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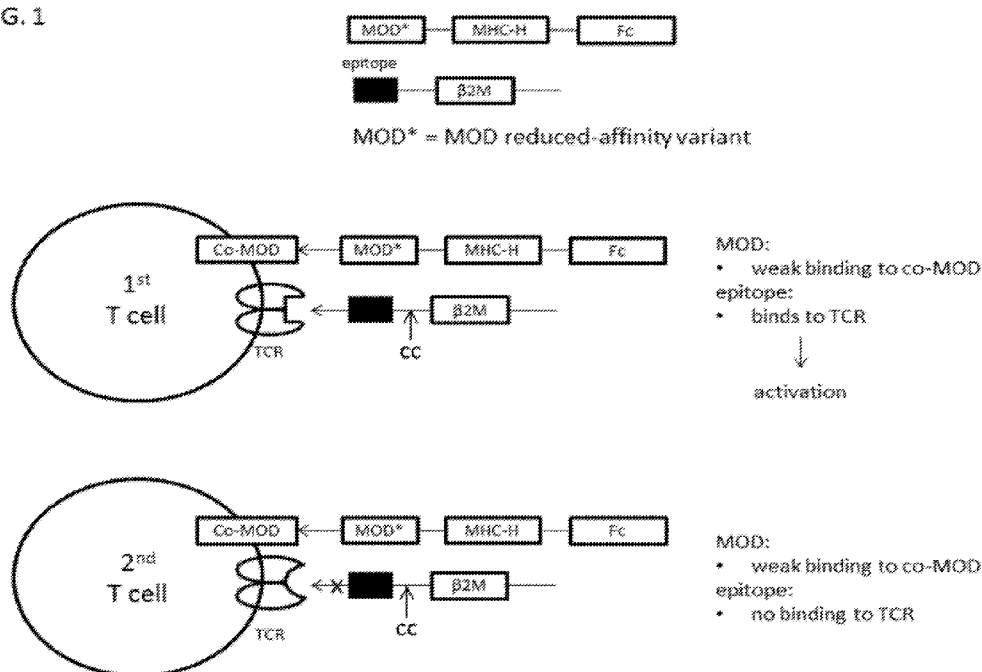
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(54) Title: T-CELL MODULATORY MULTIMERIC POLYPEPTIDES WITH CONJUGATION SITES AND METHODS OF USE THEREOF

FIG. 1



(57) **Abstract:** The present disclosure provides T-cell modulatory multimeric polypeptide epitope conjugates comprising an immunomodulatory polypeptide ("MOD") that may be selected to exhibit reduced binding affinity to a cognate co-immunomodulatory polypeptide ("Co-MOD") and a conjugated Wilms tumor-1 (WT-1) epitope presenting peptide. The T-Cell-MMP-epitope conjugates are useful for modulating the activity of a T-cell by delivering immunomodulatory peptides, such as IL-2 or IL-2 variants that exhibit reduced binding affinity for IL-2R, to the T-cells in a WT-1 epitope selective/specific manner, and accordingly, for treating individuals, particularly those with 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.



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T-Cell Modulatory Multimeric Polypeptides with Conjugation Sites and Methods of Use Thereof

[0001] This application claims the benefit of: U.S. Provisional Appln. No. 62/782,245 filed on 19 December 2018. This application contains a sequence listing submitted electronically via EFS-web, which serves as both the paper copy and the computer readable form (CRF) and consists of a file entitled “123640-8004.WO00_seqlist.txt”, which was created on December 19, 2019, which is 531,356 bytes in size, and which is herein incorporated by reference in its entirety.

Introduction

[0002] 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 on 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 is not epitope specific and instead is generally expressed on all T-cells or on large T-cell subsets.

Summary

[0003] The present disclosure provides T-cell modulatory multimeric polypeptides (a “T-Cell-MMP” or multiple “T-Cell-MMPs”) that in one embodiment comprise a portion of a MHC receptor and at least one immunomodulatory polypeptide (also referred to herein as a “MOD polypeptide” or, simply, a “MOD”). Any one or more of the MODs present in the T-Cell-MMP may be wild-type (wt) or a variant that exhibits reduced binding affinity to its cellular (e.g., T-cell surface) binding partner/receptor (generally referred to as a “Co-MOD”). The T-Cell-MMPs comprise at least one chemical conjugation site at which a molecule comprising a target epitope (e.g., a peptide or non-peptide such as a carbohydrate) may be covalently bound for presentation to a cell bearing a T-cell receptor. T-Cell-MMPs comprising a chemical conjugation site for linking an epitope are useful for rapidly preparing T-Cell-MMP-epitope conjugates that can modulate the activity of T-cells specific to the epitope presented and, accordingly, for modulating an immune response in an individual involving those T-cells. The T-Cell-MMPs described herein are suitable for production in cell expression systems where most, substantially all (e.g., greater than 85% or 90% of the T-Cell-MMP), or all of the expressed protein is in a soluble non-aggregated state (e.g., in the form of dimers) that is suitably stable at 37 °C for production in tissue culture and use at least up to that temperature. Most, substantially all (e.g., greater than 85% or 90% of the T-Cell-MMP), or all of the expressed protein remains in a soluble non-aggregated state even after conjugation to epitope

peptides and is similarly stable. The T-Cell-MMPs and their epitope conjugates may additionally comprise sites for the conjugation of bioactive substances (payloads) such as chemotherapeutic agents for co-delivery with a specific target epitope. As such, T-Cell-MMP-epitope conjugates may be considered a means by which to deliver MODs (*e.g.*, IL-2, 4-1BBL, FasL, TGF- β , CD70, CD80, CD86, OX40L, ICOS-L, ICAM, JAG1, or fragments thereof, or altered (mutated) variants thereof) and/or payloads (*e.g.*, chemotherapeutics) to cells in an epitope specific manner.

[0004] In embodiments described herein the T-Cell-MMPs may comprise modifications that assist in the stabilization of the T-Cell-MMP during intracellular trafficking and/or following secretion by cells expressing the multimeric polypeptide even in the absence of an associated epitope peptide. In embodiments described herein the T-Cell-MMPs may include modifications that link the carboxyl end of the MHC-I α_1 helix and the amino end of the MHC-I α_2 helix. Such modifications include the insertion of cysteine residues that result in the formation of disulfide linkages linking the indicated regions of those helices. For example, the insertion of cysteine residues at amino acid (aa) 84 (Y84C substitution) and 139 (A139C substitution) of MHC-I, or the equivalent positions relative to the sequences forming the helices, may form a disulfide linkage that helps stabilize the T-Cell-MMP. *See, e.g.*, Z. Hein *et al.* (2014), *Journal of Cell Science* 127:2885–2897.

Brief Description Of The Drawings

[0005] FIG. 1 depicts preferential activation of an epitope-specific T-cell to an epitope non-specific T-cell by an embodiment of a T-Cell-MMP of the present disclosure bearing a epitope attached by chemical coupling (denoted by “CC”) to a β -2 microglobulin (β 2M) polypeptide sequence.

[0006] FIGs. 2A-2G provide aa sequences of immunoglobulin Fc polypeptides (including SEQ ID NOs. 1-13).

[0007] FIGs. 3A, 3B and 3C provide aa sequences of human leukocyte antigen (HLA) Class I heavy chain polypeptides. Signal sequences, aas 1-24, are bolded and underlined. Fig. 3A 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:14; entry 3A.2 is HLA-A*1101, SEQ ID NO:15; entry 3A.3 is HLA-A*2402, SEQ ID NO:16, and entry 3A.4 is HLA-A*3303, SEQ ID NO:17. FIG. 3B provides the sequence for HLA-B*07:02:01 (HLA-B*0702) (NCBI GenBank Accession NP_005505.2 (*see, also* GenBank Accession AUV50118.1)) SEQ ID NO:18. FIG. 3C provides the sequence for 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) SEQ ID NO:19.

[0008] FIG. 3D provides an alignment of eleven mature MHC Class I heavy chain peptide sequences without all, or substantially all, of their leader, transmembrane and intracellular domain regions. The aligned sequences include human HLA-A*0101, SEQ ID NO:20 (*see also* SEQ ID NO:14); HLA-B*0702, SEQ ID NO:21; HLA-C, SEQ ID NO:22; HLA-A*0201, SEQ ID NO:23; a mouse H2K protein sequence, SEQ ID NO:24; three variants of HLA-A (var.2, var. 2C [having Y84C and A139C substitutions], and var.2CP), SEQ ID NOs:25-27; 3 human HLA-A molecules (HLA-A*1101 (HLA-A11), SEQ ID NO:28; HLA-A*2402 (HLA-A24), SEQ ID NO:29; and HLA-A*3303 (HLA-A33), SEQ

ID NO:30). HLA-A*0201 is a variant of HLA-A. The Y84A and A236C variant of HLA-A is marked as HLA-A(var. 2). The seventh HLA-A sequence, marked as HLA-A (var. 2C), shows HLA-A substituted with C residues at positions 84, 139 and 236, and the eighth sequence adds one additional proline to the C-terminus of the preceding sequence. The ninth through the eleventh sequences are from HLA-A11 (HLA-A*1101); HLA-A24 (HLA-A*2402); and HLA-A33 (HLA-A*3303), respectively, which are prevalent in certain Asian populations. Indicated in the alignment are the locations (84 and 139 of the mature proteins) where cysteine residues may be inserted in place of the aa at that position for the formation of a disulfide bond to stabilize the MHC-H - β 2M complex in the absence of a bound epitope peptide. Also shown in the alignment is position 236 (of the mature polypeptide), which may be replaced by a cysteine residue that can form an interchain disulfide bond with β 2M (e.g., at aa 12 of the mature polypeptide). An arrow appears above each of those locations and the residues are bolded. The boxes flanking residues 84, 139 and 236 show the groups of five aas on either side of those six sets of five residues, denoted aa cluster 1, aa cluster 2, aa cluster 3, aa cluster 4, aa cluster 5, and aa cluster 6 (shown in the figure as aac 1 through aac 6, respectively), that may be replaced by 1 to 5 aas selected independently from (i) any naturally occurring aa or (ii) any naturally occurring aa except proline or glycine.

[0009] FIGs. 3E-3G provide alignments of the aa sequences of mature HLA-A, -B, and, -C class I heavy chains, respectively. The sequences are provided for a portion of the mature proteins (without all or substantially all of their leader sequences, transmembrane domains or intracellular domains). As described in FIG 3D, the positions of aa residues 84, 139, and 236 and their flanking residues (aac1 to aac6) that may be replaced by 1 to 5 aas selected independently from (i) any naturally occurring aa or (ii) any naturally occurring aa except proline or glycine are also shown. A consensus sequence is also provided for each group of HLA alleles provided in the figures showing the variable aa positions as "X" residues sequentially numbered and the locations of aas 84, 139 and 236 double underlined.

[0010] FIG. 3H 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.

[0011] FIG. 3I provides an alignment of the consensus aa sequences for HLA-A, -B, -C, -E, -F, and -G, which are given in FIGs. 3E to 3H (SEQ ID NOs: 35, 43, and 53-56). The alignment shows the correspondence of aas between the different sequences. Variable residues in each sequence are listed as "X" with the sequential numbering removed. The permissible aas at each variable residue can be determined by reference to FIGs. 3E-3H. As indicated in FIG. 3D, the locations of aas 84, 139 and 236 with their flanking five-aa clusters that may be replaced by 1 to 5 aas selected independently from (i) any naturally occurring aa or (ii) any naturally occurring aa except proline or glycine are also shown.

[0012] FIG. 4 provides a multiple aa sequence alignment of β 2M precursors (i.e., including the leader sequence) from Homo sapiens (NP_004039.1; SEQ ID NO:57), Pan troglodytes (NP_001009066.1; SEQ ID NO:58), Macaca mulatta (NP_001040602.1; SEQ ID NO:59), Bos Taurus (NP_776318.1; SEQ ID NO:60) and Mus musculus (NP_033865.2; SEQ ID NO:61). Underlined aas 1-20 are the signal peptide (sometime referred to as a leader sequence).

[0013] **FIG. 5** provides six T-Cell-MMP embodiments (structures) marked as **A** through **F**. In each case the T-Cell-MMPs comprise: a first polypeptide having an N-terminus and C-terminus and which comprises a first major histocompatibility complex (MHC) polypeptide (MHC-1); and a second polypeptide having an N-terminus and C-terminus and a second MHC polypeptide (MHC-2), and optionally comprising an immunoglobulin (Fc) polypeptide or a non-Ig polypeptide scaffold. In the embodiments shown the first and second polypeptides are shown linked by a disulfide bond; however, the T-Cell-MMPs do not require a disulfide linkage or any other covalent linkage between the first and second polypeptides. The T-Cell-MMPs may also comprise independently selected linker sequences indicated by the dashed line (- - -). The first polypeptide, the second polypeptide, or both the first and second polypeptides of the T-Cell-MMP comprise at least one chemical conjugation site. Some potential locations for the first polypeptide chemical conjugation sites (CC-1) and second polypeptide chemical conjugation sites (CC-2) are shown by arrows. Locations for one or more MODs that are selected independently (e.g., a sequence comprising one, two, three or more MODs connected in sequence with optional aa linkers between the MODs) are shown by “MOD” in the stippled box. The MODs may be variant MODs as described within this disclosure. In **A** the MOD(s) are located at the C-terminus of the first polypeptide, in **B** the MOD(s) are located at the N-terminus of the second polypeptide, in **C** the MOD(s) are located at the C-terminus of the second polypeptide, in **D** the MODs are located at the C-terminus of the first peptide and the N-terminus of the second peptide, in **E** the MOD(s) are added with the epitope peptide, and in **F** the MOD(s) are between the MHC-2 and Fc peptide. Where more than one MOD is present they may be the same (e.g., two IL-2 MODs) or different, and may be placed adjacent to each other.

[0014] **FIG.** provides twelve embodiments of T-Cell-MMP-epitope conjugates, marked as **A** through **L**, that parallel the embodiments in FIG. 5. As in FIG. 5, the first polypeptide has an N-terminus and C-terminus with the first MHC polypeptide given as comprising a β -2-microglobulin polypeptide (β 2M) capable of interacting with the MHC Class I heavy chain (MHC-H) and presenting the epitope to a T-Cell receptor. The second polypeptide has an N-terminus and C-terminus and a MHC-H polypeptide, and optionally comprises an immunoglobulin (Fc) polypeptide or a non-Ig polypeptide scaffold. The optional disulfide bond joining the first and second polypeptides of the T-Cell-MMP-epitope conjugates is shown connecting the β 2M peptide sequence and MHC-H peptide sequence in **A** to **F**, and the independently selected optional linker sequences, indicated by the dashed line (- - -), are not required. In **G** to **L**, the complexes in **A** to **F** are repeated; however, a disulfide bond joining the first and second polypeptides is shown joining the MHC-H peptide sequence to a linker sequence interposed between the epitope and β 2M peptide sequence (e.g., a bond from a Cys residue at position 84 of a MHC-H chain sequence as indicated in FIG. 3 to the interposed linker). The first polypeptide, the second polypeptide, or both the first and second polypeptides of the T-Cell-MMP may also comprise one or more chemical conjugation sites in addition to the site employed for the conjugation of the epitope. The potential locations for such sites (CC-1 and CC-2) are shown by arrows. The one or more immunomodulatory polypeptides (either MODs or variant MODs) are as described in FIG. 5. The MODs (e.g., tandem IL-2 polypeptides) may be

placed on the N-terminus of the MHC-H polypeptide (Position 1 as in B and H), between the MHC-H and Fc (Position 2 as in F and L), on the C-terminus of the MHC-H (Position 3 as in C and I); N-terminal to the peptide (Position 4 as in E and K); or C-terminal to the β 2M (Position 5 as in A and G). on the N-terminus of the MHC-H polypeptide ("Position 1 as in B and H), between the MHC-H and Fc (Position 2 as in F and L), on the C-terminus of the MHC H (Position 3 as in C and I); N-terminal to the peptide (Position 4 as in E and K); or C-terminal to the β 2M (Position 5 as in A and G).

[0015] FIG. 7 provides examples of two dimers formed from T-Cell-MMPs. The dimer labeled "A" is the result of dimerizing two of the T-Cell-MMPs labeled "A" in FIG. 6. The dimer labeled "B" is the result of dimerizing two of the T-Cell-MMPs labeled "B" in FIG. 6. The embodiment as shown includes one or more disulfide bonds between the polypeptides, each of which is optional. In addition, only a subset of CC-2 sites in the Fc region or the attached optional linker are shown.

[0016] FIG. 8 shows some schematics of epitopes having a maleimide group appended for conjugation to a free nucleophile (e.g., cysteine) present in a T-Cell-MMP to form an epitope conjugate. In "a" the maleimide group is attached by an optional linker (e.g., a peptide linker sequence) to the epitope. In "b" through "e," the linker is a glycine serine polypeptide (GGGGS) repeated n times, where n is 1-5 when present, and n is 0 when the linker is absent. In "c"- "e" the attachment of a maleimide group is through a lysine (K), such as through the epsilon amino group of the lysine. In "d" and "e" the maleimide group is linked to the peptide through an alkyl amide formed with the epsilon amino group of a lysine residue, where m is 1-7.

[0017] FIG. 9 shows in part A a map of a T-Cell-MMP with the first polypeptide having a sulfatase motif (aas 26-31 bolded) between two linker sequences as the location for developing a chemical conjugation site (an fGly residue) through the action of an FGE enzyme. At B, **FIG. 9** shows a second polypeptide of a T-Cell-MMP having tandem IL-2 MODs attached to the amino end of a human MHC Class I HLA-A heavy chain polypeptide followed by a human IgG1 Fc polypeptide. Linkers are bolded, italicized and underlined.

[0018] FIG. 10A to FIG. 10 D show a series of HLA A*1101 heavy chain constructs having, from N-terminus to C-terminus, a human IL-2 signal sequence, shown in underline and bold. The signal (leader) sequence is followed by a MOD, which is indicated as a human IL-2 or an "optional peptide linker-immunomodulatory polypeptide-optional peptide linker." Where the MOD is not specified, it may be any desired MOD. The remainder of the sequence is HLA A*1101 H chain sequence with three cysteine substitutions (Y84C; A139C; A236C); a linker; and a hIgG1 Fc with two aa substitutions (L234A; L235A). The asterisks indicate stops to the sequences.

[0019] FIG. 11A to FIG. 11E provide the aa sequence of a Wilms tumor-1 (WT-1) protein isoform A, B, D, E, and F.

[0020] FIG. 12 shows a comparison of two immunomodulatory proteins having a first polypeptide (comprising a β 2M polypeptide sequence) and a second polypeptide (comprising an MHC-H chain α 1- α 3 segments and an IgFc). The immunomodulatory proteins appear as a dimer comprising two copies of both the first and second polypeptides that are associated by a disulfide bond between the β 2M and

MHC-H sequences and disulfide bonds between the Fc regions. Structure A is a control immunomodulatory protein is shown that has a 9 aa cytomegalovirus (CMV) epitope at the N-terminus of a β 2M polypeptide sequence of the first polypeptide. Structure B is a T-Cell-MMP of the present disclosure is provided where having a linker at the N-terminus of a β 2M polypeptide sequence of the first polypeptide bearing a chemical conjugation site indicated with an “*” in a linker (not shown) attached to the N-terminus of a β 2M polypeptide sequence. An SDS PAGE gel of the expressed and purified proteins under non-reducing and reducing conditions is shown at C with molecular weight markers (left lane), the non-reduced samples conjugated to MART1 and CMV peptides are in the 2nd and 3rd lanes from the left, reduced samples conjugated to MART1 and CMV peptides are in the 4th and 5th lanes from the left. The first polypeptides are labeled as “light chain” and the second polypeptides are labeled as “heavy chain.” See Example 2 for more details.

[0021] FIG. 13 shows size exclusion chromatography of T-Cell-MMPs conjugated to CMV (CMV+T-Cell-MMP) and MART-1 (MART+T-Cell-MMP) polypeptides plotted in mAU (milli-absorbance units) vs time in minutes. See example 3.

[0022] FIG. 14 shows the response of Ficoll-Paque[®] samples of leukocytes from CMV responsive donors simulated with various concentrations of immunomodulatory polypeptide constructs and control treatments measured as the number of CMV or MART-1 responsive CD8+ T-cells. See Example 4 for details.

Definitions

[0023] 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.

[0024] A polynucleotide or polypeptide has a certain percent “sequence identity” to another polynucleotide or polypeptide, meaning that, when aligned, that percentage of bases or aas is 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/, and mafft.cbrc.jp/alignment/software/. See, e.g., Altschul et al. (1990), J. Mol. Biol. 215:403-10. Unless stated otherwise, sequence alignments are prepared using BLAST.

[0025] The terms “amino acid” and “amino acids” are abbreviated as “aa” and “aas,” respectively. Naturally occurring aa or naturally occurring aas, unless stated otherwise, means: L (Leu, leucine), A (Ala, alanine), G (Gly, glycine), S (Ser, serine), V (Val, valine), F (Phe, phenylalanine), Y (Tyr, tyrosine), H (His, histidine), R (Arg, arginine), N (Asn, asparagine), E (Glu, glutamic acid), D (Asp, asparagine), C (Cys, cysteine), Q (Gln, glutamine), I (Ile, isoleucine), M (Met, methionine), P (Pro, proline), T (Thr,

threonine), K (Lys, lysine), and W (Trp, tryptophan); all of the L-configuration. Both selenocysteine and hydroxyproline are naturally occurring aas that are specifically referred to in any instance where they are intended to be encompassed.

[0026] Non-natural aas are any aa other than the naturally occurring aas recited above, selenocysteine, and hydroxyproline.

[0027] “Chemical conjugation” as used herein means formation of a covalent bond. “Chemical conjugation site” as used herein means a location in a polypeptide at which a covalent bond can be formed, including any contextual elements (*e.g.*, surrounding aa sequences) that are required or assist in the formation of a covalent bond to the polypeptide. Accordingly, a site comprising a group of aas that direct enzymatic modification, and ultimately covalent bond formation at an aa within the group, may also be referred to as a chemical conjugation site. In some instances, as will be clear from the context, the term chemical conjugation site may be used to refer to a location where covalent bond formation or chemical modification has already occurred.

[0028] The term “conservative aa substitution” refers to the interchangeability in proteins of aa residues having similar side chains. For example, a group of aas having aliphatic side chains consists of glycine, alanine, valine, leucine, and isoleucine; a group of aas having aliphatic-hydroxyl side chains consists of serine and threonine; a group of aas having amide containing side chains consists of asparagine and glutamine; a group of aas having aromatic side chains consists of phenylalanine, tyrosine, and tryptophan; a group of aas having basic side chains consists of lysine, arginine, and histidine; a group of aas having acidic side chains consists of glutamate and aspartate; and a group of aas having sulfur containing side chains consists of cysteine and methionine. Exemplary conservative aa substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine-glycine, and asparagine-glutamine.

[0029] The terms “immunological synapse” or “immune synapse” as used herein generally refer to the natural interface between two interacting immune cells of an adaptive immune response including, *e.g.*, the interface between an APC, or target T-cell, and an effector cell, *e.g.*, a lymphocyte, an effector T-cell, a natural killer cell, or 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 one or more MHC molecules, *e.g.*, as described in Bromley et al., *Ann Rev Immunol.* 2001; 19:375-96; the disclosure of which is incorporated herein by reference in its entirety.

[0030] “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.

[0031] Unless stated otherwise, as used herein, the terms “first major histocompatibility complex (MHC) polypeptide” or “first MHC polypeptide”, and the terms “second MHC polypeptide”, “MHC heavy chain”, and “MHC-H”, refer to MHC Class I receptor elements.

[0032] A “MOD” (also termed a co-immunomodulatory or co-stimulatory polypeptide), as the term is used herein, includes a polypeptide on an APC (*e.g.*, a dendritic cell, a B cell, and the like), or a portion of the polypeptide on an APC, that specifically binds a “Co-MOD” (also termed a cognate co-

immunomodulatory polypeptide or a cognate co-stimulatory 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 MHC polypeptide loaded with peptide, mediates a T-cell response including, but not limited to, proliferation, activation, differentiation, and the like. MODs include, but are 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), transforming growth factor beta (TGF- β), CD30L, CD40, CD70, CD83, HVEM, lymphotoxin beta receptor, 3/TR6, ILT3, ILT4, HVEM, an agonist or antibody that binds the Toll ligand receptor, and a ligand that specifically binds with B7-H3. A MOD also encompasses, inter alia, an antibody (or an antigen binding portion thereof, such as a Fab) that specifically binds with a Co MOD present on a T-cell, such as, but not limited to, CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, LIGHT, NKG2C, B7-H3, and a ligand that specifically binds to CD83.

[0033] An “immunomodulatory domain” (“MOD”) of a T-Cell-MMP is a polypeptide of the T-Cell-MMP or part thereof that acts as a MOD.

[0034] “Heterologous,” as used herein, means a nucleotide or polypeptide that is not found in the native nucleic acid or protein, respectively.

[0035] “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.

[0036] The terms “recombinant expression vector” and “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.

[0037] 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). Affinity can be at least 1-fold greater, at least 2-fold greater, at least 3-fold greater, at least 4-fold greater, at least 5-fold greater, at least 6-fold greater, at least 7-fold greater, at least 8-fold greater, at least 9-fold greater, at least 10-fold greater, at least 20-fold greater, at least 30-fold greater, at least 40-fold greater, at least 50-fold greater, at least 60-fold greater, at least 70-fold greater, at least 80-fold greater, at least 90-fold greater, at least 100-fold greater, or at least 1,000-fold greater, or more, than the affinity of an antibody for unrelated aa sequences. Affinity of an antibody to a target protein can be, for example, from about 100 nanomolar (nM) to about 0.1 nM, from about 100 nM to about 1 picomolar (pM), or from about 100 nM to about 1 femtomolar (fM) or more. 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.

[0038] “Binding” as used herein (e.g., with reference to binding of a molecule such as a T-cell-MMP comprising one or more MODs or its epitope conjugate to one or more polypeptides (e.g., a T-cell receptor and a Co-MOD on a T-cell) refers to a non-covalent interaction(s) between the 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. Non-covalent binding interactions are generally characterized by a dissociation constant (K_D) of less than 10^{-6} M, less than 10^{-7} M, less than 10^{-8} M, less than 10^{-9} M, less than 10^{-10} M, less than 10^{-11} M, or less than 10^{-12} M. “Affinity” refers to the strength of non-covalent binding, increased binding affinity being correlated with a lower K_D . “Specific binding” generally refers to, e.g., binding between a ligand molecule and its binding site or “receptor” with an affinity of at least about 10^{-7} M or greater (e.g., less than 5×10^{-7} M, less than 10^{-8} M, less than 5×10^{-8} M, less than 10^{-9} M, less than 10^{-10} M, less than 10^{-11} M, or less than 10^{-12} M and greater affinity, or in a range from 10^{-7} to 10^{-9} or from 10^{-9} to 10^{-12}). “Non-specific binding” generally refers to the binding of a ligand to something other than its designated binding site or “receptor,” typically with an affinity of less than about 10^{-7} M (e.g., binding with an affinity of less than about 10^{-6} M, less than about 10^{-5} M, less than about 10^{-4} M). However, in some contexts, e.g., binding between a TCR and a peptide/MHC complex, “specific binding” can be in the range of from 1 μ M to 100 μ M, or from 100 μ M to 1 mM. “Covalent binding” as used herein means the formation of one or more covalent chemical bonds between two different molecules

[0039] 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 and/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.

[0040] 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), canines, felines, *etc.*

[0041] Before the present invention is further described, it is to be understood that this invention is not limited to the 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 invention will be limited only by the appended claims.

[0042] Where a range of values is provided, it is understood that the range includes each intervening value, to the tenth 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 smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where a range includes upper and/or lower limits, ranges excluding either or both of those limits are also included in the invention.

[0043] 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 invention, 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.

[0044] 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 “multimeric T-cell modulatory polypeptide” includes a plurality of such polypeptides and reference to “the immunomodulatory polypeptide” or “the MOD” 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.

[0045] It is appreciated that certain features of the invention, 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 invention and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.

[0046] 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 invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication

provided may be different from the actual publication dates which may need to be independently confirmed.

Detailed Description

I. T-Cell Modulatory Multimeric Polypeptides (T-Cell-MMPs) With Chemical Conjugation Sites for Epitope Binding

[0047] The present disclosure provides T-Cell-MMP-epitope conjugates that comprise an epitope-presenting Wilms tumor-1 (WT-1) peptide. Such T-Cell-MMP-epitope conjugates are useful for modulating the activity of T cells, and for modulating an immune response in an individual.

[0048] The present disclosure provides T-Cell-MMPs and their epitope conjugates that are useful for modulating the activity of a T-cell, and methods of their preparation and use in modulating an immune response in an individual. The T-Cell-MMPs may comprise one or more independently selected wild-type and/or variant MOD polypeptides that exhibit reduced binding affinity to their Co-MODs and chemical conjugation sites for coupling epitopes and payloads. Included in this disclosure are T-Cell-MMPs that are heterodimeric, comprising two types of polypeptides (a first polypeptide and a second polypeptide), wherein at least one of those polypeptides comprises a chemical conjugation site for the attachment (e.g., covalent attachment) of payloads such as chemotherapeutic agents and/or materials (e.g., epitope peptides and null peptides) that can bind a TCR. Also included in this disclosure are T-Cell-MMPs which have been chemically conjugated to an epitope and/or a payload (e.g., a chemotherapeutic). Depending on the type of MOD(s) present in the T-Cell-MMP, when an epitope specific to a TCR is present on a T-Cell-MMP, the T-cell can respond by undergoing activation including, for example, clonal expansion (e.g., when activating MODs such as IL-2, 4-1BBL and/or CD80 are incorporated into the T-Cell-MMP). Alternatively, the T-cell may undergo inhibition that down regulates T-cell activity (e.g., blocking autoimmune reactions) when MODs such as CD86 and/or PD-L1 are incorporated into the T-Cell-MMPs. Because MODs are not specific to any epitope, activation or inhibition of T-cells can be biased toward epitope-specific interactions by incorporating variant MODs having reduced affinity for their Co-MOD into the T-Cell-MMPs such that the binding of a T-Cell-MMP to a T-cell is strongly affected by, or even dominated by, the MHC-epitope-TCR interaction.

[0049] A T-Cell-MMP-epitope conjugate may be considered to function as a surrogate APC, and mimics the adaptive immune response. The T-Cell-MMP-epitope conjugate does so by engaging a TCR present on the surface of a T-cell with a covalently bound epitope presented in the T-Cell-MMP-epitope conjugate complex. This engagement provides the T-Cell-MMP-epitope conjugate with the ability to achieve epitope-specific cell targeting. In embodiments described herein, T-Cell-MMP-epitope conjugates also possess at least one MOD that engages a counterpart costimulatory protein (Co-MOD) on the T-cell. Both signals – epitope/MHC binding to a TCR and MOD binding to a Co-MOD – then drive both the desired T-cell specificity and either inhibition or activation/proliferation.

[0050] The T-Cell-MMPs having chemical conjugation sites find use as a platform into which different epitopes and/or payloads may be inserted to prepare materials for therapeutic, diagnostic and research applications. Such T-Cell-MMPs comprising a chemical conjugation site permit the rapid preparation of diagnostics and therapeutics as they permit the epitope containing material (e.g., a peptide) to be rapidly inserted into the T-Cell-MMP and tested for activation or inhibition of T-cells bearing TCRs specific to the epitope.

[0051] In an embodiment, a chemical conjugation site of such a T-Cell-MMP may be utilized to attach a payload such as a chemotherapeutic agent or enzyme to the T-Cell-MMP. In the absence of an added epitope, the resulting complex may be used in a fashion similar to an antibody to deliver the payload, particularly when the T-Cell-MMPs form multimers (e.g., dimers or higher order structures) due to the incorporation of an Fc scaffold. Due to the lack of an epitope, the MODs of T-Cell-MMP-payload conjugates will dictate the cells that will receive the payload by their binding specificity and the avidity of the complex for different cells.

[0052] In an embodiment, where variant MODs that stimulate T-cell proliferation and an epitope are incorporated into a T-Cell-MMP, contacting the T-cells with at least one concentration of the T-Cell-MMP induces at least a twofold (e.g., at least a 2, 3, 4, 5, 10, 20, 30, 50, 75, or 100 fold) difference in the activation of T-cells (as measured by T-cell proliferation or ZAP-70 activity, *see e.g.*, Wang, *et al.*, *Cold Spring Harbor perspectives in biology* 2.5 (2010): a002279) having a TCR specific to the epitope, as compared to T-cells contacted with the same concentration of the T-Cell-MMP that do not have a TCR specific to the epitope.

[0053] In an embodiment where variant MODs that inhibit T-cell activation and an epitope are incorporated into a T-Cell-MMP, contacting the T-cells with at least one concentration of the T-Cell-MMP prevents activation of T-cells in an epitope specific manner as measured by T-cell proliferation.

[0054] The specificity of T-Cell-MMPs into which an epitope has been incorporated will depend on the relative contributions of the epitope and MODs to the binding. Where the MODs dominate the binding interactions, the specificity of the T-Cell-MMP of T-cells specific to the epitope will be reduced relative to T-Cell-MMP complexes where the epitope dominates the binding interactions by contributing more to the overall binding energy than the MODs. The greater the contribution of the epitope to a TCR specific to the epitope, the greater the specificity of the T-Cell-MMP will be for that T-cell type. Where an epitope has strong affinity for its TCR, the use of variant MODs with reduced affinity for their Co-MODs will favor epitope selective interactions of the T-Cell-MMP-epitope conjugates, and also facilitate selective delivery of any payload that may be conjugated to the T-Cell-MMP-epitope conjugate.

[0055] In addition to being useful as a structure into which to incorporate epitopes and prepare T-Cell-MMPs that are epitope specific, the T-Cell-MMPs described as either lacking an epitope or containing a null peptide may be employed to deliver a payload to target cells bearing receptors for the MODs and/or variant MODs present in the T-Cell-MMPs.

[0056] In an embodiment, T-Cell-MMPs bearing MODs inhibitory to T-cell activation and/or proliferation that lack an epitope (or contain a null peptide) may be used as stimulators of T-cells that

contain one or more receptors for the MOD or variant MODs present in the T-Cell-MMP. Such stimulatory T-Cell-MMPs may be used to simultaneously deliver a payload (e.g., a chemically conjugated chemotherapeutic) to the T-cells to which the T-Cell-MMPs binds.

[0057] In an embodiment, T-Cell-MMPs bearing MODs inhibitory to T-cell activation and/or proliferation that lack an epitope (or that contain a null peptide) may be used as an immunosuppressant alone or in conjunction with other immunosuppressants such as cyclosporine to suppress immune reactions (e.g., prevent graft-v-host or host-v-graft rejection). Such inhibitory T-Cell-MMPs may be used to simultaneously deliver a payload (e.g., a chemically conjugated chemotherapeutic) to the T-cells to which the T-Cell-MMPs binds

[0058] The present disclosure provides T-Cell-MMPs that are useful for modulating the activity of a T-cell and, accordingly, for modulating an immune response in an individual. The T-Cell-MMPs comprise a MOD that exhibits reduced binding affinity to a Co-MOD.

A. T-Cell-MMPs And T-Cell-MMP Epitope Conjugates

[0059] The T-Cell-MMP frameworks described herein comprise at least one chemical conjugation site on either the first polypeptide chain or the second polypeptide chain.

[0060] In an embodiment, the present disclosure provides a T-Cell-MMP comprising a heterodimer comprising: a) a first polypeptide comprising: a first MHC polypeptide; b) a second polypeptide comprising a second MHC polypeptide; c) at least one of first or second polypeptides comprises a chemical conjugation site, and d) at least one MOD, where the first and/or the second polypeptide comprises the at least one MOD (e.g., one, two, three, or more). Optionally, the first or the second polypeptide comprises an Ig Fc polypeptide or a non-Ig scaffold. One or more of the MODs, which are selected independently, may be a variant MOD that exhibits reduced affinity to a Co-MOD compared to the affinity of a corresponding wild-type MOD for the Co-MOD. The disclosure also provides T-Cell-MMPs in which an epitope (e.g., a peptide bearing an epitope) is covalently bound (directly or indirectly) to the chemical conjugation site forming a T-Cell-MMP-epitope conjugate. In such an embodiment, the epitope (e.g., epitope peptide) present in a T-Cell-MMP-epitope conjugate of the present disclosure may bind to a T-cell receptor (TCR) on a T-cell with an affinity of at least 100 micro molar (μM) (e.g., at least 10 μM , at least 1 μM , at least 100 nM, at least 10 nM, or at least 1 nM). A T-Cell-MMP-epitope conjugate may bind to a first T-cell with an affinity that is at least 25% higher than the affinity with which the T-Cell-MMP-epitope conjugate binds to a second T-cell, where the first T-cell expresses on its surface the Co-MOD and a TCR that binds the epitope with an affinity of at least 100 μM , and where the second T-cell expresses on its surface the Co-MOD but does not express on its surface a TCR that binds the epitope with an affinity of at least 100 μM (e.g., at least 10 μM , at least 1 μM , at least 100 nM, at least 10 nM, or at least 1 nM).

[0061] In an embodiment, the present disclosure provides a heterodimeric T-Cell-MMP (which may form higher level multimers, dimers, trimers, etc. of the heterodimers) comprising:

- a) a first polypeptide comprising a first MHC polypeptide;

- b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a second MHC polypeptide and ii) an optional immunoglobulin (Ig) Fc polypeptide scaffold or a non-Ig polypeptide scaffold;
- c) one or more first polypeptide chemical conjugation sites attached to or within the first polypeptide, and/or one or more second polypeptide chemical conjugation sites attached to or within the second polypeptide; and
- d) one or more immunomodulatory polypeptides (MODs), wherein at least one of the one or more MODs is

- A) at the C-terminus of the first polypeptide (see, e.g., A in FIGs 5 or 6),
- B) at the N-terminus of the second polypeptide (see, e.g., B in FIGs. 5 or 6),
- C) at the C-terminus of the second polypeptide (see, e.g., C in FIGs. 5 or 6), or
- D) at the C-terminus of the first polypeptide and at the N-terminus of the second polypeptide (see, e.g., D in FIGs. 5 or 6);

wherein each of the one or more MODs is an independently selected wild-type or variant MOD.

[0062] Such T-Cell-MMP frameworks act as a platform on which WT-1 epitopes (e.g., polypeptide epitopes) can be covalently attached through a linkage to one of the first or second chemical conjugation sites bound to at least one of the first and second MHC polypeptides forming a T-Cell-MMP-epitope conjugate. This permits facile introduction of different epitopes into the framework for presentation in the context of the T-Cell-MMP to a T-cell receptor (TCR) on a T-cell. Payload (e.g., chemotherapeutics) can similarly be attached to a T-Cell-MMP by covalent attachment to one of the first or second chemical conjugation sites (e.g., a site not employed for attachment of an epitope).

[0063] Where an immunoglobulin (Ig) Fc polypeptide or a non-Ig polypeptide scaffold that can multimerize is employed, the T-Cell-MMPs may multimerize. The complexes may be in the form of dimers (see, e.g., FIG. 7), trimers, tetramers, or pentamers. Compositions comprising multimers of T-Cell-MMPs may also comprise monomers and, accordingly may comprise monomers, dimers, trimers, tetramers, pentamers, or combinations of any thereof (e.g., a mixture of monomers and dimers).

[0064] In an embodiment, the MODs are independently selected wild-type MODs and/or variant MODs presented in a T-Cell-MMP that optionally comprises an epitope. In an embodiment, the MODs are one or more wt MODs and/or variant MODs capable of stimulating epitope-specific T-cell activation/proliferation (e.g., IL-2, 4-1BBL and/or CD80). In another embodiment, the MODs are one or more wt MODs and/or variant MODs capable of inhibiting T-cell activation/proliferation (e.g., FAS-L and/or PD-L1). When used in conjunction with a T-Cell-MMP bearing a suitable epitope, such activating or inhibitory MODs are capable of epitope-specific T-cell action, particularly where the MODs are variant MODs and the MHC-epitope-TCR interaction is sufficiently strong to dominate the interaction of the T-Cell-MMP with the T-cells.

1. Locations of the First and Second Chemical Conjugation Sites in T-Cell-MMPs

[0065] Prior to being subject to chemical conjugation reactions that incorporate an epitope (e.g., an epitope containing peptide) and/or payload, the T-Cell-MMPs described herein comprise at least one

chemical conjugation site. Where the T-Cell-MMPs comprise more than one chemical conjugation site, there may be two or more conjugation sites on the first polypeptide (first polypeptide chemical conjugation sites), two or more conjugation sites on the second polypeptide (second polypeptide chemical conjugation sites), or at least one first polypeptide chemical conjugation site and at least one second polypeptide chemical conjugation site. In each instance where more than one chemical conjugation site is present in a T-Cell-MMP molecule, the sites are independently selected and may employ the same or different chemistries, amino acid sequences, or chemical groups for conjugation. Some examples of the locations for first polypeptide chemical conjugation sites (indicated as CC-1) and second polypeptide chemical conjugation sites (indicated as CC-1) are shown in FIGs. 5-7.

[0066] In embodiments, the first polypeptide of the T-Cell-MMPs comprise: a first MHC polypeptide without a linker on its N-terminus and C-terminus; a first MHC polypeptide bearing a linker on its N-terminus; a first MHC polypeptide bearing a linker on its C-terminus, or a first MHC polypeptide bearing a linker on its N-terminus and C-terminus. At least one of the one or more first polypeptide chemical conjugation sites is: a) attached to (e.g., at the N- or C-terminus), or within, the sequence of the first MHC polypeptide when the first MHC polypeptide is without a linker on its N- and C-termini; b) attached to, or within, the sequence of the first MHC polypeptide, where the first MHC polypeptide comprises a linker on its N- and C-terminus; c) attached to, or within, the sequence of a linker on the N-terminus of the first MHC polypeptide; and/or d) attached to, or within, the sequence of a linker on the C-terminus of the first MHC polypeptide. Additional first polypeptide chemical conjugation sites of a T-Cell-MMP may be present at (attached to or within) any location on the first polypeptide (e.g., more than one enzyme modification sequence serving as a site for chemical conjugation), including the first MHC polypeptide or in any linker attached to the MHC peptide. In such embodiments, the first MHC polypeptide may comprise a β 2M polypeptide sequence as described below.

[0067] In embodiments, the second polypeptide of the T-Cell-MMPs comprise: a second MHC polypeptide without a linker on its N-terminus and C-terminus; a second MHC polypeptide bearing a linker on its N-terminus; a second MHC polypeptide bearing a linker on its C-terminus, or a second MHC polypeptide bearing a linker on its N-terminus and C-terminus. At least one of the one or more second polypeptide chemical conjugation sites is: a) attached to (e.g., at the N- or C-terminus), or within, the sequence of the second MHC polypeptide when the second MHC polypeptide is without a linker on its N- and C-termini; b) attached to, or within, the sequence of the second MHC polypeptide where the second MHC polypeptide comprises a linker on its N- and C-terminus; c) attached to, or within, the sequence of the linker on the N-terminus of the second MHC polypeptide; and/or d) attached to, or within, the sequence of the linker on the C-terminus of the second MHC polypeptide. In addition, when the second polypeptide contains an immunoglobulin (Fc) polypeptide aa sequence or a non-Ig polypeptide scaffold, along with an additional linker attached thereto, the second polypeptide chemical conjugation sites may be attached to or within the second MHC polypeptide, the immunoglobulin polypeptide, the polypeptide scaffold, or the attached linker. Additional second polypeptide chemical conjugation sites of a T-Cell-MMP may be present at (attached to or within) any location on the second polypeptide (e.g., more than

one enzyme modification sequence serving as a site for chemical conjugation), including the second MHC polypeptide or in any linker attached to it. In such embodiments, the second MHC polypeptide may comprise a MHC heavy chain (MHC-H) polypeptide sequence as described below.

[0068] In an embodiment, the first and second MHC polypeptides may be selected to be Class I MHC polypeptides, with the first MHC polypeptide comprising a β 2M polypeptide sequence and the second polypeptide comprising a MHC heavy chain sequence, wherein there is at least one chemical conjugation site on the first or second polypeptide. In an embodiment, at least one of the one or more first chemical conjugation sites in the T-Cell-MMP may be attached to (including at the N- or C-terminus) or within either the β 2M polypeptide or the linker attached to its N-terminus or C-terminus. In an embodiment, at least one of the one or more second polypeptide chemical conjugation sites in the T-Cell-MMP may be attached to (including at the N- or C-terminus) or within: the MHC-H polypeptide; a linker attached to the N- terminus or C-terminus of the MHC-H polypeptide; or, when present, attached to or within an immunoglobulin (Fc) polypeptide (or a non-Ig polypeptide scaffold) or a linker attached thereto. In another embodiment of such a Class I MHC polypeptide construct, both the first and second polypeptides comprise at least one chemical conjugation site.

[0069] Where the T-Cell-MMP comprises a β 2M polypeptide sequence, the sequence may have at least 85% amino acid sequence identity (e.g., at least 90%, 95%, 98% or 99% identity, or even 100% identity) to one of the amino acid sequences set forth in FIG. 4. The β 2M polypeptide may comprise an amino acid sequence having at least 20, 30, 40, 50, 80, 100, or 110 contiguous amino acids with identity to a portion of an amino acid sequence set forth in FIG 4. The chemical conjugation sequences can be attached to the β 2M polypeptide (e.g., at the N- and/or C- termini or linkers attached thereto) or within the β 2M polypeptide.

[0070] Where the T-Cell-MMP comprises a MHC-H polypeptide, it may be a HLA-A, -B, -C, -E, -F, or -G heavy chain. In an embodiment, the MHC-H polypeptide may comprise an amino acid sequence having at least 85% amino acid sequence identity (e.g., at least 90%, 95%, 98% or 99% identity, or even 100% identity) to the amino acid sequence set forth in one of FIGs. 3A-3H. The MHC Class I heavy chain polypeptides may comprise an amino acid sequence having at least 20, 30, 40, 50, 80, 100, 150, 200, 250, 300, or 330 contiguous amino acids with identity to a portion of an amino acid sequence set forth in FIGs. 3A-3H. The chemical conjugation sequences can be attached (e.g., at the N- and/or C-termini or linkers attached thereto) or within the MHC-H polypeptides.

[0071] The second polypeptide of the T-Cell-MMP may comprise an Ig Fc polypeptide sequence that can act as part of a molecule scaffold providing structure and the ability to multimerize to the T-Cell-MMP (or its epitope conjugate) and, in addition, potential locations for chemical conjugation. In some embodiments 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 such embodiments the Ig Fc polypeptide may comprise an amino acid sequence that has at least 85%, 90%, 95%, 98, or 99%, or even 100%, amino acid sequence identity to an amino acid sequence depicted in one of FIGs. 2A-2G. Ig Fc polypeptides may comprise a sequence having at least 20, 30, 40, 50, 60, 80, 100,

120, 140, 160, 180, 200, or 220 contiguous amino acids with identity to a portion of an amino acid sequence in any of FIGs. 2A-2G. In an embodiment where the second polypeptide comprises an IgG1 Fc polypeptide, the polypeptide may also comprise one or more amino acid substitutions selected from N297A, L234A, L235A, L234F, L235E, and P331S. In one such embodiment, the IgG1 Fc polypeptide comprises L234A and L235A substitutions either alone or in combination with a second polypeptide chemical conjugation site. The chemical conjugation sites can be located/attached at the N- and/or C-termini or to linkers attached thereto, or within the Ig Fc polypeptides.

2. Chemical Conjugation Sites of T-Cell-MMPs

[0072] The first and second polypeptide chemical conjugation sites of the T-Cell-MMPs may be any suitable site that can be modified upon treatment with a reagent and/or catalyst such as an enzyme that permits the formation of a covalent linkage to either one or both of the T-Cell-MMP polypeptides. In an embodiment, there is only one chemical conjugation site that has been introduced into either the first or second polypeptide of a T-Cell-MMP. In an embodiment, each first and second polypeptide chemical conjugations sites are selected to be either the same or different types of chemical conjugation sites, thereby permitting the same or different molecules to be selectively conjugated to each of the polypeptides. In another embodiment, each first and second polypeptide chemical conjugation site is selected such that they are different types of conjugation site on the respective polypeptides, permitting different molecules to be selectively conjugated to each of the polypeptides. In other embodiments, such as where both an epitope molecule and one or more payload molecules are to be incorporated into a T-Cell-MMP, more than one copy of a first and/or second polypeptide chemical conjugation may be introduced into the T-Cell-MMP. For example, a T-Cell-MMP may have one first polypeptide chemical conjugation site (e.g., for conjugating an epitope) and multiple second polypeptide chemical conjugation sites for delivering molecules of payload(or vice versa).

[0073] In embodiments, the first and second chemical conjugation sites may be selected independently from:

- a) peptide sequence attached to or within the first or second polypeptide that acts as an enzyme modification sequence (e.g., sulfatase, sortase, and/or transglutaminase sequences);
- b) non-natural amino acids and/or selenocysteines attached to or within the first or second polypeptide;
- c) engineered amino acid chemical conjugation sites;
- d) carbohydrate or oligosaccharide moieties attached to the first or second polypeptide; and
- e) IgG nucleotide binding sites attached to or within the first or second polypeptide.

a. Sulfatase Motifs

[0074] In those embodiments where enzymatic modification is chosen as the means of chemical conjugation, at least one of the one or more first and second chemical conjugation sites may comprise a sulfatase motif. Sulfatase motifs are usually 5 or 6 amino acids in length, and are described, for example, in U.S. Pat. No. 9,540,438 and U.S. Pat. Pub. No. 2017/0166639 A1, which are incorporated by reference. Insertion of the motif results in the formation of a protein or polypeptide that is sometimes referred to as

aldehyde tagged or having an aldehyde tag. The motif may be acted on by formylglycine generating enzyme(s) ("FGE" or "FGEs") to convert a cysteine or serine in the motif to a formylglycine residue ("fGly" although sometimes denoted "FGly"), which is an aldehyde containing amino acid that may be utilized for selective (e.g., site specific) chemical conjugation reactions. Accordingly, as used herein, "aldehyde tag" or "aldehyde tagged" polypeptides refer to an amino acid sequence comprising an unconverted sulfatase motif, as well as to an amino acid sequence comprising a sulfatase motif in which the cysteine or the serine residue of the motif has been converted to fGly by action of an FGE. In addition, where a sulfatase motif is provided in the context of an amino acid sequence, both the amino acid sequence (e.g., polypeptide) containing the unconverted motif as well as its fGly containing counterpart are disclosed. Once incorporated into a polypeptide (e.g., of a T-Cell-MMP), a fGly residue may be reacted with molecules (e.g., epitope peptides) comprising a variety of reactive groups including, but not limited to, thiosemicarbazide, aminooxy, hydrazide, and hydrazino groups to form a conjugate (e.g., a T-Cell-MMP-epitope conjugate) having a covalent bond between the peptide and the molecule via the fGly residue. Sulfatase motifs may be used to incorporate not only epitopes (e.g., epitope presenting peptides), but also to incorporate payloads (e.g., in the formation of conjugates with drugs and diagnostic molecules).

[0075] In embodiments, the sulfatase motif is at least 5 or 6 aa residues, but can be, for example, from 5 to 16 (e.g., 6-16, 5-14, 6-14, 5-12, 6-12, 5-10, 6-10, 5-8, or 6-8) aa in length. The sulfatase motif may be limited to a length less than 16, 14, 12, 10, or 8 amino acid residues.

[0076] In an embodiment, the sulfatase motif contains the sequence shown in Formula (I):

X1Z1X2Z2X3Z3 (SEQ ID NO:62), where

Z1 is cysteine or serine;

Z2 is either a proline or alanine residue (which can also be represented by "P/A");

Z3 is a basic amino acid (arginine, lysine, or histidine, usually lysine), or an aliphatic amino acid (alanine, glycine, leucine, valine, isoleucine, or proline, usually A, G, L, V, or I);

X1 is present or absent and, when present, can be any amino acid, though usually an aliphatic amino acid, a sulfur-containing amino acid, or a polar uncharged amino acid (e.g., other than an aromatic amino acid or a charged amino acid), usually L, M, V, S or T, more usually L, M, S or V, with the proviso that, when the sulfatase motif is at the N-terminus of the target polypeptide, X1 is present; and

X2 and X3 independently can be any amino acid, though usually an aliphatic amino acid, a polar, uncharged amino acid, or a sulfur containing amino acid (e.g., other than an aromatic amino acid or a charged amino acid), usually S, T, A, V, G or C, more usually S, T, A, V or G.

[0077] As indicated above, a sulfatase motif of an aldehyde tag is at least 5 or 6 amino acid residues, but can be, for example, from 5 to 16 amino acids in length. The motif can contain additional residues at one or both of the N- and C-termini, such that the aldehyde tag includes both a sulfatase motif and an "auxiliary motif." In an embodiment, the sulfatase motif includes a C-terminal auxiliary motif (i.e., following the Z3 position of the motif).

[0078] A variety of FGEs may be employed for the conversion (oxidation) of cysteine or serine in a sulfatase motif to fGly. As used herein, the term formylglycine generating enzyme, or FGE, refers to fGly-generating enzymes that catalyze the conversion of a cysteine or serine of a sulfatase motif to fGly. As discussed in U.S. Pat. No. 9,540,438, the literature often uses the term formylglycine-generating enzymes for those enzymes that convert a cysteine of the motif to fGly, whereas enzymes that convert a serine in a sulfatase motif to fGly are referred to as Ats-B-like.

[0079] Sulfatase motifs of Formula (I) amenable to conversion by a prokaryotic FGE often contain a cysteine or serine at Z1 and a proline at Z2 that may be modified either by the “SUMP I-type” FGE or the “AtsB-type” FGE, respectively. Prokaryotic FGE enzymes that may be employed include the enzymes from *Clostridium perfringens* (a cysteine type enzyme), *Klebsiella pneumoniae* (a Serine-type enzyme) or the FGE of *Mycobacterium tuberculosis*. Where peptides containing a sulfatase motif are being prepared for conversion into fGly-containing peptides by a eukaryotic FGE, for example by expression and conversion of the peptide in a eukaryotic cell or cell free system using a eukaryotic FGE, sulfatase motifs amenable to conversion by a eukaryotic FGE may advantageously be employed.

[0080] *Host* cells for production of polypeptides with unconverted sulfatase motifs, or where the cell expresses a suitable FGE for converting fGly-containing polypeptide sequences, include those of a prokaryotic and eukaryotic organism. Non-limiting examples include *Escherichia coli* strains, *Bacillus spp.* (e.g., *B. subtilis*, and the like), yeast or fungi (e.g., *S. cerevisiae*, *Pichia spp.*, and the like). Examples of other host cells, including those derived from a higher organism such as insects and vertebrates, particularly mammals, include, but are not limited to, CHO cells, HEK cells, and the like (e.g., American Type Culture Collection (ATCC) No. CCL-2), CHO cells (e.g., ATCC Nos. CRL9618 and CRL9096), CHO DG44 cells, CHO-K1 cells (ATCC CCL-61), 293 cells (e.g., ATCC No. CRL-1573), Vero cells, NIH 3T3 cells (e.g., ATCC No. CRL-1658), Hnh-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.

[0081] Sulfatase motifs may be incorporated into any desired location on the first or second polypeptide of the T-Cell-MMP (or its epitope conjugate). In an embodiment, a sulfatase motif may be added at or near the terminus of any element in the first or second polypeptide of the T-Cell-MMP (or its epitope conjugate), including the first and/or second MHC polypeptides (e.g., MHC-H and/or β 2M polypeptides), the scaffold or Ig Fc, and the linkers adjoining those elements. Accordingly, the sulfatase motif may be linked to an amino acid in the N-terminal region of β 2M (with or without a linker).

[0082] In an embodiment a sulfatase motif is incorporated into, or attached to (e.g., via a peptide linker), a T-Cell-MMP (or its epitope conjugate) in a first or second polypeptide that has a β 2M polypeptide with a sequence having at least 85% (e.g., at least 90%, 95%, 98% or 99%, or even 100%) amino acid sequence identity to a sequence shown in FIG. 4 (e.g., any of the full length sequences shown in FIG. 4, or the sequence of any of the mature β 2M polypeptides starting at amino acid 21 and ending at their C-terminus). For the purposes of this embodiment sequence identity of the β 2M polypeptide is

determined relative to the corresponding portion of a β 2M polypeptide in FIG 4 without consideration of the added sulfatase motif and any linker sequences present.

[0083] U.S. Pat. No. 9,540,438 discusses the incorporation of sulfatase motifs into the various immunoglobulin sequences, including Fc region polypeptides, and is herein incorporated by reference for its teachings on sulfatase motifs and modification of Fc polypeptides and other polypeptides. That patent is also incorporated by reference for its guidance on FGE enzymes, and their use in forming fGly residues, as well as the chemistry related to the coupling of molecules such as epitopes and payloads to fGly residues.

[0084] The incorporation of a sulfatase motif may be accomplished by incorporating a nucleic acid sequence encoding the motif at the desired location in a nucleic acid encoding the first and/or second polypeptide of the T-Cell-MMP. As discussed below, the nucleic acid sequence may be placed under the control of a transcriptional regulatory sequence(s) (a promoter) and provided with regulatory elements that direct its expression. The expressed protein may be treated with one or more FGEs after expression and partial or complete purification. Alternatively, expression of the nucleic acid in cells that express a FGE that recognizes the sulfatase motif results in the conversion of the cysteine or serine of the motif to fGly, which is sometimes called oxoalanine.

[0085] In view of the foregoing, this disclosure provides for T-Cell-MMPs comprising one or more fGly residues incorporated into the sequence of the first or second polypeptide chain as discussed above. The fGly residues may, for example, be in the context of the sequence X1(fGly)X2Z2X3Z3, where: fGly is the formylglycine residue; and Z2, Z3, X1, X2 and X3 are as defined in Formula (I) above.

[0086] After chemical conjugation the T-Cell-MMPs comprise one or more fGly' residues incorporated into the sequence of the first or second polypeptide chain in the context of the sequence X1(fGly')X2Z2X3Z3, where the fGly' residue is formylglycine that has undergone a chemical reaction and now has a covalently attached moiety (e.g., epitope or payload).

[0087] A number of chemistries and commercially available reagents can be utilized to conjugate a molecule (e.g., an epitope or payload) to a fGly residue, including, but not limited to, the use of thiosemicarbazide, aminooxy, hydrazide, or hydrazino derivatives of the molecules to be coupled at a fGly-containing chemical conjugation site. For example, epitopes (e.g., epitope peptides) and/or payloads bearing thiosemicarbazide, aminooxy, hydrazide, hydrazino or hydrazinyl functional groups (e.g., attached directly to an amino acid of a peptide or via a linker such as a PEG) can be reacted with fGly-containing first or second polypeptides of the T-Cell-MMP to form a covalently linked epitope. Similarly, payloads such as drugs and therapeutics can be incorporated using, for example, biotin hydrazide as a linking agent.

[0088] An epitope (e.g., an epitope presenting peptide, phosphopeptide, lipopeptide, or glycopeptide) such as an epitope having a length from about 4 aa to about 20 aa (e.g., 4 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, or 20 aa) in length and/or one or more payloads may be conjugated to a fGly containing polypeptide.

[0089] The disclosure provides for methods of preparing T-Cell-MMP-epitope conjugates and/or T-Cell-MMP-payload conjugates comprising:

- a) incorporating a sequence encoding a sulfatase motif including a serine or cysteine (e.g., a sulfatase motif of Formula (I) or (II) such as X1CX2PX3Z3 (SEQ ID NO:63); CX1PX2Z3 (SEQ ID NO:64) discussed above) into a nucleic acid encoding a first polypeptide and/or second polypeptide of a T-Cell-MMP;
- b) expressing the sulfatase motif-containing first polypeptide and/or second polypeptide in a cell that
 - i) expresses a FGE and converts the serine or cysteine of the sulfatase motif to a fGly and partially or completely purifying the fGly-containing first polypeptide and/or second polypeptide separately or as the T-Cell-MMP, or
 - ii) does not express a FGE that converts a serine or cysteine of the sulfatase motif to a fGly, purifying or partially purifying the T-Cell-MMP containing the fGly residue and contacting the purified or partially purified T-Cell-MMP with a FGE that converts the serine or cysteine of the sulfatase motif into a fGly residue; and
- c) contacting the fGly-containing first and/or second polypeptides separately, or as part of a T-Cell-MMP, with an epitope and/or payload that has been functionalized with a group that forms a covalent bond between the aldehyde of the fGly and epitope and/or payload; thereby forming a T-Cell-MMP-epitope conjugate and/or T-Cell-MMP payload conjugate.

In such methods the epitope (epitope containing molecule) and/or payload may be functionalized by any suitable function group that reacts selectively with an aldehyde group. Such groups may, for example, be selected from the group consisting of thiosemicarbazide, aminooxy, hydrazide, and hydrazino. In an embodiment a sulfatase motif is incorporated into a first or second polypeptide comprising a β 2M aa sequence with at least 85% (e.g., at least 90%, 95%, 98% or 99%, or even 100%) sequence identity to at least 60, 70, 80 or 90 contiguous aas of a β 2M sequence shown in FIG. 4, (e.g., with identity calculated without including or before the addition of the sulfatase motif sequence). For example, the sulfatase motif may be placed between the signal sequence and the sequence of the mature peptide, or at the N-terminus of the mature peptide, and the motif may be separated from the β 2M sequence(s) by peptide linkers.

[0090] In an embodiment, the method of preparing a T-Cell-MMP-epitope conjugate and/or T-Cell-MMP payload conjugate, a sulfatase motif is incorporated into a polypeptide comprising a sequence having at least 85% (e.g., at least 90%, 95%, 98% or 99%, or even 100%) amino acid sequence identity to at least 150, 175, 200, or 225 contiguous aas of a sequence shown in FIG. 3 (e.g., 3A-3I, with sequence identity calculated without including the addition of the sulfatase motif sequence). In one such embodiment, the sulfatase motifs may be utilized as sites for the conjugation of, for example, epitopes and/or payloads either directly or indirectly through a peptide or chemical linker.

b. Sortase A Enzyme Sites

[0091] Epitopes (e.g., peptides comprising the sequence of an epitope) and payloads may be attached at the N- and/or C-termini of the first and/or second polypeptides of a T-Cell-MMP by incorporating sites for Sortase A conjugation at those locations.

[0092] Sortase A recognizes a C-terminal pentapeptide sequence LP(X5)TG/A (SEQ ID NO 65, with X5 being any single amino acid, and G/A being a glycine or alanine), and creates an amide bond between the threonine within the sequence and glycine or alanine in the N-terminus of the conjugation partner.

[0093] For attachment of epitopes or payloads to the carboxy terminus of the first or second polypeptide of the T-Cell-MMP, an LP(X5)TG/A is engineered into the carboxy terminal portion of the desired polypeptide(s). An exposed stretch of glycines or alanines (e.g., (G)₃₋₅ (SEQ ID NOs:66 and 67 when using Sortase A from *Staphylococcus aureus* or alanines (A)₃₋₅, SEQ ID NOs:68 and 69 when using Sortase A from *Streptococcus pyogenes*) is engineered into the N-terminus of a peptide that comprises an epitope (or a linker attached thereto), a peptide payload (or a linker attached thereto), or a peptide covalently attached to a non-peptide epitope or payload.

[0094] For attachment of epitopes or payloads to the amino terminus of the first or second polypeptide of the T-Cell-MMP, an aa sequence comprising an exposed stretch of glycines (e.g., (G)_{2, 3, 4, or 5}) or alanines (e.g., (A)_{2, 3, 4, or 5}) is engineered to appear at the N-terminus of the desired polypeptide(s), and a LP(X5)TG/A is engineered into the carboxy terminal portion of a peptide that comprises an epitope (or a linker attached thereto), a peptide payload (or a linker attached thereto), or a peptide covalently attached to a non-peptide epitope or payload.

[0095] Combining Sortase A with the amino and carboxy engineered peptides results in a cleavage between the Thr and Gly/Ala residues in the LP(X5)TG/A sequence, forming a thioester intermediate with the carboxy labeled peptide. Nucleophilic attack by the N-terminal modified polypeptide results in the formation of a covalently coupled complex of the form: carboxy-modified polypeptide-LP(X5)T*G/A-amino-modified polypeptide, where the "*" represents the bond formed between the threonine of the LP(X5)TG/A motif and the glycine or alanine of the N-terminal modified peptide.

[0096] In place of LP(X5)TG/A, a LPETGG (SEQ ID NOs:70) peptide may be used for *S. aureus* Sortase A coupling, or a LPETAA (SEQ ID NOs:71) peptide may be used for *S. pyogenes* Sortase A coupling. The conjugation reaction is still between the threonine and the amino terminal oligoglycine or oligoalanine peptide to yield a carboxy-modified polypeptide-LP(X5)T*G/A-amino-modified polypeptide, where the "*" represents the bond formed between the threonine and the glycine or alanine of the N-terminal modified peptide.

[0097] In an embodiment, a A₂₋₅ or a G₂₋₅ motif is incorporated into a polypeptide comprising a sequence having at least 85% (e.g., at least 90%, 95%, 98% or 99%, or even 100%) amino acid sequence identity to at least 60, 70, 80 or 90 contiguous aas of a sequence shown in FIG. 4 (e.g., either the entire sequences shown in FIG. 4, or the sequence of the mature polypeptides starting at amino acid 21 and ending at their C-terminus), with sequence identity assessed without consideration of the added A₂₋₅ or a G₂₋₅ motif and any linker sequences present.

[0098] In an embodiment, an A₂₋₅ or a G₂₋₅ motif is incorporated into a polypeptide comprising a β2M sequence having 1 to 15 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15) amino acid deletions, insertions and/or changes compared with a sequence shown in FIG. 4 (e.g., any of the full length sequences shown in FIG. 4, or any of the mature polypeptide sequences starting at amino acid 21 and ending at their C-terminus), with amino acid deletions, insertions and/or changes assessed without consideration of the added A₂₋₅ or a G₂₋₅ motif and any linker sequences present. In one such embodiment an A₂₋₅ or a G₂₋₅ motif may either replace and/or be inserted between any of the amino terminal 15 (e.g., 1-5, 5-10 or 10-15) amino acids of a mature β2M sequence, such as those shown in FIG. 4.

c. Transglutaminase Enzyme Sites

[0099] Transglutaminases (mTGs) catalyze the formation of a covalent bond between the amide group on the side chain of a glutamine residue and a primary amine donor (e.g., a primary alkyl amine, such as is found on the side chain of a lysine residue in a polypeptide). Transglutaminases may be employed to conjugate epitopes and payloads to T-Cell-MMPs, either directly or indirectly via a linker comprising a free primary amine. As such, glutamine residues present in the first and/or second polypeptides of the T-Cell-MMP may be considered as chemical conjugation sites when they can be accessed by enzymes such as *Streptovorticillium mobaraense* transglutaminase. That enzyme (EC 2.3.2.13) is a stable, calcium-independent enzyme catalyzing the γ-acyl transfer of glutamine to the ε-amino group of lysine. Glutamine residues appearing in a sequence are, however, not always accessible for enzymatic modification. The limited accessibility can be advantageous as it limits the number of locations where modification may occur. For example, bacterial mTGs are generally unable to modify glutamine residues in native IgG1s; however, Schibli and co-workers (Jeger, S., et al. *Angew Chem (Int Engl)*. 2010;49:99957 and Dennler P, et al. *Bioconjug Chem*. 2014;25(3):569–78) found that deglycosylating IgG1s at N297 rendered glutamine residue Q295 accessible and permitted enzymatic ligation to create an antibody drug conjugate. Further, by producing a N297 to Q297 IgG1 mutant, they introduce two sites for enzymatic labeling by transglutaminase.

[00100] Where a first and/or second polypeptide of the T-Cell-MMP does not contain a glutamine that may be employed as a chemical conjugation site (e.g., it is not accessible to a transglutaminase or not placed in the desired location), a glutamine residue, or a sequence comprising an accessible glutamine that can act as a substrate of a transglutaminase (sometimes referred to as a “glutamine tag” or a “Q-tag”), may be incorporated into the polypeptide. The added glutamine or Q-tag may act as a first polypeptide chemical conjugation site or a second polypeptide chemical conjugation site. US Pat. Pub.

No.2017/0043033 A1 describes the incorporation of glutamine residues and Q-tags and the use of transglutaminase for modifying polypeptides and is incorporated herein for those teachings.

[00101] Incorporation of glutamine residues and Q-tags may be accomplished chemically where the peptide is synthesized, or by modifying a nucleic acid that encodes the polypeptide and expressing the modified nucleic acid in a cell or cell free system. In embodiments, the glutamine-containing Q-tag comprises an amino acid sequence selected from the group consisting of LQG, LLQGG (SEQ ID

NO:72), LLQG (SEQ ID NO:73), LSLSQG (SEQ ID NO:74), and LLQLQG (SEQ ID NO:75) (numerous others are available).

[00102] In an embodiment, the added glutamine residue or Q-tag is attached to (e.g., at the N- or C-terminus), or within, the sequence of the first MHC polypeptide, or, if present, a linker attached to the first MHC polypeptide. In one such embodiment, the first MHC polypeptide of a T-Cell-MMP is a β 2M polypeptide, and an added glutamine or Q-tag is incorporated within 20, 15, or 10 amino acids of the N-terminus of a mature β 2M polypeptide sequence, which exclude the 20 base pair signal sequence, provided in FIG. 4 (or a peptide having at least 85%, 90%, 95%, 98%, 99, or even 100% sequence identity to a mature β 2M polypeptide in FIG. 4). In another embodiment, the glutamine or Q-tag is present in a polypeptide linker attached to the N-terminus of one of the mature β 2M polypeptides provided in FIG. 4.

[00103] In an embodiment the added glutamine residue or Q-tag is attached to (e.g., at the N- or C-terminus), or within, the sequence of the second polypeptide of a T-Cell-MMP, for example at a terminus or within a second MHC polypeptide (e.g., a MHC-H peptide), or, if present, a Fc, scaffold peptide or linker attached directly or indirectly to the second MHC polypeptide. In one embodiment, the second MHC polypeptide is a MHC-H polypeptide, the second polypeptide comprises a Fc polypeptide, and an added glutamine or Q-tag is incorporated within the MHC-H or the Fc polypeptide sequence. In another embodiment, the glutamine or Q-tag is present within a polypeptide linker between the MHC-H and Fc polypeptides, or within a linker attached to the carboxyl terminus of the Fc polypeptide.

[00104] Payloads and epitopes that contain, or have been modified to contain, a primary amine group may be used as the amine donor in a transglutaminase catalyzed reaction forming a covalent bond between a glutamine residue (e.g., a glutamine residue in a Q-tag) and the epitope or payload.

[00105] Where an epitope or payload does not comprise a suitable primary amine to permit it to act as the amine donor, the epitope or payload may be chemically modified to incorporate an amine group (e.g., modified to incorporate a primary amine by linkage to a lysine, aminocaproic acid, cadaverine *etc.*). Where an epitope or payload comprises a peptide and requires a primary amine to act as the amine donor a lysine or another primary amine that a transglutaminase can act on may be incorporated into the peptide. Other amine containing compounds that may provide a primary amine group and that may be incorporated into, or at the end of, an alpha amino acid chain include, but are not limited to, homolysine, 2,7-diaminoheptanoic acid, and aminoheptanoic acid. Alternatively, the epitope or payload may be attached to a peptide or non-peptide linker that comprises a suitable amine group. Examples of suitable non-peptide linkers include an alkyl linker and a PEG (polyethylene glycol) linker.

[00106] Transglutaminase can be obtained from a variety of sources including enzymes from: mammalian liver (e.g., guinea pig liver); fungi (e.g., *Oomycetes*, *Actinomycetes*, *Saccharomyces*, *Candida*, *Cryptococcus*, *Monascus*, or *Rhizopus* transglutaminases); myxomycetes (e.g., *Physarum polycephalum* transglutaminase); and/or bacteria including a variety of *Streptovorticillium*, *Streptomyces*, *Actinomadura* sp., *Bacillus*, and the like.

[00107] As discussed above for other first polypeptide chemical conjugation sites and second polypeptide chemical conjugation sites, a glutamine or Q-tag may be incorporated into any desired location on the first or second polypeptide of the T-Cell-MMP. In an embodiment, a glutamine or Q-tag may be added at or near the terminus of any element in the first or second polypeptide of the T-Cell-MMP, including the first and second MHC polypeptides (e.g., MHC-H and β 2M polypeptides), the scaffold or Ig Fc, and the linkers adjoining those elements.

[00108] In one embodiment, where the first polypeptide of the T-Cell-MMP comprises a β 2M polypeptide sequence, the first polypeptide contains a glutamine or Q-tag at the N-terminus of the polypeptide, or at the N-terminus of a polypeptide linker attached to the first polypeptide (e.g., the linker is attached to the N-terminus of the first polypeptide).

[00109] In an embodiment a Q-tag motif is incorporated into a polypeptide comprising a β 2M sequence having at least 85% (e.g., at least 90%, 95%, 98% or 99%, or even 100%) amino acid sequence identity to at least 60, 70, 80 or 90 contiguous aas of a sequence shown in FIG. 4 (e.g., any of the full-length sequences shown in FIG. 4, or the sequence of any of the mature β 2M polypeptide starting at amino acid 21 and ending at their C-terminus), with identity assessed without consideration of the added Q-tag motif and any linker sequences present.

[00110] In an embodiment a Q-tag motif is incorporated into a sequence having 1 to 15 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15) amino acid deletions, insertions and/or changes compared with a sequence shown in FIG. 4 (either the entire sequences shown in FIG. 4, or the sequence of the mature polypeptides starting at amino acid 21 and ending at their C-terminus). Changes are assessed without consideration of the amino acids of the Q-tag motif and any linker sequences present. In one such embodiment a Q-tag motif may replace and/or be inserted between any of the amino terminal 15 (e.g., 1-5, 5-10, or 10-15) amino acids of a mature β 2M sequence, such as those shown in FIG. 4.

[00111] Q-tags may be created by modifying the aa sequence around any one, two, or three of the glutamine residues appearing in a β 2M and/or MHC-H chain sequence appearing in a T-Cell-MMP and used as a chemical conjugation site for addition of an epitope or payload. Similarly, Q-tags may be incorporated into the IgFc region as second polypeptide chemical conjugation sites and used for the conjugation of, for example, epitopes and/or payloads either directly or indirectly through a peptide or chemical linker bearing primary amine.

d. Selenocysteine and Non-Natural Amino Acids as Chemical Conjugation Sites

[00112] One strategy for providing site-specific chemical conjugation sites in the first and/or second polypeptides of a T-Cell-MMP employs the insertion of amino acids with reactivity distinct from the other amino acids present in the polypeptide. Such amino acids include, but are not limited to, the non-natural amino acids, acetylphenylalanine (p-acetyl-L-phenylalanine, pAcPhe), parazido phenylalanine, and propynyl-tyrosine, and the naturally occurring amino acid, selenocysteine (Sec).

[00113] Thanos *et al.* in US Pat. Publication No. 20140051836 A1 discuss some other non-natural amino acids including O-methyl-L-tyrosine, L-3-(2-naphthyl)alanine, a 3-methyl-phenylalanine, an O-4-allyl-L-tyrosine, a 4-propyl-L-tyrosine, a tri-O-acetyl-GlcNAc β -serine, L-Dopa, a fluorinated

phenylalanine, an isopropyl-L-phenylalanine, a p-acyl-L-phenylalanine, a p-benzoyl-L-phenylalanine, L-phosphoserine, a phosphoserine, a phosphonoserine, a phosphotyrosine, a p-iodo-phenylalanine, a p-bromophenylalanine, a p-amino-L-phenylalanine, an isopropyl-L-phenylalanine, and a p-propargyloxy-phenylalanine. Other non-natural amino acids include reactive groups including amino, carboxy, acetyl, hydrazino, hydrazido, semicarbazido, sulfanyl, azido and alkynyl. *See, e.g.,* US Pat. Publication No. 20140046030 A1.

[00114] In addition to directly synthesizing polypeptides in the laboratory, two methods utilizing stop codons have been developed to incorporate non-natural amino acids into proteins and polypeptides utilizing transcription-translation systems. The first incorporates selenocysteine (Sec) by pairing the opal stop codon, UGA, with a Sec insertion sequence. The second incorporates non-natural amino acids into a polypeptide generally through the use of amber, ochre, or opal stop codons. The use of other types of codons such as a unique codon, a rare codon, an unnatural codon, a five-base codon, and a four-base codon, and the use of nonsense and frameshift suppression have also been reported. *See, e.g.,* US Pat. Publication No. 20140046030 A1 and Rodriguez et al., PNAS 103(23)8650-8655(2006). By way of example, the non-natural amino acid acetylphenylalanine may be incorporated at an amber codon using a tRNA/aminoacyl tRNA synthetase pair in an *in vivo* or cell free transcription-translation system.

[00115] Incorporation of both selenocysteine and non-natural amino acids requires engineering the necessary stop codon(s) into the nucleic acid coding sequence of the first and/or second polypeptide of the T-Cell-MMP at the desired location(s), after which the coding sequence is used to express the first or second polypeptide strand of the T-Cell-MMP in an *in vivo* or cell free transcription-translation system.

[00116] *In vivo* systems generally rely on engineered cell-lines to incorporate non-natural amino acids that act as bio-orthogonal chemical conjugation sites into polypeptides and proteins. *See, e.g.,* International Published Application No. 2002/085923 entitled “*In vivo* incorporation of unnatural amino acids.” *In vivo* non-natural amino acid incorporation relies on a tRNA and an aminoacyl tRNA synthetase (aaRS) pair that is orthogonal to all the endogenous tRNAs and synthetases in the host cell. The non-natural amino acid of choice is supplemented to the media during cell culture or fermentation, making cell-permeability and stability important considerations.

[00117] Various cell-free synthesis systems provided with the charged tRNA may also be utilized to incorporate non-natural amino acids. Such systems include those described in US Published Pat. Application No. 20160115487A1; Gubens et al., *RNA*. 2010 Aug; 16(8): 1660–1672; Kim, D. M. and Swartz, J. R. *Biotechnol. Bioeng.* 66:180-8 (1999); Kim, D. M. and Swartz, J. R. *Biotechnol. Prog.* 16:385-90 (2000); Kim, D. M. and Swartz, J. R. *Biotechnol. Bioeng.* 74:309-16 (2001); Swartz et al, *Methods Mol. Biol.* 267:169-82 (2004); Kim, D. M. and Swartz, J. R. *Biotechnol. Bioeng.* 85:122-29 (2004); Jewett, M. C. and Swartz, J. R., *Biotechnol. Bioeng.* 86:19-26 (2004); Yin, G. and Swartz, J. R., *Biotechnol. Bioeng.* 86:188-95 (2004); Jewett, M. C. and Swartz, J. R., *Biotechnol. Bioeng.* 87:465-72 (2004); Voloshin, A. M. and Swartz, J. R., *Biotechnol. Bioeng.* 91:516-21 (2005).

[00118] Once incorporated into the first or second polypeptide of the T-Cell-MMP, epitopes and/or payload bearing groups reactive with the incorporated selenocysteine or non-natural amino acid are

brought into contact with the T-Cell-MMP under suitable conditions to form a covalent bond. By way of example, the keto group of the pAcPhe is reactive towards alkoxy-amines, via oxime coupling, and can be conjugated directly to alkoxyamine containing epitopes and/or payloads or indirectly to epitopes and payloads via an alkoxyamine containing linker. Selenocysteine reacts with, for example, primary alkyl iodides (e.g., iodoacetamide which can be used as a linker), maleimides, and methylsulfone phenyloxadiazole groups. Accordingly, epitopes and/or payloads bearing those groups or bound to linkers bearing those groups can be covalently bound to polypeptide chains bearing selenocysteines.

[00119] As discussed above for other first polypeptide chemical conjugation sites and second polypeptide chemical conjugation sites, selenocysteines and/or non-natural amino acids may be incorporated into any desired location in the first or second polypeptide of the T-Cell-MMP. In an embodiment, selenocysteines and/or non-natural amino acids may be added at or near the terminus of any element in the first or second polypeptide of the T-Cell-MMP, including the first and second MHC polypeptides (e.g., MHC-H and β 2M polypeptides), the scaffold or Ig Fc, and the linkers adjoining those elements. In embodiments selenocysteines and/or non-natural amino acids may be incorporated into a β 2M, class I MHC heavy chain, and/or a Fc Ig polypeptide. In an embodiment, selenocysteines and/or non-natural amino acids may be incorporated into the first polypeptide near or at the amino terminal end of the first MHC polypeptide (e.g., the β 2M polypeptide) or a linker attached to it. For example, where the first polypeptide comprises a β 2M sequence, selenocysteines and/or non-natural amino acids may be incorporated at or near the N-terminus of a β 2M sequence, permitting the chemical conjugation of, for example, an epitope either directly or through a linker. By way of example, the sequences of β 2M as shown in FIG. 4 begin with a 20 amino acid leader sequence, and the mature polypeptide begins with the initial sequence IQRTP(K/Q)IQVYS... and continues through the remainder of the polypeptide (see SEQ ID NOs:57-61).

[00120] In an embodiment selenocysteines and/or non-natural amino acids are incorporated into a polypeptide comprising a β 2M sequence having at least 85% (e.g., at least 90%, 95%, 98% or 99%, or even 100%) amino acid sequence identity to a β 2M sequence shown in FIG. 4 (e.g., any of the full length sequences shown in FIG. 4, or the sequence of any of the mature β 2M polypeptides starting at amino acid 21 and ending at their C-terminus), with sequence identity assessed without consideration of the added selenocysteines and/or non-natural amino acids and any linker sequences present.

[00121] In an embodiment selenocysteines and/or non-natural amino acids are incorporated into a polypeptide comprising a β 2M sequence having 1 to 15 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15) amino acid deletions, insertions and/or changes compared with a β 2M sequence shown in FIG. 4 (e.g., any of the full-length sequences shown in FIG. 4, or the sequence of any of the mature β 2M polypeptides starting at amino acid 21 and ending at their C-terminus). Changes are assessed without consideration of the amino acids of the selenocysteines and/or non-natural amino acids and any linker sequences present. In one such embodiment a selenocysteine and/or non-natural amino acid may replace and/or be inserted between any of the amino terminal 15 amino acids of a mature β 2M sequence, such as those shown in FIG. 4.

[00122] In other embodiments, selenocysteines and/or non-natural amino acids may be incorporated into polypeptides comprising a MHC-H chain or IgFc polypeptide sequences (including linkers attached thereto) as chemical conjugation sites. In one such embodiment they may be utilized as sites for the conjugation of, for example, epitopes and/or payloads conjugated to the T-Cell-MMP either directly or indirectly through a peptide or chemical linker.

e. Engineered Amino Acid Chemical Conjugation Sites

[00123] Any of the variety of functionalities (e.g., -SH, -NH₃, -OH, -COOH and the like) present in the side chains of naturally occurring amino acids, or at the termini of polypeptides, can be used as chemical conjugation sites. This includes the side chains of lysine and cysteine, which are readily modifiable by reagents including N-hydroxysuccinimide and maleimide functionalities, respectively. The main disadvantages of utilizing such amino acid residues is the potential variability and heterogeneity of the products. For example, an IgG has over 80 lysines, with over 20 at solvent-accessible sites. *See*, e.g., McComb and Owen, AAPS J. 117(2): 339-351. Cysteines tend to be less widely distributed; they tend to be engaged in disulfide bonds and may be inaccessible and not located where it is desirable to place a chemical conjugation site. Accordingly, it is possible to engineer the first and/or second polypeptide to incorporate non-naturally occurring amino acids at the desired locations for selective modification of the T-Cell-MMP first and/or second polypeptides. Engineering may take the form of direct chemical synthesis of the polypeptides (e.g., by coupling appropriately blocked amino acids) and/or by modifying the sequence of a nucleic acid encoding the polypeptide followed expression in a cell or cell free system. Accordingly, the specification includes and provides for the preparation of the first and/or second polypeptide of a T-Cell-MMP by transcription/translation bearing a non-natural or natural (including selenocysteine) amino acid to be used as a chemical conjugation site (e.g., for epitopes or peptides).

[00124] The specification also includes and provides for the preparation of all or part of the first and/or second polypeptide of a T-Cell-MMP by transcription/translation, and joining to the C- or N-terminus of the translated portion of the first and/or second polypeptide an engineered polypeptide bearing a non-natural or natural (including selenocysteine) amino acid to be used as a chemical conjugation site (e.g., for epitopes or peptides). The engineered peptide may be joined by any suitable method, including the use of a sortase as described for epitope peptides above and may include a linker peptide sequence. In an embodiment the engineered peptide may comprise a sequence of 2, 3, 4, or 5 alanines or glycines that may serve for sortase conjugation and/or as part of a linker sequence.

[00125] In one embodiment, a first or second polypeptide of a T-Cell-MMP contains at least one naturally occurring amino acid (e.g., a cysteine) to be used as a chemical conjugation site engineered into a β 2M sequence as shown in FIG. 4, an IgFc sequence as shown in any of FIGs. 2A-G, or a MHC Class I heavy chain polypeptide as shown in FIG. 3A-3I. In an embodiment, at least one naturally occurring amino acid to be used as a chemical conjugation site is engineered into a polypeptide having at least 85% (e.g., at least 90%, 95%, 98% or 99%, or even 100%) amino acid sequence identity to a β 2M sequence as shown in FIG. 4, an IgFc sequence as shown in FIG. 2, or a MHC Class I heavy chain polypeptide as shown in any of FIGs. 3A-3I. At least one naturally occurring amino acid (e.g., a cysteine) may be

engineered as a chemical conjugation site in a T-Cell-MMP first or second polypeptide comprising a β 2M amino acid sequence having at least 90% (e.g., at least 93%, 95%, 98% or 99%, or even 100%) amino acid sequence identity with at least the amino terminal 10, 20, 30, 40, 50 60 or 70 amino acids of a mature β 2M sequence as shown in FIG. 4. At least one naturally occurring amino acid (e.g., a cysteine) may be engineered as a chemical conjugation site in a T-Cell-MMP first or second polypeptide comprising an IgFc sequence as shown in any of FIGs. 2A-2G. At least one naturally occurring amino acid (e.g., a cysteine) may be engineered as a chemical conjugation site in a T-Cell-MMP first or second polypeptide comprising a MHC Class I heavy chain polypeptide as shown in any of FIGs. 3A to 3I. In another embodiment, at least one naturally occurring amino acid to be used as a chemical conjugation site is engineered into a first or second polypeptide comprising a contiguous sequence of at least 30, 40, 50, 60, 70, 80, 90, or 100 amino acids having 100% amino acid sequence identity a MHC Class I heavy chain sequence as shown in any of FIGs. 3A to 3I. In any of the embodiments mentioned above where a naturally occurring amino acid is engineered into a polypeptide, the amino acid may be selected from the group consisting of arginine, lysine, cysteine, serine, threonine, glutamic acid, glutamine, aspartic acid, and asparagine. Alternatively, the amino acid engineered as a conjugation site is selected from the group consisting of lysine, cysteine, serine, threonine, and glutamine. The amino acid engineered as a conjugation site may also be selected from the group consisting of lysine, glutamine, and cysteine. In an embodiment, the engineered amino acid is cysteine. In an embodiment, the engineered amino acid is lysine. In another embodiment, the engineered amino acid is glutamine.

[00126] Any method known in the art may be used to couple payloads or epitopes to amino acids engineered into the first or second polypeptides of the T-Cell-MMP. By way of example, maleimides may be utilized to couple to sulfhydryls, N-hydroxysuccinimide may be utilized to couple to amine groups, acid anhydrides or chlorides may be used to couple to alcohols or amines, and dehydrating agents may be used to couple alcohols or amines to carboxylic acid groups. Accordingly, using such chemistry an epitope or payload may be coupled directly, or indirectly through a linker (e.g., a homo- or hetero-bifunctional crosslinker), to a location on a first and/or second polypeptide. A number of bifunctional crosslinkers may be utilized, including, but not limited to those described for linking a payload to the T-Cell-MMP described herein below. For example, an epitope peptide (or a peptide-containing payload) including a maleimide group attached by way of a homo or heterobifunctional linker (see e.g., FIG. 8) or a maleimide amino acid can be conjugated to a sulfhydryl of a chemical conjugation site (e.g., a cysteine residue) that is naturally occurring or engineered into a T-Cell-MMP.

[00127] Maleimido amino acids can be incorporated directly into peptides (e.g., epitope peptides) using a Diels-Alder/retro-Diels-Alder protecting scheme, as part of a solid phase peptide synthesis. See, e.g., Koehler, Kenneth Christopher (2012), "Development and Implementation of Clickable Amino Acids," *Chemical & Biological Engineering Graduate Theses & Dissertations*, 31, https://scholar.colorado.edu/chbe_gradetds/31.

[00128] A maleimide group may also be appended to an epitope peptide using a homobifunctional or heterobifunctional linker (sometimes referred to as a crosslinker) that attaches a maleimide directly (or

indirectly, e.g., through an intervening linker that may comprise additional amino acids bound to the peptide presenting the epitope) to the epitope peptide. For example, a heterobifunctional N-hydroxysuccinimide - maleimide crosslinker can attach maleimide to an amine group of a peptide lysine. Some specific cross linkers include molecules with a maleimide functionality and either a N-hydroxysuccinimide ester (NHS) or N-succinimidyl group that can attach an maleimide to an amine (e.g., an epsilon amino group of lysine). Examples such crosslinkers include, but are not limited to, NHS-PEG4-maleimide, γ -maleimide butyric acid N-succinimidyl ester (GMBS); ϵ -maleimidocaproic acid N-hydroxysuccinimide ester (EMCS); m-maleimide benzoyl-N-hydroxysuccinimide ester (MBS); and N-(α -maleimidoacetoxy)-succinimide ester (AMAS), which offer different lengths and properties for peptide immobilization. Other amine reactive crosslinkers that incorporate a maleimide group include N-succinimidyl 4-(2-pyridyldithio)butanoate (SPDB). Additional cross linkers (bifunctional agents) are recited below. In an embodiment the epitopes coupled to the T-Cell-MMP have an maleimido alkyl carboxylic acid coupled to the peptide by an optional linker (see e.g., FIG. 8), for example by an amide formed with the epsilon amino group of a lysine. The maleimido carboxylic acid can be, for example, a maleimido ethanoic, propanoic, butanoic, pentanoic, hexanoic, heptanoic, or octanoic acid.

[00129] Accordingly, an epitope peptide may be coupled to a cysteine present (e.g., engineered into), for example, in the binding pocket of a T-Cell-MMP through a bifunctional linker comprising a maleimide or a maleimide amino acid incorporated into the peptide. An epitope peptide may be conjugated (e.g., by one or two maleimide amino acids or at least one maleimide containing bifunctional linker) to a MHC heavy chain having cysteine residues at any one or more (e.g., 1 or 2) aa positions selected from positions 5, 7, 59, 84, 116, 139, 167, 168, 170, and/or 171 (e.g., Y7C, Y59C, Y84C, Y116C, A139C, W167C, L168C, R170C, and Y171C substitutions) with the numbering as in FIGs. 3D-3I. An epitope peptide may be conjugated (e.g., by one or two maleimide amino acids or at least one maleimide containing bifunctional linker) to a MHC heavy chain having cysteine residues at any one or more (e.g., 1 or 2) aa positions selected from positions 7, 84 and/or 116, (e.g., Y7C, Y84C, and Y116C substitutions) with the numbering as in FIGs. 3D-3H. An epitope peptide may be conjugated (e.g., by one or two maleimide amino acids or at least one maleimide containing bifunctional linker) to a MHC heavy chain having cysteine residues at any one or more (e.g., 1 or 2) aa positions selected from positions 84 and/or 116 (e.g., Y84C and/or Y116C substitutions) with the numbering as in FIGs. 3D-3H.

[00130] Epitope peptides may also be coupled to a cysteine present (e.g., engineered into), a β 2M polypeptide sequence having at least 85% (e.g., at least 90%, 95% 97% or 100%) sequence identity to at least 60 contiguous amino acids (e.g., at least 70, 80, 90 or all contiguous aas) of a mature β 2M polypeptide sequence set forth in FIG. 4. Epitopes may be conjugated to cysteines at positions 2, 44, 50, 77, 85, 88, 91, or 98 of the mature polypeptides (aas 22, 64, 70, 97, 85, 108, 111, or 118 of the β 2M sequences as shown in FIG. 4). Accordingly, the β 2M sequences of a T-Cell-MMP or its epitope conjugate may contain cysteine chemical conjugation site engineered into the mature β 2M sequence selected from Q2C, E44C, E50C, E77C, V85V, S88C, K91C, and D98C. Any of those substitutions may be accompanied by an R12C substitution which that forms a disulfide bond with a cysteine engineered

into position 236 (e.g., A236C) of the MHC-H chain to form a interchain disulfide bond between the β 2M sequence and the MHC H sequence as described, for example, in Section I.A.4. The cysteine chemical conjugation sites in β 2M sequences may also be combined with Y84C and A139C substitutions made to stabilize the MHC H.

[00131] A T-Cell-MMP or its epitope conjugate may comprise a Q2C substitution in its β 2M sequence for conjugation of epitope peptides (e.g., peptides, glycopeptides, lipopeptides or phosphopeptides) to β 2M the sequence directly or indirectly via a linker). The β 2M sequence with a Q2C substitution may also include a R12C substitution. In a T-cell-MMP, a β 2M sequence comprising a Q2C substitution may be combined with an MHC-H chain comprising a Y84C and A139C. In a T-cell-MMP, a β 2M sequence comprising a Q2C substitution and an R12C substitution may be combined with an MHC-H chain comprising a Y84C, A139C, and A236C substitutions. Any of the foregoing may be used to prepare a T-Cell-MMP-epitope conjugate.

[00132] A T-Cell-MMP or its epitope conjugate may comprise an E44C substitution in its β 2M sequence for conjugation of epitope peptides (e.g., peptides, glycopeptides, lipopeptides or phosphopeptides) to β 2M the sequence directly or indirectly via a linker). The β 2M sequence with an E44C substitution may also include a R12C substitution. In a T-cell-MMP, a β 2M sequence comprising a E44C substitution may be combined with an MHC-H chain comprising a Y84C and A139C. In a T-cell-MMP, a β 2M sequence comprising a E44C substitution and an R12C substitution may be combined with an MHC-H chain comprising a Y84C, A139C, and A236C substitutions. Any of the foregoing may be used to prepare a T-Cell-MMP-epitope conjugate.

[00133] A pair of sulfhydryl groups may be employed simultaneously to create a chemical conjugate to a T-Cell-MMP. In such an embodiment a T-Cell-MMP that has a disulfide bond, or has two cysteines (or selenocysteines) engineered into locations proximate to each other, may be utilized as a chemical conjugation site by incorporation of bis-thiol linkers. Bis-thiol linkers, described by Godwin and co-workers, avoid the instability associated with reducing a disulfide bond by forming a bridging group in its place and at the same time permit the incorporation of another molecule, which can be an epitope or payload. *See, e.g.,* Badescu G, et al., (2014), *Bioconjug Chem.*, 25(6):1124–36, entitled *Bridging disulfides for stable and defined antibody drug conjugates*, describing the use of bis-sulfone reagents, which incorporate a hydrophilic linker (e.g., PEG (polyethyleneglycol) linker).

[00134] Where a T-Cell-MMP comprises a disulfide bond, the bis-thiol linker may be used to incorporate an epitope or payload by reducing the bond, generally with stoichiometric or near stoichiometric amounts of dithiol reducing agents (e.g., dithiothreitol) and allowing the linker to react with both cysteine residues. Where multiple disulfide bonds are present, the use of stoichiometric or near stoichiometric amounts of reducing agents may allow for selective modification at one site. *See, e.g.,* Brocchini, et al., *Adv. Drug. Delivery Rev.* (2008) 60:3-12. Where the first and/or second polypeptides of the T-Cell-MMP do not comprise a pair of cysteines and/or selenocysteines (e.g., a selenocysteine and a cysteine), they may be engineered into the polypeptide (by introducing one or both of the cysteines or selenocysteines) to provide a pair of residues that can interact with a bis-thiol linker. The cysteines

and/or selenocysteines should be located such that a bis-thiol linker can bridge them (*e.g.*, at a location where two cysteines could form a disulfide bond). Any combination of cysteines and selenocysteines may be employed (*i.e.* two cysteines, two selenocysteines, or a selenocysteine and a cysteine). The cysteines and/or selenocysteines may both be present on the first and/or second polypeptide of a T-Cell-MMP. Alternatively, the cysteines and/or selenocysteines may be present on the first polypeptide and their counterparts for bis-thiol linker reaction present on the second polypeptide of a T-Cell-MMP.

[00135] In an embodiment, a pair of cysteines and/or selenocysteines is incorporated into a first or second polypeptide of a T-Cell-MMP comprising a β 2M sequence having at least 85% (*e.g.*, at least 90%, 95%, 98% or 99%, or even 100%) amino acid sequence identity to a sequence shown in FIG. 4 before the addition of the pair of cysteines and/or selenocysteines, or into a peptide linker attached to one of those sequences. In one such embodiment the pair of cysteines and/or selenocysteines may be utilized as a bis-thiol linker coupling site for the conjugation of, for example, epitopes and/or payloads either directly or indirectly through a peptide or chemical linker. In one embodiment, the pair of cysteines and/or selenocysteines is located within 10, 20, 30, 40 or 50 amino acids of the amino terminus of the first polypeptide of the T-Cell-MMP.

[00136] In another embodiment, a pair of cysteines and/or selenocysteines is incorporated into an IgFc sequence incorporated into a second polypeptide to provide a chemical conjugation site. In an embodiment a pair of cysteines and/or selenocysteines is incorporated into a polypeptide comprising an IgFc sequence having at least 85% (*e.g.*, at least 90%, 95%, 98% or 99%, or even 100%) amino acid sequence identity to a sequence shown in any of FIGs. 2A-2G before the addition of the pair of cysteines or selenocysteines, or into a peptide linker attached to one of those sequences. In one such embodiment the pair of cysteines and/or selenocysteines may be utilized as a bis-thiol linker coupling site for the conjugation of, for example, epitopes and/or payloads either directly or indirectly through a peptide or chemical linker.

[00137] In another embodiment, a pair of cysteines and/or selenocysteines is incorporated into a polypeptide comprising a MHC Class I heavy chain polypeptide sequence as a chemical conjugation site. In an embodiment, a pair of cysteines and/or selenocysteines is incorporated into a polypeptide comprising a sequence having at least 85% (*e.g.*, at least 90%, 95%, 98% or 99%, or even 100%) amino acid sequence identity to a sequence shown in any of FIGs. 3A-3I before the addition of a pair of cysteines or selenocysteines, or into a peptide linker attached to one of those sequences. In one such embodiment the pair of cysteines and/or selenocysteines may be utilized as a bis-thiol linker coupling site for the conjugation of, for example, epitopes and/or payloads either directly or indirectly through a peptide or chemical linker.

Other Chemical Conjugation Sites

(i) Carbohydrate Chemical Conjugation Sites

[00138] Many proteins prepared by cellular expression contain added carbohydrates (*e.g.*, oligosaccharides of the type added to antibodies expressed in mammalian cells). Accordingly, where first and/or second polypeptides of a T-Cell-MMP are prepared by cellular expression, carbohydrates may be

present and available as site selective chemical conjugation sites in glycol-conjugation reactions.

McCombs and Owen, AAPS Journal, (2015) 17(2): 339-351, and references cited therein describe the use of carbohydrate residues for glycol-conjugation of molecules to antibodies.

[00139] The addition and modification of carbohydrate residues may also be conducted *ex vivo*, through the use of chemicals that alter the carbohydrates (e.g., periodate, which introduces aldehyde groups), or by the action of enzymes (e.g., fucosyltransferases) that can incorporate chemically reactive carbohydrates or carbohydrate analogs for use as chemical conjugation sites.

[00140] In an embodiment, the incorporation of an IgFc scaffold with known glycosylation sites may be used to introduce site specific chemical conjugation sites.

[00141] This disclosure includes and provides for T-Cell-MMPs and their epitope conjugates having carbohydrates as chemical conjugation (glycol-conjugation) sites. The disclosure also includes and provides for the use of such molecules in forming conjugates with epitopes and with other molecules such as drugs and diagnostic agents, and the use of those molecules in methods of medical treatment and diagnosis.

(ii) Nucleotide Binding Sites

[00142] Nucleotide binding sites offer site-specific functionalization through the use of a UV-reactive moiety that can covalently link to the binding site. Bilgicer *et al.*, Bioconjug Chem. 2014;25(7):1198–202, reported the use of an indole-3-butyric acid (IBA) moiety that can be covalently linked to an IgG at a nucleotide binding site. By incorporation of the sequences required to form a nucleotide binding site, chemical conjugates of T-Cell-MMP with suitably modified epitopes and/or other molecules (e.g., drugs or diagnostic agents) bearing a reactive nucleotide may be employed to prepare T-Cell-MMP-epitope conjugates.

[00143] This disclosure includes and provides for T-Cell-MMPs having nucleotide binding sites as chemical conjugation sites. The disclosure also includes and provides for the use of such molecules in forming conjugates with epitopes and with other molecules such as drugs and diagnostic agents, and the use of those molecules in methods of treatment and diagnosis.

3. Binding and Properties of T-Cell-MMPs, Epitopes and MOD

[00144] The present disclosure provides T-Cell-MMP-epitope conjugates. In one embodiment the disclosure provides for a T-Cell-MMP-epitope conjugate comprising: a) a first polypeptide; and b) a second polypeptide, wherein the first and second polypeptides of the multimeric polypeptide comprise an epitope (e.g., a WT-1 peptide); a first MHC polypeptide; a second MHC polypeptide; and optionally an immunoglobulin (Ig) Fc polypeptide or a non-Ig scaffold. In another embodiment, the present disclosure also provides a T-Cell-MMP-epitope conjugate comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) an epitope (e.g., a WT-1 peptide); 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; and ii) optionally an Ig Fc polypeptide or a non-Ig scaffold. In addition to those components recited above, at least one of the first and second polypeptides of the T-Cell-MMP-epitope conjugates of

the present disclosure comprise one or more (e.g., at least one or at least two) MODs. The one or more MODs are located at: A) the C-terminus of the first polypeptide; B) the N-terminus of the second polypeptide; C) the C-terminus of the second polypeptide; D) at the C-terminus of the first polypeptide and at the N-terminus of the second polypeptide; and/or E) between the MHC polypeptide and an Ig Fc polypeptide of the second polypeptide. In an embodiment, at least one (e.g., at least two, or at least three) of the one or more MODs is a variant MOD that exhibits reduced affinity to a Co-MOD compared to the affinity of a corresponding wild-type MOD for the Co-MOD.

[00145] In an embodiment, the epitope present in a T-Cell-MMP-epitope conjugate of the present disclosure (*see*, e.g., FIG. 6) binds to a T-cell receptor (TCR) on a T-cell with an affinity of at least 100 μM (e.g., at least 10 μM , at least 1 μM , at least 100 nM, at least 10 nM or at least 1 nM). In an embodiment, a T-Cell-MMP-epitope conjugate of the present disclosure binds to a first T-cell with an affinity that is at least 25% higher than the affinity with which the T-Cell-MMP-epitope conjugate binds to a second T-cell, where the first T-cell expresses on its surface the Co-MOD and a TCR that binds the epitope with an affinity of at least 100 μM , and where the second T-cell expresses on its surface the Co-MOD but does not express on its surface a TCR that binds the epitope with an affinity of at least 100 μM (e.g., at least 10 μM , at least 1 μM , at least 100 nM, at least 10 nM, or at least 1 nM). In some cases, the peptide presenting an epitope present in a T-Cell-MMP-epitope conjugate of the present disclosure is a WT-1 peptide.

[00146] In some cases, the epitope present in a T-Cell-MMP-epitope conjugate of the present disclosure binds to a TCR on a T-cell with an affinity of from about 10^{-4} M to about 5×10^{-4} M, from about 5×10^{-4} M to about 10^{-5} M, from about 10^{-5} M to about 5×10^{-5} M, from about 5×10^{-5} M to about 10^{-6} M, from about 10^{-6} M to about 5×10^{-6} M, from about 5×10^{-6} M to about 10^{-7} M, from about 10^{-7} M to about 10^{-8} M or from about 10^{-8} M to about 10^{-9} M. Expressed another way, in some cases, the epitope present in a T-Cell-MMP-epitope conjugate of the present disclosure binds to a TCR on a T-cell with an affinity of from about 0.1 μM to about 0.5 μM , from about 0.5 μM to about 1 μM , from about 1 μM to about 5 μM , from about 5 μM to about 10 μM , from about 10 μM to about 25 μM , from about 25 μM to about 50 μM , from about 50 μM to about 75 μM , or from about 75 μM to about 100 μM .

[00147] In an embodiment, a variant MOD present in a T-Cell-MMP-epitope conjugate of the present disclosure binds to its Co-MOD with an affinity that is 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.

[00148] In some cases, a variant MOD present in a T-Cell-MMP-epitope conjugate of the present disclosure has a binding affinity for a Co-MOD that is from 1 nM to 100 nM, or from 100 nM to 100 μM . For example, in some cases, a variant MOD present in a T-Cell-MMP-epitope conjugate of the present disclosure has a binding affinity for a Co-MOD that is from about 1 nM to about 5 nM, from about 5 nM to about 10 nM, from about 10 nM to about 50 nM, from about 50 nM to about 100 nM, from about 100

nM to about 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, from 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. In some cases, a variant MOD present in a T-Cell-MMP of the present disclosure has a binding affinity for a Co-MOD that is from about 1 nM to about 5 nM, from about 5 nM to about 10 nM, from about 10 nM to about 50 nM, or from about 50 nM to about 100 nM.

[00149] The combination of the reduced affinity of the MOD for its Co-MOD and the affinity of the epitope for a TCR provides for enhanced selectivity of a T-Cell-MMP-epitope conjugate of the present disclosure, while still allowing for activity of the MOD. For example, a T-Cell-MMP-epitope conjugate of the present disclosure binds selectively to a first T-cell that displays both: i) a TCR specific for the epitope present in the T-Cell-MMP-epitope conjugate; and ii) a Co-MOD that binds to the MOD present in the T-Cell-MMP-epitope conjugate, compared to binding to a second T-cell that displays: i) a TCR specific for an epitope other than the epitope present in the T-Cell-MMP-epitope conjugate; and ii) a Co-MOD that binds to the MOD present in the T-Cell-MMP-epitope conjugate. For example, a T-Cell-MMP-epitope conjugate 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 200% (2-fold), at least 250% (2.5-fold), at least 500% (5-fold), at least 1,000% (10-fold), at least 1,500% (15-fold), at least 2,000% (20-fold), at least 2,500% (25-fold), at least 5,000% (50-fold), at least 10,000% (100-fold), or more than 100-fold, higher than the affinity to which it binds the second T-cell.

[00150] In some cases, a T-Cell-MMP-epitope conjugate of the present disclosure, 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, the T-Cell-MMP-epitope conjugate of the present disclosure, 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 T-Cell-MMP-epitope conjugate; and ii) a Co-MOD that binds to the MOD present in the T-Cell-MMP-epitope conjugate. The T-Cell-MMP-epitope conjugate also 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 T-Cell-MMP-epitope conjugate; and ii) a Co-MOD that binds to the MOD present in the T-Cell-MMP-epitope conjugate. 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, from about 50:1 to about 100:1, or more than 100:1. “Modulating the activity” of a T-cell can include, but is not limited to, 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; and iv) inhibiting activity of an autoreactive T-cell; and the like.

[00151] The combination of the reduced affinity of the MOD for its Co-MOD and the affinity of the epitope for a TCR provides for enhanced selectivity of a T-Cell-MMP-epitope conjugate of the present disclosure. Thus, for example, a T-Cell-MMP-epitope conjugate of the present disclosure binds with higher avidity to a first T-cell that displays both: i) a TCR specific for the epitope present in the T-Cell-MMP-epitope conjugate; and ii) a Co-MOD that binds to the MOD present in the T-Cell-MMP-epitope conjugate, compared to the avidity with which it binds to a second T-cell that displays: i) a TCR specific for an epitope other than the epitope present in the T-Cell-MMP-epitope conjugate; and ii) a Co-MOD that binds to the MOD present in the T-Cell-MMP-epitope conjugate.

a. Determining binding affinity

[00152] Binding affinity between a MOD and its Co-MOD can be determined by bio-layer interferometry (BLI) using purified MOD and purified Co-MOD. Binding affinity between a T-Cell-MMP-epitope conjugate and its Co-MOD can be determined by BLI using purified T-Cell-MMP-epitope conjugate and the 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. The specific and relative binding affinities described in this disclosure between a Co-MOD and a MOD, or between a Co-MOD and a T-Cell-MMP (or its epitope conjugate), can be determined using the following procedures.

[00153] A BLI assay can be carried out using an Octet RED 96 (Pal FortéBio) instrument, or a similar instrument, as follows. For example, to determine binding affinity of a Co-MOD for a T-Cell-MMP (or its epitope conjugate) (e.g., a T-Cell-MMP-epitope conjugate of the present disclosure with a variant MOD, or a control T-Cell-MMP-epitope conjugate comprising a wild-type MOD), the T-Cell-MMP (or its epitope conjugate) is immobilized onto an insoluble support (a “biosensor”). The immobilized T-Cell-MMP (or its epitope conjugate) is the “target.” Immobilization can be effected by immobilizing a capture antibody onto the insoluble support, where the capture antibody immobilizes the T-Cell-MMP (or its epitope conjugate). For example, where the T-Cell-MMP comprises an IgFc polypeptide, immobilization can be effected by immobilizing anti-Fc (e.g., anti-human IgG Fc) antibodies onto the insoluble support, and contacting the T-Cell-MMP-epitope conjugate with the immobilized anti-Fc antibodies which will bind to and immobilize it. A Co-MOD is applied, at several different concentrations, to the immobilized T-Cell-MMP (or its immobilized epitope conjugate), 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-MOD to the immobilized T-Cell-MMP(or its epitope conjugate) 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 (mAb) 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-MOD, 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) from an internal reference layer. A change in the number of molecules (“analyte”; e.g., Co-MOD; 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_a/k_d) gives rise to the affinity constant K_D . The assay can also be conducted with purified wild-type or its variant MOD immobilized on the biosensor while the Co-MOD is applied, at several different concentrations, to determine the binding parameters between a MOD and its Co-MOD.

[00154] Determining the binding affinity of a Co-MOD (e.g., IL-2R) with both a wild-type MOD (e.g., IL-2) and a variant MOD (e.g., an IL-2 variant as disclosed herein), or with a T-Cell-MMP (or its epitope conjugate) containing wild-type or variant MODs, thus allows one to determine the relative binding affinity of the wild-type and variant molecules. That is, one can determine whether the binding affinity of a variant MOD for its receptor (its Co-MOD) is reduced as compared to the binding affinity of the wild-type MOD for the same Co-MOD, and, if so, what is the percentage reduction from the binding affinity of the wild-type Co-MOD.

[00155] 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.

[00156] Unless otherwise stated herein, the affinity of a T-Cell-MMP-epitope conjugate of the present disclosure for a Co-MOD, or the affinity of a control T-Cell-MMP-epitope conjugate (where a control T-Cell-MMP-epitope conjugate comprises a wild-type MOD) for a Co-MOD, is determined using BLI, as described above. Likewise, the affinity of a MOD and its Co-MOD polypeptide can be determined using BLI as described above.

[00157] In some cases, the ratio of: i) the binding affinity of a control T-Cell-MMP-epitope conjugate (where the control comprises a wild-type MOD) to a Co-MOD to ii) the binding affinity of a T-Cell-MMP-epitope conjugate of the present disclosure comprising a variant of the wild-type MOD to the Co-

MOD, 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 T-Cell-MMP-epitope conjugate (where the control comprises a wild-type MOD) to a Co-MOD to ii) the binding affinity of a T-Cell-MMP-epitope conjugate of the present disclosure comprising a variant of the wild-type MOD to the Co-MOD, 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 2.0:1 to 5:1, from 10:1 to 15:1, 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.

[00158] As an example, where a control T-Cell-MMP-epitope conjugate comprises a wild-type IL-2 polypeptide, and where a T-Cell-MMP-epitope conjugate 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 MOD, the ratio of: i) the binding affinity of the control T-Cell-MMP-epitope conjugate to an IL-2 receptor (the Co-MOD) to ii) the binding affinity of the T-Cell-MMP-epitope conjugate of the present disclosure to the IL-2 receptor (the Co-MOD), 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 T-Cell-MMP-epitope conjugate comprises a wild-type IL-2 polypeptide, and where a T-Cell-MMP-epitope conjugate 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 MOD, the ratio of: i) the binding affinity of the control T-Cell-MMP-epitope conjugate to IL-2 receptor (the Co-MOD) to ii) the binding affinity of the T-Cell-MMP-epitope conjugate 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.

[00159] As another example, where a control T-Cell-MMP-epitope conjugate comprises a wild-type PD-L1 polypeptide, and where a T-Cell-MMP-epitope conjugate of the present disclosure comprises a variant PD-L1 polypeptide (comprising from 1 to 10 amino acid substitutions relative to the amino acid sequence of the wild-type PD-L1 polypeptide) as the MOD, the ratio of: i) the binding affinity of the control T-Cell-MMP-epitope conjugate to a PD-1 polypeptide (i.e., the Co-MOD) to ii) the binding affinity of the T-Cell-MMP-epitope conjugate of the present disclosure to the PD-1 polypeptide, 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.

[00160] As another example, where a control T-Cell-MMP-epitope conjugate comprises a wild-type CD80 polypeptide, and where a T-Cell-MMP-epitope conjugate of the present disclosure comprises a variant CD80 polypeptide (comprising from 1 to 10 amino acid substitutions relative to the amino acid sequence of the wild-type CD80 polypeptide) as the MOD, the ratio of: i) the binding affinity of the

control T-Cell-MMP-epitope conjugate to CTLA4 polypeptide (i.e., the Co-MOD) to ii) the binding affinity of the T-Cell-MMP-epitope conjugate of the present disclosure to the CTLA4 polypeptide, 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.

[00161] As another example, where a control T-Cell-MMP-epitope conjugate comprises a wild-type CD80 polypeptide, and where a T-Cell-MMP-epitope conjugate of the present disclosure comprises a variant CD80 polypeptide (comprising from 1 to 10 amino acid substitutions relative to the amino acid sequence of the wild-type CD80 polypeptide) as the MOD, the ratio of: i) the binding affinity of the control T-Cell-MMP-epitope conjugate to a CD28 polypeptide (i.e., the Co-MOD) to ii) the binding affinity of the T-Cell-MMP-epitope conjugate of the present disclosure to the CD28 polypeptide, 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.

[00162] As another example, where a control T-Cell-MMP-epitope conjugate comprises a wild-type 4-1BBL polypeptide, and where a T-Cell-MMP-epitope conjugate of the present disclosure comprises a variant 4-1BBL polypeptide (comprising from 1 to 10 amino acid substitutions relative to the amino acid sequence of the wild-type 4-1BBL polypeptide) as the MOD, the ratio of: i) the binding affinity of the control T-Cell-MMP-epitope conjugate to a 4-1BB polypeptide (i.e., the Co-MOD) to ii) the binding affinity of the T-Cell-MMP-epitope conjugate of the present disclosure to the 4-1BB polypeptide, 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.

[00163] As another example, where a control T-Cell-MMP-epitope conjugate comprises a wild-type CD86 polypeptide, and where a T-Cell-MMP-epitope conjugate of the present disclosure comprises a variant CD86 polypeptide (comprising from 1 to 10 amino acid substitutions relative to the amino acid sequence of the wild-type CD86 polypeptide) as the MOD, the ratio of: i) the binding affinity of the control T-Cell-MMP-epitope conjugate to a CD28 polypeptide (i.e., the Co-MOD) to ii) the binding affinity of the T-Cell-MMP-epitope conjugate of the present disclosure to the CD28 polypeptide, 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.

[00164] Binding affinity of a T-Cell-MMP-epitope conjugate of the present disclosure to a target T-cell can be measured in the following manner: A) contacting a T-Cell-MMP-epitope conjugate of the present disclosure with a target T-cell expressing on its surface with: i) a Co-MOD that binds to the parental wild-type MOD; and ii) a TCR that binds to the epitope, where the T-Cell-MMP-epitope conjugate comprises an epitope tag or fluorescent label, such that the T-Cell-MMP-epitope conjugate binds to the target T-cell; B) if the T-Cell-MMP-epitope conjugate is unlabeled, contacting the target T-cell-bound T-

Cell-MMP-epitope conjugate with a fluorescently labeled binding agent (e.g., a fluorescently labeled antibody) that binds to the epitope tag, generating a T-Cell-MMP-epitope conjugate/target T-cell/binding agent complex; and C) measuring the mean fluorescence intensity (MFI) of the T-Cell-MMP-epitope conjugate/target T-cell/binding agent complex using flow cytometry. The epitope tag can be, e.g., a FLAG tag, a hemagglutinin tag, a c-myc tag, a poly(histidine) tag, *etc.* The MFI measured over a range of concentrations of the T-Cell-MMP-epitope conjugate (library member) provides a measure of the affinity. The MFI measured over a range of concentrations of the T-Cell-MMP-epitope conjugate (library member) provides a half maximal effective concentration (EC_{50}) of the T-Cell-MMP-epitope conjugate. In some cases, the EC_{50} of a T-Cell-MMP-epitope conjugate of the present disclosure for a target T-cell is in the nM range; and the EC_{50} of the T-Cell-MMP-epitope conjugate for a control T-cell (where a control T-cell expresses on its surface: i) a Co-MOD that binds the parental wild-type MOD; and ii) a T-cell receptor that does not bind to the epitope present in the T-Cell-MMP-epitope conjugate) is in the μ M range. In some cases, the ratio of the EC_{50} of a T-Cell-MMP-epitope conjugate of the present disclosure for a control T-cell to the EC_{50} of the T-Cell-MMP-epitope conjugate for a target T-cell 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. The ratio of the EC_{50} of a T-Cell-MMP-epitope conjugate of the present disclosure for a control T-cell to the EC_{50} of the T-Cell-MMP-epitope conjugate for a target T-cell is an expression of the selectivity of the T-Cell-MMP-epitope conjugate.

[00165] In some cases, when measured as described in the preceding paragraph, a T-Cell-MMP-epitope conjugate of the present disclosure exhibits selective binding to a target T-cell, compared to binding of the T-Cell-MMP-epitope conjugate (library member) to a control T-cell that comprises: i) the Co-MOD that binds the parental wild-type MOD; and ii) a TCR that binds to an epitope other than the epitope present in the T-Cell-MMP-epitope conjugate (library member).

b. Dimerized multimeric T-cell modulatory polypeptides

[00166] T-Cell-MMPs and T-Cell-MMP-epitope conjugates of the present disclosure, can be in the form of dimers, i.e., the present disclosure provides a multimeric polypeptide comprising a dimer of a multimeric T-Cell-MMP of the present disclosure. An example of a dimerized T-Cell-MMP is shown in FIG 12 structure B, and examples of dimerized T-Cell-MMP-epitope conjugates are shown FIG. 7. Thus, the present disclosure provides a multimeric T-Cell-MMP 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 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 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 heterodimers that form the dimerized T-Cell-MMPs 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 (see e.g., the disulfide bonds between the IgFc regions (“Fc”) in FIG. 7 and FIG. 12 structure B). In some cases, the first heterodimer and the second heterodimer are covalently linked to one another via an amino acid in the C-terminal region of the second polypeptide of the first heterodimer and an amino acid in 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 amino acid 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 aa to 200 aa (e.g., from 1 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).

[00167] The first MHC polypeptides of the first and second heterodimers may be β 2M polypeptides, and the second MHC polypeptides of the first and second heterodimers may be MHC Class I heavy chain polypeptides. In some cases, the MOD of the first heterodimer and the MOD of the second heterodimer comprise the same amino acid sequence. In some cases, the MOD of the first heterodimer and the MOD of the second heterodimer are variant MODs that comprise from 1 to 10 amino acid substitutions compared to a corresponding parental wild-type MOD, wherein from 1 to 10 amino acid substitutions result in reduced affinity binding of the variant MOD to a Co-MOD. In some cases, the MOD(s) of the first heterodimer and the MOD(s) of the second heterodimer are each independently selected from the group consisting of IL-2, 4-1BBL, PD-L1, CD70, CD80, CD86, ICOS-L, OX-40L, FasL, JAG1(CD339), TGF- β , ICAM, and variant MODs thereof (e.g., variant MODs having 1 to 10 amino acid substitutions compared to a corresponding parental wild-type MOD). Examples of suitable MHC polypeptides, MODs, and peptide epitopes are described below.

[00168] In some cases, the peptide epitope of the first heterodimer and the peptide epitope of the second heterodimer comprise the same amino acid sequence.

[00169] In addition to dimers, the T-Cell-MMPs and T-Cell-MMP-epitope conjugates of the present disclosure may form higher order complexes including trimers, tetramers, or pentamers. Compositions comprising multimers of T-Cell-MMPs may also comprise lower order complexes such as monomers and, accordingly, may comprise monomers, dimers, trimers, tetramers, pentamers, or combinations of any thereof (e.g., a mixture of monomers and dimers).

4. MHC polypeptides of T-Cell-MMPs

[00170] As noted above, T-Cell-MMPs and T-Cell-MMP-epitope conjugates include MHC polypeptides. For the purposes of the instant disclosure, the term “major histocompatibility complex (MHC) polypeptides” is meant to include MHC Class I 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 and/or portions thereof). In some cases, the first MHC polypeptide is a MHC Class I

β 2M (β 2M) polypeptide, and the second MHC polypeptide is a MHC Class I heavy chain (MHC-H)). In an embodiment, both the β 2M and MHC-H chain sequences in a T-Cell-MMP (or its epitope conjugate) are of human origin. Unless expressly stated otherwise, the T-Cell-MMPs and the T-Cell-MMP-epitope conjugates described herein are not intended to include membrane anchoring domains (transmembrane regions) of a MHC Class I heavy chain, or a part of that molecule sufficient to anchor the resulting T-Cell-MMP, or a peptide thereof, 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 T-Cell-MMPs and T-Cell-MMP-epitope conjugates 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 T-Cell-MMP of the present disclosure includes only the α 1, α 2, and α 3 domains of an MHC Class I heavy chain. In some cases, the MHC Class I heavy chain present in a T-Cell-MMP of the present disclosure 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 T-Cell-MMP of the present disclosure 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.

[00171] In some cases, a MHC polypeptide of a T-Cell-MMP, or a T-Cell-MMP-epitope conjugate is a human MHC polypeptide, where human MHC polypeptides are also referred to as "human leukocyte antigen" ("HLA") polypeptides, more specifically, a Class I HLA polypeptide, *e.g.*, a β 2M polypeptide, or a Class I HLA heavy chain polypeptide. Class I HLA heavy chain polypeptides that can be included in T-Cell-MMPs or their epitope conjugates include HLA-A, -B, -C, -E, -F, and/or -G heavy chain polypeptides. In an embodiment, the Class I HLA heavy chain polypeptides of T-Cell-MMPs or their epitope conjugates comprise polypeptides having 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 the amino acid sequence of any of the human HLA heavy chain polypeptides depicted in FIGs. 3A to 3I. For example, they may comprise 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 FIGs. 3E to 3I).

[00172] As an example, a MHC Class I heavy chain polypeptide of a multimeric polypeptide 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 sequences) or 25-365 (lacking the leader) of the human HLA-A heavy chain polypeptides depicted in FIGs. 3A, 3B and/or 3C.

a. MHC Class I Heavy Chains

[00173] Class I human MHC polypeptides may be drawn from the classical HLA alleles (HLA-A, B, and C), or the non-classical HLA alleles (*e.g.*, HLA-E, F and G). The following are non-limiting examples of MHC-H alleles and variants of those alleles that may be incorporated into T-Cell-MMPs and their epitope conjugates.

(i) HLA-A heavy chains

[00174] The HLA-A heavy chain peptide sequences, or portions thereof, that may be incorporated into a T-Cell-MMP or its epitope conjugate 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 3E. Any of those alleles may comprise a substitution at one or more of positions 84, 139 and/or 236 (as shown in FIG. 3E) 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-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 incorporated into a T-Cell-MMP (e.g., it may comprise 1-30, 1-5, 5-10, 10-15, 15-20, 20-25, or 25-30 amino acid insertions, deletions, and/or substitutions).

(a) HLA-A*0101 (HLA-A*01:01:01:01)

[00175] An MHC Class I heavy chain polypeptide of a T-Cell-MMP or a T-Cell-MMP-epitope conjugate may comprise an amino acid sequence of HLA-A*01:01:01:01 (HLA-A in FIG. 3D (SEQ ID NO:20) or FIG. 3E), 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 1-30, 1-5, 5-10, 10-15, 15-20, 20-25, or 25-30 amino acid insertions, deletions, and/or substitutions). In an embodiment, where the HLA-A heavy chain polypeptide of a T-Cell-MMP or its epitope conjugate has less than 100% identity to the sequence labeled HLA-A in FIG. 3D, it may comprise a substitution 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).

(b) HLA-A*0201

[00176] An MHC Class I heavy chain polypeptide of a T-Cell-MMP or a T-Cell-MMP-epitope conjugate may comprise an amino acid sequence of HLA-A*0201 (SEQ ID NO:23) provided in FIG. 3D or FIG. 3E, 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 1-30, 1-5, 5-10, 10-15, 15-20, 20-25, or 25-30 amino acid insertions, deletions, and/or substitutions). In an embodiment, where the HLA-A*0201 heavy chain polypeptide of a T-Cell-MMP or its epitope conjugate has less than 100% identity to the sequence labeled HLA-A*0201 in FIGs. 3D or 3E, it may comprise a substitution 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 an embodiment, the HLA-A*0201 heavy chain polypeptide of a

T-Cell-MMP or its epitope conjugate comprises the Y84A and A236C substitutions. In an embodiment, the HLA-A*0201 heavy chain polypeptide of a T-Cell-MMP or its epitope conjugate comprises the Y84C and A139C substitutions. In an embodiment, the HLA-A*0201 heavy chain polypeptide of a T-Cell-MMP or its epitope conjugate comprises the Y84C, A139C and A236C substitutions.

(c) HLA-A*1101

[00177] An MHC Class I heavy chain polypeptide of a T-Cell-MMP or a T-Cell-MMP-epitope conjugate may comprise an amino acid sequence of HLA-A*1101 (SEQ ID NO:28) provided in FIG. 3D or in FIG. 3E, 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 1-30, 1-5, 5-10, 10-15, 15-20, 20-25, or 25-30 amino acid insertions, deletions, and/or substitutions). The HLA-A*1101 heavy chain allele may be prominent in Asian populations, including populations of individuals of Asian descent.

[00178] In an embodiment, where the HLA-A*1101 heavy chain polypeptide of a T-Cell-MMP or its epitope conjugate has less than 100% identity to the sequence labeled HLA-A*1101 in FIGs. 3D or 3E, it may comprise a substitution 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 an embodiment, the HLA-A*1101 heavy chain polypeptide of a T-Cell-MMP or its epitope conjugate comprises the Y84A and A236C substitutions. In an embodiment, the HLA-A*1101 heavy chain polypeptide of a T-Cell-MMP or its epitope conjugate comprises the Y84C and A139C substitutions. In an embodiment, the HLA-A*1101 heavy chain polypeptide of a T-Cell-MMP or its epitope conjugate comprises the Y84C, A139C and A236C substitutions.

(d) HLA-A*2402

[00179] An MHC Class I heavy chain polypeptide of a T-Cell-MMP or a T-Cell-MMP-epitope conjugate may comprise an amino acid sequence of HLA-A*2402 (SEQ ID NO:29) provided in FIGs. 3D or 3E, 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 1-30, 1-5, 5-10, 10-15, 15-20, 20-25, or 25-30 amino acid insertions, deletions, and/or substitutions). The HLA-A*2402 heavy chain allele may be prominent in Asian populations, including populations of individuals of Asian descent.

[00180] In an embodiment, where the HLA-A*2402 heavy chain polypeptide of a T-Cell-MMP or its epitope conjugate has less than 100% identity to the sequence labeled HLA-A*2402 in FIGs. 3D or 3E, it may comprise a substitution 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 an embodiment, the HLA-A*2402 heavy chain polypeptide of a T-Cell-MMP or its epitope conjugate comprises the Y84A and A236C substitutions. In an embodiment, the HLA-A*2402 heavy chain

polypeptide of a T-Cell-MMP or its epitope conjugate comprises the Y84C and A139C substitutions. In an embodiment, the HLA-A*2402 heavy chain polypeptide of a T-Cell-MMP or its epitope conjugate comprises the Y84C, A139C and A236C substitutions.

(e) HLA-A*3303

[00181] An MHC Class I heavy chain polypeptide of a T-Cell-MMP or a T-Cell-MMP-epitope conjugate may comprise an amino acid sequence of HLA-A*3303 (SEQ ID NO:30) provided in FIGs. 3D or 3E, or a sequence having at least 75% (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 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). The HLA-A*3303 heavy chain allele may be prominent in Asian populations, including populations of individuals of Asian descent.

[00182] In an embodiment, where the HLA-A*3303 heavy chain polypeptide of a T-Cell-MMP or its epitope conjugate has less than 100% identity to the sequence labeled HLA-A*3303 in FIG. 3D, it may comprise a substitution 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 an embodiment, the HLA-A*3303 heavy chain polypeptide of a T-Cell-MMP or its epitope conjugate comprises the Y84A and A236C substitutions. In an embodiment, the HLA-A*3303 heavy chain polypeptide of a T-Cell-MMP or its epitope conjugate comprises the Y84C and A139C substitutions. In an embodiment, the HLA-A*3303 heavy chain polypeptide of a T-Cell-MMP or its epitope conjugate comprises the Y84C, A139C and A236C substitutions.

(ii) HLA-B heavy chains.

[00183] The HLA-B heavy chain peptide sequences, or portions thereof, that may be that may be incorporated into a T-Cell-MMP or its epitope conjugate 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 3F. Any of those alleles may comprise a substitution at one or more of positions 84, 139 and/or 236 (as shown in FIG. 3F) 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 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 incorporated into a T-Cell-MMP (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).

(a) HLA-B*0702

[00184] An MHC Class I heavy chain polypeptide of a T-Cell-MMP or a T-Cell-MMP-epitope conjugate may comprise an amino acid sequence of HLA-B*0702 (SEQ ID NO:21) in FIG. 3D (labeled HLA-B in

FIG. 3D), 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 an embodiment, where the HLA-B heavy chain polypeptide of a T-Cell-MMP or its epitope conjugate has less than 100% identity to the sequence labeled HLA-B in FIG. 3D, it may comprise a substitution 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 an embodiment, the HLA-B heavy chain polypeptide of a T-Cell-MMP or its epitope conjugate comprises the Y84A and A236C substitutions. In an embodiment, the HLA-B*0702 heavy chain polypeptide of a T-Cell-MMP or its epitope conjugate comprises the Y84C and A139C substitutions. In an embodiment, the HLA-B heavy chain polypeptide of a T-Cell-MMP or its epitope conjugate comprises the Y84C, A139C and A236C substitutions.

(iii) HLA-C heavy chains

[00185] The HLA-C heavy chain peptide sequences, or portions thereof, that may be that may be incorporated into a T-Cell-MMP or its epitope conjugate include, but are not limited to, the alleles: C*0102, C*0303, C*0304, C*0401, C*0602, C*0701, C*0702, C*0801, and C*1502, which are aligned without all, or substantially all, of the leader, transmembrane and cytoplasmic sequences in FIG 3G. Any of those alleles may comprise a substitution at one or more of positions 84, 139 and/or 236 (as shown in FIG. 3G) 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, an HLA-C 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 incorporated into a T-Cell-MMP (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).

(a) HLA-C*701 and HLA-C*702

[00186] In an embodiment, a MHC Class I heavy chain polypeptide of a T-Cell-MMP or a T-Cell-MMP-epitope conjugate comprises an amino acid sequence of HLA-C*701 (SEQ ID NO:49) or HLA-C*702 (SEQ ID NO:50) in FIG. 3G (labeled HLA-C in FIG. 3D), 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 those sequences (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 relative to those sequences). In an embodiment, where the HLA-C heavy chain polypeptide of a T-Cell-MMP or its epitope conjugate has less than 100% identity to the sequence labeled HLA-C in FIG. 3D, it may comprise a substitution 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 an embodiment, the HLA-C heavy chain polypeptide of a T-Cell-MMP or its epitope conjugate comprises the Y84A and A236C substitutions. In an embodiment, the HLA-C*701 heavy chain polypeptide of a T-Cell-MMP or its epitope conjugate comprises the Y84C and A139C substitutions. In an embodiment, the HLA-C heavy chain polypeptide of a T-Cell-MMP or its epitope conjugate comprises the Y84C, A139C and A236C substitutions.

(iv) Non-Classical HLA-E, F and G heavy chains

[00187] The non-classical HLA heavy chain peptide sequences, or portions thereof, that may be that may be incorporated into a T-Cell-MMP or its epitope conjugate include, but are not limited to, those of the HLA-E, F, and/or G alleles. Sequences for those alleles, (and the HLA-A, B and C alleles) may be found on the world wide web at, for example, 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 Bioecology Information (www.ncbi.nlm.nih.gov).

[00188] Some 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. Some 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. Some 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. 3H, and aligned with consensus sequences of the above-mentioned HLA-A, -B, and -C alleles provided in FIGs. 3E-G in FIG. and in 3I.

[00189] 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. 3I for the consensus sequences. In an embodiment, the substitutions may be selected from a: position 84 tyrosine to alanine (Y84A) or cysteine (Y84C), or in the case of HLA-F a R84A or R84C substitution; a position 139 alanine to cysteine (A139C), or in the case of HLA-F a V139C substitution; and an alanine to cysteine substitution at position 236 (A236C). In addition, HLA-E, -F, and /or -G sequences 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. 3I 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).

(v) Mouse H2K

[00190] An MHC Class I heavy chain polypeptide of a T-Cell-MMP or a T-Cell-MMP-epitope conjugate may comprise an amino acid sequence of MOUSE H2K (SEQ ID NO:24) (MOUSE H2K in FIG. 3D), 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 1-30, 1-5, 5-10, 10-15, 15-20, 20-25, or 25-30 amino acid insertions, deletions, and/or substitutions). In an embodiment, where the MOUSE H2K heavy chain polypeptide of a T-Cell-MMP or its epitope conjugate has less than 100% identity to the sequence labeled MOUSE H2K in FIG. 3D, it may comprise a substitution 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 an embodiment, the MOUSE H2K heavy chain polypeptide of a T-Cell-MMP or its epitope conjugate comprises the Y84A and A236C substitutions. In an embodiment, the MOUSE H2K heavy chain polypeptide of a T-Cell-MMP or its epitope conjugate comprises the Y84C and A139C substitutions. In an embodiment, the MOUSE H2K heavy chain polypeptide of a T-Cell-MMP or its epitope conjugate comprises the Y84C, A139C and A236C substitutions.

(vi) The Effect of Amino Acid Substitutions in MHC Polypeptides on T-Cell-MMPs**(a) Substitutions at Positions 84, 139 and 236**

[00191] Substitution of position 84 of the MHC H chain (see FIG. 3I), particularly when it is a tyrosine residue, with a small amino acid such as alanine (Y84A) tends to open one end of the MHC binding pocket, allowing a linker (e.g., attached to an epitope peptide) to “thread” through the end of the pocket, and accordingly, permits a greater variation in the size of the epitope (e.g., longer peptides bearing epitope sequences) that can fit into the MHC pocket and be presented by the T-Cell-MMP. In an embodiment, the HLA-A heavy chain polypeptide of a T-Cell-MMP or its epitope conjugate comprises the Y84A and A236C substitutions. In an embodiment, the HLA-A heavy chain polypeptide of a T-Cell-MMP or its epitope conjugate comprises the Y84C and A139C substitutions. When amino acids 84 and 139 are both cysteines they may form an intrachain disulfide bond which can stabilize the MHC Class 1 protein and permit translation and excretion of the T-Cell-MMP by eukaryotic cells, even when not loaded with an epitope peptide. When position 84 is a C residue, it can also form an interchain disulfide bond with a linker attached to the N-terminus of a β 2M polypeptide (e.g., e.g., epitope-linker sequence-mature β 2M polypeptide, such as epitope-GCGGS(G₄S) linker sequence (SEQ ID NO:93)-mature β 2M polypeptide, see SEQ ID NOs:57 to 61). When amino acid 236 is a cysteine it can form an interchain disulfide bond with a cysteine at amino acid 12 of a variant β 2M polypeptide that comprises R12C substitution at that position. Some possible combinations of MHC Class 1 heavy chain sequence modifications that may be incorporated into a T-Cell-MMP or its epitope conjugate are shown in the Table that follows. Any combination of substitutions provided in the table at residues 84, 139 and 236

may be combined with any combination of substitutions in the epitope binding cleft, such as those described at positions 116 and 167.

(b) Substitutions at Positions 116 and 167

[00192] Any MHC Class I heavy chain sequences (including those disclosed above for: the HLA-A*0101 (HLA-A*01:01:01:01); HLA-A*0201; HLA-A*1101; HLA-A*2402; HLA-A*3303; HLA-B; HLA-C; and Mouse H2K, or the HLA-A, B, C, E, F, and/or G) may further comprise a cysteine substitution at position 116 (e.g., Y116C), providing thiol for anchoring an epitope peptide such as by reaction with a maleimide peptide) and/or one of an alanine (W167A) or cysteine (W167C) at position 167. As with substitutions that open one end of the MHC-H binding pocket (e.g., at position 84 or its equivalent such as Y84A), substitution of an alanine or glycine at position 167 or its equivalent (e.g., a W167A substitution) opens the other end of the MHC binding pocket, creating a groove that permits greater variation (e.g., longer length) of the epitope peptides that may be presented by the T-Cell-MMP-epitope conjugates. Substitutions at positions 84 and 167 or their equivalent (e.g., Y84A in combination with W167A or W167G) may be used in combination to modify the binding pocket of MHC-H chains. The placement of a cysteine at position 167 (e.g., a W167C substitution) or its equivalent provides a thiol residue for anchoring an epitope peptide). Cysteine substitutions at positions 116 and 167 may be used separately to anchor epitopes (e.g., epitope peptides), or in combination to anchor the epitope in two locations (e.g., the ends of the epitope containing peptide. Substitutions at positions 116 and/or 167 may be combined with any one or more substitutions at positions 84, 139 and/or 236 described above.

SOME COMBINATIONS OF MHC CLASS 1 HEAVY CHAIN SEQUENCE MODIFICATIONS THAT MAY BE INCORPORATED INTO A T-CELL-MMP OR ITS EPITOPE CONJUGATE

Entry	HLA Heavy Chain Sequence From FIGs. 3D-H	Sequence Identity Range*	Specific Substitutions at aa positions 84, 139 and/or 236	Substitutions at positions 116 and/or 167
1	HLA-A Consensus FIG. 3E	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)	None; Y116C; W167A; W167C; or (Y116C & W167C)
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)	None; Y116C; W167A; W167C; or (Y116C & W167C)
3	HLA-B Consensus FIG. 3F	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	None; Y84C; Y84A; A139C; A236C; (Y84A & A236C); (Y84C & A139C); or	None; Y116C; W167A; W167C; or

Entry	HLA Heavy Chain Sequence From FIGs. 3D-H	Sequence Identity Range*	Specific Substitutions at aa positions 84, 139 and/or 236	Substitutions at positions 116 and/or 167
		insertions, deletions, and/or substitutions (not counting variable residues)	(Y84C, A139C & A236C)	(Y116C & W167C)
4	B*0702, B*0801, B*1502, B*3802, B*4001, B*4601, or B*5301	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)	None; Y116C; W167A; W167C; or (Y116C & W167C)
5	HLA-C Consensus FIG. 3G	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)	None; Y116C; W167A; W167C; or (Y116C & W167C)
6	C*0102, C*0303, C*0304, C*0401, C*0602, C*0701, C*702, C*0801, or C*1502	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)	None; Y116C; W167A; W167C; or (Y116C & W167C)
7	HLA-E, F, or G Consensus FIG. 3H	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)	None; Y116C; W167A; W167C; or (Y116C & W167C)
8	MOUSE H2K	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)	None; Y116C; W167A; W167C; or (Y116C & W167C)

* The Sequence Identity Range is the permissible range in sequence identity of a MHC-H polypeptide sequence incorporated into a T-Cell-MMP relative to the corresponding portion of the sequences listed in FIG. 3D -3H not counting the variable residues in the consensus sequences.

b. MHC Class I β 2-Microglobins and Combinations with MHC-H Polypeptides

[00193] A β 2M polypeptide of a T-Cell-MMP or its epitope conjugate 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. 4. 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. 4.

[00194] In some cases, a 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 a MHC polypeptide of a first polypeptide of a T-Cell-MMP, or its epitope conjugate, can form a disulfide bond with a cysteine residue present in a second polypeptide chain.

[00195] In some cases, a first MHC polypeptide in a first polypeptide of a T-Cell-MMP and/or a second MHC polypeptide in a second polypeptide of a T-Cell-MMP, include a substitution of 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.

[00196] For example, in some cases, one of following pairs of residues in a HLA β 2M (see FIG. 4) and a HLA Class I heavy chains (see FIGs. 3D -3I) 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, -B, or -C amino acid sequence (i.e., residue 260 of the amino acid sequence depicted in FIGs. 3A- 3C respectively) 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. 4 is substituted with a Cys.

[00197] Separately, or in addition to, the pairs of cysteine residues in a β 2M and HLA Class I heavy chain polypeptide that may be used to form interchain disulfide bonds between the first and second polypeptides of a T-Cell-MMP (discussed above), the HLA-heavy chain of a T-Cell-MMP or its epitope conjugate may be substituted with cysteines to form an intrachain disulfide bond between a cysteine substituted into the carboxyl end portion of the α 1 helix and a cysteine in the amino end portion of the α 2-1 helix. Such disulfide bonds stabilize the T-Cell-MMP and permit its cellular processing and excretion from eukaryotic cells in the absence of a bound epitope peptide (or null peptide). In one embodiment the carboxyl end portion of the α 1 helix is from about amino acid position 79 to about amino acid position 89 and the amino end portion of the α 2-1 helix is from about amino acid position 134 to about amino acid position 144 of the MHC Class I heavy chain (the amino acid positions are determined based on the sequence of the heavy chains without their leader sequence (*see, e.g.*, FIGs. 3D-3H). In one such embodiment the disulfide bond is between a cysteine located at positions 83, 84, or 85 and a cysteine located at any of positions 138, 139 or 140 of the MHC Class I heavy chain. For example, a disulfide bond may be formed from cysteines incorporated into the MHC Class I heavy chain at amino acid 83 and a cysteine at an amino acid located at any of positions 138, 139 or 140. Alternatively, a disulfide bond may be formed between a cysteine inserted at position 84 and a cysteine inserted at any of positions 138, 139 or 140, or between a cysteine inserted at position 85 and a cysteine at any one of positions 138, 139 or 140. In an embodiment, the MHC Class I heavy chain intrachain disulfide bond is between cysteines substituted into a heavy chain sequence at positions 84 and 139 (e.g., the heavy chain sequence may be one of the heavy chain sequences set forth in FIGs. 3D-3H). As noted above, any of the MHC Class I intrachain disulfide bonds, including a disulfide bond between cysteines at 84 and 139, may be combined with intrachain disulfide bonds including a bond between MHC Class I heavy position 236 and position 12 of a mature β 2M polypeptide sequence (lacking its leader) as shown, for example, in FIG. 4.

[00198] In another embodiment, an intrachain disulfide bond may be formed in a MHC-H sequence of a T-Cell-MMP, or its epitope conjugate, between a cysteine substituted into the region between amino acid positions 79 and 89 and a cysteine substituted into the region between amino acid positions 134 and 144 of the sequences given in FIGs. 3D-3H. In such an embodiment, the MHC Class I heavy chain sequence may have insertions, deletions and/or substitutions of 1 to 5 amino acids preceding or following the cysteines forming the disulfide bond between the carboxyl end portion of the α 1 helix and the amino end portion of the α 2-1 helix. Any inserted amino acids may be selected from the naturally occurring amino acids or the naturally occurring amino acids except proline and alanine.

[00199] In an embodiment, the β 2M polypeptide of a T-Cell-MMP or its epitope conjugate comprises a mature β 2M polypeptide sequence (aas 21-119) of any one of NP_004039.1, NP_001009066.1, NP_001040602.1, NP_776318.1, or NP_033865.2 (SEQ ID NOs 57-61).

[00200] In some cases, a HLA Class I heavy chain polypeptide of a T-Cell-MMP or its epitope conjugate comprises any one of the HLA-A, -B, -C, -E, F, or -G sequences in FIGs. 3D-3H. Any of the heavy chain sequences may further comprise cysteine substitutions at positions 84 and 139, which may form an intrachain disulfide bond.

[00201] In an embodiment, the β 2M polypeptide of a T-Cell-MMP, or its epitope conjugate, comprises a mature β 2M polypeptide sequence (aas 21-119) of any one of the sequences in FIG. 4, which further comprises a R12C substitution.

[00202] In an embodiment, a T-Cell-MMP, or its epitope conjugate, comprises a first polypeptide comprising a mature β 2M polypeptide sequence (e.g., aas 21-119 of any one of the sequences in FIG. 4) having a R12C substitution, and a second polypeptide comprising any one of the HLA-A, -B, -C, -E, -F, or -G sequences in FIGs. 3D-3H bearing a cysteine at position 236. In such embodiments an intrachain disulfide bond may form between the cysteines at positions 12 and 236. In addition, any of the heavy chain sequences may further comprise cysteine substitutions at positions 84 and 139, which may form an intrachain disulfide bond.

[00203] In some cases, a HLA Class I heavy chain polypeptide of a T-Cell-MMP, or its epitope conjugate, comprises the amino acid sequence of HLA-A*0201 (FIG. 3D). In some cases, a HLA Class I heavy chain polypeptide of a T-Cell-MMP, or its epitope conjugate, comprises the amino acid sequence of HLA-A*0201 having an A236C substitution (FIG. 3D). In some cases, a HLA Class I heavy chain polypeptide of a T-Cell-MMP, or its epitope conjugate, comprises the amino acid sequence of HLA-A*0201 having a Y84A and a A236C substitution (FIG. 3D).

[00204] In an embodiment, a T-Cell-MMP, or its epitope conjugate, comprises a first polypeptide comprising amino acid residues 21-119 of NP_004039.1 with a R12C substitution (see FIG. 4), and a second polypeptide comprising a HLA-A*0201 (HLA-A2) sequence in FIG 3D. In one such embodiment the HLA-A*0201 sequence has an A236C substitution. In another such embodiment, the HLA-A*0201 sequence has a Y84C and A139C substitution. In another such embodiment, the HLA-A*0201 sequence has a Y84C, A139C, and A236C substitution. As indicated, MHC-H sequences with Y84C and A139C substitutions may form a stabilizing intrachain disulfide bond, and a cysteine at position 236 of the mature MHC-H may bond to a cysteine at position 12 of a mature β 2M polypeptide.

[00205] In an embodiment, a T-Cell-MMP, or its epitope conjugate, comprises a first polypeptide comprising amino acid residues 21-119 of NP_004039.1 with a R12C substitution (see FIG. 4), and a second polypeptide, a HLA Class I heavy chain polypeptide comprises the amino acid sequence
 GSHSMRYFFTSVSRPGRGEPRIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDGET
 RKVKAHSQTHRVDL(aa cluster 1){C}(aa cluster 2)AGSHTVQRMYGCDVGSDWRFLRGYHQYAY
 DGKDYIALKEDLRSW(aa cluster 3){C}(aa cluster 4)HKWEAAHVAEQLRAYLEGTCVEWLRRLYLE
 NGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTELVETRPA
 GDGTFQKWAAVVVPSGQEQRYSCHVQHEGLPKPLTLRWEP (SEQ ID NO:76); or,
 the first polypeptide comprises the sequence

IQRTPKIQVY SCHPAENGKS NFLNCYVSGF HPSDIEVDLLKNGERIEKVE HSDLSFSKDW SFYLLYYTEF TPTEKDEYAC RVNHVTLSP KIVKWDRDM (SEQ ID NO:77), and the second polypeptide comprises the amino acid sequence,

GSHSMRYFFTSVSRPGRGEPFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDGET RKVKAHSQTHRVDL(aa cluster 1){C}(aa cluster 2)AGSHTVQRMYGCDVGS DWRFLRGYHQYA YDGKDYIALKEDLRSW(aa cluster 3){C}(aa cluster 4))HKWEAAHVAEQLRAYLEGTCVEWLRRY LENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTEL(aa cluster 5)(C)(aa cluster 6)QKWA AVVVPSGQEQRYTCHVQHEGLPKPLTLRWEP (SEQ ID NO:78); where the cysteine residues indicated as {C} form a disulfide bond between the $\alpha 1$ and $\alpha 2-1$ helices and the (C) residue forms a disulfide bond with the mature $\beta 2M$ polypeptide cysteine at position 12.

[00206] Each occurrence of aa cluster 1, aa cluster 2, aa cluster 3, aa cluster 4, aa cluster 5, and aa cluster 6 is independently selected to be 1-5 amino acid residues, wherein the amino acid residues are each selected independently from i) any naturally occurring (proteogenic) amino acid or ii) any naturally occurring amino acid except proline or glycine.

[00207] In an embodiment where the MHC Class I heavy chain is an HLA-A chain:

aa cluster 1 may be the amino acid sequence GTLRG 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);

aa cluster 2 may be the amino acid sequence YNQSE 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);

aa cluster 3 may be the amino acid sequence TAADM 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);

aa cluster 4 may be the amino acid sequence AQTTK 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);

aa cluster 5 may be the amino acid sequence VETRP 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

aa cluster 6 may be the amino acid sequence GDGTF 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).

[00208] In some cases, the $\beta 2M$ polypeptide comprises the amino acid sequence:

IQRTPKIQVYSCHPAENGKSNFLNCYVSGFHPSDIEVDLLKNGERIEKVEHSDLSFSKDW SFYLLYYTEFTPTEKDEYACRVNHVTLSPKIVKWDRDM (SEQ ID NO:9).

[00209] In some cases, the first polypeptide and the second polypeptide of a T-Cell-MMP of the present disclosure are disulfides linked to one another through: i) a Cys residue present in a linker connecting the peptide epitope and a $\beta 2M$ polypeptide in the first polypeptide chain (e.g., with the epitope placed in the

N-terminal to the linker and the β 2M sequences); and ii) a Cys residue present in a 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, where n is 1, 2, 3, 4, 5, 6, 7, 8, or 9 (SEQ ID NO:93) (e.g., epitope-GCGGS(G₄S)_n-mature β 2M polypeptide). For example, in some cases, the linker comprises the amino acid sequence GCGGSGGGSGGGSGGGGS (SEQ ID NO:95). As another example, the linker comprises the amino acid sequence GCGGSGGGSGGGGS (SEQ ID NO:96). Examples of such a disulfide-linked first and second polypeptide are depicted schematically in FIGs. 6E-6H.

5. Scaffold polypeptides

[00210] T-Cell-MMPs and T-Cell-MMP-epitope conjugates can comprise a Fc polypeptide, or can comprise another suitable scaffold polypeptide.

[00211] 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, a 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:80), 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.

[00212] Suitable scaffold polypeptides will in some cases be 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 multimeric polypeptide, compared to a control multimeric polypeptide lacking the scaffold polypeptide. For example, in some cases, a scaffold polypeptide increases the *in vivo* half-life of the multimeric polypeptide, compared to a control multimeric polypeptide 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, a Fc polypeptide increases the *in vivo* half-life (serum half-life) of the multimeric polypeptide, compared to a control multimeric polypeptide 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.

6. Fc polypeptides

[00213] In some cases, the first and/or the second polypeptide chains of a T-Cell-MMP or its corresponding T-Cell-MMP-epitope conjugate (multimeric polypeptide(s)) comprise a Fc polypeptide which may be modified to include one or more chemical conjugation sites within or attached (e.g., at a terminus or attached by a linker) to the polypeptide. The Fc polypeptide of a T-Cell-MMP or T-Cell-MMP-epitope conjugate can be, for example, from an IgA, IgD, IgE, IgG, or IgM, which may contain a human polypeptide sequence, a humanized polypeptide sequence, a Fc region polypeptide of a synthetic heavy chain constant region, or a consensus heavy chain constant region. In embodiments, the Fc polypeptide can be from a human IgG1 Fc, a human IgG2 Fc, a human IgG3 Fc, a human IgG4 Fc, a human IgA Fc, a human IgD Fc, a human IgE Fc, a human IgM Fc, *etc.* Unless stated otherwise, the Fc polypeptides used in the T-Cell-MMPs and their epitope conjugates do not comprise a trans-membrane anchoring domain or a portion thereof sufficient to anchor the T-Cell-MMP or its epitope conjugate to a cell membrane. In some cases, the Fc polypeptide comprises an amino acid sequence having at least about 70% (e.g., at least about 75%, 80%, 85%, 90%, 95%, 98, 99%, or 100%) amino acid sequence identity to an amino acid sequence of a Fc region depicted in FIGs. 2A-2G. In some cases, the Fc region comprises an amino acid sequence having at least about 70% (e.g., at least about 75%, 80%, 85%, 90%, 95%, 98, 99%, or 100%) amino acid sequence identity to the human IgG1 Fc polypeptide depicted in FIG. 2A. In some cases, the Fc region comprises an amino acid sequence having at least about 70%, (e.g., at least about 75%, 80%, 85%, 90%, 95%, 98, 99%, or 100%) amino acid sequence identity to the human IgG1 Fc polypeptide depicted in FIG. 2A; and comprises a substitution of N77, which is underlined and bolded; e.g., the Fc polypeptide comprises a **N77A** substitution. In some cases, the Fc polypeptide comprises an amino acid sequence having at least about 70% (e.g., at least about 75%, 80%, 85%, 90%, 95%, 98, 99%, or 100%) amino acid sequence identity to the human IgG2 Fc polypeptide depicted in FIG. 2A; e.g., the Fc polypeptide comprises an amino acid sequence having at least about 70% (e.g., at least about 75%, 80%, 85%, 90%, 95%, 98, 99%, or 100%) amino acid sequence identity to amino acids 99-325 of the human IgG2 Fc polypeptide depicted in FIG. 2A. In some cases, the Fc polypeptide comprises an amino acid sequence having at least about 70% (e.g., at least about 75%, 80%, 85%, 90%, 95%, 98, 99%, or 100%) amino acid sequence identity to the human IgG3 Fc polypeptide depicted in FIG. 2A; e.g., the Fc polypeptide comprises an amino acid sequence having at least about 70% (e.g., at least about 75%, 80%, 85%, 90%, 95%, 98, 99%, or 100%) amino acid sequence identity to amino acids 19-246 of the human IgG3 Fc polypeptide depicted in FIG. 2A. In some cases, the Fc polypeptide comprises an amino acid sequence having at least about 70% (e.g., at least about 75%, 80%, 85%, 90%, 95%, 98, 99%, or 100%) amino acid sequence identity to the human IgM Fc polypeptide depicted in FIG. 2B; e.g., the Fc polypeptide comprises an amino acid sequence having at least about 70% (e.g., at least about 75%, 80%, 85%, 90%, 95%, 98, 99%, or 100%) amino acid sequence identity to amino acids 1-276 to the human IgM Fc polypeptide depicted in FIG. 2B. In some cases, the Fc polypeptide comprises an amino acid sequence having at least about 70% (e.g., at least about 75%, 80%, 85%, 90%, 95%, 98, 99%, or 100%) amino acid sequence identity to the human IgA Fc polypeptide

depicted in FIG. 2C; e.g., the Fc polypeptide comprises an amino acid sequence having at least about 70% (e.g., at least about 75%, 80%, 85%, 90%, 95%, 98, 99%, or 100%) amino acid sequence identity to amino acids 1-234 to the human IgA Fc polypeptide depicted in FIG. 2C.

[00214] In some cases, the Fc polypeptide present in a multimeric polypeptide comprises the amino acid sequence depicted in FIG. 2A (human IgG1 Fc). In some cases, the Fc polypeptide present in a multimeric polypeptide comprises the amino acid sequence depicted in FIG. 2A (human IgG1 Fc), except for a substitution of N297 (N77 of the amino acid sequence depicted in FIG. 2A) with an amino acid other than asparagine. In some cases, the Fc polypeptide present in a multimeric polypeptide comprises the amino acid sequence depicted in FIG. 2C (human IgG1 Fc comprising an N297A substitution, which is N77 of the amino acid sequence depicted in FIG. 2A). In some cases, the Fc polypeptide present in a multimeric polypeptide comprises the amino acid sequence depicted in FIG. 2A (human IgG1 Fc), except for a substitution of L234 (L14 of the amino acid sequence depicted in FIG. 2A) with an amino acid other than leucine. In some cases, the Fc polypeptide present in a multimeric polypeptide comprises the amino acid sequence depicted in FIG. 2A (human IgG1 Fc), except for a substitution of L235 with an amino acid other than leucine.

[00215] In some cases, the Fc polypeptide present in a multimeric polypeptide comprises the amino acid sequence depicted in FIG. 2E. In some cases, the Fc polypeptide present in a multimeric polypeptide comprises the amino acid sequence depicted in FIG. 2F. In some cases, the Fc polypeptide present in a multimeric polypeptide comprises the amino acid sequence depicted in FIG. 2G (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. 2G). In some cases, the Fc polypeptide present in a multimeric polypeptide comprises the amino acid sequence depicted in FIG. 2A (human IgG1 Fc), except for a substitution of P331 (P111 of the amino acid sequence depicted in FIG. 2A) 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 multimeric polypeptide comprises the amino acid sequence depicted in FIG. 2A (human IgG1 Fc), except for substitutions at L234 and L235 (L14 and L15 of the amino acid sequence depicted in FIG. 2A) with amino acids other than leucine. In some cases, the Fc polypeptide present in a multimeric polypeptide comprises the amino acid sequence depicted in FIG. 2A (human IgG1 Fc), except for substitutions at L234 and L235 (L14 and L15 of the amino acid sequence depicted in FIG. 2A) with amino acids other than leucine, and a substitution of P331 (P111 of the amino acid sequence depicted in FIG. 2A) with an amino acid other than proline. In some cases, the Fc polypeptide present in a multimeric polypeptide comprises the amino acid sequence depicted in FIG. 2E (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. 2E). In some cases, the Fc polypeptide present in a multimeric polypeptide is an IgG1 Fc polypeptide that comprises L234A and L235A substitutions (substitutions of L14 and L15 of the amino acid sequence depicted in FIG. 2A with Ala), as depicted in FIG. 2G.

[00216] In some cases, the Fc polypeptide comprises an amino acid sequence having at least about 70% (e.g., at least about 75%, 80%, 85%, 90%, 95%, 98, 99%, or 100%) amino acid sequence identity to a human IgG4 Fc polypeptide depicted in FIG. 2C. In some cases, the Fc polypeptide comprises an amino acid sequence having at least about 70% (e.g., at least about 75%, 80%, 85%, 90%, 95%, 98, 99%, or 100%) amino acid sequence identity to amino acids 100 to 327 of the GenBank P01861 human IgG4 Fc polypeptide depicted in FIG. 2C.

7. Linkers

[00217] T-Cell-MMPs (and their T-Cell-MMP-epitope conjugates) can include one or more independently selected linker peptides interposed between, for example, any one or more of: i) a MHC polypeptide and an Ig Fc polypeptide, where such a linker is referred to herein as a “L1 linker”; ii) a MHC polypeptide and a MOD, where such a linker is referred to herein as a “L2 linker”; iii) a first MOD and a second MOD, where such a linker is referred to herein as a “L3 linker” (e.g., between a first variant 4-1BBL polypeptide and a second variant 4-1BBL polypeptide; or between a second variant 4-1BBL polypeptide and a third variant 4-1BBL polypeptide); iv) a conjugation site or a peptide antigen (conjugated “epitope” peptide) and a MHC Class I polypeptide (e.g., $\beta 2M$); v) a 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 IgFc polypeptide.

[00218] 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 aa to 25 aa, from 3 aa to 20 aa, from 2 aa to 15 aa, from 3 aa to 12 aa, from 4 aa to 10 aa, from 5 aa to 9 aa, from 6 aa to 8 aa, or from 7 aa to 8 aa. In embodiments, 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 aa in length. In some cases, a linker has a length of from 25 aa to 50 aa, e.g., from 25 to 30, from 30 to 35, from 35 to 40, from 40 to 45, or from 45 to 50 aa in length.

[00219] Exemplary linkers include glycine polymers (G)_n, glycine-serine polymers (including, for example, (GS), (GS₂GS) (SEQ ID NO:81 and (GGGS) (SEQ ID NO:82), any of which may be repeated from one to ten times (e.g., repeated one, two, three, four, five, six, seven, eight, nine, or ten times) glycine-alanine polymers, alanine-serine polymers, and other flexible linkers known in the art. Glycine and glycine-serine polymers can both be used; both Gly and Ser are relatively unstructured, and therefore can serve as a neutral tether between components. Glycine polymers access significantly more phi-psi space than even alanine, and are much less restricted than residues with longer side chains (*see* Scheraga, *Rev. Computational Chem.* 11173-142 (1992)). Exemplary linkers can also comprise amino acid sequences including, but not limited to, GGSG (SEQ ID NO:83), GGSGG (SEQ ID NO:84), GSGSG (SEQ ID NO:85), GSGGG (SEQ ID NO:86), GGGSG (SEQ ID NO:87), GSSSG (SEQ ID NO:88), which may be repeated from one to ten times (e.g., repeated one, two, three, four, five, six, seven, eight, nine, or ten times), combinations thereof, and the like. Exemplary linkers can comprise the sequence Gly(Ser)₄ (SEQ ID NO:89) or Gly₄Ser (SEQ ID NO:90), either of which may be repeated from one to

ten times (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 times). In one embodiment the linker comprises the amino acid sequence AAAGG (SEQ ID NO:91), which may be repeated from 1 to 10 times.

[00220] In some cases, a linker comprises the aa sequence (GGGS) (SEQ ID NO:92), which may be repeated from 1 to 10 times (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 times). In some cases, a linker polypeptide, present in a first polypeptide of a T-Cell-MMP or its epitope conjugate, includes a cysteine residue that can form a disulfide bond with a cysteine residue present in an epitope presenting polypeptide or a second polypeptide of a T-Cell-MMP or its epitope conjugate. In some cases, for example, the linker comprises the aa sequence GCGGS(G₄S) (SEQ ID NO:93) where the G₄S unit may be repeated from 1 to 10 times (e.g., repeated 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 times), GCGASGGGSGGGGS (SEQ ID NO:94), the sequence GCGGSGGGSGGGSGGGGS (SEQ ID NO:95) or the sequence GCGGSGGGSGGGGS (SEQ ID NO:96).

[00221] Linkers, including the polypeptide linkers described above, may be present between a payload coupled to the first or second polypeptide of a T-Cell-MMP (or its epitope conjugate). In addition to the polypeptide linkers recited above, the linkers used to attach a payload or epitope (e.g., peptide) to the first and/or second polypeptide can be non-peptides. Such non-peptide linkers include polymers comprising, for example, polyethylene glycol (PEG). Other linkers, including those resulting from coupling with a bifunctional crosslinking agent, such as those recited below, may also be utilized.

8. Immunomodulatory polypeptides (MODs)

[00222] In some cases, a MOD present in a T-Cell-MMP of the present disclosure is a wt. MOD. In other cases, a MOD present in a T-Cell-MMP of the present disclosure is a variant MOD that has reduced affinity for a Co-MOD, compared to the affinity of a corresponding wt. MOD for the Co-MOD. Some MOD polypeptides that may be incorporated into T-Cell-MMPs exhibit reduced affinity for Co-MODs. The MOD polypeptides can have from 1 aa to 10 aa differences from a wt. immunomodulatory domain. For example, in some cases, a variant MOD polypeptide present in a T-Cell-MMP of the present disclosure may differ in aa sequence by, for example, 1 aa, 2 aa, 3 aa, 4 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, or 20 aa (e.g., from 1aa to 5 aa, from 5 aa to 10 aa, or from 10 aa to 20 aa) from a corresponding wild-type MOD. As an example, in some cases, a variant MOD polypeptide present in a T-Cell-MMP of the present disclosure has and/or includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 (e.g., from about 1 to about 20; 1 to 2; 1 to 3; 1 to 5; 2 to 4; 2 to 5; 2 to 6; 2 to 7; 2 to 8; 2 to 9; 2 to 10; 2 to 11; 2 to 12; 2 to 13; 2 to 14; 2 to 15; 2 to 16; 2 to 17; 2 to 18; 2 to 19, 2 to 20; 5 to 10; or 10 to 20) aa substitutions, compared to a corresponding reference (e.g., wt.) MOD. In some cases, variant MOD polypeptides present in a T-Cell-MMP include a single aa substitution compared to a corresponding reference (e.g., wt.) MOD.

[00223] As discussed above, variant MODs suitable for inclusion as domains (MOD polypeptides) in T-Cell-MMPs of the present disclosure (and/or their epitope conjugates) include those that exhibit reduced affinity for a Co-MOD, compared to the affinity of a corresponding wild-type MOD for the Co-MOD. Suitable variant MODs can be identified by, for example, mutagenesis, such as scanning mutagenesis (e.g., alanine, serine, or glycine scanning mutagenesis).

[00224] Exemplary pairs of MODs and Co-MODs include, but are not limited to entries (a) to (r) listed in the following table:

Exemplary Pairs of MODs and Co-MODs

a) 4-1BBL (MOD) and 4-1BB (Co-MOD);	k) CD40 (MOD) and CD40L (Co-MOD);
b) PD-L1 (MOD) and PD1 (Co-MOD);	l) CD83 (MOD) and CD83L (Co-MOD);
c) IL-2 (MOD) and IL-2 receptor (Co-MOD);	m) HVEM (CD270) (MOD) and CD160 (Co-MOD);
d) CD80 (MOD) and CD28 (Co-MOD);	n) JAG1 (CD339) (MOD) and Notch (Co-MOD);
e) CD86 (MOD) and CD28 (Co-MOD);	o) JAG1 (CD339) (MOD) and CD46 (Co-MOD);
f) OX40L (CD252) (MOD) and OX40 (CD134) (Co-MOD);	p) CD70 (MOD) and CD27 (Co-MOD);
g) Fas ligand (MOD) and Fas (Co-MOD);	q) CD80 (MOD) and CTLA4 (Co-MOD); and
h) ICOS-L (MOD) and ICOS (Co-MOD);	r) CD86 (MOD) and CTLA4 (Co-MOD)
i) ICAM (MOD) and LFA-1 (Co-MOD);	s) PD-L1 (MOD) and CD-80 (Co-MOD)
j) CD30L (MOD) and CD30 (Co-MOD);	

[00225] In some cases, a variant MOD present in a T-Cell-MMP of the present disclosure has a binding affinity for a Co-MOD that is from 100 nM to 100 μ M. For example, in some cases, a variant MOD polypeptide present in a T-Cell-MMP of the present disclosure (or its epitope conjugate) has a binding affinity for a Co-MOD that is from about 100 nM to about 150 nM, from about 100 nM to about 500 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 500 nM to about 1 μ M, 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, from about 1 μ M to about 5 μ M, from about 1 μ M to about 25 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 25 μ M to about 100 μ M, from about 50 μ M to about 75 μ M, or from about 75 μ M to about 100 μ M.

[00226] A variant MOD present in a T-Cell-MMP of the present disclosure exhibits reduced affinity for a cognate Co-MOD. Similarly, a T-Cell-MMP of the present disclosure that comprises a variant MOD exhibits reduced affinity for a cognate Co-MOD. Thus, for example, a T-Cell-MMP of the present disclosure that comprises a variant MOD has a binding affinity for a cognate Co-MOD that is from 100 nM to 100 μ M. For example, in some cases, a T-Cell-MMP of the present disclosure that comprises a variant MOD has a binding affinity for a cognate Co-MOD that is from about 100 nM to about 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, from

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.

a. Wild-type and variant PD-L1 MODs

[00227] As one non-limiting example, a MOD or variant MOD present in a masked TGF- β construct or complex is a PD-L1 or variant PD-L1 polypeptide. Wild-type PD-L1 binds to PD1.

[00228] A wild-type human PD-L1 polypeptide can comprise the following amino acid sequence:

MRIFAVFIFM TYWHLNNAFT VTPPKDLYVV EYGSNMTIEC KFPVEKQLDL AALIVYWEME
DKNIIQFVHG EEDLKVQHSS YRQRARLLKD QLSLGNAALQ ITDVKLQDAG VYRCMISYGG
ADYKRITVKV NAPYNKINQR ILVVDPTISE HELTCQAEY PKAEVIWTSS DHQVLSGKTT
TTNSKREEKL FNVSTSLRIN TTTNEIFYCT FRRLDPEENH TAEVIPGNI LNVSIKICLT
LSPST (SEQ ID NO:97); where aas 1-18 form the signal sequence, aas 19-127 form the Ig-like, V-type, or IgV domain, and 133-225 form the Ig-like C2 type domain.

[00229] A wild-type human PD-L1 ectodomain can comprise the following amino acid sequence: FT

VTPPKDLYVV EYGSNMTIEC KFPVEKQLDL AALIVYWEME DKNIIQFVHG EEDLKVQHSS
YRQRARLLKD QLSLGNAALQ ITDVKLQDAG VYRCMISYGG ADYKRITVKV NAPYNKINQR
ILVVDPTISE HELTCQAEY PKAEVIWTSS DHQVLSGKTT TTNSKREEKL FNVSTSLRIN
TTTNEIFYCT FRRLDPEENH TAEVIPGNI LNVSIKI (SEQ ID NO:98); where aas 1-109 form the Ig-like V-type or "IgV" domain, and aas 115-207 for the Ig-like C2 type domain.

[00230] A wild-type PD-L1 IgV domain, suitable for use as a MOD may comprise all or part of the PD-L1 IgV domain (aas 19-127 of SEQ D No. 97), and a carboxyl terminal stabilization sequence, such as

for instance the last seven aas (bolded and italicized) of the sequence: AFT VTPPKDLYVV
EYGSNMTIEC KFPVEKQLDL AALIVYWEME DKNIIQFVHG EEDLKTQHSS YRQRARLLKD
QLSLGNAA***LQ*** ITDVKLQDAG VYRCMISYGG ADYKRITVKV NAPI***AAALHE H*** SEQ ID NO:99.

Where the carboxyl stabilizing sequence comprises a histidine (e.g., a histidine approximately 5 residues to the C-terminal side of the tyrosine (Y) appearing as aa 117 of SEQ ID NO:99) at about aa 122, the histidine may form a stabilizing electrostatic bond with the backbone amide at aas 82 and 83 (bolded and italicized in SEQ ID NO:99 (Q107 and L106 of SEQ ID NO:97)). As an alternative, a stabilizing disulfide bond may be formed by substituting one of aas 82 or 83 (Q107 and L106 of SEQ ID NO:97) and one of aa residues 121, 122, or 123 (equivalent to aa positions 139-141 of SEQ ID NO:97).

[00231] A wild-type PD-1 polypeptide can comprise the following amino acid sequence:

PGWFLDSPDR PWNPTFSPA LLVVTEGDNA TFTCSFSNTS ESFVLNWYRM SPSNQTDKLA
AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV VRARRNDSGT YLCGAISLAP KAQIKESLRA
ELRVTERRAE VPTAHPSPSP RPAGQFQTLV VGVVGGLLGS LVLLVWVLAV ICSRAARGTI
GARRTGQPLK EDPSAVPVFS VDYGELDFQW REKTPEPPVP CVPEQTEYAT IVFPPSGMGTS
SPARRGSADG PRSAQPLRPE DGHCSWPL (SEQ ID NO:100).

[00232] In some cases, a variant PD-L1 polypeptide (e.g., a variant of SEQ ID NO:98 or PD-L1's IgV domain SEQ ID NO:99) exhibits reduced binding affinity to PD-1 (e.g., a PD-1 polypeptide comprising

the amino acid sequence set forth in SEQ ID NO:100), compared to the binding affinity of a PD-L1 polypeptide comprising the amino acid sequence set forth in SEQ ID NO:97 or SEQ ID NO:98. For example, in some cases, a variant PD-L1 polypeptide binds PD-1 (e.g., a PD-1 polypeptide comprising the amino acid sequence set forth in SEQ ID NO:100) with a binding affinity that is at least 10% less, at least 20% less, at least 30% less, at least 40% less, at least 50% less, at least 60% less, at least 70% less, at least 80% 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:97 or SEQ ID NO:98.

[00233] In some cases, a variant PD-L1 polypeptide (e.g., a variant of SEQ ID NO:98 or its IgV domain SEQ ID NO:99) has a binding affinity to PD-1 (e.g., of SEQ ID NO:100) that is from 1 nM to 1 mM (e.g., from 1 nM to 10 nM, from 10 nM to 100 nM, from 100 nM to 1 μ M, from 1 μ M to 10 μ M, from 10 μ M to 100 μ M, or from 100 μ M to 1 mM). As another example, in some cases, a variant PD-L1 polypeptide (e.g., a variant of SEQ ID NO:98) has a binding affinity for PD-1 (e.g., a PD-1 polypeptide comprising the amino acid sequence set forth in SEQ ID NO:100) that is from about 100 nM to about 200 nM, from about 200 nM to about 300 nM, from about 300 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, from 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 20 μ M, from about 20 μ M to about 30 μ M, from about 30 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.

[00234] A number of aa substitutions may be made in the PD-L1 ectodomain sequences used as MODs, including substitutions to sequences having greater than 90% (95%, 98% or 99%) sequence identity to at least 85 contiguous aas (e.g., at least 90, at least 95, at least 100, or at least 105 contiguous aas) of any one of SEQ ID NO:97, SEQ ID NO:98, aas 19-127 (the IgV domain) of SEQ ID NO:97, and SEQ ID NO:99. The substitutions may include (a) disulfide bond substitution pairs D103C and G33C, or V104 and S34C; (b) salt bridge forming substitution pairs Q107D and K62R or Q107D and S80R; and/or (c) Pi stacking substitutions M34Y or M34F. A PD-L1 MOD sequence may comprise a sequence having at least 85 contiguous aas (e.g., at least 90, at least 95, at least 100, or at least 105 contiguous aas) of SEQ ID NO:98, and at least one disulfide, salt bridge, or Pi stacking substitution. A PD-L1 MOD sequence may comprise a sequence having at least 85 contiguous aas (e.g., at least 90, at least 95, at least 100, or at least 105 contiguous aas) of aas 19-127 (the IgV domain) of SEQ ID NO:97, and at least one disulfide, salt bridge, or Pi stacking substitution. A PD-L1 MOD sequence may comprise a sequence having at least 85 contiguous aas (e.g., at least 90, at least 95, at least 100, or at least 105 contiguous aas) of SEQ ID NO:99, and at least one disulfide, salt bridge, or Pi stacking substitution.

[00235] In some cases, a variant PD-L1 polypeptide has a single aa substitution compared to the PD-L1 amino acid sequence set forth in SEQ ID NO:1, SEQ ID NO:98 or PD-L1's IgV domain. In some cases, a variant PD-L1 polypeptide has from 2 aa to 10 aa substitutions compared to the PD-L1 amino acid sequence set forth in SEQ ID NO:97, SEQ ID NO:98 or PD-L1's IgV domain. In some cases, a variant PD-L1 polypeptide has 2 aa substitutions compared to the PD-L1 amino acid sequence set forth in SEQ

ID NO:97, SEQ ID NO:98 or PD-L1's IgV domain. In some cases, a variant PD-L1 polypeptide has 3 aa or 4 aa substitutions compared to the PD-L1 amino acid sequence set forth in SEQ ID NO:97, SEQ ID NO:98 or PD-L1's IgV domain. In some cases, a variant PD-L1 polypeptide has 5 aa or 6 aa substitutions compared to the PD-L1 amino acid sequence set forth in SEQ ID NO:97, SEQ ID NO:98 or PD-L1's IgV domain provided in SEQ ID NO:99. In some cases, a variant PD-L1 polypeptide has 7 aa or 8 aa substitutions compared to the PD-L1 amino acid sequence set forth in SEQ ID NO:97, SEQ ID NO:98 or PD-L1's IgV domain. In some cases, a variant PD-L1 polypeptide has 9 aa or 10 aa substitutions compared to the PD-L1 amino acid sequence set forth in SEQ ID NO:97, SEQ ID NO:98 or PD-L1's IgV domain.

[00236] Suitable variant PD-L1 polypeptide sequences include polypeptide sequences having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% aa sequence identity to at least 170 contiguous aa (e.g., at least 180, 190 or 200 contiguous aa) of SEQ ID NO:98 (e.g., which have at least one aa insertion, deletion or substitution). Suitable variant PD-L1 IgV polypeptide sequences include polypeptide sequences having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% aa sequence identity to at least 70 contiguous aa (e.g., at least 80, 90, 100 or 105 contiguous aas) of aas 1-109 of SEQ ID NO:98 or SEQ ID NO:99 (e.g., which have at least one aa insertion, deletion or substitution).

[00237] Variant PD-L1 polypeptide sequences include polypeptide sequences having at least 90% (e.g., at least 95%, 98%, or 99%), or 100%, aa sequence identity to SEQ ID NO:98 or SEQ ID NO:99, wherein the residue at position 8 is an aa other than D; in one such instance that residue is an A, and in another, R. Variant PD-L1 polypeptide sequences include polypeptide sequences having at least 90% (e.g., at least 95%, 98%, or 99%), or 100%, aa sequence identity to SEQ ID NO:98 or SEQ ID NO:99, wherein the residue at position 36 is an aa other than I; in one such instance that residue is an A, and in another, D. Variant PD-L1 polypeptide sequences also include polypeptide sequences having at least 90% (e.g., at least 95%, 98%, or 99%), or 100%, aa sequence identity to SEQ ID NO:98 or SEQ ID NO:99, wherein the residue at position 54 is an aa other than E; in one instance that residue is an A, and in another, R.

b. Wild-type and variant CD80 MODs

[00238] In some cases, a variant MOD polypeptide present in a T-Cell-MMP of the present disclosure is a variant CD80 polypeptide. Wild-type CD80 binds to CD28.

[00239] A wild-type amino acid sequence of the ectodomain of human CD80 can be as follows:

VIHVTK EVKEVATLSC GHNVSVEELA QTRIWQKEK KMLTMMSGD MNIWPEYKNR
TIFDITNNLS IVILALRPSD EGTYECVVLK YEKDAFKREH LAEVTLSVKA DFPTPSISDF
EIPTSNIRRI ICSTSGGFPE PHLRWLENGE ELNAINTTVS QDPETELYAV SSKLDFNMTT
NHSFMCLIKY GHLRVNQTFN WNTTKQEHFP DN (SEQ ID NO:101).

[00240] A wild-type CD28 amino acid sequence can be as follows: MLRLLALNL FPSIQVTGNK
ILVKQSPMLV AYDNAVNLSC KYSYNLFSRE FRASLHKGLD SAVEVCVVYG NYSQQLQVYS
KTGFNC DGKL GNEVTFYQL NLYVNQTDIY FCKIEVMYPP PYLDNEKSNG TIIHVGKHL
CPSPLFPGPS KPFWVLVVVG GVLACYSLLV TVAFIIFWVR SKRSRLHSD YMNMTPRRPG

PTRKHYQPYA PPRDFAAYRS (SEQ ID NO:102). In some cases, where a T-Cell-MMP of the present disclosure comprises a variant CD80 polypeptide, a Co-MOD is a CD28 polypeptide comprising the amino acid sequence of SEQ ID NO:102.

[00241] A wild-type CD28 amino acid sequence can be as follows: MLRLLLALNL FPSIQVTGNK ILVKQSPMLV AYDNAVNLSW KHLCPSPFLP GPSKPFWVLV VVGGLVACYS LLVTVAFIIF WVRSKRSRLL HSDYMNMTPR RPGPTRKHYQ PYAPPRDFAA YRS (SEQ ID NO:103).

[00242] A wild-type CD28 amino acid sequence can also be as follows: MLRLLLALNL FPSIQVTGKH LCPSPFLFPGP SKPFWVLVVV GGLVACYSLL VTVAFIIFWV RSKRSRLLHS DYMNMTPRRP GPTRKHYQPY APPRDFAAYR S (SEQ ID NO:104).

[00243] 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:102 for CD28. For example, in some cases, a variant CD80 polypeptide binds CD28 with a binding affinity that is at least 10% less (e.g., at least: 15% less, 20% less, 25% less, 30% less, 35% less, 40% less, 45% less, 50% less, 55% less, 60% less, 65% less, 70% less, 75% less, 80% less, 85% less, 90% less, 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:102 for CD28 (e.g., a CD28 polypeptide comprising the amino acid sequence set forth in one of SEQ ID NOs:102, 103, or 104).

[00244] In some cases, a variant CD80 polypeptide has a binding affinity to CD28 that is from 100 nM to 100 μ M. As another example, in some cases, a variant CD80 polypeptide of the present disclosure has a binding affinity for CD28 (e.g., a CD28 polypeptide comprising the amino acid sequence set forth in SEQ ID NO:102, SEQ ID NO:103, or SEQ ID NO:104) 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, from 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.

[00245] In some cases, a variant CD80 polypeptide has a single amino acid substitution compared to the CD80 amino acid sequence set forth in SEQ ID NO:101. In some cases, a variant CD80 polypeptide has from 2 to 10 amino acid substitutions compared to the CD80 amino acid sequence set forth in SEQ ID NO:101. In some cases, a variant CD80 polypeptide has 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions compared to the CD80 amino acid sequence set forth in SEQ ID NO:101.

[00246] Some suitable CD80 variants include a polypeptide that comprises an amino acid sequence having a sequence identity of at least 90% (less than 20 substitutions), at least 95% (less than 10 substitutions), at least 97% (less than 6 substitutions), at least 98% (less than 4 substitutions), at least 99% (less than 2 substitutions), or at least 99.5% (one substitution) amino acid sequence identity to any one of the CD80 amino acid sequences that follow.

[00247] VIHVTKEVKEVATLSC GHXVSVEELA QTRIYWQKEK KMVLTMMMSGD
 MNIWPEYKNR TIFDITNNLS IVILALRPSD EGYECVVLK YEKDAFKREH LAEVTLSVKA
 DFPTPSISDF EIPTSNIRRI ICSTSGGFPE PHLSWLENGE ELNAINTTVS QDPETELYAV
 SSKLDFNMTT NHSFMCLIKY GHLRVNQTFN WNTTKQEHFP DN (SEQ ID NO:105), where X is
 any amino acid other than Asn. In some cases, X is Ala.

[00248] VIHVTKEVKEVATLSC GHNVSVEELA QTRIYWQKEK KMVLTMMMSGD
 MNIWPEYKNR TIFDITXNLS IVILALRPSD EGYECVVLK YEKDAFKREH LAEVTLSVKA
 DFPTPSISDF EIPTSNIRRI ICSTSGGFPE PHLSWLENGE ELNAINTTVS QDPETELYAV
 SSKLDFNMTT NHSFMCLIKY GHLRVNQTFN WNTTKQEHFP DN (SEQ ID NO:106), where X is
 any amino acid other than Asn. In some cases, X is Ala.

[00249] VIHVTKEVKEVATLSC GHNVSVEELA QTRIYWQKEK KMVLTMMMSGD
 MNIWPEYKNR TIFDITNNLS XVILALRPSD EGYECVVLK YEKDAFKREH LAEVTLSVKA
 DFPTPSISDF EIPTSNIRRI ICSTSGGFPE PHLSWLENGE ELNAINTTVS QDPETELYAV
 SSKLDFNMTT NHSFMCLIKY GHLRVNQTFN WNTTKQEHFP DN (SEQ ID NO:107), where X is
 any amino acid other than Ile. In some cases, X is Ala.

[00250] VIHVTKEVKEVATLSC GHNVSVEELA QTRIYWQKEK KMVLTMMMSGD
 MNIWPEYKNR TIFDITNNLS IVILALRPSD EGYECVVLX YEKDAFKREH LAEVTLSVKA
 DFPTPSISDF EIPTSNIRRI ICSTSGGFPE PHLSWLENGE ELNAINTTVS QDPETELYAV
 SSKLDFNMTT NHSFMCLIKY GHLRVNQTFN WNTTKQEHFP DN (SEQ ID NO:108), where X is
 any amino acid other than Lys. In some cases, X is Ala.

[00251] VIHVTKEVKEVATLSC GHNVSVEELA QTRIYWQKEK KMVLTMMMSGD
 MNIWPEYKNR TIFDITNNLS IVILALRPSD EGYECVVLK YEKDAFKREH LAEVTLSVKA
 DFPTPSISDF EIPTSNIRRI ICSTSGGFPE PHLSWLENGE ELNAINTTVS XDPETELYAV
 SSKLDFNMTT NHSFMCLIKY GHLRVNQTFN WNTTKQEHFP DN (SEQ ID NO:109), where X is
 any amino acid other than Gln. In some cases, X is Ala.

[00252] VIHVTKEVKEVATLSC GHNVSVEELA QTRIYWQKEK KMVLTMMMSGD
 MNIWPEYKNR TIFDITNNLS IVILALRPSD EGYECVVLK YEKDAFKREH LAEVTLSVKA
 DFPTPSISDF EIPTSNIRRI ICSTSGGFPE PHLSWLENGE ELNAINTTVS QXPETELYAV
 SSKLDFNMTT NHSFMCLIKY GHLRVNQTFN WNTTKQEHFP DN (SEQ ID NO:110), where X is
 any amino acid other than Asp. In some cases, X is Ala.

[00253] VIHVTKEVKEVATLSC GHNVSVEEXA QTRIYWQKEK KMVLTMMMSGD
 MNIWPEYKNR TIFDITNNLS IVILALRPSD EGYECVVLK YEKDAFKREH LAEVTLSVKA
 DFPTPSISDF EIPTSNIRRI ICSTSGGFPE PHLSWLENGE ELNAINTTVS QDPETELYAV
 SSKLDFNMTT NHSFMCLIKY GHLRVNQTFN WNTTKQEHFP DN (SEQ ID NO:111), where X is
 any amino acid other than Leu. In some cases, X is Ala.

[00254] VIHVTKEVKEVATLSC GHNVSVEELA QTRIXWQKEK KMVLTMMMSGD
 MNIWPEYKNR TIFDITNNLS IVILALRPSD EGYECVVLK YEKDAFKREH LAEVTLSVKA
 DFPTPSISDF EIPTSNIRRI ICSTSGGFPE PHLSWLENGE ELNAINTTVS QDPETELYAV

SSKLDFNMTT NHSFMCLIKY GHLRVNQTFN WNTTKQEHFP DN (SEQ ID NO:112), where X is any amino acid other than Tyr. In some cases, X is Ala.

[00255] VIHVTKEVKEVATLSC GHNVSVEELA QTRIYWXKEK KMLTMMSGD
MNIWPEYKNR TIFDITNNLS IVILALRPSD EGTYESCVVLK YEKDAFKREH LAEVLTSVKA
DFPTPSISDF EIPTSNIRRI ICSTSGGFPE PHLSWLENGE ELNAINTTVS QDPETELYAV
SSKLDFNMTT NHSFMCLIKY GHLRVNQTFN WNTTKQEHFP DN (SEQ ID NO:113), where X is any amino acid other than Gln. In some cases, X is Ala.

[00256] VIHVTKEVKEVATLSC GHNVSVEELA QTRIYWQKEK KXVLTMMSGD
MNIWPEYKNR TIFDITNNLS IVILALRPSD EGTYESCVVLK YEKDAFKREH LAEVLTSVKA
DFPTPSISDF EIPTSNIRRI ICSTSGGFPE PHLSWLENGE ELNAINTTVS QDPETELYAV
SSKLDFNMTT NHSFMCLIKY GHLRVNQTFN WNTTKQEHFP DN (SEQ ID NO:114), where X is any amino acid other than Met. In some cases, X is Ala.

[00257] VIHVTKEVKEVATLSC GHNVSVEELA QTRIYWQKEK KMXLTMMSGD
MNIWPEYKNR TIFDITNNLS IVILALRPSD EGTYESCVVLK YEKDAFKREH LAEVLTSVKA
DFPTPSISDF EIPTSNIRRI ICSTSGGFPE PHLSWLENGE ELNAINTTVS QDPETELYAV
SSKLDFNMTT NHSFMCLIKY GHLRVNQTFN WNTTKQEHFP DN (SEQ ID NO:115), where X is any amino acid other than Val. In some cases, X is Ala.

[00258] VIHVTKEVKEVATLSC GHNVSVEELA QTRIYWQKEK KMLTMMSGD
MNXWPEYKNR TIFDITNNLS IVILALRPSD EGTYESCVVLK YEKDAFKREH LAEVLTSVKA
DFPTPSISDF EIPTSNIRRI ICSTSGGFPE PHLSWLENGE ELNAINTTVS QDPETELYAV
SSKLDFNMTT NHSFMCLIKY GHLRVNQTFN WNTTKQEHFP DN (SEQ ID NO:116), where X is any amino acid other than Ile. In some cases, X is Ala.

[00259] VIHVTKEVKEVATLSC GHNVSVEELA QTRIYWQKEK KMLTMMSGD
MNIWPEXKNR TIFDITNNLS IVILALRPSD EGTYESCVVLK YEKDAFKREH LAEVLTSVKA
DFPTPSISDF EIPTSNIRRI ICSTSGGFPE PHLSWLENGE ELNAINTTVS QDPETELYAV
SSKLDFNMTT NHSFMCLIKY GHLRVNQTFN WNTTKQEHFP DN (SEQ ID NO:117), where X is any amino acid other than Tyr. In some cases, X is Ala.

[00260] VIHVTKEVKEVATLSC GHNVSVEELA QTRIYWQKEK KMLTMMSGD
MNIWPEYKNR TIFXITNNLS IVILALRPSD EGTYESCVVLK YEKDAFKREH LAEVLTSVKA
DFPTPSISDF EIPTSNIRRI ICSTSGGFPE PHLSWLENGE ELNAINTTVS QDPETELYAV
SSKLDFNMTT NHSFMCLIKY GHLRVNQTFN WNTTKQEHFP DN (SEQ ID NO:118), where X is any amino acid other than Asp. In some cases, X is Ala.

[00261] VIHVTKEVKEVATLSC GHNVSVEELA QTRIYWQKEK KMLTMMSGD
MNIWPEYKNR TIFDITNNLS IVILALRPSD EGTYESCVVLK YEKDAFKREH LAEVLTSVKA
DXPTPSISDF EIPTSNIRRI ICSTSGGFPE PHLSWLENGE ELNAINTTVS QDPETELYAV
SSKLDFNMTT NHSFMCLIKY GHLRVNQTFN WNTTKQEHFP DN (SEQ ID NO:119), where X is any amino acid other than Phe. In some cases, X is Ala.

[00262] VIHVTKEVKEVATLSC GHNVSVEELA QTRIYWQKEK KMVLTMMMSGD
 MNIWPEYKNR TIFDITNNLS IVILALRPSD EGTYESCVVLK YEKDAFKREH LAEVTLSVKA
 DFPTPSISDF EIPTSNIRRI ICSTSGGFPE PHLSWLENGE ELNAINTTVX QDPETELYAV
 SSKLDFNMTT NHSFMCLIKY GHLRVNQTFN WNTTKQEHFP DN (SEQ ID NO:120), where X is
 any amino acid other than Ser. In some cases, X is Ala.

[00263] VIHVTKEVKEVATLSC GHNVSVEELA QTRIYWQKEK KMVLTMMMSGD
 MNIWPEYKNR TIFDITNNLS IVILALRPSD EGTYESCVVLK YEKDAFKREH LAEVTLSVKA
 DFPTXSISDF EIPTSNIRRI ICSTSGGFPE PHLSWLENGE ELNAINTTVS QDPETELYAV
 SSKLDFNMTT NHSFMCLIKY GHLRVNQTFN WNTTKQEHFP DN (SEQ ID NO:121), where X is
 any amino acid other than Pro. In some cases, X is Ala.

c. Wild-type and variant CD86 MODs

[00264] In some cases, a variant MOD polypeptide present in a T-Cell-MMP of the present disclosure is a variant CD86 polypeptide. Wild-type CD86 binds to CD28.

[00265] The amino acid sequence of the full ectodomain of a wild-type human CD86 can be as follows:
 APLKIQAYFNETADLPCQFANSQNQSLSELVVFWDQENLVLNEVYLGKEKFDSVHSKYMNNRT
 SFDSSDSWTLRLHNLQIKDKGLYQCIIHHKKPTGMIRIHQMNSELSVLANFSQPEIVPISNITENVYI
 NLTCSSIHGYPEPKKMSVLLRTKNSTIEYDGIMQKSQDNVTELYDVSISLSVSFPDVTSNMTIFCIL
 ETDKTRLLSSPFSIELEDPPPPDHIP (SEQ ID NO:122).

[00266] The amino acid sequence of the IgV domain of a wild-type human CD86 can be as follows:
 APLKIQAYFNETADLPCQFANSQNQSLSELVVFWDQENLVLNEVYLGKEKFDSVHSKYMNNRT
 SFDSSDSWTLRLHNLQIKDKGLYQCIIHHKKPTGMIRIHQMNSELSVL (SEQ ID NO:123).

[00267] In some cases, a variant CD86 polypeptide exhibits reduced binding affinity to CD28, compared to the binding affinity of a CD86 polypeptide comprising the amino acid sequence set forth in SEQ ID NO:122 or SEQ ID NO:123 for CD28. For example, in some cases, a variant CD86 polypeptide binds CD28 with a binding affinity that is 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 binding affinity of a CD86 polypeptide comprising the amino acid sequence set forth in SEQ ID NO:122 or SEQ ID NO:123 for CD28 (e.g., a CD28 polypeptide comprising the amino acid sequence set forth in one of SEQ ID NOs:102, 103, or 104).

[00268] In some cases, a variant CD86 polypeptide has a binding affinity to CD28 that is from 100 nM to 100 μ M. As another example, in some cases, a variant CD86 polypeptide of the present disclosure has a binding affinity for CD28 (e.g., a CD28 polypeptide comprising the amino acid sequence set forth in one of SEQ ID NOs: 102, 103, or 104) 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.

[00269] In some cases, a variant CD86 polypeptide has a single amino acid substitution compared to the CD86 amino acid sequence set forth in SEQ ID NO:122. In some cases, a variant CD86 polypeptide has from 2 to 10 amino acid substitutions compared to the CD86 amino acid sequence set forth in SEQ ID NO:122. In some cases, a variant CD86 polypeptide has 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions compared to the CD86 amino acid sequence set forth in SEQ ID NO:122.

[00270] In some cases, a variant CD86 polypeptide has a single amino acid substitution compared to the CD86 amino acid sequence set forth in SEQ ID NO:123. In some cases, a variant CD86 polypeptide has from 2 to 10 amino acid substitutions compared to the CD86 amino acid sequence set forth in SEQ ID NO:123. In some cases, a variant CD86 polypeptide has 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions compared to the CD86 amino acid sequence set forth in SEQ ID NO:123.

[00271] Suitable CD86 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 amino acid sequences that follow.

[00272] APLKIQA YFNETADLPCQFANSQNQSLSELVFWQDQENLVLNEVYLGKEKFDSVHSK YMXRTSFDSDSWTLRLHNLQIKDKGLYQCIIHHKKPTGMIRIHQMNSELSVLNFSQPEIVPISNI TENVYINLTCSSIHGYPEPKKMSVLLRTKNSTIEYDGIMQKSQDNVTELYDVSISLSVSFPDVTSN MTIFCILETDKTRLLSPPFSIELEDPPPPDHIP (SEQ ID NO:124), where X is any amino acid other than Asn. In some cases, X is Ala.

[00273] APLKIQA YFNETADLPCQFANSQNQSLSELVFWQDQENLVLNEVYLGKEKFDSVHSK YMNRTSF~~X~~SDSWTLRLHNLQIKDKGLYQCIIHHKKPTGMIRIHQMNSELSVLNFSQPEIVPISNI TENVYINLTCSSIHGYPEPKKMSVLLRTKNSTIEYDGIMQKSQDNVTELYDVSISLSVSFPDVTSN MTIFCILETDKTRLLSPPFSIELEDPPPPDHIP (SEQ ID NO:125), where X is any amino acid other than Asp. In some cases, X is Ala.

[00274] APLKIQA YFNETADLPCQFANSQNQSLSELVFWQDQENLVLNEVYLGKEKFDSVHSK YMNRTSFDSDS~~X~~TLRLHNLQIKDKGLYQCIIHHKKPTGMIRIHQMNSELSVLNFSQPEIVPISNIT ENVYINLTCSSIHGYPEPKKMSVLLRTKNSTIEYDGIMQKSQDNVTELYDVSISLSVSFPDVTSNM TIFCILETDKTRLLSPPFSIELEDPPPPDHIP (SEQ ID NO:126), where X is any amino acid other than Trp. In some cases, X is Ala.

[00275] APLKIQA YFNETADLPCQFANSQNQSLSELVFWQDQENLVLNEVYLGKEKFDSVHSK YMNRTSFDSDSWTLRLHNLQIKDKGLYQCIIH~~X~~KKPTGMIRIHQMNSELSVLNFSQPEIVPISNI TENVYINLTCSSIHGYPEPKKMSVLLRTKNSTIEYDGIMQKSQDNVTELYDVSISLSVSFPDVTSN MTIFCILETDKTRLLSPPFSIELEDPPPPDHIP (SEQ ID NO:127), where X is any amino acid other than His. In some cases, X is Ala.

[00276] APLKIQAYFNETADLPCQFANSQNQSLSELVVFWDQENLVLNEVYLGKEKFDSVHISK
YMXRTSFDSDSWTLRLHNLQIKDKGLYQCIIHHKKPTGMIRIHQMNSELSVL (SEQ ID NO:128),
where X is any amino acid other than Asn. In some cases, X is Ala.

[00277] APLKIQAYFNETADLPCQFANSQNQSLSELVVFWDQENLVLNEVYLGKEKFDSVHISK
YMNRTSF~~X~~SDSWTLRLHNLQIKDKGLYQCIIHHKKPTGMIRIHQMNSELSVL (SEQ ID NO:129),
where X is any amino acid other than Asp. In some cases, X is Ala.

[00278] APLKIQAYFNETADLPCQFANSQNQSLSELVVFWDQENLVLNEVYLGKEKFDSVHISK
YMNRTSFDSDS~~X~~TLRLHNLQIKDKGLYQCIIHHKKPTGMIRIHQMNSELSVL (SEQ ID NO:130),
where X is any amino acid other than Trp. In some cases, X is Ala.

[00279] APLKIQAYFNETADLPCQFANSQNQSLSELVVFWDQENLVLNEVYLGKEKFDSVHISK
YMNRTSFDSDSWTLRLHNLQIKDKGLYQCIIH~~X~~KKPTGMIRIHQMNSELSVL (SEQ ID NO:131),
where X is any amino acid other than His. In some cases, X is Ala.

[00280] APLKIQAYFNETADLPCQFANSQNQSLSELVVFWDQENL~~X~~LNEVYLGKEKFDSVHISK
YMNRTSFDSDSWTLRLHNLQIKDKGLYQCIIHHKKPTGMIRIHQMNSELSVLANFSQPEIVPISNI
TENVYINLTCCSIHGYPEPKKMSVLLRTKNSTIEYDGIMQKSQDNVTELYDVSISLSVSFPDVTSN
MTIFCILETDKTRLLSPPFSIELEDPPPPDHIP (SEQ ID NO:132), where X is any amino acid other
than Val. In some cases, X is Ala.

[00281] APLKIQAYFNETADLPCQFANSQNQSLSELVVFWDQENL~~X~~LNEVYLGKEKFDSVHISK
YMNRTSFDSDSWTLRLHNLQIKDKGLYQCIIHHKKPTGMIRIHQMNSELSVL (SEQ ID NO:133),
where X is any amino acid other than Val. In some cases, X is Ala.

[00282] APLKIQAYFNETADLPCQFANSQNQSLSELVVFWD~~X~~DQENLVLNEVYLGKEKFDSVHISK
YMNRTSFDSDSWTLRLHNLQIKDKGLYQCIIHHKKPTGMIRIHQMNSELSVLANFSQPEIVPISNI
TENVYINLTCCSIHGYPEPKKMSVLLRTKNSTIEYDGIMQKSQDNVTELYDVSISLSVSFPDVTSN
MTIFCILETDKTRLLSPPFSIELEDPPPPDHIP (SEQ ID NO:134), where X is any amino acid other
than Gln. In some cases, X is Ala.

[00283] APLKIQAYFNETADLPCQFANSQNQSLSELVVFWD~~X~~DQENLVLNEVYLGKEKFDSVHISK
YMNRTSFDSDSWTLRLHNLQIKDKGLYQCIIHHKKPTGMIRIHQMNSELSVL (SEQ ID NO:135),
where X is any amino acid other than Gln. In some cases, X is Ala.

[00284] APLKIQAYFNETADLPCQFANSQNQSLSELVV~~X~~WQDQENLVLNEVYLGKEKFDSVHISK
YMNRTSFDSDSWTLRLHNLQIKDKGLYQCIIHHKKPTGMIRIHQMNSELSVLANFSQPEIVPISNI
TENVYINLTCCSIHGYPEPKKMSVLLRTKNSTIEYDGIMQKSQDNVTELYDVSISLSVSFPDVTSN
MTIFCILETDKTRLLSPPFSIELEDPPPPDHIP (SEQ ID NO:136), where X is any amino acid other
than Phe. In some cases, X is Ala.

[00285] APLKIQAYFNETADLPCQFANSQNQSLSELVV~~X~~WQDQENLVLNEVYLGKEKFDSVHISK
YMNRTSFDSDSWTLRLHNLQIKDKGLYQCIIHHKKPTGMIRIHQMNSELSVL (SEQ ID NO:137),
where X is any amino acid other than Phe. In some cases, X is Ala.

[00286] APLKIQAYFNETADLPCQFANSQNQSLSELVVFWDQENLVLNEVYLGKEKFDSVHISK
YMNRTSFDSDSWT~~X~~RLHNLQIKDKGLYQCIIHHKKPTGMIRIHQMNSELSVLANFSQPEIVPISNI

TENVYINLTCCSIHGYPEPKKMSVLLRTKNSTIEYDGIMQKSQDNVTELYDVSISLSVSFPDVTSN
MTIFCILETDKTRLLSSPFSIELEDPPPPDHIP (SEQ ID NO:138), where X is any amino acid other
than Leu. In some cases, X is Ala.

[00287] APLKIQAYFNETADLPCQFANSQNQSLSELVFWQDQENLVNEVYLGKEKFDSVHKS
YMNRTSFDSDSWTXRLHNLQIKDKGLYQCIIHHKKPTGMIRIHQMNSELSVL (SEQ ID NO:139),
where X is any amino acid other than Leu. In some cases, X is Ala.

[00288] APLKIQAYFNETADLPCQFANSQNQSLSELVFWQDQENLVNEVYLGKEKFDSVHKS
XMNRTSFDSDSWTLRLHNLQIKDKGLYQCIIHHKKPTGMIRIHQMNSELSVLANFSQPEIVPISNI
TENVYINLTCCSIHGYPEPKKMSVLLRTKNSTIEYDGIMQKSQDNVTELYDVSISLSVSFPDVTSN
MTIFCILETDKTRLLSSPFSIELEDPPPPDHIP (SEQ ID NO:140), where X is any amino acid other
than Tyr. In some cases, X is Ala.

[00289] APLKIQAYFNETADLPCQFANSQNQSLSELVFWQDQENLVNEVYLGKEKFDSVHKS
XMNRTSFDSDSWTLRLHNLQIKDKGLYQCIIHHKKPTGMIRIHQMNSELSVL (SEQ ID NO:141),
where X is any amino acid other than Tyr. In some cases, X is Ala.

[00290] APLKIQAYFNETADLPCQFANSQNQSLSELVFWQDQENLVNEVYLGKEKFDSVHKS
YMX₁RTSFDSDSWTLRLHNLQIKDKGLYQCIIHX₂KKPTGMIRIHQMNSELSVLANFSQPEIVPISN
ITENVYINLTCCSIHGYPEPKKMSVLLRTKNSTIEYDGIMQKSQDNVTELYDVSISLSVSFPDVTSN
MTIFCILETDKTRLLSSPFSIELEDPPPPDHIP (SEQ ID NO:142), where X₁ is any amino acid other
than Asn and the second X₂ is any amino acid other than His. In some cases, X₁ and X₂ are both Ala.

[00291] APLKIQAYFNETADLPCQFANSQNQSLSELVFWQDQENLVNEVYLGKEKFDSVHKS
YMX₁RTSFDSDSWTLRLHNLQIKDKGLYQCIIHX₂KKPTGMIRIHQMNSELSVL (SEQ ID NO:143),
where X₁ is any amino acid other than Asn and X₂ is any amino acid other than His. In some cases, X₁
and X₂ are both Ala.

[00292] APLKIQAYFNETADLPCQFANSQNQSLSELVFWQDQENLVNEVYLGKEKFDSVHKS
YMNRTSFX₁SDSWTLRLHNLQIKDKGLYQCIIHX₂KKPTGMIRIHQMNSELSVLANFSQPEIVPISN
ITENVYINLTCCSIHGYPEPKKMSVLLRTKNSTIEYDGIMQKSQDNVTELYDVSISLSVSFPDVTSN
MTIFCILETDKTRLLSSPFSIELEDPPPPDHIP (SEQ ID NO:144), where X₁ is any amino acid other
than Asp, and X₂ is any amino acid other than His. In some cases, X₁ is Ala and X₂ is Ala.

[00293] APLKIQAYFNETADLPCQFANSQNQSLSELVFWQDQENLVNEVYLGKEKFDSVHKS
YMNRTSFX₁SDSWTLRLHNLQIKDKGLYQCIIHX₂KKPTGMIRIHQMNSELSVL (SEQ ID NO:145),
where X₁ is any amino acid other than Asn and X₂ is any amino acid other than His. In some cases, X₁
and X₂ are both Ala.

[00294] APLKIQAYFNETADLPCQFANSQNQSLSELVFWQDQENLVNEVYLGKEKFDSVHKS
YMX₁RTSFX₂SDSWTLRLHNLQIKDKGLYQCIIHX₃KKPTGMIRIHQMNSELSVLANFSQPEIVPIS
NITENVYINLTCCSIHGYPEPKKMSVLLRTKNSTIEYDGIMQKSQDNVTELYDVSISLSVSFPDVT
NMTIFCILETDKTRLLSSPFSIELEDPPPPDHIP (SEQ ID NO:146), where X₁ is any amino acid other
than Asn, X₂ is any amino acid other than Asp, and X₃ is any amino acid other than His. In some cases,
X₁ is Ala, X₂ is Ala, and X₃ is Ala.

[00295] APLKIQA YFNETADLPCQFANSQNQSLSELVFWQDQENLVNEVYLGKEKFDSVHSK YMX₁RTSF_{X₂}SDSWTLRLHNLQIKDKGLYQCIH_{X₃}KKPTGMIRIHMENSELSVL (SEQ ID NO:147), where X₁ is any amino acid other than Asn, X₂ is any amino acid other than Asp, and X₃ is any amino acid other than His. In some cases, X₁ is Ala, X₂ is Ala, and X₃ is Ala.

d. Wild-type and variant 4-1BBL MODs

[00296] In some cases, a variant MOD polypeptide present in a T-Cell-MMP of the present disclosure is a variant 4-1BBL polypeptide. Wild-type 4-1BBL binds to 4-1BB (CD137).

[00297] A wild-type 4-1BBL amino acid sequence can be as follows: MEYASDASLD PEAPWPPAPR ARACRVLP **WA LVAGLLLLLL LAAACAVFLA** CPWAVSGARA SPGSAASPRL REGPELSPDD PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS PRSE (SEQ ID NO:148).

[00298] In some cases, a variant 4-1BBL polypeptide is a variant of the tumor necrosis factor (TNF) homology domain (THD) of human 4-1BBL.

[00299] A wild-type amino acid sequence of the THD of human 4-1BBL can be, e.g., one of SEQ ID NOs:23-25, as follows:

[00300] PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS PRSE (SEQ ID NO:149);

[00301] D PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS PRSE (SEQ ID NO:150); or

[00302] D PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPA (SEQ ID NO:151).

[00303] A wild-type 4-1BB amino acid sequence can be as follows: MGNSCYNIVA TLLLVLNFER TRSLQDPCSN CPAGTFCDNN RNQICSPCPP NSFSSAGGQR TCDICRQCKG VFRTRKECSS TSNAECDCTP GFHCLGAGCS MCEQDCKQGQ ELTKKGCKDC CFGTFNDQKR GICRPWTNCS LDGKSVLVNG TKERDVVCGP SPADLSPGAS SVTPPAPARE PGHSPQIISF FLALTSTALL FLLFFLTLRF SVVKRGRKKL LYIFKQPFMR PVQTTQEEDG CSCRFPEEEE GGCEL (SEQ ID NO:152). In some cases, where a T-Cell-MMP of the present disclosure comprises a variant 4-1BBL polypeptide, a Co-MOD is a 4-1BB polypeptide comprising the amino acid sequence of SEQ ID NO:152.

[00304] 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:148-151. 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% 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 binding affinity of a 4-1BBL polypeptide comprising the amino acid sequence set forth in one of SEQ ID NOs:148-151 for a 4-1BB polypeptide (e.g., a 4-1BB polypeptide comprising the amino acid sequence set forth in SEQ ID NO:152), when assayed under the same conditions.

[00305] In some cases, a variant 4-1BBL polypeptide has a binding affinity to 4-1BB that is from 100 nM to 100 μ M. As another example, in some cases, a variant 4-1BBL polypeptide has a binding affinity for 4-1BB (e.g., a 4-1BB polypeptide comprising the amino acid sequence set forth in SEQ ID NO:152) 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.

[00306] In some cases, a variant 4-1BBL polypeptide has a single amino acid substitution compared to the 4-1BBL amino acid sequence set forth in one of SEQ ID NOs:148-151. In some cases, a variant 4-1BBL polypeptide has from 2 to 10 amino acid substitutions compared to the 4-1BBL amino acid sequence set forth in one of SEQ ID NOs:148-151. In some cases, a variant 4-1BBL polypeptide has 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions compared to the 4-1BBL amino acid sequence set forth in one of SEQ ID NOs:148-151.

[00307] Suitable 4-1BBL 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 amino acid sequences that follow.

[00308] PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL TGGLSYXEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:153), where X is any amino acid other than Lys. In some cases, X is Ala.

[00309] PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWXLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:154), where X is any amino acid other than Gln. In some cases, X is Ala.

[00310] PAGLLDLRQG XFAQLVAQNV LLIDGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS

EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:155), where X is any amino acid other than Met. In some cases, X is Ala.

[00311] PAGLLDLRQG MXXAQLVAQNV LLIDGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:156), where X is any amino acid other than Phe. In some cases, X is Ala.

[00312] PAGLLDLRQG MFAXLVAQNV LLIDGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:157), where X is any amino acid other than Gln. In some cases, X is Ala.

[00313] PAGLLDLRQG MFAQXVAQNV LLIDGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:158), where X is any amino acid other than Leu. In some cases, X is Ala.

[00314] PAGLLDLRQG MFAQLXAQNV LLIDGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:159), where X is any amino acid other than Val. In some cases, X is Ala.

[00315] PAGLLDLRQG MFAQLVAXNV LLIDGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:160), where X is any amino acid other than Gln. In some cases, X is Ala.

[00316] PAGLLDLRQG MFAQLVAQXV LLIDGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:161), where X is any amino acid other than Asn. In some cases, X is Ala.

[00317] PAGLLDLRQG MFAQLVAQNX LLIDGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:162), where X is any amino acid other than Val. In some cases, X is Ala.

[00318] PAGLLDLRQG MFAQLVAQNV XLIDGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:163), where X is any amino acid other than Leu. In some cases, X is Ala.

[00319] PAGLLDLRQG MFAQLVAQNV LXIDGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:164), where X is any amino acid other than Leu. In some cases, X is Ala.

[00320] PAGLLDLRQG MFAQLVAQNV LLXDGPLSWY SDPGLAGVSL TGGLSYKEDT
 KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
 EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
 PRSE (SEQ ID NO:165), where X is any amino acid other than Ile. In some cases, X is Ala.

[00321] PAGLLDLRQG MFAQLVAQNV LLIXGPLSWY SDPGLAGVSL TGGLSYKEDT
 KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
 EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
 PRSE (SEQ ID NO:166), where X is any amino acid other than Asp. In some cases, X is Ala.

[00322] PAGLLDLRQG MFAQLVAQNV LLIDXPLSWY SDPGLAGVSL TGGLSYKEDT
 KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
 EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
 PRSE (SEQ ID NO:167), where X is any amino acid other than Gly. In some cases, X is Ala.

[00323] PAGLLDLRQG MFAQLVAQNV LLIGGXLSWY SDPGLAGVSL TGGLSYKEDT
 KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
 EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
 PRSE (SEQ ID NO:168), where X is any amino acid other than Pro. In some cases, X is Ala.

[00324] PAGLLDLRQG MFAQLVAQNV LLIGGPXSWY SDPGLAGVSL TGGLSYKEDT
 KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
 EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
 PRSE (SEQ ID NO:169), where X is any amino acid other than Leu. In some cases, X is Ala.

[00325] PAGLLDLRQG MFAQLVAQNV LLIGGPLXWY SDPGLAGVSL TGGLSYKEDT
 KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
 EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
 PRSE (SEQ ID NO:170), where X is any amino acid other than Ser. In some cases, X is Ala.

[00326] PAGLLDLRQG MFAQLVAQNV LLIGGPLSXY SDPGLAGVSL TGGLSYKEDT
 KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
 EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
 PRSE (SEQ ID NO:171), where X is any amino acid other than Trp. In some cases, X is Ala.

[00327] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWX SDPGLAGVSL TGGLSYKEDT
 KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
 EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
 PRSE (SEQ ID NO:172), where X is any amino acid other than Tyr. In some cases, X is Ala.

[00328] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY XDPGLAGVSL TGGLSYKEDT
 KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
 EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
 PRSE (SEQ ID NO:173), where X is any amino acid other than Ser. In some cases, X is Ala.

[00329] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SXPGLAGVSL TGGLSYKEDT
 KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS

EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:174), where X is any amino acid other than Asp. In some cases, X is Ala.

[00330] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDXGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:175), where X is any amino acid other than Pro. In some cases, X is Ala.

[00331] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPXLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:176), where X is any amino acid other than Gly. In some cases, X is Ala.

[00332] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGXAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:177), where X is any amino acid other than Leu. In some cases, X is Ala.

[00333] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAXVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:178), where X is any amino acid other than Gly. In some cases, X is Ala.

[00334] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGXSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:179), where X is any amino acid other than Val. In some cases, X is Ala.

[00335] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVXL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:180), where X is any amino acid other than Ser. In some cases, X is Ala.

[00336] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSX TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:181), where X is any amino acid other than Leu. In some cases, X is Ala.

[00337] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL XGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:182), where X is any amino acid other than Thr. In some cases, X is Ala.

[00338] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TXGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:183), where X is any amino acid other than Gly. In some cases, X is Ala.

[00339] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGXLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:184), where X is any amino acid other than Gly. In some cases, X is Ala.

[00340] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGXSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:185), where X is any amino acid other than Leu. In some cases, X is Ala.

[00341] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLXYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:186), where X is any amino acid other than Ser. In some cases, X is Ala.

[00342] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSXKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:187), where X is any amino acid other than Tyr. In some cases, X is Ala.

[00343] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKXDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:188), where X is any amino acid other than Glu. In some cases, X is Ala.

[00344] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEXT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:189), where X is any amino acid other than Asp. In some cases, X is Ala.

[00345] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDX
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:190), where X is any amino acid other than Thr. In some cases, X is Ala.

[00346] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
XELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:191), where X is any amino acid other than Lys. In some cases, X is Ala.

[00347] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KXLVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:192), where X is any amino acid other than Glu. In some cases, X is Ala.

[00348] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVXFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS

EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:193), where X is any amino acid other than Phe. In some cases, X is Ala.

[00349] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFXQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:194), where X is any amino acid other than Phe. In some cases, X is Ala.

[00350] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFXLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:195), where X is any amino acid other than Gln. In some cases, X is Ala.

[00351] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQXELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:196), where X is any amino acid other than Leu. In some cases, X is Ala.

[00352] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLXLR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:197), where X is any amino acid other than Glu. In some cases, X is Ala.

[00353] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLEXR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:198), where X is any amino acid other than Leu. In some cases, X is Ala.

[00354] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELX RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:199), where X is any amino acid other than Arg. In some cases, X is Ala.

[00355] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR XVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:200), where X is any amino acid other than Arg. In some cases, X is Ala.

[00356] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RXVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:201), where X is any amino acid other than Val. In some cases, X is Ala.

[00357] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVXAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:202), where X is any amino acid other than Val. In some cases, X is Ala.

[00358] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAXEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:203), where X is any amino acid other than Gly. In some cases, X is Ala.

[00359] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGXGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:204), where X is any amino acid other than Glu. In some cases, X is Ala.

[00360] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEXSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:205), where X is any amino acid other than Gly. In some cases, X is Ala.

[00361] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGXGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:206), where X is any amino acid other than Ser. In some cases, X is Ala.

[00362] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVXLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:207), where X is any amino acid other than Asp. In some cases, X is Ala.

[00363] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDXPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:208), where X is any amino acid other than Leu. In some cases, X is Ala.

[00364] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLXPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:209), where X is any amino acid other than Pro. In some cases, X is Ala.

[00365] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPAXS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:210), where X is any amino acid other than Ser. In some cases, X is Ala.

[00366] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASX
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:211), where X is any amino acid other than Ser. In some cases, X is Ala.

[00367] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS

XARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:212), where X is any amino acid other than Glu. In some cases, X is Ala.

[00368] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EAXNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:213), where X is any amino acid other than Arg. In some cases, X is Ala.

[00369] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARXSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:214), where X is any amino acid other than Asn. In some cases, X is Ala.

[00370] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNXAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:215), where X is any amino acid other than Ser. In some cases, X is Ala.

[00371] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARN SAXGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:216), where X is any amino acid other than Phe. In some cases, X is Ala.

[00372] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARN SAFGFQ GRLLHLSAGX RLGVLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:217), where X is any amino acid other than Gln. In some cases, X is Ala.

[00373] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARN SAFGFQ GRLLHLSAGQ XLGVHLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:218), where X is any amino acid other than Arg. In some cases, X is Ala.

[00374] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARN SAFGFQ GRLLHLSAGQ RXGVHLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:219), where X is any amino acid other than Leu. In some cases, X is Ala.

[00375] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARN SAFGFQ GRLLHLSAGQ RLXVHLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:220), where X is any amino acid other than Gly. In some cases, X is Ala.

[00376] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARN SAFGFQ GRLLHLSAGQ RLGXHLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:221), where X is any amino acid other than Val. In some cases, X is Ala.

[00377] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVXLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:222), where X is any amino acid other than His. In some cases, X is Ala.

[00378] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVHXHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:223), where X is any amino acid other than Leu. In some cases, X is Ala.

[00379] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVHLXTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:224), where X is any amino acid other than His. In some cases, X is Ala.

[00380] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVHLHXEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:225), where X is any amino acid other than Thr. In some cases, X is Ala.

[00381] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVHLHTXA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:226), where X is any amino acid other than Glu. In some cases, X is Ala.

[00382] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA XARHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:227), where X is any amino acid other than Arg. In some cases, X is Ala.

[00383] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RAXHAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:228), where X is any amino acid other than Arg. In some cases, X is Ala.

[00384] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARXAWQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:229), where X is any amino acid other than His. In some cases, X is Ala.

[00385] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAXQLTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:230), where X is any amino acid other than Trp. In some cases, X is Ala.

[00386] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS

EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQXTQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:231), where X is any amino acid other than Leu. In some cases, X is Ala.

[00387] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLXQ GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:232), where X is any amino acid other than Thr. In some cases, X is Ala.

[00388] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTX GATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:233), where X is any amino acid other than Gln. In some cases, X is Ala.

[00389] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ XATVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:234), where X is any amino acid other than Gly. In some cases, X is Ala.

[00390] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GAXVLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:235), where X is any amino acid other than Thr. In some cases, X is Ala.

[00391] PAGLLDLRQG MFAQLVAQNV LLIGGPLSWY SDPGLAGVSL TGGLSYKEDT
KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS
EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATXLGLFRV TPEIPAGLPS
PRSE (SEQ ID NO:236), where X is any amino acid other than Val. In some cases, X is Ala.

e. IL-2 variants

[00392] In some cases, a variant MOD polypeptide present in a T-Cell-MMP 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 .

[00393] A wild-type IL-2 amino acid sequence can be as follows: APTSSSTKKT QLQLEEHLLLD LQMILNGINN YKNPKLTRML TFKFYMPKKA TELKHLQCLEEELKPLEEVN LAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNRWITFCQSIIS TLT (UniProt, P60568, SEQ ID NO:237).

[00394] 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.

[00395] Human IL-2R α : ELCDDDPPE IPHATFKAMA YKEGTMLNCE CKRGFRRIKS
GSLYMLCTGN SSHSSWDNQC QCTSSATRNT TKQVTPQPEE QKERKTTEMQ SPMQPVDAQS
LPGHCREPPP WENEATERIY HFVVGQMVYYY QCVQGYRALH RGPASVCKM THGKTRWTQP

QLICTGEMET SQFPGEEKPQ ASPEGRPESE TSCLVTTTDF QIQTEMAATM ETSIFTTEYQ
VAVAGCVFLL ISVLLLSGLT WQRRQRKSRR TI (SEQ ID NO:238).

[00396] Human IL-2R β : VNG TSQFTCFYNS RANISCVWSQ DGALQDTSCQ VHAWPDRRRW
NQTCELLPVS QASWACNLIL GAPDSQKLTT VDIVTLRVLC REGVRWRVMA IQDFKPFENL
RLMAPISLQV VHVETHRCNI SWEISQASHY FERHLEFEAR TLSPGHTWEE APLLTLKQKQ
EWICLETLP DTQYEFQVRV KPLQGEFTTW SPWSQPLAFR TKPAALGKDT IPWLGHLLVG
LSGAFGFIL VYLLINCRNT GPWLKKVLKC NTPDPSKFFS QLSSEHGGDV QKWLSSPFFS
SSFSPGGLAP EISPLEVLER DKVTQLLLQQ DKVPEPASLS SNHSLTSCFT NQGYFFFHLP
DALEIEACQV YFTYDPYSEE DPDEGVAGAP TGSSQPLQP LSGEDDAYCT FPSRDDLLLF
SPSLLGGPSP PSTAPGGSGA GEERMPPSLQ ERVPRDWDPO PLGPPTPGVP DLVDFQPPPE
LVLREAGEEV PDAGPREGVS FPWSRPPGQG EFRALNARLP LNTDAYLSLQ ELQGQDPTHL V
(SEQ ID NO:239).

[00397] Human IL-2R γ : LNTTILTP NGNEDTTADF FLTTMPTDSL SVSTLPLPEV QCFVFNVEYM
NCTWNSSEP QPTNLTLYW YKNSDNDKVQ KCSHYLFSEE ITSGCQLQKK EIHL YQTFVV
QLQDPREPRR QATQMLKLQN LVIPWAPENL TLHKLSESQL ELNWNRRFLN HCLEHLVQYR
TDWDHSWTEQ SVDYRHKFSL PSVDGQKRYT FRVRSRFRNPL CGSAQHWSEW SHPIHWGSNT
SKENPFLFAL EAVVISVGSM GLIISLLCVY FWLERTMPRI PTLKNLEDLV TEYHGNFSAW
SGVSKGLAES LQPDYSERLC LVSEIPPKGG ALGEGPGASP CNQHSPYWAP PCYTLPKPET (SEQ
ID NO:240).

[00398] In some cases, where a T-Cell-MMP of the present disclosure comprises a variant IL-2 polypeptide, a Co-MOD is an IL-2R comprising polypeptides comprising the amino acid sequences of SEQ ID NO:238, 239, and 240.

[00399] In some cases, a variant IL-2 polypeptide exhibits reduced binding affinity to IL-2R, compared to the binding affinity of an IL-2 polypeptide comprising the amino acid sequence set forth in SEQ ID NO:237. 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% 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 binding affinity of an IL-2 polypeptide comprising the amino acid sequence set forth in SEQ ID NO:237 for an IL-2R (e.g., an IL-2R comprising polypeptides comprising the amino acid sequences set forth in SEQ ID NOs: 238, 239, and 240), when assayed under the same conditions.

[00400] 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 sequences set forth in SEQ ID NOs: 238, 239, and 240) 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.

[00401] 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:237. 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:237. In some cases, a variant IL-2 polypeptide has 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions compared to the IL-2 amino acid sequence set forth in SEQ ID NO:237.

[00402] Suitable IL-2 variant MOD polypeptides 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 amino acid sequences that follow.

[00403] APTSSSTKKT QLQLEHLLLD LQMILNGINN YKNPKLTRML TXKFYMPKKA
TELKHLQCLE EELKPLEEVN LAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFCQSIIS TLT (SEQ ID NO:241), 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.

[00404] APTSSSTKKT QLQLEHLLLD XLQMILNGINN YKNPKLTRML TFKFYMPKKA
TELKHLQCLE EELKPLEEVN LAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFCQSIIS TLT (SEQ ID NO:242), where X is any amino acid other than Asp. In some cases, X is Ala.

[00405] APTSSSTKKT QLQLXHLLLD LQMILNGINN YKNPKLTRML TFKFYMPKKA
TELKHLQCLE EELKPLEEVN LAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFCQSIIS TLT (SEQ ID NO:243), where X is any amino acid other than Glu. In some cases, X is Ala.

[00406] APTSSSTKKT QLQLEXLLLD LQMILNGINN YKNPKLTRML TFKFYMPKKA
TELKHLQCLE EELKPLEEVN LAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFCQSIIS TLT (SEQ ID NO:244), 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.

[00407] APTSSSTKKT QLQLEXLLLD LQMILNGINN YKNPKLTRML TFKFYMPKKA
TELKHLQCLE EELKPLEEVN LAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFCQSIIS TLT (SEQ ID NO:245), where X is any amino acid other than His. In some cases, X is Ala, Asn, Arg, Asp, Cys, Glu, Gln, Gly, Ile, Lys, Leu, Met, Phe, Pro, Ser, Thr, Tyr, Trp, or Val.

[00408] APTSSSTKKT QLQLEHLLLD LQMILNGINN YKNPKLTRML TFKFXMPKKA
TELKHLQCLE EELKPLEEVN NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFCQSIIS TLT (SEQ ID NO:246), where X is any amino acid other than Tyr. In some cases, X is Ala.

[00409] APTSSSTKKT QLQLEHLLLD LQMILNGINN YKNPKLTRML TFKFYMPKKA
TELKHLQCLE EELKPLEEVN NLAQSKNFHL RPRDLISXIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFCQSIIS TLT (SEQ ID NO:247), where X (N88) is any amino acid other than Asn. In some cases, X is Ala; in some cases, X is Arg.

[00410] APTSSSTKKT QLQLEHLLLD LQMILNGINN YKNPKLTRML TFKFYMPKKA
TELKHLQCLE EELKPLEEVN NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFCXSIIS TLT (SEQ ID NO:248), where X is any amino acid other than Gln. In some cases, X is Ala.

[00411] APTSSSTKKT QLQLEX₁LLLD LQMILNGINN YKNPKLTRML TX₂KFYMPKKA
TELKHLQCLE EELKPLEEVN NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFCQSIIS TLT (SEQ ID NO:249), 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.

[00412] APTSSSTKKT QLQLEX1LLLD LQMILNGINN YKNPKLTRML TX2KFYMPKKA
TELKHLQCLE EELKPLEEVN NLAQSKNFHL RPRDLISRIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFCQSIIS TLT (SEQ ID NO:250), which comprises an additional N88R substitution, and where X1 (H16) is any amino acid other than His, and where X2 (F42) 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. In some cases, X1 is Ala; and X2 is Thr. In some cases, X1 is Thr; and X2 is Thr.

[00413] APTSSSTKKT QLQLEHLLLX₁ LQMILNGINN YKNPKLTRML TX₂KFYMPKKA
TELKHLQCLE EELKPLEEVN NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFCQSIIS TLT (SEQ ID NO:251), 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.

[00414] APTSSSTKKT QLQLX₁HLLLX₂ LQMILNGINN YKNPKLTRML TX₃KFYMPKKA
TELKHLQCLE EELKPLEEVN NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFCQSIIS TLT (SEQ ID NO:252), 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.

[00415] APTSSSTKKT QLQLEX₁LLLX₂ LQMILNGINN YKNPKLTRML TX₃KFYMPKKA
TELKHLQCLE EELKPLEEVN NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFCQSIIS TLT (SEQ ID NO:253), 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.

[00416] APTSSSTKKT QLQLEHLLLX₁ LQMILNGINN YKNPKLTRML TX₂KFYMPKKA
TELKHLQCLE EELKPLEEV L NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFCX₃SIIS TLT (SEQ ID NO:254), 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.

[00417] APTSSSTKKT QLQLEHLLLX₁ LQMILNGINN YKNPKLTRML TX₂KFX₃MPKKA
TELKHLQCLE EELKPLEEV L NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFCQSIIS TLT (SEQ ID NO:255), 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.

[00418] APTSSSTKKT QLQLEX₁LLLX₂ LQMILNGINN YKNPKLTRML TX₃KFX₄MPKKA
TELKHLQCLE EELKPLEEV L NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFCQSIIS TLT (SEQ ID NO:256), 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.

[00419] APTSSSTKKT QLQLEHLLLX₁ LQMILNGINN YKNPKLTRML TX₂KFX₃MPKKA
TELKHLQCLE EELKPLEEV L NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFCX₄SIIS TLT (SEQ ID NO:257), 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.

[00420] APTSSSTKKT QLQLEX₁LLLX₂ LQMILNGINN YKNPKLTRML TX₃KFX₄MPKKA
TELKHLQCLE EELKPLEEV L NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFCX₅SIIS TLT (SEQ ID NO:258), 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.

[00421] APTSSSTKKT QLQLEX₁LLLD LQMILNGINN YKNPKLTRML TX₂KFYMPKKA
TELKHLQCLE EELKPLEEV L NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE
TATIVEFLNR WITFCX₃SIIS TLT (SEQ ID NO:259), 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.

[00422] In any of the wild-type or variant IL-2 sequences provided herein, the cysteine at position 125 may be substituted with an alanine (a C125A substitution). In addition to any stability provided by the substitution, it may be employed where, for example, an epitope containing peptide or payload is to be conjugated to a cysteine residue elsewhere in a T-Cell-MMP first or second polypeptide, thereby avoiding competition from the C125 of the IL-2 MOD sequence.

9. Additional polypeptides

[00423] A polypeptide chain of a T-Cell-MMP or its epitope conjugate 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(s) can be included as part of a polypeptide translated by cell or cell free system at the N-terminus of a polypeptide chain of a multimeric polypeptide, at the C-terminus of a polypeptide chain of a multimeric polypeptide, or internally within a polypeptide chain of a multimeric polypeptide.

10. Epitope Tags

[00424] Suitable epitope tags include, but are not limited to, hemagglutinin (HA; e.g., YPYDVPDYA (SEQ ID NO:260)); FLAG (e.g., DYKDDDDK (SEQ ID NO:261)); c-myc (e.g., EQKLISEEDL; SEQ ID NO:262)), and the like.

11. Affinity domain

[00425] 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 include His5 (HHHHH) (SEQ ID NO:263), HisX6 (HHHHHH) (SEQ ID NO:264), C-myc (EQKLISEEDL) (SEQ ID NO:26533), Flag (DYKDDDDK) (SEQ ID NO:266), StrepTag (WSHPQFEK) (SEQ ID NO:267), hemagglutinin, (e.g., HA Tag (YPYDVPDYA) (SEQ ID NO:268)), glutathione-S-transferase (GST), thioredoxin, cellulose binding domain, RYIRS (SEQ ID NO:269), Phe-His-His-Thr (SEQ ID NO:270), chitin binding domain, S-peptide, T7 peptide, SH2 domain, C-end RNA tag, WEAAAREACCRECCARA (SEQ ID NO:271), metal binding domains, e.g., zinc binding domains or calcium binding domains such as those from calcium-binding proteins, e.g., calmodulin, troponin C, calcineurin B, myosin light chain, recoverin, S-modulin, visinin, VILIP, neurocalcin, hippocalcin, frequenin, caltractin, calpain large-subunit, S100 proteins, parvalbumin, calbindin D9K, calbindin D28K, and calretinin, inteins, biotin, streptavidin, MyoD, Id, leucine zipper sequences, and maltose binding protein.

12. Epitopes

[00426] The chemical conjugation sites and chemistries described herein permit the incorporation of WT-1 peptides (e.g., phosphopeptide, lipopeptides or glycopeptide) into a T-Cell-MMP to form a T-

Cell-MMP-epitope conjugate. Epitopes peptides of a T-Cell-MMP conjugate are not part of the first or second polypeptide as translated from mRNA, but are added to a T-Cell-MMP at a chemical conjugation site. Selection of candidate MHC allele and (e.g., phosphopeptide, lipopeptides or glycopeptide) epitope combinations for effective presentation to a TCR by a T-Cell-MMP-epitope conjugate, can be accomplished using any of a number of well-known methods to determine if the free peptide has affinity for the specific HLA allele used to construct the T-Cell-MMP in which it will be presented as part of the epitope conjugate, and to determine if the peptide in combination with the specific heavy chain allele and β can affect the T-cell in the desired manner (e.g., induction of proliferation, anergy or apoptosis). Applicable methods include binding assays and T-cell activation assays.

a. Cell-based binding assays

[00427] As one example, cell-based peptide-induced stabilization assays can be used to determine if a candidate peptide binds an HLA class I allele intended for use in a T-Cell-MMP-epitope conjugate. The binding assay can be used in the selection of peptides for incorporation into a T-Cell-MMP-epitope conjugate using the intended allele. 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, or directly where the peptide is fluorescently labeled. The stabilization and resultant increased life-span of peptide-HLA complexes on the cell surface by the addition of a peptide validates their identity. Accordingly, binding of candidate peptides for presentation by various Class I HLA heavy chain alleles can be tested by genetically modifying the T2 cells to express the HLA H allele of interest.

[00428] In 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 suspended at 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, and a non-HLA A*0201-restricted peptide, HPVGEADYF (HLA-B*3501), are included as positive and negative controls, respectively. The cell/peptide mixtures are kept at 37°C in 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.

b. Biochemical binding assays

[00429] MHC Class I complexes comprising a β 2M polypeptide complexed with an HLA heavy chain polypeptide of a specific allele intended for use in construction of a T-Cell-MMP 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 the MHC-class I complex to form an MHC-reference peptide complex. The ability of a test peptide of interest to displace the labeled reference peptide from the 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.

[00430] As another example, a peptide of interest can be incubated with a MHC Class I complex (containing an HLA heavy chain peptide and β 2M) and the stabilization of the MHC complex by bound peptide can be measured in an immunoassay format. The ability of a peptide of interest to stabilize the MHC complex 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 MHC complex bound to the peptide 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.

c. T-cell activation assays

[00431] Whether a given peptide binds a MHC Class I complex (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.

(i) ELISPOT assays

[00432] Suitable assays include, e.g., an enzyme linked immunospot (ELISPOT) assay where production of a product by target cells (e.g., IFN γ production by target CD8⁺ T) is measured following contact of the target with an antigen-presenting cell (APC) that presents a peptide of interest complexed with a class I MHC (e.g., HLA). Antibody to IFN γ is immobilized on wells of a multi-well plate. APCs are added to the wells, and the plates are 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 heavy 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.

(ii) Cytotoxicity assays

[00433] Whether a given peptide binds to a particular MHC class I heavy chain allele complexed with $\beta 2M$ and, when bound, 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 MHC class I complex comprising $\beta 2M$, and an epitope-peptide and MHC heavy chain allele combination to be tested. The target cells can be radioactively labeled, e.g., with ^{51}Cr . Whether the target cell effectively presents the 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 ^{51}Cr 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.

(iii) Detection of Antigen-specific T cells with peptide-HLA tetramers

[00434] As another example, multimers (e.g., tetramers) of peptide-MHC 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.

d. Peptides Presenting WT-1 Epitopes

[00435] In some cases, an epitope (a peptide presenting one or more epitopes) present in a T-Cell-MMP-epitope conjugate of the present disclosure is a WT-1 peptide (e.g., a WT-1 peptide that, together with a MHC, presents an epitope to a TCR). Amino acid sequences of WT-1 isoforms are presented in FIGs. 11A-11E. A portion of a WT-1 protein 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 peptide presenting an epitope (or the epitope presenting portion of the peptide) present in a T-Cell-MMP-epitope conjugate of the present disclosure can be a peptide of from 4 to 25 contiguous amino acids (aa) (e.g., 4 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 FIGs. 11A-11E. In some cases, a WT-1 epitope present in a T-Cell-MMP-epitope conjugate of the present disclosure can be a peptide of from 4 to 25 contiguous aas (e.g., 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. 11A. In some cases, a WT-1 epitope present in a T-Cell-MMP-epitope conjugate of the present disclosure can be a peptide of from 4 to 25 contiguous aas (e.g., 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. 11B. In some cases, a WT-1 epitope present in a T-Cell-MMP-epitope conjugate of the present disclosure can be a peptide of from 4 to 25 contiguous aas (e.g., 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. 11C. In some cases, a WT-1 epitope present in a T-Cell-MMP-epitope conjugate of the present disclosure can be a peptide of from 6 to 25 contiguous aas (e.g., 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. 11D. In some cases, a WT-1 epitope present in a T-Cell-MMP-epitope conjugate of the present disclosure can be a peptide of from 4 to 25 contiguous aas (e.g., 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. 11E. Peptide epitopes from post-translational modified polypeptides/proteins may also serve as epitopes, including phosphopeptides, glycopeptides and lipopeptides (e.g., peptides modified with fatty acids, isoprenoids, sterols, phospholipids, or glycosylphosphatidyl inositol).

[00436] In some cases, the epitope peptide present in a T-Cell-MMP-epitope conjugate of the present disclosure presents an epitope specific to an HLA-A, -B, -C, -E, -F or -G allele. In an embodiment, the epitope peptide present in a T-Cell-MMP-epitope conjugate presents an epitope restricted to HLA-A*0101, A*0201, A*0301, A*1101, A*2301, A*2402, A*2407, A*3303, and/or A*3401. In an embodiment, the epitope peptide present in a T-Cell-MMP-epitope conjugate presents an epitope restricted to HLA-B*0702, B*0801, B*1502, B*3802, B*4001, B*4601, and/or B*5301. In an embodiment, the epitope peptide present in a T-Cell-MMP-epitope conjugate presents an epitope restricted to C*0102, C*0303, C*0304, C*0401, C*0602, C*0701, C*702, C*0801, and/or C*1502.

[00437] An epitope (or the epitope presenting sequence of the peptide) present in a T-Cell-MMP-epitope conjugate can be a peptide of from 4 to 25 contiguous aas (e.g., 4 aa, 5 aa, 6 aa, 7 aa, 8 aa, 9 aa, 10 aa, 11aa, 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, or from 7 to 25 aa, from 7 to 12, from 7 to 25, from 10 aa to 15 aa, from 15 aa to 20 aa, or from 20 aa to 25 aa).

[00438] In an embodiment, an epitope presenting peptide present in a T-Cell-MMP-epitope conjugate of the present disclosure can have a length of from about 4 aas (aa) to about 25aa, e.g., the epitope can have a length of from 4 aa to 10 aa, from 7 to 12, from 7 to 25, from 10 aa to 15 aa, from 15 aa to 20 aa, or from 20 aa to 25 aa.

[00439] In an embodiment, a WT-1 epitope present in a T-Cell-MMP-epitope conjugate of the present disclosure 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.

[00440] Examples of WT-1 peptides suitable for inclusion in a T-Cell-MMP of the present disclosure include, but are not limited to, CMTWNQMNLGATLKG (SEQ ID NO:272), WNQMNLGATLKGVAA (SEQ ID NO:273), CMTWNYMNLGATLKG (SEQ ID NO:274), WNYMNLGATLKGVAA (SEQ ID NO: 275), MTWNQMNLGATLKGV (SEQ ID NO:276), TWNQMNLGATLKGVA (SEQ ID NO:277), CMTWNLMNLGATLKG (SEQ ID NO:279), MTWNLMNLGATLKGV (SEQ ID NO:280), TWNLMNLGATLKGVA (SEQ ID NO:281), WNLNMNLGATLKGVAA (SEQ ID NO:282), MNLGATLK (SEQ ID NO:283), MTWNYMNLGATLKGV (SEQ ID NO:284), TWNYMNLGATLKGVA (SEQ ID NO:285), CMTWNQMNLGATLKGVA (SEQ ID NO:286), CMTWNLMNLGATLKGVA (SEQ ID NO:287), CMTWNYMNLGATLKGVA (SEQ ID NO:288), GYLRNPTAC (SEQ ID NO:289), GALRNPTAL (SEQ ID NO:290), YALRNPTAC (SEQ ID NO:291), GLLRNPTAC (SEQ ID NO:292), NQMNLGATL (SEQ ID NO:293), RYRPHPGAL (SEQ ID NO:294), YQRPHPGAL (SEQ ID NO:295), RLRPHPGAL (SEQ ID NO:296), RIRPHPGAL (SEQ ID NO:297), QFPNHSFKHEDPMGQ (SEQ ID NO:298), HSFKHEDPY (SEQ ID NO:299), QFPNHSFKHEDPM (SEQ ID NO:300), QFPNHSFKHEDPY (SEQ ID NO:301), KRPFMCAYPGCK (SEQ ID NO:302), KRPFMCAYPGCKY (SEQ ID NO:303), FMCAYPGCKY (SEQ ID NO:304), FMCAYPGCK (SEQ ID NO:305), KRPFMCAYPGCKRY (SEQ ID NO:306), SEKRPFMCAYPGCK (SEQ ID NO:307), KRPFMCAYPGCKRY (SEQ ID NO:308), NLMNLGATL (SEQ ID NO:309), VLDFAPPGA (SEQ ID NO:310); RMFPNAPYL (SEQ ID NO:311); CMTWNQMN (SEQ ID NO:312); CYTWNQMNL (SEQ ID NO:313); NYMNLGATL (SEQ ID NO:314); YMFPNAPYL (SEQ ID NO:315); SLGEQQYSV (SEQ ID NO:316); and CMTWNQMNL (SEQ ID NO:317). In some cases, the WT-1 peptide present in a T-Cell-MMP-epitope conjugate of the present disclosure is CMTWNQMN (SEQ ID NO:312). In some cases, the WT-1 peptide present in a T-Cell-MMP-epitope conjugate of the present disclosure is CYTWNQMNL (SEQ ID NO:313).

[00441] In some cases, the WT-1 peptide present in a T-Cell-MMP-epitope conjugate of the present disclosure is CMTWNQMN (SEQ ID NO:312); NYMNLGATL (SEQ ID NO:314) (WT-1 239-247; Q240Y); CYTWNQMNL (SEQ ID NO:313) (WT-1 235-243); CMTWNQMNL (SEQ ID NO:317) (WT-1 235-243); NQMNLGATL (SEQ ID NO:293 (WT-1 239-247); NYMNLGATL (SEQ ID NO:314) (WT-1 239-247; Q240L).

[00442] In some cases, the WT-1 peptide present in a T-Cell-MMP-epitope conjugate of the present disclosure is CYTWNQMNL (SEQ ID NO:312).

[00443] In some cases, the WT-1 peptide present in a T-Cell-MMP-epitope conjugate of the present disclosure presents an HLA-A*2402-restricted epitope. WT-1 peptides that present an HLA-A*2402-restricted epitope include, e.g., NQMNLGATL (SEQ ID NO:293) (WT-1 239-247); CMTWNQMN (SEQ ID NO:312); NYMNLGATL (SEQ ID NO:314) (WT-1 239-247; Q240Y); CYTWNQMNL (SEQ ID NO:313) (WT-1 235-243); NLMNLGATL (SEQ ID NO:314) (WT-1 239-247; Q240L); and CMTWNQMNL (SEQ ID NO:317) (WT-1 235-243).

[00444] In some cases, the WT-1 peptide present in a T-Cell-MMP-epitope conjugate of the present disclosure presents an HLA-A*0201-restricted epitope. WT-1 peptides that present an HLA-A*0201-

restricted epitope include, e.g., NLMNLGATL (SEQ ID NO:309) (WT-1 239-247; Q240L); VLDFAPPGA (SEQ ID NO:310) (WT-1 37-45); RMFPNAPYL (SEQ ID NO:311) (WT-1 126-134); YMFNPAPYL (SEQ ID NO:315) (WT-1 126-134; R126Y); and SLGEQQYSV (SEQ ID NO:316) (WT-1 187-195).

13. Payloads

[00445] A broad variety of payloads may be associated with T-Cell-MMPs and T-Cell-MMP-epitope conjugates, which may incorporate more than one type of payload in addition to epitopes conjugated (covalently) to the T-Cell-MMPs at a first or second chemical conjugation site. In addition, where the T-Cell-MMP molecules or their epitope conjugates multimerize, it may be possible to incorporate monomers labeled with different payloads into a multimer. Accordingly, it is possible to introduce one or more payloads selected, for example, from the group consisting of: therapeutic agents, chemotherapeutic agents, diagnostic agents, labels and the like. It will be apparent that some payloads may fall into more than one category (e.g., a radio label may be useful as a diagnostic and as a therapeutic for selectively irradiating specific tissue or cell type).

[00446] As noted above, T-Cell-MMP polypeptides (e.g., a scaffold or Fc polypeptide) can be modified with crosslinking reagents to conjugate payloads and/or epitopes to chemical conjugation sites attached to or in the first or second polypeptide of the T-Cell-MMPs (e.g., at a chemical conjugation site such as an engineered cysteine or lysine). Such crosslinking agents include succinimidyl 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (SMCC), sulfo-SMCC, maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), sulfo-MBS or succinimidyl-iodoacetate. Introducing payloads using an excess of such crosslinking agents can result in multiple molecules of payload being incorporated into the T-Cell-MMP. Some bifunctional linkers for introducing payloads into T-Cell-MMPs and their epitope conjugates include cleavable linkers and non-cleavable linkers. In some cases, the payload linker is a protease-cleavable linker. Suitable payload linkers include, e.g., peptides (e.g., from 2 to 10 amino acids in length; e.g., 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids in length), alkyl chains, poly(ethylene glycol), disulfide groups, thioether groups, acid labile groups, photolabile groups, peptidase labile groups, and esterase labile groups. Non-limiting examples of suitable linkers are: N-succinimidyl-[(N-maleimidopropionamido)-tetraethyleneglycol]ester (NHS-PEG4-maleimide); N-succinimidyl 4-(2-pyridyldithio)butanoate (SPDB); disuccinimidyl suberate (DSS); disuccinimidyl glutarate (DGS); dimethyl adipimidate (DMA); N-succinimidyl 4-(2-pyridyldithio)2-sulfobutanoate (sulfo-SPDB); κ -maleimidoundecanoic acid N-succinimidyl ester (KMUA); γ -maleimide butyric acid N-succinimidyl ester (GMBS); ϵ -maleimidocaproic acid N-hydroxysuccinimide ester (EMCS); m-maleimide benzoyl-N-hydroxysuccinimide ester (MBS); N-(α -maleimidoacetoxymethyl)-succinimide ester (AMAS); succinimidyl-6-(β -maleimidopropionamide)hexanoate (SMPH); N-succinimidyl 4-(p-maleimidophenyl)butyrate (SMPB); N-(p-maleimidophenyl)isocyanate (PMPD); N-succinimidyl 4(2-pyridylthio)pentanoate (SPP); N-succinimidyl(4-iodo-acetyl)aminobenzoate (SIAB); 6-maleimidocaproyl (MC); maleimidopropanoyl (MP); p-aminobenzyloxycarbonyl (PAB); N-succinimidyl 4-(maleimidomethyl)cyclohexanecarboxylate (SMCC); succinimidyl 3-(2-pyridyldithio)propionate (SPDP); PEG4-SPDP (PEGylated, long-chain

SPDP crosslinker); BS(PEG)₅ (PEGylated bis(sulfosuccinimidyl)suberate); BS(PEG)₉ (PEGylated bis(sulfosuccinimidyl)suberate); maleimide-PEG₆-succinimidyl ester; maleimide-PEG₈-succinimidyl ester; maleimide-PEG₁₂-succinimidyl ester; PEG₄-SPDP (PEGylated, long-chain SPDP crosslinker); PEG₁₂-SPDP (PEGylated, long-chain SPDP crosslinker); N-succinimidyl-4-(N-maleimidomethyl)-cyclohexane-1-carboxy-(6-amidocaproate), a “long chain” analog of SMCC (LC-SMCC); 3-maleimidopropanoic acid N-succinimidyl ester (BMPS); N-succinimidyl iodoacetate (SIA); N-succinimidyl bromoacetate (SBA); and N-succinimidyl 3-(bromoacetamido)propionate (SBAP).

[00447] Control of the stoichiometry of the reaction may result in some selective modification where engineered sites with chemistry orthogonal to all other groups in the molecule is not utilized. Reagents that display far more selectivity, such as the bis-thio linkers discussed above, tend to permit more precise control of the location and stoichiometry than reagents that react with single lysine, or cysteine residues.

[00448] Where a T-Cell-MMP of the present disclosure comprises a Fc polypeptide, the Fc polypeptide can comprise one or more covalently attached molecules of payload that are attached directly or indirectly through a linker. By way of example, where a T-Cell-MMP of the present disclosure comprises a Fc polypeptide, the polypeptide chain comprising the Fc polypeptide can be of the formula (A)-(L)-(C), where (A) is the polypeptide chain comprising the Fc polypeptide; where (L), if present, is a linker; and where (C) is a payload (e.g., a cytotoxic agent). (L), if present, links (A) to (C). In some cases, the polypeptide chain comprising the Fc polypeptide can comprise more than one molecule of payload (e.g., 2, 3, 4, 5, or more than 5 cytotoxic agent molecules).

[00449] In an embodiment, the payload is selected from the group consisting of: biologically active agents or drugs, diagnostic agents or labels, nucleotide or nucleoside analogs, nucleic acids or synthetic nucleic acids (e.g., antisense nucleic acids, small interfering RNA, double stranded (ds)DNA, single stranded (ss)DNA, ssRNA, dsRNA), toxins, liposomes (e.g., incorporating a chemotherapeutic such as 5-fluorodeoxyuridine), nanoparticles (e.g., gold or other metal bearing nucleic acids or other molecules, lipids, particle bearing nucleic acids or other molecules), and combinations thereof.

[00450] In an embodiment, the payload is selected from biologically active agents or drugs selected independently from the group consisting of: therapeutic agents (e.g., drugs or prodrugs), chemotherapeutic agents, cytotoxic agents, antibiotics, antivirals, cell cycle synchronizing agents, ligands for cell surface receptor(s), immunomodulatory agents (e.g., immunosuppressants such as cyclosporine), pro-apoptotic agents, anti-angiogenic agents, cytokines, chemokines, growth factors, proteins or polypeptides, antibodies or antigen binding fragments thereof, enzymes, proenzymes, hormones and combinations thereof.

[00451] In an embodiment, the payload is selected from biologically active agents or drugs selected independently from therapeutic diagnostic agents or labels, selected independently from the group consisting of photodetectable labels (e.g., dyes, fluorescent labels, phosphorescent labels, luminescent labels), contrast agents (e.g., iodine or barium containing materials), radiolabels, imaging agents, paramagnetic labels/imaging agents (gadolinium containing magnetic resonance imaging labels), ultrasound labels and combinations thereof.

a. Therapeutic Agents and Chemotherapeutic Agents

[00452] As discussed above, a polypeptide chain of a T-Cell-MMP or its epitope conjugate can comprise a payload including, but not limited, a small molecule drug, such as a therapeutic or chemotherapeutic agent, linked (e.g., covalently attached) to the first or second polypeptide chain at chemical conjugation sites. The linkage between a payload and a first or second polypeptide chain of a T-Cell-MMP or its epitope conjugate may be a direct or indirect linkage. Direct linkage can involve linkage directly to an amino acid side chain. Indirect linkage can be linkage via a linker. A drug (e.g., a payload such as a cancer chemotherapeutic agent) can be linked to a polypeptide chain (e.g., a Fc polypeptide) of a T-Cell-MMP of the present disclosure via a thioether bond, an amide bond, a carbamate bond, a disulfide bond, or an ether bond.

[00453] Suitable therapeutic agents include, e.g., rapamycin, retinoids, such as all-trans retinoic acid (ATRA); vitamin D3; vitamin D3 analogs; and the like. As noted above, in some cases, a drug is a cytotoxic agent. Cytotoxic agents are known in the art. A suitable cytotoxic agent can be any compound that results in the death of a cell, induces cell death, or in some manner decreases cell viability, and includes, for example, maytansinoids and maytansinoid analogs, benzodiazepines, taxoids, CC-1065 and CC-1065 analogs, duocarmycins and duocarmycin analogs, enediynes, such as calicheamicins, dolastatins and dolastatin analogs including auristatins, tomaymycin derivatives, leptomycin derivatives, methotrexate, cisplatin, carboplatin, daunorubicin, doxorubicin, vincristine, vinblastine, melphalan, mitomycin C, chlorambucil and morpholino doxorubicin.

[00454] For example, in some cases, the cytotoxic agent is a compound that inhibits microtubule formation in eukaryotic cells. Such agents include, e.g., maytansinoid, benzodiazepine, taxoid, CC-1065, duocarmycin, a duocarmycin analog, calicheamicin, dolastatin, a dolastatin analog, auristatin, tomaymycin, and leptomycin, or a pro-drug of any one of the foregoing. Maytansinoid compounds include, e.g., N(2')-deacetyl-N(2')-(3-mercapto-1-oxopropyl)-maytansine (DM1); N(2')-deacetyl-N(2')-(4-mercapto-1-oxopentyl)-maytansine (DM3); and N(2')-deacetyl-N2-(4-mercapto-4-methyl-1-oxopentyl)-maytansine (DM4). Benzodiazepines include, e.g., indolinobenzodiazepines and oxazolidinobenzodiazepines.

[00455] Cytotoxic agents include taxol; cytochalasin B; gramicidin D; ethidium bromide; emetine; mitomycin; etoposide; tenoposide; vincristine; vinblastine; colchicin; doxorubicin; daunorubicin; dihydroxy anthracin dione; maytansine or an analog or derivative thereof; an auristatin or a functional peptide analog or derivative thereof; dolastatin 10 or 15 or an analogue thereof; irinotecan or an analogue thereof; mitoxantrone; mithramycin; actinomycin D; 1-dehydrotestosterone; a glucocorticoid; procaine; tetracaine; lidocaine; propranolol; puromycin; calicheamicin or an analog or derivative thereof; an antimetabolite; 6 mercaptopurine; 6 thioguanine; cytarabine; fludarabin; 5 fluorouracil; decarbazine; hydroxyurea; asparaginase; gemcitabine; cladribine; an alkylating agent; a platinum derivative; duocarmycin A; duocarmycin SA; rachelmycin (CC-1065) or an analog or derivative thereof; an antibiotic; pyrrolo[2,1-c][1,4]-benzodiazepines (PDB); diphtheria toxin; ricin toxin; cholera toxin; a Shiga-like toxin; LT toxin; C3 toxin; Shiga toxin; pertussis toxin; tetanus toxin; soybean Bowman-Birk

protease inhibitor; *Pseudomonas* exotoxin; alorin; saporin; modeccin; gelatin; abrin A chain; modeccin A chain; alpha-sarcin; *Aleurites fordii* proteins; dianthin proteins; *Phytolacca americana* proteins; momordica charantia inhibitor; curcin; crotin; sapaonaria officinalis inhibitor; gelonin; mitogellin; restrictocin; phenomycin; enomycin toxins; ribonuclease (RNase); DNase I; Staphylococcal enterotoxin A; pokeweed antiviral protein; diphtherin toxin; and *Pseudomonas* endotoxin.

b. Diagnostic Agents and Labels

[00456] The first and/or second polypeptide chains of a T-Cell-MMP can comprise one or more molecules of payload of photodetectable labels (e.g., dyes, fluorescent labels, phosphorescent labels, luminescent labels), contrast agents (e.g., iodine or barium containing materials), radiolabels, imaging agents, spin labels, Forster Resonance Energy Transfer (FRET)-type labels, paramagnetic labels/imaging agents (e.g., gadolinium containing magnetic resonance imaging labels), ultrasound labels and combinations thereof.

[00457] In some embodiments, the conjugate moiety comprises a label that is or includes a radioisotope. Examples of radioisotopes or other labels include, but are not limited to, ^3H , ^{11}C , ^{14}C , ^{15}N , ^{35}S , ^{18}F , ^{32}P , ^{33}P , ^{64}Cu , ^{68}Ga , ^{89}Zr , ^{90}Y , ^{99}Tc , ^{123}I , ^{124}I , ^{125}I , ^{131}I , ^{111}In , ^{131}In , ^{153}Sm , ^{186}Re , ^{188}Re , ^{211}At , ^{212}Bi , and ^{213}Pb .

II. Methods of Generating T-cell-MMP Polypeptides

[00458] The present disclosure provides a method of obtaining T-Cell-MMPs and/or T-Cell-MMP-epitope conjugates, including those comprising one or more variant MODs that exhibit lower affinity for a Co-MOD compared to the affinity of the corresponding parental wild-type MOD for the Co-MOD, the method comprising:

A) generating a T-Cell-MMP by introducing nucleic acids encoding a first polypeptide and a second polypeptide of the T-Cell-MMP in cells or cell free systems, wherein:

- a) the first polypeptide comprises: i) a first MHC Class I polypeptide (e.g., a $\beta 2\text{M}$ polypeptide); and
- b) the second polypeptide comprises: i) a second MHC polypeptide (e.g., a MHC Class I heavy chain polypeptide); and ii) optionally an Ig Fc polypeptide or a non-Ig scaffold,

wherein the first polypeptide comprises a first chemical conjugation site and/or the second polypeptide comprises a second chemical conjugation site, and at least one of the first polypeptide or second polypeptide comprises one or more independently selected MODs (e.g., 1, 2, 3 or more wild-type and/or variant MODs); and

B) contacting the first polypeptide and second polypeptide (if co-expressed in the same cell or cell-free system the polypeptides may come into contact as they are translated) to form a T-Cell-MMP;

wherein when the T-Cell-MMP comprises one or more nascent (e.g., unactivated) chemical conjugation sites, the nascent chemical conjugation site may be optionally activated to produce a T-Cell-MMP with the first and/or second chemical conjugation site (e.g., reacting sulfatase motifs with a formyl glycine generating enzyme if the cells expressing the T-Cell-MMP do not express a formylglycine generating enzyme).

The method may be stopped at this point and the T-Cell-MMP obtained by purification; alternatively, where a T-Cell-MMP-epitope conjugate is desired, the method may be continued with the reaction of the T-Cell-MMP with an epitope presenting molecule:

- C) providing an epitope (e.g., an epitope presenting peptide) suitable for conjugation with the first and/or second chemical conjugation site (e.g., a hydrazinyl or hydrazinyl indole modified peptide for reaction with a formyl glycine of a sulfatase motif) and contacting the epitope with the T-Cell-MMP (e.g., under suitable reaction conditions) to produce a T-Cell-MMP-epitope conjugate.

[00459] Where it is desirable for a T-Cell-MMP to contain a payload (e.g., a small molecule drug, radio label, etc.), the payload may be reacted with the T-Cell-MMP in place of the epitope conjugate as described above. Where it is desirable for a T-Cell-MMP-epitope conjugate to contain a payload, the payload may be reacted with the chemical conjugation site(s) either before or after the epitope is contacted and reacted with its chemical reaction site(s). The selectivity of the epitope and the payload for different conjugation sites (e.g., first and second chemical conjugation sites) may be controlled through the use of orthogonal chemistries and/or control of stoichiometry in the conjugation reactions. In embodiments, linkers (e.g., polypeptides or other bifunctional chemical linkers) may be used to attach the epitope and/or payloads to their conjugation sites.

[00460] The present disclosure provides a method of obtaining a T-Cell-MMP and/or T-Cell-MMP-epitope conjugate comprising one or more variant MODs that exhibit lower affinity for a Co-MOD compared to the affinity of the corresponding parental wild-type MOD for the Co-MOD, the method comprising:

- A) generating a library of T-Cell-MMP-epitope conjugates comprising a plurality of members, wherein each member comprises: a) a first polypeptide comprising: i) an epitope; and ii) a first MHC polypeptide (e.g., a β 2M polypeptide); and b) a second polypeptide comprising: i) a second MHC polypeptide (e.g., a MHC Class I heavy chain polypeptide); and ii) optionally an Ig Fc polypeptide or a non-Ig scaffold, wherein each member comprises a different variant MOD on the first polypeptide, the second polypeptide, or both the first and the second polypeptide;
- B) determining the affinity of each member of the library for a Co-MOD; and
- C) selecting a library member that exhibits reduced affinity for the Co-MOD.

In some cases, the affinity is determined by BLI using purified T-Cell-MMP or T-Cell-MMP-epitope conjugate library members and the Co-MOD. BLI methods are well known to those skilled in the art. A BLI assay is described above. See, e.g., Lad et al. (2015) *J. Biomol. Screen.* 20(4): 498-507; and Shah and Duncan (2014) *J. Vis. Exp.* 18:e51383.

[00461] The present disclosure provides a method of obtaining a T-Cell-MMP-epitope conjugate that exhibits selective binding to a T-cell, the method comprising:

- A) generating a library of T-Cell-MMP-epitope conjugates comprising a plurality of members, wherein each member comprises:
 - a) a first polypeptide comprising i) a first MHC polypeptide, and

- b) a second polypeptide comprising i) a second MHC polypeptide, and ii) optionally an immunoglobulin (Ig) Fc polypeptide or a non-Ig scaffold,
- wherein each member comprises a different variant MOD on the first polypeptide, the second polypeptide, or both the first and the second polypeptide, wherein the variant MOD differs in amino acid sequence by from 1 aa to 10 aa from a parental wild-type MOD,
- wherein the T-Cell-MMP-epitope conjugate library members further comprise an epitope tag or a fluorescent label), and
- wherein one of the first or second polypeptides comprises an epitope covalently bound through a chemical conjugation site, either directly or indirectly through a linker, to the first and/or second polypeptide;
- B) contacting a T-Cell-MMP-epitope conjugate library member with a target T-cell expressing on its surface with: i) a Co-MOD that binds the parental wild-type MOD; and ii) a TCR that binds to the epitope;
- C) when the T-Cell-MMP-epitope conjugate comprises an epitope tag, contacting the T-Cell-MMP-epitope conjugate library member bound to the target T-cell with a fluorescently labeled binding agent that binds to the epitope tag (which is unnecessary with a fluorescently labeled T-Cell-MMP-epitope conjugate), generating a library member/target T-cell/binding agent complex;
- D) measuring the mean fluorescence intensity (MFI) of the T-Cell-MMP-epitope conjugate library member/target T-cell/binding agent complex using flow cytometry, wherein the MFI measured over a range of concentrations of the T-Cell-MMP-epitope conjugate library member provides a measure of the affinity and apparent avidity; and
- E) selecting a T-Cell-MMP-epitope conjugate library member that selectively binds the target T-cell, compared to binding of the T-Cell-MMP-epitope conjugate library member to a control T-cell that comprises: i) the Co-MOD that binds the parental wild-type MOD; and ii) a TCR that binds to an epitope other than the epitope present in the T-Cell-MMP library member.

[00462] In some cases, a T-Cell-MMP library member that is identified as selectively binding to a target T-cell is isolated from the library. In some cases, parental wild-type MOD and Co-MOD pairs are selected from: IL-2 and IL-2 receptor; 4-1BBL and 4-1BB; PD-L1 and PD-1; FasL and Fas; TGF- β and TGF- β receptor; CD80 and CD28; CD86 and CD28; OX40L and OX40; ICOS-L and ICOS; ICAM and LFA-1; JAG1 and Notch; JAG1 and CD46; CD70 and CD27; CD80 and CTLA4; and CD86 and CTLA4.

[00463] The present disclosure provides a method of obtaining a T-Cell-MMP-epitope conjugate comprising one or more variant MODs that exhibit reduced affinity for a Co-MOD compared to the affinity of the corresponding parental wild-type MOD for the Co-MOD, the method comprising selecting, from a library of T-Cell-MMP-epitope conjugates comprising a plurality of members, a member that exhibits reduced affinity for the Co-MOD, wherein each of the plurality of members comprises: a) a first polypeptide comprising: i) an epitope covalently bound to a chemical conjugation site; and ii) a first MHC polypeptide; and b) a second polypeptide comprising: i) a second MHC polypeptide; and ii) optionally an Ig Fc polypeptide or a non-Ig scaffold, wherein the members of the library comprise a

plurality of variant MODs present in the first polypeptide, the second polypeptide, or both the first and the second polypeptide. In some cases, the selecting step comprises determining the affinity, using BLI, of binding between T-Cell-MMP-epitope conjugate library members and the Co-MOD. In some cases, the T-Cell-MMP-epitope conjugate is as described above.

[00464] In some cases, the method of obtaining T-Cell-MMP-epitope conjugates comprising one or more variant MODs that exhibit reduced affinity for a Co-MOD compared to the affinity of the corresponding parental wild-type MODs for the Co-MOD further comprises: a) contacting the selected T-Cell-MMP-epitope conjugate library member with a target T-cell expressing on its surface: i) a Co-MOD that binds the parental wild-type MOD; and ii) a TCR that binds to the epitope, wherein the T-Cell-MMP-epitope conjugate library member comprises an epitope tag, such that the T-Cell-MMP-epitope conjugate library member binds to the target T-cell; b) contacting the selected T-Cell-MMP-epitope conjugate library member bound to the target T-cell with a fluorescently labeled binding agent that binds to the epitope tag, generating a selected T-Cell-MMP-epitope conjugate library member/target T-cell/binding agent complex; and c) measuring the MFI of the selected T-Cell-MMP-epitope conjugate library member/target T-cell/binding agent complex using flow cytometry, wherein the MFI measured over a range of concentrations of the selected T-Cell-MMP-epitope conjugate library member provides a measure of the affinity and apparent avidity. A selected T-Cell-MMP-epitope conjugate library member that selectively binds the target T-cell, compared to binding of the T-Cell-MMP-epitope conjugate library member to a control T-cell that comprises: i) the Co-MOD that binds the parental wild-type MOD; and ii) a TCR that binds to an epitope other than the epitope present in the T-Cell-MMP-epitope conjugate library member, is identified as selectively binding to the target T-cell. In some cases, the binding agent is an antibody specific for the epitope tag. In some cases, the variant MOD comprises from 1 to 20 amino acid substitutions (e.g., 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 corresponding parental wild-type MOD. In some cases, the T-Cell-MMP-epitope conjugate comprises two variant MODs. In some cases, the two variant MODs comprise the same amino acid sequence. In some cases, the first polypeptide comprises one of the two variant MODs and the second polypeptide comprises the second of the two variant MODs. In some cases, the two variant MODs are on the same polypeptide chain of the T-Cell-MMP-epitope conjugate. In some cases, the two variant MODs are on the first polypeptide of the T-Cell-MMP-epitope conjugate. In some cases, the two variant MODs are on the second polypeptide of the T-Cell-MMP-epitope conjugate.

[00465] In some cases, the method of obtaining a T-Cell-MMP-epitope conjugate comprising one or more variant MODs that exhibit reduced affinity for a Co-MOD compared to the affinity of the corresponding parental wild-type MOD for the Co-MOD further comprises isolating the selected T-Cell-MMP-epitope conjugate library member from the library. In some cases, the method further comprises providing a nucleic acid comprising a nucleotide sequence encoding a T-Cell-MMP with at least one chemical conjugation site used to prepare the selected library member. In some cases, the nucleic acid is present in a recombinant expression vector. In some cases, the nucleotide sequence is operably linked to a transcriptional control element that is functional in a eukaryotic cell. In some cases, the method further

comprises introducing the nucleic acid into a eukaryotic host cell, and culturing the cell in a liquid medium to synthesize the encoded T-Cell-MMP with at least one chemical conjugation site in the cell, isolating the synthesized T-Cell-MMP with at least one chemical conjugation site from the cell or from liquid culture medium, and conjugating it to at least one epitope to form the selected T-Cell-MMP-epitope conjugate. In some cases, the selected T-Cell-MMP with at least one chemical conjugation site comprises an Ig Fc polypeptide. In some cases, the method further comprises conjugating a drug to the Ig Fc polypeptide. In some cases, the drug is a cytotoxic agent that is selected from maytansinoid, benzodiazepine, taxoid, CC-1065, duocarmycin, a duocarmycin analog, calicheamicin, dolastatin, a dolastatin analog, auristatin, tomaymycin, and leptomycin, or a pro-drug of any one of the foregoing. In some cases, the drug is a retinoid. In some cases, the parental wild-type MOD and the Co-MODs are selected from: IL-2 and IL-2 receptor; 4-1BBL and 4-1BB; PD-L1 and PD-1; FasL and Fas; TGF- β and TGF- β receptor; CD70 and CD27; CD80 and CD28; CD86 and CD28; OX40L and OX40; FasL and Fas; ICOS-L and ICOS; ICAM and LFA-1; and JAG1 and Notch; JAG1 and CD46; CD80 and CTLA4; and CD86 and CTLA4.

[00466] The present disclosure provides a method of obtaining a T-Cell-MMP-epitope conjugate comprising one or more variant MODs that exhibit reduced affinity for a Co-MOD compared to the affinity of the corresponding parental wild-type MOD for the Co-MOD, the method comprising:
A) providing a library of T-Cell-MMP-epitope conjugates comprising a plurality of members, wherein the plurality of members comprise: a) a first polypeptide comprising: i) an epitope covalently bound at a chemical conjugation site; and ii) a first MHC polypeptide; and b) a second polypeptide comprising: i) a second MHC polypeptide; and ii) optionally an Ig Fc polypeptide or a non-Ig scaffold, wherein the members of the library comprise a plurality of variant MODs present in the first polypeptide, the second polypeptide, or both the first and the second polypeptide; and B) selecting from the library a member that exhibits reduced affinity for the Co-MOD. In some cases, the selecting step comprises determining the affinity, using BLI, of binding between T-Cell-MMP-epitope conjugate library members and the Co-MOD. In some cases, the T-Cell-MMP-epitope conjugate is as described above.

[00467] In some cases, the method further comprises: a) contacting the selected T-Cell-MMP-epitope conjugate library member with a target T-cell expressing on its surface: i) a Co-MOD that binds the parental wild-type MOD; and ii) a T-cell receptor that binds to the epitope, wherein the T-Cell-MMP-epitope conjugate library member comprises an epitope tag, such that the T-Cell-MMP-epitope conjugate library member binds to the target T-cell; b) contacting the selected T-Cell-MMP-epitope conjugate library member bound to the target T-cell with a fluorescently labeled binding agent that binds to the epitope tag, generating a selected T-Cell-MMP-epitope conjugate library member/target T-cell/binding agent complex; and c) measuring the MFI of the selected T-Cell-MMP-epitope conjugate library member/target T-cell/binding agent complex using flow cytometry, wherein the MFI measured over a range of concentrations of the selected T-Cell-MMP-epitope conjugate library member provides a measure of the affinity and apparent avidity. A selected T-Cell-MMP-epitope conjugate library member that selectively binds the target T-cell, compared to binding of the T-Cell-MMP-epitope conjugate library

member to a control T-cell that comprises: i) the Co-MOD that binds the parental wild-type MOD; and ii) a T-cell receptor that binds to an epitope other than the epitope present in the T-Cell-MMP-epitope conjugate library member, is identified as selectively binding to the target T-cell. In some cases, the binding agent is an antibody specific for the epitope tag. In some cases, the variant MOD comprises from 1 to 20 amino acid substitutions (e.g., 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 corresponding parental wild-type MOD. In some cases, the T-Cell-MMP-epitope conjugate comprises two variant MODs. In some cases, the two variant MODs comprise the same amino acid sequence. In some cases, the first polypeptide comprises one of the two variant MODs and the second polypeptide comprises the second of the two variant MODs. In some cases, the two variant MODs are on the same polypeptide chain of the T-Cell-MMP-epitope conjugate. In some cases, the two variant MODs are on the first polypeptide of the T-Cell-MMP-epitope conjugate. In some cases, the two variant MODs are on the second polypeptide of the T-Cell-MMP-epitope conjugate.

[00468] In some cases, the method further comprises isolating the selected T-Cell-MMP-epitope conjugate library member from the library. In some cases, the method further comprises providing a nucleic acid comprising a nucleotide sequence encoding a T-Cell-MMP with at least one chemical conjugation site used to prepare the selected library member. In some cases, the nucleic acid is present in a recombinant expression vector. In some cases, the nucleotide sequence is operably linked to a transcriptional control element that is functional in a eukaryotic cell. In some cases, the method further comprises introducing the nucleic acid into a eukaryotic host cell, and culturing the cell in a liquid medium to synthesize the encoded T-Cell-MMP with at least one chemical conjugation site in the cell, isolating the synthesized selected T-Cell-MMP with at least one chemical conjugation site from the cell or from the liquid culture medium, and conjugating it to at least one epitope to form the selected T-Cell-MMP-epitope conjugate. In some cases, the selected T-Cell-MMP library member comprises an Ig Fc polypeptide. In some cases, the method further comprises conjugating a drug to the Ig Fc polypeptide. In some cases, the drug is a cytotoxic agent selected from maytansinoid, benzodiazepine, taxoid, CC-1065, duocarmycin, a duocarmycin analog, calicheamicin, dolastatin, a dolastatin analog, auristatin, tomaymycin, and leptomycin, or a pro-drug of any one of the foregoing. In some cases, the drug is a retinoid. In some cases, the parental wild-type MODs and the co-MODs are selected from: IL-2 and IL-2 receptor; 4-1BBL and 4-1BB; PD-L1 and PD-1; FasL and Fas; TGF- β and TGF- β receptor; CD70 and CD27; CD80 and CD28; CD86 and CD28; OX40L and OX40; FasL and Fas; ICOS-L and ICOS; ICAM and LFA-1; and JAG1 and Notch; JAG1 and CD46; CD80 and CTLA4; and CD86 and CTLA4.

III. Nucleic Acids

[00469] The present disclosure provides a nucleic acid comprising a nucleotide sequence encoding a T-Cell-MMP of the present disclosure. The present disclosure provides a nucleic acid comprising a nucleotide sequence encoding a T-Cell-MMP of the present disclosure including chemical conjugation sites that are engineered into the polypeptides of the T-Cell-MMP.

[00470] The present disclosure provides nucleic acids comprising nucleotide sequences encoding the T-Cell-MMPs described herein. In some cases, the individual polypeptide chains of a T-Cell-MMP of the present disclosure are encoded in separate nucleic acids. In some cases, all polypeptide chains of a T-Cell-MMP of the present disclosure are encoded in a single nucleic acid. In some cases, a first nucleic acid comprises a nucleotide sequence encoding a first polypeptide of a T-Cell-MMP of the present disclosure; and a second nucleic acid comprises a nucleotide sequence encoding a second polypeptide of a T-Cell-MMP of the present disclosure. In some cases, a single nucleic acid comprises a nucleotide sequence encoding a first polypeptide of a T-Cell-MMP of the present disclosure and a second polypeptide of a T-Cell-MMP of the present disclosure.

A. Separate nucleic acids encoding individual polypeptide chains of a multimeric polypeptide

[00471] The present disclosure provides nucleic acids comprising nucleotide sequences encoding a T-Cell-MMP. As noted above, in some cases, the individual polypeptide chains of a T-Cell-MMP are encoded in separate nucleic acids. In some cases, nucleotide sequences encoding the separate polypeptide chains of a T-Cell-MMP 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.

[00472] 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 T-Cell-MMP of the present disclosure, where the first polypeptide comprises, in order from N-terminus to C-terminus: a) a first MHC polypeptide; and b) a MOD (e.g., a reduced-affinity variant MOD polypeptide as described above); and where the second nucleic acid comprises a nucleotide sequence encoding a second polypeptide of a T-Cell-MMP, 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 epitopes, MHC polypeptides, MODs, and Ig Fc polypeptides are described above. At least one of the first and second polypeptides comprises a chemical conjugation site (or a nascent site that can be converted to a chemical conjugation site). In some cases, the nucleotide sequences encoding the first and 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.

[00473] 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 T-Cell-MMP, where the first polypeptide comprises a first MHC polypeptide; and where the second nucleic acid comprises a nucleotide sequence encoding a second polypeptide of a T-Cell-MMP, where the second polypeptide comprises, in order from N-terminus to C-terminus: a) a MOD (e.g., a reduced-affinity variant MOD polypeptide as described above); b) a second MHC polypeptide; and c) an Ig Fc polypeptide. Suitable MHC polypeptides, MODs, and Ig Fc polypeptides are described above. At least one of the first and second polypeptides comprises a chemical conjugation site. In some cases, the nucleotide sequences

encoding the first and 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.

B. Nucleic acid encoding two or more polypeptides present in a T-Cell-MMP

[00474] The present disclosure provides a nucleic acid comprising nucleotide sequences encoding at least the first polypeptide and the second polypeptide of a T-Cell-MMP. In some cases, where a T-Cell-MMP 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 T-Cell-MMP include 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 T-Cell-MMP include 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 T-Cell-MMP include a ribosome skipping signal (or *cis*-acting hydrolase element, CHYSEL) 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 T-Cell-MMP; 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.

[00475] In some cases provided for herein, 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 T-Cell-MMP; 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 the T-Cell-MMP. In some cases, the nucleotide sequence encoding the first polypeptide and the 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.

[00476] 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 the elements: a) a first MHC polypeptide; b) a MOD (e.g., a reduced-affinity variant as described above); c) a proteolytically cleavable linker; d) a second MHC polypeptide; and e) an immunoglobulin (Ig) Fc polypeptide; wherein at least one of the elements comprises a chemical conjugation site that is not removed during cellular processing. 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 the elements: a) a first leader peptide; b) a first MHC polypeptide; c) a MOD (e.g., a reduced-affinity variant as described above); d) a

proteolytically cleavable linker; e) a second leader peptide; f) a second MHC polypeptide; and g) an Ig Fc polypeptide; wherein at least one of the elements comprises a chemical conjugation site that is not removed during cellular processing. 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, the elements: a) a first MHC polypeptide; b) a proteolytically cleavable linker; c) a MOD (e.g., a reduced-affinity variant as described above); d) a second MHC polypeptide; and e) an Ig Fc polypeptide; wherein at least one of the elements comprises a chemical conjugation site that is not removed during cellular processing. In some cases, the first leader peptide and the second leader peptide are β 2M leader peptides. 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.

[00477] Suitable MHC polypeptides are described above. In some cases, the first MHC polypeptide comprises a β 2-microglobulin (β 2M) polypeptide; and the second MHC polypeptide comprises a MHC Class I heavy chain polypeptide. In some cases, the β 2M polypeptide comprises an amino acid sequence having at least about 85% (e.g., at least about 90%, 95%, 98%, 99%, or even 100%) amino acid sequence identity to a β 2M amino acid sequence depicted in FIG. 4. In some cases, the MHC Class I heavy chain polypeptide is a 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 FIGs. 3A-3D. In such an embodiment the MHC Class I heavy chain polypeptide may not comprise a transmembrane anchoring domain (e.g., the heavy chain polypeptide comprises a sequence in FIG. 3D).

[00478] 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 FIGs. 2A-2G.

[00479] Suitable immunomodulatory polypeptides (MODs) are described above.

[00480] In addition to any other proteolytically cleavable linkers, in some cases, the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of: a) LEVLFQGP (SEQ ID NO:318); b) ENLYTQS (SEQ ID NO:319); c) DDDDK (SEQ ID NO:320); d) LVPR (SEQ ID NO:321); and e) GSGATNFSLLKQAGDVEENPGP (SEQ ID NO:322).

[00481] In some cases, a linker comprising a first Cys residue attached to the first MHC polypeptide is provided, and the second MHC polypeptide comprises an amino acid substitution to provide a second (engineered) Cys residue, such that the first and second Cys residues provide for a disulfide linkage between the linker and the second MHC polypeptide. In some cases, the first MHC polypeptide comprises an amino acid substitution to provide a first engineered Cys residue, and the second MHC polypeptide comprises an amino acid substitution to provide a second engineered 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. As discussed above, where disulfide bridges are provided, it is possible to use either thiol reactive agents or bis-thiol linkers to incorporate payloads or epitopes.

C. Recombinant expression vectors

[00482] 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 embodiments, 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.*

[00483] 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, lentivirus, human immunodeficiency virus, myeloproliferative sarcoma virus, and mammary tumor virus); and the like.

[00484] 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.

[00485] 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).

[00486] In some embodiments, 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 embodiments, 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 the DNA-targeting RNA and/or site-directed modifying polypeptide in both prokaryotic and eukaryotic cells.

[00487] 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.

IV. Genetically Modified Host cells

[00488] The present disclosure provides a genetically modified host cell, where the host cell is genetically modified with a nucleic acid of the present disclosure.

[00489] 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. CRL-9618™, CCL-61™, 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. CCL-10™), PC12 cells (ATCC No. CRL-1721™), 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.

[00490] In some cases, the host cell is a mammalian cell that has been genetically modified such that it does not synthesize endogenous MHC β 2M and/or such that it does not synthesize endogenous MHC Class I heavy chains (MHC-H). In addition to the foregoing, host cells expressing formylglycine generating enzyme (FGE) activity are discussed above for use with T-Cell-MMPs comprising a sulfatase motif, and such cells may advantageously be modified such that they do not express at least one, if not both, of the endogenous MHC β 2M and MHC-H proteins.

V. Compositions

[00491] The present disclosure provides compositions, including pharmaceutical compositions, comprising one or more T-Cell-MMPs and/or T-Cell-MMP-epitope conjugates, wherein the pharmaceutical compositions may comprise one or more pharmaceutically acceptable excipients as provided below. The present disclosure also provides compositions, including pharmaceutical compositions, comprising a nucleic acid or a recombinant expression vector of the present disclosure.

A. Compositions comprising T-Cell-MMPs

[00492] A composition of the present disclosure can comprise, in addition to a T-Cell-MMP or its epitope conjugate 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.

[00493] A composition comprising a T-Cell-MMP or its epitope conjugate may further 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.

[00494] A pharmaceutical composition can comprise a T-Cell-MMP of the present disclosure, 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 embodiments, 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.

[00495] 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.

[00496] For example, compositions may include (e.g., be in the form of) aqueous or other solutions, powders, granules, tablets, pills, suppositories, capsules, suspensions, sprays, and the like. The composition may be formulated according to the various routes of administration described below.

[00497] Where a T-Cell-MMP of the present disclosure 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, a non-aqueous form (e.g., a reconstitutable storage-stable powder) or an 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 subject protein following administration. For example, the protein 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.

[00498] 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.

[00499] The concentration of a T-Cell-MMP and/or T-Cell-MMP-epitope conjugate 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.

[00500] 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.

[00501] The present disclosure provides compositions, including pharmaceutical compositions, comprising a T-Cell-MMP or its epitope conjugate. A composition can comprise: a) a T-Cell-MMP and/or a T-Cell-MMP-epitope conjugate; and b) an excipient, as described above for the T-Cell-MMPs and their epitope conjugates. In some cases, the excipient is a pharmaceutically acceptable excipient.

[00502] In some cases, a T-Cell-MMP and/or T-Cell-MMP-epitope conjugate is present in a liquid composition. Thus, the present disclosure provides compositions (e.g., liquid compositions, including pharmaceutical compositions) comprising a T-Cell-MMP and/or T-Cell-MMP-epitope conjugate of the present disclosure. In some cases, a composition of the present disclosure comprises: a) a T-Cell-MMP and/or T-Cell-MMP-epitope conjugate of the present disclosure; and b) saline (e.g., 0.9% or about 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 T-Cell-MMP and/or T-Cell-MMP-epitope conjugate; and b) saline (e.g., 0.9% or about 0.9% NaCl), where the composition is sterile and is free of detectable pyrogens and/or other toxins.

B. Compositions comprising a nucleic acid or a recombinant expression vector

[00503] 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.

[00504] 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 T-Cell-MMP; 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, (for example) 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.*

[00505] 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 embodiments, a subject formulation comprises a nucleic acid or recombinant expression vector of the present disclosure.

[00506] 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.

[00507] 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.

[00508] 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 one or more spherical 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.

[00509] 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.

[00510] The formulations and compositions of the present disclosure may also include surfactants. The use of surfactants in drug products, formulations and emulsions is well known in the art. Surfactants and their uses are further described in U.S. Pat. No. 6,287,860.

[00511] 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.

[00512] 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 minitables. 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™.

VI. Methods of Modulating T-cell Activity

[00513] The present disclosure provides a method of selectively modulating the activity of a T cell, the method comprising contacting the T cell with a MODs on a T-Cell-MMP-epitope conjugate, and in some instances the payload the T-Cell-MMP-epitope conjugate may be carrying. Where the T-Cell-MMP has been conjugated to an epitope (*i.e.* it is a T-Cell-MMP-epitope conjugate), contacting the conjugate to a T-cell can result in epitope-specific T-cell modulation. In some cases, the contacting occurs *in vivo* (*e.g.*,

in a mammal such as a human, rat, mouse, dog, cat, pig, horse, or primate). In some cases, the contacting occurs *in vitro*. In some cases, the contacting occurs *ex vivo*. In some cases, the contacting occurs *ex vivo*. In some cases, the T-cell is a CD8+ T-cell, CD4+ T-cell, a NK-T-Cell, or a T (Treg) cell as described below under Treatment Methods.

[00514] The present disclosure provides a method of selectively modulating the activity of a WT-1 epitope-specific T-cell, the method comprising contacting the T-cell with a T-Cell-MMP-epitope conjugate of the present disclosure bearing the epitope recognized by the epitope-specific T-Cell, where contacting the T-cell with a T-Cell-MMP-epitope conjugate of the present disclosure 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*. In some cases, the T-cell is a CD8+ T-cell, CD4+ T-cell, a NK-T-cell, or a Treg cell as described below under Treatment Methods. In some cases, the T-cell is a CD8+ T-cell as described below under Treatment Methods.

[00515] Where a T-Cell-MMP-epitope conjugate of the present disclosure includes a MOD that is an activating polypeptide, contacting the T-cell with the T-Cell-MMP-epitope conjugate 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 T-Cell-MMP-epitope conjugate 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 T-Cell-MMP-epitope conjugate increases the number of the epitope-specific T-cells.

[00516] Where a T-Cell-MMP-epitope conjugate of the present disclosure includes a MOD that is an inhibiting polypeptide, contacting the T-cell with the multimer 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.

[00517] 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 T-Cell-MMP-epitope conjugate of the present disclosure. Administering the T-Cell-MMP-epitope conjugate 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.

[00518] The present disclosure also provides a method of detecting, in a mixed population of cells (e.g., a mixed population of T cells) obtained from an individual, the presence of a target T cells that binds an epitope of interest (e.g., a WT-1 epitope), the method comprising: a) contacting *in vitro* the mixed population of cell (e.g., mixed population of T cells) with a T-Cell-MMP-epitope conjugate of the present disclosure, wherein the T-Cell-MMP-epitope conjugate 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.

VII. Methods of Selectively Delivering a Costimulatory Polypeptide (MOD)

[00519] The present disclosure provides a method of delivering one or more independently selected MODs and/or MODs or a reduced-affinity variant of a naturally occurring MODs (such as a variant disclosed herein) to a selected T-cell or a selected T-cell population, *e.g.*, in a manner such that a TCR specific for a given WT-1 epitope is targeted. The present disclosure provides a method of delivering a MOD or a reduced-affinity variant of a naturally occurring MOD disclosed herein, selectively to a target T-cell bearing a TCR specific for the epitope (e.g., an epitope of WT-1) present in a T-Cell-MMP-epitope conjugate of the present disclosure. The method comprises contacting a population of T-cells with a T-Cell-MMP-epitope conjugate of the present disclosure. The population of T-cells can be a mixed population that comprises: i) the target T-cell; and ii) non-target T-cells that are not specific for the epitope (*e.g.*, T-cells that are specific for an epitope(s) other than the epitope to which the epitope-specific T-cell binds). The epitope-specific T-cell is specific for the epitope-presenting peptide (e.g., a peptide presenting an epitope of WT-1) present in the T-Cell-MMP-epitope conjugate and binds to the peptide HLA complex or peptide MHC complex provided by the T-Cell-MMP-epitope conjugate. Accordingly, contacting the population of T-cells with the T-Cell-MMP-epitope conjugate delivers the costimulatory polypeptide (e.g., a wild-type MOD or a reduced-affinity variant of the wild-type MOD, as described herein) selectively to the T-cell(s) that are specific for the epitope present in the T-Cell-MMP-epitope conjugate. 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 T-Cell-MMP-epitope conjugate 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(s), 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.

[00520] Thus, the present disclosure provides a method of delivering a MOD (such as IL-2), or a reduced-affinity variant of a naturally occurring MOD (such as an IL-2 variant) disclosed herein, or a combination of both, selectively to a target T-cell, the method comprising contacting a mixed population of T-cells with a T-Cell-MMP-epitope conjugate of the present disclosure. The mixed population of T-cells comprises the target T-cell and non-target T-cells. The target T-cell is specific for the epitope present

within the T-Cell-MMP-epitope conjugate. Contacting the mixed population of T-cells with a T-Cell-MMP-epitope conjugate of the present disclosure delivers the MOD(s) present within the T-Cell-MMP-epitope conjugate to the target T-cell.

VIII. Treatment Methods

[00521] The present disclosure provides a method of selectively modulating the activity of an epitope-specific T-cell in an individual (e.g., treat an individual), the method comprising administering to the individual an amount of a T-Cell-MMP-epitope conjugate of the present disclosure. Also provided is a T-Cell-MMP-epitope conjugate of the present disclosure for use in a method of treatment of the human or animal body. A treatment method of the present disclosure may comprise administering to an individual in need thereof a T-Cell-MMP-epitope conjugate of the present disclosure. Conditions that can be treated include cancers, examples of some of which are described below.

[00522] In some cases, a T-cell-MMP-epitope conjugate of the present disclosure, 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 T-cell-MMP-epitope conjugate of the present disclosure, 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 T-Cell-MMP; and ii) a Co-MOD that binds to the MOD present in the T-Cell-MMP-epitope conjugate; 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 T-Cell-MMP; and ii) a Co-MOD that binds to the MOD present in the T-Cell-MMP. 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, 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.

[00523] The combination of the reduced affinity of the MOD for its Co-MOD, and the affinity of the epitope for a TCR, provides for enhanced selectivity of a T-Cell-MMP-epitope conjugate of the present disclosure. Thus, for example, a T-Cell-MMP-epitope conjugate of the present disclosure binds with higher avidity to a first T-cell that displays both: i) a TCR specific for the epitope present in the T-Cell-MMP-epitope conjugate; and ii) a Co-MOD that binds to the MOD present in the T-Cell-MMP-epitope conjugate, 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 T-Cell-MMP-epitope conjugate; and ii) a Co-MOD that binds to the MOD present in the T-Cell-MMP-epitope conjugate.

[00524] 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 T-Cell-MMP-epitope conjugate of the present disclosure, where the T-Cell-MMP-epitope conjugate 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 T-Cell-MMP-epitope conjugate.

[00525] In some cases, the MOD is an activating polypeptide, and the T-Cell-MMP-epitope conjugate activates the epitope-specific T cell. In some cases, the epitope is a cancer-associated epitope, and the T-Cell-MMP-epitope conjugate increases the activity of a T cell specific for the cancer-associated epitope. In some cases, the MOD is an activating polypeptide, and the T-Cell-MMP-epitope conjugate activates an 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 an WT-1 epitope, and the T-Cell-MMP-epitope conjugate 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.

[00526] In some cases, a T-Cell-MMP-epitope conjugate (WT-1 peptide epitope conjugate) 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 T-Cell-MMP-epitope conjugate of the present disclosure comprises an inhibitory MOD (e.g., PD-L1, FasL, and the like), the T-Cell-MMP-epitope conjugate reduces the proliferation and/or activity of a Treg.

[00527] Where a T-Cell-MMP-epitope conjugate of the present disclosure comprises a WT-1 peptide epitope, it 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), a non-small cell lung cancer (NSCLC), a myeloma, pancreatic cancer, colorectal cancer, a mesothelioma, a soft tissue sarcoma, a neuroblastoma, and a nephroblastoma.

[00528] Where a T-Cell-MMP-epitope conjugate of the present disclosure comprises a WT-1 peptide epitope, it can be administered to an individual in need thereof to treat acute myeloid leukemia (AML) in the individual. Where a T-Cell-MMP-epitope conjugate of the present disclosure comprises a WT-1 peptide epitope, it can be administered to an individual in need thereof to treat a myeloma in the

individual. Where a T-Cell-MMP-epitope conjugate of the present disclosure comprises a WT-1 peptide epitope, it can be administered to an individual in need thereof to treat ovarian cancer in the individual. Where a T-Cell-MMP-epitope conjugate of the present disclosure comprises a WT-1 peptide epitope, it can be administered to an individual in need thereof to treat pancreatic cancer in the individual. Where a T-Cell-MMP-epitope conjugate of the present disclosure comprises a WT-1 peptide epitope, it can be administered to an individual in need thereof to treat non-small cell lung cancer (NSCLC) in the individual. Where a T-Cell-MMP-epitope conjugate of the present disclosure comprises a WT-1 peptide epitope, it can be administered to an individual in need thereof to treat colorectal cancer (CRC) in the individual. Where a T-Cell-MMP-epitope conjugate of the present disclosure comprises a WT-1 peptide epitope, it can be administered to an individual in need thereof to treat breast cancer in the individual. Where a T-Cell-MMP-epitope conjugate of the present disclosure comprises a WT-1 peptide epitope, it can be administered to an individual in need thereof to treat a Wilms tumor in the individual. Where a T-Cell-MMP-epitope conjugate of the present disclosure comprises a WT-1 peptide epitope, it can be administered to an individual in need thereof to treat mesothelioma in the individual. Where a T-Cell-MMP-epitope conjugate of the present disclosure comprises a WT-1 peptide epitope, it can be administered to an individual in need thereof to treat soft tissue sarcoma in the individual. Where a T-Cell-MMP-epitope conjugate of the present disclosure comprises a WT-1 peptide epitope, it can be administered to an individual in need thereof to treat a neuroblastoma in the individual. Where a T-Cell-MMP-epitope conjugate of the present disclosure comprises a WT-1 peptide epitope, it can be administered to an individual in need thereof to treat a nephroblastoma in the individual.

[00529] The present disclosure provides a method of treating a WT-1 expressing cancer in an individual, the method comprising administering to the individual an effective amount of a T-Cell-MMP-epitope conjugate of the present disclosure where the T-Cell-MMP-epitope conjugate may comprise a T-cell epitope that is a cancer epitope, and where the T-Cell-MMP-epitope conjugate comprises a stimulatory MOD. In some cases, an “effective amount” of a T-Cell-MMP-epitope conjugate 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 T-Cell-MMP or T-Cell-MMP-epitope conjugate 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%, at least 95%, or to undetectable levels compared to the number of cancer cells in the individual before administration of the T-Cell-MMP or T-Cell-MMP-epitope conjugate, or in the absence of administration with the T-Cell-MMP-epitope conjugate. In some cases, an “effective amount” of a T-Cell-MMP or T-Cell-MMP-epitope conjugate of the present disclosure is an amount that, when administered in one or more doses to an individual in need thereof (an individual having a tumor), reduces either the number of cancer cells in the individual compared to the number of cancer cells in the individual before administration of the T-Cell-MMP-epitope conjugate, or in the absence of administration with the T-Cell-MMP-epitope conjugate.

[00530] In some cases, an “effective amount” of a T-Cell-MMP or T-Cell-MMP-epitope conjugate of the present disclosure is an amount that, when administered in one or more doses to an individual in need thereof, 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%, at least 95%, or to an undetectable level compared to the total tumor mass in the individual before administration of the T-Cell-MMP or T-Cell-MMP-epitope conjugate, or in the absence of administration of the T-Cell-MMP or T-Cell-MMP-epitope conjugate. In some cases, an “effective amount” of a T-Cell MMP-epitope conjugate 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 to undetectable levels.

[00531] In another embodiment, the “effective amount” of a T-Cell-MMP-epitope conjugate of the present disclosure 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 of at least one tumor in the individual. For example, in some cases, an “effective amount” of a multimeric polypeptide of the present disclosure 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 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%, at least 95%, or to undetectable levels (volume) compared to the tumor volume in the individual before administration of the T-Cell-MMP-epitope conjugate, or in the absence of administration of the T-Cell-MMP-epitope conjugate. In such an embodiment the mass may be calculated based on tumor density and volume.

[00532] In some cases, an “effective amount” of a T-Cell-MMP-epitope conjugate of the present disclosure 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 T-Cell-MMP-epitope conjugate of the present disclosure 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 T-Cell-MMP-epitope conjugate.

[00533] In some cases, an “effective amount” of a T-Cell-MMP-epitope conjugate of the present disclosure is an amount that, when administered in one or more doses to individuals in a population of individuals in need thereof, increases average survival time of the population. For example, in some cases, an “effective amount” of a T-Cell-MMP-epitope conjugate of the present disclosure is an amount that, when administered in one or more doses to individuals in a population of individuals suffering from a specified disease (e.g., type of cancer) in need thereof, increases the average survival time of the population of individuals receiving the T-Cell-MMP-epitope conjugate 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 average survival time of the individuals suffering from the specified disease not receiving the T-Cell-MMP-epitope

conjugate; wherein the population is an age, gender, weight, and/or disease state (disease and degree of progression) matched population.

[00534] As noted above, in some cases, in carrying out a subject treatment method, a T-Cell-MMP-epitope conjugate of the present disclosure is administered to an individual in need thereof, as the polypeptide *per se*.

IX. Formulations

Suitable formulations are described above, where suitable formulations include a pharmaceutically acceptable excipient. In some cases, a suitable formulation comprises: a) a T-Cell-MMP-epitope conjugate (e.g., comprising a peptide presenting a WT-1 epitope) of the present disclosure; and b) a pharmaceutically acceptable excipient. Suitable pharmaceutically acceptable excipients are described above.

A. Dosages

[00535] 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, route of administration, general health, and other drugs being administered concurrently. A T-Cell-MMP-epitope conjugate 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 µg to 10 mg per kilogram of body weight per minute. A T-Cell-MMP-epitope conjugate 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.

[00536] In some cases, a suitable dose of a T-Cell-MMP-epitope conjugate of the present disclosure is from 0.01 µg to 100 g per kg of body weight, from 0.1 µg to 10 g per kg of body weight, from 1 µg to 1 g per kg of body weight, from 10 µg to 100 mg per kg of body weight, from 100 µg to 10 mg per kg of body weight, or from 100 µg 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 T-

Cell-MMP-epitope conjugate of the present disclosure is administered in maintenance doses, ranging from 0.01 µg to 100 g per kg of body weight, from 0.1 µg to 10 g per kg of body weight, from 1 µg to 1 g per kg of body weight, from 10 µg to 100 mg per kg of body weight, from 100 µg to 10 mg per kg of body weight, or from 100 µg to 1 mg per kg of body weight.

[00537] Those of skill will readily appreciate that dose levels can vary as a function of the specific T-Cell-MMP-epitope conjugate, 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.

[00538] In some embodiments, multiple doses of a T-Cell-MMP-epitope conjugate of the present disclosure. The frequency of administration of a T-Cell-MMP-epitope conjugate of the present disclosure 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 embodiments T-Cell-MMP-epitope conjugate 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).

[00539] The duration of administration of a T-Cell-MMP-epitope conjugate of the present disclosure e.g., the period of time over which a T-Cell-MMP-epitope conjugate of the present disclosure is administered can vary, depending on any of a variety of factors, e.g., patient response, *etc.* For example, a T-Cell-MMP-epitope conjugate 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.

B. Routes of administration

[00540] AT-Cell-MMP-epitope conjugate of the present disclosure of the present disclosure) may be 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.

[00541] Conventional and pharmaceutically acceptable routes of administration include intratumoral, peritumoral, intramuscular, intralymphatic, intratracheal, intracranial, subcutaneous, intradermal, topical, 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 T-Cell-MMP-epitope conjugate and/or the desired effect. A T-Cell-MMP-epitope conjugate of the present disclosure can be administered in a single dose or in multiple doses.

[00542] In some embodiments, a T-Cell-MMP-epitope conjugate of the present disclosure is administered intravenously. In some embodiments, aT-Cell-MMP-epitope conjugate of the present is administered intramuscularly. In some embodiments, aT-Cell-MMP-epitope conjugate of the present is administered intralymphatically. In some embodiments, aT-Cell-MMP-epitope conjugate of the present is administered

locally. In some embodiments, aT-Cell-MMP-epitope conjugate of the present is administered intratumorally. In some embodiments, aT-Cell-MMP-epitope conjugate of the present is administered peritumorally. In some embodiments, aT-Cell-MMP-epitope conjugate of the present is administered intracranially. In some embodiments, a T-Cell-MMP-epitope conjugate of the present disclosure, is administered subcutaneously.

[00543] In some embodiments, T-Cell-MMP-epitope conjugate of the present disclosure is administered intravenously. In some embodiments, T-Cell-MMP-epitope conjugate of the present disclosure is administered intramuscularly. In some embodiments, a T-Cell-MMP-epitope conjugate of the present disclosure is administered locally. In some embodiments, a T-Cell-MMP-epitope conjugate of the present disclosure is administered intratumorally. In some embodiments, a T-Cell-MMP-epitope conjugate of the present disclosure is administered peritumorally. In some embodiments, a T-Cell-MMP-epitope conjugate of the present disclosure is administered intracranially. In some embodiments, a T-Cell-MMP-epitope conjugate is administered subcutaneously. In some embodiments, a T-Cell-MMP-epitope conjugate is administered intralymphatically.

[00544] A T-Cell-MMP-epitope conjugate of the present disclosure 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.

[00545] 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 out to effect systemic or local delivery of a T-Cell-MMP-epitope conjugate of the present disclosure. Where systemic delivery is desired, administration typically involves invasive or systemically absorbed topical or mucosal administration of pharmaceutical preparations.

C. Subjects suitable for treatment

[00546] Subjects suitable for treatment with a method or T-Cell-MMP-epitope conjugate 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.

X. Certain Embodiments

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,

and/or 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.

1. A T-Cell-MMP-epitope conjugate comprising:

- a) a first polypeptide having an N-terminus and a C-terminus, the first polypeptide comprising,
 - i) a first major histocompatibility complex (MHC) polypeptide having an N-terminus and a C-terminus, and an optional linker at the N-terminus or the C-terminus;
- b) a second polypeptide having an N-terminus and a C-terminus, the second polypeptide comprising, (e.g., in order from N-terminus to C-terminus),
 - i) a second MHC polypeptide;
 - ii) optionally an immunoglobulin (Ig) Fc polypeptide or a non-Ig polypeptide scaffold, and
 - iii) an optional linker at the N-terminus or the C-terminus of the second polypeptide;
- c) one or more first polypeptide chemical conjugation sites attached to (e.g., at the N- or C-terminus) or within the first polypeptide, and/or one or more second polypeptide chemical conjugation sites attached to (e.g., at the N- or C-terminus) or within the second polypeptide;
- d) one or more (e.g., two or more) immunomodulatory polypeptides (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,
 - D) at the C-terminus of the first polypeptide and at the N-terminus of the second polypeptide or
 - E) within the first or second polypeptide; and
- e) a Wilms tumor-1 (WT-1) peptide epitope covalently bound, directly or indirectly (e.g., through a linker) to at least one of the one of the one or more first polypeptide chemical conjugation sites or the one or more second polypeptide chemical conjugation sites, the WT-1 peptide epitope comprising four (4) or more (e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16) contiguous amino acids of any of the WT-1 sequences set forth as SEQ ID NO.335, SEQ ID NO.336, SEQ ID NO.337, SEQ ID NO.338, or SEQ ID NO.339 (see FIGs. 11A-11E);

wherein each of the one or more MODs is an independently selected wild-type or variant MOD.

The T-Cell-MMP-epitope conjugate of embodiment 1 may be subject to the proviso that neither the first nor the second polypeptide comprises an MHC-H polypeptide explicitly disclosed (e.g., as a sequence) in International Appln. PCT /US2018/049803, which published as WO 2019/051127. The T-Cell-MMP-epitope conjugate of embodiment 1, may be subject to the proviso that it does not include a T-Cell-MMP and/or T-Cell-MM-epitope conjugate disclosed in International Appln. PCT /US2018/049803.

2. A T-Cell-MMP-epitope conjugate comprising:

- a) a first polypeptide having an N-terminus and a C-terminus, the first polypeptide comprising,
 - i) a first major histocompatibility complex (MHC) polypeptide having an N-terminus and a C-terminus, and an optional linker at its N-terminus or C-terminus;

- b) a second polypeptide having an N-terminus and a C-terminus, the second polypeptide comprising (e.g., in order from N-terminus to C-terminus),
- i) a second MHC polypeptide;
 - ii) optionally an immunoglobulin (Ig) Fc polypeptide or a non-Ig polypeptide scaffold, and
 - (iii) an optional linker at the N-terminus or the C-terminus of the second polypeptide;
- c) one or more first polypeptide chemical conjugation sites attached to (e.g., at the N- or C-terminus) or within the first polypeptide, and/or one or more second polypeptide chemical conjugation sites attached to (e.g., at the N- or C-terminus) or within the second polypeptide;
- d) one or more (e.g., two or more) immunomodulatory polypeptides (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,
- D) at the C-terminus of the first polypeptide and at the N-terminus of the second polypeptide or
- E) within the first or second polypeptide; and

- e) a Wilms tumor-1 (WT-1) peptide epitope covalently bound, directly or indirectly (e.g., through a linker) to at least one of the one of the one or more first polypeptide chemical conjugation sites or the one or more second polypeptide chemical conjugation sites, the WT-1 peptide epitope comprising four (4) or more (e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16) contiguous amino acids of any of the WT-1 sequences set forth as SEQ ID NO.335, SEQ ID NO.336, SEQ ID NO.337, SEQ ID NO.338, or SEQ ID NO.339 (see FIGs. 11A-11E;

wherein each of the one or more MODs is an independently selected wild-type or variant MOD; and

wherein the first or second polypeptide comprises an MHC-H polypeptide sequence at least 85% (e.g., at least 90%, at least 95%, at least 97%, at least 98% at least 99% or at least 100%) sequence identity to at least a portion (e.g., 20-250, 20-100, 30-100, 40-120, 50-150, 70-170, 100-150, 100-200, 150-200, 200-250 aas, more than 250 contiguous aas, or all) of an MHC-H chain polypeptide selected from the group consisting of: HLA-A*0301 (SEQ ID NO:31), HLA-A*2407 (SEQ ID NO:33), HLA-A*3401 (SEQ ID NO:34), HLA-B*0801 (SEQ ID NO:37); HLA-B*1502 (SEQ ID NO:38), HLA-B*3802 (SEQ ID NO:39), HLA-B*4001 (SEQ ID NO:40), HLA-B*4601 (SEQ ID NO:41), HLA-B*5301 (SEQ ID NO:42), HLA-C*0102 (SEQ ID NO:44) HLA-C*0303 (SEQ ID NO:45) HLA-C*0304 (SEQ ID NO:46) HLA-C*0401 (SEQ ID NO:47) HLA-C*0602 (SEQ ID NO:48) HLA-C*0701 (SEQ ID NO:49) HLA-C*0702 (SEQ ID NO:50) HLA-C*0801 (SEQ ID NO:51) and HLA-C*1502 (SEQ ID NO:52, an HLA-E polypeptide (SEQ ID NO: 54), an HLA-F polypeptide (SEQ ID NO: 55), and HLA-G polypeptide (SEQ ID NO:56).

The T-Cell-MMP-epitope conjugate of embodiment 2 may be subject to the proviso that neither the first nor the second polypeptide comprises an MHC-H polypeptide explicitly disclosed (e.g., as a sequence) in

International Appln. PCT /US2018/049803, which published as WO 2019/051127. The T-Cell-MMP-epitope conjugate of embodiment 2, may be subject to the proviso that it does not include a T-Cell-MMP and/or T-Cell-MM-epitope conjugate disclosed in International Appln. PCT /US2018/049803.

3. A T-Cell-MMP-epitope conjugate comprising:

- a) a first polypeptide having an N-terminus and a C-terminus, the first polypeptide comprising,
 - i) a first major histocompatibility complex (MHC) polypeptide having an N-terminus and a C-terminus, and an optional linker at its N-terminus or C-terminus;
 - b) a second polypeptide having an N-terminus and a C-terminus, the second polypeptide comprising (e.g., in order from N-terminus to C-terminus),
 - i) a second MHC polypeptide;
 - ii) optionally an immunoglobulin (Ig) Fc polypeptide or a non-Ig polypeptide scaffold, and
 - (iii) an optional linker at the N-terminus or the C-terminus of the second polypeptide;
 - c) one or more first polypeptide chemical conjugation sites attached to (e.g., at the N- or C-terminus) or within the first polypeptide, and/or one or more second polypeptide chemical conjugation sites attached to (e.g., at the N- or C-terminus) or within the second polypeptide;
 - d) one or more (e.g., two or more) immunomodulatory polypeptides (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,
 - D) at the C-terminus of the first polypeptide and at the N-terminus of the second polypeptide or
 - E) within the first or second polypeptide; and
 - e) a Wilms tumor-1 (WT-1) peptide epitope covalently bound, directly or indirectly (e.g., through a linker) to at least one of the one of the one or more first polypeptide chemical conjugation sites or the one or more second polypeptide chemical conjugation sites, the WT-1 peptide epitope comprising four (4) or more (e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16) contiguous amino acids of any of the WT-1 sequences set forth as SEQ ID NO.335, SEQ ID NO.336, SEQ ID NO.337, SEQ ID NO.338, or SEQ ID NO.339 (see FIGs. 11A-11E);
- wherein each of the one or more MODs is an independently selected wild-type or variant MOD; and

wherein the first or second polypeptide comprises an MHC-H polypeptide sequence having at least 85% (e.g., at least 90%, at least 95%, at least 97%, at least 98% at least 99% or a 100%) sequence identity to at least a portion (e.g., 20-250, 20-100, 30-100, 40-120, 50-150, 70-170, 100-150, 100-200, 150-200, 200-250 contiguous aas, more than 250 contiguous aas, or all) of an MHC-H chain polypeptide selected from the group consisting of: an HLA-A polypeptide of SEQ ID NO:35, an HLA-B polypeptide of SEQ ID NO: 43, an HLA-C polypeptide of SEQ ID NO 53, an HLA-E polypeptide of SEQ ID NO: 54, an HLA-F polypeptide of SEQ ID NO: 55, and an HLA-G polypeptide of SEQ ID NO:56.

The T-Cell-MMP-epitope conjugate of embodiment 3 may be subject to the proviso that neither the first nor the second polypeptide comprises an MHC-H polypeptide explicitly disclosed (e.g., as a sequence) in International Appln. PCT /US2018/049803, which published as WO 2019/051127. The T-Cell-MMP-epitope conjugate of embodiment 3, may be subject to the proviso that it does not include a T-Cell-MMP and/or T-Cell-MM-epitope conjugate disclosed in International Appln. PCT /US2018/049803.

4. The T-Cell-MMP-epitope conjugate of any of embodiments 1-3, wherein the first polypeptide comprises:

- a first MHC polypeptide without a linker (e.g., a polypeptide linker) on its N-terminus and C-terminus,
- a first MHC polypeptide bearing a linker (e.g., a polypeptide linker) on its N-terminus,
- a first MHC polypeptide bearing a linker (e.g., a polypeptide linker) on its C-terminus, or
- a first MHC polypeptide bearing a linker (e.g., a polypeptide linker) on its N-terminus and C-terminus.

5. The T-Cell-MMP-epitope conjugate of any one of embodiments 1 to 4, wherein at least one of the one or more first polypeptide chemical conjugation sites is:

- a) attached to (e.g., at the N- or C-terminus), or within, the sequence of the first MHC polypeptide, where the first MHC polypeptide is without a linker on its N- and C-termini;
- b) attached to (e.g., at the N- or C-terminus), or within, the sequence of the first MHC polypeptide where the first MHC polypeptide comprises a linker on its N- and C-terminus;
- c) attached to (e.g., at the N- or C-terminus) or within, the sequence of the linker on the N-terminus of the first MHC polypeptide; and/or
- d) attached to (e.g., at the N- or C-terminus) or within, the sequence of the linker on the C-terminus of the first MHC polypeptide.

6. The T-Cell-MMP-epitope conjugate of any one of embodiments 1 to 5, wherein the first and second MHC polypeptides are Class I MHC polypeptides, and the first MHC polypeptide comprises:

- a beta-2-microglobulin ("β2M") polypeptide (see e.g., FIG. 4) having an N-terminus and a C-terminus with or without a linker on its N- and/or C-termini,
- a β2M polypeptide bearing a linker on its N-terminus,
- a β2M polypeptide bearing a linker on its C-terminus, or
- a β2M polypeptide bearing a linker on its N-terminus and C-terminus.

7. The T-Cell-MMP-epitope conjugate of embodiment 6, wherein at least one of the one or more first polypeptide chemical conjugation sites is:

- a) attached to (e.g., at the N- or C-terminus) or within the sequence of the β2M polypeptide without a linker on its N- or C-terminus;
- b) attached to (e.g., at the N- or C-terminus) or within the sequence of the β2M polypeptide where the β2M polypeptide comprises a linker on its N- and C-termini;
- c) attached to (e.g., at the N- or C-terminus) or within the sequence of the linker on the N-terminus of the β2M polypeptide; and/or

- d) attached to (e.g., at the N- or C-terminus) or within, the sequence of the linker on the C-terminus of the β 2M polypeptide.
8. The T-Cell-MMP-epitope conjugate of any one of embodiments 1 to 7, comprising:
 - a second MHC polypeptide without a linker on its N-terminus and C-terminus,
 - a second MHC polypeptide bearing a linker on its N-terminus,
 - a second MHC polypeptide bearing a linker on its C-terminus, or
 - a second MHC polypeptide bearing a linker on its N-terminus and C-terminus.
 9. The T-Cell-MMP-epitope conjugate of embodiment 8, wherein the second polypeptide further comprises an immunoglobulin (Ig) Fc polypeptide or a non-Ig polypeptide scaffold.
 10. The T-Cell-MMP-epitope conjugate of embodiment 9, wherein the second polypeptide comprises, in order from N-terminus to C-terminus:
 - a second MHC polypeptide bearing a linker on its C-terminus followed by an immunoglobulin (Ig) Fc polypeptide or a non-Ig polypeptide scaffold; or
 - a second MHC polypeptide bearing a linker on its N-terminus and/or C-terminus followed by an immunoglobulin (Ig) Fc polypeptide or a non-Ig polypeptide scaffold.
 11. The T-Cell-MMP-epitope conjugate of any one of embodiments 1 to 10, wherein at least one of the one or more second polypeptide chemical conjugation sites is:
 - a) attached to (e.g., at the N- or C-terminus) or within the sequence of the second MHC polypeptide, wherein the second MHC polypeptide is without a linker on its N- and C-termini;
 - b) attached to (e.g., at the N- or C-terminus) or within the sequence of the second MHC polypeptide wherein the second MHC polypeptide comprises a linker on its N- and/or C-terminus;
 - c) attached to (e.g., at the N- or C-terminus) or within the sequence of the linker on the N-terminus of the second MHC polypeptide;
 - d) attached to (e.g., at the N- or C-terminus) or within the sequence of the linker on the C-terminus of the second MHC polypeptide; and/or
 - e) attached to (e.g., at the N- or C-terminus) or within the sequence of an immunoglobulin (Ig) Fc polypeptide or a non-Ig polypeptide scaffold when the second MHC polypeptide is followed by an immunoglobulin (Ig) Fc polypeptide or a non-Ig polypeptide scaffold.
 12. The T-Cell-MMP-epitope conjugate of any one of embodiments 1 to 11, wherein the second MHC polypeptide comprises: a MHC Class I heavy chain ("MHC-H" see e.g., FIGs. 3A-3D) polypeptide having an N-terminus and a C-terminus without a linker on its N- and C-termini, a MHC-H polypeptide bearing a linker on its N-terminus, a MHC-H polypeptide bearing a linker on its C-terminus, or a MHC-H polypeptide bearing a linker on its N-terminus and C-terminus.
 13. The T-Cell-MMP-epitope conjugate of any one of embodiments 6-12, wherein at least one of the one or more first polypeptide chemical conjugation sites is:
 - a) attached to (e.g., at the N- or C-terminus), or within, the sequence of the β 2M polypeptide without a linker on its N- or C-terminus;

- b) attached to (e.g., at the N- or C-terminus), or within, the sequence of the β 2M polypeptide where the β 2M polypeptide comprises a linker on its N- and C-termini;
 - c) attached to (e.g., at the N- or C-terminus), or within, the sequence of the linker on the N-terminus of the β 2M polypeptide; and/or
 - d) attached to (e.g., at the N- or C-terminus), or within, the sequence of the linker on the C-terminus of the β 2M polypeptide.
14. The T-Cell-MMP-epitope conjugate of any one of embodiments 6-12, wherein at least one of the one or more first polypeptide chemical conjugation sites replaces and/or is inserted between any of the amino terminal 15 amino acids of a mature β 2M polypeptide sequence lacking its signal sequence (e.g., a β 2M polypeptide sequence shown in FIG. 4).
 15. The T-Cell-MMP-epitope conjugate of any one of embodiments 1 to 14, wherein the second polypeptide comprises an Ig Fc polypeptide.
 16. The T-Cell-MMP-epitope conjugate of embodiment 15, wherein 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 (e.g., an immunoglobulin sequence in any of FIGs. 2A to 2G).
 17. The T-Cell-MMP-epitope conjugate of embodiment 16, wherein the Ig Fc polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity (e.g., at least 90%, 95%, 98% or 99% identity, or even 100% identity) to an amino acid sequence depicted in one of FIGs. 2A-2D, or a portion of a sequence (at least about 50, 75, 100, 125 or 150 amino acids in length) in one of FIGs. 2A-2D corresponding to the IgFc polypeptide.
 18. The T-Cell-MMP-epitope conjugate of embodiment 17, wherein the IgFc polypeptide is an IgG1 Fc polypeptide.
 19. The T-Cell-MMP-epitope conjugate of embodiment 18, wherein the IgG1 Fc polypeptide comprises one or more amino acid substitutions selected from N297A, L234A, L235A, L234F, L235E, and P331S.
 20. The T-Cell-MMP-epitope conjugate of embodiment 19, wherein the IgG1 Fc polypeptide comprises L234A and L235A substitutions.
 21. The T-Cell-MMP-epitope conjugate of any one of embodiments 1 to 20, wherein at least one (e.g., at least two, or each) of the one or more MODs is a wild-type or variant MOD selected independently from the group consisting of CD7, CD80, CD86, PD-L1, PD-L2, 4-1BBL, OX40L, Fas ligand (FasL), ICOS-L, ICAM, TGF- β , CD30L, CD40, CD70, CD83, HVEM, lymphotoxin beta receptor, 3/TR6, ILT3, ILT4, HVEM, ILCD70, JAG1, and TGF- β .
 22. The T-Cell-MMP-epitope conjugate of any one of embodiments 1 to 21, wherein at least one (e.g., at least two) of the one or more MODs is a wild-type or variant MOD selected independently from the group consisting of: IL-2, 4-1BBL, PD-L1, CD80, and CD86.
 23. The T-Cell-MMP-epitope conjugate of any one of embodiments 1 to 21, wherein the T-Cell-MMP-epitope conjugate comprises one or more independently selected wild type or variant IL-2 MODs.

24. The T-Cell-MMP-epitope conjugate of any one of embodiments 1 to 21, wherein the T-Cell-MMP-epitope conjugate comprises one or more independently selected wild type or variant 4-1BBL MODs.
25. The T-Cell-MMP-epitope conjugate of any one of embodiments 1 to 21, wherein the T-Cell-MMP-epitope conjugate comprises one or more independently selected wild type or variant PD-L1 MODs.
26. The T-Cell-MMP-epitope conjugate of any one of embodiments 1 to 21, wherein the T-Cell-MMP-epitope conjugate comprises one or more independently selected wild type or variant CD80 MODs.
27. The T-Cell-MMP-epitope conjugate of any one of embodiments 1 to 21, wherein the T-Cell-MMP-epitope conjugate comprises one or more independently selected wild type or variant CD86 MODs.
28. The T-Cell-MMP-epitope conjugate of any one of embodiments 1 to 27, wherein the T-Cell-MMP-epitope conjugate comprises one or more independently selected wild-type and/or variant MOD polypeptides; wherein at least one of the one or more variant MOD polypeptides exhibits a reduced affinity to a Co-MOD (its Co-MOD) compared to the affinity of a corresponding wild-type MOD for the Co-MOD (e.g., the ratio of i) the binding affinity of a control T-Cell-MMP-epitope conjugate (where the control comprises a wild-type MOD) to a Co-MOD to ii) the binding affinity of a T-Cell-MMP-epitope conjugate of the present disclosure comprising a variant of the wild-type MOD to the Co-MOD, 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 102:1, at least 5 x 102:1, at least 103:1, at least 5 x 103:1, at least 104:1, at least 105:1, or at least 106:1).
29. The T-Cell-MMP-epitope conjugate of embodiment 28, wherein the variant MOD polypeptides comprise from 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions, insertions, or deletions relative to a corresponding wild-type immunomodulatory polypeptide, or comprises an amino acid sequence having at least 85% amino acid sequence identity (e.g., at least 90%, 95%, 98% or 99% identity, or even 100% identity) to an amino acid sequence of the corresponding wild-type MOD, or a portion of the sequence of a wild-type MOD (e.g., at least about 50, 75, 100, 125 or 150 contiguous amino acids of the wild-type MOD in length).
30. The T-Cell-MMP-epitope conjugate of any one of embodiments 1 to 29, wherein the first MHC polypeptide comprises a β 2M polypeptide; and wherein the second MHC polypeptide comprises a MHC Class I heavy chain polypeptide.
31. The T-Cell-MMP-epitope conjugate of any one of embodiments 6 to 30, wherein the β 2M polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity (e.g., at least 90%, 95%, 98% or 99% identity, or even 100% identity) to one of the amino acid sequences set forth in FIG. 4, or at least 60 contiguous amino acids of a mature sequence β 2M polypeptide in FIG. 4 (e.g., at least about 60, 70, 80, or 90 amino acids).
32. The T-Cell-MMP-epitope conjugate of any one of embodiments 6 to 31, wherein the β 2M polypeptide comprises, consists essentially of, or consists of a sequence of at least 20, 30, 40, 50, 60, 70, 80, 90 or 99 contiguous amino acids having identity with at least a portion of one of the amino acid sequences set forth in FIG. 4 (e.g., a sequence having 20-99, 20-40, 30-50, 40-60, 40-90, 50-70,

- 60 to 80, 60-99, 70-90, or 79-99 contiguous amino acids with identity to a sequence of mature β 2M polypeptide lacking its signal sequence set forth in FIG. 4).
33. The T-Cell-MMP-epitope conjugate of any one of embodiments 12 to 32, wherein the MHC Class I heavy chain polypeptide comprises all or part of a HLA-A, HLA-B, HLA-C, HLA-E, HLA-F or HLA-G heavy chain (e.g., from FIGs. 3A to 3I).
34. The T-Cell-MMP-epitope conjugate of embodiment 33, wherein the MHC Class I heavy chain polypeptide sequence comprises an amino acid sequence having at least 85% amino acid sequence identity (e.g., at least 90%, 95%, 98% or 99% identity, or even 100% identity) to all or part (e.g., 20-250, 20-40, 20-100, 30-50, 40-60, 40-90, 50-70, 60-80, 60-90, 70-100, 80-120, 100-150, 120-200, 150-200, 200-250, or more than 250 contiguous amino acids) of the amino acid sequences set forth in one of FIGs. 3D-3I,
35. The T-Cell-MMP-epitope conjugate of embodiment 33, wherein the MHC Class I heavy chain polypeptide sequence comprises all or part (e.g., 20-250, 20-40, 20-100, 30-50, 40-60, 40-90, 50-70, 60-80, 60-90, 70-100, 80-120, 100-150, 120-200, 150-200, 200-250, or more than 250 contiguous amino acids) of an HLA-A polypeptide sequence having greater than 85%, 90%, 95%, or 98% sequence identity to an HLA-A allele sequence set forth in FIG. 3E.
36. The T-Cell-MMP-epitope conjugate of embodiment 35, wherein the MHC Class I heavy chain polypeptide sequence comprises all or part (e.g., 20-250, 20-40, 20-100, 30-50, 40-60, 40-90, 50-70, 60-80, 60-90, 70-100, 80-120, 100-150, 120-200, 150-200, 200-250, or more than 250 contiguous amino acids) of an HLA-A polypeptide sequence having greater than 90%, 95%, 98% or 99% sequence identity to the HLA-A allele consensus sequence set forth in FIG. 3E (excluding positions X1 to X45 with defined amino acid variations in the consensus sequence).
37. The T-Cell-MMP-epitope conjugate of embodiment 33, wherein the MHC Class I heavy chain polypeptide sequence comprises all or part (e.g., 20-250, 20-40, 20-100, 30-50, 40-60, 40-90, 50-70, 60-80, 60-90, 70-100, 80-120, 100-150, 120-200, 150-200, 200-250, or more than 250 contiguous amino acids) of an HLA-B polypeptide sequence having greater than 90%, 95%, 98% or 99% sequence identity to an HLA-B allele sequence set forth in FIG. 3F.
38. The T-Cell-MMP-epitope conjugate of embodiment 37, wherein the MHC Class I heavy chain polypeptide sequence comprises all or part (e.g., 20-250, 20-40, 20-100, 30-50, 40-60, 40-90, 50-70, 60-80, 60-90, 70-100, 80-120, 100-150, 120-200, 150-200, 200-250, or more than 250 contiguous amino acids) of an HLA-B polypeptide sequence having greater than 90%, 95%, 98% or 99% sequence identity to the HLA-B allele consensus sequence set forth in FIG. 3F (excluding positions X1 to X34 with defined amino acid variations in the consensus sequence)
39. The T-Cell-MMP-epitope conjugate of embodiment 33, wherein the MHC Class I heavy chain polypeptide sequence comprises all or part (e.g., 20-250, 20-40, 20-100, 30-50, 40-60, 40-90, 50-70, 60-80, 60-90, 70-100, 80-120, 100-150, 120-200, 150-200, 200-250, or more than 250 contiguous amino acids) of an HLA-C polypeptide sequence having greater than 85%, 90%, 95%, or 98% sequence identity to an HLA-C allele sequence set forth in FIG. 3G.

40. The T-Cell-MMP-epitope conjugate of embodiment 39, wherein the MHC Class I heavy chain polypeptide sequence comprises all or part (e.g., 20-250, 20-40, 20-100, 30-50, 40-60, 40-90, 50-70, 60-80, 60-90, 70-100, 80-120, 100-150, 120-200, 150-200, 200-250, or more than 250 contiguous amino acids) of an HLA-C polypeptide sequence having greater than 90%, 95%, 98% or 99% sequence identity to the HLA-C allele consensus sequence set forth in FIG. 3G (excluding positions X1 to X37 with defined amino acid variations in the consensus sequence).
41. The T-Cell-MMP-epitope conjugate of embodiment 33, wherein the MHC Class I heavy chain polypeptide sequence comprises all or part (e.g., 20-250, 20-40, 20-100, 30-50, 40-60, 40-90, 50-70, 60-80, 60-90, 70-100, 80-120, 100-150, 120-200, 150-200, 200-250, or more than 250 contiguous amino acids) of an HLA-E, F, or G polypeptide sequence having greater than 85%, 90%, 95%, or 98% sequence identity to an HLA-E, F, or G allele sequence set forth in FIG. 3H.
42. The T-Cell-MMP-epitope conjugate of embodiment 41, wherein the MHC Class I heavy chain polypeptide sequence comprises all or part (e.g., 20-250, 20-40, 20-100, 30-50, 40-60, 40-90, 50-70, 60-80, 60-90, 70-100, 80-120, 100-150, 120-200, 150-200, 200-250, or more than 250 contiguous amino acids) of an HLA-E polypeptide sequence having greater than 90%, 95%, 98% or 99% sequence identity to an HLA-E allele consensus sequence set forth in FIG. 3H (excluding positions indicated by an "X" with defined amino acid variations in the consensus sequences).
43. The T-Cell-MMP-epitope conjugate of embodiment 41, wherein the MHC Class I heavy chain polypeptide sequence comprises all or part (e.g., 20-250, 20-40, 20-100, 30-50, 40-60, 40-90, 50-70, 60-80, 60-90, 70-100, 80-120, 100-150, 120-200, 150-200, 200-250, or more than 250 contiguous amino acids) of an HLA-F polypeptide sequence having greater than 90%, 95%, 98% or 99% sequence identity to an HLA-F allele consensus sequence set forth in FIG. 3H (excluding positions indicated by an "X" with defined amino acid variations in the consensus sequences).
44. The T-Cell-MMP-epitope conjugate of embodiment 41, wherein the MHC Class I heavy chain polypeptide sequence comprises all or part (e.g., 20-250, 20-40, 20-100, 30-50, 40-60, 40-90, 50-70, 60-80, 60-90, 70-100, 80-120, 100-150, 120-200, 150-200, 200-250, or more than 250 contiguous amino acids) of an HLA-G polypeptide sequence having greater than 90%, 95%, 98% or 99% sequence identity to an HLA-G allele consensus sequence set forth in FIG. 3H (excluding positions indicated by an "X" with defined amino acid variations in the consensus sequences).
45. The T-Cell-MMP-epitope conjugate of any one of embodiments 12 to 44, wherein the MHC Class I heavy chain polypeptide sequence comprises a disulfide bond between a cysteine at the carboxyl end portion of the MHC heavy chain α 1 helix and a cysteine in the amino end portion of the MHC heavy chain α 2-1 helix, and/or a cysteine or a cysteine substitution at any one or more (two, three, four, etc.) of amino acid residues 2, 7, 84, 5, 59, 116, 139, 167, 168, 170, or 171.
46. The T-Cell-MMP-epitope conjugate of embodiment 45, wherein the carboxyl end portion of the MHC heavy chain α 1 helix is from about amino acid position 79 to about amino acid position 89 and the amino end portion of the MHC heavy chain α 2-1 helix is from about amino acid position 134 to amino acid position 144 of the MHC Class I heavy chain, wherein the amino acid positions are

- determined based on the sequence of the heavy chains without their leader sequence (*see, e.g.*, FIGs. 3D to 3I).
47. The T-Cell-MMP-epitope conjugate of any one of embodiments 45 to 46, wherein the disulfide bond is between a cysteine located at positions 83, 84, or 85 and a cysteine located at position 138, 139 or 140 (e.g., from position 83 to position 138, 139 or 140, from position 84 to position 138, 139 or 140, or from position 85 to position 138, 139 or 140).
48. The T-Cell-MMP-epitope conjugate of any one of embodiments 45 to 47, wherein the disulfide bond is between a cysteine located at positions 84 and a cysteine located at position 139.
49. The T-Cell-MMP-epitope conjugate of any of embodiments 45 to 48, wherein the MHC Class I heavy chain sequence may have insertions, deletions and/or substitutions of 1 to 5 aas (e.g., 1, 2, 3 or 4 aas) preceding and/or following either or both cysteines forming the disulfide bond between the carboxyl end portion of the $\alpha 1$ helix and the amino end portion of the $\alpha 2$ -1 helix.
50. The T-Cell-MMP-epitope conjugate of embodiment 49, wherein, when substitutions and/or insertions are present, the amino acids may be selected from any naturally occurring amino acid, or any naturally occurring amino acid except glycine and/or proline.
51. The T-Cell-MMP-epitope conjugate of any one of embodiments 33 to 50, wherein the MHC Class I heavy chain polypeptide amino acid sequence at positions 1 to 79 has at least 85% amino acid sequence identity (e.g., at least 90%, 95%, 98% or 99% identity, or even 100% identity) to the corresponding portion of at least one sequence set forth in FIGs. 3D to 3H (e.g., the sequence has 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid insertions, deletions, or substitutions relative to a sequence in FIGs. 3D to 3H).
52. The T-Cell-MMP-epitope conjugate of any one of embodiments 33 to 50, wherein the MHC Class I heavy chain polypeptide amino acid sequence from position 89 to 134 (inclusive of those positions) has at least 85% amino acid sequence identity (e.g., at least 90%, 95%, 98% or 99% identity, or even 100% identity) to the corresponding portion of at least one sequence set forth in FIGs. 3D to 3H (e.g., the sequence has 1, 2, 3, 4, 5, or 6 amino acid insertions, deletions, or substitutions relative to a sequence in FIGs. 3D to 3H).
53. The T-Cell-MMP-epitope conjugate of any one of embodiments 33 to 52, wherein the MHC Class I heavy chain polypeptide amino acid sequence from position 144 to 230 (inclusive of those positions) has at least 85% amino acid sequence identity (e.g., at least 90%, 95%, 98% or 99% identity, or even 100% identity) to the corresponding portion of at least one sequence set forth in FIGs. 3D to 3H (e.g., the sequence has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 amino acid insertions, deletions, or substitutions relative to a sequence in FIGs. 3D to 3H).
54. The T-Cell-MMP-epitope conjugate of any one of embodiments 33 to 53, wherein the MHC Class I heavy chain polypeptide amino acid sequence from positions 242 to 274 (inclusive of those positions) has at least 85% amino acid sequence identity (e.g., at least 90%, 95%, 98% or 99% identity, or even 100% identity) to the corresponding portion of at least one sequence set forth in FIGs. 3D to 3H (e.g.,

the sequence has 1, 2, 3, or 4 amino acid insertions, deletions, or substitutions relative to a sequence in FIGs. 3D to 3H).

55. The T-Cell-MMP-epitope conjugate of any one of embodiments 1 to 54, wherein the first polypeptide and the second polypeptide are non-covalently associated.
56. The T-Cell-MMP-epitope conjugate of any one of embodiments 1 to 54, wherein the first polypeptide and the second polypeptide are covalently linked to one another.
57. The T-Cell-MMP-epitope conjugate of embodiment 56, wherein the covalent linkage is via a disulfide bond.
58. The T-Cell-MMP-epitope conjugate of any one of embodiments 1 to 57, comprising two or more, three or more, or four or more independently selected MODs.
59. The T-Cell-MMP-epitope conjugate of any one of embodiments 1 to 58, comprising a peptide linker between any two or more, three or more, or four or more of the two or more (e.g., two, three or four) wild-type and/or variant MODs.
60. The T-Cell-MMP-epitope conjugate of any one of embodiments 1 to 59, wherein the first polypeptide comprises a peptide linker between the first MHC polypeptide and at least one wild-type or variant MOD.
61. The T-Cell-MMP-epitope conjugate of any one of embodiments 1 to 58, wherein the second polypeptide comprises a peptide linker between the second MHC polypeptide and at least one wild-type or variant MOD.
62. The T-Cell-MMP-epitope conjugate of any one of embodiments 1 to 61, comprising at least one peptide linker in the first and/or second polypeptide chain, wherein the linker has a length of from 5 amino acids to 30 amino acids (e.g., 5-10, 10-20, or 20-30 amino acids).
63. The T-Cell-MMP-epitope conjugate of embodiment 62, wherein the linker comprises a peptide of the formula AAAGG or GGGGS, which may be repeated from one to ten times (e.g., from 1 to 4, 3 to 6, or 4 to 8 times).
64. The T-Cell-MMP-epitope conjugate of any one of embodiments 1 to 63, wherein the first and second chemical conjugation sites are independently selected from:
 - a) peptide sequences that act an enzymatic modification sequenc (*e.g.*, a sulfatase motif);
 - b) non-natural amino acids and/or selenocysteines;
 - c) engineered amino acid chemical conjugation sites;
 - d) carbohydrate or oligosaccharide moieties; and/or
 - e) IgG nucleotide binding sites.
65. The T-Cell-MMP-epitope conjugate of any one of embodiments 1 to 64, wherein at least one of the one or more first and second chemical conjugation sites comprises an enzymatic modification sequence.
66. The T-Cell-MMP-epitope conjugate of any of embodiments 1 to 65, wherein at least one of the one or more first or second chemical conjugation site is a sulfatase motif.

67. The T-Cell-MMP-epitope conjugate of embodiment 66, wherein the sulfatase motif comprises the sequence X1Z1X2Z2X3Z3, wherein
- Z1 is cysteine or serine;
 - Z2 is either a proline or alanine residue;
 - Z3 is a basic amino acid (arginine, lysine, or histidine, usually lysine), or an aliphatic amino acid (alanine, glycine, leucine, valine, isoleucine, or proline, usually A, G, L, V, or I);
 - X1 is present or absent and, when present, can be any amino acid, though usually an aliphatic amino acid, a sulfur-containing amino acid, or a polar, uncharged amino acid (i.e., other than an aromatic amino acid or a charged amino acid), usually L, M, V, S or T, more usually L, M, S or V, with the proviso that, when the sulfatase motif is at the N-terminus of the target polypeptide, X1 is present; and
 - X2 and X3 independently can be any amino acid, though usually an aliphatic amino acid, a polar, uncharged amino acid, or a sulfur containing amino acid (i.e., other than an aromatic amino acid or a charged amino acid), usually S, T, A, V, G or C, more usually S, T, A, V or G.
68. The T-Cell-MMP-epitope conjugate of embodiment 67, comprising one or more fGly amino acid residue in the amino acid sequence of the first polypeptide or the second polypeptide.
69. The T-Cell-MMP-epitope conjugate of any one of embodiments 1 to 68, wherein at least one of the one or more first or second chemical conjugation site is a Sortase A enzyme site comprising the amino acid sequence LP(X5)TG, LP(X5)TA, or LPETGG positioned at the C-terminus of the first and/or second polypeptide and wherein X5 is any amino acid.
70. The T-Cell-MMP-epitope conjugate of any one of embodiments 1 to 69, wherein at least one of the one or more first or second chemical conjugation sites is a Sortase A enzyme site comprising at least one oligoglycine (e.g., (G)_{2, 3, 4, or 5}) at the amino terminus of the first and/or second polypeptides, and/or at least one oligo alanine (e.g., (A)_{2, 3, 4, or 5}) at the amino terminus of the first and/or second polypeptides.
71. The T-Cell-MMP-epitope conjugate of any one of embodiments 1 to 70, wherein at least one of the one or more first or second chemical conjugation sites is a transglutaminase site.
72. The T-Cell-MMP-epitope conjugate of embodiment 71, wherein at least one of the one or more transglutaminase sites is selected from the group consisting of: LQG, LLQGG, LLQG, LSLSQG, and LLQLQG.
73. The T-Cell-MMP-epitope conjugate of any one of embodiments 1 to 72, wherein at least one of the one or more first and second chemical conjugation sites comprises a selenocysteine or an amino acid sequence containing one or more independently selected non-natural amino acids.
74. The T-Cell-MMP-epitope conjugate of embodiment 73, wherein at least one of the one or more non-natural amino acids is selected from the group consisting of para-acetylphenylalanine, para-azido phenylalanine and propynyl-tyrosine.

75. The T-Cell-MMP-epitope conjugate of any one of embodiments 1 to 74, wherein at least one of the one or more first and second chemical conjugation sites comprises an engineered amino acid site (e.g., a cysteine engineered into the first or second polypeptide).
76. The T-Cell-MMP-epitope conjugate of any one of embodiments 1 to 75, wherein at least one of the one or more first and second chemical conjugation sites comprises one or more sulfhydryl or amine groups (e.g., a cysteine substitution at any one or more (two, three, four, etc.) of amino acid residues 2, 5, 7, 59, 84, 116, 139, 167, 168, 170, or 171).
77. The T-Cell-MMP-epitope conjugate of embodiment 76, wherein at least one of the one or more sulfhydryl or amine groups results from the presence of a lysine or cysteine in the first and or second polypeptide.
78. The T-Cell-MMP-epitope conjugate of any one of embodiments 1 to 77, wherein at least one of the one or more first and second chemical conjugation sites comprises an independently selected carbohydrate, monosaccharide, disaccharide and/or oligosaccharide.
79. The T-Cell-MMP-epitope conjugate of any one of embodiments 1 to 78, wherein at least one of the one or more first and second chemical conjugation sites comprises one or more IgG nucleotide antibody binding sites.
80. The T-Cell-MMP-epitope conjugate of any of embodiments 1 to 79, wherein:
- a) the first polypeptide comprises a β 2M polypeptide sequence,
 - b) the second polypeptide comprises (e.g., in order from N-terminus to C-terminus),
 - i) a MHC-H polypeptide and an immunoglobulin (Ig) Fc polypeptide;
 - c) one or more first polypeptide chemical conjugation sites within the β 2M polypeptide or on a peptide linker are attached (located) at the N-terminal to the β 2M polypeptide sequence; and
 - d) 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,
 - D) at the C-terminus of the first polypeptide and at the N-terminus of the second polypeptide and/or
 - E) within the first or second polypeptide;
- wherein each of the one or more MODs is an independently selected wild-type or variant MOD; and wherein the first and second polypeptide are optionally joined by an interpeptide covalent bond.
- The T-Cell-MMP of embodiment 80, which does not contain an epitope peptide as part of the translated sequence or chemically conjugated (covalently linked) to it, may be subject to the proviso that neither the first nor the second polypeptide comprises an MHC-H polypeptide explicitly disclosed (e.g., as a sequence) in International Appln. PCT /US2018/049803, which published as WO 2019/051127.
- The T-Cell-MMP of embodiment 80, may also be subject to the proviso that it does not include a T-Cell-MMP and/or T-Cell-MM-epitope conjugate disclosed in International Appln. PCT /US2018/049803.
81. The T-Cell-MMP-epitope conjugate of embodiment 80, comprising at least one MOD at;

- A) the C-terminus of the first polypeptide;
 - B) the N-terminus of the second polypeptide; and/or
 - C) the C-terminus of the second polypeptide.
82. The T-Cell-MMP of embodiment 80,
- wherein the first and second polypeptides are joined by a disulfide bond between the MHC-H polypeptide and the β 2M polypeptide; or
- wherein the first and second polypeptides are joined by a disulfide bond between the MHC-H polypeptide and a peptide linker attached at the N-terminal to the β 2M polypeptide sequence.
83. The T-Cell-MMP of any of embodiments 80-82, wherein the disulfide bond between the MHC-H polypeptide and the β 2M polypeptide is between an aa of the MHC-H polypeptide at about position 236 (e.g., A236C) and an aa of the β 2M polypeptide at about position 12 (e.g., R12C).
84. The T-Cell-MMP of any of embodiments 80 to 83, wherein at least one chemical conjugation site comprises a cysteine engineered into the first or second polypeptide.
85. The T-Cell-MMP of any of embodiments 80 to 84, wherein at least one chemical conjugation sites is a cysteine engineered into the β 2M polypeptide sequence at position 2, 44, 50, 77, 85, 88, 91 or 98 (e.g., a Q2C, E44C, E50C, E77C, V85V, S88C, K91C, or D98C substitution) in a mature β 2M polypeptide.
86. The T-Cell-MMP of any of embodiments 80-85, wherein the chemical conjugation site is a cysteine engineered into the β 2M polypeptide sequence as a Q2C or E44C substitution of a mature β 2M polypeptide (e.g., the polypeptide of SEQ ID NOs: 57-60, which lacks the signal sequence).
87. The T-Cell-MMP-epitope conjugate of any one of embodiments 84-86, wherein the epitope is conjugated through the cysteine engineered into the first or second polypeptide.
88. The T-Cell-MMP- epitope conjugate of any one of embodiments 88-87, wherein the epitope is conjugated to a chemical conjugation site through a linker, selected from a peptide or non-peptide polymer.
89. The T-Cell-MMP-epitope conjugate of embodiment 88, wherein the epitope is conjugated through a linker that comprises a peptide having a length of from 4 amino acids to 30 amino acids (e.g., 4-10, 10-20 or 20-30 amino acids), including, but not limited to polypeptides comprising from 1-10 repeating units of: glycine (polyG); glycine-serine polymer repeating units (including, for example, (GS), (GSGGS), (GGGS), (GGSG), (GGSGG), (GSGSG), (GSGGG), (GGGSG), (GSSSG), and (GGGGS)_n); glycine-alanine polymer repeating units such as (AAAGG); alanine-serine polymers; cysteine containing linkers (e.g., GCGASGGGSGGGGS, GCGGSGGGSGGGSGGGGS, GCGGSGGGSGGGGS, or GCGGS(G4S)_n, where n is an integer of at least one, (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10).
90. The T-Cell-MMP-epitope conjugate of embodiment 89, wherein the epitope is conjugated through a linker that comprises a peptide of the formula (AAAGG) or (GGGGS), which may be repeated from 1 to 8 times(e.g., 1, 2, 3, 4, 5, 6, 7, or 8 time, or in a range selected from 1 to 4, 3 to 6, or 4 to 8 times.

91. The T-Cell-MMP-epitope conjugate of any of embodiments 84-90, where the epitope is conjugated through an engineered cysteine by a maleimide group incorporated into the peptide or a linker attached to the peptide bearing a maleimide group.
92. The T-Cell-MMP-epitope conjugate of embodiment 91, wherein the maleimide group attached to the epitope through a linker comprising a peptide (e.g., a GGGGS sequence repeated from 1 to 8 times and a maleimide group).
93. The T-Cell-MMP-epitope conjugate of any of embodiments 88-92, wherein the epitope conjugated to the T-Cell-MMP has the structure, from N-terminus to C-terminus (epitope)-(peptide linker)-(optional alkyl linker)-(maleimide) or (epitope)-(peptide linker)-(lysine)-(alkyl linker bound to the epsilon amino group of the lysine)-(maleimide).
93. The T-Cell-MMP of any of embodiments 80-93, wherein the MHC-H polypeptide comprises a cysteine at positions 84 and 139 (e.g., Y84C and A139C substitutions) that form an intrachain disulfide bond (e.g., stabilizing the protein for expression).
94. A T-Cell-MMP-epitope conjugate comprising a T-Cell-MMP of any one of embodiments 1 to 93, wherein the T-Cell-MMP-epitope conjugate comprises a structure selected from structures A, B, C, D, E, F, G, H, I, J, K, or L of FIG. 6, wherein the first polypeptide and the second polypeptide are each organized from N-terminus to C-terminus as in the selected structure.
95. The T-Cell-MMP-epitope conjugate of any of embodiments 1-92 comprising at least one variant MOD, wherein:
 - (a) the T-Cell-MMP-epitope conjugate binds to a first T-cell with an affinity that is at least 25% higher (1.25 times higher) than the affinity with which the T-Cell-MMP binds to a second T-cell, wherein the first T-cell expresses on its surface a Co-MOD and a TCR that binds the epitope with an affinity of at least 10^{-7} M (e.g., 10^{-8} or 10^{-9} M), and wherein the second T-cell expresses on its surface the Co-MOD but does not express on its surface a TCR that binds the epitope with an affinity of at least 10^{-7} M (e.g., an affinity less than 10^{-7} M, such as 10^{-6} or 10^{-5} M); or
 - (b) wherein the T-Cell-MMP-epitope conjugate binds to a first T-cell with an affinity that is at least 10% (e.g., 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 2-fold (e.g., 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, wherein the first T-cell displays both i) a TCR specific for the epitope present in the T-Cell-MMP-epitope conjugate, and ii) a Co-MOD that binds to the MOD present in the T-Cell-MMP-epitope conjugate, and wherein the second T-cell displays: i) a TCR specific for an epitope other than the epitope present in the T-Cell-MMP-epitope conjugate; and ii) a Co-MOD that binds to the MOD present in the T-Cell-MMP-epitope conjugate.

96. The T-Cell-MMP of any of embodiments 1-95, wherein the epitope is a peptide that is not post-translationally modified (e.g., it is not a glycopeptide, phosphopeptide, or lipopeptide).
97. The T-Cell-MMP of any of embodiments 1-95, wherein the epitope is a peptide that has been post-translationally modified (e.g., it is a glycopeptide, phosphopeptide, or lipopeptide).
98. The T-Cell-MMP-epitope conjugate of any one of embodiments 1-97, wherein the epitope is a peptide that comprises 4 to 25 contiguous aas (e.g., a range of 4-10 aas, 7-12 aas, 10-15 aas, 15-20 aas, 20-25aas, or 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 contiguous aas) of a protein set forth in section I.A.12 (entitled "Epitopes")
99. The T-Cell-MMP-epitope conjugate of any one of embodiments 1-97, wherein the WT-1 peptide epitope is selected from the group consisting of: CMTWNQMNLGATLKG (SEQ ID NO:272), WNQMNLGATLKGVAA (SEQ ID NO:273), CMTWNYMNLGATLKG (SEQ ID NO:274), WNYMNLGATLKGVAA (SEQ ID NO: 275), MTWNQMNLGATLKG (SEQ ID NO:276), TWNQMNLGATLKGVA (SEQ ID NO:277), CMTWNLMNLGATLKG (SEQ ID NO:279), MTWNLMNLGATLKG (SEQ ID NO:280), TWNLMNLGATLKGVA (SEQ ID NO:281), WNLNMNLGATLKGVAA (SEQ ID NO:282), MNLGATLKG (SEQ ID NO:283), MTWNYMNLGATLKG (SEQ ID NO:284), TWNYMNLGATLKGVA (SEQ ID NO:285), CMTWNQMNLGATLKGVA (SEQ ID NO:286), CMTWNLMNLGATLKGVA (SEQ ID NO:287), CMTWNYMNLGATLKGVA (SEQ ID NO:288), GYLRNPTAC (SEQ ID NO:289), GALRNPTAL (SEQ ID NO:290), YALRNPTAC (SEQ ID NO:291), GLLRNPTAC (SEQ ID NO:292), NQMNLGATL (SEQ ID NO:293), RYRPHPGAL (SEQ ID NO:294), YQRPHPGAL (SEQ ID NO:295), RLRPHPGAL (SEQ ID NO:296), RIRPHPGAL (SEQ ID NO:297), QFPNHSFKHEDPMGQ (SEQ ID NO:298), HSFKHEDPY (SEQ ID NO:299), QFPNHSFKHEDPM (SEQ ID NO:300), QFPNHSFKHEDPY (SEQ ID NO:301), KRPFMCAYPGCNK (SEQ ID NO:302), KRPFMCAYPGCYK (SEQ ID NO:303), FMCAYPGCY (SEQ ID NO:304), FMCAYPGCK (SEQ ID NO:305), KRPFMCAYPGCNKRY (SEQ ID NO:306), SEKRPFMCAYPGCNK (SEQ ID NO:307), KRPFMCAYPGCYKRY (SEQ ID NO:308), NLMNLGATL (SEQ ID NO:309), VLDFAPPGA (SEQ ID NO:310); RMFPNAPYL (SEQ ID NO:311); CMTWNQMN (SEQ ID NO:312); CYTWNQMNL (SEQ ID NO:313); NYMNLGATL (SEQ ID NO:314); YMFPNAPYL (SEQ ID NO:315); SLGEQQYSV (SEQ ID NO:316); and CMTWNQMNL (SEQ ID NO:317).
100. The T-Cell-MMP-epitope conjugate of any one of embodiments 1-97, wherein the WT-1 peptide epitope is selected from the group consisting of CMTWNQMN (SEQ ID NO:312); NYMNLGATL (SEQ ID NO:314) (WT-1 239-247; Q240Y); CYTWNQMNL (SEQ ID NO:313) (WT-1 235-243); CMTWNQMNL (SEQ ID NO:317) (WT-1 235-243); NQMNLGATL (SEQ ID NO:293) (WT-1 239-247); NYMNLGATL and (SEQ ID NO:314) (WT-1 239-247; Q240L).
101. The T-Cell-MMP-epitope conjugate of any one of embodiments 1-97, wherein the MHC-H polypeptide comprises the sequence of HLA-A*2402, and the WT-1 peptides that presents an epitope is selected from the group consisting of NQMNLGATL (SEQ ID NO:293) (WT-1 239-247); CMTWNQMN (SEQ ID NO:312); NYMNLGATL (SEQ ID NO:314) (WT-1 239-247; Q240Y);

- CYTWNQMNL (SEQ ID NO:313) (WT-1 235-243); NLMNLGATL (SEQ ID NO:314) (WT-1 239-247; Q240L); and CMTWNQMNL (SEQ ID NO:317) (WT-1 235-243).
102. The T-Cell-MMP-epitope conjugate of any one of embodiments 1-97, wherein the MHC-H polypeptide comprises the sequence of HLA-A*0201, and the WT-1 peptides that presents an epitope is selected from the group consisting of NLMNLGATL (SEQ ID NO:309) (WT-1 239-247; Q240L); VLDFAPPGA (SEQ ID NO:310) (WT-1 37-45); RMFPNAPYL (SEQ ID NO:311) (WT-1 126-134); YMFNAPYL (SEQ ID NO:315) (WT-1 126-134; R126Y); and SLGEQQYSV (SEQ ID NO:316) (WT-1 187-195).
103. The T-Cell-MMP-epitope conjugate of any of embodiments 1-102, wherein the T-Cell-MMP-epitope conjugate is in the form of a dimer.
104. The T-Cell-MMP-epitope conjugate of embodiment 103, wherein and the T-Cell-MMP-epitope conjugate comprises an (Ig) Fc polypeptide or a non-Ig polypeptide scaffold through which the T-Cell-MMP-epitope conjugate dimerizes, wherein the dimer is optionally stabilized by one or two disulfide bonds between the Ig Fc or non-Ig polypeptide chains.
105. The T-Cell-MMP or T-Cell-MMP-epitope conjugate of any one of embodiments 1 to 104 (e.g., embodiments 109-114), further comprising one or more independently selected payloads covalently bound to one or more first and/or second chemical conjugation sites either directly or indirectly through a spacer or linker, wherein the spacer or linker is optionally cleavable (e.g., in an endosome of a mammalian cell).
106. The T-Cell-MMP or T-Cell-MMP-epitope conjugate of embodiment 105, wherein the payload is conjugated via linker have from 1 to 20, (e.g., 1-2, 2-4, 5-10 or 10-20) independently selected alpha, beta, delta, gamma amino acids, or a combination thereof; or wherein the linker is a peptide of the formula poly-glycine poly-alanine, a random poly glycine/alanine copolymer, or poly(GGGGS)_n where n is 1, 2, 3, 4, 5, 6, 7, or 8.
107. The T-Cell-MMP or T-Cell-MMP-epitope conjugate of embodiment 105, wherein the payload is attached to a chemical conjugations site by a spacer, wherein the spacer results from the action of a homofunctional (e.g., homobifunctional) crosslinker or a heterofunctional (e.g., heterobifunctional) crosslinker.
108. A composition comprising a T-Cell-MMP-epitope conjugate of any of embodiments 1-107.
109. A composition comprising:
- a) the T-Cell-MMP-epitope conjugate of any one of embodiments 1 to 107; and
 - b) a pharmaceutically acceptable excipient.
110. A method of delivering an immunomodulatory polypeptide (MOD) to a target T-cell (e.g., a regulatory T-cell or cytotoxic T-cell) in an epitope-selective or epitope-selective/specific manner *in vitro*, or to an individual *in vivo*, comprising:
- contacting a T-Cell-MMP-epitope conjugate of any one of embodiments 1-107 with the target T-cell or a mixed population of T-cells comprising the target T-cell *in vitro*; or

- administering the T-Cell-MMP-epitope conjugate of any one of embodiments 1-107 or a composition comprising the T-Cell-MMP-epitope conjugate of any one of embodiments 108-109 to the individual (e.g., patient or subject);
- wherein the target T-cells are specific for the epitope present in the T-Cell-MMP-epitope conjugate.
111. A method of modulating the activity (e.g., activation or proliferation) of a target T-cell (e.g., a regulatory T-cell or cytotoxic T-cell) in an epitope-selective or epitope-selective/specific manner *in vitro*, or in an individual *in vivo*, comprising:
- contacting a T-Cell-MMP-epitope conjugate of any one of embodiments 1-107 with the target T-cell or a mixed population of T-cells comprising the target T-cell *in vitro*; or
- administering the T-Cell-MMP-epitope conjugate of any one of embodiments 1-107 or a composition comprising the T-Cell-MMP-epitope conjugate of any one of embodiments 108-109 to the individual (e.g., patient or subject);
- wherein the target T-cells are specific for the epitope present in the T-Cell-MMP-epitope conjugate.
112. The method of embodiment 111, wherein said modulating comprises increasing a cytotoxic T-cell response to a cancer cell.
113. A method of treating a patient having a cancer, the method comprising administering to the patient an effective amount of composition according to any one of embodiments 108-109.
114. The method of embodiment 113, wherein the cancer is selected from the cancers in group (i) or group (ii):
- (i) 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), a non-small cell lung cancer (NSCLC), a myeloma, pancreatic cancer, colorectal cancer, a mesothelioma, a soft tissue sarcoma, a neuroblastoma, or a nephroblastoma; or
 - (ii) 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.
115. The method of embodiment 112 or embodiment 113, wherein said administering is intramuscular.
116. The method of embodiment 112 or embodiment 113, wherein said administering is intravenous.
117. The method of any one of embodiments 110 to 116, wherein the individual is a human.
118. The method of any one of embodiments 110 to 117, wherein said administering is rectal, nasal, oral, and other enteral and/or parenteral routes of administration.
119. The method of any one of embodiments 110 to 118, wherein said administering is intratumoral, peritumoral, intramuscular, intratracheal, intracranial, subcutaneous, intralymphatic, intradermal, topical, intravenous, and/or intraarterial.
110. The T-Cell-MMP-epitope conjugate of any of embodiments 1 to 108, wherein the chemical conjugation site to which the epitope was covalently bound to create the T-Cell-MMP-epitope

conjugate is not located in or immediately adjacent to (within one amino acid) an amino acid sequence having 100% amino acid identity to:

the Fc polypeptide sequence in FIGs. 2A-2G;

the MHC Class I heavy chain polypeptides sequences in FIGs. 3A-3H; or

the β -2 microglobulin polypeptide sequences in FIG. 4.

111. The T-Cell-MMP-epitope conjugate of any of embodiments 1 to 87 (e.g., 70 to 94), wherein the chemical conjugation site to which the epitope was covalently bound to create the T-Cell-MMP-epitope conjugate is not located in or immediately adjacent to (within one amino acid) a 10, 20, 30, 40, or 50 amino acid long sequence having 100% amino acid identity to any portion of any one of:

the Fc polypeptide sequence in FIGs. 2A-2G;

the MHC Class I heavy chain polypeptides sequences in FIGs. 3A-3H; or

the β -2 microglobulin polypeptide sequences in FIG. 4.

112. The T-Cell-MMP-epitope conjugate of any of embodiments 1 to 108, wherein the chemical conjugation site to which the epitope was covalently bound to create the T-Cell-MMP-epitope conjugate is not an amino acid appearing in a 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, or 70 amino acid long sequence having 100% amino acid identity to any portion of any one of:

the Fc polypeptides in FIGs. 2A-2G;

the MHC Class I heavy chain polypeptides in FIGs. 3A-3H; or

the β -2 microglobulin polypeptide sequences in FIG. 4.

113. The T-Cell-MMP-epitope conjugate of any of embodiments 1 to 108, wherein the chemical conjugation site to which the epitope was covalently bound to create the T-Cell-MMP-epitope conjugate is not a lysine, cysteine, serine, threonine, arginine, aspartic acid, glutamic acid, asparagine, or glutamine located in an 10, 20, 30, 40, 50, 60, or 70 amino acid long sequence having 100% amino acid identity to any portion of any one of:

the Fc polypeptide sequence in FIGs. 2A-2G;

the MHC Class I heavy chain polypeptides sequences in FIGs. 3A-3H; or

the β -2 microglobulin polypeptide sequences in FIG. 4.

114. A method of modulating an immune response in an individual, the method comprising administering to the individual an effective amount of the T-cell MMP-epitope conjugate of any one of embodiments 1-108, 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 T-cell-MMP epitope conjugate) 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.

115. The method of embodiment 114, wherein the ratio of the epitope-specific T cell response to the epitope-non-specific T cell response is at least 5:1.

116. The method of embodiment 114, wherein the ratio of the epitope-specific T cell response to the epitope-non-specific T cell response is at least 10:1.

117. The method of embodiment 114, wherein the ratio of the epitope-specific T cell response to the epitope-non-specific T cell response is at least 25:1.
118. The method of embodiment 114, wherein the ratio of the epitope-specific T cell response to the epitope-non-specific T cell response is at least 50:1 or at least 100:1.
119. The method of any one of embodiments 114-40, wherein the individual is a human.
120. The method of any one of embodiments 114-118, wherein said modulating comprises increasing a cytotoxic T-cell response to a cancer cell (e.g., a WT-1-expressing cancer cell).
121. The method of any one of embodiments 114-120, wherein said administering is intravenous, subcutaneous, intramuscular, systemic, intralymphatic, distal to a treatment site, local, or at or near a treatment site.
122. The method of any one of embodiments 114-121, 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 MMP-epitope conjugate comprising a corresponding wild-type immunomodulatory polypeptide.
123. 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 MMP-epitope conjugate of any one of embodiments 1-107, wherein the T-Cell-MMP-epitope conjugate 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.
124. A polypeptide construct comprising a polypeptide comprising a mature β 2M polypeptide sequence (lacking its signal sequence) having an N-terminus and a C-terminus and at least one optional linker; and a WT-1 peptide epitope that comprises 6 or more contiguous amino acids of the WT-1 sequence set forth in FIG. 11A-11E as SEQ ID NO.:335 through SEQ ID NO.:339.
125. The polypeptide of embodiment 124, further comprising one or more chemical conjugation sites within or at the ends of the sequence of the mature β 2M polypeptide, or covalently attached to the mature β 2M polypeptide via the optional linker, wherein the WT-1 peptide epitope is covalently bound, directly or indirectly, to at least one of the one or more chemical conjugation sites.
126. The polypeptide of embodiment 124 or 125, wherein the mature β 2M polypeptide has a sequence with at least 85%, (e.g., at least 90%, 95%, 98% or 99% identity, or even 100%) amino acid sequence identity to at least 60 contiguous aas (at least 70, 80, 90 or all aas) of the of a mature β 2M polypeptide provided in FIG. 4; wherein identity between the β 2M polypeptide and the corresponding sequences in FIG. 4..
127. The polypeptide of any of embodiments 124 to 126, wherein the β 2M polypeptide sequence comprises, consists essentially of, or consists of a sequence of at least 20, 30, 40, 50, 60, 70, 80, 90 or 99 contiguous amino acids having identity with at least a portion of one of the amino acid sequence set forth in FIG. 4 (e.g., a sequence having 20-99, 20-40, 30-50, 40-60, 40-90, 50-70, 60 to 80, 60-99, 70-90, or 79-99 contiguous amino acids with identity to a sequence of mature β 2M lacking its signal sequence set forth in FIG. 4).

128. The polypeptide of any one of embodiments 124 to 127, wherein the β 2M polypeptide sequence comprises a cysteine at one, two or more of amino acid positions 2, 44, 50, 77, 85, 88, 91 or 98 (e.g., a Q2C, E44C, E50C, E77C, V85V, S88C, K91C, or D98C substitution) of the mature β 2M polypeptide sequence.
129. The polypeptide of embodiment 128, wherein the first 12 amino acids of the β 2M polypeptide sequence are IQRTPKIQVYSC.
130. The polypeptide of any one of embodiments 125 to 127, wherein the chemical conjugation site is a sulfatase motif
131. The polypeptide of embodiment 130, wherein the sulfatase motif is linked directly, or indirectly via a linker, to the N-terminus of the β 2M polypeptide sequence.
132. The polypeptide of any one of embodiments 130 to 131, comprising a sulfatase motif, or a sulfatase motif wherein serine or cysteine of the sulfatase motif has been converted to an fGly (formylglycine) residue.
133. The polypeptide of embodiment 132, wherein the WT-1 peptide epitope is covalently bound to the polypeptide through a chemical reaction with the fGly residue (e.g., the reaction of a thiosemicarbazide, aminooxy, hydrazide, or hydrazino modified WT-1 epitope polypeptide with the aldehyde of the fGly),,
135. The polypeptide of any of embodiments 124 to 134, further comprising a signal sequence, or a signal sequence and a linker, wherein the signal sequence is the amino terminal most element of the polypeptide.
136. A composition comprising a polypeptide of any one of embodiments 124 to 135 optionally comprising a pharmaceutically acceptable carrier.
137. A polypeptide construct comprising a mature MHC Class I heavy chain polypeptide sequence (lacking its signal sequence) and an optional linker; and
a WT-1 polypeptide that comprises 6 or more contiguous amino acids of the WT-1 sequence set forth in FIG. 11A - FIG. 11E as SEQ ID NO.:335 through SEQ ID NO:339;
and optionally an immunoglobulin (Ig) Fc polypeptide or a non-Ig polypeptide scaffold.
138. The polypeptide of embodiment 137, further comprising one or more chemical conjugation sites within or at the ends of the sequence of the mature MHC Class I heavy chain polypeptide, or covalently attached to the mature MHC Class I heavy chain polypeptide via the optional linker, wherein the WT-1 peptide epitope is covalently bound, directly or indirectly, to at least one of the one or more chemical conjugation sites.
139. The polypeptide of embodiment 137 or 138, wherein the MHC Class I heavy chain polypeptide has a sequence with at least 85% (e.g., at least 90%, 95%, 98%, 99%, or even 100%) amino acid sequence identity to any of the sequences provided in FIGs. 3D-3H; wherein identity between the MHC Class I heavy chain polypeptide and the corresponding sequences in FIGs. 3D-3H is determined without consideration of the (Ig) Fc polypeptide and any optional linker present.

140. The polypeptide of any of embodiments 137 to 139, wherein the MHC Class I heavy chain polypeptide comprises, consists essentially of, or consists of a sequence of at least 20, 30, 40, 50, 60, 70, 80, 90 or 100 contiguous amino acids having identity with at least a portion of one of the amino acid sequence set forth in any of FIGs. 3D-3H (e.g., a sequence having 20-100, 20-40, 30-50, 40-60, 40-90, 50-70, 60-80, 60-90, 70-90, or 80-100 contiguous amino acids with identity to a MHC Class I heavy chain polypeptide sequence set forth in any of FIGs. 3D-3H).
141. The polypeptide of embodiment 140, wherein the MHC Class I heavy chain polypeptide comprises one, two or three sequences selected from the group consisting of:
- i) a sequence from about amino acid position 79 to about amino acid position 89;
 - ii) a sequence from about amino acid position 134 to about amino acid position 144; and
 - iii) a sequence from about amino acid position 231 to about amino acid position 241 of the MHC Class I heavy chain sequences set forth in any of FIGs. 3D-3H.
142. The polypeptide of embodiment 141, wherein the MHC Class I heavy chain polypeptide comprises:
- i) the sequence from about amino acid position 79 to about amino acid position 89; and
 - ii) the sequence from about amino acid position 134 to about amino acid position 144;
- wherein one positions 83,84, or 85 have been substituted with cysteine that forms an intrachain disulfide bond with a cysteine substituted at one of positions 138, 139, or 140.
143. The polypeptide of any of embodiments 141 to 142, wherein the polypeptide comprises a MHC Class I heavy chain polypeptide sequence from about amino acid position 231 to about amino acid position 241 of the MHC Class I heavy chain sequences set forth in any of FIGs. 3D-3H wherein one of positions 235, 236 or 237 have been substituted by a cysteine.
144. The polypeptide of any one of embodiments 137 to 143, wherein any one or more of the linkers is selected independently from peptides of formula (AAAGG)_n or (GGGGS)_n, where n is from 1 to 8 (e.g., 1, 2, 3, 4, 5, 6, 7, or 8, or in a range selected from 1 to 4, 3 to 6, or 4 to 8).
145. A composition comprising a polypeptide of any one of embodiments 137 to 144.
146. A composition comprising a polypeptide of any one of embodiments 137 to 144 and a pharmaceutically acceptable carrier.

The subject matter of this disclosure and any of embodiments 1-146 may be subject to the proviso that neither the T-Cell-MMPs nor their epitope conjugates comprise an MHC-H polypeptide explicitly disclosed (e.g., as a sequence) in International Appln. PCT /US2018/049803, which published as WO 2019/051127. The subject matter of this disclosure and any of embodiments 1-146 may be subject to the proviso that neither the T-Cell-MMPs nor their epitope conjugates include T-Cell-MMP and/or T-Cell-MM-epitope conjugate disclosed in International Appln. PCT /US2018/049803.

XII. EXAMPLES

Example 1

[00547] This example describes and provides for the preparation of a T-Cell-MMP having a first polypeptide (see **FIG. 9 A**) containing a sulfatase motif (bolded but not underlined) that can be acted on

by an FGE to provide a fGly chemical conjugation site and a second polypeptide. The first and second polypeptides taken together form a T-Cell-MMP into which an epitope can be conjugated, and which can be dimerized through the IgFc regions. At **B, FIG. 9** shows a second polypeptide of a T-Cell-MMP having tandem IL-2 MODs attached to the amino end of a human MHC Class I HLA-A heavy chain polypeptide followed by a human IgG1 Fc polypeptide.

[00548] The polypeptides are prepared by assembling the coding sequences of the first and second polypeptides in expression cassettes that include constitutive or inducible promoter elements for driving the expression of mRNA molecules encoding the first and second polypeptides along with polyadenylation and stop codons. The expression cassettes are assembled into separate vectors (plasmid, viral *etc.*), or a single vector, for transient expression from a suitable cell line (e.g., CHO, HEK, Vero, COS, yeast *etc.*). Alternatively, the assembled cassettes are stably integrated into such cells for constitutive or induced expression of the first and second polypeptides.

1A. First Polypeptides

[00549] The first polypeptide of this example comprises from the N-terminus to the C-terminus a) a leader sequence, b) a sulfatase motif to introduce an fGly chemical coupling site, c) an optional linker, and d) a β 2M polypeptide. Following the action of a FGE, the first peptide has a cysteine in the motif converted to a formylglycine (fGly) residue.

[00550] Within the above-mentioned first peptide, the first 20 aas serve as the signal sequence and are removed during cellular processing during maturation of the polypeptide. The residues of the sulfatase motif (X1, Z1, X2, Z2, X3, and Z3), here LCTPSR, are described in Section I.A above flanked by the linker sequence GGGGS (SEQ ID NO:76) to emphasize that linkers may be placed before and/or after the motif. The map also indicates by double underlining the location of a potential amino acid substitution at position 12 in the β 2M polypeptide changing an arginine to a cysteine (R12C).

1B. Second Polypeptides

[00551] The second polypeptide of this example comprises from N-terminus to C-terminus a) a leader sequence, b) a MOD polypeptide(s), c) an optional linker, d) a MHC Class 1 heavy chain polypeptide, e) an optional linker, and f) an immunoglobulin Fc region.

[00552] The mRNAs encode the second polypeptide polypeptides having the overall structure: signal sequence-linker-tandem IL-2 (IL2 polypeptide-optional linker-IL2 polypeptide)-linker-MHC Class 1 heavy chain polypeptide-linker-immunoglobulin heavy chain Fc polypeptide where the signal sequence is a 20 aa human IL2 signal sequence. The polypeptide also contains a human HLA-A polypeptide and a human IgG1 Fc polypeptide. Indicated below the map are the locations of potential amino acid substitutions including the location of the Y84C, A139C, and the A236C cysteine substitutions. The Y84C and A139C substitutions permit a stabilizing disulfide bond to form between the region near the carboxyl end of the HLA α 1 helix and the region around the amino terminus of the HLA α 2-1 helix. The cysteine resulting from the A236C substitution can form an interchain disulfide bond with a cysteine at, for example, position 12 of the β 2M polypeptide in the first polypeptide. Below the map appears an exemplary peptide sequence for a second polypeptide including the leader sequence.

[00553] Additional polypeptides that could be used to prepare T-Cell-MMPs and their epitope conjugates are provided in FIG. 10. In addition, although exemplified principally with the A11 MHC Class I heavy chain HLA-A*1101, any of the other MHC heavy chains set forth in FIGs. 3A to 3D, or portions thereof, could have been employed, including, but not limited to, HLA-A*0201; HLA-A*2401; HLA-A*2402 (SEQ ID NO:136) and HLA-A*3303 (SEQ ID NO:137).

1C. Expression and Maturation of the First Second Polypeptides

[00554] As indicated above, first and second polypeptides are prepared by transient or stable expression in a suitable cell line (*e.g.*, a eukaryotic or mammalian cell line). Processing in the cell removes the signal sequence and forms a fGly residue when the cells employed for polypeptide expression also express an FGE that is capable of converting a cysteine or serine of the sulfatase motif to a formylglycine (fGly) residue.

[00555] T-Cell-MMPs can be processed by cells as a complex that includes the first and second polypeptides and a bound (non-covalently associated) epitope or null polypeptide. The introduction of the disulfide bond in the HLA heavy chain polypeptide between the region at the carboxyl end of the $\alpha 1$ helix and the region at the amino terminus of the $\alpha 2$ -1 helix permits expression in the absence of an epitope polypeptide associated with the first and second polypeptides. In addition, as the T-Cell-MMP complexes do not contain a membrane anchor region, the complex is released from the expressing cell in soluble form.

[00556] Cell culture media containing the expressed T-Cell-MMP is collected after suitable levels of the expressed T-Cell-MMP have been attained. Where the cells used for expression did not have FGE activity, the T-Cell-MMPs are treated with an FGE capable of forming the fGly residue at the sulfatase motif. Isolation and concentration of the T-Cell-MMP from the media (*e.g.*, serum free media) is conducted using, for example, chromatographic methods to produce a purified T-Cell-MMP having a fGly chemical conjugation site at or near the amino terminus of the first polypeptide of the complex. The resulting T-Cell-MMP has the general structure shown in FIG. 5, part B, where the MHC-1 in the first polypeptide is the $\beta 2$ M polypeptide, the second polypeptide "MOD" is the pair of IL2 polypeptides, the MHC-2 is a HLA-A polypeptide, and Fc is a IgG1 heavy chain constant region. The disulfide bond between the first and second polypeptides results from the cysteines arising from the $\beta 2$ M polypeptide R12C and HLA-A A236C substitutions.

1.D. Preparation of T-Cell-MMP-Epitope Conjugates

[00557] Epitope polypeptides are conjugated to the fGly-containing T-Cell-MMP prepared above by forming on the epitope peptide a group capable of reacting with the fGly aldehyde. While thiosemicarbazide, aminooxy, hydrazide, or hydrazino aldehyde reactive groups can be utilized, this example is illustrated by the use of a hydrazinyl group (e.g., attached to an indole) where the epitope peptide is covalently bound, directly or indirectly, to the nitrogen of the indole ring. Contacting the epitope peptide with the fGly containing polypeptide of the T-Cell-MMP results in the T-Cell-MMP and epitope becoming covalently linked, thereby forming the T-Cell-MMP-epitope conjugates.

1.E. Epitopes for T-Cell-MMPs Conjugates

[00558] Non-limiting examples of WT-1 epitope peptides that can be used to form T-Cell-MMP-epitope conjugates include those recited in section I.A.12, such as the epitope peptides set forth in Section I.A.12.d. Such epitope peptides the HLA-A*2402 and HLA-A*0201 restricted epitopes (used in combination with corresponding MHC- H chains), and other epitopes of WT-1 polypeptides (see e.g., FIGs. 11A-11E). In embodiments, the epitope may be CMTWNQMNL (SEQ ID NO:317) or CYTWNQMNL (SEQ ID NO:313). In another embodiment it is not CMTWNQMNL (SEQ ID NO:317) or CYTWNQMNL (SEQ ID NO:313).

Example 2. Preparation of a T-Cell-MMP-Epitope Conjugate

[00559] Example 2 illustrates the ability to produce T-Cell-MMPs and conjugate them to a peptide resulting in a protein that is not aggregated (a dimer of T-cell-MMP's), displays suitable stability for use at 37 °C, and can be purified. The example is conducted with a CMV peptide; however, a WT-1 epitope peptide can equally be utilized to form the epitope conjugates providing similar results. Any of the WT-1 peptides in Section I.A.12.d, including the HLA-A*2402 and HLA-A*0201 restricted epitopes may be used in combination with corresponding MHC- H chains.

[00560] In the Example two immunomodulatory proteins are prepared by cellular expression in Expi-CHO cells using transient transfection using an expression vector containing a nucleic acid construct encoding the proteins. The proteins were purified over Protein A (MabSelect SuRe™; GE), followed by further purification by size exclusion chromatography.

[00561] The first immunomodulatory protein, having structure A set forth in FIG. 12 (generically termed an IL-2 Control Construct), is not a T-Cell-MMP or an epitope conjugate thereof. The protein acts as a control for the T-Cell-MMP of the present disclosure. That control protein comprises a first polypeptide having a 9 aa cytomegalovirus (CMV) epitope at the N-terminus of a β 2M polypeptide sequence:
 NLVPMVATV**GGGASGGGSGGG**SIQRTPKIQVYSCHPAENGKSNFLNCYVSGFHPS
 DIEVDLLKNGERIEKVEHSDLSFSKDWSFYLLYYTEFTPTKDEYACRVNHVTLSPKIVKWDR
 DM (SEQ ID NO: 323). Linkers are shown in bold and italics.

[00562] The second polypeptide of the control protein has, from N-terminus to C-terminus: two copies of an IL-2 immunomodulatory sequence (with H16A F42A substitutions) in tandem; a linker; a HLA-A*0201 polypeptide (with Y84A and A236C substitutions); a linker; and a human IgG1-Fc polypeptide having a LALA (L234A/L235A, see e.g., FIG. 2G) substitution:

APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEEELK
 PLEEVNLNAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL
TGGGGS**GGGGS****GGGGS****GGGGS**APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAK
 KFYMPKKATELKHLQCLEEEELKPLEEVNLNAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY
 ADETATIVEFLNRWITFCQSIISTL**TGGGGS****GGGGS****GGGGS****GGGGS**SGSHSMRYFFTSVSRPGRGEP
 RFIAVGYYDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRG
 AYNQSEAGSHTVQRMYGCDVGS DWRFLRGYHQYAYDGKDIALKEDLRSWTAADMAAQTTK
 HKWEAAHVAEQLRAYLEGTCVEWLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWA
 LSFYPAEITLTWQRDGEDQTQDTEL VETRPCGDGTFQKWA AAVVPSGQEQRYTCHVQHEGLPK
 PLTLRWEAA**AGG**DKTHTCPPCPAPEAAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
 KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI
 SKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
 SFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:324).

Linkers are shown in bold and italics.

[00563] The second immunomodulatory protein, having structure B set forth in FIG. 12 is an IL-2 containing T-Cell-MMP of the present disclosure having tandem IL-2 polypeptide sequences. The T-Cell-MMP comprises a first polypeptide having a linker bearing cysteine at aa 44 of the mature β 2M polypeptide (E44C substitution marked as C*) that acts as a chemical conjugation site located at the N-terminus of the β 2M polypeptide sequence:

IQRTPKIQVYSCHPAENGKSNFLNCYVSGFHPSDIEVDLLKNGC*RIEKVEHSDLSFSKD
 WSFYLLYYTEFTPEKDEYACRVNHVTLSPKIVKWDRDM (SEQ ID NO:325).

[00564] The second polypeptide of the T-Cell-MMP has, from N-terminus to C-terminus: two copies of an IL-2 immunomodulatory sequence (with H16A F42A substitutions) in tandem; a linker; a HLA-A*0201 polypeptide (with Y84C, A139C, and A236C substitutions); a linker; and a human IgG1-Fc polypeptide having a LALA (L234A/L235A, see e.g., FIG. 2G) substitution.

[00565] APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEEELKPLEEVNLNAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL**TGGGGS****GGGGS****GGGGS****GGGGS**APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEEELKPLEEVNLNAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL**TGGGGS****GGGGS****GGGGS****GGGGS**SGSHSMRYFFTSVSRPGRGEPRIAVGYYDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRGCYNQSEAGSHTVQRMYGCDVGS DWRFLRGYHQYAYDGKDIALKEDLRSWTAADMC AQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTEL VETRPCGDGTFQKWA AAVVPSGQEQRYTCHVQHEGLPKPLTLRWEAA**AGG**DKTHTCPPCPAPEAAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID

NO:326). Linkers are shown in bold and italics. The Y84C and A139C substitutions are shown as forming a disulfide bond between the $\alpha 1$ and $\alpha 2$ segments (helices) of the Class I heavy chain.

[00566] As shown in FIG. 12 at C, the expressed and purified proteins were subject to reducing SDS PAGE gel electrophoresis with both the control and T-Cell-MMP providing a light chain (first polypeptide) and heavy chain (second polypeptide).

Example 3

[00567] A T-Cell-MMP similar to Example 2 structure B was expressed and purified as described in Example 2. The purified T-Cell-MMP (labeled “IL-2 T-Cell-MMP”), which has an engineered cysteine residue as a chemical conjugation site, was conjugated to a CMV polypeptide (“CMV+T-Cell-MMP”) or a melanoma antigen MART-1 (“MART+T-Cell-MMP”) via a maleimide reactive linker attached to the peptide. The T-Cell-MMP-epitope conjugates were subjected to LC MS. The upper LCMS plot in FIG. 13 shows the IL-2 T-Cell-MMP + CMV light chain parent ion at 13,505.50 mass units with substantially complete conjugation. The lower LCMS plot in FIG. 13 shows the IL-2 T-Cell-MMP + MART light chainparent ion at 13,548.0010 mass units, with a minor amount of unconjugated T-Cell-MMP at 11,653.0010 mass units. Size exclusion chromatography indicates that at least 93% of the T-Cell-MMP conjugated to the CMV polypeptide and 91% of the T-Cell-MMP conjugated to the MART-1 polypeptide is in the form of an unaggregated dimer comprised of two first and two second polypeptides (see FIGs. 12 and 13 for reference).

Example 4

[00568] Control Constructs (see FIG 12 structure A) comprising a first polypeptide having at the N-terminus of a $\beta 2M$ polypeptide sequence either a CMV epitope peptide as in Example 2 (“CMV+ContCON”) or a MART-1 epitope peptide (“MART+ContCON”) were prepared by cell expression and purification as described in Examples 2 and 3.

[00569] T-Cell-MMP-epitope-conjugates with tandem IL-2 MODs having a conjugated CMV peptide (“CMV+T-Cell-MMP”) or conjugated MART-1 peptide (“MART+T-Cell-MMP”) were prepared as in Example 3.

[00570] Ficoll-Paque® samples of leukocytes from three CMV responsive donors (Leukopak Transforms 1-3) and from three MART-1 (MART) responsive donors (Leukopak Transforms 4-6) were used. Responsiveness of the donor cells was determined based on the ability to expand CMV or MART-1 specific T-Cells upon CMV or MART-1 peptide stimulation in the presence of IL-2 as determined by flow cytometry. For the test shown in FIG. 15 the leukocytes were suspended at 2.5×10^6 cells per ml in Immunocult media containing the indicated amounts of the control constructs or T-Cell-MMPs. As an additional control, cells grown without stimulation were stained with CMV or MART-1 tetramers purchased from MBL International Corp. After 10 days in culture the number of cells responsive to CMV or MART-1 were assessed by Flowcytometry. The results indicate that both the CMV+ContCON (having a CMV epitope expressed as part of the protein) and CMV+IL-2 T-Cell-MMPs (having a conjugated CMV epitope peptide) stimulate the expansion of CMV responsive CD8+ T-Cells in CMV responsive donors in a concentration dependent manner. Similarly, both the MART-1 control construct

(having a MART-1 epitope expressed as part of the protein) and MART-1 IL-2 T-Cell-MMPs (having a conjugated MART-1 epitope peptide) stimulate the expansion of MART-1 responsive CD8+ T-Cells in MART-1 responsive donors in a concentration dependent manner.

[00571] The antigen specificity in the responses are evidenced by the fact that CMV Control Construct and IL-2 T-Cell-MMP molecules did not stimulate expansion of MART-1 responsive CD8+ T-cells.

Likewise, MART-1 Control Construct and IL-2 T-Cell-MMP molecules did not stimulate expansion of CMV responsive CD8+ T-cells. Accordingly, the presence of IL-2 polypeptide sequences present in each of the molecules were not responsible for nonspecific expansion of the leukocytes.

What is Claimed Is:

1. A T-cell modulatory multimeric polypeptide epitope conjugate (T-Cell-MMP-epitope conjugate) comprising:

- a) a first polypeptide having an N-terminus and a C-terminus, the first polypeptide comprising,
 - i) a first major histocompatibility complex (MHC) polypeptide having an N-terminus and a C-terminus, and an optional linker at its N-terminus or C-terminus;
- b) a second polypeptide having an N-terminus and a C-terminus, the second polypeptide comprising,
 - i) a second MHC polypeptide;
 - ii) optionally an immunoglobulin (Ig) Fc polypeptide or a non-Ig polypeptide scaffold, and an optional linker at the N-terminus or the C-terminus of the second polypeptide;
- c) one or more first polypeptide chemical conjugation sites attached to or within the first polypeptide, and/or one or more second polypeptide chemical conjugation sites attached to or within the second polypeptide; and
- d) one or more immunomodulatory polypeptides (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,
 - D) at the C-terminus of the first polypeptide and at the N-terminus of the second polypeptide or
 - E) within the first or second polypeptide; and
- e) a Wilms tumor-1 (WT-1) peptide epitope covalently bound, directly or indirectly to at least one of the one of the one or more first polypeptide chemical conjugation sites or the one or more second polypeptide chemical conjugation sites, the WT-1 peptide epitope comprising four (4) or more contiguous amino acids of any of the WT-1 sequences set forth as SEQ ID NO.335, SEQ ID NO.336, SEQ ID NO.337, SEQ ID NO.338, or SEQ ID NO.339;

wherein each of the one or more MODs is an independently selected wild-type or variant MOD.

2. A T-cell modulatory multimeric polypeptide epitope conjugate (T-Cell-MMP-epitope conjugate) comprising:

- a) a first polypeptide having an N-terminus and a C-terminus, the first polypeptide comprising,
 - i) a first major histocompatibility complex (MHC) polypeptide having an N-terminus and a C-terminus, and an optional linker at the N-terminus or the C-terminus;
- b) a second polypeptide having an N-terminus and a C-terminus, the second polypeptide comprising,
 - i) a second MHC polypeptide;
 - ii) optionally an immunoglobulin (Ig) Fc polypeptide or a non-Ig polypeptide scaffold, and an optional linker at the N-terminus or the C-terminus of the second polypeptide;

- c) one or more first polypeptide chemical conjugation sites attached to or within the first polypeptide, and/or one or more second polypeptide chemical conjugation sites attached to or within the second polypeptide; and
- d) one or more immunomodulatory polypeptides (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,
 - D) at the C-terminus of the first polypeptide and at the N-terminus of the second polypeptide or
 - E) within the first or second polypeptide; and
- e) a Wilms tumor-1 (WT-1) peptide epitope covalently bound, directly or indirectly to at least one of the one of the one or more first polypeptide chemical conjugation sites or the one or more second polypeptide chemical conjugation sites, the WT-1 peptide epitope comprising four (4) or more contiguous amino acids of any of the WT-1 sequences set forth as SEQ ID NO.335, SEQ ID NO.336, SEQ ID NO.337, SEQ ID NO.338, or SEQ ID NO.339;

wherein each of the one or more MODs is an independently selected wild-type or variant MOD;
and

wherein the first or second polypeptide comprises an MHC-H polypeptide sequence having at least 85% sequence identity to 200-250 aas of an MHC-H chain polypeptide selected from the group consisting of: HLA-A*0301 (SEQ ID NO:31), HLA-A*2407 (SEQ ID NO:33), HLA-A*3401 (SEQ ID NO:34), HLA-B*0801 (SEQ ID NO:37); HLA-B*1502 (SEQ ID NO:38), HLA-B*3802 (SEQ ID NO:39), HLA-B*4001 (SEQ ID NO:40), HLA-B*4601 (SEQ ID NO:41), HLA-B*5301 (SEQ ID NO:42), HLA-C*0102 (SEQ ID NO:44), HLA-C*0303 (SEQ ID NO:45), HLA-C*0304 (SEQ ID NO:46), HLA-C*0401 (SEQ ID NO:47), HLA-C*0602 (SEQ ID NO:48), HLA-C*0701 (SEQ ID NO:49), HLA-C*0702 (SEQ ID NO:50), HLA-C*0801 (SEQ ID NO:51), HLA-C*1502 (SEQ ID NO:52), an HLA-E polypeptide (SEQ ID NO: 54), an HLA-F polypeptide (SEQ ID NO: 55), and an HLA-G polypeptide (SEQ ID NO:56).

3. A T-cell modulatory multimeric polypeptide epitope conjugate (T-Cell-MMP-epitope conjugate) comprising:

- a) a first polypeptide having an N-terminus and a C-terminus, the first polypeptide comprising,
 - i) a first major histocompatibility complex (MHC) polypeptide having an N-terminus and a C-terminus, and an optional linker at the N-terminus or the C-terminus;
- b) a second polypeptide having an N-terminus and a C-terminus, the second polypeptide comprising,
 - i) a second MHC polypeptide;
 - ii) optionally an immunoglobulin (Ig) Fc polypeptide or a non-Ig polypeptide scaffold, and an optional linker at the N-terminus or the C-terminus of the second polypeptide;

- c) one or more first polypeptide chemical conjugation sites attached to or within the first polypeptide, and/or one or more second polypeptide chemical conjugation sites attached to or within the second polypeptide; and
- d) one or more immunomodulatory polypeptides (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,
 - D) at the C-terminus of the first polypeptide and at the N-terminus of the second polypeptide or
 - E) within the first or second polypeptide;
- e) a Wilms tumor-1 (WT-1) peptide epitope covalently bound, directly or indirectly to at least one of the one of the one or more first polypeptide chemical conjugation sites or the one or more second polypeptide chemical conjugation sites, the WT-1 peptide epitope comprising four (4) or more contiguous amino acids of any of the WT-1 sequences set forth as SEQ ID NO.335, SEQ ID NO.336, SEQ ID NO.337, SEQ ID NO.338, or SEQ ID NO.339;

wherein each of the one or more MODs is an independently selected wild-type or variant MOD;
and

wherein the first or second polypeptide comprises an MHC-H polypeptide sequence having at least 85% sequence identity to 200-250 aas of an MHC-H chain polypeptide selected from the group consisting of: an HLA-A polypeptide of SEQ ID NO:35, an HLA-B polypeptide of SEQ ID NO: 43, an HLA-C polypeptide of SEQ ID NO 53, an HLA-E polypeptide of SEQ ID NO: 54, an HLA-F polypeptide of SEQ ID NO: 55, and an HLA-G polypeptide of SEQ ID NO:56.

4. A T-Cell-MMP-epitope conjugate comprising a T-Cell-MMP of any of claims 1-3, wherein the first and second MHC polypeptides are Class I MHC polypeptides, and the first MHC polypeptide comprises:

a beta-2-microglobulin ("β2M") polypeptide having an N-terminus and a C-terminus without a linker on its N-terminus and C-terminus,

a β2M polypeptide bearing a linker on its N-terminus,

a β2M polypeptide bearing a linker on its C-terminus, or

a β2M polypeptide bearing a linker on its N-terminus and C-terminus.

5. The T-Cell-MMP-epitope conjugate of claim 4, wherein the second polypeptide comprises: a second MHC polypeptide comprising a MHC Class I heavy chain ("MHC-H") polypeptide.

6. The T-Cell-MMP-epitope conjugate of claim 5, wherein the second polypeptide further comprises an immunoglobulin (Ig) Fc polypeptide or a non-Ig polypeptide scaffold.

7. The T-Cell-MMP-epitope conjugate of claim 6, wherein the T-Cell-MMP-epitope conjugate comprises one, two, or more independently selected wild-type and/or variant MOD polypeptides; wherein, if at least one variant MOD polypeptide is present, the variant MOD polypeptide exhibits a

reduced affinity to a Co-MOD (its Co-MOD) compared to the affinity of a corresponding wild-type MOD for the Co-MOD; and wherein the ratio of i) the binding affinity of a control T-Cell-MMP-epitope conjugate (where the control comprises a wild-type MOD) to the Co-MOD to ii) the binding affinity of a T-Cell-MMP-epitope conjugate of the present disclosure comprising a variant of the wild-type MOD to the Co-MOD, when measured by bio-layer interferometry ("BLI"), is at least 1.5:1 or in a range of from 1.5:1 to 10^6 :1.

8. The T-Cell-MMP-epitope conjugate of claim 7, wherein the wild-type MOD polypeptides are selected independently from the group consisting of IL-2, 4-1BBL, PD-L1, CD70, CD80, CD86, ICOS-L, OX-40L, FasL, JAG1, TGF- β , ICAM, and PD-L2, and the variant MOD polypeptides are variants thereof.

9. The T-Cell-MMP-epitope conjugate of any one of claims 1 to 3, wherein the first and second chemical conjugation sites are independently selected from:

- a) peptide sequences that act as enzymatic modification sequences;
- b) non-natural amino acids and/or selenocysteines;
- c) engineered amino acid chemical conjugation sites;
- d) carbohydrate or oligosaccharide moieties; and/or
- e) IgG nucleotide binding sites.

10. The T-Cell-MMP-epitope conjugate of claim 8, wherein the first and second chemical conjugation sites are independently selected from:

- a) peptide sequences that act as enzymatic modification sequences;
- b) non-natural amino acids and/or selenocysteines;
- c) engineered amino acid chemical conjugation sites;
- d) carbohydrate or oligosaccharide moieties; and/or
- e) IgG nucleotide binding sites.

11. The T-Cell-MMP-epitope conjugate of claim 10, wherein at least one chemical conjugation site to which the epitope is attached is a cysteine engineered into the β 2M polypeptide or as an amino acid of a linker at the N-terminus of the β 2M polypeptide.

12. The T-Cell-MMP-epitope conjugate of claim 11, wherein at least one chemical conjugation site to which the epitope is attached is a cysteine engineered into the β 2M polypeptide sequence of the T-Cell-MMP-epitope conjugate as an aa substitution selected from Q2C, E44C, E50C, E77C, V85V, S88C, K91C, and/or D98C; and wherein the β 2M polypeptide has a sequence with at least 85% sequence identity to at least 80 contiguous amino acids of a mature β 2M polypeptide set forth in any of SEQ ID NOs: 57-61.

13. The T-Cell-MMP-epitope conjugate of claim 11, wherein the epitope is a peptide, glycopeptide, lipopeptide, or phosphopeptide.

14. The T-Cell-MMP-epitope conjugate of claim 13, wherein the epitope is a peptide that comprises 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous amino acids of a protein set forth in section I.A.12.d.
15. The T-Cell-MMP-epitope conjugate of embodiment 14, wherein the epitope is a peptide of the Wilms tumor-1 (WT-1) protein isoform A, B, D, E, or F (SEQ ID NO:335 to SEQ ID NO:339).
16. The T-Cell-MMP-epitope conjugate of embodiment 15, wherein the peptide epitope is selected from the group consisting of: CMTWNQMNLGATLKG (SEQ ID NO:272), WNQMNLGATLKGVAA (SEQ ID NO:273), CMTWNYMNLGATLKG (SEQ ID NO:274), WNYMNLGATLKGVAA (SEQ ID NO: 275), MTWNQMNLGATLKG (SEQ ID NO:276), TWNMNLGATLKGVA (SEQ ID NO:277), CMTWNLMNLGATLKG (SEQ ID NO:279), MTWNLMNLGATLKG (SEQ ID NO:280), TWNLNMNLGATLKGVA (SEQ ID NO:281), WNLNMNLGATLKGVAA (SEQ ID NO:282), MNLGATLKG (SEQ ID NO:283), MTWNYMNLGATLKG (SEQ ID NO:284), TWNYMNLGATLKGVA (SEQ ID NO:285), CMTWNQMNLGATLKGVA (SEQ ID NO:286), CMTWNLMNLGATLKGVA (SEQ ID NO:287), CMTWNYMNLGATLKGVA (SEQ ID NO:288), GYLRNPTAC (SEQ ID NO:289), GALRNPTAL (SEQ ID NO:290), YALRNPTAC (SEQ ID NO:291), GLLRNPTAC (SEQ ID NO:292), NQMNLGATL (SEQ ID NO:293), RYRPHPGAL (SEQ ID NO:294), YQRPHPGAL (SEQ ID NO:295), RLRPHPGAL (SEQ ID NO:296), RIRPHPGAL (SEQ ID NO:297), QFPNHSFKHEDPMGQ (SEQ ID NO:298), HSFKHEDPY (SEQ ID NO:299), QFPNHSFKHEDPM (SEQ ID NO:300), QFPNHSFKHEDPY (SEQ ID NO:301), KRPFMCAYPGCK (SEQ ID NO:302), KRPFMCAYPGCKY (SEQ ID NO:303), FMCAYPGCKY (SEQ ID NO:304), FMCAYPGCK (SEQ ID NO:305), KRPFMCAYPGCKRY (SEQ ID NO:306), SEKRPFMCAYPGCK (SEQ ID NO:307), KRPFMCAYPGCKRY (SEQ ID NO:308), NLMNLGATL (SEQ ID NO:309), VLDFAPPGA (SEQ ID NO:310); RMFPNAPYL (SEQ ID NO:311); CMTWNQMN (SEQ ID NO:312); CYTWNQMNL (SEQ ID NO:313); NYMNLGATL (SEQ ID NO:314); YMFPNAPYL (SEQ ID NO:315); SLGEQQYSV (SEQ ID NO:316); and CMTWNQMNL (SEQ ID NO:317).
17. The T-Cell-MMP-epitope conjugate of embodiment 15, wherein the peptide epitope is selected from the group consisting of CMTWNQMN (SEQ ID NO:312); NYMNLGATL (SEQ ID NO:314) (WT-1 239-247; Q240Y); CYTWNQMNL (SEQ ID NO:313) (WT-1 235-243); CMTWNQMNL (SEQ ID NO:317) (WT-1 235-243); NQMNLGATL (SEQ ID NO:293 (WT-1 239-247); NYMNLGATL and (SEQ ID NO:314) (WT-1 239-247; Q240L).
18. The T-Cell-MMP-epitope conjugate of embodiment 15, wherein the MHC-H polypeptide comprises the sequence of HLA-A*2402, and the epitope is selected from the group consisting of NQMNLGATL (SEQ ID NO:293) (WT-1 239-247); CMTWNQMN (SEQ ID NO:312); NYMNLGATL (SEQ ID NO:314) (WT-1 239-247; Q240Y); CYTWNQMNL (SEQ ID NO:313) (WT-1 235-243); NLMNLGATL (SEQ ID NO:314) (WT-1 239-247; Q240L); and CMTWNQMNL (SEQ ID NO:317) (WT-1 235-243).

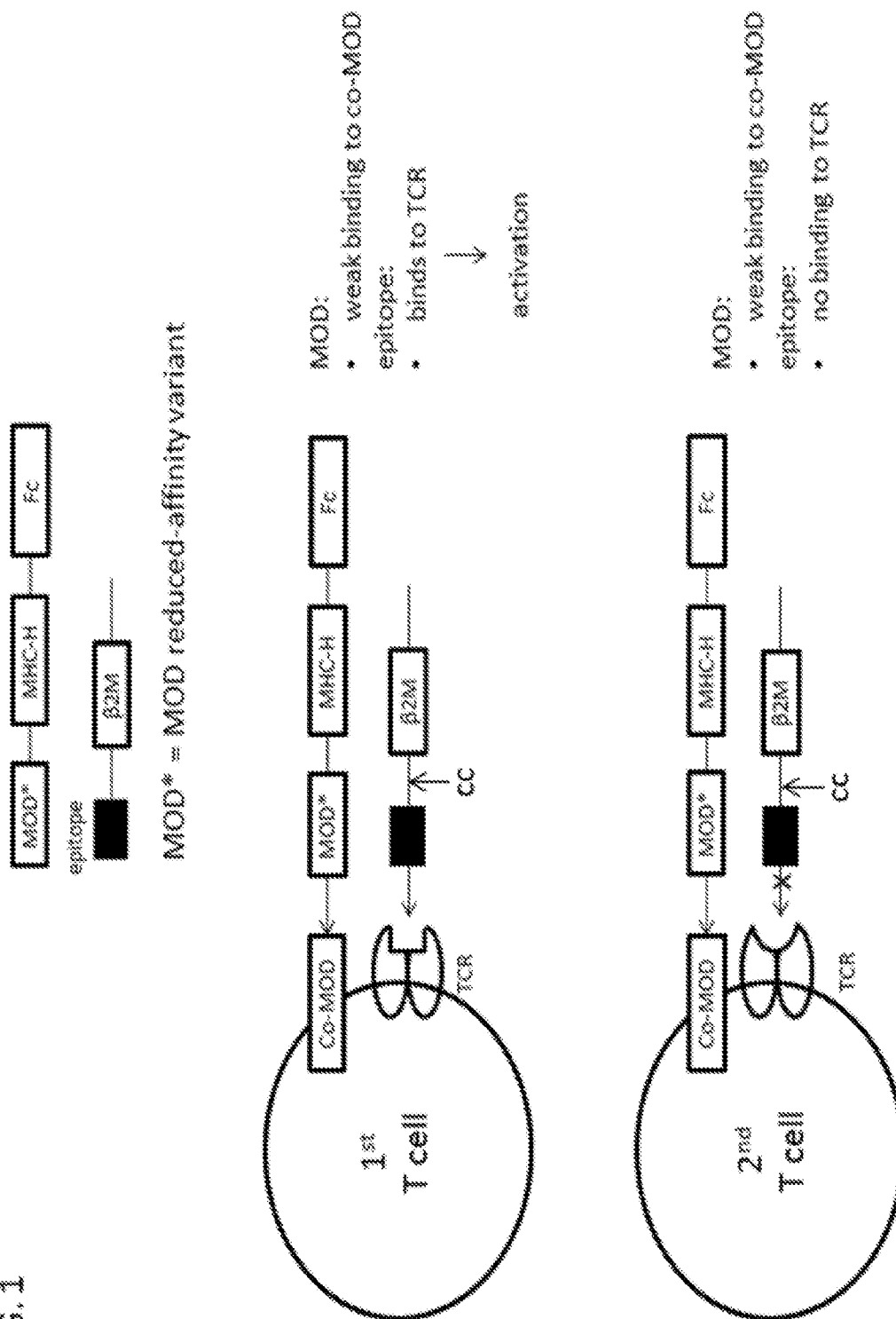
19. The T-Cell-MMP-epitope conjugate of embodiment 15, wherein the MHC-H polypeptide comprises the sequence of HLA-A*0201, and epitope is selected from the group consisting of NLMNLGATL (SEQ ID NO:309) (WT-1 239-247; Q240L); VLDFAPPGA (SEQ ID NO:310) (WT-1 37-45); RMFPNAPYL (SEQ ID NO:311) (WT-1 126-134); YMFPNAPYL (SEQ ID NO:315) (WT-1 126-134; R126Y); and SLGEQQYSV (SEQ ID NO:316) (WT-1 187-195).
20. The T-Cell-MMP-epitope conjugate of claim 15, wherein the epitope is conjugated via a linker peptide covalently bound to the epitope to the cysteine engineered into the β 2M polypeptide or the cysteine as an amino acid of a linker at the N-terminus of the β 2M polypeptide.
21. The T-Cell-MMP-epitope conjugate of claim 20, wherein the linker peptide covalently bound to the epitope comprises a maleimide reacted with the cysteine engineered into the β 2M polypeptide sequence as a Q2C, E44C, E50C, E77C, V85V, S88C, K91C, and/or D98C amino acid substitution.
22. A T-Cell-MMP-epitope conjugate of any one of claims 1 to 3, wherein the T-Cell-MMP-epitope conjugate has a structure selected from structure A, B, C, D, E, F, G, H, I, J, K, or L of FIG. 6.
23. The T-Cell-MMP-epitope conjugate of claim 22, wherein the second MHC polypeptide comprises an immunoglobulin (Ig) Fc polypeptide or a non-Ig polypeptide scaffold; and wherein the T-Cell-MMP-epitope conjugate forms a dimer through covalent or non-covalent bonds between the (Ig) Fc polypeptide or the non-Ig polypeptide scaffold.
24. A composition comprising:
- a) the T-Cell-MMP-epitope conjugate of claim 13; and
 - b) a pharmaceutically acceptable excipient.
25. The use of the T-Cell-MMP-epitope conjugate of claim 13 for the manufacture of a medicament for administering to an individual in need thereof an effective amount of the T-Cell-MMP-epitope conjugate.
26. The use of a T-Cell-MMP-epitope conjugate of claim 13 for the manufacture of a medicament for use in a method of delivering an immunomodulatory polypeptide (MOD) to a target T-cell in an epitope-selective or epitope-selective/specific manner *in vitro*, or to an individual *in vivo*, comprising:
- contacting the medicament with the T-Cell *in vitro*, or
 - administering the medicament to the individual;
- wherein the target T-cells are specific for the epitope present in the T-Cell-MMP-epitope conjugate.
27. A method of treating a patient or individual, the method comprising administering to the patient or individual an effective amount of the T-Cell-MMP-epitope conjugate of claim 13 or of a pharmaceutical composition comprising the T-Cell MMP-epitope conjugate of claim 13.

28. The method of claim 27, wherein the patient or individual is being treated for a WT-1-expressing cancer, wherein the cancer is selected from:

(i) 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), a non-small cell lung cancer (NSCLC), a myeloma, pancreatic cancer, colorectal cancer, a mesothelioma, a soft tissue sarcoma, a neuroblastoma, and/or a nephroblastoma; or

(ii) 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.

FIG. 1



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Figure 2A

***Homo sapiens* IgG1 Fc GenBank 3S7G_A (SEQ ID NO:1) 227 aa**

1 DKTHTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
 61 GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK
 121 GQPREPQVYT LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTTPVLDS
 181 DGSFFLYSKL TVDKSRWQQG NVFSCSVME ALHNHYTQKS LSLSPGK

***Homo sapiens* IgG2 Fc GenBank AAN76044 (amino acids 99-325) (SEQ ID NO:2) 227 aa**

1 STKGPSVFPL APCSRSTSES TAALGCLVKD YFPEPVTVSW NSGALTSGVH TTPAVLQSSG
 61 LYSLSSTVTV PSSNFGTQTY TCNVDHKPSN TKVDKIVERK CCVECPPCA PPVAGPSVFL
 121 FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VQFNWYVDGV EVHNAKTKPR EEQFNSTFRV
 181 VSVLTVVHQD WLNGKEYKCK VSNKGLPAPI EKTISKTKGQ PREPQVYTLF PSREEMTKNQ
 241 VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPMLDSDG SFFLYSKLTV DKSRWQQGNV
 301 FSCSVMEAL HNHYTQKSLS LSPGK

***Homo sapiens* IgG3 Fc GenBank AAW65947(amino acids 19-246) (SEQ ID NO:3) 238 aa**

1 HKPSNTKVDK RVELKTPLGD THTCPPCA PELLGGPSVF LFPPKPKDTL MISRTPEVTC
 61 VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC
 121 KVSNAKALPA IEKTISKAKG QPREPQVYTL PPSRDELTKN QVSLTCLVKG FYPSDIAVEW
 181 ESNGQPENNY KTTTPVLDSG GSFFLYSKLT VDKSRWQQGN VFSCSVMEAL LHNHYTQKSL
 241 SLSPGK

Figure 2B

***Homo sapiens* IgD Fc GenBank AAA52770 (amino acids 162-383) (SEQ ID NO:4) 222 aa**

1 PTKAPDVFP IISGCRHPKDN SPVVLACLIT GYHPTSVTVT WYMGTSQSPQ RTFPEIQRRD
 61 SYMTSSQLS TPLQQWRQGE YKCVVQHTAS KSKKEIFRWP ESPKAQASSV PTAQPQAEQS
 121 LAKATTAPAT TRNTGRGGEE KKKEKEKEEQ EERETKTPEC PSHTQPLGVY LLTPAVQDLW
 181 LRDKATFTCF VVGSDLKDAH LTWEVAGKVP TGGVEEGLLE RHSNGSQSQH SRLTLPRSLW
 241 NAGTSVTCTL NHPSLPPQRL MALREPAAQA PVKLSLNLAA SSDPPEAASW LLCEVSGFSP
 301 PNILLMWLED QREVNTSGFA PARPPPQRS TTFWAWSVLR VPAPPSPQPA TYTCVVSHE
 361 SRTLLNASRS LEVSYVTDHG PMK

***Homo sapiens* IgM Fc GenBank 0308221A (SEQ ID NO:5) 276 aa**

1 VTSTLTIKZS DWLGESMFTC RVDHRGLTFQ QNASMCVDP QDTAIRVFAI PPSFASIFLT
 61 KSTKLTLCLVT DLTITYBSVTI SWTREENGAV KTHTNISESH PNATFSAVGE ASICEDBDWS
 121 GERFTCTVTH TDLPSPLKQT ISRPKGVALH RPBVYLLPPA RZZLNLRESA TITCLVTGFS
 181 PADVFVEWMQ RGEPLSPQKY VTSAPMPEPQ APGRYFAHSI LTVSEEEWNT GGTYTCTVAH
 241 EALPNRVTER TVDKSTGKPT LYNVSLVMSD TAGTCY

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Figure 2C***Homo sapiens* IgA Fc GenBank P01876 (amino acids 120-353) (SEQ ID NO:6) 234 aa**

1 ASPTSPKVFP LSLCSTQPDG NVVIACLVQG FFPQEPLSVT WSESGQGVTA RNFPPSQDAS
 61 GDLYTTSSQL TLPATQCLAG KSVTCHVKHY TNPSQDVTVP CPVPSTPPTP SPSTPPTPSP
 121 SCCHPRLSLH RPALEDLLLG SEANLTCTLT GLRDASGVTF TWTPSSGKSA VQGPPERDLC
 181 GCYSVSSVLP GCAEPWNHGK TFTCTAAYPE SKTPLTATLS KSGNTRPEV HLLPPPSEEL
 241 ALNELVT LTC LARGFSPKDV LVRWLQGSQE LPREKYLTWA SRQEPSQGT TFAVTSILRV
 301 AAEDWKKGDT FSCMVGHEAL PLAFTQKTID RLAGKPTHVN VSVVMAEVDG TCY

***Homo sapiens* IgE Fc GenBank 1F6A_B (amino acids 6-222) (SEQ ID NO:7) 212 aa**

1 ADPCDSNPRG VSAYLSRPSP FDLFIRKSPT ITCLVVDLAP SKGTVNLTWS RASGKPVNHS
 61 TRKEEKQRNG TLTVTSTLPV GTRDWIEGET YQCRVTHPHL PRALMRSTTK TSGPRAAPEV
 121 YAFATPEWPG SRDKRTLACL IQNFMPEDIS VQWLHNEVQL PDARHSTTQP RKTKGSGFFV
 181 FSRLEVTRAE WEQKDEFICR AVHEAASPSQ TVQRAVSVNP GK

***Homo sapiens* IgG4 Fc GenBank P01861 (amino acids 100-327) (SEQ ID NO:8) 228 aa**

1 ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS
 61 GLYSLSSVVT VPSSSLGTKT YTCNVDHKPS NTKVDKRVES KYGPPCPSCP APEFLGGPSV
 121 FLFPPKPKDT LMISRTPEVT CVVVDVSQED PEVQFNWYVD GVEVHNAKTK PREEQFNSTY
 181 RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS SIEKTISKAK GQPREPQVYT LPPSQEEMTK
 241 NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTTPVLD DGSFFLYSRL TVDKSRWQEG
 301 NVFSCSVME ALHNHYTQKS LSLSLGK

***Homo sapiens* IgG4 Fc Segment (SEQ ID NO:9) 223 aa**

1 PPCPSCPAPPE FLGGPSVFLF PPKPKDTLMI SRTPEVTCVV VDVSDQEDPEV QFNWYVDGVE
 61 VHNAKTKPRE EQFNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKGLPSSIE KTISKAKGQP
 121 REPQVYTLPP SQEEMTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT TTPVLDSDGS
 181 FFLYSRLTVD KSRWQEGNVF SCSVMHEALH NHYTQKSLSL SPG

FIG. 2D***WT Human IgG1 Fc Sequence (SEQ ID NO:10)***

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
 HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPEIEKTISKAKGQPREPQV
 YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV
 DKSRWQQGNVFSVMHEALHNHYTQKSLSLSPGK

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FIG. 2E***Human IgG1 Fc Mutant: L234F/L235E/P331S (Triple Mutant "TM") (SEQ ID NO:11)***

DKTHTCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPSIEKTISKAKGQPREPQV
YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV
DKSRWQQGNVFSQVMHEALHNHYTQKSLSLSPGK

FIG. 2F***Human IgG1 Fc Mutant: N297A (SEQ ID NO:12)***

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV
YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV
DKSRWQQGNVFSQVMHEALHNHYTQKSLSLSPGK

FIG. 2G***Human IgG1 Fc Mutant: L234A/L235A ("LALA") (SEQ ID NO:13)***

DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV
YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV
DKSRWQQGNVFSQVMHEALHNHYTQKSLSLSPGK

Residue numbered according to EU index (Kabat Numbering)

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Fig. 3A *Homo sapiens* HLA-A**3A.1 *Homo sapiens* HLA-A*01:01:01:01 NCBI (National Center for Biotechnology Information) Accession NP_001229687.1 (SEQ ID NO:14)**

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1  MAVMAPRTLL LLLSGALALT QTWAGSHSMR YFFTSVSRPG RGEPRFIAVG YVDDTQFVRF
61 DSDAASQKME PRAPWIEQEG PEYWDQETRN MKAHSQTDRA NLGTLRGYYN QSEDGSHTIQ
121 IMYGCDVGPD GRFLRGYRQD AYDGKDYIAL NEDLRSWTAA DMAAQITKRK WEAVHAAEQR
181 RVYLEGRGCV DGLRRYLENGK ETLQRTDPPK THMTHHPISD HEATLRCWAL GFYPAEITLT
241 WQRDGEDQTQ DTELVEPTRPA GDGTFQKWAA VVVPSGEEQR YTCHVQHEGL PKPLTLRWEL
301 SSQPTIPIVG ILAGLVLLGA VITGAVVAAV MWRRKSSDRK GGSYTAQASS DSAQGSVDVSL
361 TACKV

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3A.2 *Homo sapiens* HLA-A*1101 NCBI Accession P13746.1 (SEQ ID NO:15)

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1  MAVMAPRTLL LLLSGALALT QTWAGSHSMR YFYTSVSRPG RGEPRFIAVG YVDDTQFVRF
61 DSDAASQRME PRAPWIEQEG PEYWDQETRN VKAQSQTDRV DLGTLRGYYN QSEDGSHTIQ
121 IMYGCDVGPD GRFLRGYRQD AYDGKDYIAL NEDLRSWTAA DMAAQITKRK WEAHAAAEQQ
181 RAYLEGRGCV WLRRYLENGK ETLQRTDPPK THMTHHPISD HEATLRCWAL GFYPAEITLT
241 WQRDGEDQTQ DTELVEPTRP GDGTFQKWAA VVVPSGEEQR YTCHVQHEGL PKPLTLRWEL
301 SSQPTIPIVG ILAGLVLLGA VITGAVVAAV MWRRKSSDRK GGSYTAQASS DSAQGSVDVSL
361 TACKV

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3A.3 *Homo sapiens* HLA-A*2402 NCBI Accession P05534.2 (SEQ ID NO:16)

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1  MAVMAPRTLV LLLSGALALT QTWAGSHSMR YFSTSVSRPG RGEPRFIAVG YVDDTQFVRF
61 DSDAASQRME PRAPWIEQEG PEYWDEETGK VKAHSQTDRE NLRIALRYYN QSEAGSHTLQ
121 MMFGCDVGSD GRFLRGYHQY AYDGKDYIAL KEDLRSWTAA DMAAQITKRK WEAHVAEQQ
181 RAYLEGTCVD GLRRYLENGK ETLQRTDPPK THMTHHPISD HEATLRCWAL GFYPAEITLT
241 WQRDGEDQTQ DTELVEPTRP GDGTFQKWAA VVVPSGEEQR YTCHVQHEGL PKPLTLRWEP
301 SSQPTVPIVG ILAGLVLLGA VITGAVVAAV MWRRNSSDRK GGSYSQAASS DSAQGSVDVSL
361 TACKV

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3A.4 *Homo sapiens* HLA-A*3303 NCBI Accession AAA79865.1 SEQ ID NO:17

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1  MAVMAPRTLL LLLLGALALT QTWAGSHSMR YFTTSVSRPG RGEPRFIAVG YVDDTQFVRF
61 DSDAASQRME PRAPWIEQEG PEYWDNRNTRN VKAHSQIDRV DLGTLRGYYN QSEAGSHTIQ
121 MMYGCDVGSD GRFLRGYQQD AYDGKDYIAL NEDLRSWTAA DMAAQITQRK WEAAARVAEQL
181 RAYLEGTCVE WLRRYLENGK ETLQRTDPPK THMTHHAVSD HEATLRCWAL SFYPAEITLT
241 WQRDGEDQTQ DTELVEPTRP GDGTFQKWA VVVPSGQEQY YTCHVQHEGL PKPLTLRWEP
301 SSQPTIPIVG ILAGLVLFGA VFAGAVVAAV RWRRKSSDRK GGSYSQAASS DSAQGSMSL
361 TACKV

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Fig. 3B

***Homo sapiens* HLA-B*07:02:01 GenBank Accession NP_005505.2 Amino Acids 25-362 (SEQ ID NO:18)**

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1  MLVMAPRTVL LLLSAALALT ETWAGSHSMR YFYTSVSRPG RGEPRFISVG YVDDTQFVRF
61 DSDAASPREE PRAPWIEQEG PEYWDNRNTQI YKAQAQTDRE SLRNLRGYYN QSEAGSHTLQ
121 SMYGCDVGPD GRLLRGHDQY AYDGKDYIAL NEDLRSWTAA DTAAQITQRK WEAAREAEQR
181 RAYLEGECVE WLRRYLENGK DKLERADPPK THVTHHPISD HEATLRCWAL GFYPAEITLT
241 WQRDGEDQTQ DTELVEPTRPA GDRTFQKWAA VVVPSGEEQR YTCHVQHEGL PKPLTLRWEP
301 SSQSTVPIVG IVAGLAVLAV VVIGAVVAAV MCRRKSSGGK GGSYSQAACS DSAQGSVDVSL
361 TA

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Fig. 3C *Homo sapiens* HLA-C GenBank Accession NP_001229971.1, Amino acids 25-366 (SEQ ID NO:19)

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1  MRVMAPRALL LLLSGGLALT ETWACSHSMR YFDTAVSRPG RGEPRFISVG YVDDTQFVRF
61 DSDAASPRGE PRAPWVEQEG PEYWDRETQN YKRQAQADRV SLRNLRGYYN QSEDGSHTLQ
121 RMYGCDLGPD GRLLRGYDQS AYDGKDYIAL NEDLRSWTAA DTAAQITQRK LEAARAAEQLE
181 RAYLEGTCEVE WLRRLYENGK ETLQRAEPPK THVTHHPLSD HEATLRCWAL GFYPAEITLT
241 WORDGEDQTQ DTELVETRPA GDGTFQKWAA VVVPSCQEQR YTCHMQHEGL QEPLTSLWEP
301 SSQPTIPIMG IVAGLAVLVV LAVLGAVVTA MMCRKSSGG KGGSCSQAAC SNSAQGSDES
361 LITCK

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Fig. 3D.

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HLA-A      GSHSMRYFFTSVSRPGRGEPRFIAVG YVDDTQFVRFSDAASQKMEPRAPWIEQEGPEYW
HLA-B      GSHSMRYFFTSVSRPGRGEPRFISVG YVDDTQFVRFSDAASQKMEPRAPWIEQEGPEYW
HLA-C      GSHSMRYFFTSVSRPGRGEPRFISVG YVDDTQFVRFSDAASQKMEPRAPWIEQEGPEYW
HLA-A*0201 GSHSMRYFFTSVSRPGRGEPRFIAVG YVDDTQFVRFSDAASQKMEPRAPWIEQEGPEYW
Mouse H2K  GPHSLRYFVTAVSRPGLGEPRFIAVG YVDDTQFVRFSDAASQKMEPRAPWIEQEGPEYW
HLA_A (var. 2) GSHSMRYFFTSVSRPGRGEPRFIAVG YVDDTQFVRFSDAASQKMEPRAPWIEQEGPEYW
HLA_A (var. 2C) GSHSMRYFFTSVSRPGRGEPRFIAVG YVDDTQFVRFSDAASQKMEPRAPWIEQEGPEYW
HLA-A(var.2CP) GSHSMRYFFTSVSRPGRGEPRFIAVG YVDDTQFVRFSDAASQKMEPRAPWIEQEGPEYW
HLA-A*1101  GSHSMRYFFTSVSRPGRGEPRFIAVG YVDDTQFVRFSDAASQKMEPRAPWIEQEGPEYW
HLA-A*2402  GSHSMRYFFTSVSRPGRGEPRFIAVG YVDDTQFVRFSDAASQKMEPRAPWIEQEGPEYW
HLA-A*3303  GSHSMRYFFTSVSRPGRGEPRFIAVG YVDDTQFVRFSDAASQKMEPRAPWIEQEGPEYW
          **;*** *:***** *****;***** . : *****;*****

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HLA-A      DQETRMNKAHSQTDRANLSTLRGYNQSEAGSHTIQIMYGCDVSPDGRFLRGYRQDAYDG
HLA-B      DRNTQIYKAQAQTDRESLNLRGYNQSEAGSHTLQSMYGCDFGPDGRLLRGYHQAAYDG
HLA-C      DRETQNYKRAQAQADRVSLNLRGYNQSEAGSHTLQRMYGCDLGPDGRLRGYDQSAAYDG
HLA-A*0201 DGETRKVKKAHSQTHRVDLSTLRGYNQSEAGSHTVQRMYGCDVGSDFWFLRGYHQAAYDG
MOUSE H2K  EEQTQRAKSDQWFRVSLRTAQRNLNQSEAGSHTVQRMFGCDVGSDFWFLRGYHQAAYDG
HLA_A (var. 2) DGETRKVKKAHSQTHRVDLSTLRGYNQSEAGSHTVQRMYGCDVGSDFWFLRGYHQAAYDG
HLA_A (var. 2C) DGETRKVKKAHSQTHRVDLSTLRGYNQSEAGSHTVQRMYGCDVGSDFWFLRGYHQAAYDG
HLA-A (var.2CP) DGETRKVKKAHSQTHRVDLSTLRGYNQSEAGSHTVQRMYGCDVGSDFWFLRGYHQAAYDG
HLA-A*1101  DQETRMNKAHSQTDRVLDLSTLRGYNQSEAGSHTIQIMYGCDVSPDGRFLRGYRQDAYDG
HLA-A*2402  DEETGKVKKAHSQTDRENIALALNQSEAGSHTLQMMFGCDVGSDFGFLRGYHQAAYDG
HLA-A*3303  DRNTRNVKAHSQILFVDLSTLRGYNQSEAGSHTIQMMYGCDVGSDFGFLRGYHQAAYDG
          : : * * . * * . * *****; *****; *****; *****; *****

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aac1 aac2

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HLA-A      KDYIALNEDLRSWTAADMAQITTKWEAAHVAEQRRAYLEGCVEWLRRLYENGKETLQ
HLA-B      KDYIALNEDLRSWTAADMAQITTKWEAAHVAEQRRAYLEGCVEWLRRLYENGKETLQ
HLA-C      KDYIALNEDLRSWTAADMAQITTKWEAAHVAEQRRAYLEGCVEWLRRLYENGKETLQ
HLA-A*0201 KDYIALNEDLRSWTAADMAQITTKWEAAHVAEQRRAYLEGCVEWLRRLYENGKETLQ
MOUSE H2K  RDYIALNEDLKTWTAADMAQITTKWEAAHVAEQRRAYLEGCVEWLRRLYENGKETLQ
HLA_A (var. 2) KDYIALKEDLRSWTAADMAQITTKWEAAHVAEQRRAYLEGCVEWLRRLYENGKETLQ
HLA_A (var. 2C) KDYIALKEDLRSWTAADMAQITTKWEAAHVAEQRRAYLEGCVEWLRRLYENGKETLQ
HLA-A (var.2CP) KDYIALKEDLRSWTAADMAQITTKWEAAHVAEQRRAYLEGCVEWLRRLYENGKETLQ
HLA-A*1101  KDYIALNEDLRSWTAADMAQITTKWEAAHVAEQRRAYLEGCVEWLRRLYENGKETLQ
HLA-A*2402  KDYIALKEDLRSWTAADMAQITTKWEAAHVAEQRRAYLEGCVEWLRRLYENGKETLQ
HLA-A*3303  KDYIALNEDLRSWTAADMAQITTKWEAAHVAEQRRAYLEGCVEWLRRLYENGKETLQ
          : *****;*****;***** * :;***** * * *****;*****;*****

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aac3 aac4

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Fig. 3D. Continued

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HLA-A	RTDPPKTHMTNHFISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTEL	VETRP	SDGTF
HLA-B	RADPPKTHVTNHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTEL	VETRP	SDRTF
HLA-C	RAEPPKTHVTNHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTEL	VETRP	SDGTF
HLA-A*0201	RTDAPKTHMTNHAIVSDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTEL	VETRP	SDGTF
MOUSE H2K	RTDSEKAVTYTHFERSQVDVTLRCWALGFYPADITLTWQLNGEHLTQDMEL	VETRP	SDGTF
HLA_A (var.2)	RTDAPKTHMTNHAIVSDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTEL	VETRP	SDGTF
HLA_A (var.2C)	RTDAPKTHMTNHAIVSDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTEL	VETRP	SDGTF
HLA-A(var.2CP)	RTDAPKTHMTNHAIVSDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTEL	VETRP	SDGTF
HLA-A*1101	RTDPPKTHMTNHFISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTEL	VETRP	SDGTF
HLA-A*2402	RTDPPKTHMTNHFISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTEL	VETRP	SDGTF
HLA-A*3303	RTDPPKTHMTNHAIVSDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTEL	VETRP	SDGTF

*:: **::**::* *; 2.*****.****;***** :*** ** ***** ** **

aac5	aac6
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HLA-A	QKWAAVVVPSGEEQRYTCHVQHEGLFKPLTLRWE
HLA-B	QKWAAVVVPSGEEQRYTCHVQHEGLFKPLTLRWE
HLA-C	QKWAAVVVPSGEEQRYTCHVQHEGLFKPLTLRWE
HLA-A*0201	QKWAAVVVPSGEEQRYTCHVQHEGLFKPLTLRWE
MOUSE H2K	QKWAAVVVPLGKEQNYTCHVHNGLPEDTLRW
HLA_A (var.2)	QKWAAVVVPSGEEQRYTCHVQHEGLFKPLTLRWE
HLA_A (var.2C)	QKWAAVVVPSGEEQRYTCHVQHEGLFKPLTLRWE
HLA-A(var.2CP)	QKWAAVVVPSGEEQRYTCHVQHEGLFKPLTLRWE
HLA-A*1101	QKWAAVVVPSGEEQRYTCHVQHEGLFKPLTLRWEL
HLA-A*2402	QKWAAVVVPSGEEQRYTCHVQHEGLFKPLTLRWE
HLA-A*3303	QKWAASVVPSGEEQRYTCHVQHEGLFKPLTLRWE

****;**** *:***,****;*:*** :**** *

HLA-A*0101, SEQ ID NO:20; HLA-B*0702, SEQ ID NO:21; HLA-C, SEQ ID NO:22; HLA-A*0201, SEQ ID NO:23; a mouse H2K protein sequence, SEQ ID NO:24; three variants of HLA-A (var.2, var. 2C, and var.2CP, SEQ ID NOs:25, 26, and 27); (HLA-A*1101 (HLA-A11), SEQ ID NO:28; HLA-A*2402 (HLA-A24), SEQ ID NO:29; HLA-A*3303 (HLA-A33), SEQ ID NO:30.

[illegible]

HLA-A*0101, SEQ ID NO:20; HLA-A*0201, SEQ ID NO:23; HLA-A*0301, SEQ ID NO:31; HLA-A*1101, SEQ ID NO:28; HLA-A*2301, SEQ ID NO:32; HLA-A*2402, SEQ ID NO:29; HLA-A*2407, SEQ ID NO:33; HLA-A*3303, SEQ ID NO:30; and HLA-A*3401, SEQ ID NO:34

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FIG. 3E (continued) HLA-A ALLELE CONSENSUS SEQUENCE

GSHSMRYFX1TSVSRPGRGEPRFIAVGIVDDTQFVRFSDAASQX2MEPRAPWIEQEGPEYWDX
3X4TX5X6X7KAX8SQX9X10RX11X12LX13X14X15X16X17YYNQSEXX18GSHTX19QX20
MX21GCDVGX22DX23RFLRGYX24QX25AYDGKDYIALX26EDLRSWTAADMAAAQX27TX287
X29KWEXX30X31X32EAEQX33RX34YLX35GX36CVX37X38LRRLYLENGKETLQRTDX39PK
 THMTHHX40X41SDHEATLRCWALX42FYPAEITLTWQRDGEDQTQDTELVETRPAGDGTFQKW
AX43VVVPSGX44EQRYTCHVQHEGLPKPLTLRWEXX45

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.

SEQ ID NO:35;

HLA-B*0702, SEQ ID NO:36; HLA-B*0801, SEQ ID NO:37; HLA-B*1502, SEQ ID NO:38; HLA-B*3802, SEQ ID NO:39; HLA-B*4001, SEQ ID NO:40; HLA-B*4601, SEQ ID NO:41; and HLA-B*5301, SEQ ID NO:42;

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FIG. 3F (continued) HLA-B ALLELE CONSENSUS

GSHSMRYFX1TX2X3SRPGRGEPREX4VGYVDDTX5FVRFDSDAX6SPRX7X8PRAPWIEQEG
 PEYWDRX9TQX10X11KTX12X13TQX14YX15X16NLX17X18X19X20YYNQSEAGSHX21X
22QX23MYGCDLGPDGRLLRGHDQSAYDGKDYIALNEDLX24SWTAADTAAQIX25QRKX26EA
 ARX27AEQX28RX29YLEGX30CVEWLRRYLENGKX31X32LX33RADPPKTHVTHHPX34SDH
 EATLRCWALGFYPAEITLTWQRDGEDQTQDTELVEPTRAGDRTFQKWAAVVVP SGEEQRYTCHV
 QHEGLPKPLTLRWEF

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.

SEQ ID NO:43;

SUBSTITUTE SHEET (RULE 26)

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FIG. 3G (continued)

HLA-C*0102, SEQ ID NO:44; HLA-C*0303, SEQ ID NO:45; HLA-C*0304, SEQ ID NO:46; HLA-C*0401, SEQ ID NO:47; HLA-C*0602, SEQ ID NO:48; HLA-C*0701, SEQ ID NO:49; HLA-C*0702, SEQ ID NO:50; HLA-C*0801, SEQ ID NO:51; and HLA-C*1502, SEQ ID NO:52

HLA-C ALLELE CONSENSUS

X1SHSMX2YFX3TAVSX4PGRGEPX5FIX6VG YVDDTQFVX7FDSDAASPRGEPRX8PWVEQEG
 PEYWDRETQX9YKRQAQX10DRVX11LRX12LRGYYNQSEX13X14SHX15X16QX17MX18GC
 DX19GPDGRLLRGX20X21QX22AYDGKD YIALNEDLRSWTAADTAAQITQRKX23EAARX24A
 EQX25RAYLEGX26CVEWLRRYLX27NGKX28TLQRAEX29PKTHVTHHPX30SDHEATLRCWA
 LGFYPAEITLTWQX31DGEDQTQDTELVETRPAGDGTFQKWA AVX32VPSGX33EQRYTCHX34
 QHEGLX35EPLTLX36WX37P

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.

SEQ ID NO:53

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FIG. 3H - HLA-E, HLA-F, and HLA-G ALLELE CONSENSUS SEQUENCES

SEQUENCE				
HLA-E				
GSHSLKYFHT	SVSRPGRGEP	RFISVGYVDD	TQFVRFDNDA	ASPRMVPRAP
WMEQEGSEYW	DRETRSARDT	AQIFRVNLRT	LRG <u>Y</u> YNQ <u>S</u> X1 A	GSHTLQWMHG
CELGPD <u>X2</u> RFL	RGYEQFAYDG	KDYLTILNEDL	RSWTAVDT <u>AA</u>	QISEQKSND
SEAEHQ <u>X3X4</u> YL	EDTCVEWLHK	YLEKGKETLL	HLEPPKTHVT	HHPISDHEAT
LRCWALGFYP	AEITLTWQOD	GEGHTQDTEL	VETRP <u>AGDGT</u>	FQKWAAVVVP
SGEE <u>X5</u> RYTCH	VQHEGL <u>X6</u> EPV	TLRWKPASQP	TIP	
X1= K or E; X2= R or G; X3= R or G; X4= A or V; X5= Q or P; and X6= P or S SEQ ID NO:54				
Encompasses: 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; HLA-E*01:10				
HLA-F				
GSHSLR <u>X1</u> FST	AVSRPGRGEP	RYIAVEYVDD	TQFLRFDSDA	AIPRMEPRE <u>X2</u>
WVEQEGPQYW	EWTTGYAKAN	AQTDRVALRN	LLR <u>R</u> YNQSEA	GSHTLQGMNG
CDMGPDGRLL	RGYHQHAYDG	KDYISLNEDL	RSWTAADT <u>VA</u>	QITQRFYEA
EYAEERFTYL	EGECLELLRR	YLENGKETLQ	RADPPKAHVA	HHPISDHEAT
LRCWALGFYP	AEITLTWQOD	GEEQTQDTEL	VETRP <u>AGDGT</u>	FQKWAAVVVP
<u>X3</u> GEEQRYTCH	VQHEGLPQPL	ILRWEQ <u>S</u> X4 QP	TIP	
X1= Y or F; X2= P or Q; X3= S or P; and X4= P or L SEQ ID NO:55				
Encompasses: 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; HLA-F*01:06;				
HLA-G				
GSHSMRYFSA	AV <u>X1</u> RPGRGEP	RFIAMG <u>X2</u> VDD	<u>X3</u> QF <u>X4</u> RFDSDS	ACPRMEPRAP
WVE <u>X5</u> EGPEYW	EEETRNTKAH	AQTDRMNLQT	<u>X6</u> RG <u>Y</u> YNQSEA	
SSHTLQWM <u>X7</u>	CDL <u>X8X9</u> DGRL <u>X10</u>	RGYEQYAYDG	KDYLA LNEDL	
RSWTAADT <u>AA</u>	QISKRKCEAA	NVAEQRRAX <u>X11</u> L	EGTCVEWL <u>X12</u> R	
<u>X13</u> LENGKE <u>X14</u> LQ	RADP <u>X15</u> KTHVT	HHPVFDYEAT	LRCWALGFYP	
AEIILTQW <u>X16</u> D	GEDQTQDVEL	VETRP <u>AGDGT</u>	FQKWAAVVVP	SGEEQRY <u>X17</u> CH
VQHEGLPEPL	MLRW <u>X18</u> QSSLP	TIP		
X1= S or F; X2= Y or H; X3= T, S, or M; X4= L or V; X5= Q or R; X6= P or L; X7= G or D; X8= G or V; X9= S or C; X10= L or I; X11= Y or H; X12= H or R; X13= Y or H; X14= M or T; X15= P or A; X16= R, W, or Q; X17= T or M; X18= K or E; SEQ ID NO:56				
Encompasses: 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; HLA-G*01:22				

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FIG. 3I

CONSENSUS SEQUENCE ALIGNMENT

HLA-A	GSHSMRYFXTSVSRPGRGEPFRFIAVGYVDDTQFVRFDSDAASQXMEPRAPWIEQEGPEYW	60
HLA-B	GSHSMRYFXTXXSRPGRGEPFRFIXVGYVDDTQFVRFDSDAASPRXXPRAPWIEQEGPEYW	60
HLA-C	XSHSMXYFXTAVSXPGRGEPFXFIXVGYVDDTQFVXFDSDAASPRGEPXPWPVEQEGPEYW	60
HLA-E	GSHSLKYFHTSVSRPGRGEPFRFISVGYVDDTQFVRFDNDAASPRMVPRAPWMEQEGSEYW	60
HLA-F	GSHSLRXFSTAVSRPGRGEPFRYIAVEYVDDTQFLRFDSDAALPRMEPREXWVEQEGPQYW	60
HLA-G	GSHSMRYFSAAVXRPGRGEPFRFIAMGXVDDXQFXRFDSDSACPRMEPRAPWVEXEGPEYW	60
	: * : ** : * : *** * *.*: ** *: * ** : **	
84		
HLA-A	DXXTXXXKAXSQXXRXIXXXXXYINQSEKXSHTXQXMXGCDVGXDXRFLRGYXQXAYDG	120
HLA-B	DRXTQXXKTXXTQXYXXNLXXXXYINQSEAGSHXXQXMYGCDLGPDRLLRGHDQSAYDG	120
HLA-C	DRETQXYKRQAQXDRVXILRXLRGYINQSEKXSHXXQXMXGCDXGPDGRLLRGXXQXAYDG	120
HLA-E	DRETRSARDTAQIFRVNIRTLRGYINQSKAGSHTLQWMHGCELGPDXRFLRGYEQFAYDG	120
HLA-F	EWTGTGYAKANAQTDRAIRNLLRYINQSEAGSHTLQGMNGCDMGPDRLLRGYHQHAYDG	120
HLA-G	EEETRNTKAHAQTDRMNIQTXRGYINQSEASSHTLQWMIXCDLXXDGRLLRGYEQYAYDG	120
	: * : ***** ** * * *: * *: ** * *****	
	aac1 aac2	
139		
HLA-A	KDYIALXEDLRSWTAADMAAQXTXKKWEXXXEAEQXRXYLXGXCVXXLRRYLENGKETLQ	180
HLA-B	KDYIALNEDLRSWTAADIAAQIXQRKXEAARXAEQXRXYLEGXCVEWLRRYLENGKXXLX	180
HLA-C	KDYIALNEDLRSWTAADIAAQITQRKXEAARXAEQXRXYLEGXCVEWLRRYLNKGKXTLQ	180
HLA-E	KDYLTLNEDLRSWTAVDTAAQISEQKSNDASEAHHQXXYLEDTCEVWLHKYLEKGKETLL	180
HLA-F	KDYISLNEDLRSWTAADTVAQITQRFYEAEEYAEFRTYLEGECLELLRRYLENGKETLQ	180
HLA-G	KDYIALNEDLRSWTAADIAAQISKRKCEAANVAEQRRAXLEGTCVEWLXXRLENGKEXLQ	180
	***: * ** * ** * * : ** . * . *: * : * : ** *	
	aac3 aac4	
236		
HLA-A	RTDXPKTHMTHHXXSDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTEIVETRPAGDGTG	241
HLA-B	RADPPKTHVTHHPXSDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTEIVETRPAGDRTG	241
HLA-C	RAEXPKTHVTHHPXSDHEATLRCWALGFYPAEITLTWQXDGEDQTQDTEIVETRPAGDGTG	241
HLA-E	HLEPPKTHVTHHPISDHEATLRCWALGFYPAEITLTWQDGEDGTQDTEIVETRPAGDGTG	241
HLA-F	RADPPKAHVHHPISDHEATLRCWALGFYPAEITLTWQRDGEETQDTEIVETRPAGDGTG	241
HLA-G	RADPKXTHVTHHPVFDYEATLRCWALGFYPAEIILTQXDGEDQTQDVEIVETRPAGDGTG	241
	: : *: *: ** *: ***** ***** ***** ** : ** . ** ***** ** *	
	ac5 aac6	
HLA-A	QKWAXVVVPSGXEQRYTCHVQHEGLPKPLTLRWEX-----	276
HLA-B	QKWAAVVVPSGEEQRYTCHVQHEGLPKPLTLRWEP-----	276
HLA-C	QKWAADVVPXGXEQRYTCHVQHEGLXEPPLTLXWXP-----	276
HLA-E	QKWAADVVPXGEEQRYTCHVQHEGLXEPVTLRWKPPASQPTIPI	284
HLA-F	QKWAADVVPXGEEQRYTCHVQHEGLPQPLILRWEXSXQPTIPI	284
HLA-G	QKWAADVVPXGEEQRYXCHVQHEGLPEPLMLRWXQSSLPTIPI	284
	***** * * * * * * * * * * : * *	

FIG. 4

NP_004039.1	MSRSVALAVLALLSLSGLEAIQRTPKIQVYSRHPAENGKSNFLNCYVSGHFPSDIEVDLL	60
NP_001009066.1	MSRSVALAVLALLSLSGLEAIQRTPKIQVYSRHPAENGKSNFLNCYVSGHFPSDIEVDLL	60
NP_001040602.1	MSRSVALAVLALLSLSGLEAIQRTPKIQVYSRHPAENGKSNFLNCYVSGHFPSDIEVDLL	60
NP_776318.1	MAKFAVALLGLLSLGLDAIQRPPIQVYSRHPPEDEGKPNYLNLCYVYGFHPPQIEIDL	60
NP_033865.2	MARSVTLVFLVLSLGLYAIQRTFQIQVYSRHPPEDEGKPNYLNLCYVYGFHPPHIEIQML	60
	:	
NP_004039.1	KNGERIEKVEHSDLSFSKDWSFYLLYYTEFTPTTEKDEYACRVNHVTLSQLPKIVKWRDM	119
NP_001009066.1	KNGERIEKVEHSDLSFSKDWSFYLLYYTEFTPTTEKDEYACRVNHVTLSQLPKIVKWRDM	119
NP_001040602.1	KNGERIEKVEHSDLSFSKDWSFYLLYYTEFTPTTEKDEYACRVNHVTLSQLPKIVKWRDM	119
NP_776318.1	KNGERT-KSEQSDLSFSKDWSFYLLSHAEFTENSKIQYSCRVKHVTLSQLPKIVKWRDL	118
NP_033865.2	KNGKKIPKVENMSDMSFSKDWSFYLLAHTEFTPTTEDTYACRVKHASMAEPKTVYNDRDM	119
	***:::*	

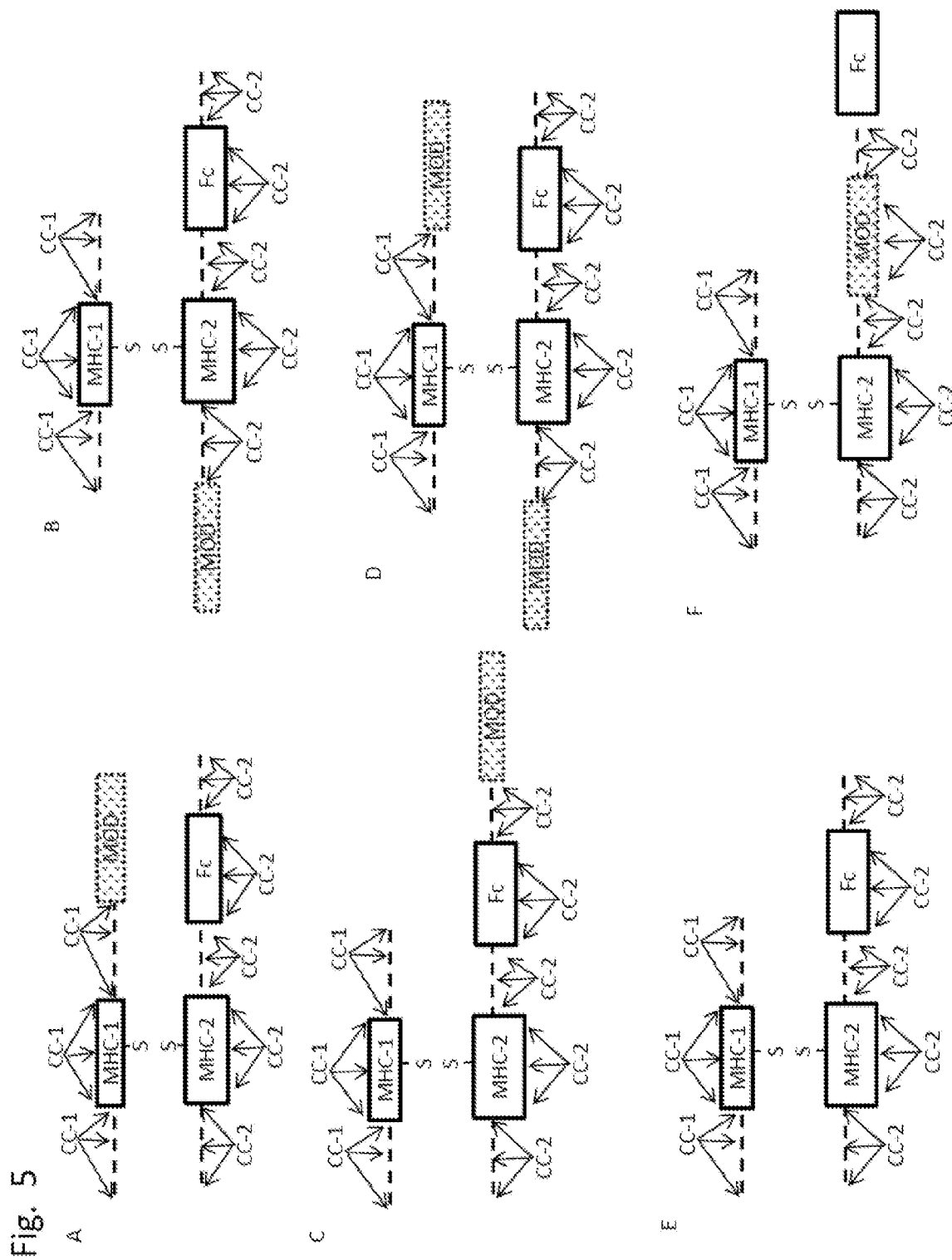


Fig. 6

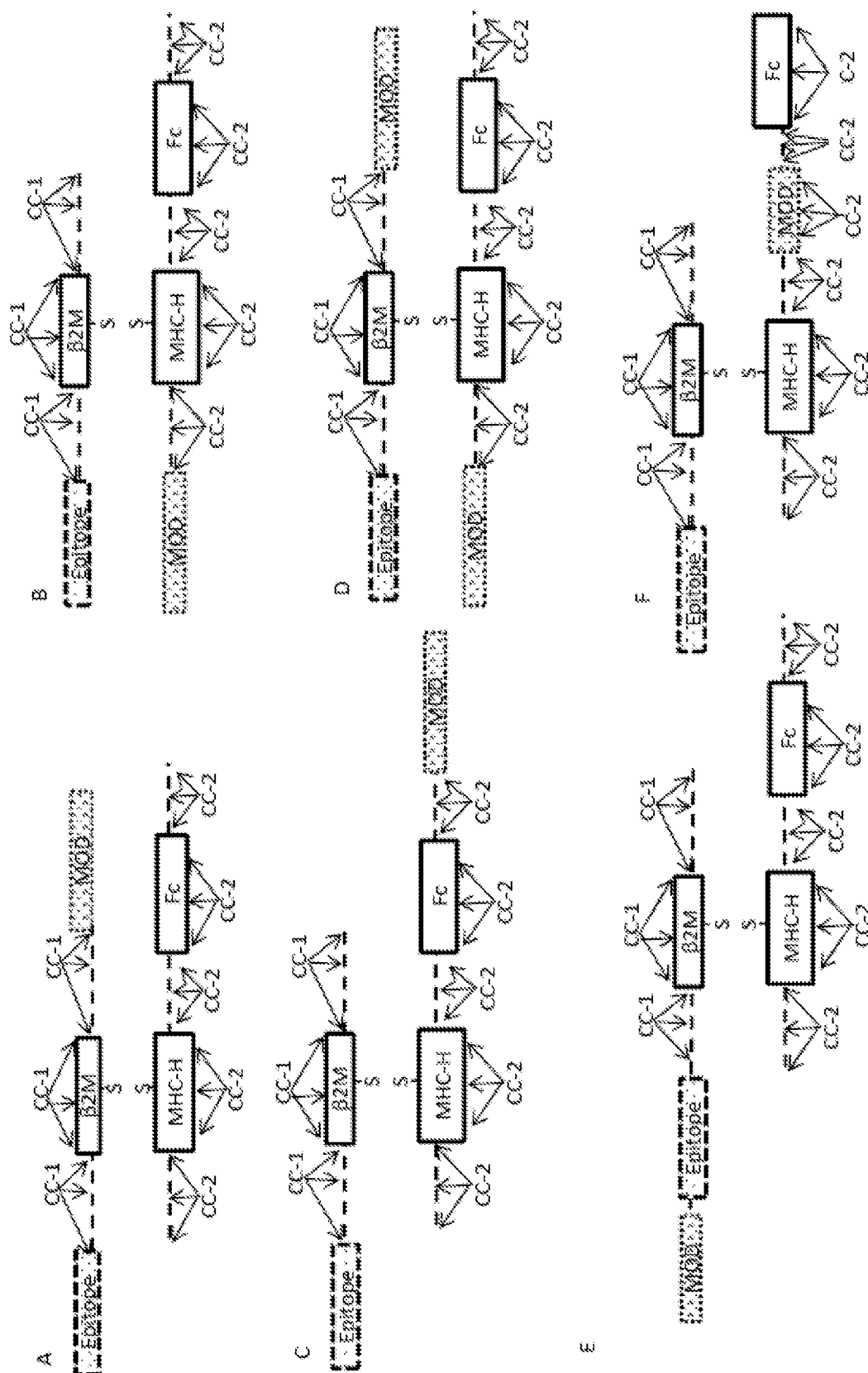
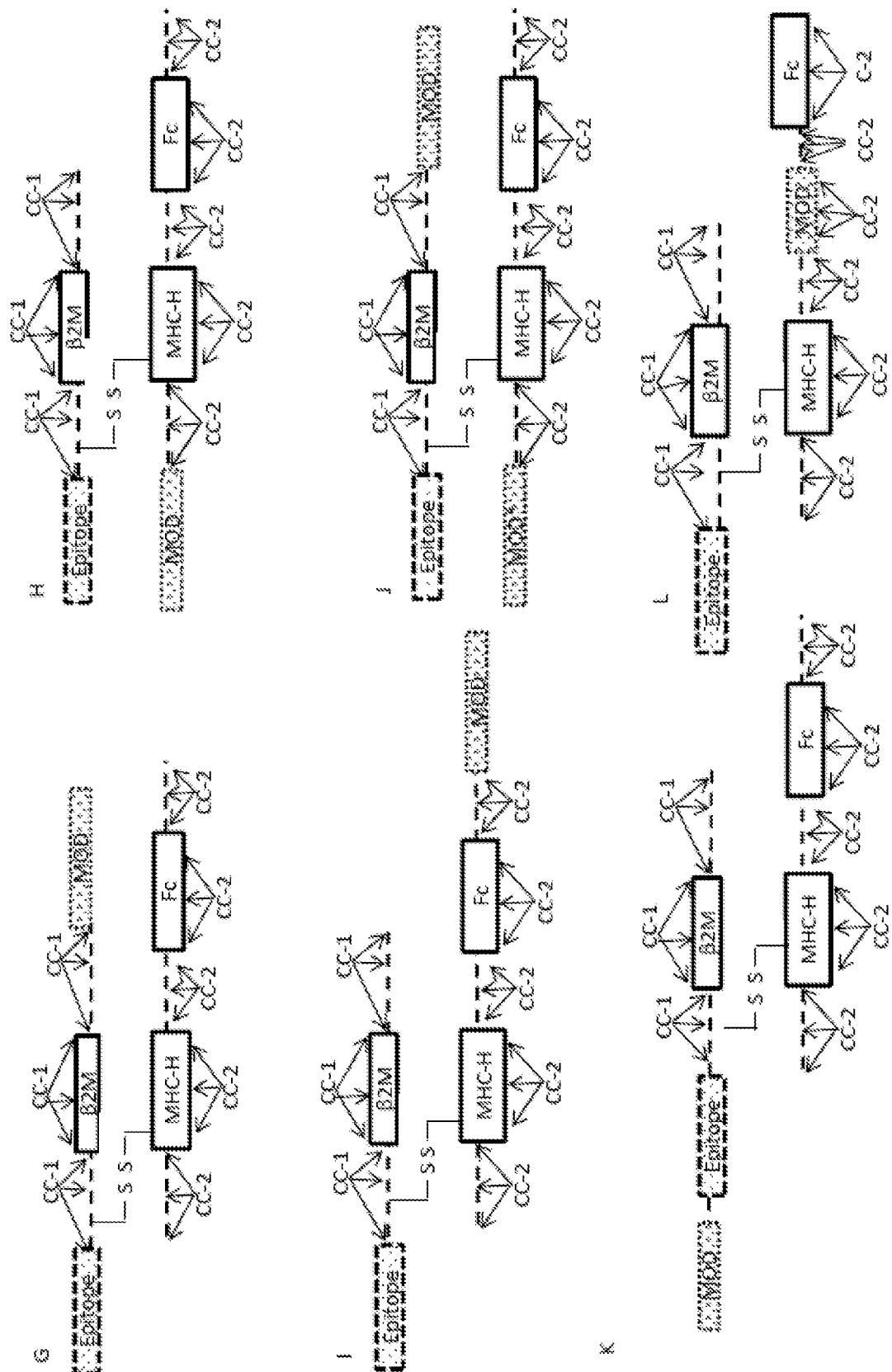


Fig. 6 (continued)



60
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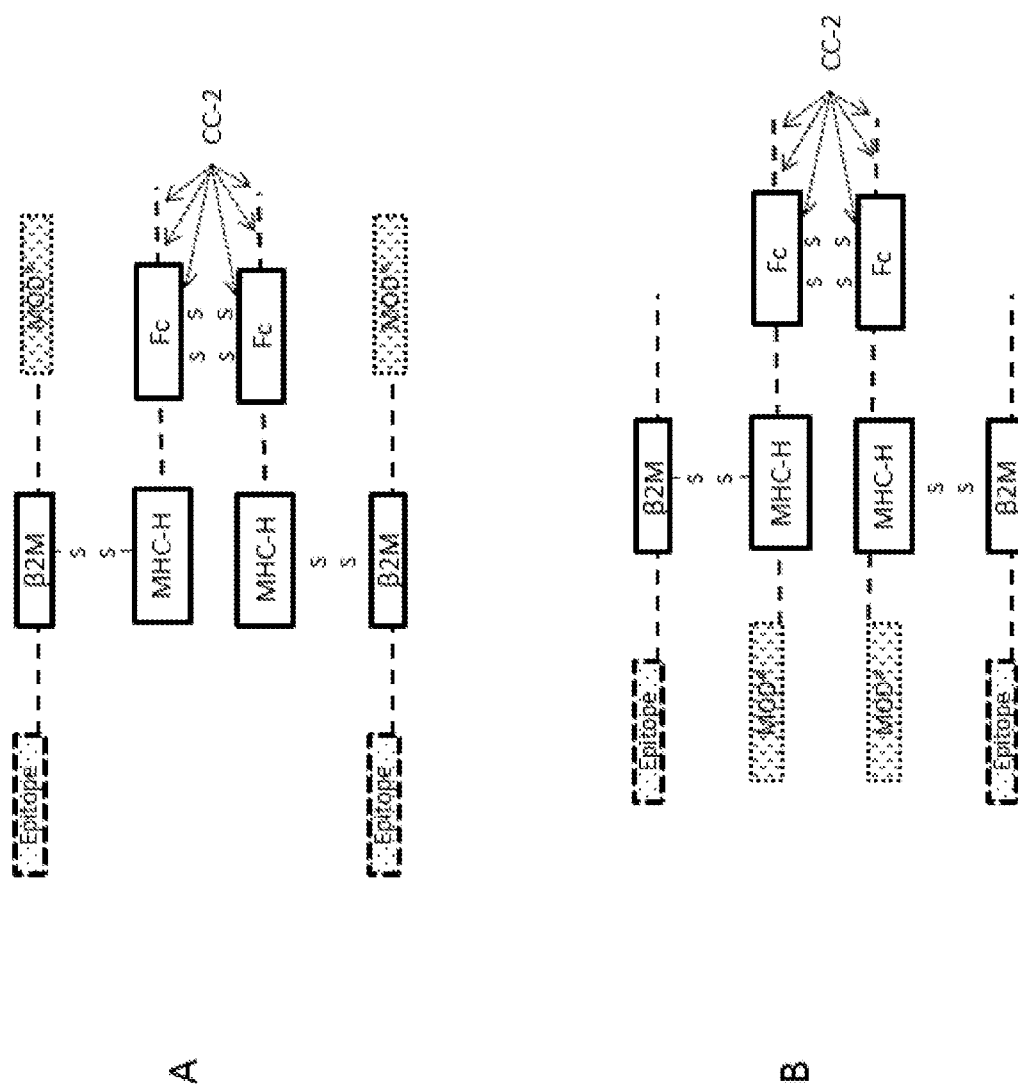


FIG. 8

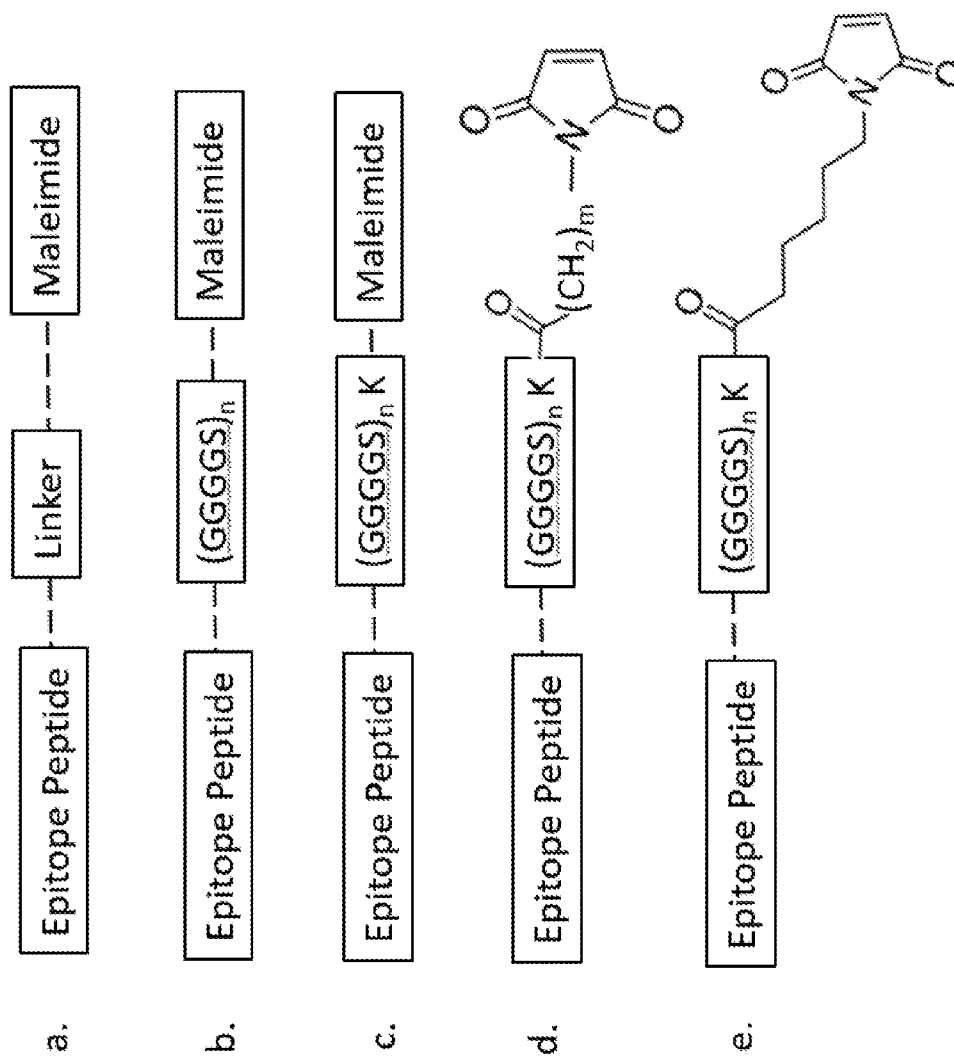
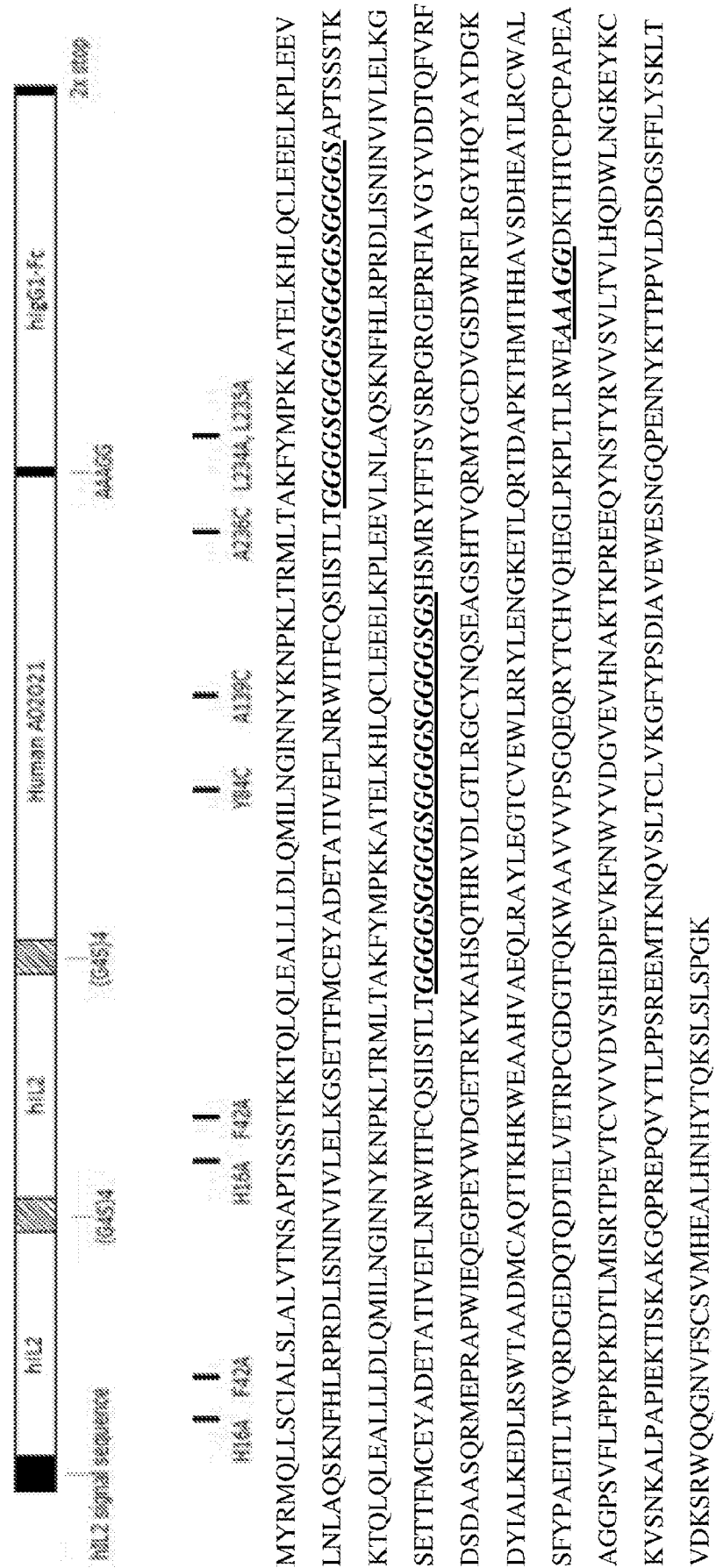


FIG. 9

A. (SEQ ID NO:327)

MSRSVALAVALLSLGLEAGGGGSLCTPSRGGGSIQRTPKIQVYS $\overline{\text{CHPAENGKSNFLNCYVSGFHPSDIEVDLLKNGERIEKVEHSD}}$
LSFSKDWSFYLLYYTEFTPTTEKDEYACRVNHVTLSPKIVKWDRDM

B. (SEQ ID NO:328)



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FIG. 10A

1775' hIL-2 signal; optional linker; immunomodulatory peptide (MOD); optional linker; HLA-A11 H chain (Y84C; A139C; A236C); AAAGG linker; hIgG1 Fc (L234A; L235A) (SEQ ID NO:329 and SEQ ID NO:330)

MYRMOLLSCIALSLALVTNS(optional linker)immunomodulatory peptide(optional linker)
 GSHSMRYFYTSVSRPGRGEPRFIAVGYYDDTQFVRFDSDAASQRMEPRAPWIEQEGPE
 YWDQETRNKVAQSQTDRVDLGTLRGCYNQSEDGSHTIQIMYGCDVGPDGRFLRGYR
 QDAYDGKDYIALNEDLRSWTAADMCAQITKRKWEAAHAAEQQRAYLEGTCVEWLR
 RYLENGKETLQRTDPPKTHMTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQD
 TELVETRPCGDGTFQKWA^{AA}VVPSGEEQRYTCHVQHEGLPKPLTLRWE^{AA}AGGDKTH
 TCPPCPAPE^{AA}AGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
 VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
 GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVL
 DSDGSFFLYSKLTVDKSRWQQGNVFC^{SV}MHEALHNHYTQKSLSLSPGK**

HLA-A11 (Y84C; A139C; A236C) (SEQ ID NO:331)

GSHSMRYFYTSVSRPGRGEPRFIAVGYYDDTQFVRFDSDAASQRMEPRAPWIEQEGPE
 YWDQETRNKVAQSQTDRVDLGTLRGCYNQSEDGSHTIQIMYGCDVGPDGRFLRGYR
 QDAYDGKDYIALNEDLRSWTAADMCAQITKRKWEAAHAAEQQRAYLEGTCVEWLR
 RYLENGKETLQRTDPPKTHMTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQD
 TELVETRPCGDGTFQKWA^{AA}VVPSGEEQRYTCHVQHEGLPKPLTLRWE

FIG. 10B

1777' hIL-2 signal; hIL2 (H16A; F42A); (G4S)₄ linker; hIL2 (H16A; F42A); (G4S)₄ linker;
 HLA A11 H chain (Y84C; A139C; A236C); AAAGG linker; hIgG1 Fc (L234A; L235A) (SEQ
 ID NO:329 and SEQ ID NO:332)

MYRMOLLSCIALSLALVTNSAPTSSSTKKTQLQLE^{ALLLDLQ}MILNGINNYKNPKLTR
 MLT^AKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELK
 GSETTFMCEYADETATIVEFLNRWITFCQSIISTLTGGGGSGGGGSGGGGSGGGGSAPTS
 SSTKKTQLQLE^{ALLLDLQ}MILNGINNYKNPKLTRMLT^AKFYMPKKATELKHLQCLEEE
 LKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWI
 TFCQSIISTLTGGGGSGGGGSGGGGSGGGGSGSHSMRYFYTSVSRPGRGEPRFIAVGYY
 DDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDQETRNKVAQSQTDRVDLGTLRGCY
 NQSEDGSHTIQIMYGCDVGPDGRFLRGYRQDAYDGKDYIALNEDLRSWTAADMCAQI
 TKRKWEAAHAAEQQRAYLEGTCVEWLRRLYLENGKETLQRTDPPKTHMTHHPISDHEA
 TLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPCGDGTFQKWA^{AA}VVPSGEEQR
 YTCHVQHEGLPKPLTLRWE^{AA}AGGDKTHTCPPCPAPE^{AA}AGGPSVFLFPPKPKDTLMISR
 TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
 DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLV
 KGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFC^{SV}
 MHEALHNHYTQKSLSLSPGK

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FIG. 10C

1779' hIL-2 signal; optional linker; immunomodulatory peptide (MOD); optional linker; HLA-A A11 (Y84C; A139C; A236C); (G4S)6 linker; hIgG1 Fc (L234A; L235A) (SEQ ID NO:329 and SEQ ID NO:333)

MYRMQLLSICIALSLALVTNS(optional linker)immunomodulatory peptide(optional linker)
 GSHSMRYFYTSVSRPGRGEPRFIAVGYYDDTQFVRFDSDAASQRMPEPRAPWIEQEGPE
 YWDQETRNVKAQSQTDRVDLGTLRG**C**YNQSEDGSHTIQIMYGCDVGPDGRFLRGYR
 QDAYDGKDYLALNEDLRSWTAADM**C**AQITKRKWEAAHAAEQQRAYLEGTCVEWLR
 RYLENGKETLQRTDPPKTHMTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQD
 TELVETRPCGDGTFQKWA⁶AVVPSGEEQRYTCHVQHEGLPKPLTLRWE**GGGGSGGG**
GSGGGSGGGSGGGSGGGSGGGSDKTHTCPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPE
 VTCCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW
 LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF
 YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHE
 ALHNHYTQKSLSLSPGK

FIG. 10D

1781' hIL-2 signal; hIL2 (H16A; F42A); (G4S)4 linker; hIL2 (H16A; F42A); (G4S)4 linker;
 HLA-A A11 (Y84C; A139C; A236C); (G4S)6 linker; hIgG1 Fc (L234A; L235A) (SEQ ID
 NO:329 and SEQ ID NO:334)

MYRMQLLSICIALSLALVTNSAPTSSSTKKTQLQLE**A**ALLDLQMILNGINNYKNPKLTR
 MLT**A**KFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELK
 GSETTFMCEYADETATIVEFLNRWITFCQSIISTLT**GGGGSGGGSGGGSGGGSGGG**SAPTS
 SSTKKTQLQLE**A**ALLDLQMILNGINNYKNPKLTRMLT**A**KFYMPKKATELKHLQCLEE
 LKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWI
 TFCQSIISTLT**GGGGSGGGSGGGSGGGSGGGSG**SHSMRYFYTSVSRPGRGEPRFIAVGYY
 DDTQFVRFDSDAASQRMPEPRAPWIEQEGPEYWDQETRNVKAQSQTDRVDLGTLRG**C**Y
 NQSEDGSHTIQIMYGCDVGPDGRFLRGYRQDAYDGKDYLALNEDLRSWTAADM**C**AQI
 TKRKWEAAHAAEQQRAYLEGTCVEWLRRYLENGKETLQRTDPPKTHMTHHPISDHEA
 TLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPCGDGTFQKWA⁶AVVPSGEEQRY
 TCHVQHEGLPKPLTLRWE**GGGGSGGGSGGGSGGGSGGGSGGGSDK**THTCPC
 PCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCCVVVDVSHEDPEVKFNWYVDGVEVH
 NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE
 PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
 GSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

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FIG. 11A

WT-1 isoform A (SEQ ID NO:335)

```

1  MDFLLLQDPA  STCVPEPASQ  HTLRSGPGCL  QQPEQQGVVD  PGGIWAKLGA  AEASAERLQG
61  RRSRGASGSE  PQQMGSDVRD  LNALLPAVPS  LGGGGGCALP  VSGAAQWAPV  LDFAPPGASA
121 YGSLGGPAPP  PAPPPPPPPP  PHSFIKQEPS  WGAEPHEEQ  CLSAFTVHFS  GQFTGTAGAC
181 RYGPFGPAPP  SQASSGQARM  FPNAPYLPSC  LESQPAIRNQ  GYSTVTFDGT  PSYGHTPSHH
241 AAQFPNHSFK  HEDPMGQQGS  LGEQQYSVPP  PVYGCHTPTD  SCTGSQALLL  RTPYSSDNLY
301 QMTSQLECMT  WNQMNLGATL  KGHSTGYESD  NHTTPILCGA  QYRIHTHGVF  RGIQDVRRVP
361 GVAPTLVRSR  SETSEKRPFM  CAYPGCNKRY  FKLSHLQMS  RKHTGEKPYQ  CDFKDCERRE
421 SRSDQLKRHQ  RRHTGVKPFQ  CKTCQRKFSR  SDHLKTHTRT  HTGEKPFSCR  WPSCQKKFAR
481 SDELVRHHNM  HQRNMTKLQL  AL

```

FIG. 11B

WT-1 isoform B (SEQ ID NO:336)

```

1  MDFLLLQDPA  STCVPEPASQ  HTLRSGPGCL  QQPEQQGVVD  PGGIWAKLGA  AEASAERLQG
61  RRSRGASGSE  PQQMGSDVRD  LNALLPAVPS  LGGGGGCALP  VSGAAQWAPV  LDFAPPGASA
121 YGSLGGPAPP  PAPPPPPPPP  PHSFIKQEPS  WGAEPHEEQ  CLSAFTVHFS  GQFTGTAGAC
181 RYGPFGPAPP  SQASSGQARM  FPNAPYLPSC  LESQPAIRNQ  GYSTVTFDGT  PSYGHTPSHH
241 AAQFPNHSFK  HEDPMGQQGS  LGEQQYSVPP  PVYGCHTPTD  SCTGSQALLL  RTPYSSDNLY
301 QMTSQLECMT  WNQMNLGATL  KGVAAGSSSS  VKWTEGQSNH  STGYESDNHT  TPILCGAQYR
361 IHTHGVFRGI  QDVRRVPGVA  PTLVRSASET  SEKRPFMCAY  PGCNKRYFKL  SHLQMSRKH
421 TGEKPYQCDF  KDCERRFSRS  DQLKRHQRRH  TGVPKFQCKT  CQRKFSRSDH  LKTHTRHTGT
481 EKPFSRWPS  CQKKFARSDE  LVRHHNMHQR  NMTKLQLAL

```

FIG. 11C

WT-1 isoform D (SEQ ID NO:337)

```

1  MDFLLLQDPA  STCVPEPASQ  HTLRSGPGCL  QQPEQQGVVD  PGGIWAKLGA  AEASAERLQG
61  RRSRGASGSE  PQQMGSDVRD  LNALLPAVPS  LGGGGGCALP  VSGAAQWAPV  LDFAPPGASA
121 YGSLGGPAPP  PAPPPPPPPP  PHSFIKQEPS  WGAEPHEEQ  CLSAFTVHFS  GQFTGTAGAC
181 RYGPFGPAPP  SQASSGQARM  FPNAPYLPSC  LESQPAIRNQ  GYSTVTFDGT  PSYGHTPSHH
241 AAQFPNHSFK  HEDPMGQQGS  LGEQQYSVPP  PVYGCHTPTD  SCTGSQALLL  RTPYSSDNLY
301 QMTSQLECMT  WNQMNLGATL  KGVAAGSSSS  VKWTEGQSNH  STGYESDNHT  TPILCGAQYR
361 IHTHGVFRGI  QDVRRVPGVA  PTLVRSASET  SEKRPFMCAY  PGCNKRYFKL  SHLQMSRKH
421 TGEKPYQCDF  KDCERRFSRS  DQLKRHQRRH  TGVPKFQCKT  CQRKFSRSDH  LKTHTRHTGT
481 KTSEKPFSCR  WPSCQKKFAR  SDELVRHHNM  HQRNMTKLQL  AL

```

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FIG. 11D

WT-1 isoform E (SEQ ID NO:338)

```
1  MEKGYSTVTF DGTPSYGHTP SHHAAQFPNH SFKHEDPMGQ QGSLGEQQYS VPPFVYGCHT
61 PTDSCITGSQA LLLRTPYSSD NLYQMTSQLE CMTWNQMNLG ATLKGVAAGS SSSVKWIEGQ
121 SNHSTGYESD NHTTPILCGA QYRIHTHGVF RGIQDVRRVP GVAPTLVRSV SETSEKRPFM
181 CAYPGCNKRY FKLSHLQMHV RKHTGEKPYQ CDFKDCERRF SRSDQLKRHQ RRHTGVKPFQ
241 CKTCQRKFSR SDHLKTHTRT HTGEKPFSCR WPSQCQKKFAR SDELVRHHNM HQRNMTKLQL
301 AL
```

FIG. 11E

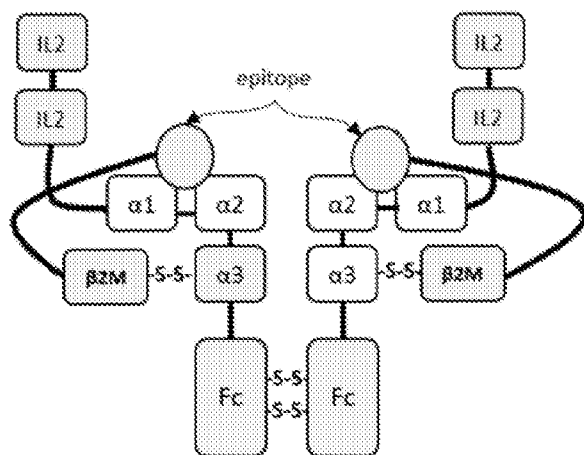
WT-1 isoform F (SEQ ID NO:339)

```
1  MEKGYSTVTF DGTPSYGHTP SHHAAQFPNH SFKHEDPMGQ QGSLGEQQYS VPPFVYGCHT
61 PTDSCITGSQA LLLRTPYSSD NLYQMTSQLE CMTWNQMNLG ATLKGHSTGY ESDNHTTPIL
121 CGAQYRIHTH GVFRGIQDVR RVPGVAPTLV RSASETSEKR PFMCAYPGCN KRYFKLSHLQ
181 MHSRKHTGEK PYQCDFKDCE RRFSRSDQLK RHQRRHTGVK PFQCKTCQRK FSRSDHLKTH
241 TRHTGKTSE KPFSCRWPSQ QKKFARSDEL VRHHNMHQRN MTKLQLAL
```

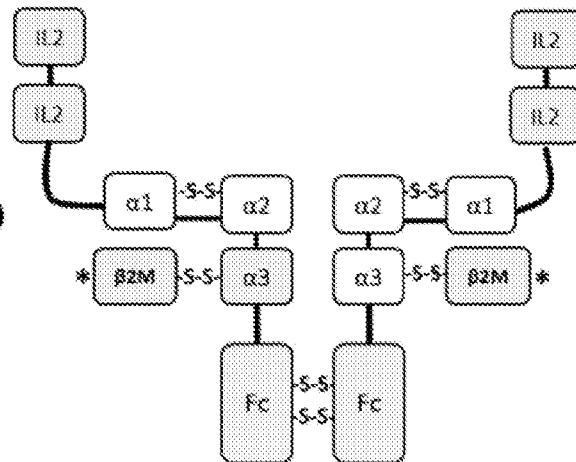
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FIG. 12

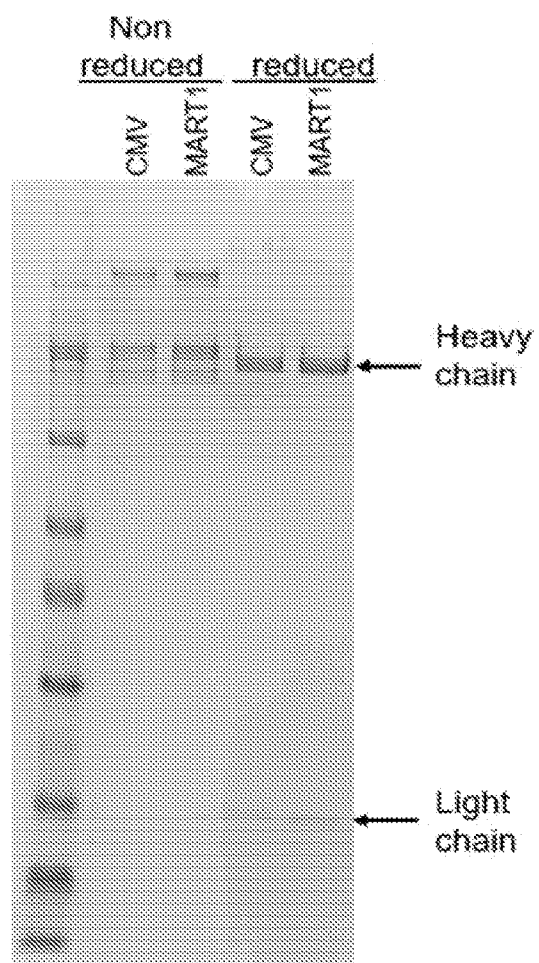
A



B

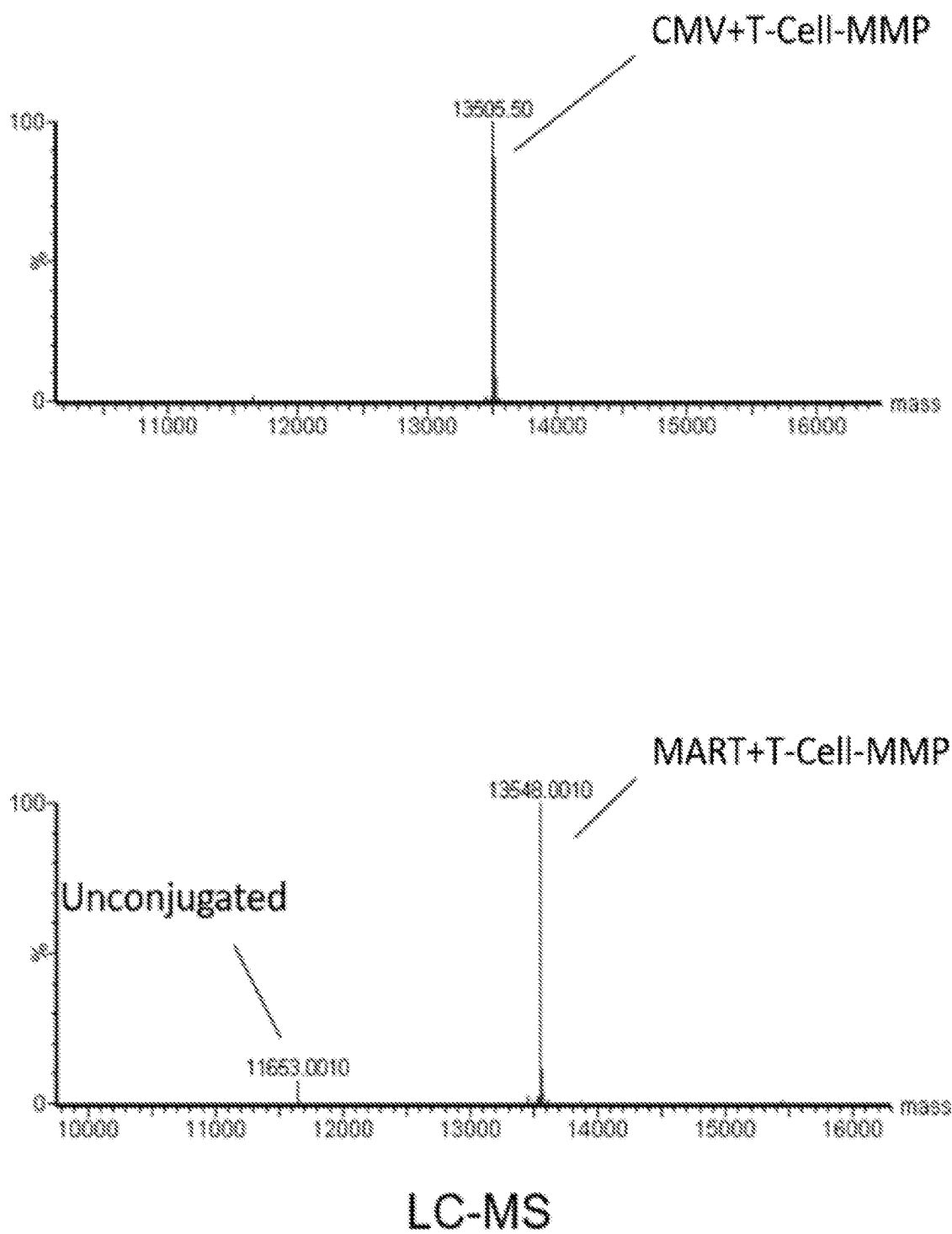


C



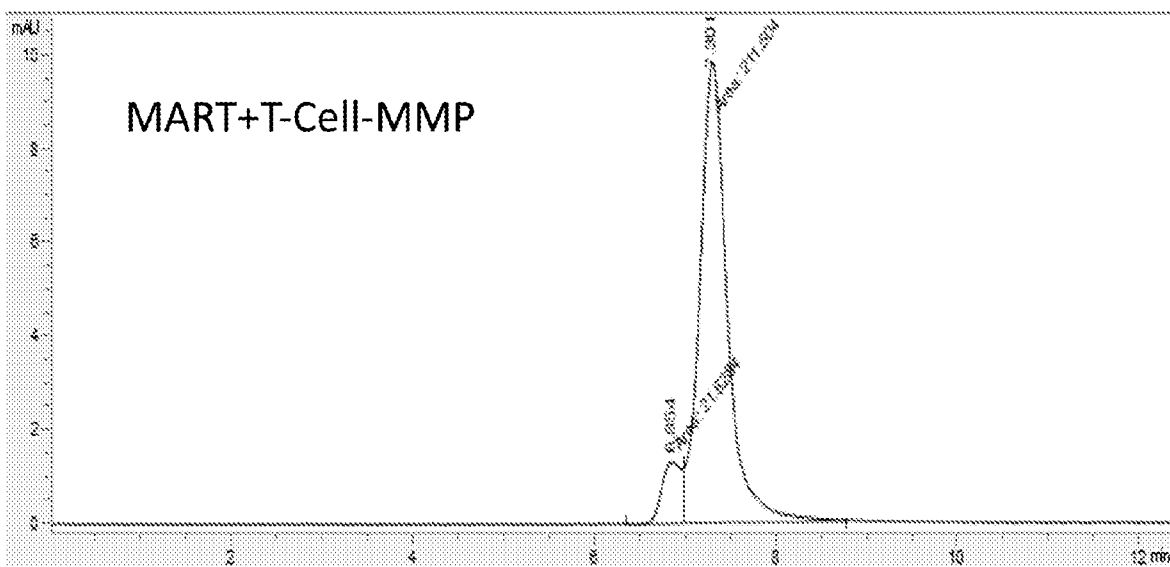
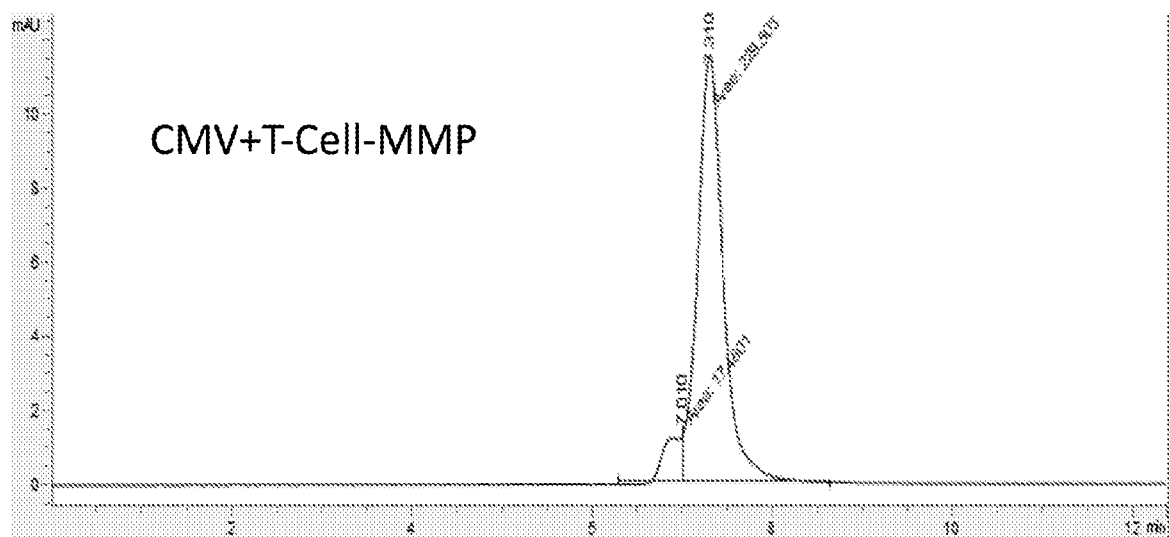
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FIG. 13



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FIG. 13 (Continued)



Size Exclusion Chromatography

FIG. 14

