The present disclosure provides compositions, including pharmaceutical compositions, comprising a TMMP. The present disclosure provides compositions, including pharmaceutical compositions, comprising a nucleic acid or a recombinant expression vector of the present disclosure.

Compositions comprising a multimeric polypeptide

[00458] A composition of the present disclosure can comprise, in addition to a TMMP of the present disclosure, one or more of: a salt, e.g., NaCl, MgCl₂, KCl, MgSO₄, etc.; a buffering agent, e.g., a Tris buffer, N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES), 2-(N-Morpholino)ethanesulfonic acid (MES), 2-(N-Morpholino)ethanesulfonic acid sodium salt (MES), 3-(N-Morpholino)propanesulfonic acid (MOPS), N-tris[Hydroxymethyl]methyl-3-aminopropanesulfonic acid (TAPS), etc.; a solubilizing agent; a detergent, e.g., a non-ionic detergent such as Tween-20, etc.; a protease inhibitor; glycerol; and the like.

[00459] The composition may comprise a pharmaceutically acceptable excipient, a variety of which are known in the art and need not be discussed in detail herein. Pharmaceutically acceptable excipients have been amply described in a variety of publications, including, for example, "Remington: The Science and Practice of Pharmacy", 19th Ed. (1995), or latest edition, Mack Publishing Co; A. Gennaro (2000) "Remington: The Science and Practice of Pharmacy", 20th edition, Lippincott, Williams, & Wilkins; Pharmaceutical Dosage Forms and Drug Delivery Systems (1999) H.C. Ansel et al., eds 7th

ed., Lippincott, Williams, & Wilkins; and Handbook of Pharmaceutical Excipients (2000) A.H. Kibbe et al., eds., 3rd ed. Amer. Pharmaceutical Assoc.

[00460] A pharmaceutical composition can comprise a TMMP, and a pharmaceutically acceptable excipient. In some cases, a subject pharmaceutical composition will be suitable for administration to a subject, e.g., will be sterile. For example, in some cases, a subject pharmaceutical composition will be suitable for administration to a human subject, e.g., where the composition is sterile and is free of detectable pyrogens and/or other toxins.

[00461] The protein compositions may comprise other components, such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium, carbonate, and the like. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, hydrochloride, sulfate salts, solvates (e.g., mixed ionic salts, water, organics), hydrates (e.g., water), and the like.

[00462] For example, compositions may include aqueous solution, powder form, granules, tablets, pills, suppositories, capsules, suspensions, sprays, and the like. The composition may be formulated according to the various routes of administration described below.

[00463] Where a TMMP is administered as an injectable (e.g. subcutaneously, intraperitoneally, intramuscularly, and/or intravenously) directly into a tissue, a formulation can be provided as a ready-to-use dosage form, or as non-aqueous form (e.g. a reconstitutable storage-stable powder) or aqueous form, such as liquid composed of pharmaceutically acceptable carriers and excipients. The protein-containing formulations may also be provided so as to enhance serum half-life of the TMMP following administration. For example, the TMMP may be provided in a liposome formulation, prepared as a colloid, or other conventional techniques for extending serum half-life. A variety of methods are available for preparing liposomes, as described in, e.g., Szoka et al. 1980 *Ann. Rev. Biophys. Bioeng.* 9:467, U.S. Pat. Nos. 4,235,871, 4,501,728 and 4,837,028. The preparations may also be provided in controlled release or slow-release forms.

[00464] Other examples of formulations suitable for parenteral administration include isotonic sterile injection solutions, anti-oxidants, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. For example, a subject pharmaceutical composition can be present in a container, e.g., a sterile container, such as a syringe. The formulations can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid excipient, for example, water, for injections, immediately

prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets.

[00465] The concentration of a TMMP in a formulation can vary widely (e.g., from less than about 0.1%, usually at or at least about 2% to as much as 20% to 50% or more by weight) and will usually be selected primarily based on fluid volumes, viscosities, and patient-based factors in accordance with the particular mode of administration selected and the patient's needs.

[00466] The present disclosure provides a container comprising a composition of the present disclosure, e.g., a liquid composition. The container can be, e.g., a syringe, an ampoule, and the like. In some cases, the container is sterile. In some cases, both the container and the composition are sterile.

[00467] The present disclosure provides compositions, including pharmaceutical compositions, comprising a TMMP. A composition can comprise: a) a TMMP of the present disclosure; and b) an excipient, as described above. In some cases, the excipient is a pharmaceutically acceptable excipient.

[00468] In some cases, a TMMP is present in a liquid composition. Thus, the present disclosure provides compositions (e.g., liquid compositions, including pharmaceutical compositions) comprising a TMMP. In some cases, a composition of the present disclosure comprises: a) a TMMP of the present disclosure; and b) saline (e.g., 0.9% NaCl). In some cases, the composition is sterile. In some cases, the composition is suitable for administration to a human subject, e.g., where the composition is sterile and is free of detectable pyrogens and/or other toxins. Thus, the present disclosure provides a composition comprising: a) a TMMP of the present disclosure; and b) saline (e.g., 0.9% NaCl), where the composition is sterile and is free of detectable pyrogens and/or other toxins.

Compositions comprising a nucleic acid or a recombinant expression vector

[00469] The present disclosure provides compositions, e.g., pharmaceutical compositions, comprising a nucleic acid or a recombinant expression vector of the present disclosure. A wide variety of pharmaceutically acceptable excipients is known in the art and need not be discussed in detail herein. Pharmaceutically acceptable excipients have been amply described in a variety of publications, including, for example, A. Gennaro (2000) "Remington: The Science and Practice of Pharmacy", 20th edition, Lippincott, Williams, & Wilkins; Pharmaceutical Dosage Forms and Drug Delivery Systems (1999) H. C. Ansel et al., eds 7th ed., Lippincott, Williams, & Wilkins; and Handbook of Pharmaceutical Excipients (2000) A. H. Kibbe et al., eds., 3rd ed. Amer. Pharmaceutical Assoc.

[00470] A composition of the present disclosure can include: a) one or more nucleic acids or one or more recombinant expression vectors comprising nucleotide sequences encoding a TMMP; and b) one or more of: a buffer, a surfactant, an antioxidant, a hydrophilic polymer, a dextrin, a chelating agent, a suspending agent, a solubilizer, a thickening agent, a stabilizer, a bacteriostatic agent, a wetting agent, and a preservative. Suitable buffers include, but are not limited to, (such as N,N-bis(2-hydroxyethyl)-2-

aminoethanesulfonic acid (BES), bis(2-hydroxyethyl)amino-tris(hydroxymethyl)methane (BIS-Tris), N-(2-hydroxyethyl)piperazine-N'-3-propanesulfonic acid (EPPS or HEPPS), glycylglycine, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 3-(N-morpholino)propane sulfonic acid (MOPS), piperazine-N,N'-bis(2-ethane-sulfonic acid) (PIPES), sodium bicarbonate, 3-(N-tris(hydroxymethyl)-methyl-amino)-2-hydroxy-propanesulfonic acid) TAPSO, (N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES), N-tris(hydroxymethyl)methyl-glycine (Tricine), tris(hydroxymethyl)-aminomethane (Tris), etc.). Suitable salts include, e.g., NaCl, MgCl₂, KCl, MgSO₄, etc.

[00471] A pharmaceutical formulation of the present disclosure can include a nucleic acid or recombinant expression vector of the present disclosure in an amount of from about 0.001% to about 90% (w/w). In the description of formulations, below, "subject nucleic acid or recombinant expression vector" will be understood to include a nucleic acid or recombinant expression vector of the present disclosure. For example, in some cases, a subject formulation comprises a nucleic acid or recombinant expression vector of the present disclosure.

[00472] A subject nucleic acid or recombinant expression vector can be admixed, encapsulated, conjugated or otherwise associated with other compounds or mixtures of compounds; such compounds can include, e.g., liposomes or receptor-targeted molecules. A subject nucleic acid or recombinant expression vector can be combined in a formulation with one or more components that assist in uptake, distribution and/or absorption.

[00473] A subject nucleic acid or recombinant expression vector composition can be formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, gel capsules, liquid syrups, soft gels, suppositories, and enemas. A subject nucleic acid or recombinant expression vector composition can also be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions may further contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

[00474] A formulation comprising a subject nucleic acid or recombinant expression vector can be a liposomal formulation. As used herein, the term "liposome" means a vesicle composed of amphiphilic lipids arranged in a spherical bilayer or bilayers. Liposomes are unilamellar or multilamellar vesicles which have a membrane formed from a lipophilic material and an aqueous interior that contains the composition to be delivered. Cationic liposomes are positively charged liposomes that can interact with negatively charged DNA molecules to form a stable complex. Liposomes that are pH sensitive or negatively charged are believed to entrap DNA rather than complex with it. Both cationic and noncationic liposomes can be used to deliver a subject nucleic acid or recombinant expression vector.

[00475] Liposomes also include "sterically stabilized" liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Examples of sterically stabilized liposomes are those in which part of the vesicle-forming lipid portion of the liposome comprises one or more glycolipids or is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol (PEG) moiety. Liposomes and their uses are further described in U.S. Pat. No. 6,287,860, which is incorporated herein by reference in its entirety.

[00476] The formulations and compositions may also include surfactants. The use of surfactants in drug products, formulations and in emulsions is well known in the art. Surfactants and their uses are further described in U.S. Pat. No. 6,287,860.

[00477] In one embodiment, various penetration enhancers are included, to effect the efficient delivery of nucleic acids. In addition to aiding the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also enhance the permeability of lipophilic drugs. Penetration enhancers may be classified as belonging to one of five broad categories, i.e., surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants. Penetration enhancers and their uses are further described in U.S. Pat. No. 6,287,860, which is incorporated herein by reference in its entirety.

[00478] Compositions and formulations for oral administration include powders or granules, microparticulates, nanoparticulates, suspensions or solutions in water or non-aqueous media, capsules, gel capsules, sachets, tablets, or minitabets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable. Suitable oral formulations include those in which a subject antisense nucleic acid is administered in conjunction with one or more penetration enhancers surfactants and chelators. Suitable surfactants include, but are not limited to, fatty acids and/or esters or salts thereof, bile acids and/or salts thereof. Suitable bile acids/salts and fatty acids and their uses are further described in U.S. Pat. No. 6,287,860. Also suitable are combinations of penetration enhancers, for example, fatty acids/salts in combination with bile acids/salts. An exemplary suitable combination is the sodium salt of lauric acid, capric acid, and UDCA. Further penetration enhancers include, but are not limited to, polyoxyethylene-9-lauryl ether, and polyoxyethylene-20-cetyl ether. Suitable penetration enhancers also include propylene glycol, dimethylsulfoxide, triethanolamine, N,N-dimethylacetamide, N,N-dimethylformamide, 2-pyrrolidone and derivatives thereof, tetrahydrofurfuryl alcohol, and AZONE™.

METHODS OF MODULATING T CELL ACTIVITY

[00479] The present disclosure provides a method of selectively modulating the activity of an epitope-specific T cell, the method comprising contacting the T cell with a TMMP of the present disclosure, where contacting the T cell with a TMMP selectively modulates the activity of the epitope-

specific T cell. In some cases, the contacting occurs *in vitro*. In some cases, the contacting occurs *in vivo*. In some cases, the contacting occurs *ex vivo*.

[00480] In some cases, e.g., where the target T cell is a CD8⁺ T cell, the TMMP comprises Class I MHC polypeptides (e.g., β 2-microglobulin and Class I MHC heavy chain).

[00481] Where a TMMP includes an immunomodulatory polypeptide that is an activating polypeptide, contacting the T cell with the TMMP activates the epitope-specific T cell. In some instances, the epitope-specific T cell is a T cell that is specific for an epitope present on a cancer cell, and contacting the epitope-specific T cell with the TMMP increases cytotoxic activity of the T cell toward the cancer cell. In some instances, the epitope-specific T cell is a T cell that is specific for an epitope present on a cancer cell, and contacting the epitope-specific T cell with the TMMP increases the number of the epitope-specific T cells.

[00482] In some instances, the epitope-specific T cell is a T cell that is specific for an epitope present on a virus-infected cell, and contacting the epitope-specific T cell with the TMMP increases cytotoxic activity of the T cell toward the virus-infected cell. In some instances, the epitope-specific T cell is a T cell that is specific for an epitope present on a virus-infected cell, and contacting the epitope-specific T cell with the TMMP increases the number of the epitope-specific T cells.

[00483] Where a TMMP includes an immunomodulatory polypeptide that is an inhibiting polypeptide, contacting the T cell with the TMMP inhibits the epitope-specific T cell. In some instances, the epitope-specific T cell is a self-reactive T cell that is specific for an epitope present in a self antigen, and the contacting reduces the number of the self-reactive T cells.

[00484] The present disclosure provides a method of modulating an immune response in an individual, the method comprising administering to the individual an effective amount of a TMMP. Administering the TMMP induces an epitope-specific T cell response (e.g., a WT-1 epitope-specific T-cell response) and an epitope-non-specific T cell response, where the ratio of the epitope-specific T cell response to the epitope-non-specific T cell response is at least 2:1. In some cases, the ratio of the epitope-specific T cell response to the epitope-non-specific T cell response is at least 5:1. In some cases, the ratio of the epitope-specific T cell response to the epitope-non-specific T cell response is at least 10:1. In some cases, the ratio of the epitope-specific T cell response to the epitope-non-specific T cell response is at least 25:1. In some cases, the ratio of the epitope-specific T cell response to the epitope-non-specific T cell response is at least 50:1. In some cases, the ratio of the epitope-specific T cell response to the epitope-non-specific T cell response is at least 100:1. In some cases, the individual is a human. In some cases, the modulating increases a cytotoxic T-cell response to a cancer cell, e.g., a WT-1-expressing cancer cell. In some cases, the administering is intravenous, subcutaneous, intramuscular, systemic, intralymphatic, distal to a treatment site, local, or at or near a treatment site.

[00485] The present disclosure provides a method of delivering a costimulatory (i.e., immunomodulatory) polypeptide selectively to target T cell, the method comprising contacting a mixed population of T cells with a TMMP of the present disclosure, where the mixed population of T cells comprises the target T cell and non-target T cells, where the target T cell is specific for the epitope present within the TMMP (e.g., where the target T cell is specific for the WT-1 epitope present within the TMMP), and where the contacting step delivers the one or more costimulatory polypeptides (immunomodulatory polypeptides) present within the TMMP to the target T cell. In some cases, the population of T cells is *in vitro*. In some cases, the population of T cells is *in vivo* in an individual. In some cases, the method comprises administering the TMMP to the individual. In some case, the T cell is a cytotoxic T cell. In some cases, the mixed population of T cells is an *in vitro* population of mixed T cells obtained from an individual, and the contacting step results in activation and/or proliferation of the target T cell, generating a population of activated and/or proliferated target T cells; in some of these instances, the method further comprises administering the population of activated and/or proliferated target T cells to the individual.

[00486] The present disclosure provides a method of detecting, in a mixed population of T cells obtained from an individual, the presence of a target T cell that binds an epitope of interest (e.g., a WT-1 epitope), the method comprising: a) contacting *in vitro* the mixed population of T cells with a TMMP, wherein the TMMP comprises the epitope of interest (e.g., the WT-1 epitope); and b) detecting activation and/or proliferation of T cells in response to said contacting, wherein activated and/or proliferated T cells indicates the presence of the target T cell.

TREATMENT METHODS

[00487] The present disclosure provides a method of treatment of an individual, the method comprising administering to the individual an amount of a TMMP of the present disclosure, or one or more nucleic acids encoding the TMMP, effective to treat the individual. Also provided is a TMMP for use in a method of treatment of the human or animal body. In some cases, a treatment method of the present disclosure comprises administering to an individual in need thereof one or more recombinant expression vectors comprising nucleotide sequences encoding a TMMP. In some cases, a treatment method of the present disclosure comprises administering to an individual in need thereof one or more mRNA molecules comprising nucleotide sequences encoding a TMMP. In some cases, a treatment method of the present disclosure comprises administering to an individual in need thereof a TMMP of the present disclosure. Conditions that can be treated include, e.g., cancer and autoimmune disorders, as described below.

[00488] In some cases, a TMMP, when administered to an individual in need thereof, induces both an epitope-specific T cell response and an epitope non-specific T cell response. In other words, in some cases, a TMMP, when administered to an individual in need thereof, induces an epitope-specific T

cell response by modulating the activity of a first T cell that displays both: i) a TCR specific for the epitope present in the TMMP; ii) a co-immunomodulatory polypeptide that binds to the immunomodulatory polypeptide present in the TMMP; and induces an epitope non-specific T cell response by modulating the activity of a second T cell that displays: i) a TCR specific for an epitope other than the epitope present in the TMMP; and ii) a co-immunomodulatory polypeptide that binds to the immunomodulatory polypeptide present in the TMMP. The ratio of the epitope-specific T cell response to the epitope-non-specific T cell response is at least 2:1, at least 5:1, at least 10:1, at least 15:1, at least 20:1, at least 25:1, at least 50:1, or at least 100:1. The ratio of the epitope-specific T cell response to the epitope-non-specific T cell response is from about 2:1 to about 5:1, from about 5:1 to about 10:1, from about 10:1 to about 15:1, from about 15:1 to about 20:1, from about 20:1 to about 25:1, from about 25:1 to about 50:1, or from about 50:1 to about 100:1, or more than 100:1. "Modulating the activity" of a T cell can include one or more of: i) activating a cytotoxic (e.g., CD8⁺) T cell; ii) inducing cytotoxic activity of a cytotoxic (e.g., CD8⁺) T cell; iii) inducing production and release of a cytotoxin (e.g., a perforin; a granzyme; a granulysin) by a cytotoxic (e.g., CD8⁺) T cell; iv) inhibiting activity of an autoreactive T cell; and the like.

[00489] The combination of the reduced affinity of the MOD for its cognate co-MOD, and the affinity of the epitope for a TCR, provides for enhanced selectivity of a TMMP of the present disclosure. Thus, for example, a TMMP binds with higher avidity to a first T cell that displays both: i) a TCR specific for the epitope present in the TMMP; and ii) a co-immunomodulatory polypeptide that binds to the immunomodulatory polypeptide present in the TMMP, compared to the avidity to which it binds to a second T cell that displays: i) a TCR specific for an epitope other than the epitope present in the TMMP; and ii) a co-MOD that binds to the immunomodulatory polypeptide present in the TMMP.

[00490] The present disclosure provides a method of selectively modulating the activity of an epitope-specific T cell in an individual, the method comprising administering to the individual an effective amount of a TMMP, or one or more nucleic acids (e.g., expression vectors; mRNA; etc.) comprising nucleotide sequences encoding the TMMP, where the TMMP selectively modulates the activity of the epitope-specific T cell in the individual. Selectively modulating the activity of an epitope-specific T cell can treat a disease or disorder in the individual. Thus, the present disclosure provides a treatment method comprising administering to an individual in need thereof an effective amount of a TMMP.

[00491] In some cases, the MOD is an activating polypeptide, and the TMMP activates the epitope-specific T cell. In some cases, the epitope is a cancer-associated epitope, and the TMMP increases the activity of a T cell specific for the cancer-associated epitope. In some cases, the MOD is an activating polypeptide, and the TMMP activates a WT-1 epitope-specific T-cell. In some cases, the T cells are T-helper cells (CD4⁺ cells), cytotoxic T-cells (CD8⁺ cells), or NK-T-cells. In some cases, the

epitope is a WT-1 epitope, and the TMMP increases the activity of a T-cell specific for a cancer cell expressing the WT-1 epitope (e.g., T-helper cells (CD4⁺ cells), cytotoxic T-cells (CD8⁺ cells), and/or NK-T-cells). Activation of CD4⁺ T cells can include increasing proliferation of CD4⁺ T cells and/or inducing or enhancing release cytokines by CD4⁺ T cells. Activation of NK-T-cells and/or CD8⁺ cells can include: increasing proliferation of NK-T-cells and/or CD8⁺ cells; and/or inducing release of cytokines such as interferon γ by NK-T-cells and/or CD8⁺ cells. In some cases, a TMMP of the present disclosure reduces proliferation and/or activity of a regulatory T (Treg) cell. Tregs are FoxP3⁺, CD4⁺ T cells. In some cases, e.g., where a TMMP of the present disclosure comprises an inhibitory immunomodulatory polypeptide (e.g., PD-L1, FasL, and the like), the TMMP reduces the proliferation and/or activity of a Treg.

[00492] In some cases, the MOD is an activating polypeptide, and the TMMP activates the epitope-specific T cell. In some cases, the epitope is a cancer-associated epitope, and the TMMP increases the activity of a T cell specific for the cancer-associate epitope.

[00493] Where a TMMP comprises a WT-1 peptide epitope, the TMMP can be administered to an individual having a WT-1-expressing cancer. WT1-expressing cancers include a leukemia, a desmoplastic small round cell tumor, a gastric cancer, a colon cancer, a lung cancer, a breast cancer, a germ cell tumor, an ovarian cancer, a uterine cancer, a thyroid cancer, a liver cancer, a renal cancer, a Kaposi's sarcoma, a sarcoma, a hepatocellular carcinoma, a Wilms' tumor, an acute myelogenous leukemia (AML), a myelodysplastic syndrome (MDS), an a non-small cell lung cancer (NSCLC), a myeloma, pancreatic cancer, colorectal cancer, a mesothelioma, a soft tissue sarcoma, a neuroblastoma, and a nephroblastoma.

[00494] Where a TMMP comprises a WT-1 peptide epitope, the TMMP can be administered to an individual in need thereof to treat acute myeloid leukemia (AML) in the individual. Where a TMMP comprises a WT-1 peptide epitope, the TMMP can be administered to an individual in need thereof to treat a myeloma in the individual. Where a TMMP comprises a WT-1 peptide epitope, the TMMP can be administered to an individual in need thereof to treat ovarian cancer in the individual. Where a TMMP of the present disclosure comprises a WT-1 peptide epitope, the TMMP can be administered to an individual in need thereof to treat pancreatic cancer in the individual. Where a TMMP comprises a WT-1 peptide epitope, the TMMP can be administered to an individual in need thereof to treat non-small cell lung cancer (NSCLC) in the individual. Where a TMMP comprises a WT-1 peptide epitope, the TMMP can be administered to an individual in need thereof to treat colorectal cancer (CRC) in the individual. Where a TMMP comprises a WT-1 peptide epitope, the TMMP can be administered to an individual in need thereof to treat breast cancer in the individual. Where a TMMP comprises a WT-1 peptide epitope, the TMMP can be administered to an individual in need thereof to treat a Wilms tumor in the individual. Where a TMMP of the present disclosure comprises a WT-1 peptide epitope, the TMMP can be

administered to an individual in need thereof to treat mesothelioma in the individual. Where a TMMP comprises a WT-1 peptide epitope, the TMMP can be administered to an individual in need thereof to treat soft tissue sarcoma in the individual. Where a TMMP comprises a WT-1 peptide epitope, the TMMP can be administered to an individual in need thereof to treat a neuroblastoma in the individual. Where a TMMP comprises a WT-1 peptide epitope, the TMMP can be administered to an individual in need thereof to treat a nephroblastoma in the individual.

[00495] The present disclosure provides a method of treating cancer in an individual, the method comprising administering to the individual an effective amount of a TMMP of the present disclosure, or one or more nucleic acids (e.g., expression vectors; mRNA; etc.) comprising nucleotide sequences encoding the TMMP, where the TMMP comprises a T-cell epitope that is a cancer epitope, and where the TMMP comprises a stimulatory MOD. In some cases, an “effective amount” of a TMMP is an amount that, when administered in one or more doses to an individual in need thereof, reduces the number of cancer cells in the individual. For example, in some cases, an “effective amount” of a TMMP of the present disclosure is an amount that, when administered in one or more doses to an individual in need thereof, reduces the number of cancer cells in the individual by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%, compared to the number of cancer cells in the individual before administration of the TMMP, or in the absence of administration with the TMMP. In some cases, an “effective amount” of a TMMP is an amount that, when administered in one or more doses to an individual in need thereof, reduces the number of cancer cells in the individual to undetectable levels.

[00496] In some cases, an “effective amount” of a TMMP is an amount that, when administered in one or more doses to an individual in need thereof, reduces the tumor mass in the individual. For example, in some cases, an “effective amount” of a TMMP is an amount that, when administered in one or more doses to an individual in need thereof (an individual having a tumor), reduces the tumor mass in the individual by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%, compared to the tumor mass in the individual before administration of the TMMP, or in the absence of administration with the TMMP. In some cases, an “effective amount” of a TMMP is an amount that, when administered in one or more doses to an individual in need thereof (an individual having a tumor), reduces the tumor volume in the individual. For example, in some cases, an “effective amount” of a TMMP is an amount that, when administered in one or more doses to an individual in need thereof (an individual having a tumor), reduces the tumor volume in the individual by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%, compared to the tumor volume in the individual before administration of the TMMP, or in the absence of administration with the TMMP. In some cases, an “effective amount” of a TMMP is an

amount that, when administered in one or more doses to an individual in need thereof, increases survival time of the individual. For example, in some cases, an “effective amount” of a TMMP is an amount that, when administered in one or more doses to an individual in need thereof, increases survival time of the individual by at least 1 month, at least 2 months, at least 3 months, from 3 months to 6 months, from 6 months to 1 year, from 1 year to 2 years, from 2 years to 5 years, from 5 years to 10 years, or more than 10 years, compared to the expected survival time of the individual in the absence of administration with the TMMP.

[00497] In some cases, an “effective amount” of a TMMP is an amount that, when administered in one or more doses to an individual in need thereof, either as a monotherapy or as part of a combination therapy (e.g., as part of a combination therapy with an immune checkpoint inhibitor), as discussed below, reduces the overall tumor burden in the individual, i.e., the amount of cancer in the body, or alternatively, causes the total tumor burden in the patient to remain relatively stable for a sufficient period of time for the patient to have a confirmed “stable disease” as determined by standard RECIST criteria. See, e.g., Aykan and Özatli (2020) *World J. Clin. Oncol.* 11:53.

[00498] In some cases, an effective amount of a TMMP is an amount that, when administered in one or more doses to an individual in need thereof, either as a monotherapy or as part of a combination therapy, e.g., with an immune checkpoint inhibitor, as discussed below, causes the tumor size to be reduced by a sufficient amount, and for a sufficient period of time, for the patient to have a confirmed “partial response” as determined by standard RECIST criteria.

[00499] In some cases, an effective amount of a TMMP is an amount that, when administered in one or more doses to an individual in need thereof, either as a monotherapy or as part of a combination therapy, e.g., with an immune checkpoint inhibitor, causes the tumor size to be reduced by a sufficient amount, and for a sufficient period of time, for the patient to have a confirmed “complete response” as determined by standard RECIST criteria.

[00500] In some instances, the epitope-specific T cell is a T cell that is specific for an epitope present on a virus-infected cell, and contacting the epitope-specific T cell with the TMMP increases cytotoxic activity of the T cell toward the virus-infected cell. In some instances, the epitope-specific T cell is a T cell that is specific for an epitope present on a virus-infected cell, and contacting the epitope-specific T cell with the TMMP increases the number of the epitope-specific T cells.

[00501] Thus, the present disclosure provides a method of treating a virus infection in an individual, the method comprising administering to the individual an effective amount of a TMMP, or one or more nucleic acids comprising nucleotide sequences encoding the TMMP, where the TMMP comprises a T-cell epitope that is a viral epitope, and where the TMMP comprises a stimulatory MOD. In some cases, an “effective amount” of a TMMP is an amount that, when administered in one or more

doses to an individual in need thereof, reduces the number of virus-infected cells in the individual. For example, in some cases, an “effective amount” of a TMMP is an amount that, when administered in one or more doses to an individual in need thereof, reduces the number of virus-infected cells in the individual by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%, compared to the number of virus-infected cells in the individual before administration of the TMMP, or in the absence of administration with the TMMP. In some cases, an “effective amount” of a TMMP is an amount that, when administered in one or more doses to an individual in need thereof, reduces the number of virus-infected cells in the individual to undetectable levels.

[00502] Thus, the present disclosure provides a method of treating an infection in an individual, the method comprising administering to the individual an effective amount of a TMMP, or one or more nucleic acids comprising nucleotide sequences encoding the TMMP, where the TMMP comprises a T-cell epitope that is a pathogen-associated epitope, and where the TMMP comprises a stimulatory immunomodulatory polypeptide. In some cases, an “effective amount” of a TMMP of the present disclosure is an amount that, when administered in one or more doses to an individual in need thereof, reduces the number of pathogens in the individual. For example, in some cases, an “effective amount” of a TMMP is an amount that, when administered in one or more doses to an individual in need thereof, reduces the number of pathogens in the individual by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%, compared to the number of pathogens in the individual before administration of the TMMP, or in the absence of administration with the TMMP. In some cases, an “effective amount” of a TMMP is an amount that, when administered in one or more doses to an individual in need thereof, reduces the number of pathogens in the individual to undetectable levels. Pathogens include viruses, bacteria, protozoans, and the like.

[00503] In some cases, the MOD is an inhibitory polypeptide, and the TMMP inhibits activity of the epitope-specific T cell. In some cases, the epitope is a self-epitope, and the TMMP selectively inhibits the activity of a T cell specific for the self-epitope.

[00504] The present disclosure provides a method of treating an autoimmune disorder in an individual, the method comprising administering to the individual an effective amount of a TMMP, or one or more nucleic acids comprising nucleotide sequences encoding the TMMP, where the TMMP comprises a T-cell epitope that is a self epitope, and where the TMMP comprises an inhibitory MOD. In some cases, an “effective amount” of a TMMP is an amount that, when administered in one or more doses to an individual in need thereof, reduces the number self-reactive T cells by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%, compared to number of self-reactive T cells in the individual

before administration of the TMMP, or in the absence of administration with the TMMP. In some cases, an “effective amount” of a TMMP is an amount that, when administered in one or more doses to an individual in need thereof, reduces production of Th2 cytokines in the individual. In some cases, an “effective amount” of a TMMP is an amount that, when administered in one or more doses to an individual in need thereof, ameliorates one or more symptoms associated with an autoimmune disease in the individual.

[00505] As noted above, in some cases, in carrying out a subject treatment method, a TMMP is administered to an individual in need thereof, as the TMMP *per se*. In other instances, in carrying out a subject treatment method, one or more nucleic acids comprising nucleotide sequences encoding a TMMP is/are administering to an individual in need thereof. Thus, in other instances, one or more nucleic acids of the present disclosure, e.g., one or more recombinant expression vectors of the present disclosure, is/are administered to an individual in need thereof.

Formulations

[00506] Suitable formulations are described above, where suitable formulations include a pharmaceutically acceptable excipient. In some cases, a suitable formulation comprises: a) a TMMP of; and b) a pharmaceutically acceptable excipient. In some cases, a suitable formulation comprises: a) a nucleic acid comprising a nucleotide sequence encoding a TMMP; and b) a pharmaceutically acceptable excipient; in some instances, the nucleic acid is an mRNA. In some cases, a suitable formulation comprises: a) a first nucleic acid comprising a nucleotide sequence encoding the first polypeptide of a TMMP; b) a second nucleic acid comprising a nucleotide sequence encoding the second polypeptide of a TMMP; and c) a pharmaceutically acceptable excipient. In some cases, a suitable formulation comprises: a) a recombinant expression vector comprising a nucleotide sequence encoding a TMMP; and b) a pharmaceutically acceptable excipient. In some cases, a suitable formulation comprises: a) a first recombinant expression vector comprising a nucleotide sequence encoding the first polypeptide of a TMMP; b) a second recombinant expression vector comprising a nucleotide sequence encoding the second polypeptide of a TMMP; and c) a pharmaceutically acceptable excipient.

[00507] Suitable pharmaceutically acceptable excipients are described above.

Dosages

[00508] A suitable dosage can be determined by an attending physician or other qualified medical personnel, based on various clinical factors. As is well known in the medical arts, dosages for any one patient depend upon many factors, including the patient's size, body surface area, age, the particular polypeptide or nucleic acid to be administered, sex of the patient, time, and route of administration, general health, and other drugs being administered concurrently. A TMMP of the present disclosure may be administered in amounts between 1 ng/kg body weight and 20 mg/kg body weight per dose, e.g. between 0.1 mg/kg body weight to 10 mg/kg body weight, e.g. between 0.5 mg/kg body weight to 5

mg/kg body weight; however, doses below or above this exemplary range are envisioned, especially considering the aforementioned factors. If the regimen is a continuous infusion, it can also be in the range of 1 μ g to 10 mg per kilogram of body weight per minute. A TMMP of the present disclosure can be administered in an amount of from about 1 mg/kg body weight to 50 mg/kg body weight, e.g., from about 1 mg/kg body weight to about 5 mg/kg body weight, from about 5 mg/kg body weight to about 10 mg/kg body weight, from about 10 mg/kg body weight to about 15 mg/kg body weight, from about 15 mg/kg body weight to about 20 mg/kg body weight, from about 20 mg/kg body weight to about 25 mg/kg body weight, from about 25 mg/kg body weight to about 30 mg/kg body weight, from about 30 mg/kg body weight to about 35 mg/kg body weight, from about 35 mg/kg body weight to about 40 mg/kg body weight, or from about 40 mg/kg body weight to about 50 mg/kg body weight.

[00509] In some cases, a suitable dose of a TMMP is from 0.01 μ g to 100 g per kg of body weight, from 0.1 μ g to 10 g per kg of body weight, from 1 μ g to 1 g per kg of body weight, from 10 μ g to 100 mg per kg of body weight, from 100 μ g to 10 mg per kg of body weight, or from 100 μ g to 1 mg per kg of body weight. Persons of ordinary skill in the art can easily estimate repetition rates for dosing based on measured residence times and concentrations of the administered agent in bodily fluids or tissues. Following successful treatment, it may be desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state, wherein a TMMP is administered in maintenance doses, ranging from 0.01 μ g to 100 g per kg of body weight, from 0.1 μ g to 10 g per kg of body weight, from 1 μ g to 1 g per kg of body weight, from 10 μ g to 100 mg per kg of body weight, from 100 μ g to 10 mg per kg of body weight, or from 100 μ g to 1 mg per kg of body weight.

[00510] Those of skill will readily appreciate that dose levels can vary as a function of the specific TMMP, the severity of the symptoms and the susceptibility of the subject to side effects. Preferred dosages for a given compound are readily determinable by those of skill in the art by a variety of means.

[00511] In some cases, multiple doses of a TMMP, a nucleic acid of the present disclosure, or a recombinant expression vector of the present disclosure are administered. The frequency of administration of a TMMP, a nucleic acid of the present disclosure, or a recombinant expression vector of the present disclosure can vary depending on any of a variety of factors, e.g., severity of the symptoms, etc. For example, in some cases, a TMMP, a nucleic acid of the present disclosure, or a recombinant expression vector of the present disclosure is administered once per month, twice per month, three times per month, every other week (qow), once per week (qw), twice per week (biw), three times per week (tiw), four times per week, five times per week, six times per week, every other day (qod), daily (qd), twice a day (qid), or three times a day (tid).

[00512] The duration of administration of a TMMP, a nucleic acid of the present disclosure, or a recombinant expression vector of the present disclosure, e.g., the period of time over which a TMMP, a nucleic acid of the present disclosure, or a recombinant expression vector of the present disclosure is

administered, can vary, depending on any of a variety of factors, e.g., patient response, etc. For example, a TMMP, a nucleic acid of the present disclosure, or a recombinant expression vector of the present disclosure can be administered over a period of time ranging from about one day to about one week, from about two weeks to about four weeks, from about one month to about two months, from about two months to about four months, from about four months to about six months, from about six months to about eight months, from about eight months to about 1 year, from about 1 year to about 2 years, or from about 2 years to about 4 years, or more.

Routes of administration

[00513] An active agent (a TMMP of the present disclosure, a nucleic acid of the present disclosure, or a recombinant expression vector of the present disclosure) is administered to an individual using any available method and route suitable for drug delivery, including *in vivo* and *ex vivo* methods, as well as systemic and localized routes of administration.

[00514] Conventional and pharmaceutically acceptable routes of administration include intratumoral, peritumoral, intramuscular, intralymphatic, intratracheal, intracranial, subcutaneous, intradermal, topical application, intravenous, intraarterial, rectal, nasal, oral, and other enteral and parenteral routes of administration. Routes of administration may be combined, if desired, or adjusted depending upon the TMMP and/or the desired effect. A TMMP, or a nucleic acid or recombinant expression vector of the present disclosure, can be administered in a single dose or in multiple doses.

[00515] In some cases, a TMMP, a nucleic acid, or a recombinant expression vector is administered intravenously. In some cases, a TMMP of the present disclosure, a nucleic acid of the present disclosure, or a recombinant expression vector of the present disclosure is administered intramuscularly. In some cases, a TMMP, a nucleic acid, or a recombinant expression vector is administered intralymphatically. In some cases, a TMMP, a nucleic acid, or a recombinant expression vector is administered locally. In some cases, a TMMP, a nucleic acid, or a recombinant expression vector is administered intratumorally. In some cases, a TMMP, a nucleic acid, or a recombinant expression vector is administered peritumorally. In some cases, a TMMP, a nucleic acid, or a recombinant expression vector is administered intracranially. In some cases, a TMMP, a nucleic acid, or a recombinant expression vector is administered subcutaneously.

[00516] In some cases, a TMMP is administered intravenously. In some cases, a TMMP is administered intramuscularly. In some cases, a TMMP is administered locally. In some cases, a TMMP is administered intratumorally. In some cases, a TMMP is administered peritumorally. In some cases, a TMMP is administered intracranially. In some cases, a TMMP is administered subcutaneously. In some cases, a TMMP is administered intralymphatically.

[00517] A TMMP, a nucleic acid, or a recombinant expression vector can be administered to a host using any available conventional methods and routes suitable for delivery of conventional drugs, including systemic or localized routes. In general, routes of administration contemplated for use in a method of the present disclosure include, but are not necessarily limited to, enteral, parenteral, and inhalational routes.

[00518] Parenteral routes of administration other than inhalation administration include, but are not necessarily limited to, topical, transdermal, subcutaneous, intramuscular, intraorbital, intracapsular, intraspinal, intrasternal, intratumoral, intralymphatic, peritumoral, and intravenous routes, *i.e.*, any route of administration other than through the alimentary canal. Parenteral administration can be carried to effect systemic or local delivery of a TMMP of the present disclosure, a nucleic acid of the present disclosure, or a recombinant expression vector of the present disclosure. Where systemic delivery is desired, administration typically involves invasive or systemically absorbed topical or mucosal administration of pharmaceutical preparations.

Combination therapies

[00519] In some cases, a method of the present disclosure for treating cancer in an individual comprises: a) administering a TMMP; and b) administering at least one additional therapeutic agent or therapeutic treatment. Suitable additional therapeutic agents include, but are not limited to, a small molecule cancer chemotherapeutic agent, and an immune checkpoint inhibitor. Suitable additional therapeutic treatments include, *e.g.*, radiation, surgery (*e.g.*, surgical resection of a tumor), and the like.

[00520] A treatment method of the present disclosure can comprise co-administration of a TMMP and at least one additional therapeutic agent. By “co-administration” is meant that both a TMMP and at least one additional therapeutic agent are administered to an individual, although not necessarily at the same time, in order to achieve a therapeutic effect that is the result of having administered both the TMMP and the at least one additional therapeutic agent. The administration of the TMMP and the at least one additional therapeutic agent can be substantially simultaneous, *e.g.*, the TMMP can be administered to an individual within about 1 minute to about 24 hours (*e.g.*, within about 1 minute, within about 5 minutes, within about 15 minutes, within about 30 minutes, within about 1 hour, within about 4 hours, within about 8 hours, within about 12 hours, or within about 24 hours) of administration of the at least one additional therapeutic agent. In some cases, a TMMP of the present disclosure is administered to an individual who is undergoing treatment with, or who has undergone treatment with, the at least one additional therapeutic agent. The administration of the TMMP can occur at different times and/or at different frequencies.

[00521] As an example, a treatment method of the present disclosure can comprise co-administration of a TMMP and an immune checkpoint inhibitor such as an antibody specific for an

immune checkpoint. By “co-administration” is meant that both a TMMP and an immune checkpoint inhibitor (e.g., an antibody specific for an immune checkpoint polypeptide) are administered to an individual, although not necessarily at the same time, in order to achieve a therapeutic effect that is the result of having administered both the TMMP and the immune checkpoint inhibitor (e.g., an antibody specific for an immune checkpoint polypeptide). The administration of the TMMP and the immune checkpoint inhibitor (e.g., an antibody specific for an immune checkpoint polypeptide) can be substantially simultaneous, e.g., the TMMP can be administered to an individual within about 1 minute to about 24 hours (e.g., within about 1 minute, within about 5 minutes, within about 15 minutes, within about 30 minutes, within about 1 hour, within about 4 hours, within about 8 hours, within about 12 hours, or within about 24 hours) of administration of the immune checkpoint inhibitor (e.g., an antibody specific for an immune checkpoint polypeptide). In some cases, a TMMP of the present disclosure is administered to an individual who is undergoing treatment with, or who has undergone treatment with, an immune checkpoint inhibitor (e.g., an antibody specific for an immune checkpoint polypeptide). The administration of the TMMP and the immune checkpoint inhibitor (e.g., an antibody specific for an immune checkpoint polypeptide) can occur at different times and/or at different frequencies.

[00522] Exemplary immune checkpoint inhibitors include inhibitors that target an immune checkpoint polypeptide such as CD27, CD28, CD40, CD122, CD96, CD73, CD47, OX40, GITR, CSF1R, JAK, PI3K delta, PI3K gamma, TAM, arginase, CD137 (also known as 4-1BB), ICOS, A2AR, B7-H3, B7-H4, BTLA, CTLA-4, LAG3, TIM3, VISTA, CD96, TIGIT, CD122, PD-1, PD-L1 and PD-L2. In some cases, the immune checkpoint polypeptide is a stimulatory checkpoint molecule selected from CD27, CD28, CD40, ICOS, OX40, GITR, CD122 and CD137. In some cases, the immune checkpoint polypeptide is an inhibitory checkpoint molecule selected from A2AR, B7-H3, B7-H4, BTLA, CTLA-4, IDO, KIR, LAG3, PD-1, TIM3, CD96, TIGIT and VISTA.

[00523] In some cases, the immune checkpoint inhibitor is an antibody specific for an immune checkpoint polypeptide. In some cases, the anti-immune checkpoint antibody is a monoclonal antibody. In some cases, the anti-immune checkpoint antibody is humanized, or de-immunized such that the antibody does not substantially elicit an immune response in a human. In some cases, the anti-immune checkpoint antibody is a humanized monoclonal antibody. In some cases, the anti-immune checkpoint antibody is a de-immunized monoclonal antibody. In some cases, the anti-immune checkpoint antibody is a fully human monoclonal antibody. In some cases, the anti-immune checkpoint antibody inhibits binding of the immune checkpoint polypeptide to a ligand for the immune checkpoint polypeptide. In some cases, the anti-immune checkpoint antibody inhibits binding of the immune checkpoint polypeptide to a receptor for the immune checkpoint polypeptide.

[00524] Suitable anti-immune checkpoint antibodies include, but are not limited to, nivolumab (Bristol-Myers Squibb), pembrolizumab (Merck), pidilizumab (Curetech), AMP-224

(GlaxoSmithKline/Amplimmune), MPDL3280A (Roche), MDX-1105 (Medarex, Inc./Bristol Myer Squibb), MEDI-4736 (Medimmune/AstraZeneca), arelumab (Merck Serono), ipilimumab (YERVOY, (Bristol-Myers Squibb), tremelimumab (Pfizer), pidilizumab (CureTech, Ltd.), IMP321 (Immutep S.A.), MGA271 (MacroGenics), BMS-986016 (Bristol-Meyers Squibb), lirilumab (Bristol-Myers Squibb), urelumab (Bristol-Meyers Squibb), PF-05082566 (Pfizer), IPH2101 (Innate Pharma/Bristol-Myers Squibb), MEDI-6469 (MedImmune/AZ), CP-870,893 (Genentech), Mogamulizumab (Kyowa Hakko Kirin), Varlilumab (Celldex Therapeutics), Avelumab (EMD Serono), Galiximab (Biogen Idec), AMP-514 (Amplimmune/AZ), AUNP 12 (Aurigene and Pierre Fabre), Indoximod (NewLink Genetics), NLG-919 (NewLink Genetics), INCB024360 (Incyte); KN035; and combinations thereof. For example, in some cases, the immune checkpoint inhibitor is an anti-PD-1 antibody. Suitable anti-PD-1 antibodies include, e.g., nivolumab, pembrolizumab (also known as MK-3475), pidilizumab, SHR-1210, PDR001, and AMP-224. In some cases, the anti-PD-1 monoclonal antibody is nivolumab, pembrolizumab or PDR001. Suitable anti-PD1 antibodies are described in U.S. Patent Publication No. 2017/0044259. For pidilizumab, see, e.g., Rosenblatt et al. (2011) *J. Immunother.* 34:409-18. In some cases, the immune checkpoint inhibitor is an anti-CTLA-4 antibody. In some cases, the anti-CTLA-4 antibody is ipilimumab or tremelimumab. For tremelimumab, see, e.g., Ribas et al. (2013) *J. Clin. Oncol.* 31:616-22. In some cases, the immune checkpoint inhibitor is an anti-PD-L1 antibody. In some cases, the anti-PD-L1 monoclonal antibody is BMS-935559, MEDI4736, MPDL3280A (also known as RG7446), KN035, or MSB0010718C. In some embodiments, the anti-PD-L1 monoclonal antibody is MPDL3280A (atezolizumab) or MEDI4736 (durvalumab). For durvalumab, see, e.g., WO 2011/066389. For atezolizumab, see, e.g., U.S. Patent No. 8,217,149.

Subjects suitable for treatment

[00525] Subjects suitable for treatment with a method of the present disclosure include individuals who have cancer, including individuals who have been diagnosed as having cancer, individuals who have been treated for cancer but who failed to respond to the treatment, and individuals who have been treated for cancer and who initially responded but subsequently became refractory to the treatment. Subjects suitable for treatment with a method of the present disclosure include individuals who have an infection (e.g., an infection with a pathogen such as a bacterium, a virus, a protozoan, etc.), including individuals who have been diagnosed as having an infection, and individuals who have been treated for an infection but who failed to respond to the treatment. Subjects suitable for treatment with a method of the present disclosure include individuals who have bacterial infection, including individuals who have been diagnosed as having a bacterial infection, and individuals who have been treated for a bacterial infection but who failed to respond to the treatment. Subjects suitable for treatment with a method of the present disclosure include individuals who have a viral infection, including individuals who have been diagnosed as having a viral infection, and individuals who have been treated for a viral infection but who failed to

respond to the treatment. Subjects suitable for treatment with a method of the present disclosure include individuals who have an autoimmune disease, including individuals who have been diagnosed as having an autoimmune disease, and individuals who have been treated for an autoimmune disease but who failed to respond to the treatment.

Examples of Non-Limiting Aspects of the Disclosure

ASPECTS SET A

[00526] Aspects, including embodiments, of the present subject matter described above may be beneficial alone or in combination, with one or more other aspects or embodiments. Without limiting the foregoing description, certain non-limiting aspects of the disclosure numbered 1-95 are provided below. As will be apparent to those of skill in the art upon reading this disclosure, each of the individually numbered aspects may be used or combined with any of the preceding or following individually numbered aspects. This is intended to provide support for all such combinations of aspects and is not limited to combinations of aspects explicitly provided below:

[00527] Aspect 1. T-cell modulatory multimeric polypeptide comprising: at least one heterodimer comprising: a) a first polypeptide comprising: i) a Wilms tumor-1 (WT-1) peptide epitope; and ii) first major histocompatibility complex (MHC) polypeptide; b) a second polypeptide comprising a second MHC polypeptide, and c) at least one immunomodulatory polypeptide, wherein the first and/or the second polypeptide comprises the immunomodulatory polypeptide.

[00528] Aspect 2. A T-cell modulatory multimeric polypeptide of aspect 1, wherein at least one of the one or more immunomodulatory domains is a variant immunomodulatory polypeptide that exhibits reduced affinity to a cognate co-immunomodulatory polypeptide compared to the affinity of a corresponding wild-type immunomodulatory polypeptide for the cognate co-immunomodulatory polypeptide, and wherein the epitope binds to a T-cell receptor (TCR) on a T cell with an affinity of at least 10^{-7} M, such that: i) the T-cell modulatory multimeric polypeptide binds to a first T cell with an affinity that is at least 25% higher than the affinity with which the T-cell modulatory multimeric polypeptide binds a second T cell, wherein the first T cell expresses on its surface the cognate co-immunomodulatory polypeptide and a TCR that binds the epitope with an affinity of at least 10^{-7} M, and wherein the second T cell expresses on its surface the cognate co-immunomodulatory polypeptide but does not express on its surface a TCR that binds the epitope with an affinity of at least 10^{-7} M; and/or ii) the ratio of the binding affinity of a control T-cell modulatory multimeric polypeptide, wherein the control comprises a wild-type immunomodulatory polypeptide, to a cognate co-immunomodulatory polypeptide to the binding affinity of the T-cell modulatory multimeric polypeptide comprising a variant of the wild-type immunomodulatory polypeptide to the cognate co-immunomodulatory polypeptide, when measured by bio-layer interferometry, is in a range of from 1.5:1 to 10^6 :1.

[00529] Aspect 3. A T-cell modulatory multimeric polypeptide of aspect 2, wherein: a) the T-cell modulatory multimeric polypeptide binds to the first T cell with an affinity that is at least 50%, at least 2-fold, at least 5-fold, or at least 10-fold higher than the affinity with which it binds the second T cell; and/or b) the variant immunomodulatory polypeptide binds the co-immunomodulatory polypeptide with an affinity of from about 10^{-4} M to about 10^{-7} M, from about 10^{-4} M to about 10^{-6} M, from about 10^{-4} M to about 10^{-5} M; and/or c) wherein the ratio of the binding affinity of a control T-cell modulatory multimeric polypeptide, wherein the control comprises a wild-type immunomodulatory polypeptide, to a cognate co-immunomodulatory polypeptide to the binding affinity of the T-cell modulatory multimeric polypeptide comprising a variant of the wild-type immunomodulatory polypeptide to the cognate co-immunomodulatory polypeptide, when measured by bio-layer interferometry, is at least 10:1, at least 50:1, at least 10^2 :1, or at least 10^3 :1.

[00530] Aspect 4. A T-cell modulatory multimeric polypeptide of any one of aspects 1-3, wherein the first or the second polypeptide comprises an immunoglobulin (Ig) Fc polypeptide.

[00531] Aspect 5. A T-cell modulatory multimeric polypeptide of aspect 4, wherein the Ig Fc polypeptide is an IgG1 Fc polypeptide.

[00532] Aspect 6. A T-cell modulatory multimeric polypeptide of aspect 5, wherein IgG1 Fc polypeptide comprises one or more amino acid substitutions selected from N297A, L234A, L235A, L234F, L235E, and P331S.

[00533] Aspect 7. A T-cell modulatory multimeric polypeptide of any one of aspects 1-6, wherein: a1) the first polypeptide comprises, in order from N-terminus to C-terminus: i) the WT-1 peptide epitope; ii) the first MHC polypeptide; and iii) at least one immunomodulatory polypeptide; and b2) the second polypeptide comprises, in order from N-terminus to C-terminus: i) the second MHC polypeptide; and ii) an immunoglobulin (Ig) Fc polypeptide; or a2) the first polypeptide comprises, in order from N-terminus to C-terminus: i) the WT-1 peptide epitope; and ii) the first MHC polypeptide; and b2) the second polypeptide comprises, in order from N-terminus to C-terminus: i) at least one immunomodulatory polypeptide; ii) the second MHC polypeptide; and iii) an Ig Fc polypeptide; or a3) the first polypeptide comprises, in order from N-terminus to C-terminus: i) the WT-1 peptide epitope; and ii) the first MHC polypeptide; and b3) the second polypeptide comprises, in order from N-terminus to C-terminus: i) the second MHC polypeptide; and ii) an Ig Fc polypeptide; and iii) at least one immunomodulatory polypeptide; or a4) the first polypeptide comprises, in order from N-terminus to C-terminus: i) the WT-1 peptide epitope; and ii) the first MHC polypeptide; and b4) the second polypeptide comprises, in order from N-terminus to C-terminus: i) the second MHC polypeptide; and ii) at least one immunomodulatory polypeptide; or a5) the first polypeptide comprises, in order from N-terminus to C-terminus: i) the WT-1 peptide epitope; and ii) the first MHC polypeptide; and b5) a second polypeptide

comprises, in order from N-terminus to C-terminus: i) at least one immunomodulatory polypeptide; and ii) the second MHC polypeptide; or a6) the first polypeptide comprises, in order from N-terminus to C-terminus: i) the WT-1 peptide epitope; ii) the first MHC polypeptide; and iii) at least one immunomodulatory polypeptide; and b6) the second polypeptide comprises: i) the second MHC polypeptide.

[00534] Aspect 8. A T-cell modulatory multimeric polypeptide of any one of aspects 1-7, wherein the first polypeptide comprises a peptide linker between the WT-1 epitope and the first MHC polypeptide and/or wherein the second polypeptide comprises a peptide linker between the immunomodulatory polypeptide and the second MHC polypeptide.

[00535] Aspect 9. A T-cell modulatory multimeric polypeptide of aspect 8, wherein the peptide linker comprises the amino acid sequence (GGGGS)_n (SEQ ID NO:284), where n is an integer from 1 to 10.

[00536] Aspect 10. A T-cell modulatory multimeric polypeptide of any one of aspects 1-9, wherein the first MHC polypeptide is a β 2-microglobulin polypeptide; and wherein the second MHC polypeptide is an MHC class I heavy chain polypeptide.

[00537] Aspect 11. A T-cell modulatory multimeric polypeptide of any one of aspects 1-10, wherein the at least one immunomodulatory polypeptide is selected from the group consisting of a cytokine (e.g., an IL2 polypeptide, an IL7 polypeptide, an IL12 polypeptide, an IL15 polypeptide, an IL17 polypeptide, an IL21 polypeptide, an IL27 polypeptide, an IL-23 polypeptide, a TGF β polypeptide, and the like; and including all family members, e.g., IL17A, IL-17B, IL-17C, IL-17D, IL-17E, IL-17F, IL-17E), a 4-1BBL polypeptide, an ICOS-L polypeptide, an OX-40L polypeptide, a CD80 polypeptide, a CD86 polypeptide, (CD80 and CD86 are also known as B7-1 and B7-2, respectively), a CD40 polypeptide, a CD70 polypeptide, a JAG1 (CD339) polypeptide, an ICAM (CD540 polypeptide, a PD-L1 polypeptide, a FasL polypeptide, a PD-L2 polypeptide, a PD-1H (VISTA) polypeptide, an ICOS-L (CD275) polypeptide, a GITRL polypeptide, an HVEM polypeptide, a CXCL10 polypeptide, a CXCL9 polypeptide, a CXCL11 polypeptide, a CXCL13 polypeptide, and a CX3CL1 polypeptide, a Galectin-9 polypeptide, a CD83 polypeptide, a CD30L polypeptide, a HLA-G polypeptide, a MICA polypeptide, a MICB polypeptide, a HVEM (CD270) polypeptide, a lymphotoxin beta receptor polypeptide, a 3/TR6 polypeptide, an ILT3 polypeptide, an ILT4 polypeptide, a CXCL10 polypeptide, a CXCL9 polypeptide, a CXCL11 polypeptide, a CXCL13 polypeptide, and a CX3CL1 polypeptide, and combinations thereof.

[00538] Aspect 12. A T-cell modulatory multimeric polypeptide of any one of aspects 1-11, wherein the at least one immunomodulatory polypeptide is an IL-2 polypeptide.

[00539] Aspect 13. A T-cell modulatory multimeric polypeptide of any one of aspects 1-12, wherein the multimeric polypeptide comprises at least two immunomodulatory polypeptides, and wherein at least two of the immunomodulatory polypeptides are the same.

[00540] Aspect 14. A T-cell modulatory multimeric polypeptide of aspect 13, wherein the 2 or more immunomodulatory polypeptides are in tandem.

[00541] Aspect 15. A T-cell modulatory multimeric polypeptide of any one of aspects 1-14, wherein the first polypeptide and the second polypeptide are covalently linked to one another.

[00542] Aspect 16. A T-cell modulatory multimeric polypeptide of aspect 15, wherein the covalent linkage is via a disulfide bond.

[00543] Aspect 17. A T-cell modulatory multimeric polypeptide of any one of aspects 1-16, wherein the first MHC polypeptide or a linker between the epitope and the first MHC polypeptide comprises an amino acid substitution to provide a first Cys residue, wherein the second MHC polypeptide comprises an amino acid substitution to provide a second Cys residue, and wherein the disulfide linkage is between the first and the second Cys residues.

[00544] Aspect 18. A T-cell modulatory multimeric polypeptide of any one of aspects 1-17, wherein the WT-1 peptide epitope has a length of from about 4 amino acids to about 25 amino acids.

[00545] Aspect 19. A T-cell modulatory multimeric polypeptide of any one of aspects 1-18, wherein the WT-1 peptide epitope comprises an amino acid sequence selected from the group consisting of: NLMNLGATL (SEQ ID NO:258), NYMNLGATL (SEQ ID NO:263), CMTWNQMNLGATLKG (SEQ ID NO:223), WNQMNLGATLKGVA (SEQ ID NO:224), CMTWNYMNLGATLKG (SEQ ID NO:225), WNYMNLGATLKGVA (SEQ ID NO:226), MTWNQMNLGATLKG (SEQ ID NO:227), TWNMNLGATLKGVA (SEQ ID NO:228), CMTWNLMNLGATLKG (SEQ ID NO:229), MTWNLMNLGATLKG (SEQ ID NO:230), TWNLMNLGATLKGVA (SEQ ID NO:231), WNLNMNLGATLKGVA (SEQ ID NO:232), MNLGATLKG (SEQ ID NO:233), MTWNYMNLGATLKG (SEQ ID NO:234), TWNYMNLGATLKGVA (SEQ ID NO:235), CMTWNQMNLGATLKGVA (SEQ ID NO:236), CMTWNLMNLGATLKGVA (SEQ ID NO:237), CMTWNYMNLGATLKGVA (SEQ ID NO:238), GYLRNPTAC (SEQ ID NO:239), GALRNPTAL (SEQ ID NO:240), YALRNPTAC (SEQ ID NO:241), GLLRNPTAC (SEQ ID NO:242), RYRPHPGAL (SEQ ID NO:243), YQRPHPGAL (SEQ ID NO:244), RLRPHPGAL (SEQ ID NO:245), RIRPHPGAL (SEQ ID NO:246), QFPNHSFKHEDPMGQ (SEQ ID NO:247), HSFKHEDPY (SEQ ID NO:248), QFPNHSFKHEDPM (SEQ ID NO:249), QFPNHSFKHEDPY (SEQ ID NO:250), KRPFMCAYPGCNK (SEQ ID NO:251), KRPFMCAYPGCYK (SEQ ID NO:252), FMCAYPGCY (SEQ ID NO:253), FMCAYPGCK (SEQ ID NO:254), KRPFMCAYPGCNKRY (SEQ ID NO:255), SEKRPFMCAYPGCNK (SEQ ID NO:256), KRPFMCAYPGCYKRY (SEQ ID NO:257),

NLMNLGATL (SEQ ID NO:258), VLDFAPPGA (SEQ ID NO:259), RMFPNAPYL (SEQ ID NO:260), CMTWNQMN (SEQ ID NO:261), CYTWNQMNL (SEQ ID NO:262), NYMNLGATL (SEQ ID NO:263), YMFPNAPYL (SEQ ID NO:264), SLGEQQYSV (SEQ ID NO:265), CMTWNQMNL (SEQ ID NO:266), and NQMNLGATL (SEQ ID NO:267).

[00546] Aspect 20. A T-cell modulatory multimeric polypeptide of any one of aspects 1-18, wherein the WT-1 peptide comprises the amino acid sequence CMTWNQMNL (SEQ ID NO:266) or CYTWNQMNL (SEQ ID NO:262).

[00547] Aspect 21. A T-cell modulatory multimeric polypeptide of any one of aspects 1-20, wherein the first or the second MHC polypeptide comprises: a) an amino acid sequence having at least 95% amino acid sequence identity to the HLA-A*0101, HLA-A*0201, HLA-A*0201, HLA-A*1101, HLA-A*2301, HLA-A*2402, HLA-A*2407, HLA-A*3303, or HLA-A*3401 amino acid sequence depicted in FIG. 9A; or b) an amino acid sequence having at least 95% amino acid sequence identity to the HLA-B*0702, HLA-B*0801, HLA-B*1502, HLA-B*3802, HLA-B*4001, HLA-B*4601, or HLA-B*5301 amino acid sequence depicted in FIG. 10A; or c) an amino acid sequence having at least 95% amino acid sequence identity to the HLA-C*0102, HLA-C*0303, HLA-C*0304, HLA-C*0401, HLA-C*0602, HLA-C*0701, HLA-C*0702, HLA-C*0801, or HLA-C*1502 depicted in FIG. 11A.

[00548] Aspect 22. A T-cell modulatory multimeric polypeptide of any one of aspects 1-20, wherein the first MHC polypeptide is a β 2M polypeptide, and wherein the second MHC polypeptide comprises an amino acid sequence having at least 95% amino acid sequence identity to an HLA-A*2402 polypeptide.

[00549] Aspect 23. A T-cell modulatory multimeric polypeptide of any one of aspects 1-20, wherein the first MHC polypeptide is a β 2M polypeptide, and wherein the second MHC polypeptide is an HLA-A*1101 polypeptide.

[00550] Aspect 24. A T-cell modulatory multimeric polypeptide of any one of aspects 1-20, wherein the first MHC polypeptide is a β 2M polypeptide, and wherein the second MHC polypeptide comprises an amino acid sequence having at least 95% amino acid sequence identity to an HLA-A*3303 polypeptide.

[00551] Aspect 25. A T-cell modulatory multimeric polypeptide of any one of aspects 1-20, wherein the first MHC polypeptide is a β 2M polypeptide, and wherein the second MHC polypeptide comprises an amino acid sequence having at least 95% amino acid sequence identity to an HLA-A*0201 polypeptide.

[00552] Aspect 26. A T-cell modulatory multimeric polypeptide of any one of aspects 21-25, wherein the MHC heavy chain polypeptide comprises a Cys at position 236.

[00553] Aspect 27. A T-cell modulatory multimeric polypeptide of any one of aspects 21-26, wherein the β 2M polypeptide comprises a Cys at position 12.

[00554] Aspect 28. A T-cell modulatory multimeric polypeptide of any one of aspects 1-27, wherein the immunomodulatory polypeptide is a variant IL-2 polypeptide comprising: i) an H16A substitution and an F42A substitution; or ii) an H16T substitution and an F42A substitution.

[00555] Aspect 29. A T-cell modulatory multimeric polypeptide of any one of aspects 4-28, wherein the multimeric polypeptide comprises a first and a second heterodimer, and wherein the first and second heterodimers are covalently bound by one or more disulfide bonds between the Ig Fc polypeptides of the first and second heterodimers.

[00556] Aspect 30. A nucleic acid comprising a nucleotide sequence encoding a first or second polypeptide according to any one of aspects 1-28, wherein the first or second polypeptide comprises at least one immunomodulatory domain.

[00557] Aspect 31. An expression vector comprising the nucleic acid of aspect 30.

[00558] Aspect 32. A method of selectively modulating the activity of T cell specific for a Wilms tumor-1 (WT-1) epitope, the method comprising contacting the T cell with a T-cell modulatory multimeric polypeptide according to any one of aspects 1-29, wherein said contacting selectively modulates the activity of the WT-1 epitope-specific T cell.

[00559] Aspect 33. A method of treating a patient having a cancer, the method comprising administering to the patient an effective amount of a pharmaceutical composition comprising T-cell modulatory multimeric polypeptide according to any one of aspects 1-29.

[00560] Aspect 34. The method of aspect 33, wherein the cancer is hepatocellular carcinoma, pancreatic cancer, stomach cancer, colorectal cancer, hepatoblastoma, or an ovarian yolk sac tumor.

[00561] Aspect 35. The method of aspect 33 or aspect 34, wherein said administering is intramuscular.

[00562] Aspect 36. The method of aspect 33 or aspect 34, wherein said administering is intravenous.

[00563] Aspect 37. A method of modulating an immune response in an individual, the method comprising administering to the individual an effective amount of the T-cell modulatory multimeric polypeptide (TMMP) of any one of aspects 1-29, wherein said administering induces an epitope-specific T cell response (e.g., a T cell response specific for the WT-1 epitope present in the TMMP) and an epitope-non-specific T cell response, wherein the ratio of the epitope-specific T cell response to the epitope-non-specific T cell response is at least 2:1.

[00564] Aspect 38 The method of aspect 37, wherein the ratio of the epitope-specific T cell response to the epitope-non-specific T cell response is at least 5:1.

- [00565] Aspect 39. The method of aspect 37, wherein the ratio of the epitope-specific T cell response to the epitope-non-specific T cell response is at least 10:1.
- [00566] Aspect 40. The method of aspect 37, wherein the ratio of the epitope-specific T cell response to the epitope-non-specific T cell response is at least 25:1.
- [00567] Aspect 41. The method of aspect 37, wherein the ratio of the epitope-specific T cell response to the epitope-non-specific T cell response is at least 50:1.
- [00568] Aspect 42. The method of aspect 37, wherein the ratio of the epitope-specific T cell response to the epitope-non-specific T cell response is at least 100:1.
- [00569] Aspect 43. The method of any one of aspects 37-42, wherein the individual is a human.
- [00570] Aspect 44. The method of any one of aspects 37-43, wherein said modulating comprises increasing a cytotoxic T-cell response to a cancer cell (e.g., a WT-1-expressing cancer cell).
- [00571] Aspect 45. The method of any one of aspects 37-44, wherein said administering is intravenous, subcutaneous, intramuscular, systemic, intralymphatic, distal to a treatment site, local, or at or near a treatment site.
- [00572] Aspect 46. The method of any one of aspects 37-45, wherein the epitope non-specific T-cell response is less than the epitope non-specific T-cell response that would be induced by a control T-cell modulatory multimeric polypeptide comprising a corresponding wild-type immunomodulatory polypeptide.
- [00573] Aspect 47. A method of delivering a costimulatory (i.e., immunomodulatory) polypeptide selectively to target T cell, the method comprising contacting a mixed population of T cells with a T-cell modulatory multimeric polypeptide (TMMP) of any one of aspects 1-29, wherein the mixed population of T cells comprises the target T cell and non-target T cells, wherein the target T cell is specific for the epitope present within the TMMP (e.g., wherein the target T cell is specific for the WT-1 epitope present within the TMMP), and wherein said contacting delivers the one or more costimulatory polypeptides present within the TMMP to the target T cell.
- [00574] Aspect 48. The method of aspect 47, wherein the population of T cells is *in vitro*.
- [00575] Aspect 49. The method of aspect 47, wherein the population of T cells is *in vivo* in an individual.
- [00576] Aspect 50. The method of aspect 49, comprising administering the multimeric polypeptide to the individual.
- [00577] Aspect 51. The method of any one of aspects 47-50, wherein the target T cell is a cytotoxic T cell.
- [00578] Aspect 52. The method of aspect 47, wherein the mixed population of T cells is an *in vitro* population of mixed T cells obtained from an individual, and wherein said contacting results in

activation and/or proliferation of the target T cell, generating a population of activated and/or proliferated target T cells.

[00579] Aspect 53. The method of aspect 52, further comprising administering the population of activated and/or proliferated target T cells to the individual.

[00580] Aspect 54. A method of detecting, in a mixed population of T cells obtained from an individual, the presence of a target T cell that binds a WT-1 epitope of interest, the method comprising: a) contacting *in vitro* the mixed population of T cells with T-cell modulatory multimeric polypeptide (TMMP) of any one of aspects 1-29, wherein the TMMP comprises the WT-1 epitope of interest; and b) detecting activation and/or proliferation of T cells in response to said contacting, wherein activated and/or proliferated T cells indicates the presence of the target T cell.

[00581] Aspect 55. A T-cell modulatory multimeric polypeptide comprising: at least one heterodimer comprising: a) a first polypeptide comprising: i) a Wilms tumor-1 (WT-1) peptide epitope, wherein the WT-1 peptide has a length of from about 4 amino acids to about 25 amino acids; and ii) first major histocompatibility complex (MHC) class I polypeptide; b) a second polypeptide comprising a second MHC class I polypeptide, and c) at least one immunomodulatory polypeptide, wherein the first and/or the second polypeptide comprises the immunomodulatory polypeptide, and wherein the first and the second polypeptides are covalently linked to one another via at least 2 disulfide bonds.

[00582] Aspect 56. A T-cell modulatory multimeric polypeptide of aspect 55, wherein at least one of the at least one immunomodulatory polypeptides is a variant immunomodulatory polypeptide that exhibits reduced affinity to a cognate co-immunomodulatory polypeptide compared to the affinity of a corresponding wild-type immunomodulatory polypeptide for the cognate co-immunomodulatory polypeptide, and wherein the epitope binds to a T-cell receptor (TCR) on a T cell with an affinity of at least 10^{-7} M, such that: i) the T-cell modulatory multimeric polypeptide binds to a first T cell with an affinity that is at least 25% higher than the affinity with which the T-cell modulatory multimeric polypeptide binds a second T cell, wherein the first T cell expresses on its surface the cognate co-immunomodulatory polypeptide and a TCR that binds the epitope with an affinity of at least 10^{-7} M, and wherein the second T cell expresses on its surface the cognate co-immunomodulatory polypeptide but does not express on its surface a TCR that binds the epitope with an affinity of at least 10^{-7} M; and/or ii) the ratio of the binding affinity of a control T-cell modulatory multimeric polypeptide, wherein the control comprises a wild-type immunomodulatory polypeptide, to a cognate co-immunomodulatory polypeptide to the binding affinity of the T-cell modulatory multimeric polypeptide comprising a variant of the wild-type immunomodulatory polypeptide to the cognate co-immunomodulatory polypeptide, when measured by bio-layer interferometry, is in a range of from 1.5:1 to 10^6 :1.

[00583] Aspect 57. A T-cell modulatory multimeric polypeptide of aspect 56, wherein: a) the T-cell modulatory multimeric polypeptide binds to the first T cell with an affinity that is at least 50%, at least 2-fold, at least 5-fold, or at least 10-fold higher than the affinity with which it binds the second T cell; and/or b) the variant immunomodulatory polypeptide binds the co-immunomodulatory polypeptide with an affinity of from about 10^{-4} M to about 10^{-7} M, from about 10^{-4} M to about 10^{-6} M, from about 10^{-4} M to about 10^{-5} M; and/or c) wherein the ratio of the binding affinity of a control T-cell modulatory multimeric polypeptide, wherein the control comprises a wild-type immunomodulatory polypeptide, to a cognate co-immunomodulatory polypeptide to the binding affinity of the T-cell modulatory multimeric polypeptide comprising a variant of the wild-type immunomodulatory polypeptide to the cognate co-immunomodulatory polypeptide, when measured by bio-layer interferometry, is at least 10:1, at least 50:1, at least 10^2 :1, or at least 10^3 :1.

[00584] Aspect 58. A T-cell modulatory multimeric polypeptide of any one of aspects 55-57, wherein the first or the second polypeptide comprises an immunoglobulin (Ig) Fc polypeptide.

[00585] Aspect 59. A T-cell modulatory multimeric polypeptide of aspect 58, wherein the Ig Fc polypeptide is an IgG1 Fc polypeptide.

[00586] Aspect 60. A T-cell modulatory multimeric polypeptide of aspect 58, wherein the Ig Fc polypeptide is an IgG4 Fc polypeptide.

[00587] Aspect 61. T-cell modulatory multimeric polypeptide of aspect 59, wherein IgG1 Fc polypeptide comprises one or more amino acid substitutions selected from N297A, L234A, L235A, L234F, L235E, and P331S.

[00588] Aspect 62. A T-cell modulatory multimeric polypeptide of any one of aspects 55-61, wherein

a1) the first polypeptide comprises, in order from N-terminus to C-terminus:

- i) the WT-1 peptide epitope;
- ii) the first class I MHC polypeptide; and
- iii) at least one immunomodulatory polypeptide; and

b1) the second polypeptide comprises, in order from N-terminus to C-terminus:

- i) the second class I MHC polypeptide; and
- ii) an immunoglobulin (Ig) Fc polypeptide; or

a2) the first polypeptide comprises, in order from N-terminus to C-terminus:

- i) the WT-1 peptide epitope; and
- ii) the first class I MHC polypeptide; and

b2) the second polypeptide comprises, in order from N-terminus to C-terminus:

- i) at least one immunomodulatory polypeptide;

- ii) the second class I MHC polypeptide; and
 - iii) an Ig Fc polypeptide; or
- a3) the first polypeptide comprises, in order from N-terminus to C-terminus:
 - i) the WT-1 peptide epitope; and
 - ii) the first class I MHC polypeptide; and
- b3) the second polypeptide comprises, in order from N-terminus to C-terminus:
 - i) the second class I MHC polypeptide; and
 - ii) an Ig Fc polypeptide; and
 - iii) at least one immunomodulatory polypeptide; or
- a4) the first polypeptide comprises, in order from N-terminus to C-terminus:
 - i) the WT-1 peptide epitope; and
 - ii) the first class I MHC polypeptide; and
- b4) the second polypeptide comprises, in order from N-terminus to C-terminus:
 - i) the second class I MHC polypeptide; and
 - ii) at least one immunomodulatory polypeptide; or
- a5) the first polypeptide comprises, in order from N-terminus to C-terminus:
 - i) the WT-1 peptide epitope; and
 - ii) the first class I MHC polypeptide; and
- b5) a second polypeptide comprises, in order from N-terminus to C-terminus:
 - i) at least one immunomodulatory polypeptide; and
 - ii) the second class I MHC polypeptide; or
- a6) the first polypeptide comprises, in order from N-terminus to C-terminus:
 - i) the WT-1 peptide epitope;
 - ii) the first class I MHC polypeptide; and
 - iii) at least one immunomodulatory polypeptide; and
- b6) the second polypeptide comprises:
 - i) the second class I MHC polypeptide.

[00589] Aspect 63. A T-cell modulatory multimeric polypeptide of any one of aspects 55-62, wherein the first MHC polypeptide is a β 2-microglobulin polypeptide; and wherein the second MHC polypeptide is an MHC class I heavy chain polypeptide.

[00590] Aspect 64. A T-cell modulatory multimeric polypeptide of any one of aspects 55-63, wherein the at least one immunomodulatory polypeptide is selected from the group consisting of a cytokine (e.g., an IL2 polypeptide, an IL7 polypeptide, an IL12 polypeptide, an IL15 polypeptide, an IL17 polypeptide, an IL21 polypeptide, an IL27 polypeptide, an IL-23 polypeptide, a TGF β polypeptide, and the like; and including all family members, e.g., IL17A, IL-17B, IL-17C, IL-17D, IL-17E, IL-17F,

IL-17E), a 4-1BBL polypeptide, an ICOS-L polypeptide, an OX-40L polypeptide, a CD80 polypeptide, a CD86 polypeptide, (CD80 and CD86 are also known as B7-1 and B7-2, respectively), a CD40 polypeptide, a CD70 polypeptide, a JAG1 (CD339) polypeptide, an ICAM (CD540 polypeptide, a PD-L1 polypeptide, a FasL polypeptide, a PD-L2 polypeptide, a PD-1H (VISTA) polypeptide, an ICOS-L (CD275) polypeptide, a GITRL polypeptide, an HVEM polypeptide, a CXCL10 polypeptide, a CXCL9 polypeptide, a CXCL11 polypeptide, a CXCL13 polypeptide, and a CX3CL1 polypeptide, a Galectin-9 polypeptide, a CD83 polypeptide, a CD30L polypeptide, a HLA-G polypeptide, a MICA polypeptide, a MICB polypeptide, a HVEM (CD270) polypeptide, a lymphotoxin beta receptor polypeptide, a 3/TR6 polypeptide, an ILT3 polypeptide, an ILT4 polypeptide, a CXCL10 polypeptide, a CXCL9 polypeptide, a CXCL11 polypeptide, a CXCL13 polypeptide, and a CX3CL1 polypeptide, and combinations thereof.

[00591] Aspect 65. A T-cell modulatory multimeric polypeptide of any one of aspects 55-63, wherein the at least one immunomodulatory polypeptide is an IL-2 polypeptide.

[00592] Aspect 66. A T-cell modulatory multimeric polypeptide of any one of aspects 55-65, wherein the multimeric polypeptide comprises at least two immunomodulatory polypeptides, and wherein at least two of the immunomodulatory polypeptides are the same.

[00593] Aspect 67. A T-cell modulatory multimeric polypeptide of aspect 66, wherein the 2 or more immunomodulatory polypeptides are in tandem.

[00594] Aspect 68. The T-cell modulatory multimeric polypeptide of any one of aspects 55-67, wherein: a) a first disulfide bond is between: i) a Cys present in a linker between the WT-1 peptide epitope and the first MHC class I polypeptide, wherein the first MHC class I polypeptide is a β 2M polypeptide; and ii) a Cys residue introduced via a Y84C substitution in the second MHC class I polypeptide, wherein the second MHC class I polypeptide is a MHC Class I heavy chain polypeptide; and b) a second disulfide bond is between: i) a Cys residue introduced into the β 2M polypeptide via an R12C substitution; and ii) a Cys residue introduced into the MHC Class I heavy chain polypeptide via an A236C substitution.

[00595] Aspect 69. A T-cell modulatory multimeric polypeptide of aspect 68, wherein the linker comprises the amino acid sequence GCGGS (SEQ ID NO:318).

[00596] Aspect 70. A T-cell modulatory multimeric polypeptide of aspect 69, wherein the linker comprises the amino acid sequence GCGGS(GGGGS)_n (SEQ ID NO:319), where n is an integer from 1 to 10.

[00597] Aspect 71. A T-cell modulatory multimeric polypeptide of any one of aspects 55-70, wherein the WT-1 peptide epitope has a length of from about 4 amino acids to about 15 amino acids.

[00598] Aspect 72. A T-cell modulatory multimeric polypeptide of any one of aspects 55-71, wherein the WT-1 peptide epitope comprises an amino acid sequence selected from the group consisting

of: NLMNLGATL (SEQ ID NO:258), NYMNLGATL (SEQ ID NO:263), CMTWNQMNLGATLKG (SEQ ID NO:223), WNQMNLGATLKGVAA (SEQ ID NO:224), CMTWNYMNLGATLKG (SEQ ID NO:225), WNYMNLGATLKGVAA (SEQ ID NO:226), MTWNQMNLGATLKG (SEQ ID NO:227), TWNQMNLGATLKGVA (SEQ ID NO:228), CMTWNLMNLGATLKG (SEQ ID NO:229), MTWNLMNLGATLKG (SEQ ID NO:230), TWNLMNLGATLKGVA (SEQ ID NO:231), WNLMLNLGATLKG (SEQ ID NO:232), MNLGATLKG (SEQ ID NO:233), MTWNYMNLGATLKG (SEQ ID NO:234), TWNYMNLGATLKGVA (SEQ ID NO:235), CMTWNQMNLGATLKGVA (SEQ ID NO:236), CMTWNLMNLGATLKGVA (SEQ ID NO:237), CMTWNYMNLGATLKGVA (SEQ ID NO:238), GYLRNPTAC (SEQ ID NO:239), GALRNPTAL (SEQ ID NO:240), YALRNPTAC (SEQ ID NO:241), GLLRNPTAC (SEQ ID NO:242), RYRPHPGAL (SEQ ID NO:243), YQRPHPGAL (SEQ ID NO:244), RLRPHPGAL (SEQ ID NO:245), RIRPHPGAL (SEQ ID NO:246), QFPNHSFKHEDPMGQ (SEQ ID NO:247), HSFKHEDPY (SEQ ID NO:248), QFPNHSFKHEDPM (SEQ ID NO:249), QFPNHSFKHEDPY (SEQ ID NO:250), KRPFMCAYPGCNK (SEQ ID NO:251), KRPFMCAYPGCYK (SEQ ID NO:252), FMCAYPGCY (SEQ ID NO:253), FMCAYPGCK (SEQ ID NO:254), KRPFMCAYPGCNKRY (SEQ ID NO:255), SEKRPFMCAYPGCNK (SEQ ID NO:256), KRPFMCAYPGCYKRY (SEQ ID NO:257), NLMNLGATL (SEQ ID NO:258), VLDFAPPGA (SEQ ID NO:259), RMFPNAPYL (SEQ ID NO:260), CMTWNQMN (SEQ ID NO:261), CYTWNQMN (SEQ ID NO:262), NYMNLGATL (SEQ ID NO:263), YMFPNAPYL (SEQ ID NO:264), SLGEQQYSV (SEQ ID NO:265), CMTWNQMN (SEQ ID NO:266), and NQMNLGATL (SEQ ID NO:267).

[00599] Aspect 73. A T-cell modulatory multimeric polypeptide of any one of aspects 55-71, wherein the WT-1 peptide comprises the amino acid sequence VLDFAPPGA (SEQ ID NO:259) or RMFPNAPYL (SEQ ID NO:260).

[00600] Aspect 74. A T-cell modulatory multimeric polypeptide of any one of aspects 55-73, wherein the first or the second MHC class I polypeptide comprises: a) an amino acid sequence having at least 95% amino acid sequence identity to the HLA-A*0101, HLA-A*0201, HLA-A*0201, HLA-A*1101, HLA-A*2301, HLA-A*2402, HLA-A*2407, HLA-A*3303, or HLA-A*3401 amino acid sequence depicted in FIG. 9A; or b) an amino acid sequence having at least 95% amino acid sequence identity to the HLA-B*0702, HLA-B*0801, HLA-B*1502, HLA-B*3802, HLA-B*4001, HLA-B*4601, or HLA-B*5301 amino acid sequence depicted in FIG. 10A; or c) an amino acid sequence having at least 95% amino acid sequence identity to the HLA-C*0102, HLA-C*0303, HLA-C*0304, HLA-C*0401, HLA-C*0602, HLA-C*0701, HLA-C*0702, HLA-C*0801, or HLA-C*1502 depicted in FIG. 11A.

[00601] Aspect 75. A T-cell modulatory multimeric polypeptide of any one of aspects 55-74, wherein the first MHC polypeptide is a β 2M polypeptide, and wherein the second MHC polypeptide

comprises an amino acid sequence having at least 95% amino acid sequence identity to an HLA-A*2402 polypeptide.

[00602] Aspect 76. A T-cell modulatory multimeric polypeptide of any one of aspects 55-74, wherein the first MHC polypeptide is a β 2M polypeptide, and wherein the second MHC polypeptide is an HLA-A*1101 polypeptide.

[00603] Aspect 77. A T-cell modulatory multimeric polypeptide of any one of aspects 55-74, wherein the first MHC polypeptide is a β 2M polypeptide, and wherein the second MHC polypeptide comprises an amino acid sequence having at least 95% amino acid sequence identity to an HLA-A*3303 polypeptide.

[00604] Aspect 78. A T-cell modulatory multimeric polypeptide of any one of aspects 55-74, wherein the first MHC polypeptide is a β 2M polypeptide, and wherein the second MHC polypeptide comprises an amino acid sequence having at least 95% amino acid sequence identity to an HLA-A*0201 polypeptide.

[00605] Aspect 79. A T-cell modulatory multimeric polypeptide of any one of aspects 55-78, wherein the at least one immunomodulatory polypeptide is a variant IL-2 polypeptide comprising: i) an H16A substitution and an F42A substitution; or ii) an H16T substitution and an F42A substitution.

[00606] Aspect 80. A T-cell modulatory multimeric polypeptide of any one of aspects 55-79, wherein the multimeric polypeptide comprises a first and a second heterodimer.

[00607] Aspect 81. A nucleic acid comprising a nucleotide sequence encoding a first or second polypeptide according to any one of aspects 55-80, wherein the first or second polypeptide comprises at least one immunomodulatory domain.

[00608] Aspect 82. An expression vector comprising the nucleic acid of aspect 81.

[00609] Aspect 83. A method of selectively modulating the activity of T cell specific for a Wilms tumor-1 (WT-1) epitope, the method comprising contacting the T cell with a T-cell modulatory multimeric polypeptide according to any one of aspects 55-80, wherein said contacting selectively modulates the activity of the WT-1 epitope-specific T cell.

[00610] Aspect 84. A method of treating a patient having a cancer, the method comprising administering to the patient an effective amount of a pharmaceutical composition comprising T-cell modulatory multimeric polypeptide according to any one of aspects 55-80.

[00611] Aspect 85. The method of aspect 84, wherein the cancer expresses a WT-1 protein.

[00612] Aspect 86. The method of aspect 84 or aspect 85, wherein the cancer is acute myeloid leukemia, myeloma, ovarian cancer, pancreatic cancer, non-small cell lung cancer, colorectal cancer, breast cancer, Wilms tumor, mesothelioma, soft tissue sarcoma, neuroblastoma, or nephroblastoma.

[00613] Aspect 87. The method of any one of aspects 84-86, wherein said administering is intramuscular.

[00614] Aspect 88. The method of any one of aspects 84-86, wherein said administering is intravenous.

[00615] Aspect 89. A method of any one of aspects 84-88, further comprising administering one or more checkpoint inhibitors to the individual.

[00616] Aspect 90. A method according to aspect 89, wherein the checkpoint inhibitor is an antibody that binds to a polypeptide selected from the group consisting of CD27, CD28, CD40, CD122, CD96, CD73, CD47, OX40, GITR, CSF1R, JAK, PI3K delta, PI3K gamma, TAM, arginase, CD137, ICOS, A2AR, B7-H3, B7-H4, BTLA, CTLA-4, LAG3, TIM3, VISTA, CD96, TIGIT, CD122, PD-1, PD-L1, and PD-L2.

[00617] Aspect 91. A method according to aspect 90, wherein the checkpoint inhibitor is an antibody specific for PD-1, PD-L1, or CTLA4.

[00618] Aspect 92. A method according to aspect 89, wherein the one or more checkpoint inhibitors is selected from the group consisting of nivolumab, pembrolizumab, pidilizumab, AMP-224, MPDL3280A, MDX-1105, MEDI-4736, avelumab, ipilimumab, tremelimumab, pidilizumab, IMP321, MGA271, BMS-986016, lirilumab, urelumab, PF-05082566, IPH2101, MEDI-6469, CP-870,893, Mogamulizumab, Varlilumab, Avelumab, Galiximab, AMP-514, AUNP 12, Indoximod, NLG-919, INCB024360, KN035, and combinations thereof.

[00619] Aspect 93. A method of modulating an immune response in an individual, the method comprising administering to the individual an effective amount of the T-cell modulatory multimeric polypeptide of any one of aspects 55-80, wherein said administering induces an epitope-specific T cell response and an epitope-non-specific T cell response, wherein the ratio of the epitope-specific T cell response to the epitope-non-specific T cell response is at least 2:1.

[00620] Aspect 94. A method of delivering an immunomodulatory polypeptide selectively to a target T cell, the method comprising contacting a mixed population of T cells with a T-cell modulatory multimeric polypeptide of any one of aspects 55-80, wherein the mixed population of T cells comprises the target T cell and non-target T cells, wherein the target T cell is specific for the WT-1 epitope present within the T-cell modulatory multimeric polypeptide, and wherein said contacting delivers the one or more immunomodulatory polypeptides present within the T-cell modulatory multimeric polypeptide to the target T cell.

[00621] Aspect 95. A method of detecting, in a mixed population of T cells obtained from an individual, the presence of a target T cell that binds a Wilms tumor-1 (WT-1) epitope, the method comprising: a) contacting *in vitro* the mixed population of T cells with the T-cell modulatory multimeric

polypeptide of any one of aspects 55-80, wherein the T-cell modulatory multimeric polypeptide comprises the WT-1 epitope; and b) detecting activation and/or proliferation of T cells in response to said contacting, wherein activated and/or proliferated T cells indicates the presence of the target T cell.

ASPECTS SET B

[00622] Aspects, including embodiments, of the present subject matter described above may be beneficial alone or in combination, with one or more other aspects or embodiments. Without limiting the foregoing description, certain non-limiting aspects of the disclosure numbered 1-36 are provided below. As will be apparent to those of skill in the art upon reading this disclosure, each of the individually numbered aspects may be used or combined with any of the preceding or following individually numbered aspects. This is intended to provide support for all such combinations of aspects and is not limited to combinations of aspects explicitly provided below:

[00623] Aspect 1. A T-cell modulatory multimeric polypeptide comprising: at least one heterodimer comprising: a) a first polypeptide comprising: i) a Wilms tumor-1 (WT-1) peptide epitope, wherein the WT-1 peptide has a length of at least 4 amino acids; and ii) first class I major histocompatibility complex (MHC) polypeptide; b) a second polypeptide comprising a second class I MHC polypeptide, and c) at least one activating immunomodulatory, wherein the first and/or the second polypeptide comprises the immunomodulatory polypeptide, and wherein the WT-1 peptide epitope comprises an amino acid sequence selected from the group consisting of: NLMNLGATL (SEQ ID NO:258), NYMNLGATL (SEQ ID NO:263), CMTWNQMNLGATLKG (SEQ ID NO:223), WNQMNLGATLKGVA (SEQ ID NO:224), CMTWNYMNLGATLKG (SEQ ID NO:225), WNYMNLGATLKGVA (SEQ ID NO:226), MTWNQMNLGATLKG (SEQ ID NO:227), TWNMNLGATLKGVA (SEQ ID NO:228), CMTWNLMNLGATLKG (SEQ ID NO:229), MTWNLMNLGATLKG (SEQ ID NO:230), TWNLMNLGATLKGVA (SEQ ID NO:231), WNLNMNLGATLKGVA (SEQ ID NO:232), MNLGATLKG (SEQ ID NO:233), MTWNYMNLGATLKG (SEQ ID NO:234), TWNYMNLGATLKGVA (SEQ ID NO:235), CMTWNQMNLGATLKGVA (SEQ ID NO:236), CMTWNLMNLGATLKGVA (SEQ ID NO:237), CMTWNYMNLGATLKGVA (SEQ ID NO:238), GYLRNPTAC (SEQ ID NO:239), GALRNPTAL (SEQ ID NO:240), YALRNPTAC (SEQ ID NO:241), GLLRNPTAC (SEQ ID NO:242), RYRPHPGAL (SEQ ID NO:243), YQRPHPGAL (SEQ ID NO:244), RLRPHPGAL (SEQ ID NO:245), RIRPHPGAL (SEQ ID NO:246), QFPNHSFKHEDPMGQ (SEQ ID NO:247), HSFKHEDPY (SEQ ID NO:248), QFPNHSFKHEDPM (SEQ ID NO:249), QFPNHSFKHEDPY (SEQ ID NO:250), KRPFMCAYPGCNK (SEQ ID NO:251), KRPFMCAYPGCYK (SEQ ID NO:252), FMCAYPGCY (SEQ ID NO:253), FMCAYPGCK (SEQ ID NO:254), KRPFMCAYPGCNKRY (SEQ ID NO:255), SEKRPFMCAYPGCNK (SEQ ID NO:256), KRPFMCAYPGCYKRY (SEQ ID NO:257), NLMNLGATL (SEQ ID NO:258), VLDFAPPGA (SEQ ID NO:259), RMFPNAPYL (SEQ ID NO:260),

CMTWNQMN (SEQ ID NO:261), CYTWNQMNL (SEQ ID NO:262), NYMNLGATL (SEQ ID NO:263), YMFPNAPYL (SEQ ID NO:264), SLGEQQYSV (SEQ ID NO:265), CMTWNQMNL (SEQ ID NO:266), and NQMNLGATL (SEQ ID NO:267), optionally wherein the first or the second polypeptide comprises an immunoglobulin (Ig) Fc polypeptide.

[00624] Aspect 2. A T-cell modulatory multimeric polypeptide of aspect 1, wherein at least one of the one or more immunomodulatory domains is a variant immunomodulatory polypeptide that exhibits reduced affinity to a cognate co-immunomodulatory polypeptide compared to the affinity of a corresponding wild-type immunomodulatory polypeptide for the cognate co-immunomodulatory polypeptide, and wherein the epitope binds to a T-cell receptor (TCR) on a T cell with an affinity of at least 10^{-7} M, such that:

[00625] i) the T-cell modulatory multimeric polypeptide binds to a first T cell with an affinity that is at least 25% higher than the affinity with which the T-cell modulatory multimeric polypeptide binds a second T cell, wherein the first T cell expresses on its surface the cognate co-immunomodulatory polypeptide and a TCR that binds the epitope with an affinity of at least 10^{-7} M, and wherein the second T cell expresses on its surface the cognate co-immunomodulatory polypeptide but does not express on its surface a TCR that binds the epitope with an affinity of at least 10^{-7} M; and/or

[00626] ii) the ratio of the binding affinity of a control T-cell modulatory multimeric polypeptide, wherein the control comprises a wild-type immunomodulatory polypeptide, to a cognate co-immunomodulatory polypeptide to the binding affinity of the T-cell modulatory multimeric polypeptide comprising a variant of the wild-type immunomodulatory polypeptide to the cognate co-immunomodulatory polypeptide, when measured by bio-layer interferometry, is in a range of from 1.5:1 to $10^6:1$.

[00627] Aspect 3. A T-cell modulatory multimeric polypeptide of aspect 2, wherein:

[00628] a) the T-cell modulatory multimeric polypeptide binds to the first T cell with an affinity that is at least 50%, at least 2-fold, at least 5-fold, or at least 10-fold higher than the affinity with which it binds the second T cell; and/or

[00629] b) the variant immunomodulatory polypeptide binds the co-immunomodulatory polypeptide with an affinity of from about 10^{-4} M to about 10^{-7} M, from about 10^{-4} M to about 10^{-6} M, from about 10^{-4} M to about 10^{-5} M; and/or

[00630] c) wherein the ratio of the binding affinity of a control T-cell modulatory multimeric polypeptide, wherein the control comprises a wild-type immunomodulatory polypeptide, to a cognate co-immunomodulatory polypeptide to the binding affinity of the T-cell modulatory multimeric polypeptide comprising a variant of the wild-type immunomodulatory polypeptide to the cognate co-

immunomodulatory polypeptide, when measured by bio-layer interferometry, is at least 10:1, at least 50:1, at least 10^2 :1, or at least 10^3 :1.

- [00631] Aspect 4. A T-cell modulatory multimeric polypeptide of any one of aspects 1-3, wherein
- [00632] a1) the first polypeptide comprises, in order from N-terminus to C-terminus:
- [00633] i) the WT-1 peptide epitope;
- [00634] ii) the first MHC polypeptide; and
- [00635] iii) at least one immunomodulatory polypeptide; and
- [00636] b1) the second polypeptide comprises, in order from N-terminus to C-terminus:
- [00637] i) the second MHC polypeptide; and
- [00638] ii) an immunoglobulin (Ig) Fc polypeptide; or
- [00639] a2) the first polypeptide comprises, in order from N-terminus to C-terminus:
- [00640] i) the WT-1 peptide epitope; and
- [00641] ii) the first MHC polypeptide; and
- [00642] b2) the second polypeptide comprises, in order from N-terminus to C-terminus:
- [00643] i) at least one immunomodulatory polypeptide;
- [00644] ii) the second MHC polypeptide; and
- [00645] iii) an Ig Fc polypeptide; or
- [00646] a3) the first polypeptide comprises, in order from N-terminus to C-terminus:
- [00647] i) the WT-1 peptide epitope; and
- [00648] ii) the first MHC polypeptide; and
- [00649] b3) the second polypeptide comprises, in order from N-terminus to C-terminus:
- [00650] i) the second MHC polypeptide; and
- [00651] ii) an Ig Fc polypeptide; and
- [00652] iii) at least one immunomodulatory polypeptide; or
- [00653] a4) the first polypeptide comprises, in order from N-terminus to C-terminus:
- [00654] i) the WT-1 peptide epitope; and
- [00655] ii) the first MHC polypeptide; and
- [00656] b4) the second polypeptide comprises, in order from N-terminus to C-terminus:
- [00657] i) the second MHC polypeptide; and
- [00658] ii) at least one immunomodulatory polypeptide; or
- [00659] a5) the first polypeptide comprises, in order from N-terminus to C-terminus:
- [00660] i) the WT-1 peptide epitope; and
- [00661] ii) the first MHC polypeptide; and

- [00662] b5) a second polypeptide comprises, in order from N-terminus to C-terminus:
- [00663] i) at least one immunomodulatory polypeptide; and
- [00664] ii) the second MHC polypeptide; or
- [00665] a6) the first polypeptide comprises, in order from N-terminus to C-terminus:
- [00666] i) the WT-1 peptide epitope;
- [00667] ii) the first MHC polypeptide; and
- [00668] iii) at least one immunomodulatory polypeptide; and
- [00669] b6) the second polypeptide comprises:
- [00670] i) the second MHC polypeptide.
- [00671] Aspect 5. A T-cell modulatory multimeric polypeptide of any one of aspects 1-4, wherein:
- [00672] a) the first MHC polypeptide is a β 2-microglobulin polypeptide; and the second MHC polypeptide is an MHC class I heavy chain polypeptide; or
- [00673] b) the first MHC polypeptide is an MHC class I heavy chain polypeptide; and the second MHC polypeptide is a β 2-microglobulin polypeptide.
- [00674] Aspect 6. A T-cell modulatory multimeric polypeptide of aspect 5, wherein:
- [00675] a) the first polypeptide comprises, in order from N-terminus to C-terminus:
- [00676] i) the WT-1 peptide epitope; and
- [00677] ii) the β 2-microglobulin polypeptide; and
- [00678] b) the second polypeptide comprises, in order from N-terminus to C-terminus:
- [00679] i) at least one immunomodulatory polypeptide;
- [00680] ii) the MHC class I heavy chain polypeptide; and
- [00681] iii) an Ig Fc polypeptide.
- [00682] Aspect 7. A T-cell modulatory multimeric polypeptide of aspect 5, wherein:
- [00683] a) the first polypeptide comprises, in order from N-terminus to C-terminus:
- [00684] i) the WT-1 peptide epitope; and
- [00685] ii) the β 2-microglobulin polypeptide; and
- [00686] b) the second polypeptide comprises, in order from N-terminus to C-terminus:
- [00687] i) the MHC class I heavy chain polypeptide; and
- [00688] ii) an Ig Fc polypeptide; and
- [00689] iii) at least one immunomodulatory polypeptide
- [00690] Aspect 8. A T-cell modulatory multimeric polypeptide of any one of aspects 1-7, wherein the at least one immunomodulatory polypeptide is selected from the group consisting of a cytokine, a 4-

1BBL polypeptide, an ICOS-L polypeptide, an OX-40L polypeptide, a CD80 polypeptide, a CD86 polypeptide, a CD40 polypeptide, a CD70 polypeptide, and combinations thereof.

[00691] Aspect 9. A T-cell modulatory multimeric polypeptide of any one of aspects 1-8, wherein the at least one immunomodulatory polypeptide is an IL-2 polypeptide.

[00692] Aspect 10. A T-cell modulatory multimeric polypeptide of any one of aspects 1-9, wherein the multimeric polypeptide comprises at least two immunomodulatory polypeptides, and wherein at least two of the immunomodulatory polypeptides are the same, optionally wherein the 2 or more immunomodulatory polypeptides are in tandem.

[00693] Aspect 11. A T-cell modulatory multimeric polypeptide of any one of aspects 1-10, wherein the immunomodulatory polypeptide is a variant IL-2 polypeptide that exhibits reduced affinity to an IL-2 receptor compared to the affinity of a wild-type IL-2 polypeptide for the IL-2 receptor.

[00694] Aspect 12. A T-cell modulatory multimeric polypeptide of aspect 11, wherein the variant IL-2 polypeptide comprises: i) an H16A substitution and an F42A substitution; or ii) an H16T substitution and an F42A substitution.

[00695] Aspect 13. A T-cell modulatory multimeric polypeptide of any one of aspects 1-12, wherein the first polypeptide and the second polypeptide are covalently linked to one another, optionally wherein the covalent linkage is via a disulfide bond.

[00696] Aspect 14. A T-cell modulatory multimeric polypeptide of any one of aspects 1-13, wherein the first MHC polypeptide or a linker between the epitope and the first MHC polypeptide comprises an amino acid substitution to provide a first Cys residue, wherein the second MHC polypeptide comprises an amino acid substitution to provide a second Cys residue, and wherein the disulfide linkage is between the first and the second Cys residues.

[00697] Aspect 15. The T-cell modulatory multimeric polypeptide of any one of aspects 1-14, wherein the polypeptide comprises a disulfide bond between: i) a Cys present in a linker between the WT-1 peptide epitope and the first MHC class I polypeptide, wherein the first MHC class I polypeptide is a β 2M polypeptide; and ii) a Cys residue introduced via a Y84C substitution in the second MHC class I polypeptide, wherein the second MHC class I polypeptide is a MHC Class I heavy chain polypeptide.

[00698] Aspect 16. The T-cell modulatory multimeric polypeptide of any one of aspects 1-14, wherein the polypeptide comprises a disulfide bond between i) a Cys residue introduced into the first MHC class I polypeptide via an R12C substitution, wherein the first MHC class I polypeptide is a β 2M polypeptide; and ii) a Cys residue introduced into the second MHC class I polypeptide, via an A236C substitution, wherein second MHC class I polypeptide is an MHC Class I heavy chain polypeptide.

[00699] Aspect 17. The T-cell modulatory multimeric polypeptide of any one of aspects 1-14, wherein the polypeptide comprises a first disulfide bond between: i) a Cys present in a linker between

the WT-1 peptide epitope and the first MHC class I polypeptide, wherein the first MHC class I polypeptide is a β 2M polypeptide; and ii) a Cys residue introduced via a Y84C substitution in the second MHC class I polypeptide, wherein the second MHC class I polypeptide is a MHC Class I heavy chain polypeptide, and a second disulfide bond between i) a Cys residue introduced into the β 2M polypeptide via an R12C substitution; and ii) a Cys residue introduced into the MHC Class I heavy chain polypeptide via an A236C substitution.

[00700] Aspect 18. A T-cell modulatory multimeric polypeptide of aspect 15 or aspect 17, wherein the linker between the WT-1 peptide epitope and the first MHC is GCGGS(G4S)_n (SEQ ID NO:315), where n is 1, 2, 3, 4, 5, 6, 7, 8, or 9.

[00701] Aspect 19. A T-cell modulatory multimeric polypeptide of any one of aspects 1-18, wherein the WT-1 peptide epitope has a length of from about 4 amino acids to about 25 amino acids.

[00702] Aspect 20. A T-cell modulatory multimeric polypeptide of any one of aspects 1-19, wherein the WT-1 peptide comprises the amino acid sequence CMTWNQMNL (SEQ ID NO: 266), CYTWNQMNL (SEQ ID NO:262), NYMNLGATL (SEQ ID NO:263), VLDFAPPGA (SEQ ID NO:259), YMFPNAPYL (SEQ ID NO:264), SLGEQQYSV (SEQ ID NO:265), RMFPNAPYL (SEQ ID NO:260), and NLMNLGATL (SEQ ID NO:258).

[00703] Aspect 21. A T-cell modulatory multimeric polypeptide of any one of aspects 1-20, wherein the first or the second MHC polypeptide comprises:

[00704] a) an amino acid sequence having at least 95% amino acid sequence identity to the HLA-A*0101, HLA-A*0201, HLA-A*0201, HLA-A*1101, HLA-A*2301, HLA-A*2402, HLA-A*2407, HLA-A*3303, or HLA-A*3401 amino acid sequence depicted in FIG. 9A; or

[00705] b) an amino acid sequence having at least 95% amino acid sequence identity to the HLA-B*0702, HLA-B*0801, HLA-B*1502, HLA-B*3802, HLA-B*4001, HLA-B*4601, or HLA-B*5301 amino acid sequence depicted in FIG. 10A; or

[00706] c) an amino acid sequence having at least 95% amino acid sequence identity to the HLA-C*0102, HLA-C*0303, HLA-C*0304, HLA-C*0401, HLA-C*0602, HLA-C*0701, HLA-C*0702, HLA-C*0801, or HLA-C*1502 depicted in FIG. 11A.

[00707] Aspect 22. A T-cell modulatory multimeric polypeptide of any one of aspects 1-21, wherein the first MHC polypeptide is a β 2M polypeptide, and wherein the second MHC polypeptide comprises an amino acid sequence having at least 95% amino acid sequence identity to an HLA-A*2402 polypeptide, and wherein the epitope is selected from the group consisting of: RMFPNAPYL (SEQ ID NO:260), CYTWNQMNL (SEQ ID NO:262), and NYMNLGATL (SEQ ID NO:263).

[00708] Aspect 23. A T-cell modulatory multimeric polypeptide of any one of aspects 1-21, wherein the first MHC polypeptide is a β 2M polypeptide, and wherein the second MHC polypeptide

comprises an amino acid sequence having at least 95% amino acid sequence identity to an HLA-A*0201 polypeptide, and wherein the epitope is selected from the group consisting of: VLDFAPPGA (SEQ ID NO:259), RMFPNAPYL (SEQ ID NO:260), and YMFNPAPYL (SEQ ID NO:264).

[00709] Aspect 24. A T-cell modulatory multimeric polypeptide of any one of aspects 1-23, wherein the multimeric polypeptide comprises a first and a second heterodimer, and wherein the first and second heterodimers are covalently bound by one or more disulfide bonds between the Ig Fc polypeptides of the first and second heterodimers.

[00710] Aspect 25. A nucleic acid comprising a nucleotide sequence encoding a first or second polypeptide according to any one of aspects 1-24.

[00711] Aspect 26. An expression vector comprising the nucleic acid of aspect 25.

[00712] Aspect 27. A method of selectively modulating the activity of T cell specific for a Wilms tumor-1 (WT-1) epitope, the method comprising contacting the T cell with a T-cell modulatory multimeric polypeptide according to any one of aspects 1-24, wherein said contacting selectively modulates the activity of the WT-1 epitope-specific T cell.

[00713] Aspect 28. A method of treating a patient having a cancer, the method comprising administering to the patient an effective amount of a pharmaceutical composition comprising T-cell modulatory multimeric polypeptide according to any one of aspects 1-24.

[00714] Aspect 29. The method of aspect 28, wherein the cancer is acute myeloid leukemia, myeloma, ovarian cancer, pancreatic cancer, non-small cell lung cancer, colorectal cancer, breast cancer, Wilms tumor, mesothelioma, soft tissue sarcoma, neuroblastoma, or nephroblastoma.

[00715] Aspect 30. A method of aspect 28 or aspect 29, further comprising administering one or more checkpoint inhibitors to the individual.

[00716] Aspect 31. A method according to aspect 30, wherein the checkpoint inhibitor is an antibody that binds to a polypeptide selected from the group consisting of CD27, CD28, CD40, CD122, CD96, CD73, CD47, OX40, GITR, CSF1R, JAK, PI3K delta, PI3K gamma, TAM, arginase, CD137, ICOS, A2AR, B7-H3, B7-H4, BTLA, CTLA-4, LAG3, TIM3, VISTA, CD96, TIGIT, CD122, PD-1, PD-L1, and PD-L2.

[00717] Aspect 32. A method according to aspect 31, wherein the checkpoint inhibitor is an antibody specific for PD-1, PD-L1, or CTLA4.

[00718] Aspect 33. A method according to aspect 30, wherein the one or more checkpoint inhibitors is selected from the group consisting of nivolumab, pembrolizumab, pidilizumab, AMP-224, MPDL3280A, MDX-1105, MEDI-4736, avelumab, ipilimumab, tremelimumab, pidilizumab, IMP321, MGA271, BMS-986016, lirilumab, urelumab, PF-05082566, IPH2101, MEDI-6469, CP-870,893,

Mogamulizumab, Varlilumab, Avelumab, Galiximab, AMP-514, AUNP 12, Indoximod, NLG-919, INCB024360, KN035, and combinations thereof.

[00719] Aspect 34. A method of modulating an immune response in an individual, the method comprising administering to the individual an effective amount of the T-cell modulatory multimeric polypeptide of any one of aspects 1-24, wherein said administering induces an epitope-specific T cell response and an epitope-non-specific T cell response, and wherein the ratio of the epitope-specific T cell response to the epitope-non-specific T cell response is at least 2:1.

[00720] Aspect 35. A method of delivering an immunomodulatory polypeptide selectively to a target T cell, the method comprising contacting a mixed population of T cells with a T-cell modulatory multimeric polypeptide of any one of aspects 1-24, wherein the mixed population of T cells comprises the target T cell and non-target T cells, wherein the target T cell is specific for the WT-1 epitope present within the T-cell modulatory multimeric polypeptide, and wherein said contacting delivers the one or more immunomodulatory polypeptides present within the T-cell modulatory multimeric polypeptide to the target T cell.

[00721] Aspect 36. A method of detecting, in a mixed population of T cells obtained from an individual, the presence of a target T cell that binds a WT-1 epitope, the method comprising: a) contacting *in vitro* the mixed population of T cells with the T-cell modulatory multimeric polypeptide of any one of aspects 1-24, wherein the T-cell modulatory multimeric polypeptide comprises the WT-1 epitope; and b) detecting activation and/or proliferation of T cells in response to said contacting, wherein activated and/or proliferated T cells indicates the presence of the target T cell.

ASPECTS SET C

[00722] Aspects, including embodiments, of the present subject matter described above may be beneficial alone or in combination, with one or more other aspects or embodiments. Without limiting the foregoing description, certain non-limiting aspects of the disclosure numbered 1-37 are provided below. As will be apparent to those of skill in the art upon reading this disclosure, each of the individually numbered aspects may be used or combined with any of the preceding or following individually numbered aspects. This is intended to provide support for all such combinations of aspects and is not limited to combinations of aspects explicitly provided below:

[00723] Aspect 1. A T-cell modulatory multimeric polypeptide comprising:

[00724] at least one heterodimer comprising:

[00725] a) a first polypeptide comprising:

[00726] i) a Wilms tumor-1 (WT-1) peptide epitope comprising the amino acid sequence X₁X₂X₃TWNQMNL (SEQ ID NO:460) or X₂X₃TWNQMNL (SEQ ID NO:461), wherein each

of X₁, X₂, and X₃ is independently any amino acid, with the proviso that the N-terminal amino acid is not a Cys, and wherein the WT-1 peptide epitope has a length from 9 to 25 amino acids; and

[00727] ii) a first Class I major histocompatibility complex (MHC) polypeptide;

[00728] b) a second polypeptide comprising a second class I MHC polypeptide, and

[00729] c) at least one activating immunomodulatory polypeptide,

[00730] wherein the first and/or the second polypeptide comprises the at least one immunomodulatory polypeptide, and optionally wherein the first or the second polypeptide comprises an immunoglobulin (Ig) Fc polypeptide.

[00731] Aspect 2. A T-cell modulatory multimeric polypeptide of aspect 1, wherein at least one of the one or more immunomodulatory polypeptides is a variant immunomodulatory polypeptide that exhibits reduced affinity to a cognate co-immunomodulatory polypeptide compared to the affinity of a corresponding wild-type immunomodulatory polypeptide for the cognate co-immunomodulatory polypeptide.

[00732] Aspect 3. A T-cell modulatory multimeric polypeptide of aspect 2, wherein the ratio of the binding affinity of the wild-type immunomodulatory polypeptide to a cognate co-immunomodulatory polypeptide to the binding affinity of the variant immunomodulatory polypeptide to the cognate co-immunomodulatory polypeptide, when measured by bio-layer interferometry, is at least 1.5:1.

[00733] Aspect 4. A T-cell modulatory multimeric polypeptide of aspect 2 or 3, wherein the variant immunomodulatory polypeptide binds the co-immunomodulatory polypeptide with an affinity selected from the group consisting of from about 10⁻⁴ M to about 10⁻⁷ M, from about 10⁻⁴ M to about 10⁻⁶ M, and from about 10⁻⁴ M to about 10⁻⁵ M.

[00734] Aspect 5. A T-cell modulatory multimeric polypeptide of any one of aspects 1-4, wherein:

[00735] a1) the first polypeptide comprises, in order from N-terminus to C-terminus:

[00736] i) the WT-1 peptide epitope; and

[00737] ii) the first MHC polypeptide; and

[00738] b1) the second polypeptide comprises, in order from N-terminus to C-terminus:

[00739] i) the at least one immunomodulatory polypeptide;

[00740] ii) the second MHC polypeptide; and

[00741] iii) an Ig Fc polypeptide; or

[00742] a2) the first polypeptide comprises, in order from N-terminus to C-terminus:

[00743] i) the WT-1 peptide epitope; and

[00744] ii) the first MHC polypeptide; and

[00745] b2) the second polypeptide comprises, in order from N-terminus to C-terminus:

- [00746] i) the second MHC polypeptide;
- [00747] ii) the at least one immunomodulatory polypeptide; and
- [00748] iii) an Ig Fc polypeptide; or
- [00749] a3) the first polypeptide comprises, in order from N-terminus to C-terminus:
 - [00750] i) the WT-1 peptide epitope; and
 - [00751] ii) the first MHC polypeptide; and
- [00752] b3) the second polypeptide comprises, in order from N-terminus to C-terminus:
 - [00753] i) the second MHC polypeptide;
 - [00754] ii) an Ig Fc polypeptide; and
 - [00755] iii) the at least one immunomodulatory polypeptide; or
- [00756] a4) the first polypeptide comprises, in order from N-terminus to C-terminus:
 - [00757] i) the at least one immunomodulatory polypeptide;
 - [00758] ii) the WT-1 peptide epitope;
 - [00759] ii) the first MHC polypeptide; and
- [00760] b4) the second polypeptide comprises, in order from N-terminus to C-terminus:
 - [00761] i) the second MHC polypeptide; and
 - [00762] ii) the Ig Fc polypeptide; or
- [00763] a5) the first polypeptide comprises, in order from N-terminus to C-terminus:
 - [00764] i) the WT-1 peptide epitope;
 - [00765] ii) the first MHC polypeptide; and
 - [00766] iii) the at least one immunomodulatory polypeptide; and
- [00767] b5) the second polypeptide comprises, in order from N-terminus to C-terminus:
 - [00768] i) the second MHC polypeptide; and
 - [00769] ii) an immunoglobulin (Ig) Fc polypeptide.
- [00770] Aspect 6. A T-cell modulatory multimeric polypeptide of any one of aspects 1-4, wherein:
 - [00771] a) the first MHC polypeptide is a β 2-microglobulin polypeptide; and the second MHC polypeptide is an MHC class I heavy chain polypeptide; or
 - [00772] b) the first MHC polypeptide is an MHC class I heavy chain polypeptide; and the second MHC polypeptide is a β 2-microglobulin polypeptide.
- [00773] Aspect 7. A T-cell modulatory multimeric polypeptide of aspect 6, wherein:
 - [00774] a) the first polypeptide comprises, in order from N-terminus to C-terminus:
 - [00775] i) the WT-1 peptide epitope; and

- [00776] ii) the β 2-microglobulin polypeptide; and
- [00777] b) the second polypeptide comprises, in order from N-terminus to C-terminus:
- [00778] i) the at least one immunomodulatory polypeptide;
- [00779] ii) the MHC class I heavy chain polypeptide; and
- [00780] iii) an Ig Fc polypeptide.
- [00781] Aspect 8. A T-cell modulatory multimeric polypeptide of aspect 6, wherein:
- [00782] a) the first polypeptide comprises, in order from N-terminus to C-terminus:
- [00783] i) the WT-1 peptide epitope; and
- [00784] ii) the β 2-microglobulin polypeptide; and
- [00785] b) the second polypeptide comprises, in order from N-terminus to C-terminus:
- [00786] i) the MHC class I heavy chain polypeptide; and
- [00787] ii) an Ig Fc polypeptide; and
- [00788] iii) at least one immunomodulatory polypeptide
- [00789] Aspect 9. A T-cell modulatory multimeric polypeptide of any one of aspects 1-8, wherein the at least one immunomodulatory polypeptide is selected from the group consisting of a cytokine, a 4-1BBL polypeptide, an ICOS-L polypeptide, an OX-40L polypeptide, a CD80 polypeptide, a CD86 polypeptide, a CD40 polypeptide, a CD70 polypeptide, and combinations thereof.
- [00790] Aspect 10. A T-cell modulatory multimeric polypeptide of any one of aspects 1-9, wherein the at least one immunomodulatory polypeptide comprises an IL-2 polypeptide.
- [00791] Aspect 11. A T-cell modulatory multimeric polypeptide of any one of aspects 1-10, wherein the multimeric polypeptide comprises at least two immunomodulatory polypeptides, and wherein at least two of the immunomodulatory polypeptides are the same, optionally wherein the 2 or more immunomodulatory polypeptides are in tandem.
- [00792] Aspect 12. A T-cell modulatory multimeric polypeptide of any one of aspects 1-11, wherein one or more of the at least one immunomodulatory polypeptide is a variant IL-2 polypeptide that exhibits reduced affinity to an IL-2 receptor compared to the affinity of a wild-type IL-2 polypeptide for the IL-2 receptor.
- [00793] Aspect 13. A T-cell modulatory multimeric polypeptide of aspect 12, wherein the one or more variant IL-2 polypeptides comprises: i) an H16A substitution and an F42A substitution; or ii) an H16T substitution and an F42A substitution.
- [00794] Aspect 14. A T-cell modulatory multimeric polypeptide of any one of aspects 1-13, wherein the first polypeptide and the second polypeptide are covalently linked to one another, optionally wherein the covalent linkage is via a disulfide bond.

[00795] Aspect 15. A T-cell modulatory multimeric polypeptide of any one of aspects 1-14, wherein the first MHC polypeptide or a linker between the epitope and the first MHC polypeptide comprises an amino acid substitution to provide a first Cys residue, wherein the second MHC polypeptide comprises an amino acid substitution to provide a second Cys residue, and wherein the disulfide linkage is between the first and the second Cys residues.

[00796] Aspect 16. The T-cell modulatory multimeric polypeptide of any one of aspects 1-15, wherein the polypeptide comprises a disulfide bond between: i) a Cys present in a linker between the WT-1 peptide epitope and the first MHC class I polypeptide, wherein the first MHC class I polypeptide is a β 2M polypeptide; and ii) a Cys residue introduced via a Y84C substitution in the second MHC class I polypeptide, wherein the second MHC class I polypeptide is a MHC Class I heavy chain polypeptide.

[00797] Aspect 17. The T-cell modulatory multimeric polypeptide of any one of aspects 1-15, wherein the polypeptide comprises a disulfide bond between i) a Cys residue introduced into the first MHC class I polypeptide via an R12C substitution, wherein the first MHC class I polypeptide is a β 2M polypeptide; and ii) a Cys residue introduced into the second MHC class I polypeptide, via an A236C substitution, wherein second MHC class I polypeptide is an MHC Class I heavy chain polypeptide.

[00798] Aspect 18. The T-cell modulatory multimeric polypeptide of any one of aspects 1-15, wherein the polypeptide comprises a first disulfide bond between: i) a Cys present in a linker between the WT-1 peptide epitope and the first MHC class I polypeptide, wherein the first MHC class I polypeptide is a β 2M polypeptide; and ii) a Cys residue introduced via a Y84C substitution in the second MHC class I polypeptide, wherein the second MHC class I polypeptide is a MHC Class I heavy chain polypeptide, and a second disulfide bond between i) a Cys residue introduced into the β 2M polypeptide via an R12C substitution; and ii) a Cys residue introduced into the MHC Class I heavy chain polypeptide via an A236C substitution.

[00799] Aspect 19. A T-cell modulatory multimeric polypeptide of aspect 16 or aspect 18, wherein the linker between the WT-1 peptide epitope and the first MHC is GCGGS(GGGGS)_n (SEQ ID NO:319), where n is 1, 2, 3, 4, 5, 6, 7, 8, or 9.

[00800] Aspect 20. A T-cell modulatory multimeric polypeptide of any one of aspects 1-19, wherein the WT-1 peptide epitope has a length of 9 or 10 amino acids.

[00801] Aspect 21. A T-cell modulatory multimeric polypeptide of any one of aspects 1-20, wherein the Ig Fc polypeptide comprises one of the amino acid sequences depicted in FIG. 5D, FIG. 5E, FIG. 5F, FIG. 4G, and FIG. 5H.

[00802] Aspect 22. A T-cell modulatory multimeric polypeptide of any one of aspects 1-21, wherein the WT-1 peptide comprises the amino acid sequence SMTWNQMNL (SEQ ID NO:451),

GCMTWNQMNL (SEQ ID NO:452), SYTWNQMNL (SEQ ID NO:453), or GCYTWNQMNL (SEQ ID NO:454).

[00803] Aspect 23. A T-cell modulatory multimeric polypeptide of any one of aspects 1-21, wherein the first or the second MHC polypeptide comprises an amino acid sequence having at least 95% amino acid sequence identity to amino acids 25-299 of the HLA-A*2402 amino acid sequence depicted in FIG. 7A.3.

[00804] Aspect 24. A T-cell modulatory multimeric polypeptide of any one of aspects 1-23, wherein the first MHC polypeptide is a β 2M polypeptide, and wherein the second MHC polypeptide comprises an amino acid sequence having at least 95% amino acid sequence identity to an HLA-A24 polypeptide, wherein the epitope is selected from the group consisting of: SMTWNQMNL (SEQ ID NO:451), GCMTWNQMNL (SEQ ID NO:452), SYTWNQMNL (SEQ ID NO:453), and GCYTWNQMNL (SEQ ID NO:454), and wherein the Ig Fc polypeptide comprises the amino acid sequence depicted in FIG. 5G or FIG. 5H.

[00805] Aspect 25. A T-cell modulatory multimeric polypeptide of aspect 1, wherein: a) the first polypeptide comprises the amino acid sequence depicted in FIG. 37B; and b) the second polypeptide comprises the amino acid sequence depicted in FIG. 20B.

[00806] Aspect 26. A T-cell modulatory multimeric polypeptide of any one of aspects 1-25, wherein the multimeric polypeptide comprises a first and a second heterodimer, and wherein the first and second heterodimers are covalently bound by one or more disulfide bonds between the Ig Fc polypeptides of the first and second heterodimers.

[00807] Aspect 27. A nucleic acid comprising a nucleotide sequence encoding a first or second polypeptide according to any one of aspects 1-26.

[00808] Aspect 28. An expression vector comprising the nucleic acid of aspect 27.

[00809] Aspect 29. A method of selectively modulating the activity of T cell specific for a Wilms tumor-1 (WT-1) epitope, the method comprising contacting the T cell with a T-cell modulatory multimeric polypeptide according to any one of aspects 1-26, wherein said contacting selectively modulates the activity of the WT-1 epitope-specific T cell.

[00810] Aspect 30. A method of treating a patient having a cancer, the method comprising administering to the patient an effective amount of a pharmaceutical composition comprising T-cell modulatory multimeric polypeptide according to any one of aspects 1-26.

[00811] Aspect 31. The method of aspect 30, wherein the cancer is acute myeloid leukemia, myeloma, ovarian cancer, pancreatic cancer, non-small cell lung cancer, colorectal cancer, breast cancer, Wilms tumor, mesothelioma, soft tissue sarcoma, neuroblastoma, or nephroblastoma.

[00812] Aspect 32. A method of aspect 30 or 31, further comprising administering one or more checkpoint inhibitors to the individual.

[00813] Aspect 33. A method according to aspect 32, wherein the checkpoint inhibitor is an antibody that binds to a polypeptide selected from the group consisting of CD27, CD28, CD40, CD122, CD96, CD73, CD47, OX40, GITR, CSF1R, JAK, PI3K delta, PI3K gamma, TAM, arginase, CD137, ICOS, A2AR, B7-H3, B7-H4, BTLA, CTLA-4, LAG3, TIM3, VISTA, CD96, TIGIT, CD122, PD-1, PD-L1, and PD-L2.

[00814] Aspect 34. A method according to aspect 33, wherein the checkpoint inhibitor is an antibody specific for PD-1, PD-L1, or CTLA4.

[00815] Aspect 35. A method according to aspect 33 or 34, wherein the one or more checkpoint inhibitors is selected from the group consisting of nivolumab, pembrolizumab, pidilizumab, AMP-224, MPDL3280A, MDX-1105, MEDI-4736, arelumab, ipilimumab, tremelimumab, pidilizumab, IMP321, MGA271, BMS-986016, lirilumab, urelumab, PF-05082566, IPH2101, MEDI-6469, CP-870,893, Mogamulizumab, Varlilumab, Avelumab, Galiximab, AMP-514, AUNP 12, Indoximod, NLG-919, INCB024360, KN035, and combinations thereof.

[00816] Aspect 36. A method of modulating an immune response in an individual, the method comprising administering to the individual an effective amount of the T-cell modulatory multimeric polypeptide of any one of aspects 1-26, wherein said administering induces an epitope-specific T cell response and an epitope-non-specific T cell response, and wherein the ratio of the epitope-specific T cell response to the epitope-non-specific T cell response is at least 2:1.

[00817] Aspect 37. A method of delivering an immunomodulatory polypeptide selectively to a target T cell, the method comprising contacting a mixed population of T cells with a T-cell modulatory multimeric polypeptide of any one of aspects 1-26, wherein the mixed population of T cells comprises the target T cell and non-target T cells, wherein the target T cell is specific for the WT-1 epitope present within the T-cell modulatory multimeric polypeptide, and wherein said contacting delivers the one or more immunomodulatory polypeptides present within the T-cell modulatory multimeric polypeptide to the target T cell.

[00818] Aspect 38. A method of detecting, in a mixed population of T cells obtained from an individual, the presence of a target T cell that binds a WT-1 epitope, the method comprising:

[00819] a) contacting *in vitro* the mixed population of T cells with the T-cell modulatory multimeric polypeptide of any one of aspects 1-26, wherein the T-cell modulatory multimeric polypeptide comprises the WT-1 epitope; and

[00820] b) detecting activation and/or proliferation of T cells in response to said contacting, wherein activated and/or proliferated T cells indicates the presence of the target T cell.

[00821] Aspect 39. A T-cell modulatory multimeric polypeptide (TMMP) comprising: at least one heterodimer comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a Wilms tumor-1 (WT-1) peptide, wherein the WT-1 peptide has the amino acid sequence VLDFAPPGA (SEQ ID NO:259); ii) a linker having the amino acid sequence GCGGSGGGGSGGGGS (SEQ ID NO:317); and iii) a β 2-microglobulin polypeptide comprising the amino acid sequence set forth in SEQ ID NO:311; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a variant IL-2 polypeptide comprising the amino acid sequence set forth in SEQ ID NO:188, where X₁ is Ala and where X₂ is Ala; ii) a (GGGS)₄ linker; iii) a variant IL-2 polypeptide comprising the amino acid sequence set forth in SEQ ID NO:188, where X₁ is Ala and where X₂ is Ala; iv) a major histocompatibility complex (MHC) heavy chain polypeptide comprising the amino acid sequence set forth in SEQ ID NO:341; v) a linker comprising the amino acid sequence AAAGG; and vi) an immunoglobulin (Ig) Fc polypeptide.

[00822] Aspect 40. A TMMP of aspect 39, wherein the Ig Fc polypeptide is a variant Ig Fc polypeptide comprising one or more sequence variations relative to the wild type polypeptide, wherein the ability of the Ig Fc polypeptide to induce cell lysis through complement-dependent cytotoxicity (CDC) and/or antibody-dependent cellular cytotoxicity (ADCC) is reduced or substantially eliminated, optionally wherein the Ig Fc polypeptide comprises an amino acid sequence having at least at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to an amino acid sequence of an Fc region depicted in FIG. 5A-5G or 5H.

[00823] Aspect 41. A TMMP of aspect 40, wherein the Ig Fc polypeptide is a variant human IgG1 Fc polypeptide comprising comprises an L234A and/or L235A substitutions (L14 and L15 in the amino acid sequence depicted in FIG. 5H).

[00824] Aspect 42. A TMMP of any one of aspects 39-41, wherein the first polypeptide comprises construct 2380 having the amino acid sequence set forth in FIG. 14B and SEQ ID NO:423; and where the second polypeptide comprises construct 1715 Δ having the amino acid sequence set forth in FIG. 14J and SEQ ID NO:486.

[00825] Aspect 43. A TMMP comprising a homodimer of the heterodimer of any one of aspects 39-42.

[00826] Aspect 44. A nucleic acid comprising a nucleotide sequence encoding the first and/or the second polypeptide of any one of aspects 39-42.

- [00827] Aspect 45. An expression vector comprising the nucleic acid of aspect 44.
- [00828] Aspect 46. A genetically modified host cell, wherein the host cell is genetically modified with a nucleic acid of aspect 44 or an expression vector of aspect 45.
- [00829] Aspect 47. A method of making a T-cell modulatory multimeric polypeptide (TMMP), the method comprising culturing the genetically modified host cell of aspect 46 *in vitro* in a culture medium under conditions such that the host cell synthesizes the TMMP.
- [00830] Aspect 48. A method of selectively modulating the activity of T cell specific for a Wilms tumor-1 (WT-1) epitope, the method comprising contacting the T cell with a T-cell modulatory multimeric polypeptide according to any one of aspects 39-43, wherein said contacting selectively modulates the activity of the WT-1 epitope-specific T cell.
- [00831] Aspect 49. A method of treating a patient having a cancer associated with WT-1, the method comprising administering to the patient an effective amount of a pharmaceutical composition comprising T-cell modulatory multimeric polypeptide according to any one of aspects 39-43.
- [00832] Aspect 50. A method of aspect 49, wherein the cancer is acute myeloid leukemia, myeloma, ovarian cancer, pancreatic cancer, non-small cell lung cancer, colorectal cancer, breast cancer, Wilms tumor, mesothelioma, soft tissue sarcoma, neuroblastoma, or nephroblastoma.
- [00833] Aspect 51. A method of 49 or 50, further comprising administering one or more immune checkpoint inhibitors to the individual.
- [00834] Aspect 52. A method according to aspect 51, wherein the immune checkpoint inhibitor is an antibody that binds to a polypeptide selected from the group consisting of CD27, CD28, CD40, CD122, CD96, CD73, CD47, OX40, GITR, CSF1R, JAK, PI3K delta, PI3K gamma, TAM, arginase, CD137, ICOS, A2AR, B7-H3, B7-H4, BTLA, CTLA-4, LAG3, TIM3, VISTA, CD96, TIGIT, CD122, PD-1, PD-L1, and PD-L2.
- [00835] Aspect 53. A method according to aspect 52, wherein the immune checkpoint inhibitor is an antibody specific for PD-1, PD-L1, or CTLA4.
- [00836] Aspect 54. A method according to aspect 52 or 53, wherein the one or more immune checkpoint inhibitors is selected from the group consisting of nivolumab, pembrolizumab, pidilizumab, AMP-224, MPDL3280A, MDX-1105, MEDI-4736, avelumab, ipilimumab, tremelimumab, pidilizumab, IMP321, MGA271, BMS-986016, lirilumab, urelumab, PF-05082566, IPH2101, MEDI-6469, CP-870,893, Mogamulizumab, Varlilumab,

Avelumab, Galiximab, AMP-514, AUNP 12, Indoximod, NLG-919, INCB024360, KN035, and combinations thereof.

EXAMPLES

[00837] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Celsius, and pressure is at or near atmospheric. Standard abbreviations may be used, e.g., bp, base pair(s); kb, kilobase(s); pl, picoliter(s); s or sec, second(s); min, minute(s); h or hr, hour(s); aa, amino acid(s); kb, kilobase(s); bp, base pair(s); nt, nucleotide(s); i.m., intramuscular(ly); i.p., intraperitoneal(ly); s.c., subcutaneous(ly); and the like.

Example 1

[00838] The effect of linking the two polypeptide chains of a TMMP heterodimer via two disulfide bonds on stability and production was tested.

[00839] The following TMMPs were generated: a) a TMMP comprising 1715 + 2380 polypeptides; and b) a TMMP comprising 1715 and 2381 polypeptides. The amino acid sequences of the polypeptide chains are provided in FIG. 14A-14C. As shown in FIG. 15 and FIG. 16, the TMMPs included: i) Class I HLA-A heavy chain polypeptides of the A02:01 allele; and ii) two copies of IL2 (H16A; F42A) immunomodulatory ("MOD") polypeptides. The 2380 polypeptide comprises the WT1 peptide WT1(37-45), while the 2381 polypeptide comprises the WT1 peptide WT1(126-134). The 1715-2380 TMMP is a homodimer of a heterodimer comprising the 1715 polypeptide and the 2380 polypeptide. Likewise, the 1715-2381 TMMP is a homodimer of a heterodimer comprising the 1715 polypeptide and the 2381 polypeptide. Thus, the TMMPs included: i) 2 copies of the 1715 + 2380 heterodimer, linked by 2 disulfide bonds between the IgFc polypeptide present in the 1715 polypeptides; or ii) 2 copies of the 1715 + 2381 heterodimer, linked by 2 disulfide bonds between the IgFc polypeptide present in the 1715 polypeptides. This arrangement is depicted schematically in FIG. 17C.

[00840] TMMP 1715 + 2380 is a double disulfide-linked heterodimer: a) a first disulfide linkage is between: i) the Cys present in the linker between the WT1 peptide and the β 2M chain in the 2380 polypeptide; and ii) the Cys introduced by the Y84C substitution in the Class I heavy chain present in the 1715 polypeptide; and b) a second disulfide linkage is between: i) the Cys introduced by the R12C

substitution in the β 2M polypeptide present in the 2380 polypeptide; and ii) the Cys introduced by the A236C substitution in the Class I heavy chain present in the 1715 polypeptide.

[00841] TMMP 1715 + 2381 is a double disulfide-linked heterodimer: a) a first disulfide linkage is between: i) the Cys present in the linker between the WT1 peptide and the β 2M chain in the 2381 polypeptide; and ii) the Cys introduced by the Y84C substitution in the Class I heavy chain present in the 1715 polypeptide; and b) a second disulfide linkages is between: i) the Cys introduced by the R12C substitution in the β 2M polypeptide present in the 2381 polypeptide; and ii) the Cys introduced by the A236C substitution in the Class I heavy chain present in the 1715 polypeptide.

[00842] The TMMPs were produced in ExpiCHO cells (adapted from Chinese hamster ovary (CHO) cells; ThermoFisher; see, e.g., Jain et al. (2017) *Protein Expr. Purif.* 134:38) and were purified from the cell culture medium in which the cells were grown. Two purification steps were carried out. In a first step, the cell culture medium was clarified, and the clarified cell culture medium was subjected to Protein A column purification. In the second purification step, the eluate from the Protein A column was subjected to size exclusion chromatography.

[00843] The stability of the purified TMMPs was tested. The amount of heterodimeric TMMP present was determined after storage of the purified TMMPs in a liquid solution (phosphate-buffered saline (PBS) containing 365 mM NaCl, pH 7.4) for 28 days at 37°C or for 28 days at 42°C.

[00844] In addition, the purified TMMPs were subjected to 3 freeze/thaw cycles.

[00845] The results are depicted in FIG. 15 and FIG. 16.

[00846] As shown in FIG. 15, a homodimer of the 1715-2380 heterodimer (referred to in FIG. 15 as “monomer”) represented 80% of the eluate from the Protein A column. As shown in FIG. 16, a homodimer of the 1715-2381 heterodimer (referred to in FIG. 16 as “monomer”) represented 79% of the eluate from the Protein A column.

[00847] Homodimers of heterodimers 1715-2380 and 1715-2381 were found to be stable to 3 freeze/thaw cycles.

[00848] Unfolding temperatures of the peptide/HLA, IL-2, and Fc domains of various TMMPs, expressed as T_m (°C), are provided in FIG. 15 and FIG. 16. In addition, the temperature at which aggregation occurs (T_m °C) is provided in FIG. 15 and FIG. 16.

[00849] Stability assays (10-day *in vitro* stability at 4°C, 37°C, and 42°C) were conducted, comparing the stability of double-disulfide-bonded TMMPs to that of single disulfide-bonded TMMPs. The data are shown in Tables 2-4, below.

[00850] Table 2 below illustrates the results obtained with TMMPs containing the WT-1 peptide VLDFAPPGA (SEQ ID NO:259):

Table 2

Engineered disulfide	R12C-A236C	R12C-A236C; G2C-Y84C	G2C-Y84C
Titer (mg/L)	157	281	243
% Monomer (post ProtA)	<47	61	NA
% Monomer (post SEC)		98	99
Stability (10d @ 4°C/37°C/42°C Monomer recovery%)		100/88/24	99/83/14
Freeze-thaw (0x/1x/3x)		98/98/98	99/94/90
T _m (°C)		51 66 81	55 66 82
Intact mass (LC-MS)	Confirmed	Confirmed	Confirmed

NA = data not available

[00851] The middle data column of Table 2 presents *in vitro* stability data for a double-disulfide-bonded TMMP comprising the WT-1 peptide VLDFAPPGA (SEQ ID NO:259), compared to single-disulfide TMMPs that have only one of those two disulfide bonds (left-hand data column and right-hand data column).

[00852] The single-disulfide TMMP that has only the disulfide bond between the Cys at position 12 in the β 2M polypeptide ("R12C") and the Cys at amino acid 236 of the MHC class I heavy chain ("A236C") was so unstable that it could not be purified in sufficient quantities to conduct stability assays. These data are presented in the left-hand data column in Table 2.

[00853] The single-disulfide TMMP that has only the disulfide bond between: i) the linker between the WT-1 epitope and the β 2M polypeptide ("G2C"); and ii) the Cys at amino acid 84 of the MHC class I heavy chain ("Y84C") could be produced; however, it was less stable *in vitro* than the double-disulfide-bonded TMMP. For example, whereas the freeze/thaw values (representing the % monomer remaining after the indicated number of freeze/thaw cycles and thus the stability of the TMMP) for double-disulfide TMMP remained stable and unchanged over successive freeze/thaw cycles, the freeze/thaw values for this single-disulfide TMMP decreased over successive freeze/thaw cycles, indicating instability. Furthermore, the freeze/thaw value for the third freeze/thaw cycle was significantly lower than that of the double-disulfide-bonded TMMP. These data are presented in the right-hand data column in Table 2.

[00854] Similar results were obtained with TMMPs made with TMMPs containing the WT-1 peptide RMFPNAPYL (SEQ ID NO:260). The results are provided in Table 3, below.

Table 3

Engineered disulfide	R12C-A236C	R12C-A236C; G2C-Y84C	G2C-Y84C
Titer (mg/L)	228	280	236
% Monomer (post ProtA)	32	58	61
% Monomer (post SEC)	91	98	99
Stability (10d @ 4°C/37°C/42°C Monomer recovery%)	100/11/7	100/85/11	100/80/7
Freeze-thaw (0x/1x/3x)	91/91/91	98/98/98	99/98/92
T _m (°C)	41 66 81	50 67 81	48 66 81
Intact mass (LC-MS)	Confirmed	Confirmed	Confirmed

[00855] The middle data column of Table 3 presents *in vitro* stability data for a double-disulfide-bonded TMMP comprising the WT-1 peptide RMFPNAPYL (SEQ ID NO:260), compared to single-disulfide TMMPs that have only one of those two disulfide bonds (left-hand data column and right-hand data columns). The data show that the double-disulfide-bonded TMMP is more stable *in vitro* than either of the single-disulfide TMMPs.

[00856] The data in the left-hand data column is for the single-disulfide TMMP that has only the disulfide bond between the Cys at position 12 in the β 2M polypeptide ("R12C") and the Cys at amino acid 236 of the MHC class I heavy chain ("A236C"). This single-disulfide TMMP exhibited instability at 37°C, compared to the double-disulfide-bonded TMMP.

[00857] The data in the right-hand data column is for the single-disulfide TMMP that has only the disulfide bond between: i) the linker between the WT-1 epitope and the β 2M polypeptide ("G2C"); and ii) the Cys at amino acid 84 of the MHC class I heavy chain ("Y84C"). Again, whereas the freeze/thaw values for double-disulfide TMMP remained stable and unchanged over successive freeze/thaw cycles, the freeze/thaw values for this single-disulfide TMMP decreased over successive freeze/thaw cycles, indicating instability. Furthermore, the freeze/thaw value for the third freeze/thaw cycle was significantly lower than that of the double-disulfide-bonded TMMP.

[00858] Similar results were obtained with TMMPs made with TMMPs containing the WT-1 peptide YMFNPAPYL (SEQ ID NO:264). The results are provided in Table 4, below.

Table 4

Engineered disulfide	R12C-A236C	R12C-A236C; G2C-Y84C	G2C-Y84C
Titer (mg/L)	280	300	290

Engineered disulfide	R12C-A236C	R12C-A236C; G2C-Y84C	G2C-Y84C
% Monomer (post ProtA)	40	48	60
% Monomer (post SEC)	97	97	98
Stability (10d @ 4°C/37°C/42°C Monomer recovery%)	100/41/0	100/87/27	100/86/29
Freeze-thaw (0x/1x/3x)	97/97/97	97/97/97	98/96/91
T _m (°C)	44 67 81	55 66 82	51 66 80
Intact mass (LC-MS)	Confirmed	Confirmed	Confirmed

[00859] The middle data column of Table 4 presents *in vitro* stability data for a double-disulfide-bonded TMMP comprising the WT-1 peptide YMFNPAPYL (SEQ ID NO:264), compared to single-disulfide TMMPs that have only one of those two disulfide bonds (left-hand data column and right-hand data columns). The data show that the double-disulfide-bonded TMMP is more stable *in vitro* than either of the single-disulfide TMMPs.

[00860] The data in the left-hand data column is for the single-disulfide TMMP that has only the disulfide bond between the Cys at position 12 in the β 2M polypeptide ("R12C") and the Cys at amino acid 236 of the MHC class I heavy chain ("A236C"). This single-disulfide TMMP exhibited instability at 37°C, compared to the double-disulfide-bonded TMMP.

[00861] The data in the right-hand data column is for the single-disulfide TMMP that has only the disulfide bond between: i) the linker between the WT-1 epitope and the β 2M polypeptide ("G2C"); and ii) the Cys at amino acid 84 of the MHC class I heavy chain ("Y84C"). Again, whereas the freeze/thaw values for double-disulfide TMMP remained stable and unchanged over successive freeze/thaw cycles, the freeze/thaw values for this single-disulfide TMMP decreased over successive freeze/thaw cycles, indicating instability. Furthermore, the freeze/thaw value for the third freeze/thaw cycle was significantly lower than that of the double-disulfide-bonded TMMP.

Example 2: Biochemical characterization of TMMPs comprising WT1 epitopes, HLA-A*02 heavy chains, either one or two disulfide bonds between the 2 polypeptide chains of the heterodimer, and variant IL-2 immunomodulatory polypeptides at position 1

[00862] The constructs used in this study are summarized in Table 5.

Table 5

Constructs	Epitope	S-S bond(s)	IL-2 position
2405 + 2762	WT-1 (37-45)	G2C	1

Constructs	Epitope	S-S bond(s)	IL-2 position
1715 + 2380	WT-1 (37-45)	G2C + R12C	1
2405 + 2763	WT-1 (126-134)	G2C	1
1715 + 2381	WT-1 (126-134)	G2C + R12C	1
2405 + 3626	WT-1 (126-134 (R126Y))	G2C	1
1715 + 3625	WT-1 (126-134 (R126Y))	G2C + R12C	1

[00863] Amino acid sequences of the polypeptide chains of the constructs are provided in FIG. 14A-14I.

[00864] “G2C” indicates that the TMMP includes a disulfide bond between: i) a Cys in the peptide linker between the peptide epitope and the β 2M polypeptide; and ii) a Cys at position 84 of the MHC class I heavy chain, where the MHC class I heavy chain has a Y84C substitution.

[00865] “R12C” indicates that the TMMP includes a disulfide bond between: i) a Cys at position 12 in the β 2M polypeptide, where the β 2M polypeptide has an R12C substitution; and ii) a Cys at position 236 of the MHC class I heavy chain, where the MHC class I heavy chain has an A236C substitution.

[00866] “G2C + R12C” indicates that the TMMP includes both the “G2C” disulfide bond and the “R12C” disulfide bond.

[00867] WT-1 (37-45) is VLDFAPPGA (SEQ ID NO:259).

[00868] WT-1 (126-134) is RMFPNAPYL (SEQ ID NO:260).

[00869] WT-1 (126-134 (R126Y)) is YMFPNAPYL (SEQ ID NO:264) and is also referred to as “126-134 mimotope.”

[00870] IL-2 “position 1” is depicted schematically in FIG. 19.

RESULTS

[00871] The ability of TMMPs to stimulate antigen-specific proliferation of CD8⁺ T cells was tested. The TMMPs included, as the epitope: i) WT1 37-45; ii) WT1 126-134; or iii) WT1 126-134 (R126Y). All TMMPs included A02 allele MHC class I heavy chains.

[00872] Peripheral blood mononuclear cells (PBMCs) obtained from human donors were incubated *in vitro* with the TMMPs at various concentrations (0 nM, 10 nM, 100 nM, 300 nM, or 1000 nM) for 10 days. After the 10-day incubation period, the number of cells specific for the epitope was determined. Data from PBMCs from two donors (“Leukopak 7 and Leukopak 12”) are shown in **FIG. 21**.

[00873] The data presented in FIG. 21 demonstrate, in two donors, that WT1-specific TMMPs can induce expansion of WT1-specific CD8⁺ T cells from total PBMCs over a course of a 10-day stimulation culture. This expansion was achieved in PBMCs that have low or no detectable WT1-specific T cell precursors, indicating that the TMMPs were able to induce antigen-specific responses in donors from an unprimed or naïve repertoire. The data demonstrate that TMMPs specific for any of the 3 selected WT1 peptides (37-45, 126-134, and 126-134 R126Y) and on either of the two tested disulfide frameworks (G2C and R12C/G2C) induce expansion of WT1-specific CD8⁺ T cells from total PBMCs.

[00874] PBMCs from different human donors (L7, L10, and L12) were stimulated for 10 days *in vitro* with the indicated WT1 peptides in the presence of recombinant human IL-2 and then re-stimulated for 8 days with TMMPs, containing the same peptides, and containing either the G2C disulfide bond or both the R12C and G2C disulfide bonds. The data are depicted in **FIG. 22**.

[00875] The data presented in FIG. 22 demonstrate, in PBMCs from three donors, that WT1-containing TMMPs can expand WT1-specific CD8⁺ T cells from total PBMCs over a course of an 8-day re-stimulation culture following a 10-day priming culture. This expansion occurred from cells that have a detectable number of WT1-specific T cell precursors, indicating that the TMMPs were able to expand antigen-specific T cells in donors with a primed/preexisting WT-1 specific T cell repertoire. The data demonstrate that TMMPs specific for any of the 3 WT1 peptides (37-45, 126-134, and 126-134 R126Y) and on either of the two tested disulfide frameworks (G2C and R12C/G2C) induce such expansions.

[00876] The ability of the CD8⁺ T cells expanded by contacting with TMMPs containing the WT1 37-45 peptide and containing either the G2C disulfide bond or both the R12C and G2C disulfide bonds, to produce TNF- α and IFN- γ in response to target cells (T2 cells) presenting the WT1-37-45 peptide or an irrelevant peptide (SL9) was tested. Phorbol 12-myristate 13-acetate (PMA) and ionomycin (“iono”) were used as a positive control. CD8⁺ T cells treated with media were used as a negative control. CD8⁺ T cells expanded by TMMPs were incubated with ‘target cells’ (T2 cells) that were loaded with either a WT1 peptide or with an irrelevant peptide (the SL9 peptide from HIV). The response, as indicated by production of IFN- γ and TNF- α , of the CD8⁺ T cells to the T2 cells pulsed with WT 37-45 peptide was compared to the response to T2 cells pulsed with SL9 peptide. The data are shown in **FIG. 23**.

[00877] The data presented in FIG. 23 demonstrate the selective polyfunctionality of the WT1 37-45-specific CD8⁺ T cells expanded with WT1 37-45 containing TMMPs having either the G2C or R12C/G2C framework. The response measured (TNF- α and IFN- γ production) was observed only upon recognition of target cells presenting the WT1 37-45 peptide but not the SL9 peptide. The positive and negative control wells show that there is no baseline activity in the CD8⁺ T cells in the absence of stimulation (as seen in the media-only wells) and that both antigen-specific and non-antigen-specific

cells are capable of showing functional responses upon strong, antigen-non-specific stimulation (PMA + ionomycin).

[00878] Using the same assay, the ability of the CD8⁺ T cells expanded by TMMPs containing the WT1 126-134 peptide ("WT1 126") and containing the R12C and G2C disulfide bonds, to produce TNF- α and IFN- γ in response to target cells (T2 cells) presenting the WT1-126-134 peptide, WT1-126-134 R126Y peptide or an irrelevant peptide (SL9) was tested. The data are shown in **FIG. 24**.

[00879] The data presented in FIG. 24 demonstrate the selective polyfunctionality of the WT1 126-134-specific CD8⁺ T cells expanded with WT1 126-134 specific Immuno-STATs on the R12C/G2C framework. The response measured (TNF- α and IFN- γ production) was observed only upon recognition of target cells presenting the WT1 126-134 peptide or the WT1 126-134 R126Y peptide but not the SL9 peptide. The positive and negative control wells show that there is no baseline activity in the CD8⁺ T cells in the absence of stimulation (as seen in the media-only wells) and that both antigen-specific and non-antigen-specific cells are capable of showing functional responses upon strong, antigen-non-specific stimulation (PMA + ionomycin).

[00880] The effect of disulfide bonds on IL-2-driven immune cell activation was tested. CD69 is an early activation marker on most lymphocytes and some other immune cells. Cells upregulate CD69 upon different types of stimulatory conditions, including IL-2 stimulation. CD69 upregulation on both NK cells, CD4⁺ T cells, and CD8⁺ T cells was assessed. CD69 upregulation demonstrates that the IL-2 polypeptides present in the TMMPs are active and that their function is attenuated compared to control (recombinant human IL-2). PBMCs from different human donors were incubated with various TMMPs ("ISTs") set out in the table. TMMPs comprising a CMV epitope or a MART-1 epitope were included as controls. Data from one human donor (Leukopak 6) are shown. The data are shown in **FIG. 25**. FIG. 25 shows the upregulation of CD69 on NK cells as a relevant and representative example of a cell that readily upregulates CD69 in response to IL-2. Similar data was observed on CD8⁺ T cell gates and CD4⁺ T cell gates.

[00881] The data presented in FIG. 25 demonstrates the IL-2 immunomodulatory polypeptide engineered on position 1 into HLA-A02-specific Immuno-STATs built on various disulfide frameworks (R12C, G2C and R12C/G2C) is functional (as observed by the induction of CD69 on the surface of a relevant immune cell) and attenuated compared to wild-type recombinant human IL-2.

[00882] To evaluate the potency of the variant IL-2 immunomodulatory polypeptides present in the TMMPs, a CTLL-2 proliferation assay was carried out. CTLL-2 cells are dependent on IL-2 for growth; thus, CTLL-2 proliferation is a measure of the amount and/or potency of IL-2 present in the culture medium (e.g., where the IL-2 is produced by T cells contacted with a TMMP). Gillis et al. (1978) *J. Immunol.* 120:2027.

[00883] The data are shown in **FIG. 26**. The data presented in FIG. 26 demonstrate that the IL-2 immunomodulatory polypeptide engineered on position 1 into HLA-A02-specific Immuno-STATs (TMMPs) built on various disulfide frameworks (R12C, G2C and R12C/G2C) is functional (as observed by the induction of CTLL-2 proliferation) and attenuated compared to proleukin.

[00884] The ability of the TMMPs used in these experiments to bind to FcRn was tested. The TMMPs include an Ig Fc region that can bind to FcRn. Binding to FcRn is an indication of prolonged *in vivo* half-life. Souders et al. (2015) *MAbs* 7:912. The data for the “1715 + 2380” TMPP are shown in **FIG. 27**.

[00885] The ability of the “1715 + 2380” TMPP to bind to other Fc receptors was tested. The “1715 + 2380” TMPP includes an Ig Fc region with LALA substitutions, which reduce binding of the Ig Fc to FcRI, RIIA, IIB, IIIA-F, and IIIB, thereby reducing Ig Fc-mediated effector functions. The data are shown in **FIG. 27**.

MATERIALS AND METHODS

FIG. 21

[00886] Leukopaks from two healthy donors were obtained using apheresis machines. Leukopaks were diluted with an equal volume of room temperature phosphate-buffered saline (PBS). PBMCs were isolated from diluted leukopaks by density gradient centrifugation as follows: 30 mL of diluted leukopak was underlayered with 13 mL of Ficoll-Paque in a 50 mL conical tube and centrifuged at 400 g for 30 minutes at room temperature in a swinging bucket rotor without brake. Mononuclear cell layer (lymphocytes, monocytes and thrombocytes) was collected from the plasma-Ficoll interface, transferred to new 50 mL conical tube and washed with 3-fold excess PBS by centrifugation at 300 g for 10 minutes at room temperature. After careful removal of supernatant, cells were resuspended and washed with 50 mL of PBS by centrifugation at 200 g for 10 minutes at room temperature to remove platelets. Upon washing and platelet removal, obtained PBMCs were pooled from the 50 mL tubes, resuspended in PBS, counted, pelleted by centrifugation at 300 g for 10 minutes and resuspended at a final concentration of 50×10^6 cells per ml in cell freezing media.

[00887] Human healthy donor PBMCs were prepared from two leukopaks as described above. On the day of the experiment, the cells were thawed in a 37°C water bath and washed in warm ImmunoCult™-XF Cell Expansion Media (Stemcell Technologies) by centrifugation at 350 x g for 6 minutes. The supernatant was removed, and the cells were resuspended in ImmunoCult™ media. Live cell count was assessed using the Countess automated cell counter (Invitrogen, CA). The media volume was adjusted to bring the cell concentration to 5×10^6 cells/ml and 2 mL of cells (equivalent to 10×10^6 cells) were seeded per well in a 6-well plate. PBMCs were stimulated with the indicated amounts of Immuno-STATs or with media alone in a total volume of 4 ml of media. Cells were stimulated for 10

days at 37°C, 5% CO₂ with media replacement on days 5 and 7 by aspirating 2 mL of culture supernatant from the wells and adding back 2 mL of fresh media.

[00888] Upon culture, the cells were harvested and pelleted by centrifugation at 350 x g for 5 minutes, live cell counts were determined by the Countess automated cell counter (Invitrogen, CA), and cells were processed for flow cytometry by staining with: a viability stain, appropriate WT1-peptide-specific HLA-A*0201 tetramers (MBL International) and antibodies against CD69, CD3, CD14, CD19, CD127, CD56, CD4 (Biolegend), CD8, CD25 (BD Biosciences) Stained cells were washed and analyzed by flow cytometry.

[00889] Data acquisition was performed using the Attune NxT flow cytometer instrument (Invitrogen). The acquired data was exported as fcs files and analyzed using the Flowjo software (Tree Star, OR).

[00890] Based on the frequency of antigen-specific T cells, volumes and events analyzed by flow cytometry and total volume and number of cells harvested at the end of the culture, the number of antigen specific T cells per well was calculated and plotted in the graphs shown.

FIG. 22

[00891] PBMCs from two donors were expanded for 10 days with 10 micrograms/ml of the indicated peptides and 50U/ml of recombinant human IL-2. Expansion was done in 6-well plates with a total of 10 million cells in 4 ml of Immunocult media per well. Cells were stimulated for 10 days at 37°C, 5% CO₂ with media replacement on days 5 and 7 by aspirating 2 mL of culture supernatant from the wells and adding back 2 mL of fresh media with 50 U/ml of recombinant human IL-2. Upon culture, the cells were harvested and pelleted by centrifugation at 350 g for 5 minutes, live cell counts were determined by the Countess automated cell counter (Invitrogen, CA). Each stimulation condition was performed in at least 3 wells of a six well plate. PBMCs from one well were used to estimate the frequency/amount of WT1-specific CD8+ T cells in the culture by flow cytometry upon staining with: a viability stain, appropriate WT1-peptide-specific HLA-A*0201 tetramers (MBL International) and antibodies against CD69, CD3, CD14, CD19, CD127, CD56, CD4 (Biolegend), CD8, CD25 (BD Biosciences). Stained cells were washed and analyzed by flow cytometry. At least two wells were used to enrich for CD8+ T cells using a CD8+ T cell negative selection kit from Stem Cell Technologies. The purified CD8+ T cells were restimulated for 8 days with the indicated TMMPs in the presence of autologous PBMCs, previously treated with mitomycin C, in a 1:2 ratio, with a final 5-10 million cells in a volume of 4 ml of Immunocult media per well. TMMPs were used at concentrations previously established to be optimal for the combination of a donor and a particular TMMP.

[00892] Upon culture, the cells were harvested and pelleted by centrifugation at 350 g for 5 minutes, live cell counts were determined by the Countess automated cell counter (Invitrogen, CA) and

processed for flow cytometry by staining with: a viability stain, appropriate WT1-peptide-specific HLA-A*0201 tetramers (MBL International) and antibodies against CD69, CD3, CD14, CD19, CD127, CD56, CD4 (Biolegend), CD8, CD25 (BD Biosciences) Stained cells were washed and analyzed by flow cytometry.

[00893] Data acquisition was performed using the Attune NxT flow cytometer instrument (Invitrogen). The acquired data was exported as fcs files and analyzed using the Flowjo software (Tree Star, OR).

[00894] Based on the frequency of antigen-specific T cells, volumes and events analyzed by flow cytometry and total volume and number of cells harvested at the end of the culture, the number of antigen specific T cells per well was calculated and plotted in the graphs shown.

FIG. 23

[00895] PBMCs from two donors were expanded for 10 days with 10 micrograms/ml of the WT1 37-45 peptide and 50U/ml of recombinant human IL-2. Expansion was done in 6-well plates with a total of 10 million cells in 4 ml of Immunocult media per well. Cells were stimulated for 10 days at 37°C, 5% CO₂ with media replacement on days 5 and 7 by aspirating 2 mL of culture supernatant from the wells and adding back 2 mL of fresh media with 50 U/ml of recombinant human IL-2. Upon culture, the cells were harvested and pelleted by centrifugation at 350 g for 5 minutes, live cell counts were determined by the Countess automated cell counter (Invitrogen, CA). The stimulation was performed in at least 3 wells of a six well plate. PBMCs from one well were used to estimate the frequency/amount of WT1 37-45 peptide-specific CD8+ T cells in the culture by flow cytometry upon staining with: a viability stain, appropriate WT1 37-45-peptide-specific HLA-A*0201 tetramers (MBL International) and antibodies against CD69, CD3, CD14, CD19, CD127, CD56, CD4 (Biolegend), CD8, CD25 (BD Biosciences). Stained cells were washed and analyzed by flow cytometry. Data acquisition was performed using the Attune NxT flow cytometer instrument (Invitrogen). The acquired data was exported as fcs files and analyzed using the Flowjo software (Tree Star, OR).

[00896] At least two wells were used to enrich for CD8+ T cells using a CD8+ T cell negative selection kit from Stem Cell Technologies. The purified CD8+ T cells were restimulated for 8 days with the indicated WT1 37-45 specific Immuno-STATs on either the G2C or the R12C/G2C framework in the presence of autologous PBMCs, previously treated with mitomycin C, in a 1:2 ratio, with a final 5-10 million cells in a volume of 4 ml of Immunocult media per well. WT1 37-45 specific Immuno-STAT on either the G2C or the R12C/G2C framework was used at the concentration previously established to be optimal for that donor.

[00897] Upon culture, the cells were harvested and pelleted by centrifugation at 350 g for 5 minutes, live cell counts were determined by the Countess automated cell counter (Invitrogen, CA) and CD8⁺ T cells were enriched using a CD8⁺ T cell negative selection kit from Stem Cell Technologies.

[00898] Target cells, T2 cells (ATCC), were pulsed with 5 µg/mL of the WT1 37-45 peptide or the human immunodeficiency virus-1 (HIV-1) Gag₇₇₋₈₅ SL9 peptide for 2 hours at 37°C, 5% CO₂. Post-peptide loading, the T2 cells were washed twice and resuspend in ImmunoCult™-XF Cell Expansion Media (Stemcell Technologies).

[00899] The enriched CD8⁺ T cells and the peptide-loaded T2 cells were mixed at a 1:1 ratio (1x10⁶ cells each) in a final volume of 200 µL per well in 96-well plates. Media and Phorbol 12-myristate; 13-acetate (PMA)/ionomycin was added to control wells as negative and positive controls, respectively. At 0.5 to 1 hour post-stimulation, the staining antibody against CD107a was added directly to the cells. Cell were stimulated for 5 hours, washed with PBS and stained for viability using the FVS780 for 10 minutes on ice. The cells were washed stained with the WT1 37-45 peptide-specific tetramers (labeled with APC and PE) for 15 minutes at room temperature. Subsequently, the cells were washed and stained antibodies against CD3 and CD8 for 30 minutes on ice. Stained cells were washed twice and resuspended in intracellular (IC) fixation buffer overnight at 4°C. The following day, the cells were washed and resuspended in permeabilization buffer and incubated for 5 minutes at room temperature. Permeabilized cells were washed and stained with antibodies against interferon-γ (IFN-γ), tumor-necrosis factor-α (TNF-α), resuspended in permeabilization buffer, for 30 minutes at room temperature. Stained cells were washed, resuspended in 2 mL of FACS buffer, and transferred to a 96-well deep plate. Data acquisition was performed using the Attune NxT flow cytometer instrument (Thermofisher Scientific, MA). The acquired data was exported as fcs files and analyzed using the Flowjo software (Tree Star, OR).

FIG. 24

[00900] PBMCs from two donors were expanded for 10 days with 10 micrograms/ml of the WT1 126-134 peptide and 50U/ml of recombinant human IL-2. Expansion was done in 6-well plates with a total of 10 million cells in 4 ml of Immunocult media per well. Cells were stimulated for 10 days at 37°C, 5% CO₂ with media replacement on days 5 and 7 by aspirating 2 mL of culture supernatant from the wells and adding back 2 mL of fresh media with 50 U/ml of recombinant human IL-2. Upon culture, the cells were harvested and pelleted by centrifugation at 350 g for 5 minutes, live cell counts were determined by the Countess automated cell counter (Invitrogen, CA). The stimulation was performed in at least 3 wells of a six well plate. PBMCs from one well were used to estimate the frequency/amount of WT1 126-134 peptide-specific CD8⁺ T cells in the culture by flow cytometry upon staining with: a viability stain, appropriate WT1 126-134-peptide-specific HLA-A*0201 tetramers (MBL International) and antibodies against CD69, CD3, CD14, CD19, CD127, CD56, CD4 (Biolegend), CD8, CD25 (BD

Biosciences). Stained cells were washed and analyzed by flow cytometry. Data acquisition was performed using the Attune NxT flow cytometer instrument (Invitrogen). The acquired data was exported as fcs files and analyzed using the Flowjo software (Tree Star, OR).

[00901] At least two wells were used to enrich for CD8⁺ T cells using a CD8⁺ T cell negative selection kit from Stem Cell Technologies. The purified CD8⁺ T cells were restimulated for 8 days with the WT1 126-134 specific Immuno-STATs on the R12C/G2C framework in the presence of autologous PBMCs, previously treated with mitomycin C, in a 1:2 ratio, with a final 5-10 million cells in a volume of 4 ml of Immunocult media per well. WT1 126-134 specific Immuno-STAT on the R12C/G2C framework was used at the concentration previously established to be optimal for that donor.

[00902] Upon culture, the cells were harvested and pelleted by centrifugation at 350 g for 5 minutes, live cell counts were determined by the Countess automated cell counter (Invitrogen, CA) and CD8⁺ T cells were enriched using a CD8⁺ T cell negative selection kit from Stem Cell Technologies.

[00903] Target cells, T2 cells (ATCC), were pulsed with 5 µg/mL of the WT1 126-134 peptide, the WT1 126-134 R126Y peptide or the human immunodeficiency virus-1 (HIV-1) Gag⁷⁷⁻⁸⁵ SL9 peptide for 2 hours at 37°C, 5% CO₂. Post-peptide loading, the T2 cells were washed twice and resuspend in ImmunoCult™-XF Cell Expansion Media (Stemcell Technologies).

[00904] The enriched CD8⁺ T cells and the peptide-loaded T2 cells were mixed at a 1:1 ratio (1x10⁶ cells each) in a final volume of 200 µL per well in 96-well plates. Media and Phorbol 12-myristate; 13-acetate (PMA)/ionomycin was added to control wells as negative and positive controls, respectively. At 0.5 to 1 hour post-stimulation, the staining antibody against CD107a was added directly to the cells. Cell were stimulated for 5 hours, washed with PBS and stained for viability using the FVS780 for 10 minutes on ice. The cells were washed stained with the WT1 126-134 peptide-specific tetramers (labeled with APC and PE) for 15 minutes at room temperature. Subsequently, the cells were washed and stained antibodies against CD3 and CD8 for 30 minutes on ice. Stained cells were washed twice and resuspended in intracellular (IC) fixation buffer overnight at 4°C. The following day, the cells were washed and resuspended in permeabilization buffer and incubated for 5 minutes at room temperature. Permeabilized cells were washed and stained with antibodies against interferon-γ (IFN-γ), tumor-necrosis factor-α (TNF-α), resuspended in permeabilization buffer, for 30 minutes at room temperature. Stained cells were washed, resuspended in 2 mL of FACS buffer, and transferred to a 96-well deep plate. Data acquisition was performed using the Attune NxT flow cytometer instrument (Thermofisher Scientific, MA). The acquired data was exported as fcs files and analyzed using the Flowjo software (Tree Star, OR).

FIG. 25

[00905] Human healthy donor PBMCs were prepared from leukopaks obtained from Hemacare (Northridge, CA) and kept cryopreserved at -150°C until the day of experiment.

[00906] The cells were thawed on the day of the experiment in a water bath for 1 minute, washed with 10 mL of warm ImmunoCult™-XF Cell Expansion Media (Stemcell Technologies, Vancouver, Canada), pelleted by centrifugation (350 g, 5 minutes), and resuspended in 10 mL media. Cells were counted using the Countess automated cell counter (Invitrogen, CA), the media volume was adjusted to bring the cell concentration to 3.8×10^6 cell/mL and 237.5 uL of the cell suspensions were added into round bottom 96-well plates.

[00907] 20X dilution series of the indicated Immuno-STATs and of rh-IL-2, were prepared in Immuncult media. To stimulate the PBMCs, 12.5 µL of the 20X dilution series was added to the wells containing the cells and mixed to obtain the final assay drug concentrations. The PBMCs were incubated at 37°C, 5% CO₂ for 20 to 24 hours.

[00908] Upon stimulation, the cells were pelleted by centrifugation at 350 g for 5 minutes. Supernatants were collected, frozen and stored at -20°C until further analysis. Pelleted PBMCs were washed twice with PBS and stained for 10 minutes at 4°C in 50 µL of Fixable live/dead FVS780 stain. The staining was quenched with 200 µL of stain buffer and the cells were pelleted by centrifugation (350 x g, 5 minutes). The cells were stained for 30 minutes at 4°C with antibodies against CD3, CD4, CD8, CD14, CD19, CD56, and CD69 in 50 µL volume. Upon staining cells were washed with stain buffer, pelleted by centrifugation (350 x g, 5 minutes), resuspended in 130 µL of stain buffer and analyzed by Flow Cytometry using the Attune® Flow Cytometer. The acquired data was exported as fcs files and analyzed using the Flowjo software (Tree Star, OR).

[00909] CD69 upregulation was assessed on different cell subsets that are sensitive to upregulate CD69 upon IL-2 stimulation. Based on expression levels of the surface markers used in the staining, gates were made to identify NK cells, CD8+ T cells, CD4+ T cells.

FIG. 26

[00910] One day before the assay, CTLL-2 cells were washed with media and cultured at 1×10^5 cells /ml in a 75-T flask for 24 hours at 37C, 5% CO₂ for IL-2 starvation. After the 24 hour starvation culture, cells were seeded at 5000 cells per well in 100 microliters/well of a 96 well cell culture cluster flat bottom plate with lid (Costar corning, Cat # 3599). Cell viability and count were checked before stimulation using Vi cell viability analyzer (Beckman-Coulter).

[00911] Dilution series (10 points; 3-fold dilution steps) of the indicated Immuno-STATs (TMMPs) or proleukin (Prometheus Therapeutics) were prepared in complete RPMI supplemented with 10% HI FBS as 2X stocks of the final assay concentrations. 100 µL of this 2X dilution series were added

to cells previously seeded in 96 well plates and mixed to obtain the final assay drug concentrations. Each concentration was tested in triplicates. Cells were incubated for three days at 37°C, 5% CO₂.

[00912] After three days in culture 100 µL of cells from each well was transferred into a flat bottom white tissue culture treated 96 well plate. 100 µL of CellTiter-Glo® Reagent was prepared using CellTiter-Glo Luminescent Cell viability assay kit (Promega cat # G7571) following instructions provided by the manufacturer and were added to the cells. Cells and CellTiter-Glo® Reagent were mixed by placing the plates on an orbital shaker for 2 minutes to induce cell lysis. Then plates were incubated at room temperature for 10 minutes to stabilize luminescent signal. The luminescence was measured and recorded on Biotek synergy neo2 multimode reader, software Gen5 3.04.

FIG. 27

[00913] All experiments were performed on the Octet HTX system (ForteBio). Anti-penta-his (HIS1K) kinetic grade biosensors (ForteBio, #18-5122) were hydrated in assay buffer and preconditioned in pH 1.7 glycine. The assay buffer was used for all assays except for FcRn. The buffer used for FcRn was PBS, 0.1% bovine serum albumin (BSA), 0.02% Tween-20, pH 7.2. The assay buffer used for the FcRn reagents was PBS, 0.1% BSA, 0.02% Tween-20, pH 6.

[00914] Each His-tagged receptor was immobilized onto HIS1K biosensors at a concentration of 5 µg/mL (except for FcRI: 10 µg/mL) for 120 seconds. The antigen-loaded HIS1K biosensors were then dipped into a 7-point, 1:3 dilution series of each individual antibody starting from 300 nM. A well containing only assay buffer was used to test for non-specific binding between the buffer and loaded biosensors. Association was observed for 60 seconds, followed by 60 seconds of dissociation. A short baseline (60 seconds) was established using dissociation buffer after HIS1K loading.

Example 3: Biochemical characterization of TMMPs comprising WT1 epitopes, HLA-A*24 heavy chains, either one or two disulfide bonds between the 2 polypeptide chains of the heterodimer, and variant IL-2 immunomodulatory polypeptides at position 1, 3, or 5.

[00915] The constructs used in this study are summarized in Table 6.

Table 6

Constructs	Epitope	S-S bond(s)	IL-2 position
3593 + 3192	WT1 (235-243 (M236Y))	G2C	1
3425 + 3188	WT1 (235-243 (M236Y))	R12C + G2C	3
3426 + 3192	WT1 (235-243 (M236Y))	G2C	3
3593 + 3530	WT1 (239-247 (Q240Y))	G2C	1
3592 + 3529	WT1 (239-247 (Q240Y))	R12C + G2C	1
3426 + 3530	WT1 (239-247 (Q240Y))	G2C	3
3425 + 3529	WT1 (239-247 (Q240Y))	R12C + G2C	3

3426 + 3530	WT1 (239-247 (Q240Y))	R12C	3
3197 + 3710	WT1 (239-247 (Q240Y))	G2C	5
3196 + 3709	WT1 (239-247 (Q240Y))	R12C + G2C	5
2764 + 3708	WT1 (239-247 (Q240Y))	R12C	5

[00916] Amino acid sequences of the polypeptide chains of the constructs are provided in FIG. 10A-10R.

[00917] “G2C” indicates that the TMMP includes a disulfide bond between: i) a Cys in the peptide linker between the peptide epitope and the β 2M polypeptide; and ii) a Cys at position 84 of the MHC class I heavy chain, where the MHC class I heavy chain has a Y84C substitution.

[00918] “R12C” indicates that the TMMP includes a disulfide bond between: i) a Cys at position 12 in the β 2M polypeptide, where the β 2M polypeptide has an R12C substitution; and ii) a Cys at position 236 of the MHC class I heavy chain, where the MHC class I heavy chain has an A236C substitution.

[00919] “G2C + R12C” indicates that the TMMP includes both the “G2C” disulfide bond and the “R12C” disulfide bond.

[00920] WT1 (235-243 (M236Y)) is CYTWNQMNL (SEQ ID NO:262), and is also referred to as “235 mimotope.”

[00921] WT1 (239-247 (Q240Y)) is NYMNLGATL (SEQ ID NO:263).

[00922] IL-2 “position 1,” “position 3,” and “position 5” are depicted schematically in FIG. 19.

MATERIALS AND METHODS

Effect of TMMP on number of antigen-specific (AgS) CD8⁺ T cells

[00923] Leukopaks from healthy donors were obtained using apheresis machines. Leukopaks were diluted with an equal volume of room temperature phosphate-buffered saline (PBS). PBMCs were isolated from diluted leukopaks by density gradient centrifugation as follows: 30 mL of diluted leukopak was underlaid with 13 mL of Ficoll-Paque in a 50 mL conical tube and centrifuged at 400 g for 30 minutes at room temperature in a swinging bucket rotor without brake. Mononuclear cell layer (lymphocytes, monocytes and thrombocytes) was collected from the plasma-Ficoll interface, transferred to new 50 mL conical tube and washed with 3-fold excess PBS by centrifugation at 300 g for 10 minutes at room temperature. After careful removal of supernatant, cells were resuspended and washed with 50 mL of PBS by centrifugation at 200 g for 10 minutes at room temperature to remove platelets. Upon washing and platelet removal, obtained PBMCs were pooled from the 50 mL tubes, resuspended in PBS, counted, pelleted by centrifugation at 300 g for 10 minutes and resuspended at a final concentration of 50×10^6 cells per ml in cell freezing media.

[00924] On the day of the experiment, the cells were thawed in a 37°C water bath and washed in warm ImmunoCult™-XF Cell Expansion Media (Stemcell Technologies) by centrifugation at 350 x g for 6 minutes. The supernatant was removed, and the cells were resuspended in ImmunoCult™ media. Live cell count was assessed using the Countess automated cell counter (Invitrogen, CA). The media volume was adjusted to bring the cell concentration to 5x10⁶ cells/ml and 2 mL of cells (equivalent to 10x10⁶ cells) were seeded per well in a 6-well plate. PBMCs were stimulated with the indicated amounts of Immuno-STATs or with media alone in a total volume of 4 ml of media. Cells were stimulated for 10 days at 37°C, 5% CO₂ with media replacement on days 5 and 7 by aspirating 2 mL of culture supernatant from the wells and adding back 2 mL of fresh media.

[00925] Upon culture, the cells were harvested and pelleted by centrifugation at 350 x g for 5 minutes, live cell counts were determined by the Countess automated cell counter (Invitrogen, CA), and cells were processed for flow cytometry by staining with: a viability stain, appropriate WT1-peptide-specific HLA-A*2402 tetramers (MBL International) and antibodies against CD69, CD3, CD14, CD19, CD127, CD56, CD4 (Biolegend), CD8, CD25 (BD Biosciences). Stained cells were washed and analyzed by flow cytometry.

[00926] Data acquisition was performed using the Attune NxT flow cytometer instrument (Invitrogen). The acquired data was exported as fcs files and analyzed using the Flowjo software (Tree Star, OR).

[00927] Based on the frequency of antigen-specific T cells, volumes and events analyzed by flow cytometry and total volume and number of cells harvested at the end of the culture, the number of antigen specific T cells per well was calculated and plotted in the graphs shown.

[00928] Peripheral blood mononuclear cells (PBMCs) obtained from human donors (designated “Leukopak 7”, “Leukopak 18”, and “Leukopak 6”) were incubated *in vitro* with the TMMPs at various concentrations (0 nM, 10 nM, 100 nM, 300 nM, or 1000 nM) for 10 days. After the 10-day incubation period, the number of cells specific for the epitope was determined.

Effect of disulfide bonds on IL-2-driven immune cell activation

[00929] The effect of disulfide bonds on IL-2-driven immune cell activation was tested. CD69 is an early activation marker on most lymphocytes and some other immune cells. Cells upregulate CD69 upon different types of stimulatory conditions, including IL-2 stimulation. CD69 upregulation on both NK cells, CD4⁺ T cells, and CD8⁺ T cells was assessed. CD69 upregulation demonstrates that the IL-2 polypeptides present in the TMMPs are active and that their function is attenuated compared to control (recombinant human IL-2). PBMCs from different human donors were incubated with various TMMPs (“ISTs”) set out in the table in FIG. 13 and FIG. 14. TMMPs comprising a CMV epitope or a MART-1 epitope were included as controls.

Effect of variant IL-2 on CTLL-2 proliferation

[00930] To evaluate the potency of the variant IL-2 immunomodulatory polypeptides present in the TMMPs, a CTLL-2 proliferation assay was carried out. CTLL-2 cells are dependent on IL-2 for growth; thus, CTLL-2 proliferation is a measure of the amount and/or potency of IL-2 present in the culture medium (e.g., where the IL-2 is produced by T cells contacted with a TMMP). Gillis et al. (1978) *J. Immunol.* 120:2027.

[00931] One day before the assay, CTLL-2 cells were washed with media and cultured at 1×10^5 cells /ml in a 75-T flask for 24 hours at 37C, 5% CO₂ for IL-2 starvation. After the 24 hour starvation culture, cells were seeded at 5000 cells per well in 100 microliters/well of a 96 well cell culture cluster flat bottom plate with lid (Costar corning, Cat # 3599). Cell viability and count were checked before stimulation using Vi cell viability analyzer (Beckman-Coulter).

[00932] Dilution series (10 points; 3-fold dilution steps) of the indicated Immuno-STATs (TMMPs) or proleukin (Prometheus Therapeutics) were prepared in complete RPMI supplemented with 10% HI FBS as 2X stocks of the final assay concentrations. 100 μ L of this 2X dilution series were added to cells previously seeded in 96 well plates and mixed to obtain the final assay drug concentrations. Each concentration was tested in triplicates. Cells were incubated for three days at 37C, 5% CO₂.

[00933] After three days in culture 100 μ L of cells from each well was transferred into a flat bottom white tissue culture treated 96 well plate. 100 μ L of CellTiter-Glo® Reagent was prepared using CellTiter-Glo Luminescent Cell viability assay kit (Promega cat # G7571) following instructions provided by the manufacturer and were added to the cells. Cells and CellTiter-Glo® Reagent were mixed by placing the plates on an orbital shaker for 2 minutes to induce cell lysis. Then plates were incubated at room temperature for 10 minutes to stabilize luminescent signal. The luminescence was measured and recorded on Biotek synergy neo2 multimode reader, software Gen5 3.04.

Binding to Fc receptors

[00934] All experiments were performed on the Octet HTX system (ForteBio). Anti-penta-his (HIS1K) kinetic grade biosensors (ForteBio, #18-5122) were hydrated in assay buffer and preconditioned in pH 1.7 glycine. The assay buffer was used for all assays except for FcRn. The buffer used for FcRn was PBS, 0.1% bovine serum albumin (BSA), 0.02% Tween-20, pH 7.2. The assay buffer used for the FcRn reagents was PBS, 0.1% BSA, 0.02% Tween-20, pH 6.

[00935] Each His-tagged receptor was immobilized onto HIS1K biosensors at a concentration of 5 μ g/mL (except for FcRI: 10 μ g/mL) for 120 seconds. The antigen-loaded HIS1K biosensors were then dipped into a 7-point, 1:3 dilution series of each individual antibody starting from 300 nM. A well containing only assay buffer was used to test for non-specific binding between the buffer and loaded

biosensors. Association was observed for 60 seconds, followed by 60 seconds of dissociation. A short baseline (60 seconds) was established using dissociation buffer after HIS1K loading.

RESULTS

[00936] The results are shown in **FIG. 28-34**.

[00937] The data presented in FIG. 28 and FIG. 29 demonstrate that TMMPs comprising WT1 235-243 R236Y, with immunomodulatory polypeptides (IL-2) at various positions and with either a single disulfide bond or with two disulfide bonds, can expand WT1-specific CD8+ T cells from total PBMCs over a course of a 10-day stimulation culture. This expansion occurred from PBMCs that have low or no detectable WT1-specific T cell precursors, indicating the ability of these TMMPs to induce antigen-specific responses in donors from an unprimed or naïve repertoire.

[00938] The data presented in FIG. 30 demonstrate that the IL-2 polypeptide engineered at position 1 or 3 of WT1 235-243 M236Y -specific HLA-A24 TMMPs built on various disulfide frameworks (G2C and R12C/G2C) is functional (as observed by the induction of CD69 on the surface of a relevant immune cell), comparable to the activity of CMV and MART-1 specific HLA-A02 TMMPs and attenuated compared to wild-type recombinant human IL-2.

[00939] The data presented in FIG. 31 demonstrate the IL-2 polypeptide engineered at position 1, 3 or 5 of WT1 239-247 Q240Y -specific HLA-A24 TMMPs built on various disulfide frameworks (R12C, G2C and R12C/G2C) is functional (as observed by the induction of CD69 on the surface of a relevant immune cell) comparable to the activity of CMV and MART-1 specific HLA-A02 Immuno-STATs and attenuated compared to wild-type recombinant human IL-2.

[00940] The data presented in FIG. 32 demonstrate that the IL-2 polypeptide engineered at position 1 or 3 of WT1 235-243 M236Y -specific HLA-A24 TMMPs built on various disulfide frameworks (G2C and R12C/G2C) is functional (as observed by the induction of CTLL-2 proliferation) and attenuated compared to proleukin and attenuated compared to wild-type recombinant human IL-2.

[00941] The data presented in FIG. 33 demonstrate that the IL-2 polypeptide engineered at position 1, 3 or 5 of WT1 239-247 Q240Y -specific HLA-A24 TMMPs built on various disulfide frameworks (R12C, G2C and R12C/G2C) is functional (as observed by the induction of CTLL-2 proliferation), and attenuated compared to wild-type recombinant human IL-2.

[00942] The data presented in FIG. 34 demonstrate the ability of the “3425 + 3529” TMPP to bind to FcRn. The “3425 + 3529” TMPP includes an Ig Fc region with LALA substitutions. As shown in FIG. 34, the “3425 + 3529” TMPP exhibits reduced binding of the Ig Fc to FcRI, RIIA, IIB, IIIA-F, and IIIB.

Example 4

[00943] A TMMP comprising 1715 (without C-terminal Lys, i.e., 1715Δ having the sequence set forth in SEQ ID NO:486) + 2380 polypeptides was tested. The amino acid sequences of the polypeptide chains are provided in **FIG. 14J** (1715 without C-terminal lysine, i.e., 1715Δ) and **FIG. 14B** (2380). The 1715Δ + 2380 TMMP includes: i) Class I HLA-A heavy chain polypeptides of the A02:01 allele; and ii) two copies of IL2 (H16A; F42A) immunomodulatory (“MOD”) polypeptides. The 2380 polypeptide comprises the WT1 peptide WT1(37-45). The 1715Δ -2380 TMMP is a homodimer of a heterodimer comprising the 1715Δ polypeptide and the 2380 polypeptide. Thus, the TMMP included: i) 2 copies of the 1715Δ + 2380 heterodimer, linked by 2 disulfide bonds between the IgFc polypeptide present in the 1715Δ polypeptides.

[00944] TMMP 1715Δ + 2380 is a double disulfide-linked heterodimer: a) a first disulfide linkage is between: i) the Cys present in the linker between the WT1 peptide and the β2M chain in the 2380 polypeptide; and ii) the Cys introduced by the Y84C substitution in the Class I heavy chain present in the 1715Δ polypeptide; and b) a second disulfide linkages is between: i) the Cys introduced by the R12C substitution in the β2M polypeptide present in the 2380 polypeptide; and ii) the Cys introduced by the A236C substitution in the Class I heavy chain present in the 1715Δ polypeptide.

[00945] The effect of TMMP 1715Δ + 2380 on PBMCs was tested. The data are shown in **FIG. 39-42**. TMMP 1715Δ + 2380 is also referred to below as “CUE-102/A02 WT1₃₇₋₄₅ Immuno-STAT,” “CUE-102/A02 WT1₃₇₋₄₅ IST,” or simply “CUE-102/A02.”

[00946] As shown in **FIG. 39**, CUE-102/A02 WT1₃₇₋₄₅ IST induces expansion of WT1₃₇₋₄₅-specific CD8⁺ T cells from unprimed PBMCs. Healthy donor PBMCs were stimulated for 10 days with the CUE-102/A02 WT1₃₇₋₄₅ Immuno-STAT (IST) in Immunocult™ media. Cells cultured in the absence of CUE-102/A02 were used as a negative control. Peptide-specific CD8⁺ T cells were detected by flow cytometry upon staining with WT1₃₇₋₄₅-specific tetramers.

[00947] As shown in **FIG. 40A-40B**, CUE-102/A02 WT1₃₇₋₄₅ IST induces expansion of WT1₃₇₋₄₅-specific CD8⁺ T cells from primed PBMCs. Healthy donor PBMCs were primed for 10 days with WT1₃₇₋₄₅ peptide in the presence of recombinant human IL-2. CD8⁺ T cells were then enriched by magnetic separation and restimulated with the CUE-102/A02 WT1₃₇₋₄₅ IST in Immunocult™ media in the presence of mitomycin C-treated autologous PBMCs for 8 days. Cells restimulated in the absence of CUE-102/A02 were used as a negative control. Peptide-specific CD8⁺ T cells were detected by flow cytometry upon staining with WT1₃₇₋₄₅-specific tetramers.

[00948] As shown in **FIG. 41A-41B**, the reduced affinity IL-2-containing CUE-102/A02 WT1₃₇₋₄₅ IST mitigates the risk associated with systemic IL-2 activation, compared to wild-type IL-2. Five healthy donor PBMCs were stimulated with Proleukin® (IL-2), or CUE-102/A02 WT1₃₇₋₄₅ IST, in

Immunocult™ media for 18 hours. Upon stimulation, supernatants were harvested, and levels of TNF- α , IL-6, and IFN- γ were assessed by immunoassay (FIG. 41A). NK cell, CD4⁺ T cell, and CD8⁺ T cell CD69 upregulation was assessed by flow cytometry on cells from the same culture wells (FIG. 41B). Cells cultured in the absence of Proleukin® (IL-2), or CUE-102/A02 WT1₃₇₋₄₅ IST were used as negative control.

[00949] As shown in **FIG. 42A-42B**, CUE-102/A02 WT1₃₇₋₄₅ IST-expanded T cells are polyfunctional CTLs that recognize and kill WT1₃₇₋₄₅ peptide-presenting target cells. Healthy donor PBMCs were primed for 10 days with WT1₃₇₋₄₅ peptide in the presence of recombinant human IL-2 and expanded for 8 days with WT1₃₇₋₄₅ peptide or with CUE-102/A02 WT1₃₇₋₄₅ IST in Immunocult™ media in the presence of mitomycin C-treated autologous PBMCs. WT1₃₇₋₄₅-specific CD8⁺ T cells were enriched by magnetic bead-based separation using WT1₃₇₋₄₅-specific PE-labeled tetramers. FIG. 42A: CUE-102/A02 WT1₃₇₋₄₅ IST-expanded WT1₃₇₋₄₅-specific T cells expressed effector molecules IFN- γ and TNF- α ; and up-regulated the degranulation marker CD107a upon 4 hours of interaction with target T2 cells pulsed with the cognate WT1₃₇₋₄₅ peptide, but not with a control, irrelevant peptide (SL9). FIG. 42B: Expanded WT1₃₇₋₄₅-specific T cell killed cognate WT1₃₇₋₄₅ peptide-pulsed T2 cells, but not control peptide-pulsed T2 cells, in overnight cultures performed at different T cell effector:target cell ratios. Specific killing was assessed by flow cytometry comparing the ratio of viable T2 cell pulsed with cognate peptide vs. control peptide upon overnight culture.

Example 5

[00950] The ability of TMMPs to stimulate antigen-specific proliferation of CD8⁺ T cells was tested. The TMMPs included, as the epitope: i) WT1 235-243(C235S; M236Y); or ii) WT1 239-247(Q240Y). All TMMPs included A24 allele MHC class I heavy chains.

[00951] The ability of TMMPs to stimulate antigen-specific proliferation of CD8⁺ T cells was tested. The TMMPs included, as the epitope: i) WT1 235-243(C235S; M236Y); or ii) WT1 239-247(Q240Y). All TMMPs included A24 allele MHC class I heavy chains. The TMMPs included: a) a “heavy chain” polypeptide comprising: i) a Class I HLA-A heavy chain polypeptide of the A24:02 allele comprising Y84C and A236C substitutions; ii) two copies of IL2 (H16A; F42A) immunomodulatory (“MOD”) polypeptides; and iii) IgG1 Fc polypeptide comprising L234A and L235A substitutions; and b) a “light chain” polypeptide construct 3973 (FIG. 37B) or 3529 (FIG. 20M) comprising: i) either WT1 235-243(C235S; M236Y) or WT1 239-247(Q240Y); and ii) a beta-2 microglobulin polypeptide comprising an R12C substitution. The heavy and light chain polypeptides were joined by 2 disulfide bonds. The “heavy chain” comprised the amino acid sequence of chain 3425 as depicted in FIG. 20B. The TMMPs comprised homodimers of the “heavy” and “light” chain heterodimers, joined by disulfide bonds formed between the respective IgG1 Fc regions.

[00952] Peripheral blood mononuclear cells (PBMCs) obtained from human donors were incubated *in vitro* with the TMMPs at various concentrations (0 nM, 10 nM, 100 nM, 300 nM, or 1000 nM) for 10 days. After the 10-day incubation period, the number of cells specific for the epitope was determined. Data from PBMCs from healthy human donors (Leukopak 22 (“L22”); Leukopak 24 (“L24”); Leukopak 29 (“L29”); and Leukopak 31 (“L31”)) are shown in **FIG. 43**. Data from PBMCs from healthy human donors (Leukopak 24 (“L24”); Leukopak 30 (“L30”); and Leukopak 31 (“L31”)) are shown in **FIG. 44**.

[00953] The data presented in FIG. 43 and FIG. 44 demonstrate that WT1-specific TMMPs can induce expansion of WT1-specific T cells from total PBMCs over a course of a 10-day stimulation culture.

MATERIALS AND METHODS

[00954] Leukopaks from healthy donors were obtained using apheresis machines. Leukopaks were diluted with an equal volume of room temperature phosphate-buffered saline (PBS). PBMCs were isolated from diluted leukopaks by density gradient centrifugation as follows: 30 mL of diluted leukopak was underlaid with 13 mL of Ficoll-Paque in a 50 mL conical tube and centrifuged at 400 g for 30 minutes at room temperature in a swinging bucket rotor without brake. Mononuclear cell layer (lymphocytes, monocytes and thrombocytes) was collected from the plasma-Ficoll interface, transferred to new 50 mL conical tube and washed with 3-fold excess PBS by centrifugation at 300 g for 10 minutes at room temperature. After careful removal of supernatant, cells were resuspended and washed with 50 mL of PBS by centrifugation at 200 g for 10 minutes at room temperature to remove platelets. Upon washing and platelet removal, obtained PBMCs were pooled from the 50 mL tubes, resuspended in PBS, counted, pelleted by centrifugation at 300 g for 10 minutes and resuspended at a final concentration of 50×10^6 cells per ml in cell freezing media.

[00955] Human healthy donor PBMCs were prepared from two leukopaks as described above. On the day of the experiment, the cells were thawed in a 37°C water bath and washed in warm ImmunoCult™-XF Cell Expansion Media (Stemcell Technologies) by centrifugation at 350 x g for 6 minutes. The supernatant was removed, and the cells were resuspended in ImmunoCult™ media. Live cell count was assessed using the Vi-Cell XR automated cell counter (Beckman-Coulter). The media volume was adjusted to bring the cell concentration to 5×10^6 cells/ml and 2 mL of cells (equivalent to 10×10^6 cells) were seeded per well in a 6-well plate. PBMCs were stimulated with the indicated amounts of Immuno-STATs, peptide (10 µg/mL) and IL-2 (50 IU/mL), or with media alone in a total volume of 4 mL of media. Cells were stimulated for 10 days at 37°C, 5% CO₂ with media replacement on days 5 and 7 by aspirating 2 mL of culture supernatant from the wells and adding back 2 mL of fresh media.

[00956] Upon culture, the cells were harvested and pelleted by centrifugation at 350 x g for 5 minutes, live cell counts were determined by the Vi-Cell XR automated cell counter (Beckman-Coulter). and cells were processed for flow cytometry by staining with: a viability stain, appropriate WT1-peptide-specific HLA-A*24:02 tetramers (MBL International) and antibodies against CD3, CD14, CD19, CD56, CD4 (Biolegend), CD8, (BD Biosciences) Stained cells were washed and analyzed by flow cytometry.

[00957] Data acquisition was performed using the Attune NxT flow cytometer instrument (Invitrogen). The acquired data was exported as fcs files and analyzed using the Flowjo software (Tree Star, OR).

[00958] The absolute number of antigen specific CD8⁺ T cells was plotted in the graphs shown, depicting expansion of antigen specific cells as a function of Immuno-STAT concentration.

Example 6

[00959] Naïve HLA-A2 (AAD) transgenic mice were dosed intravenously once weekly with 30 mg/kg of TMMPs comprising homodimers formed from heterodimers 1715-2380 ("TMMP 1715-2380") for a total of three doses. The first dose consisted of a TMMP 1715-2380 generated from a transiently transfected cell line, while the subsequent two doses consisted of TMMP 1715-2380 generated from a stable cell line. The frequency of WT1 37-45-specific CD8⁺ T cells was then measured in peripheral blood mononuclear cells (PBMCs) 7 days after the last dose. Isolated PBMCs were re-stimulated with WT1 37-45 peptide for 5 hours at 37°C in the presence of protein transport inhibitors and anti-CD107a antibody to measure degranulation. Cells were then surface stained with WT1 37-45/A02 tetramer, viability dye, and cell surface markers including CD3, CD4, CD8, CD45, CD11b, CD19, and CD44, followed by intracellular staining for IFN- γ , TNF- α , and granzyme B. Antigen-specific cells were detected by analyzing the frequency of tetramer⁺ cells within the CD8⁺ T cell population (defined as single, live, CD11b⁻, CD19⁻, CD45⁺, CD3⁺, CD4⁻). The frequency of WT1 37-45-specific (" % AgS") CD8⁺ T cells in treated mice was found to be greater than that observed in naïve mice that were not treated with TMMP 1715-2380 (see FIG. 45).

[00960] While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

CLAIMS

What is claimed is:

1. A T-cell modulatory multimeric polypeptide comprising:
at least one heterodimer comprising:
 - a) a first polypeptide comprising:
 - i) a Wilms tumor-1 (WT-1) peptide epitope comprising the amino acid sequence $X_1X_2X_3\text{TNQMNL}$ (SEQ ID NO:460) or $X_2X_3\text{TNQMNL}$ (SEQ ID NO:461), wherein each of X_1 , X_2 , and X_3 is independently any amino acid, with the proviso that the N-terminal amino acid is not a Cys, and wherein the WT-1 peptide epitope has a length from 9 to 25 amino acids; and
 - ii) a first Class I major histocompatibility complex (MHC) polypeptide;
 - b) a second polypeptide comprising a second class I MHC polypeptide, and
 - c) at least one activating immunomodulatory polypeptide,wherein the first and/or the second polypeptide comprises the at least one immunomodulatory polypeptide, and optionally wherein the first or the second polypeptide comprises an immunoglobulin (Ig) Fc polypeptide.
2. A T-cell modulatory multimeric polypeptide of claim 1, wherein at least one of the one or more immunomodulatory polypeptides is a variant immunomodulatory polypeptide that exhibits reduced affinity to a cognate co-immunomodulatory polypeptide compared to the affinity of a corresponding wild-type immunomodulatory polypeptide for the cognate co-immunomodulatory polypeptide.
3. A T-cell modulatory multimeric polypeptide of claim 2, wherein the ratio of the binding affinity of the wild-type immunomodulatory polypeptide to a cognate co-immunomodulatory polypeptide to the binding affinity of the variant immunomodulatory polypeptide to the cognate co-immunomodulatory polypeptide, when measured by bio-layer interferometry, is at least 1.5:1.
4. A T-cell modulatory multimeric polypeptide of claim 2 or 3, wherein the variant immunomodulatory polypeptide binds the co-immunomodulatory polypeptide with an affinity selected from the group consisting of from about 10^{-4} M to about 10^{-7} M, from about 10^{-4} M to about 10^{-6} M, and from about 10^{-4} M to about 10^{-5} M.
5. A T-cell modulatory multimeric polypeptide of any one of claims 1-4, wherein:
 - a1) the first polypeptide comprises, in order from N-terminus to C-terminus:

- i) the WT-1 peptide epitope; and
 - ii) the first MHC polypeptide; and
- b1) the second polypeptide comprises, in order from N-terminus to C-terminus:
 - i) the at least one immunomodulatory polypeptide;
 - ii) the second MHC polypeptide; and
 - iii) an Ig Fc polypeptide; or
- a2) the first polypeptide comprises, in order from N-terminus to C-terminus:
 - i) the WT-1 peptide epitope; and
 - ii) the first MHC polypeptide; and
- b2) the second polypeptide comprises, in order from N-terminus to C-terminus:
 - i) the second MHC polypeptide;
 - ii) the at least one immunomodulatory polypeptide; and
 - iii) an Ig Fc polypeptide; or
- a3) the first polypeptide comprises, in order from N-terminus to C-terminus:
 - i) the WT-1 peptide epitope; and
 - ii) the first MHC polypeptide; and
- b3) the second polypeptide comprises, in order from N-terminus to C-terminus:
 - i) the second MHC polypeptide;
 - ii) an Ig Fc polypeptide; and
 - iii) the at least one immunomodulatory polypeptide; or
- a4) the first polypeptide comprises, in order from N-terminus to C-terminus:
 - i) the at least one immunomodulatory polypeptide;
 - ii) the WT-1 peptide epitope;
 - iii) the first MHC polypeptide; and
- b4) the second polypeptide comprises, in order from N-terminus to C-terminus:
 - i) the second MHC polypeptide; and
 - ii) the Ig Fc polypeptide; or
- a5) the first polypeptide comprises, in order from N-terminus to C-terminus:
 - i) the WT-1 peptide epitope;
 - ii) the first MHC polypeptide; and
 - iii) the at least one immunomodulatory polypeptide; and

- b5) the second polypeptide comprises, in order from N-terminus to C-terminus:
- i) the second MHC polypeptide; and
 - ii) an immunoglobulin (Ig) Fc polypeptide.
6. A T-cell modulatory multimeric polypeptide of any one of claims 1-4, wherein:
- a) the first MHC polypeptide is a β 2-microglobulin polypeptide; and the second MHC polypeptide is an MHC class I heavy chain polypeptide; or
 - b) the first MHC polypeptide is an MHC class I heavy chain polypeptide; and the second MHC polypeptide is a β 2-microglobulin polypeptide.
7. A T-cell modulatory multimeric polypeptide of claim 6, wherein:
- a) the first polypeptide comprises, in order from N-terminus to C-terminus:
 - i) the WT-1 peptide epitope; and
 - ii) the β 2-microglobulin polypeptide; and
 - b) the second polypeptide comprises, in order from N-terminus to C-terminus:
 - i) the at least one immunomodulatory polypeptide;
 - ii) the MHC class I heavy chain polypeptide; and
 - iii) an Ig Fc polypeptide.
8. A T-cell modulatory multimeric polypeptide of claim 6, wherein:
- a) the first polypeptide comprises, in order from N-terminus to C-terminus:
 - i) the WT-1 peptide epitope; and
 - ii) the β 2-microglobulin polypeptide; and
 - b) the second polypeptide comprises, in order from N-terminus to C-terminus:
 - i) the MHC class I heavy chain polypeptide; and
 - ii) an Ig Fc polypeptide; and
 - iii) at least one immunomodulatory polypeptide
9. A T-cell modulatory multimeric polypeptide of any one of claims 1-8, wherein the at least one immunomodulatory polypeptide is selected from the group consisting of a cytokine, a 4-1BBL polypeptide, an ICOS-L polypeptide, an OX-40L polypeptide, a CD80 polypeptide, a CD86 polypeptide, a CD40 polypeptide, a CD70 polypeptide, and combinations thereof.
10. A T-cell modulatory multimeric polypeptide of any one of claims 1-9, wherein the at least one immunomodulatory polypeptide comprises an IL-2 polypeptide.

11. A T-cell modulatory multimeric polypeptide of any one of claims 1-10, wherein the multimeric polypeptide comprises at least two immunomodulatory polypeptides, and wherein at least two of the immunomodulatory polypeptides are the same, optionally wherein the 2 or more immunomodulatory polypeptides are in tandem.
12. A T-cell modulatory multimeric polypeptide of any one of claims 1-11, wherein one or more of the at least one immunomodulatory polypeptide is a variant IL-2 polypeptide that exhibits reduced affinity to an IL-2 receptor compared to the affinity of a wild-type IL-2 polypeptide for the IL-2 receptor.
13. A T-cell modulatory multimeric polypeptide of claim 12, wherein the one or more variant IL-2 polypeptides comprises: i) an H16A substitution and an F42A substitution; or ii) an H16T substitution and an F42A substitution.
14. A T-cell modulatory multimeric polypeptide of any one of claims 1-13, wherein the first polypeptide and the second polypeptide are covalently linked to one another, optionally wherein the covalent linkage is via a disulfide bond.
15. A T-cell modulatory multimeric polypeptide of any one of claims 1-14, wherein the first MHC polypeptide or a linker between the epitope and the first MHC polypeptide comprises an amino acid substitution to provide a first Cys residue, wherein the second MHC polypeptide comprises an amino acid substitution to provide a second Cys residue, and wherein the disulfide linkage is between the first and the second Cys residues.
16. A T-cell modulatory multimeric polypeptide of any one of claims 1-15, wherein the polypeptide comprises a disulfide bond between: i) a Cys present in a linker between the WT-1 peptide epitope and the first MHC class I polypeptide, wherein the first MHC class I polypeptide is a β 2M polypeptide; and ii) a Cys residue introduced via a Y84C substitution in the second MHC class I polypeptide, wherein the second MHC class I polypeptide is a MHC Class I heavy chain polypeptide.
17. A T-cell modulatory multimeric polypeptide of any one of claims 1-15, wherein the polypeptide comprises a disulfide bond between i) a Cys residue introduced into the first MHC class I polypeptide via an R12C substitution, wherein the first MHC class I polypeptide is a β 2M polypeptide; and ii) a Cys residue introduced into the second MHC class I polypeptide, via an A236C substitution, wherein second MHC class I polypeptide is an MHC Class I heavy chain polypeptide.

18. A T-cell modulatory multimeric polypeptide of any one of claims 1-15, wherein the polypeptide comprises a first disulfide bond between: i) a Cys present in a linker between the WT-1 peptide epitope and the first MHC class I polypeptide, wherein the first MHC class I polypeptide is a β 2M polypeptide; and ii) a Cys residue introduced via a Y84C substitution in the second MHC class I polypeptide, wherein the second MHC class I polypeptide is a MHC Class I heavy chain polypeptide, and a second disulfide bond between i) a Cys residue introduced into the β 2M polypeptide via an R12C substitution; and ii) a Cys residue introduced into the MHC Class I heavy chain polypeptide via an A236C substitution.
19. A T-cell modulatory multimeric polypeptide of claim 16 or claim 18, wherein the linker between the WT-1 peptide epitope and the first MHC is GCGGS(GGGGS)_n (SEQ ID NO:319), where n is 1, 2, 3, 4, 5, 6, 7, 8, or 9.
20. A T-cell modulatory multimeric polypeptide of any one of claims 1-19, wherein the WT-1 peptide epitope has a length of 9 or 10 amino acids.
21. A T-cell modulatory multimeric polypeptide of any one of claims 1-20, wherein the Ig Fc polypeptide comprises one of the amino acid sequences depicted in FIG. 5D, FIG. 5E, FIG. 5F, FIG. 5G, and FIG. 5H.
22. A T-cell modulatory multimeric polypeptide of any one of claims 1-21, wherein the WT-1 peptide comprises the amino acid sequence SMTWNQMNL (SEQ ID NO:451), GCMTWNQMNL (SEQ ID NO:452), SYTWNQMNL (SEQ ID NO:453), or GCYTWNQMNL (SEQ ID NO:454).
23. A T-cell modulatory multimeric polypeptide of any one of claims 1-21, wherein the first or the second MHC polypeptide comprises an amino acid sequence having at least 95% amino acid sequence identity to amino acids 25-299 of the HLA-A*2402 amino acid sequence depicted in FIG. 7A.3.
24. A T-cell modulatory multimeric polypeptide of any one of claims 1-23, wherein the first MHC polypeptide is a β 2M polypeptide, and wherein the second MHC polypeptide comprises an amino acid sequence having at least 95% amino acid sequence identity to an HLA-A24 polypeptide, wherein the epitope is selected from the group consisting of: SMTWNQMNL (SEQ ID NO:451), GCMTWNQMNL (SEQ ID NO:452), SYTWNQMNL (SEQ ID NO:453), and GCYTWNQMNL (SEQ ID NO:454), and wherein the Ig Fc polypeptide comprises the amino acid sequence depicted in FIG. 5G or FIG. 5H.

25. A T-cell modulatory multimeric polypeptide of claim 1, wherein:
- a) the first polypeptide comprises the amino acid sequence depicted in FIG. 37B; and
 - b) the second polypeptide comprises the amino acid sequence depicted in FIG. 20B.
26. A T-cell modulatory multimeric polypeptide of any one of claims 1-25, wherein the multimeric polypeptide comprises a first and a second heterodimer, and wherein the first and second heterodimers are covalently bound by one or more disulfide bonds between the Ig Fc polypeptides of the first and second heterodimers.
27. A nucleic acid comprising a nucleotide sequence encoding a first or second polypeptide according to any one of claims 1-26.
28. An expression vector comprising the nucleic acid of claim 27.
29. A method of selectively modulating the activity of T cell specific for a Wilms tumor-1 (WT-1) epitope, the method comprising contacting the T cell with a T-cell modulatory multimeric polypeptide according to any one of claims 1-26, wherein said contacting selectively modulates the activity of the WT-1 epitope-specific T cell.
30. A method of treating a patient having a cancer, the method comprising administering to the patient an effective amount of a pharmaceutical composition comprising T-cell modulatory multimeric polypeptide according to any one of claims 1-26.
31. The method of claim 30, wherein the cancer is acute myeloid leukemia, myeloma, ovarian cancer, pancreatic cancer, non-small cell lung cancer, colorectal cancer, breast cancer, Wilms tumor, mesothelioma, soft tissue sarcoma, neuroblastoma, or nephroblastoma.
32. A method of 30 or 31, further comprising administering one or more checkpoint inhibitors to the individual.
33. A method according to claim 32, wherein the checkpoint inhibitor is an antibody that binds to a polypeptide selected from the group consisting of CD27, CD28, CD40, CD122, CD96, CD73, CD47, OX40, GITR, CSF1R, JAK, PI3K delta, PI3K gamma, TAM, arginase, CD137, ICOS, A2AR, B7-H3, B7-H4, BTLA, CTLA-4, LAG3, TIM3, VISTA, CD96, TIGIT, CD122, PD-1, PD-L1, and PD-L2.

34. A method according to claim 33, wherein the checkpoint inhibitor is an antibody specific for PD-1, PD-L1, or CTLA4.

35. A method according to claim 33 or 34, wherein the one or more checkpoint inhibitors is selected from the group consisting of nivolumab, pembrolizumab, pidilizumab, AMP-224, MPDL3280A, MDX-1105, MEDI-4736, arelumab, ipilimumab, tremelimumab, pidilizumab, IMP321, MGA271, BMS-986016, lirilumab, urelumab, PF-05082566, IPH2101, MEDI-6469, CP-870,893, Mogamulizumab, Varlilumab, Avelumab, Galiximab, AMP-514, AUNP 12, Indoximod, NLG-919, INCB024360, KN035, and combinations thereof.

36. A method of modulating an immune response in an individual, the method comprising administering to the individual an effective amount of the T-cell modulatory multimeric polypeptide of any one of claims 1-26, wherein said administering induces an epitope-specific T cell response and an epitope-non-specific T cell response, and wherein the ratio of the epitope-specific T cell response to the epitope-non-specific T cell response is at least 2:1.

37. A method of delivering an immunomodulatory polypeptide selectively to a target T cell, the method comprising contacting a mixed population of T cells with a T-cell modulatory multimeric polypeptide of any one of claims 1-26, wherein the mixed population of T cells comprises the target T cell and non-target T cells, wherein the target T cell is specific for the WT-1 epitope present within the T-cell modulatory multimeric polypeptide, and wherein said contacting delivers the one or more immunomodulatory polypeptides present within the T-cell modulatory multimeric polypeptide to the target T cell.

38. A method of detecting, in a mixed population of T cells obtained from an individual, the presence of a target T cell that binds a WT-1 epitope, the method comprising:

a) contacting *in vitro* the mixed population of T cells with the T-cell modulatory multimeric polypeptide of any one of claims 1-26, wherein the T-cell modulatory multimeric polypeptide comprises the WT-1 epitope; and

b) detecting activation and/or proliferation of T cells in response to said contacting, wherein activated and/or proliferated T cells indicates the presence of the target T cell.

39. A T-cell modulatory multimeric polypeptide (TMMP) comprising:
at least one heterodimer comprising:

- a) a first polypeptide comprising, in order from N-terminus to C-terminus:
 - i) a Wilms tumor-1 (WT-1) peptide, wherein the WT-1 peptide has the amino acid sequence VLDFAPPGA (SEQ ID NO:259);
 - ii) a linker having the amino acid sequence GCGGSGGGGSGGGGS (SEQ ID NO:317); and
 - iii) a β 2-microglobulin polypeptide comprising the amino acid sequence set forth in SEQ ID NO:311; and
- b) a second polypeptide comprising, in order from N-terminus to C-terminus:
 - i) a variant IL-2 polypeptide comprising the amino acid sequence set forth in SEQ ID NO:188, where X_1 is Ala and where X_2 is Ala;
 - ii) a (GGGGS)₄ linker;
 - iii) a variant IL-2 polypeptide comprising the amino acid sequence set forth in SEQ ID NO:188, where X_1 is Ala and where X_2 is Ala;
 - iv) a major histocompatibility complex (MHC) heavy chain polypeptide comprising the amino acid sequence set forth in SEQ ID NO:341;
 - v) a linker comprising the amino acid sequence AAAGG; and
 - vi) an immunoglobulin (Ig) Fc polypeptide.

40. A TMMP of claim 39, wherein the Ig Fc polypeptide is a variant Ig Fc polypeptide comprising one or more sequence variations relative to the wild type polypeptide, wherein the ability of the Ig Fc polypeptide to induce cell lysis through complement-dependent cytotoxicity (CDC) and/or antibody-dependent cellular cytotoxicity (ADCC) is reduced or substantially eliminated, optionally wherein the Ig Fc polypeptide comprises an amino acid sequence having at least at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to an amino acid sequence of an Fc region depicted in FIG. 5A-5G or 5H.

41. A TMMP of claim 40, wherein the Ig Fc polypeptide is a variant human IgG1 Fc polypeptide comprising comprises an L234A and/or L235A substitutions (L14 and L15 in the amino acid sequence depicted in FIG. 5H).

42. A TMMP of any one of claims 39-41, wherein the first polypeptide comprises construct 2380 having the amino acid sequence set forth in FIG. 14B and SEQ ID NO:423; and where the second

polypeptide comprises construct 1715Δ having the amino acid sequence set forth in FIG. 14J and SEQ ID NO:486.

43. A TMMP comprising a homodimer of the heterodimer of any one of claims 39-42.
44. A nucleic acid comprising a nucleotide sequence encoding the first and/or the second polypeptide of any one of claims 39-42.
45. An expression vector comprising the nucleic acid of claim 44.
46. A genetically modified host cell, wherein the host cell is genetically modified with a nucleic acid of claim 44 or an expression vector of claim 45.
47. A method of making a T-cell modulatory multimeric polypeptide (TMMP), the method comprising culturing the genetically modified host cell of claim 46 *in vitro* in a culture medium under conditions such that the host cell synthesizes the TMMP.
48. A method of selectively modulating the activity of T cell specific for a Wilms tumor-1 (WT-1) epitope, the method comprising contacting the T cell with a T-cell modulatory multimeric polypeptide according to any one of claims 39-43, wherein said contacting selectively modulates the activity of the WT-1 epitope-specific T cell.
49. A method of treating a patient having a cancer associated with WT-1, the method comprising administering to the patient an effective amount of a pharmaceutical composition comprising T-cell modulatory multimeric polypeptide according to any one of claims 39-43.
50. A method of claim 49, wherein the cancer is acute myeloid leukemia, myeloma, ovarian cancer, pancreatic cancer, non-small cell lung cancer, colorectal cancer, breast cancer, Wilms tumor, mesothelioma, soft tissue sarcoma, neuroblastoma, or nephroblastoma.
51. A method of 49 or 50, further comprising administering one or more immune checkpoint inhibitors to the individual.
52. A method according to claim 51, wherein the immune checkpoint inhibitor is an antibody that binds to a polypeptide selected from the group consisting of CD27, CD28, CD40, CD122, CD96, CD73,

CD47, OX40, GITR, CSF1R, JAK, PI3K delta, PI3K gamma, TAM, arginase, CD137, ICOS, A2AR, B7-H3, B7-H4, BTLA, CTLA-4, LAG3, TIM3, VISTA, CD96, TIGIT, CD122, PD-1, PD-L1, and PD-L2.

53. A method according to claim 52, wherein the immune checkpoint inhibitor is an antibody specific for PD-1, PD-L1, or CTLA4.

54. A method according to claim 52 or 53, wherein the one or more immune checkpoint inhibitors is selected from the group consisting of nivolumab, pembrolizumab, pidilizumab, AMP-224, MPDL3280A, MDX-1105, MEDI-4736, arelumab, ipilimumab, tremelimumab, pidilizumab, IMP321, MGA271, BMS-986016, lirilumab, urelumab, PF-05082566, IPH2101, MEDI-6469, CP-870,893, Mogamulizumab, Varlilumab, Avelumab, Galiximab, AMP-514, AUNP 12, Indoximod, NLG-919, INCB024360, KN035, and combinations thereof.