(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 May 2022 (12.05.2022)

WIPO | PCT

(10) International Publication Number
WO 2022/099156 A2

(51) International Patent Classification:
Not classified

(21) International Application Number:
PCT/US2021/058490

(22) International Filing Date:
08 November 2021 (08.11.2021)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/110,946 06 November 2020 (06.11.2020) US

(71) Applicant: **CUE BIOPHARMA, INC.** [US/US]; 21 Erie Street, Cambridge, Massachusetts 02139 (US).

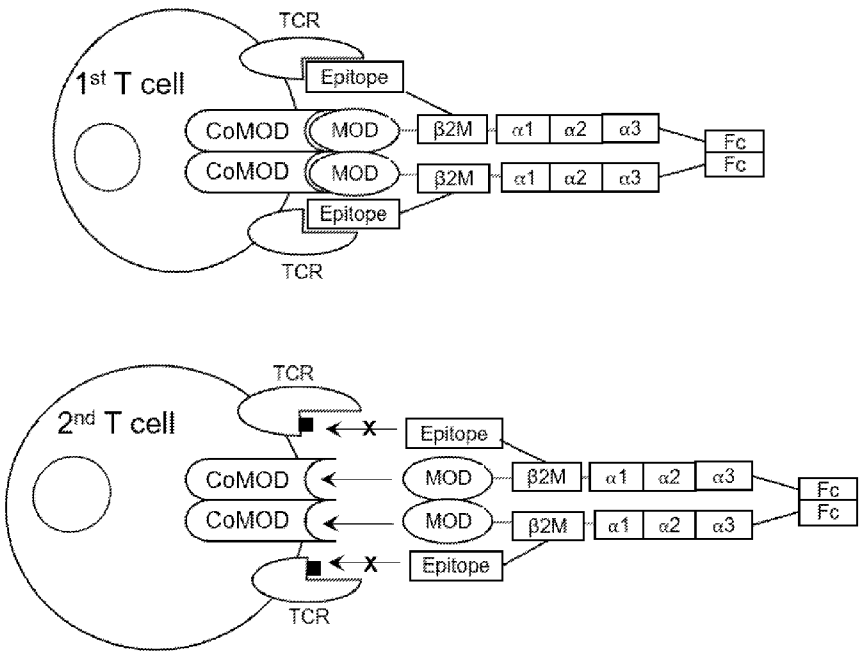
(72) Inventors: **SEIDEL III, Ronald D.**; 12 Lincoln Street, Unit 2, Natick, Massachusetts 01760 (US). **CHAPARRO, Rodolfo J.**; 244 Pearl St., Cambridge, Massachusetts 02139 (US). **ROSS, John F.**; c/o Cue Biopharma, Inc., 21 Erie Street, Cambridge, Massachusetts 02139 (US). **LOW, Chee Meng**; c/o Cue Biopharma, Inc., 21 Erie Street, Cambridge, Massachusetts 02139 (US). **SURI, Anish**; c/o Cue Biopharma, Inc., 21 Erie Street, Cambridge, Massachusetts 02139 (US). **CEMERSKI, Saso**; c/o Cue Biopharma, Inc., 21 Erie Street, Cambridge, Massachusetts 02139 (US).

(74) Agent: **RICIGLIANO, Joseph W.**; Hoffmann & Baron LLP, 6900 Jericho Turnpike, Syosset, New York 11791 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

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

FIG. 1



(57) **Abstract:** The present disclosure provides T cell modulatory polypeptides (T-Cell-MPs) comprising a chemical conjugation site at which a KRAS epitope has been conjugated and at least one immunomodulatory polypeptide sequence that may be selected to exhibit reduced binding affinity to its cognate co-immunomodulatory polypeptide. The T-Cell-epitope conjugates are useful for modulating (e.g., increasing proliferation or cytotoxic activity) the activity of T cells specific to the conjugate epitope, and accordingly for use as therapeutics. The T-Cell-epitope conjugates find use in treating a variety of cancers associated with KRAS.

WO 2022/099156 A2

[Continued on next page]

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

— *with sequence listing part of description (Rule 5.2(a))*

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

[0001] This application claims the benefit of U.S. Provisional Application No. 62/814,842 filed on March 6, 2019, which is incorporated herein by reference in its entirety. 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 "2910-24PCT_seqlist.txt", which was created on November 8, 2021, which is 457,736 bytes in size, and which is herein incorporated by reference in its entirety.

Introduction

[0002] The ability to induce 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 or non-peptide molecule (e.g., an epitope of a molecule such as a polypeptide) presented by a major histocompatibility complex (MHC; also referred to in humans as a human leukocyte antigen (HLA) complex) that is located on the surface of an antigen presenting cell (APC). 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 response of targeted T cells is dictated by the presence of immunomodulatory molecules (some of which are found on the surface of the APC) that act through engagement of counterpart receptors on the T cells. Both signals – epitope/TCR binding and engagement of immunomodulatory molecules with their counterpart receptors on T cells – are required to drive activation or inhibition of target T cell functions. The TCR is specific for a given epitope; however, the counterpart receptors for immunomodulatory molecules are not epitope-specific, and instead, are generally expressed on all T cells or on large T cell subsets.

Summary

[0003] The present disclosure provides T cell modulatory polypeptides (a "T-Cell-MP" or multiple "T-Cell-MPs") that find use in, among other things, methods of in vivo, ex vivo, and in vitro treatment of various diseases benign neoplasms and malignant neoplasms (cancers) and other disorders of mammals (e.g., humans) and the preparation of medicaments for such treatments. In one aspect, the T-Cell-MPs described herein comprise a portion of a class I MHC-H polypeptide, a β2M polypeptide, a chemical conjugation site for covalently attaching an epitope presenting molecule (e.g., a peptide) presenting a KRAS epitope, 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-MP may be wild-type ("wt.") or a variant that exhibits an altered binding affinity to its cellular binding partner/receptor (e.g., T cell surface), referred to as a Co-MOD.

[0004] T-Cell-MPs may be unconjugated or conjugated to a molecule comprising a target antigenic determinate (e.g., a peptide, glycopeptide, or non-peptide such as a carbohydrate presenting an epitope). When T-Cell-MPs are unconjugated, in which case they comprise at least one chemical conjugation site

at which a molecule comprising a target antigenic determinate (e.g., a peptide, glycopeptide, or non-peptide such as a carbohydrate presenting an epitope) may be covalently bound to form a T-Cell-MP-epitope conjugate for presentation to a cell bearing a T cell receptor. Unconjugated T-Cell-MPs comprising a chemical conjugation site to which a molecule presenting a KRAS epitope may be bound are useful for rapidly preparing T-Cell-MP-KRAS-epitope conjugates. The T-Cell-MP-KRAS-epitope conjugates can modulate the activity of T cells specific to the KRAS epitope presented and, accordingly, modulate an immune response involving those T cells in an individual.

[0005] The T-Cell-MPs described herein are suitable for production in cell-based expression systems where most, or substantially all (e.g., greater than 75%, 85% or 90%) or all, of the expressed unconjugated T-Cell-MP polypeptide/protein is in a soluble non-aggregated state that is suitably stable at 37 °C for production in tissue culture and use at least up to that temperature. The T-Cell-MPs can advantageously be produced as a single polypeptide encoded by a nucleic acid sequence contained in a single vector. The T-Cell-MPs may form higher order structures, such as duplexes (see, e.g., FIG. 1), which may be homodimeric as in FIG. 9, or heterodimeric when formed from two T-Cell-MPs, e.g., as illustrated in FIGs. 10 and 11. Unconjugated T-Cell-MPs can be expressed in high yield, e.g., greater than 25, 40, 60, or 80 mg/liter (e.g. about 25 to about 40, about 40 to 60, or about 60 to about 80 mg/l in CHO cells). Yields can be high especially when a disulfide bond is present between the carboxyl end of the MHC-H chain α_1 helix and the MHC-H chain $\alpha_{2\text{-}1}$ helix (e.g. a Y84C to A139C disulfide bond), and the linker between the MHC-H polypeptide sequence and the β2M polypeptide is of sufficient length (e.g., from about 10 to about 50 aa long). With the disulfide bond present between the α_1 and α_2 helices unconjugated T-Cell-MP expression levels may exceed 80 mg/l (e.g., from about 80 to about 100, about 100 to about 120, about 120 to about 140, about 140 to about 160, about 160 to about 180, or about 180 to about 200 mg/l).

[0006] Once purified, most, substantially all (e.g., greater than 85% or 90% of the T-Cell-MP), or all of the expressed unconjugated T-Cell-MP protein remains in a soluble non-aggregated state even after conjugation to an epitope (e.g., peptide epitopes) and is similarly stable compared to the unconjugated T-Cell-MP. The unconjugated T-Cell-MPs and their epitope conjugates may additionally comprise a targeting sequence that can direct a T-Cell-MP-epitope conjugate to a particular cell or tissue (e.g., a tumor). Payloads (e.g., bioactive substances or labels), such as a therapeutic (e.g., chemotherapeutic agents) for co-delivery with a specific target epitope, may also be covalently attached to a T-Cell-MP, such as by a crosslinking agent. Accordingly, T-Cell-MP-KRAS-epitope conjugates may be considered a means by which to deliver MODs (*e.g.*, IL-2, 4-1BBL, FasL, TGF-β, CD70, CD80, CD86, or variants thereof) and/or payloads (e.g., chemotherapeutics) to T cells in an epitope-specific manner optionally with the assistance of a targeting sequence.

[0007] The T-Cell-MPs may comprise modifications that assist in the stabilization of the unconjugated T-Cell-MP during intracellular trafficking and/or following secretion by cells expressing the multimeric polypeptide even in the absence of an associated epitope (e.g., a peptide epitope). One such modification is a bond (e.g., disulfide bond) formed between amino acid position 84 at the carboxyl end of the MHC-

class I α_1 helix (or its flanking amino acid sequences aac1 and aac2) and amino acid position 139 at the amino end of the MHC- class I $\alpha_{2\text{-}1}$ helix (or its flanking amino acid sequences aac3 and aac4). For example, the insertion of cysteine residues at amino acids 84 (Y84C substitution) and 139 (A139C substitution) of MHC-H, or the equivalent positions (see, e.g., FIG. 3I), may form a disulfide linkage that helps stabilize the T-Cell-MP. See, e.g., Z. Hein et al. (2014), *Journal of Cell Science* 127:2885–2897.

[0008] One aspect of the T-Cell-MP molecules described herein is broadly directed to an unconjugated T-Cell-MP, the polypeptide comprising (e.g., from N-terminus to C-terminus):

(i) optionally one or more MOD polypeptide sequences (e.g., two or more MOD polypeptide sequences, such as in tandem, wherein when there are two or more MOD polypeptide sequences they are optionally joined to each other by independently selected L1 linkers);

(ii) an optional linker L2 polypeptide sequence joining the one or more optional MOD polypeptide sequences to a β2M polypeptide sequence;

(iii) the β2M polypeptide sequence;

(iv) an optional L3 linker polypeptide sequence (e.g., from 10-50 aa in length);

(v) a class I MHC-H polypeptide sequence;

(vi) an optional L4 linker polypeptide sequence;

(vii) a scaffold polypeptide sequence (e.g., an immunoglobulin Fc sequence);

(viii) an optional L5 linker polypeptide sequence; and

(ix) optionally one or more MOD polypeptide sequences (e.g., two or more MOD polypeptide sequences, such as in tandem, wherein when there are two or more MOD polypeptide sequences they are optionally joined to each other by independently selected L6 linkers);

wherein the unconjugated T cell modulatory polypeptide comprises at least one MOD polypeptide sequence (e.g., the MOD(s) of element (i) and/or (ix)); and

wherein at least one of the β2M polypeptide sequence, L3 linker polypeptide sequence, and/or the MHC-H polypeptide sequence comprises a chemical conjugation site (e.g., provided by protein engineering, such as a cysteine substitution) for epitope conjugation.

[0009] It is understood that such unconjugated T-Cell-MPs do not comprise a covalently attached epitope (e.g., peptide epitope); however, the disclosure includes and provides for T-Cell-MP-epitope conjugates that further comprise a covalently attached epitope. The covalently attached epitope can be positioned within the binding cleft of the MHC-H/β2M polypeptide sequences and presented to a TCR, thereby permitting use of the molecules as agents for clinical testing and diagnostics, and as therapeutics.

Brief Description Of The Drawings

[0010] **FIG. 1** depicts preferential activation of T cells by an embodiment of a duplex T-Cell-MP-KRAS-epitope conjugate with an indirect (via a linker) covalent attachment of the epitope to the β2M polypeptides and bearing MODs, which can be wt. and/or variant MODs, e.g., having reduced affinity for their receptors (Co-MODs). The 1st epitope-specific T cell is activated due to productive engagement of both the TCRs and Co-MODs. In contrast, the 2nd epitope-non-specific T cell is not activated as the

epitope cannot engage the TCR, and thus the MODs by themselves do not lead to productive engagement. Linkers and the location of optional linkers are represented by black lines joining T-Cell-MP elements.

[0011] **FIGs. 2A-2H** provide amino acid sequences of immunoglobulin heavy chain polypeptides (including SEQ ID NOs:1-13).

[0012] **FIG. 2I** provides the sequence of a human immunoglobulin J-chain (SEQ ID NO:14).

[0013] **FIG. 2J** provides the sequence of an Ig CH1 domain sequence (SEQ ID NO:15).

[0014] **FIG. 2K** provides sequences of Ig κ and Ig λ chains (SEQ ID NOs:16-17).

[0015] **FIGs. 3A, 3B and 3C** provide amino acid sequences of major histocompatibility complex class I heavy chain (MHC-H; also known as 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:18; entry 3A.2 is HLA-A*1101, SEQ ID NO:19; entry 3A.3 is HLA-A*2402, SEQ ID NO:20, and entry 3A.4 is HLA-A*3303, SEQ ID NO:21. FIG. 3B provides the sequence for HLA-B*07:02:01 (HLA-B*0702) (NCBI GenBank Accession NP_005505.2, SEQ ID NO:22. 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:23.

[0016] **FIG. 3D** provides an alignment of all, or substantially all, of the α1, α2, and α3 domains of eleven mature MHC-H polypeptide 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:24 (see also SEQ ID NO:18); HLA-B*0702, SEQ ID NO:25; HLA-C, SEQ ID NO:26; HLA-A*0201, SEQ ID NO:27; a mouse H2K protein sequence, SEQ ID NO:28; three variants of HLA-A (var. 2, var. 2C [having Y84C and A139C substitutions], and var. 2CP), SEQ ID NOs:29-31; 3 human HLA-A molecules (HLA-A*1101 (HLA-A11), SEQ ID NO:32; HLA-A*2402 (HLA-A24), SEQ ID NO:33; and HLA-A*3303 (HLA-A33), SEQ ID NO:34). HLA-A*0201 is an allelic 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 seventh 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 - β2M complex in the absence of a bound peptide epitope. 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 β2M (e.g., at aa 12 of the mature polypeptide, forming for example, an HLA-A*0201 A236C to β2M R12C disulfide bond). 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 clusters 1, 2, 3, 4, 5, and 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.

[0017] **FIGs. 3E-3G** provide alignments of the aa sequences of all, or substantially all, of the α1, α2, and α3 domains of several 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 (aac 1 to aac 6) 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.

[0018] **FIG. 3H** provides a consensus sequence for all, or substantially all, of the α1, α2, and α3 domains of 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.

[0019] **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: 39, 47, and 57-60). 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.

[0020] **FIG. 4** provides a multiple aa sequence alignment of β2M precursors (i.e., including the leader sequence) from Homo sapiens (NP_004039.1; SEQ ID NO:61), Pan troglodytes (NP_001009066.1; SEQ ID NO:62), Macaca mulatta (NP_001040602.1; SEQ ID NO:63), Bos Taurus (NP_776318.1; SEQ ID NO:64) and Mus musculus (NP_033865.2; SEQ ID NO:65). Underlined aas 1-20 are the signal peptide (sometime referred to as a leader sequence).

[0021] **FIG. 5** provides six unconjugated T-Cell-MP embodiments (structures) marked as **A** through **F**. In each case the T-Cell-MPs comprise: at least one MOD polypeptide sequence; a core structure that comprises the elements, in the N-terminus to C-terminus direction: a β2M polypeptide sequence, a Class I MHC-H polypeptide sequence comprising MHC-H α1, α2, and α3 domain sequences; and a scaffold polypeptide sequence (e.g., an Ig Fc polypeptide sequence). In the embodiments shown the α1 and α2 polypeptide sequences are linked by an intra-peptide bond between cysteines substituted, for example, with Tyr 84 and Ala 139 (Y84C and A139C substitutions). One or more MODs are located at the amino and/or carboxy side of the core structure. Optional linker polypeptides that are selected independently, denoted as L1 to L6, are indicated by the line segments. The optional linker polypeptides may appear at either the ends of the T-Cell-MP polypeptide or joining the indicated polypeptide sequences. While the chemical conjugation site for coupling the epitope can be located at any location on the T-Cell-MP, potential locations in the β2M polypeptide sequence and the MHC-H polypeptide sequence for the

chemical conjugation sites are indicated by asterisks. Although not shown, chemical conjugation sites may also be located in the L3 linker joining the β2M polypeptide sequence and MHC-H polypeptide sequence.

[0022] **FIG. 6** provides six embodiments of unconjugated T-Cell-MPs, marked as **A** through **F**, that parallel the embodiments in FIG. 5. In the embodiments shown, the chemical conjugation site is indicated as being present in the β2M polypeptide sequence (e.g., comprising an E44C substitution) and the scaffold is an immunoglobulin Fc region, which may be interspecific, thereby permitting two different unconjugated T-Cell-MPs to specifically combine to form a heteroduplex.

[0023] **FIG. 7** provides examples of unconjugated T-Cell-MPs having different MOD substitutions (e.g., tandem IL-2 MODs in structure A). The chemical conjugation sites are indicated as being present in the β2M polypeptide sequence (e.g., an E44C substitution); however, they could be in the MHC-H polypeptide (the α1, α2, and α3 sequence), or in the linker joining the β2M and MHC polypeptides. The Fc scaffold may be replaced by any other scaffold sequence such as an interspecific Fc polypeptide sequence that can form a heterodimer with its counterpart sequence, and the specific linkers listed are only exemplary and may be replaced by other linker polypeptide sequences.

[0024] **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-MP 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 (SEQ ID NO:120) 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) on the end of the (GGGGS)n linker, 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 (K) residue, where m is 1-7.

[0025] **FIG. 9** depicts the formation of a conjugated T-Cell-MP homoduplex from an unconjugated T-Cell-MP having a scaffold (in this case an Ig Fc scaffold) shown at (A). The conjugated T-Cell-MP polypeptide from (A) forms a homoduplex as shown in (B) via interactions between the scaffold sequences. The unconjugated homoduplex may be isolated from cells stably or transiently expressing the T-Cell-MP protein. The unconjugated homoduplex, generally in a purified form, is subjected to chemical conjugation by coupling an epitope to the conjugation sites, which is exemplified by the reaction between a cysteine in the β2M polypeptide sequence (e.g., comprising an E44C substitution) and a maleimide labeled peptide to yield the T-Cell-MP-KRAS-epitope conjugate shown in (C). Excess reactive peptide can be removed or substoichiometric amounts of the reactive epitope (relative to the amount of conjugation sites) can be utilized to produce the conjugated T-Cell-MP homoduplex. The constructs are not limited to the linker sequences shown, which are exemplary of the linkers that may be employed.

[0026] **FIG. 10** depicts the formation of a conjugated T-Cell-MP heteroduplex from unconjugated T-Cell-MPs having scaffolds that selectively form heteroduplexes (in this case interspecific knob-in-hole Ig Fc scaffolds) shown at (A). The conjugated T-Cell-MP polypeptides form a heteroduplex as shown in (B) via interactions between the interspecific scaffold sequences. The unconjugated heteroduplex may be

isolated from cells stably or transiently expressing the protein. The unconjugated heteroduplex, generally in a purified form, is subjected to chemical conjugation by coupling an epitope to the conjugation sites, which is exemplified by the reaction between a cysteine in the β2M polypeptide sequence (e.g., an E44C substitution) and a maleimide labeled peptide to yield the T-Cell-MP-KRAS-epitope conjugate shown in (C). Excess reactive peptide can be removed or substoichiometric amounts of the reactive epitope (relative to the amount of conjugation sites) can be utilized to produce the conjugated T-Cell-MP heteroduplex, which as shown may comprise different MODs on each of the T-Cell-MP polypeptides. The constructs are not limited to the linker sequences shown, which are exemplary of the linkers that may be employed.

[0027] **FIG. 11** shows three heterodimeric T-Cell-MP-KRAS-epitope conjugate duplexes. Each has a scaffold comprising an interspecific Ig Fc polypeptide pair, however the scaffold polypeptides may be replaced by any other interspecific polypeptide pair. The constructs are not limited to the linker sequences shown, which are exemplary of the linkers that may be employed.

[0028] **FIG. 12** shows comparative results for the expression of a series of molecules including T-Cell-MPs in cultured CHO cells, described in Example 1, with the molecules (constructs) having varied substitutions in the L3 linker and at other locations. The overall structure of the molecules is provided at A, B, and C. The titer (amount of protein) of the molecules and fraction of the molecules that are unaggregated (e.g., existing as soluble duplexes) are provided in histograms D and E respectively.

[0029] **FIG. 13** shows the production and stability in culture of an unconjugated T-Cell-MP (construct 3861, which has an L3 linker consisting of a Gly_4Ser repeated three times $(G_4S)_3$) at 2, 4, and 6 million cells per ml at both 32 and 28° over several days (A and B). The chromatograms show protein A purified material from a culture before (C) and after (D) further purification by size exclusion chromatography. The Coomassie blue gel (E) shows that materials run against molecular weight standards (Mw) at 103128 Daltons for reduced (R) and 206213 Daltons for the non-reduced samples. See Example 2 for details.

[0030] **FIG. 14** at A demonstrates the specificity of the T-Cell-MP-KRAS-epitope conjugates for T cells specific to the conjugated epitope. At B, FIG. 14 shows an electrophoresis gel of non-reduced and reduced samples of epitope conjugates. See Example 3 for details.

[0031] **FIG. 15 and FIG. 16** show the response of CD8+ T cells present in Leukopak samples from CMV and MART-1 response donors to T-Cell-MP-epitope conjugates and control treatments as described in Example 4.

[0032] **FIG. 17** shows the effect of L3 linker length on the CHO cell expression of two series of unconjugated T-Cell-MPs, providing the titer in culture media by Octet analysis at A, and the fraction of unaggregated (duplex) molecules present in the samples at B following purification on protein A magnetic beads.

[0033] Fig. 18 provides the amino acid sequences of certain constructs discussed in this disclosure. Linker sequences (e.g., AAAGG (SEQ ID NO:122) and GGGGS (SEQ ID NO:120)) may be bolded, italicized and underlined to permit their identification. The indicated single amino acid substitutions in the MHC class I heavy chain are shown in bold with underlining. Human IL2 sequences are indicated by

hIL2, beta-2-microglobin sequences are indicated by β2M, and HLA-A02 sequences are indicated by HLA-A*0201, with each bearing the indicated aa substitutions.

Definitions

[0034] The term T-Cell-MP is generic to, and includes, both unconjugated T-Cell-MPs and T-Cell-MP-KRAS-epitope conjugates. The term "unconjugated T-Cell-MP (or "MPs" when plural) refers to T-Cell-MPs that have not been conjugated (covalently linked) to an epitope and/or payload (e.g., a non-epitope molecule such as a label), and therefore comprise at least one chemical conjugation site. Unconjugated T-Cell-MP polypeptides also do not comprise a fused peptide epitope that can be positioned within the MHC-H binding cleft and in conjunction with the β2M polypeptide sequence and presented to a TCR. The terms "T-Cell-MP-KRAS-epitope conjugate" (or "conjugates" when plural) refers to T-Cell-MPs that have been conjugated (covalently linked) to a peptide, with or without post translational modifications, presenting a KRAS epitope at a chemical conjugation site that permits the epitope to be present in the MHC binding cleft and presented to a TCR with specificity for the epitope expressed on a T Cell (an epitope specific T cell). "T-Cell-MP-payload conjugate" and "T-Cell-MP-payload conjugates" refer to T-Cell-MPs that have been conjugated (covalently linked) to one or more independently selected payloads. The term "T-Cell-MP" also includes unconjugated T-Cell-MPs and T-Cell MP-epitope conjugates that either comprise one or more independently selected MODs or are MOD-less. In those instances where this disclosure specifically refers to a T-Cell-MP that does not contain a MOD, terms such as "MOD-less T-Cell-MP" or a "T-Cell-MP without a MOD" and the like are employed. The term "T-Cell-MP" also includes unconjugated T-Cell-MPs and T-Cell MP-epitope conjugates that comprise either one or more independently selected targeting sequences (discussed below).

[0035] The terms "polynucleotide" and "nucleic acid," used interchangeably herein, refer to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. Thus, these terms include, but are 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.

[0036] The terms "polypeptide" and "protein," used interchangeably herein, refer to a polymeric form of amino acids, which unless stated otherwise are the naturally occurring proteinogenic L-amino acids that are incorporated biosynthetically into proteins during translation in a mammalian cell. Furthermore, as used herein, these terms may refer to polypeptides or proteins that include modifications, such as deletions, additions, and substitutions (generally conservative in nature as would be known to a person in the art) to the native sequence, as long as the protein maintains the desired activity. These modifications can be deliberate, as through site-directed mutagenesis, or can be accidental, such as through mutations of hosts that produce the proteins, or errors due to polymerase chain reaction (PCR) amplification or other recombinant DNA methods. References herein to a specific residue or residue number in a known polypeptide, e.g., a human MHC class I polypeptide, are understood to refer to the amino acid at that position in the wild-type polypeptide. To the extent that the sequence of the wild-type polypeptide is altered, either by addition or deletion of one or more amino acids, one of ordinary skill will understand

that a reference to the specific residue or residue number will be correspondingly altered so as to refer to the same specific amino acid in the altered polypeptide, which would be understood to reside at an altered position number. A reference to a "non-naturally occurring Cys residue" or "engineered" Cys residue in a polypeptide, e.g., an MHC class I polypeptide, means that the polypeptide comprises a Cys residue in a location where there is no Cys in the corresponding wild-type polypeptide. This can be accomplished through routine protein engineering in which a cysteine is substituted for the amino acid that occurs in the wild-type sequence

[0037] A nucleic acid or polypeptide has a certain percent "sequence identity" to another nucleic acid or polypeptide, meaning that, when aligned, that percentage of bases or amino acids are the same, and in the same relative position, when comparing the two sequences. Sequence identity can be determined in a number of different ways. To determine sequence identity, sequences can be aligned using various convenient methods and computer programs (e.g., BLAST, T-COFFEE, MUSCLE, MAFFT, etc.), available over the world wide web at sites including blast.ncbi.nlm.nih.gov/Blast.cgi for BLAST+2.10.0, 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.

[0038] As used herein amino acid ("aa" singular or "aas" plural) means the naturally occurring proteinogenic amino acids incorporated into polypeptides and proteins in mammalian cell translation. Unless stated otherwise, these are: 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). Amino acid also includes the amino acids hydroxyproline and selenocysteine, which appear in some proteins found in mammalian cells; however, unless their presence is expressly indicated they are not understood to be included.

[0039] The term "conservative amino acid 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.

[0040] The term "binding" (or "bound") refers generically to a direct association between molecules and/or atoms, due to, for example, covalent, electrostatic, hydrophobic, ionic and/or hydrogen-bond interactions, including interactions such as salt bridges and water bridges.

[0041] The term "binding" (or "bound") as used with reference to a T-Cell-MP binding to a polypeptide (e.g., a T cell receptor on a T cell), refers to a non-covalent interaction between two molecules. A non-covalent interaction 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, less than 10^{-12} M, less than 10^{-13} M, less than 10^{-14} M, or less than 10^{-15} M. "Covalent bonding" or "covalent binding" as used herein refers to the formation of one or more covalent chemical bonds between two different molecules.

[0042] "Affinity" as used herein generally refers to the strength of non-covalent binding, increased binding affinity being correlated with a lower K_D. As used herein, the term "affinity" may be described by the dissociation constant (K_D) for the reversible binding of two agents (e.g., an antibody and an antigen. Affinity can be at least 1-fold greater to at least 1,000-fold greater, (e.g., at least 2-fold to at least 5-fold greater, at least 3-fold to at least 6-fold greater, at least 4-fold to at least 8-fold greater, at least 5-fold to at least 10-fold greater, at least 6-fold to at least 15-fold greater, at least 7-fold to at least 20-fold greater, at least 8-fold to at least 30-fold greater, at least 9-fold to at least 35-fold greater, at least 10-fold to at least 40-fold greater, at least 20-fold to at least 60-fold greater, at least 40-fold to at least 80-fold greater, at least 60-fold to at least 180-fold greater, at least 80-fold to at least 240-fold greater, at least 100-fold to at least 1,000-fold greater, or at least 1,000-fold greater, than the affinity of an antibody or receptor for an unrelated aa sequence (e.g., ligand). 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.

[0043] The term "immunological synapse" or "immune synapse" as used herein generally refers to the natural interface between two interacting immune cells of an adaptive immune response including, e.g., the interface between an antigen-presenting cell (APC) or target cell and an effector cell, e.g., a lymphocyte, an effector T cell, a natural killer cell, and the like. An immunological synapse between an APC and a T cell is generally initiated by the interaction of a T cell antigen receptor and 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.

[0044] "T cell" includes all types of immune cells expressing CD3, including T-helper cells ($CD4^+$ cells), cytotoxic T cells ($CD8^+$ cells), regulatory T cells (T reg), and NK-T cells.

[0045] The term "immunomodulatory polypeptide" (also referred to as a "costimulatory polypeptide" or, as noted above, a "MOD") as used herein includes a polypeptide or portion thereof (e.g., an ectodomain) on an APC (e.g., a dendritic cell, a B cell, and the like), or otherwise available to interact with the T cell, that specifically binds a cognate co-immunomodulatory polypeptide ("Co-MOD") present on a T cell, thereby providing a signal. The signal provided by the MOD engaging its Co-MOD, in addition to the primary signal provided by, for instance, binding of a TCR/CD3 complex with a MHC

polypeptide loaded with a peptide epitope, mediates (e.g., directs) a T cell response. The responses include, but are not limited to, proliferation, activation, differentiation, and the like. A MOD can include, but is not limited to, CD7, B7-1 (CD80), B7-2 (CD86), 4-1BBL, OX40L, inducible costimulatory ligand (ICOS-L), intercellular adhesion molecule (ICAM), CD30L, CD40, CD70, CD83, HLA-G, lymphotoxin beta receptor, 3/TR6, ILT3, ILT4, HVEM, an agonist or antibody that binds Toll-Like Receptor (TLR), and a ligand that specifically binds with B7-H3. A MOD also encompasses, inter alia, an antibody or antibody fragment that specifically binds with and activates a Co-MOD molecule present on a T cell such as, but not limited, to antibodies against the receptors for any of IL-2, CD27, CD28, 4-1BB, OX40, CD30, CD40, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, LIGHT (also known as tumor necrosis factor superfamily member 14 (TNFSF14)), NKG2C, B7-DC, B7-H2, B7-H3, and CD83.

[0046] "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.

[0047] The terms "recombinant expression vector" or "DNA construct," used interchangeably herein, 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.

[0048] The terms "treatment," "treating" and the like are used herein to generally mean obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. "Treatment" as used herein covers any treatment of a disease or symptom in a mammal, and includes: (a) preventing the disease or symptom from occurring in a subject which may be predisposed to acquiring the disease or symptom but has not yet been diagnosed as having it; (b) inhibiting the disease or symptom, i.e., arresting its development; and/or (c) relieving the disease, i.e., causing regression of the disease. The therapeutic agent may be administered before, during or after the onset of disease or injury. The treatment of ongoing disease, where the treatment stabilizes or reduces the undesirable clinical symptoms of the patient, is of particular interest. Such treatment is desirably performed prior to complete loss of function in the affected tissues. The subject therapy will desirably be administered during the symptomatic stage of the disease and, in some cases, after the symptomatic stage of the disease.

[0049] 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

humans and non-human primates, and in addition include rodents (e.g., rats; mice), lagomorphs (e.g., rabbits), ungulates (e.g., cows, sheep, pigs, horses, goats, and the like), felines, canines, etc.

[0050] 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 limit the scope of the invention.

[0051] Unless indicated otherwise, the term "substantially" is intended to encompass both "wholly" and "largely but not wholly." For example, an Ig Fc that "substantially does not induce cell lysis" means an Ig Fc that induces no cell lysis at all or that largely does not induce cell lysis.

[0052] As used herein, the term "about" used in connection with an amount indicates that the amount can vary by 10% of the stated amount. For example, "about 100" means an amount of from 90-110. Where about is used in the context of a range, the "about" used in reference to the lower amount of the range means that the lower amount includes an amount that is 10% lower than the lower amount of the range, and "about" used in reference to the higher amount of the range means that the higher amount includes an amount 10% higher than the higher amount of the range. For example, from about 100 to about 1000 means that the range extends from 90 to 1100.

[0053] As used herein the term "in vivo" refers to any process or procedure occurring inside of the body, e.g., of a patient having a cancer caused by a KRAS mutation.

[0054] As used herein, "in vitro" and "ex vivo" refer to processes or procedures occurring outside of the body. Although the terms may be used interchangeably, the term ex vivo is generally used to indicate a process where an animal-derived (e.g., human-derived) tissue is subjected to a process outside of the body and then the acted-upon tissue is re-inserted into the animal.

[0055] Where a range of values is provided, it is understood that each intervening value between the upper and lower limit of that range to a tenth of the lower limit of the range is encompassed within the disclosure along with any other stated or intervening value in the range. Upper and lower limits may independently be included in smaller ranges that are also encompassed within the disclosure subject to any specifically excluded limit in the stated range. Where the stated range has a value (e.g., an upper or lower limit), ranges excluding those values are also included in the invention.

[0056] 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.

[0057] It must be noted that, as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a T reg" includes a plurality of such T regs and reference to "the MHC Class I heavy chain" includes reference to one or more MHC Class I heavy chains 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.

[0058] 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.

[0059] 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 publications 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 Polypeptides (T-Cell-MPs) With Chemical Conjugation Sites for Epitope Binding

[0060] The present disclosure includes and provides for T-Cell-MPs (both unconjugated T-Cell-MPs having a chemical conjugation site suitable for attaching an epitope and T-Cell-MP-epitope conjugates to which an epitope has been conjugated). Such T-Cell-MP are useful for modulating the activity of T cells to, for example, modulate an immune response in vitro, ex vivo, or in vivo, and accordingly to effect therapeutic treatments. The present disclosure specifically provides methods of T-Cell MP-epitope conjugate preparation and use in modulating an immune response in vitro, ex vivo, or in vivo in an individual that may be a human or non-human test subject or patient. The human or non-human test subject or patient may be suffering from one or more tumors, one or more cancers. In addition to the other elements present the T-Cell-MPs may comprise one or more independently selected wt. and/or variant MOD polypeptides that exhibit reduced binding affinity to their Co-MODs and one or more payloads.

[0061] Included in this disclosure are T-Cell-MPs that are homodimeric, comprising identical first and second T-Cell-MP polypeptides. Also included in this disclosure are T-Cell-MPs that are heterodimeric, comprising a first and a second T-Cell-MP polypeptide), wherein at least one of those polypeptides comprises a chemical conjugation site for the attachment of an epitope. Optionally at least one of the heterodimers may comprise a payload such as chemotherapeutic agent and/or a targeting sequence. Included in this disclosure are T-Cell-MPs which have been chemically conjugated to a KRAS epitope to

form a T-Cell-MP-KRAS-epitope conjugate and which optionally comprise a targeting sequence and/or a payload.

[0062] Depending on the type of MOD(s) present in a T-Cell-MP-KRAS-epitope conjugate, a T cell bearing a TCR specific to the epitope is present on a T-Cell-MP can respond by undergoing activation including, for example, clonal expansion (e.g., when activating MODs such as wt. and/or variants of IL-2, 4-1BBL and/or CD80 are incorporated into the T-Cell-MP). Alternatively, the T cell may undergo inhibition that down regulates T cell activity when MODs such as wt. and/or variants of FASL and/or PD-L1 are incorporated into the T-Cell-MPs. The incorporation of combinations of MODs such as wt. and/or variants of IL-2 and CD80 or IL2 and PD-L1 into T-Cell-MPs (e.g., T-Cell-MP-KRAS-epitope conjugates) may lead to synergistic effects where the T cell response more than exceeds the sum of the responses of T cells to otherwise identical T-Cell-MPs lacking one of the MOD. 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-MPs such that the binding of a T-Cell-MP to a T cell is strongly affected by, or even dominated by, the MHC-epitope-TCR interaction.

[0063] A T-Cell-MP-KRAS-epitope conjugate bearing MODs may be considered to function as a surrogate APC, and by interacting with a T-Cell mimic the presentation of epitope in an adaptive immune response. The T-Cell-MP-KRAS-epitope conjugate does so by engaging and presenting to a TCR present on the surface of a T cell with a covalently bound epitope (e.g., a peptide presenting a KRAS epitope). This engagement provides the T-Cell-MP-KRAS-epitope conjugate with the ability to achieve epitope-specific cell targeting. In embodiments described herein, T-Cell-MP-KRAS-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/apoptosis or activation/proliferation.

[0064] Unconjugated T-Cell-MPs, which have chemical conjugation sites, find use as a platform into which different epitopes may be introduced, either alone or along combination with one or more any additional payloads added to the T-Cell-MP, in order to prepare materials for therapeutic, diagnostic and research applications. Because T-Cell-MPs, including duplexes comprised of homodimers, and higher order homomeric complexes require only a single polypeptide sequence, they can advantageously be introduced and expressed by cells using a single vector with a single expression cassette. Similarly, heterodimeric duplex T-Cell-MPs can be introduced into cell using single vector with: two separate expression cassettes, a bicistronic expression cassette (e.g. with the proteins separated by a 2A protein sequence or internal ribosome entry sequence (IRES)); or by using two vectors each bearing a cassette coding one heterodimeric subunits. Where duplex or higher order T-Cell-MPs contain interspecific scaffold sequences, the different T-Cell-MPs may bear different MODS permitting the duplex or higher order structure to contain different MODs, or MODs at different locations on each polypeptide of the heterodimer. The modular nature of T-Cell-MPs enables the rapid preparation and testing of diagnostics and therapeutics candidates by coupling an epitope containing molecule (e.g., a peptide) into prepared T-

Cell-MP polypeptides that can then be tested for activation or inhibition of T cells bearing TCRs specific to the epitope. The ability to construct unconjugated T-Cell-MPs, and in particular heterodimer T-Cell-MP duplexes with different MODs, permits rapid assembly and assessment of different combinations of MODs with one or more epitope relevant to a disease state or condition. Further to the foregoing, the ability to rapidly attach to the T-Cell-MP various targeting sequences and/or payloads, such as chemotherapeutics, facilitates both preparation of T-Cell-MPs for screening and as therapeutics.

[0065] Where one or more activating wt. MOD or variant MOD polypeptide sequences are incorporated into a T-Cell-MP-KRASepitope conjugate, contacting the T cells with a TCR specific to the epitope with at least one concentration of the T-Cell-MP-KRAS-epitope conjugate can result in T cell activation. T cell activation may result in one or more of the following: an increase the activity of ZAP70 protein kinase activity, induction in the proliferation of the T-cell(s), granule-dependent effector actions (e.g., the release of granzymes, perforin, and/or granulysin from cytotoxic T-cells), and/or release of T cell cytokines (e.g., interferon γ from CD8+ cells). Where the MOD polypeptide sequence(s) induce T cell proliferation, the T-Cell-MP-KRAS epitope-conjugate may induce 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 having a TCR specific to the epitope as compared to T cells contacted with the same concentration of the T-Cell-MP-KRAS-epitope conjugate that do not have a TCR specific to the epitope (see FIG. 1). Activation of T-cells may be measured by, for example, ZAP-70 activity or T cell proliferation, *see e.g.*, Wang, *et al., Cold Spring Harbor perspectives in biology 2.5 (2010): a002279*, or cytokine release. Where one or more wt. or variant MOD polypeptide sequences that inhibit T cell activation are incorporated into a T-Cell-MP-KRAS-epitope conjugate, contacting the T cells having a TCR specific to the epitope with at least one concentration of the T-Cell-MP-KRAS-epitope conjugate may result in one or more of the following: prevention or inhibition of the of T cell's activation, reduction in the response of activated T cells, and/or down regulation of the epitope-specific T-Cell. In some cases, inhibitory MODs present in a T-Cell-MP-KRAS-epitope conjugate may result in apoptosis of T cell(s) with a TCR specific to the epitope. The effects of inhibitory MOD sequences may be measured by, for example, one or more of their: effect on T cell proliferation, ZAP-70 activity, reduction in granule-dependent effector actions, and/or cell death.

[0066] The specificity of T-Cell-MP-KRAS-epitope conjugates depends on the relative contributions of the epitope and its MODs to the binding. Where the affinity of the MOD(s) for the Co-MOD(s) is relatively high such that the MOD(s) dominate the T-Cell-MPs in the binding interactions, the specificity of the T-Cell-MP-KRAS-epitope conjugates will be reduced relative to T-Cell-MP complexes where the epitope dominates the binding interactions by contributing more to the overall binding energy than the MODs. The greater the contribution of binding energy between an epitope and a TCR specific to the epitope, the greater the specificity of the T-Cell-MP will be for the T cell bearing that type of TCR. Where an epitope MHC complex has strong affinity for its TCR, the use of wt. MODs that have relatively low affinity and/or variant MODs with reduced affinity for their Co-MODs will favor epitope selective interactions of the T-Cell-MP-KRAS-epitope conjugates with specific T cells, and also facilitate selective

delivery of any payload that may be conjugated to the T-Cell-MP-KRAS-epitope conjugate to the T cell and/or locations where the T cell is located.

[0067] The present disclosure provides T-Cell-MP-KRAS-epitope conjugates presenting epitopes of KRAS that are useful for modulating the activity of T cells in an epitope-specific manner and, accordingly, for modulating an immune response to those disease states in an individual. The T-Cell-MPs comprise one or more MODs that are wt. and/or exhibit reduced binding affinity to a Co-MOD.

A. Unconjugated T-Cell-MPs and T-Cell-MP-Epitope Conjugates (T-Cell-MP-KRAS-Epitope Conjugates)

1 The Structure and Composition of Unconjugated T-Cell-MPs and T-Cell-MP-Epitope Conjugate Components

[0068] The unconjugated T-Cell-MPs described herein comprise a chemical conjugation site for coupling an epitope directly, or indirectly through a linker. The chemical conjugation site can be situated at any location on the T-Cell-MP. One aspect of the disclosure is directed to T-Cell-MPs that comprise a chemical conjugation site for the attachment of a peptide epitope within the scaffold (e.g., Ig Fc), β2M, or MHC-H polypeptide sequences, or the linker (L3) joining the β2M and MHC-H polypeptide sequences, and higher order complexes of those T-Cell-MPs. Another aspect of the disclosure is directed to T-Cell-MPs that comprise a chemical conjugation site for the attachment of a peptide epitope within the β2M, or MHC-H polypeptide sequences, or the linker (L3) joining the β2M and MHC-H polypeptide sequences, and higher order complexes of those T-Cell-MPs. A chemical conjugation site for coupling an epitope directly, or indirectly through a linker, can be situated in the β2M polypeptide sequence. A chemical conjugation site for coupling an epitope directly, or indirectly through a linker, can be situated in the MHC-H polypeptide sequence. A chemical conjugation site for coupling an epitope directly, or indirectly through a linker, can be situated in the linker (L3) joining the β2M polypeptide sequence and MHC-H polypeptide sequence. A chemical conjugation site for coupling an epitope directly, or indirectly through a linker, can be situated in the within the scaffold (e.g., Ig Fc). Where a chemical conjugation site for coupling an epitope to an unconjugated T-Cell-MP appears in a scaffold (e.g., an Ig Fc), β2M, or MHC-H polypeptide sequence, the chemical conjugation site may be limited to an amino acid or sequence of amino acids not naturally appearing in any of those sequences, but instead involves one or more amino acids introduced into one of those sequences. In addition, while it is possible to utilize the N-terminal amino group or C-terminal carboxyl group of a T-Cell-MP polypeptide as a chemical conjugation site for epitope attachment, those sites may be excluded as conjugation sites from any of the T-Cell-MPs or their higher order complexes described herein. Indeed, the chemical conjugation site of a T-Cell-MP may be excluded from the N-terminal 10 or 20 aas and/or the C-terminal 10 or 20 aas.

[0069] T-Cell-MPs may form higher order complexes (e.g., duplexes, triplexes, etc.). The higher order complexes may be homomeric (e.g., homodimers or homoduplexes) or heteromeric (heterodimers or heteroduplexes). Pairs of interspecific sequences may be employed as scaffold sequences where the complexes are intended to be heterodimeric as they permit two different T-Cell-MPs to form a specific

heteroduplex, as opposed to a mixture of homoduplexes and heteroduplexes that can form if two T-Cell-MPs not having a pair of interspecific bind sequences are mixed.

[0070] A first group of T-Cell-MP molecules described herein are broadly directed to T-Cell-MPs that may form a duplex that associates through interactions in their scaffold sequences. Such T-Cell-MPs may have at least a first T-Cell-MP polypeptide sequence (e.g., duplexed as a homodimer), or non-identical first and second T-Cell-MP polypeptide sequences (e.g., duplexed as a heterodimer) with one or both of the T-Cell-MPs comprising (e.g., from N-terminus to C-terminus):

(i) optionally one or more MOD polypeptide sequences (e.g., two or more MOD polypeptide sequences, such as in tandem, wherein when there are two or more MOD polypeptide sequences they are optionally joined to each other by independently selected L1 linkers);

(ii) an optional linker L2 polypeptide sequence joining the one or more MOD polypeptide sequences to a β2M polypeptide sequence;

(iii) the β2M polypeptide sequence;

(iv) an optional L3 linker polypeptide sequence (e.g., from 10-50 aa in length);

(v) a class I MHC-H polypeptide sequence;

(vi) an optional L4 linker polypeptide sequence;

(vii) a scaffold polypeptide sequence (e.g., an immunoglobulin Fc sequence);

(viii) an optional L5 linker polypeptide sequence; and

(ix) optionally one or more MOD polypeptide sequence (e.g., two or more MOD polypeptide sequences, such as in tandem, wherein when there are two or more MOD polypeptide sequences they are optionally joined to each other by independently selected L6 linkers);

wherein the unconjugated T cell modulatory polypeptide comprises at least one MOD polypeptide sequence (e.g., the MOD(s) of element (i) and/or (ix)); and

wherein at least one of the β2M polypeptide sequence, L3 linker polypeptide sequence, and/or the MHC-H polypeptide sequence comprises at least one chemical conjugation site.

[0071] A second group of unconjugated T-Cell-MPs described herein may form a duplex between a first T-Cell-MP and a second T-Cell-MP that associate through interactions in their scaffold sequences. Such unconjugated duplex T-Cell-MPs may have an identical first and second T-Cell-MP polypeptide sequence duplexed as a homodimer, or non-identical first and second T-Cell-MP polypeptide sequences duplexed as a heterodimer with one or both of the T-Cell-MPs comprising from N-terminus to C-terminus:

(i) optionally one or more MOD polypeptide sequences (e.g., two or more MOD polypeptide sequences, such as in tandem, wherein when there are two or more MOD polypeptide sequences they are optionally joined to each other by independently selected L1 linkers);

(ii) an optional linker L2 polypeptide sequence joining the one or more optional MOD polypeptide sequences to a β2M polypeptide sequence;

(iii) a β2M polypeptide sequence;

(iv) an optional L3 linker polypeptide sequence (e.g., from 10-50 aa in length);

(v) a class I MHC-H polypeptide sequence;

(vi) an optional L4 linker polypeptide sequence;

(vii) a scaffold polypeptide sequence (e.g., an immunoglobulin Fc sequence);

(viii) an optional L5 linker polypeptide sequence; and

(ix) optionally one or more MOD polypeptide sequence (e.g., two or more MOD polypeptide sequences, such as in tandem, wherein when there are two or more MOD polypeptide sequences they are optionally joined to each other by independently selected L6 linkers);

wherein the unconjugated T cell modulatory polypeptide comprises at least one MOD polypeptide sequence (e.g., the MOD(s) of element (i) and/or (ix)); and

wherein at least one of the β2M polypeptide sequence, L3 linker polypeptide sequence, and/or the MHC-H polypeptide sequence comprises at least one chemical conjugation site, e.g., for epitope conjugation and/or payload conjugation.

[0072] A third group of unconjugated T-Cell-MPs described herein appears as a duplex between a first T-Cell-MP and a second T-Cell-MP that associate through interactions in their scaffold sequences. Such unconjugated duplex T-Cell-MP may have an identical first and second T-Cell-MP polypeptide sequence duplexed as a homodimer, or non-identical first and second T-Cell-MP polypeptide sequences duplexed as a heterodimer with one or both of the T-Cell-MPs comprising from N-terminus to C-terminus:

(i) optionally one or more MOD polypeptide sequences (e.g., two or more MOD polypeptide sequences, such as in tandem, wherein when there are two or more MOD polypeptide sequences they are optionally joined to each other by independently selected L1 linkers);

(ii) an optional L2 polypeptide sequence joining the one or more optional MOD polypeptide sequences to a β2M polypeptide sequence;

(iii) a β2M polypeptide sequence;

(iv) an L3 linker polypeptide sequence comprising from 10 to 50 amino acids;

(v) a class I MHC-H polypeptide sequence comprising cysteines substituted at positions 84 and 139 (see FIGS 3E-3H, e.g., Y84C and A139C substitutions) and forming a disulfide bond;

(vi) an L4 linker polypeptide sequence;

(vii) an interspecific or non-interspecific immunoglobulin Fc scaffold sequence;

(viii) an L5 linker polypeptide sequence; and

(ix) optionally one or more MOD polypeptide sequence (e.g., two or more MOD polypeptide sequences, such as in tandem, wherein when there are two or more MOD polypeptide sequences they are optionally joined to each other by independently selected L6 linkers);

wherein at least one of the β2M polypeptide sequence, L3 linker polypeptide sequence, and/or the MHC-H polypeptide sequence comprises at least one chemical conjugation site, e.g., for epitope conjugation and/or payload conjugation wherein at least one of the β2M polypeptide sequence, L3 linker polypeptide sequence, or the MHC-H polypeptide sequence comprises a chemical conjugation site that does not appear in a wt. sequence. The site should be suitable for epitope conjugation in that

it does not interfere with the interactions of the T-Cell-MP with a TCR and is preferably solvent accessible permitting its conjugation to the epitope; and

wherein the first and second T-Cell-MPs are optionally covalently linked through at least one disulfide bond between their Ig Fc scaffold sequence.

[0073] The chemical conjugation site for epitope conjugation to T-Cell-MPs, including those of the above-mentioned first, second, and third groups of unconjugated T-Cell-MPs, permit the covalent attachment of an epitope presenting molecule (e.g., a peptide epitope) to the T-Cell-MP such that it can be bound (located in the binding cleft) by the MHC-H polypeptide and presented to a TCR. The chemical conjugation sites of an unconjugated T-Cell-MP may be one that does not appear in a wt. sequence (e.g., it is created using the techniques of protein engineering based in biochemistry and/or molecular biology). The chemical conjugation site should also be suitable for epitope conjugation in that it does not interfere with the interactions of the T-Cell-MP with a TCR, and is preferably solvent accessible permitting its conjugation to the epitope.

[0074] It is understood that the unconjugated T-Cell-MPs do not comprise a peptide epitope (either covalently attached to, or as a fusion with, the T-Cell-MP polypeptide) that can be located in the binding cleft of the MHC-H/β2M polypeptide sequences and presented to a TCR. The disclosure does, however, include and provide for T-Cell-MP-epitope conjugates further comprising a molecule presenting an epitope of KRAS (a T-Cell-MP-KRAS-epitope conjugate) that is directly or indirectly (e.g., through a peptide or non-peptide linker) covalently attached to the T-Cell-MP at a chemical conjugation site; where the epitope can also be associated with (located in or positioned in) the binding cleft of the T-Cell-MP MHC-H polypeptide sequence and functionally presented to a T cell bearing a TCR specific for the epitope, leading to TCR mediated activation or inhibition of the T cell.

[0075] The disclosure also provides T-Cell-MPs in which the epitope present in a T-Cell-MP-KRAS-epitope conjugate of the present disclosure may bind to a TCR (e.g., 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).

[0076] A T-Cell-MP-KRAS-epitope conjugate may bind to a first T cell with an affinity that is higher than the affinity with which the T-Cell-MP-KRAS-epitope conjugate binds to a second T cell; where the first T cell expresses on its surface a Co-MOD and a TCR that binds the epitope, and where the second T cell expresses on its surface the same Co-MOD present on the first T cell, but does not express on its surface a TCR that binds the epitope (e.g., as tightly as the TCR of the first cell if it binds at all). See FIG. 1. The increased affinity may be measured in binding assays or inferred from the concentration of the T-Cell-MP-epitope conjugate required to stimulate the first as compared to the second T cell. The increased affinity for epitope-specific T cells permits the use of the epitope conjugates as agents for clinical testing, diagnostics, and as therapeutics capable of directing epitope-specific T cell actions.

[0077] MODs present in T cell-MPs are independently selected wt. MODs and/or variant MODs. Where the T cell-MP forms a heteromeric complex, such as through the use of interspecific scaffold polypeptide sequences, of the MODs presented in at least one of the T-Cell-MPs of the heteromer may be

selected independently from the other T-Cell-MPs of the heteromeric complex. Accordingly, a heterodimeric duplex T-Cell-MP may have independently selected MODs that are different in the first and second T-Cell-MP of the duplex. MODs in one aspect are selected to be one or more activating wt. MODs and/or variant MODs capable of stimulating epitope-specific T cell activation/proliferation (e.g., wt. and/or variant IL-2, 4-1BBL and/or CD80). In another embodiment, the MODs are one or more inhibitory 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-MP 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-MP with the T cells.

2 Chemical Conjugation Sites of Unconjugated T-Cell-MPs

[0078] The term "chemical conjugation site" means any suitable site of a T-Cell-MP that permits the selective formation of a direct or indirect (through an intervening linker or spacer) covalent linkage between the T-Cell-MP and an epitope- or payload-containing molecule. Chemical conjugation sites of unconjugated T-Cell-MPs may be (i) active, i.e., capable of forming a direct or indirect (through an intervening linker or spacer) covalent linkage between the T-Cell-MP and an epitope or payload without an additional chemical reaction or transformation of the chemical conjugation site (e.g., a solvent-accessible cysteine sulfhydryl), or (ii) nascent, i.e., requiring a further chemical reaction or enzymatic transformation of the chemical conjugation site to become an active chemical conjugation site (e.g., a sulfatase sequence not yet activated by an fGly enzyme).

[0079] The term "selectively formation" means that when an epitope- or payload-containing molecule bearing a moiety that is reactive with an active chemical conjugation site of a T-Cell-MP, the epitope- or payload-containing molecule will be covalently bound to the chemical conjugation site in an amount higher than to any other site in the T-Cell-MP.

[0080] Chemical conjugation sites may be introduced into a T-Cell-MP using protein engineering techniques (e.g., by use of an appropriate nucleic acid sequence) to achieve a T-Cell-MP having a desired aa sequence. Chemical conjugation sites can be individual aas (e.g., a cysteine or lysine) or aa sequences (e.g., sulfatase, sortase or transglutaminase sequences) in a protein or polypeptide sequence of the T-Cell-MP.

[0081] Where the protein or polypeptide sequence of the T-Cell-MP is derived from a naturally occurring protein (e.g., the B2M, MHC-H or an IgG scaffold), the chemical conjugation site may be a site not appearing in the naturally occurring sequence, such as a site resulting from amino acid substitutions (e.g., cysteine substitutions), insertions, and or deletions. The chemical conjugation site may also be a sequence, or part of a sequence, that is not derived from a naturally occurring protein, such as a linker sequence (e.g., the L3 linker of a T-Cell-MP connecting the β2M and MHC-H polypeptide sequences of a T-Cell-MP).

[0082] In some embodiments, there is only one chemical conjugation site (e.g., one chemical conjugation site added by protein engineering) in each unconjugated T-Cell-MP polypeptide that permits

an epitope to be covalently attached such that it can be located in the MHC polypeptide binding cleft and presented to a TCR. Each individual unconjugated T-Cell-MP may comprise more than one chemical conjugation sites that are selected to be either the same or different types of chemical conjugation sites, thereby permitting the same or different molecules (e.g., an epitope and one or more payloads) to be selectively conjugated to each of the chemical conjugation sites. Accordingly, each individual or duplexed unconjugated T-Cell-MP may comprise one or more chemical conjugations sites that 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 chemical conjugation sites. The chemical conjugations sites (e.g., for the conjugation of epitope) generally will be the same (e.g., of the same type) so that epitope presenting molecules can be covalently attached to all of the desired sites in, for example, a duplex unconjugated T-Cell-MP, using a single reaction. T-Cell-MP's may contain chemical conjugation sites in addition to those for the conjugation to an epitope, including conjugation sites for the incorporation of, for example, targeting sequences and/or payloads such as labels.

[0083] Chemical conjugation sites used to incorporate molecules other than epitopes presenting molecules will, in most instances, be of a different type (e.g., utilize different chemical reactions) and in different locations than the sites used to incorporate epitopes, thereby permitting different molecules to be selectively conjugated to each of the polypeptides. Where a T-Cell-MP is to comprise a targeting sequence and/or one or more payload molecules, the unconjugated T-Cell-MP may comprise more than one copy of a chemical conjugation site (e.g., chemical conjugation sites added by protein engineering) to permit attachment and of multiple molecules of targeting sequence and/or payload.

[0084] Chemical conjugation sites that may be incorporated into unconjugated T cell-MP polypeptides, include, but are not limited to:

a) peptide sequences that acts as an enzyme modification sequence (e.g., sulfatase, sortase, and/or transglutaminase sequences);

b) non-natural aas and/or selenocysteines;

c) chemical conjugation sites comprising individual amino acids;

d) carbohydrate or oligosaccharide moieties; and

e) IgG nucleotide binding sites.

a. Sulfatase Motifs

[0085] In those embodiments where enzymatic modification is chosen as the means of chemical conjugation, the chemical conjugation site(s) may comprise a sulfatase motif. Sulfatase motifs are usually 5 or 6 aas in length, and are described, for example, in U.S. Pat. No. 9,540,438 and U.S. Pat. Pub. No. 2017/0166639 Al, 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 aa, sometimes referred to as oxoalanine, 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 aa sequence comprising an unconverted sulfatase motif, as well as to an aa 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. Where the term sulfatase motif is utilized in the context of an aa sequence, both the nascent chemical conjugation sequence (e.g., a polypeptide containing the unconverted motif) as well as its fGly containing the active chemical conjugation site counterpart are disclosed. Once present in a polypeptide (e.g., of a T-Cell-MP), a fGly residue may be reacted with molecules (e.g., peptide epitopes with or without an intervening linker) comprising a variety of reactive groups including, but not limited to, thiosemicarbazide, aminooxy, hydrazide, and hydrazino groups to form a T-Cell-MP-KRAS-epitope conjugate having a covalent bond between the T-Cell-MP polypeptide and the now conjugated KRAS epitope via the fGly residue. Sulfatase motifs may be used to incorporate not only epitopes (e.g., epitope presenting peptides), but also to incorporate targeting sequences and/or payloads (e.g., in the formation of conjugates with drugs and diagnostic molecules).

[0086] 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) aas in length. The sulfatase motif may be limited to a length less than 16, 14, 12, 10, or 8 aa residues.

[0087] In an embodiment, the sulfatase motif comprises the sequence of in Formula (I): X1Z1X2Z2X3Z3 (SEQ ID NO:66), 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 aa (arginine, lysine, or histidine, usually lysine), or an aliphatic aa (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 aa, though usually an aliphatic aa, a sulfur-containing aa, or a polar uncharged aa (e.g., other than an aromatic aa or a charged aa), 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 aa, though usually an aliphatic aa, a polar, uncharged aa, or a sulfur containing aa (e.g., other than an aromatic aa or a charged aa), usually S, T, A, V, G or C, more usually S, T, A, V or G.

[0088] As indicated above, a sulfatase motif of an aldehyde tag is at least 5 or 6 aa residues, but can be, for example, from 5 to 16 aas 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).

[0089] 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.

[0090] 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-like" 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.

[0091] 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-Kl 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. CCLlO), PC12 cells (ATCC No. CRL1721), COS cells, COS-7 cells (ATCC No. CRL1651), RAT1 cells, mouse L cells (ATCC No. CCLI.3), human embryonic kidney (HEK) cells (ATCC No. CRL1573), HLHepG2 cells, and the like.

[0092] Sulfatase motifs may be incorporated into any desired location of a T-Cell-MP. In an embodiment they may be excluded from the amino or carboxyl terminal 10 or 20 amino acids. In an embodiment, a sulfatase motif may be added in (e.g., at or near the terminus) of any T-Cell-MP element, including the MHC-H or β2M polypeptide sequences or any linker sequence joining them (the L3 linker). Sulfatase motifs may also be added to the scaffold polypeptide (e.g., the Ig Fc) or any of the linkers present in the T-Cell-MP (e.g., L1 to L6).

[0093] A sulfatase motif may be incorporated into, or attached to (e.g., via a peptide linker) a β2M polypeptide in a T-Cell-MP with a sequence having at least 85% (e.g., at least 90%, 95%, 98% or 99%, or even 100%) aa sequence identity to at least 50 (e.g., at least 60, 70, 80, 90, 96, 97, 98 or all) contiguous aas of a mature β2M polypeptide sequence shown in FIG. 4 (e.g., the sequences shown in FIG. 4 starting at aa 21 and ending at their C-terminus). The mature human β2M polypeptide sequence in FIG. 4, may be selected for incorporation of the sulfatase motif. Sequence identity to the β2M polypeptides is determined relative to the corresponding portion of a β2M polypeptide in FIG. 4 without consideration of the added sulfatase motif or any linker or other sequences present.

[0094] In an embodiment, a sulfatase motif may be incorporated into a β2M polypeptide sequence having 1 to 15 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15) aa deletions, insertions and/or

changes compared with a sequence shown in FIG. 4 (either an entire sequence shown in FIG. 4, or the sequence of a mature polypeptides starting at aa 21 and ending at its C-terminus). Changes are assessed without consideration of the aas of the sulfatase motif and any linker sequences present. In one such embodiment a sulfatase motif may be place and/or be inserted within aas 1-15, 15-35, 35-55, 40-50, or 50-70 of a mature β2M sequence, such as those shown in FIG. 4. In one embodiment, sulfatase motifs may be located between aas 35-55 (e.g., between aas 40 to 50) of the human mature β2M polypeptide sequence of FIG. 4 and having 0 to 15 aa substitutions compared with a sequence shown in FIG. 4 (either an entire sequence shown in FIG. 4, or the sequence of a mature polypeptides starting at aa 21 and ending at its C-terminus).

[0095] A sulfatase motif may be incorporated into, or attached to (e.g., via a peptide linker) a MHC Class I heavy chain polypeptide sequence having at least 85% (e.g., at least 90%, 95%, 98% or 99%, or even 100%) aa sequence identity to at least 150, 175, 200, or 225 contiguous aas of a MHC-H sequence shown in FIGs. 3A to 3I before the addition of the sulfatase motif.

[0096] In an embodiment, the added sulfatase motif is attached to the N- or C-terminus of a T-Cell-MP or, if present, attached to or within a linker located at the N- or C-terminus of the T-Cell-MP

[0097] 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.

[0098] 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 a T-Cell-MP. 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.

[0099] In view of the foregoing, this disclosure provides for T-Cell-MPs comprising one or more fGly residues incorporated into a T-Cell-MP 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. Epitopes and/or payloads may be conjugated either directly or indirectly to the reactive formyl glycine of the sulfatase motif directly or through a peptide or chemical linker. After chemical conjugation the T-Cell-MPs comprise one or more fGly' residues incorporated 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 epitope or payload.

[00100] 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., peptide epitopes) and/or payloads bearing thiosemicarbazide, aminooxy, hydrazide, hydrazino or hydrazinyl functional groups (e.g., attached directly to an aa of a peptide or via a linker such as a PEG) can be reacted with fGly-containing T-Cell-MP polypeptides to form a covalently linked epitope. Similarly, targeting sequences and/or payloads such as drugs and therapeutics can be incorporated using, for example, biotin hydrazide as a linking agent.

[00101] The disclosure provides for methods of preparing conjugated T-Cell-MPs including T-Cell-MP-epitope conjugates (e.g., T-Cell-MP-KRAS-epitope conjugates) and/or T-Cell-MP-payload conjugates comprising:

a) incorporating a nucleotide 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:67); CX1PX2Z3 (SEQ ID NO:68) discussed above) into a nucleic acid encoding an unconjugated T-Cell-MP;

b) expressing the sulfatase motif-containing unconjugated T-Cell-MP 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 unconjugated T-Cell-MP, or

ii) does not express a FGE that converts a serine or cysteine of the sulfatase motif to a fGly, and purifying or partially purifying the T-Cell-MP containing the sulfatase motif and contacting the purified or partially purified T-Cell-MP with a FGE that converts the serine or cysteine of the sulfatase motif into a fGly residue; and

c) contacting the fGly-containing polypeptides 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-MP-epitope conjugate and/or T-Cell-MP 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 second-Cell-MP polypeptide comprising a β2M 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 β2M sequence shown in FIG. 4 (e.g., a mature β2M polypeptide with identity calculated without including or before the addition of the sulfatase motif sequence).

[00102] In an embodiment, the method of preparing a T-Cell-MP-epitope conjugate and/or T-Cell-MP 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%) aa sequence identity to at least 150, 175, 200, or 225 contiguous aas of a sequence shown in FIGs. 3A-3I, with sequence identity calculated without including the addition of the sulfatase motif sequence).

b. Sortase A Enzyme Sites

[00103] Epitopes (e.g., peptides comprising the sequence of an epitope) and payloads may be attached at the N- and/or C-termini T-Cell-MP by incorporating sites for Sortase A conjugation at those locations.

[00104] Sortase A recognizes a C-terminal pentapeptide sequence LP(X5)TG/A (SEQ ID NO:69, 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.

[00105] For attachment of epitopes or payloads to the C-terminal portion of a T-Cell-MP polypeptide an LP(X5)TG/A is provided in the carboxy terminal portion of the desired polypeptide(s), such as in an exposed L5 linker (see FIG 5 structure A). An exposed stretch of glycines or alanines (e.g., $(G)_{3\text{-}5}$ (SEQ ID NOs:70 and 71 when using Sortase A from Staphylococcus aureus or alanines $(A)_{3\text{-}5}$, SEQ ID NOs:72 and 73 when using Sortase A from Streptococcus pyogenes) is provided at the N-terminus of a peptide that comprises an epitope (e.g., in a linker attached to the epitope), a peptide payload (or a linker attached thereto), or a peptide covalently attached to a non-peptide epitope or payload.

[00106] For attachment of epitopes or payloads to the amino terminus of a T-Cell-MP polypeptide an aa sequence comprising an exposed stretch of glycines (e.g., $(G)_{2, 3, 4, \text{or } 5}$) or alanines (e.g., $(A)_{2, 3, 4, \text{or } 5}$) is provided at the N-terminus, and a LP(X5)TG/A is provided in 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.

[00107] Combining Sortase A with the amino and carboxy modified peptides described above results in a cleavage between the Thr and Gly/Ala residues in the LP(X5)TG/A sequence and 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.

[00108] In place of LP(X5)TG/A (SEQ ID NO:69), a LPETGG (SEQ ID NO:74) peptide may be used for *S. aureus* Sortase A coupling, or a LPETAA (SEQ ID NO:75) peptide may be used for *S. pyogenes* Sortase A coupling. The conjugation reaction still occurs 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.

c. Transglutaminase Enzyme Sites

[00109] 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-MPs, either directly through a free amine, or indirectly via a linker comprising a free amine. As such, glutamine residues added to a T-Cell-MP in the context of a transglutaminase site may be considered as chemical conjugation sites when they can be accessed by enzymes such as *Streptoverticillium 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. Modification at N297 also offer the potential to reduce the interaction of the IgG Fc reaction with complement C1q protein.

[00110] Where a T-Cell-MP 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 may be added to a sequence to form a transglutaminase site, or a sequence comprising a transglutaminase accessible glutamine (sometimes referred to as a "glutamine tag" or a "Q-tag"), may be incorporated through protein engineering into the polypeptide. The added glutamine or Q-tag may act as chemical conjugation site for epitopes or payloads. 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.

[00111] 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 aa sequence selected from the group consisting of LQG, LLQGG (SEQ ID NO:76), LLQG (SEQ ID NO:77), LSLSQG (SEQ ID NO:78), and LLQLQG (SEQ ID NO:79) (numerous others are available).

[00112] Glutamine residues and Q-tags may be incorporated into any desired location of a T-Cell-MP. In an embodiment, a glutamine residue or Q-tag may be added in (e.g., at or near the terminus) of any T-Cell-MP element, including the MHC-H or β2M polypeptide sequences or any linker sequence joining them (the L3 linker). Glutamine residues and Q-tags may also be added to the scaffold polypeptide (e.g., the Ig Fc) or any of the linkers present in the T-Cell-MP (e.g., L1 to L6).

[00113] A glutamine residue or Q-tag may be incorporated into, or attached to (e.g., via a peptide linker) a β2M polypeptide in a T-Cell-MP with a sequence having at least 85% (e.g., at least 90%, 95%, 98% or 99%, or even 100%) aa sequence identity to at least 50 (e.g., at least 60, 70, 80, 90, 96, 97, 98 or all) contiguous aas of a mature β2M polypeptide sequence shown in FIG. 4 (e.g., the sequences shown in FIG. 4 starting at aa 21 and ending at their C-terminus). The mature human β2M polypeptide sequence in FIG. 4, may be selected for incorporation of the glutamine residue or Q-tag. Sequence identity to the β2M polypeptides is determined relative to the corresponding portion of a β2M polypeptide in FIG. 4 without consideration of the added glutamine residue, Q-tag, or any linker or other sequences present.

[00114] In an embodiment, a glutamine residue or Q-tag may be incorporated into a β2M polypeptide sequence having 1 to 15 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15) aa deletions, insertions

and/or changes compared with a sequence shown in FIG. 4 (either an entire sequence shown in FIG. 4, or the sequence of a mature polypeptides starting at aa 21 and ending at its C-terminus). Changes are assessed without consideration of the aas of the glutamine residue, Q-tag and any linker sequences present. In one such embodiment a glutamine residue or Q-tag may be place and/or be inserted within aas 1 aas 1-15, 15-35, 35-55, 40-50, or 50-70 of a mature β2M sequence, such as those shown in FIG. 4. In one embodiment, glutamine residue or Q-tag may be located between aas 35-55 (e.g., 40 to 50) of the human mature β2M polypeptide sequence of FIG. 4 and having 0 to 15 aa substitutions.

[00115] A glutamine residue or Q-tag may be incorporated into, or attached to (e.g., via a peptide linker) a MHC Class I heavy chain polypeptide sequence having at least 85% (e.g., at least 90%, 95%, 98% or 99%, or even 100%) aa sequence identity to at least 150, 175, 200, or 225 contiguous aas of a MHC-H sequence shown in FIGs. 3A to 3I before the addition of the glutamine residue or Q-tag.

[00116] In an embodiment, the added glutamine residue or Q-tag is attached to the N- or C-terminus of a T-Cell-MP or, if present, attached to or within a linker located at the N- or C-terminus of the T-Cell-MP.

[00117] 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.

[00118] 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.

[00119] 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 *Streptoverticillium, Streptomyces, Actinomadura* sp., *Bacillus,* and the like.

[00120] Q-tags may be created by inserting a glutamine or by modifying the aa sequence around a glutamine residues appearing in a Ig Fc, β2M, and/or MHC-H chain sequence appearing in a T-Cell-MP and used as a chemical conjugation site for addition of an epitope or payload. Similarly, Q-tags may be incorporated into the Ig Fc region as chemical conjugation sites that may be used for the conjugation of, for example, epitopes and/or payloads either directly or indirectly through a peptide or chemical linker bearing a primary amine.

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

[00121] One strategy for providing site-specific chemical conjugation sites into a T-Cell-MP polypeptide employs the insertion of aas with reactivity distinct from the naturally occurring proteinogenic L-amino acids aas present in the polypeptide. Such aas include, but are not limited to, the, selenocysteine (Sec), and the non-natural aas: acetylphenylalanine (p-acetyl-L-phenylalanine, pAcPhe); parazido phenylalanine; and propynyl-tyrosine.Thanos *et al.* in US Pat. Publication No. 20140051836 A1 discuss some other non-natural aas including O-methyl-L-tyrosine, O-4-allyl-L-tyrosine, tri-O-acetyl-GlcNAcβ-serine, isopropyl-L-phenylalanine, p-benzoyl-L-phenylalanine, L-phosphoserine, and a p-propargyloxy-phenylalanine. Other non-natural aas include reactive groups such as, for example, amino, carboxy, acetyl, hydrazino, hydrazido, semicarbazido, sulfanyl, azido and alkynyl. *See*, e.g., US Pat. Publication No. 20140046030 A1.

[00122] In addition to directly synthesizing polypeptides in the laboratory, two methods utilizing stop codons have been developed to incorporate non-natural aas 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 aas 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.

[00123] Incorporation of both selenocysteine and non-natural aas requires engineering the necessary stop codon(s) into the nucleic acid coding sequence of the T-Cell MP polypeptide at the desired location(s), after which the coding sequence is used to express the T-Cell-MP in an *in vivo* or cell-free transcription-translation system.

[00124] *In vivo* systems generally rely on engineered cell-lines to incorporate non-natural aas 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 aa incorporation relies on a tRNA and an aminoacyl tRNA synthetase pair that is orthogonal to all the endogenous tRNAs and synthetases in the host cell. The non-natural aa of choice is supplemented to the media during cell culture or fermentation, making cell-permeability and stability important considerations.

[00125] Various cell-free synthesis systems provided with the charged tRNA may also be utilized to incorporate non-natural aas. Such systems include those described in US Pat. Publication 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).

[00126] Once incorporated into the T-Cell-MP, epitopes and/or payload bearing groups reactive with the incorporated selenocysteine or non-natural aa are brought into contact with the T-Cell-MP under suitable conditions to form a covalent bond. By way of example, the keto group of the pAcPhe is reactive towards alkoxyamines, and via oxime coupling 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.

[00127] As discussed above for other chemical conjugation sites, selenocysteines and/or non-natural aas may be incorporated into any desired location in the T-Cell-MP. In an embodiment, selenocysteines and/or non-natural aas may be added in (e.g., at or near the terminus) of any T-Cell-MP element, including the MHC-H or β2M polypeptide sequences or any linker sequence joining them (the L3 linker). Selenocysteines and/or non-natural aas may also be added to the scaffold polypeptide (e.g., the Ig Fc) or any of the linkers present in the T-Cell-MP (e.g., L1 to L6).

[00128] Selenocysteines and non-natural aas may be incorporated into, or attached to (e.g., via a peptide linker) a β2M polypeptide in a T-Cell-MP with a sequence having at least 85% (e.g., at least 90%, 95%, 98% or 99%, or even 100%) aa sequence identity to at least 50 (e.g., at least 60, 70, 80, 90, 96, 97, 98 or all) contiguous aas of a mature β2M polypeptide sequence shown in FIG. 4 (e.g., the sequences shown in FIG. 4 starting at aa 21 and ending at their C-terminus). The mature human β2M polypeptide sequence in FIG. 4, may be selected for incorporation of the selenocysteines and non-natural aas. Sequence identity to the β2M polypeptides is determined relative to the corresponding portion of a β2M polypeptide in FIG. 4 without consideration of the added selenocysteines, non-natural aas, or any linker or other sequences present.

[00129] In an embodiment, a selenocysteine(s) or non-natural aa(s) may be incorporated into a β2M polypeptide sequence having 1 to 15 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15) aa deletions, insertions and/or changes compared with a sequence shown in FIG. 4 (either an entire sequence shown in FIG. 4, or the sequence of a mature polypeptides starting at aa 21 and ending at its C-terminus). Changes are assessed without consideration of the selenocysteine(s), non-natural aa(s), and any linker sequences present. In one such embodiment, a selenocysteines or non-natural aa may be place and/or be inserted within aas 1-15, 15-35, 35-55, 40-50, or 50-70 of a mature β2M sequence, such as those shown in FIG. 4. In one embodiment, selenocysteines or non-natural aa may be located between aas 35-55 (e.g., 40 to 50) of the human mature β2M polypeptide sequence of Fig 4 and having 0 to 15 aa substitutions.

[00130] A selenocysteines or non-natural aa may be incorporated into, or attached to (e.g., via a peptide linker) a MHC Class I heavy chain polypeptide sequence having at least 85% (e.g., at least 90%, 95%,

98% or 99%, or even 100%) aa sequence identity to at least 150, 175, 200, or 225 contiguous aas of a MHC-H sequence shown in FIGs. 3A to 3I before the addition of the selenocysteines or non-natural aas.

[00131] In an embodiment, the added selenocysteine(s or non-natural aa(s) is attached to the N- or C-terminus of a T-Cell-MP or, if present, attached to or within a linker located at the N- or C-terminus of the T-Cell-MP. In one such embodiment they may be utilized as sites for the conjugation of, for example, epitopes, targeting sequences, and/or payloads conjugated to the T-Cell-MP either directly or indirectly through a peptide or chemical linker.

e. Amino Acid Chemical Conjugation Sites

[00132] Any of the variety of functionalities (e.g., -SH, $-NH_3$, -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 (e.g., not accessible by solvent or to molecules used to modify the cysteines, and not located where it is desirable to place a chemical conjugation site. It is, however, possible to selectively modify T-Cell-MP polypeptides to provide naturally occurring and, as discussed above, non-naturally occurring amino acids at the desired locations for placement of a chemical conjugation site. Modification 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, this disclosure includes and provides for the preparation of the T-Cell-MP polypeptides by transcription/translation systems capable of incorporating a non-natural aa or natural aa (including selenocysteine) to be used as a chemical conjugation site for epitope or payload conjugation.

[00133] This disclosure includes and provides for the preparation of a portion of a T-Cell-MP by transcription/translation systems and joining to its C- or N-terminus a polypeptide bearing a non-natural aa or natural aa (including selenocysteine) prepared by, for example, chemical synthesis. The polypeptide, which may include a linker, may be joined by any suitable method including the use of a sortase as described above for peptide epitopes. In an embodiment, the polypeptide 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.

[00134] A naturally occurring aa (e.g., a cysteine) to be used as a chemical conjugation site may be provided at any desired location of a T-Cell-MP. In an embodiment, a the naturally occurring aa may be provided (e.g., at or near the terminus) of any T-Cell-MP element, including the MHC-H or β2M polypeptide sequences or any linker sequence joining them (the L3 linker). Naturally occurring aa(s) may also be provided in the scaffold polypeptide (e.g., the Ig Fc) or any of the linkers present in the T-Cell-MP (e.g., L1 to L6).

[00135] A naturally occurring aa (e.g., a cysteine) may also be provided, e.g., via protein engineering, in, or attached to (e.g., via a peptide linker), a β2M polypeptide in a T-Cell-MP with a sequence having at least 85% (e.g., at least 90%, 95%, 98% or 99%, or even 100%) aa sequence identity to at least 50 (e.g., at least 60, 70, 80, 90, 96, 97, 98 or all) contiguous aas of a mature β2M polypeptide sequence shown in FIG. 4 (e.g., the sequences shown in FIG. 4 starting at aa 21 and ending at their C-terminus). The mature human β2M polypeptide sequence in FIG. 4, may be selected for incorporation of the naturally occurring aa. Sequence identity to the β2M polypeptides is determined relative to the corresponding portion of a β2M polypeptide in FIG. 4 without consideration of the added naturally occurring aa, any linker, or any other sequences present.

[00136] In an embodiment, a naturally occurring aa (e.g., a cysteine) may be provided, e.g., via protein engineering in a β2M polypeptide sequence having 1 to 15 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15) aa deletions, insertions and/or changes compared with a sequence shown in FIG. 4 (either an entire sequence shown in FIG. 4, or the sequence of a mature polypeptides starting at aa 21 and ending at its C-terminus). Changes are assessed without consideration of the aas of the naturally occurring aa, any linker, or other sequences present. In one such embodiment a naturally occurring aa (e.g., a cysteine) may be engineered i.e., provided (e.g., using the techniques of molecular biology) within aas 1-15, 15-35, 35-55, 40-50, or 50-70 of a mature β2M sequence, such as those shown in FIG. 4. In one embodiment, naturally occurring aa (e.g., a cysteine) may be provided between aas 35-55 (e.g., between 40 and 50, between 42 and 48, between 43 and 45, or at aa 44) of the human mature β2M polypeptide sequence of Fig 4 and having 0 to 15 aa substitutions.

[00137] A naturally occurring aa (e.g., a cysteine) may be provided in, or attached to (e.g., via a peptide linker) a MHC Class I heavy chain polypeptide sequence having at least 85% (e.g., at least 90%, 95%, 98% or 99%, or even 100%) aa sequence identity to at least 150, 175, 200, or 225 contiguous aas of a MHC-H sequence shown in FIGs. 3A to 3I before the addition of the naturally occurring aa.

[00138] In an embodiment, the naturally occurring aa (e.g., a cysteine) may be attached to the N- or C-terminus of a T-Cell-MP, or attached to or within a linker, if present, located at the N- or C-terminus of the T-Cell-MP.

[00139] In one embodiment, a T-Cell-MP contains at least one naturally occurring aa (e.g., a cysteine) to be used as a chemical conjugation site provided, e.g., via protein engineering, in a β2M sequence as shown in FIG. 4, an Ig Fc sequence as shown in any of FIGs. 2A-G, or a MHC Class I heavy chain polypeptide as shown in FIGs. 3A-3I. In an embodiment, at least one naturally occurring aa to be used as a chemical conjugation site is provided in a polypeptide having at least 85% (e.g., at least 90%, 95%, 98% or 99%, or even 100%) aa sequence identity to at least 50 (e.g., at least 60, 70, 80, 90, 96, 97, o 98 or all) contiguous aas of a mature β2M sequence as shown in FIG. 4, an Ig Fc sequence as shown in FIG. 2, or at least 85% (e.g., at least 90%, 95%, 98% or 99%, or even 100%) aa sequence identity to at least 150, 175, 200, or 225 contiguous aas of a MHC Class I heavy chain polypeptide as shown in any of FIGs. 3A-3I. At least one naturally occurring aa (e.g., a cysteine) may be provided as a chemical conjugation site in a T-Cell-MP a β2M aa sequence having at least 90% (e.g., at least 93%, 95%, 98% or 99%, or even 100%)

aa sequence identity with at least the amino terminal 10, 20, 30, 40, 50, 60 or 70 aas of a mature β2M sequence as shown in FIG. 4. At least one naturally occurring aa (e.g., a cysteine) may be provided as a chemical conjugation site in a T-Cell-MP Ig Fc sequence (e.g., as shown in any of FIGs. 2A-2G). At least one naturally occurring aa (e.g., a cysteine) may be provided as a chemical conjugation site in a T-Cell-MP MHC Class I heavy chain polypeptide sequence having at least 85% (e.g., at least 90%, 95%, 98% or 99%, or even 100%) aa sequence identity to at least 150, 175, 200, or 225 contiguous aas of a MHC H polypeptide sequence provided in any of FIGs. 3A to 3I. In another embodiment, at least one naturally occurring aa to be used as a chemical conjugation site is provided in a T-Cell-MP polypeptide comprising at least 30, 40, 50, 60, 70, 80, 90, or 100 contiguous aas having 100% aa sequence identity to a MHC Class I heavy chain sequence as shown in any of FIGs. 3A to 3I or a mature β2M sequence as shown in FIG. 4.

[00140] In any of the embodiments mentioned above where a naturally occurring aa is provided, e.g., via protein engineering, in a polypeptide, the aa may be selected from the group consisting of arginine, lysine, cysteine, serine, threonine, glutamic acid, glutamine, aspartic acid, and asparagine. Alternatively, the aa provided as a conjugation site is selected from the group consisting of lysine, cysteine, serine, threonine, and glutamine. The aa provided as a conjugation site may also be selected from the group consisting of lysine, glutamine, and cysteine. In one instance, the provided aa is cysteine. In another instance, the provided aa is lysine. In still another instance, the provided aa is glutamine.

[00141] Any method known in the art may be used to couple payloads or epitopes to amino acids provided in an unconjugated T-Cell-MP. 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 an unconjugated T-Cell-MP polypeptide. A number of bifunctional crosslinkers may be utilized, including, but not limited to, those described for linking a payload to a T-Cell-MP described herein below. For example, a peptide epitope (or a peptide-containing payload) including a maleimide group attached by way of a homo- or hetero-bifunctional linker (see, e.g., FIG. 9) 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 provided in a T-Cell-MP.

[00142] Maleimido amino acids can be incorporated directly into peptides (e.g., peptide epitopes) 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.

[00143] A maleimide group may also be appended to an epitope (e.g., a peptide epitope) using a homo- or hetero-bifunctional linker (sometimes referred to as a crosslinker) that attaches a maleimide directly (or indirectly, e.g., through an intervening linker that may comprise additional aas bound to the epitope) to

the epitope (e.g., peptide epitope). 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 a maleimide to an amine (e.g., an epsilon amino group of lysine). Examples of 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 crosslinkers (bifunctional agents) are recited below. In an embodiment the epitopes coupled to the T-Cell-MP have a maleimido alkyl carboxylic acid coupled to the peptide by an optional linker (see, e.g., FIG. 9), coupled, 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.

[00144] A KRAS peptide epitope may be coupled to a naturally occurring cysteine present or provided in (e.g., engineered into) for example, the binding pocket of a T-Cell-MP through a bifunctional linker comprising a maleimide or a maleimide amino acid incorporated into the peptide, thereby forming a T-Cell-MP-KRAS-epitope conjugate. A peptide epitope 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 locations within or adjacent to the MHC-H binding pocket. By way of example, a peptide epitope comprising maleimido amino acids or bearing a maleimide group as part of a crosslinker attached to the peptide may be covalently attached at 1 or 2 aas (e.g., cysteines) at MHC-H positions 2, 5, 7, 59, 84, 116, 139, 167, 168, 170, and/or 171 (e.g., Y7C, Y59C, Y116C, A139C, W167C, L168C, R170C, and Y171C substitutions) with the numbering as in FIGs. 3D-3I. A peptide epitope may also 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 and/or 116, (e.g., Y7C and Y116C substitutions) with the numbering as in FIGs. 3D-3H. Cysteine substitution at positions 116 (e.g., Y116C) and/or 167 (e.g., W167C), with the numbering as in FIGs. 3D-3H, may be used separately or in combination to anchor epitopes (e.g., peptide epitopes) with one or two bonds for by maleimide groups (e.g., at one or both of the ends of the epitope containing peptide).

[00145] Peptide epitopes may also be coupled to a naturally occurring cysteine present or provided in (e.g., engineered into) a β2M 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 β2M polypeptide sequence set forth in FIG. 4. Some solvent accessible positions of mature β2M polypeptides that may be substituted by a cysteine to create a chemical conjugation site include: 2, 14, 16, 34, 36, 44, 45, 47, 48, 50, 58, 74, 77, 85, 88, 89, 91, 94, and 98 (Gln 2, Pro 14, Glu 16, Asp 34, Glu 36, Glu 44, Arg 45, Glu 47, Arg 48, Glu 50, Lys 58, Glu 74, Glu 77, Val 85, Ser 88, Gln 89,

Lys 91, Lys 94, and Asp 98) of the mature peptide from NP_004039.1, or their corresponding amino acids in other β2M sequences (see the sequence alignment in FIG. 4). For example, epitopes may be conjugated to cysteines at positions 2, 44, 50, 77, 85, 88, 91, or 98 of the mature β2M polypeptides (aas 22, 64, 70, 97, 105, 108, 111, or 118 of the mature β2M sequences as shown in FIG. 4). Accordingly, the β2M sequences of a T-Cell-MP may contain cysteine chemical conjugation site provided (e.g., by protein engineering) in the mature β2M sequence selected from Q2C, E44C, E50C, E77C, V85V, S88C, K91C, and D98C. The cysteine chemical conjugation sites in β2M sequences may also be combined with MHC-H Y84C and A139C substitutions made to stabilize the MHC H by forming an intrachain disulfide bond between MHC-H sequences. In one instance, the cysteine chemical conjugation site provided in the mature β2M is located at E44 (an E44C substitution). In another instance, the cysteine chemical conjugation site provided in the mature β2M is located at E44 (an E44C substitution) and the β2M sequences also be comprises MHC-H Y84C and A139C substitutions that form an intrachain disulfide bond.

[00146] Where conjugation of an epitope, targeting sequences and/or, payload is to be conducted through a cysteine chemical conjugation site present in an unconjugated T-cell-MP (e.g., using a maleimide modified epitope or payload) a variety of process conditions may affect the conjugation efficiency and the quality (e.g., the amount/fraction of unaggregated duplex T-Cell-MP-epitope conjugate resulting from the reaction) resulting from the conjugation reaction. Conjugation process conditions that may be individually optimized including, but not limited to, (i) prior to conjugation unblocking of cysteine sulfhydryls (e.g., potential blocking groups may be present and removed), (ii) the ratio of the T-Cell-MP to the epitope or payload, reaction pH, (iii) the buffer employed, (iv) additives present in the reaction, (v) the reaction temperature, and (vi) the reaction time.

[00147] Prior to conjugation T-Cell-MPs may be treated with a disulfide reducing agent such as dithiothreitol (DTT), mercaptoethanol, or tris(2-carboxyethyl)phosphine (TCEP) to reduce and free cysteines sulfhydryls that may be blocked. Treatment may conducted using relatively low amounts of reducing agent, for example from about 0.5 to 2.0 reducing equivalents per cysteine conjugation site for relatively short periods, and the cysteine chemical conjugation site of the unconjugated T-Cell MP may be available as a reactive nucleophile for conjugation from about 10 minutes to about 1 hour, or from about 1 hour to 5 hours.

[00148] The ratio of the unconjugated T-Cell-MP to the epitope or payload being conjugated may be varied from about 1:2 to about 1:100, such as from about 1:2 to about 1:3, from about 1:3 to about 1:10, from about 1:10 to about 1:20, from about 1:20 to about 1:40, or from about 1:40 to about 1:100. The use of sequential additions of the reactive epitope or payload may be made to drive the coupling reaction to completion (e.g., multiple does of maleimide or N-hydroxy succinimide modified epitopes may be added to react with the T-Cell-MP).

[00149] As previously indicated, conjugation reaction may be affected by the buffer, its pH, and additives that may be present. For maleimide coupling to reactive cysteines present in a T-Cell-MP the reactions are typically carried out from about pH 6.5 to about pH 8.0 (e.g., from about pH 6.5 to about pH

7.0, from about pH 7.0 to about pH 7.5, from about pH 7.5 to about pH 8.0, or from about pH 8.0 to about pH 8.5. Any suitable buffer not containing active nucleophiles (e.g., reactive thiols) and preferably degassed to avoid reoxidation of the sulfhydryl may be employed for the reaction. Some suitable traditional buffers include phosphate buffered saline (PBS), Tris-HCl, and (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) HEPES. As an alternative traditional buffers, maleimide conjugation reactions may be conducted in buffers/reaction mixtures comprising amino acids such as arginine, glycine, lysine, or histidine. The use of high concentrations of amino acids, e.g., from about 0.1 M (molar) to about 1.5 M (e.g., from about 0.1 to about 0.25, from about 0.25 to about 0.5 from about 0.3 to about 0.6, from about 0.4 to about 0.7, from about 0.5 to about 0.75, from about 0.75 to about 1.0, from about 1.0 to about 1.25 M, or from about 1.25 to about 1.5 M may stabilize the unconjugated and/or unconjugated T-Cell-MP.

[00150] Additives useful for maleimide and other conjugation reactions include, but are not limited to: protease inhibitors; metal chelator (e.g., EDTA) that can block unwanted side reactions and inhibit metal dependent proteases if they are present, detergents; detergents (e.g., polysorbate 80 sold as TWEEN 80®, or nonylphenoxypolyethoxyethanol sold under the names NP40 and Tergitol™ NP); and polyols such a sucrose or glycerol that can add to protein stability.

[00151] Conjugation of T-Cell-MPs with epitopes, targeting sequences and/or payloads, and particularly conjugation at cysteines using maleimide chemistry, can be conducted over a range of temperatures, such as 0° to 40° C. For example, conjugation reactions, including cysteine -maleimide reactions, can be conducted from about 0° to about 10° C, from about 10° to about 20° C, from about 20° to about 30° C, from about 25° to about 37° C, or from about 30° to about 40° C (e.g., at about 20° C, at about ° C or at about 37° C).

[00152] Where a pair of sulfhydryl groups are present, they may be employed simultaneously for chemical conjugation to a T-Cell-MP. In such an embodiment, an unconjugated T-Cell-MP that has a disulfide bond, or that has two cysteines (or selenocysteines) provided at 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 (polyethylene glycol) linker).

[00153] Generally, stoichiometric or near stoichiometric amounts of dithiol reducing agents (*e.g.*, dithiothreitol) are employed to reduce the disulfide bond and allow the bis-thiol linker to react with both cysteine and/or selenocysteine 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 a T-Cell-MP or duplexed T-Cell-MP does not comprise a pair of cysteines and/or selenocysteines (e.g., a selenocysteine

and a cysteine), they may be provided in 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 a T-Cell-MP. Alternatively, in a duplex T-Cell-MP the first cysteine and/or selenocysteine is present in the first T-Cell-MP of the duplex and a second cysteine and/or selenocysteine is present in the second T-Cell-MP of the duplex, with the bis-thiol linker acting as a covalent bridge between the duplexed T-Cell-MPs.

[00154] In an embodiment, a pair of cysteine and/or selenocysteine residues is incorporated into a β2M sequence of a T-Cell-MP having at least 85% (e.g., at least 90%, 95%, 98% or 99%, or even 100%) aa sequence identity to at least 50 (e.g., at least 60, 70, 80, 90, 96, 97, 98 or all) contiguous aas of a mature β2M polypeptide sequence shown in FIG. 4 before the addition of the pair of cysteines and/or selenocysteines, and/or into an L2 or L3 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 an epitope and/or payload through a peptide or chemical linker attached to the bis-thiol group.

[00155] In another embodiment, a pair of cysteines and/or selenocysteines is incorporated into a MHC-H polypeptide sequence of a T-Cell-MP 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%) aa sequence identity to a sequence having at least 150, 175, 200, or 225 contiguous aas of a MHC-H 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 an epitope and/or payload through a peptide or chemical linker attached to the bis-thiol linker. Where the MHC-H sequence includes a Y84C and A139C substitutions the bis-thiol linker may be used to form a covalent bridge between those sites for the covalent coupling of an epitope (e.g., a peptide epitope).

[00156] In another embodiment, a pair of cysteines and/or selenocysteines is incorporated into an Ig Fc sequence of a T-Cell-MP to provide a chemical conjugation site. In an embodiment a pair of cysteines and/or selenocysteines is incorporated into a polypeptide comprising an Ig Fc sequence having at least 85% (e.g., at least 90%, 95%, 98% or 99%, or even 100%) aa sequence identity to a sequence shown in any of the Fc sequences of FIGs. 2A-2G before the addition of the pair of cysteines or selenocysteines. In one such embodiment the pair of cysteines and/or selenocysteines is utilized as a bis-thiol linker coupling site for the conjugation of an epitope and/or payload through a peptide or chemical linker attached to the bis-thiol group. The bis-thiol linker may be used to form a covalent bridge between scaffold polypeptides of a duplex T-Cell-MP. In such a case the cysteines of the lower hinge region that form interchain

disulfide bonds, if present in the Ig Fc scaffold polypeptide sequence, may be used to insert the bis-thiol linker.

f. Other Chemical Conjugation Sites

(i) Carbohydrate Chemical Conjugation Sites

[00157] Many proteins prepared by cellular expression contain added carbohydrates (e.g., oligosaccharides of the type added to antibodies expressed in mammalian cells). Accordingly, where a T-Cell-MP is prepared by cellular expression, carbohydrates may be present and available as selective chemical conjugation sites in, for example, glycol-conjugation reactions, particularly where the T-Cell-MP comprises an Ig Fc scaffold. 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.

[00158] 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. In an embodiment, the incorporation of an Ig Fc scaffold with known glycosylation sites may be used to introduce site specific chemical conjugation sites.

[00159] This disclosure includes and provides for T-Cell-MPs having carbohydrates as chemical conjugation (e.g., glycol-conjugation) sites.

[00160] The disclosure also includes and provides for the use of such molecules in forming conjugates with epitopes and with other molecules such as targeting sequences, drugs, and diagnostic agent payloads.

(ii) Nucleotide Binding Sites

[00161] 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-MP with suitably modified epitopes and/or other molecules (e.g., payload drugs or diagnostic agents) bearing a reactive nucleotide may be employed to prepare T-Cell-MP-KRAS-epitope conjugates. The epitope or payload may be coupled to the nucleotide binding site through the reactive entity (e.g., an IBA moiety) either directly or indirectly though an interposed linker.

~~[00162]~~ This disclosure includes and provides for T-Cell-MPs 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 MHC polypeptides of T-Cell-MPs

[00163] As noted above, T-Cell-MPs 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). Both the β2M and MHC-H chain sequences in a T-Cell-MP (may be of human origin. Unless expressly stated otherwise, the T-Cell-MPs and the T-Cell-MP-KRAS-epitope conjugates described herein are not intended to include membrane anchoring domains (transmembrane regions) of a MHC-H chain, or a part of that molecule sufficient to anchor a T-Cell-MP, or a peptide thereof, to a cell (*e.g.*, eukaryotic cell such as a mammalian cell) in which it is expressed. In addition, the MHC-H chain present in T-Cell-MPs 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-H chain present in a T-Cell-MP includes only the α1, α2, and α3 domains of an MHC Class I heavy chain. The MHC Class I heavy chain present in a T-Cell-MP may have a length of from about 270 amino acids (aa) to about 290 aa. The MHC Class I heavy chain present in a T-Cell-MP may have 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.

[00164] In some cases, the MHC-H and/or β2M polypeptide of a T-Cell-MP is a humanized or 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 β2M polypeptide, or a Class I HLA heavy chain polypeptide. Class I HLA heavy chain polypeptides that can be included in T-Cell-MPs include HLA-A, -B, -C, -E, -F, and/or -G heavy chain polypeptides. The Class I HLA heavy chain polypeptides of T-Cell-MPs may comprise polypeptide sequences having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% aa sequence identity to all or part (e.g., 50, 75, 100, 150, 200, 225, 250, or 260 contiguous aas) of the aa sequence of any of the human HLA heavy chain polypeptides depicted in FIGs. 3A to 3I (e.g., the sequences encompassing the α1, α2, and α3 domains). For example, they may comprise 1-30, 1-5, 5-10, 10-15, 15-20, 20-25 or 25-30 aa 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).

[00165] As an example, a MHC Class I heavy chain polypeptide of a multimeric polypeptide can comprise an aa 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% aa sequence identity to aas 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

[00166] 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-MPs and their epitope conjugates.

(i) HLA-A heavy chains

[00167] The HLA-A heavy chain peptide sequences, or portions thereof, that may be incorporated into a T-Cell-MP 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 further comprise a substitution at one or more of positions 84 and/or 139 (as shown in FIG. 3E) selected from: a tyrosine to alanine at position 84 (Y84A); a tyrosine to cysteine at position 84 (Y84C); and an alanine to cysteine at position 139 (A139C). 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%) aa sequence identity to all or part (e.g., 50, 75, 100, 150, 200, 225, 250, or 260 contiguous aas) of the sequence of those HLA-A alleles may also be incorporated into a T-Cell-MP (e.g., it may comprise 1-30, 1-5, 5-10, 10-15, 15-20, 20-25, or 25-30 aa insertions, deletions, and/or substitutions). The HLA-A heavy chain polypeptide sequence of a T-Cell-MP may comprise the Y84C and A139C substitutions.

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

[00168] An MHC Class I heavy chain polypeptide of a T-Cell-MP or a T-Cell-MP-KRAS-epitope conjugate may comprise aa sequence of HLA-A*01:01:01:01 (HLA-A*0101 or HLA-A*01:01, listed as HLA-A in FIG. 3D (SEQ ID NO:24) and in 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%) aa sequence identity to all or part (e.g., 50, 75, 100, 150, 200, 225, 250, or 260 contiguous aas) of that sequence (e.g., it may comprise 1-30, 1-5, 5-10, 10-15, 15-20, 20-25, or 25-30 aa insertions, deletions, and/or substitutions). In an embodiment, where the HLA-A heavy chain polypeptide of a T-Cell-MP 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 and/or 139 selected from: a tyrosine to alanine at position 84 (Y84A); a tyrosine to cysteine at position 84 (Y84C); and an alanine to cysteine at position 139 (A139C). The HLA-A*0101 heavy chain polypeptide sequence of a T-Cell-MP may comprise the Y84C and A139C substitutions.

(b) HLA-A*0201 (HLA-A*02:01)

[00169] An MHC Class I heavy chain polypeptide of a T-Cell-MP or a T-Cell-MP-KRAS-epitope conjugate may comprise an aa sequence of HLA-A*0201 (SEQ ID NO:27) 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%) aa sequence identity to all or part (e.g., 50, 75, 100, 150, 200, 225, 250, or 260 contiguous aas) of that sequence (e.g., it may comprise 1-30, 1-5, 5-10, 10-15, 15-20, 20-25, or 25-30 aa insertions, deletions, and/or substitutions). In an embodiment, where the HLA-A*0201 heavy chain polypeptide of a T-Cell-MP 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 and/or 139selected from: a tyrosine to alanine at position 84 (Y84A); a tyrosine to cysteine at position 84 (Y84C); and an alanine to

cysteine at position 139 (A139C). The HLA-A*0201 heavy chain polypeptide sequence of a T-Cell-MP may comprise the Y84C and A139C substitutions.

(c) HLA-A*1101 (HLA-A*11:01)

[00170] An MHC Class I heavy chain polypeptide of a T-Cell-MP or a T-Cell-MP-KRAS-epitope conjugate may comprise an aa sequence of HLA-A*1101 (SEQ ID NO:32) 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%) aa sequence identity to all or part (e.g., 50, 75, 100, 150, 200, 225, 250, or 260 contiguous aas) of that sequence (e.g., it may comprise 1-30, 1-5, 5-10, 10-15, 15-20, 20-25, or 25-30 aa 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.

[00171] In an embodiment, where the HLA-A*1101 heavy chain polypeptide of a T-Cell-MP 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 and/or 139 selected from: a tyrosine to alanine at position 84 (Y84A); a tyrosine to cysteine at position 84 (Y84C); and an alanine to cysteine at position 139 (A139C). The HLA-A*1101 heavy chain polypeptide sequence of a T-Cell-MP may comprise the Y84C and A139C substitutions.

(d) HLA-A*2402 (HLA-A*24:02)

[00172] An MHC Class I heavy chain polypeptide of a T-Cell-MP or a T-Cell-MP-KRAS-epitope conjugate may comprise an aa sequence of HLA-A*2402 (SEQ ID NO:33) 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%) aa sequence identity to all or part (e.g., 50, 75, 100, 150, 200, 225, 250, or 260 contiguous aas) of that sequence (e.g., it may comprise 1-30, 1-5, 5-10, 10-15, 15-20, 20-25, or 25-30 aa 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.

[00173] In an embodiment, where the HLA-A*2402 heavy chain polypeptide of a T-Cell-MP 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 and/or selected from: a tyrosine to alanine at position 84 (Y84A); a tyrosine to cysteine at position 84 (Y84C); and an alanine to cysteine at position 139 (A139C). The HLA-A*2402 heavy chain polypeptide sequence of a T-Cell-MP may comprise the Y84C and A139C substitutions.

(e) HLA-A*3303 (HLA-A*33:03) or HLA-A*3401 (HLA-A*34:01)

[00174] An MHC Class I heavy chain polypeptide of a T-Cell-MP or a T-Cell-MP-KRAS-epitope conjugate may comprise an aa sequence of HLA-A*3303 (SEQ ID NO:34) or HLA-A*3401 (SEQ ID NO:38) 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% aa sequence identity to all or part (e.g., 50, 75, 100, 150, 200, 225, 250, or 260 contiguous aas) of that sequence (e.g., it may comprise 1-25, 1-5, 5-10, 10-15, 15-20, 20-25, or 25-30 aa 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.

[00175] In an embodiment, where the HLA-A*3303 or HLA-A*3401 heavy chain polypeptide of a T-Cell-MP 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 and/or139 selected from: a tyrosine to alanine at position 84 (Y84A); a tyrosine to cysteine at position 84 (Y84C); and an alanine to cysteine at position 139 (A139C). The HLA-A*3303 or HLA-A*3401 heavy chain polypeptide sequence of a T-Cell-MP may comprise the Y84C and A139C substitutions.

(ii) HLA-B heavy chains.

[00176] The HLA-B heavy chain peptide sequences, or portions thereof, that may be incorporated into a T-Cell-MP 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 and/or139 (as shown in FIG. 3F) selected from: a tyrosine to alanine at position 84 (Y84A); a tyrosine to cysteine at position 84 (Y84C); and an alanine to cysteine at position 139 (A139C). 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% aa sequence identity to all or part (e.g., 50, 75, 100, 150, 200, 225, 250, or 260 contiguous aas) of the sequence of those HLA-B alleles may also be incorporated into a T-Cell-MP (e.g., it may comprise 1-25, 1-5, 5-10, 10-15, 15-20, 20-25, or 25-30 aa insertions, deletions, and/or substitutions). The HLA-B heavy chain polypeptide sequence of a T-Cell-MP may comprise the Y84C and A139C substitutions.

(a) HLA-B*0702 (HLA-B*07:02)

[00177] An MHC Class I heavy chain polypeptide of a T-Cell-MP or a T-Cell-MP-KRAS-epitope conjugate may comprise an aa sequence of HLA-B*0702 (SEQ ID NO:25) in FIG. 3D (labeled HLA-B in FIG. 3D), HLA-B*03501, HLA-B*4402, HLA-B*4403, HLA-B*5801 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% aa sequence identity to all or part (e.g., 50, 75, 100, 150, 200, 225, 250, or 260 contiguous aas) of any of those sequences (e.g., it may comprise 1-25, 1-5, 5-10, 10-15, 15-20, 20-25, or 25-30 aa insertions, deletions, and/or substitutions). In an embodiment, where the HLA-B heavy chain polypeptide of a T-Cell-MP 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 and/or 139 selected from: a tyrosine to alanine at position 84 (Y84A); a tyrosine to cysteine at position 84 (Y84C); and an alanine to cysteine at position 139 (A139C). The HLA-B*0702 heavy chain polypeptide sequence of a T-Cell-MP may comprise the Y84C and A139C substitutions.

(b) HLA-B*3501 (HLA-B*35:01)

[00178] An MHC Class I heavy chain polypeptide of a T-Cell-MP or a T-Cell-MP-KRAS-epitope conjugate may comprise an aa sequence of HLA-B*3501: GSHSMRYFYTAMSRPGRGEPRFIAVGYV DDTQFVRFDSDAASPRTEPRAPWIEQEGPEYWDRNTQIFKTNTQTYRESLRNLRGYYNQSEAGS

HIIQRMYGCDLGPDGRLLRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQ LRAYLEGLCVEWLRRYLENGKETLQRADPPKTHVTHHPVSDHEATLRCWALGFYPAEITLTWQ RDGEDQTQDTELVETRPAGDRTFQKWAAVVVPSGEEQRYTCHVQHEGLPKPLTLRWEP (shown lacking its signal sequence and transmembrane/intracellular regions SEQ ID NO:80), 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% aa sequence identity to all or part (e.g., 50, 75, 100, 150, 200, 225, 250, or 260 contiguous aas) of that sequence (e.g., it may comprise 1-25, 1-5, 5-10, 10-15, 15-20, 20-25, or 25-30 aa insertions, deletions, and/or substitutions). In an embodiment, the sequence may comprise a substitution at one or more of positions 84 and/or 139 selected from: a tyrosine to alanine at position 84 (Y84A); a tyrosine to cysteine at position 84 (Y84C); and an alanine to cysteine at position 139 (A139C). The HLA-B*3501 heavy chain polypeptide sequence of a T-Cell-MP may comprise the Y84C and A139C substitutions.

(c) HLA-B*4402 (HLA-B*44:02)

[00179] An MHC Class I heavy chain polypeptide of a T-Cell-MP or a T-Cell-MP-KRAS-epitope conjugate may comprise an aa sequence of HLA-B*4402: GSHSMRYFYTAMSRPGRGEPRFITVGYVDDTL-FVRFDSDATSPRKEPRAPWIEQEGPEYWDRETQISKTNTQTYRENLRTALRYYNQSEAGSHIIQR MYGCDVGPDGRLLRGYDQDAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQDRA YLEGLCVESLRRYLENGKETLQRADPPKTHVTHHPISDHEVTLRCWALGFYPAEITLTWQRDGE DQTQDTELVETRPAGDRTFQKWAAVVVPSGEEQRYTCHVQHEGLPKPLTLRWEP (shown lacking its signal sequence and transmembrane/intracellular regions SEQ ID NO:81), 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% aa sequence identity to all or part (e.g., 50, 75, 100, 150, 200, 225, 250, or 260 contiguous aas) of that sequence (e.g., it may comprise 1-25, 1-5, 5-10, 10-15, 15-20, 20-25, or 25-30 aa insertions, deletions, and/or substitutions). In an embodiment, the sequence may comprise a substitution at one or more of positions 84 and/or 139 selected from: a tyrosine to alanine at position 84 (Y84A); a tyrosine to cysteine at position 84 (Y84C); and an alanine to cysteine at position 139 (A139C). The HLA-B*4402 heavy chain polypeptide sequence of a T-Cell-MP may comprise the Y84C and A139C substitutions.

(d) HLA-B*4403 (HLA-B*44:03)

[00180] An MHC Class I heavy chain polypeptide of a T-Cell-MP or a T-Cell-MP-KRAS-epitope conjugate may comprise an aa sequence of HLA-B*4403: GSHSMRYFYTAMSRPGRGEPRFITVGYV-DDTLFVRFDSDATSPRKEPRAPWIEQEGPEYWDRETQISKTNTQTYRENLRTALRYYNQSEAGSH IIQRMYGCDVGPDGRLLRGYDQDAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQL RAYLEGLCVESLRRYLENGKETLQRADPPKTHVTHHPISDHEVTLRCWALGFYPAEITLTWQRD GEDQTQDTELVETRPAGDRTFQKWAAVVVPSGEEQRYTCHVQHEGLPKPLTLRWEP (shown lacking its signal sequence and transmembrane/intracellular regions SEQ ID NO:82), 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% aa sequence identity to all or part (e.g., 50, 75, 100, 150, 200, 225, 250, or 260 contiguous aas) of that sequence (e.g., it may comprise 1-25, 1-5, 5-10, 10-15, 15-20, 20-25, or 25-30 aa insertions,

deletions, and/or substitutions). In an embodiment, the sequence may comprise a substitution at one or more of positions 84 and/or 139 selected from: a tyrosine to alanine at position 84 (Y84A); a tyrosine to cysteine at position 84 (Y84C); and an alanine to cysteine at position 139 (A139C). The HLA-B*4403 heavy chain polypeptide sequence of a T-Cell-MP may comprise the Y84C and A139C substitutions.

(e) HLA-B*5801 (HLA-B*58:01)

[00181] An MHC Class I heavy chain polypeptide of a T-Cell-MP or a T-Cell-MP-KRAS-epitope conjugate may comprise an aa sequence of HLA-B*5801: GSHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRTEPRAPWIEQEGPEYWDGE TRNMKASAQTYRENLRIALRYYNQSEAGSHIIQRMYGCDLGPDGRLLRGHDQSAYDGKDYIAL NEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGLCVEWLRRYLENGKETLQRADPPKTH VTHHPVSDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDRTFQKWAAVVVPS GEEQRYTCHVQHEGLPKPLTLRWEP (shown lacking its signal sequence and transmembrane/intracellular regions SEQ ID NO:83), 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% aa sequence identity to all or part (e.g., 50, 75, 100, 150, 200, 225, 250, or 260 contiguous aas) of that sequence (e.g., it may comprise 1-25, 1-5, 5-10, 10-15, 15-20, 20-25, or 25-30 aa insertions, deletions, and/or substitutions). In an embodiment, the sequence may comprise a substitution at one or more of positions 84 and/or 139 selected from: a tyrosine to alanine at position 84 (Y84A); a tyrosine to cysteine at position 84 (Y84C); and an alanine to cysteine at position 139 (A139C). The HLA-B*5901 heavy chain polypeptide sequence of a T-Cell-MP may comprise the Y84C and A139C substitutions.

(iii) HLA-C heavy chains

[00182] The HLA-C heavy chain peptide sequences, or portions thereof, that may be incorporated into a T-Cell-MP 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); and an alanine to cysteine at position 139 (A139C). 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% aa sequence identity to all or part (e.g., 50, 75, 100, 150, 200, 225, 250, or 260 contiguous aas) of the sequence of those HLA-C alleles may also be incorporated into a T-Cell-MP (e.g., it may comprise 1-25, 1-5, 5-10, 10-15, 15-20, 20-25, or 25-30 aa insertions, deletions, and/or substitutions). The HLA-C heavy chain polypeptide sequence of a T-Cell-MP may comprise the Y84C and A139C substitutions.

(a) HLA-C*701 (HLA-C*07:01) and HLA-C*702 (HLA-C*07:02)

[00183] An MHC Class I heavy chain polypeptide of a T-Cell-MP or a T-Cell-MP-KRAS-epitope conjugate may comprise an aa sequence of HLA-C*701 (SEQ ID NO:23) or HLA-C*702 (SEQ ID NO:54) 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% aa sequence identity to all or

part (e.g., 50, 75, 100, 150, 200, 225, 250, or 260 contiguous aas) of those sequences (e.g., it may comprise 1-25, 1-5, 5-10, 10-15, 15-20, 20-25, or 25-30 aa insertions, deletions, and/or substitutions relative to those sequences). In an embodiment, where the HLA-C heavy chain polypeptide of a T-Cell-MP 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 and/or 139 selected from: a tyrosine to alanine at position 84 (Y84A); a tyrosine to cysteine at position 84 (Y84C); and an alanine to cysteine at position 139 (A139C). The HLA-C*701 or HLA-C*0702 heavy chain polypeptide sequence of a T-Cell-MP may comprise the Y84C and A139C substitutions.

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

[00184] The non-classical HLA heavy chain peptide sequences, or portions thereof, that may be incorporated into a T-Cell-MP 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 the National Center for Bioecology Information (www.ncbi.nlm.nih.gov).

[00185] 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*01:11, 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-3G and in FIG. 3I.

[00186] Any of the above-mentioned HLA-E, F and/or G alleles may comprise a substitution at one or more of positions 84 and/or 139 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; and/or a position 139 alanine to cysteine (A139C), or in the case of HLA-F a V139C substitution. 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% aa sequence identity to all or part (e.g., 50, 75, 100, 150, 200, 225, 250, or 260 contiguous aas) of any of the consensus sequences 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 aa insertions, deletions, and/or substitutions in addition to changes at variable residues listed therein). The HLA-E, F, or G heavy chain polypeptide sequence of a T-Cell-MP may comprise a cysteine at both position 84 and 139.

(v) Mouse H2K

[00187] An MHC Class I heavy chain polypeptide of a T-Cell-MP or a T-Cell-MP-KRAS-epitope conjugate may comprise an aa sequence of MOUSE H2K (SEQ ID NO:28) (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% aa sequence identity to all or part (e.g., 50, 75, 100, 150, 200, 225, 250, or 260 contiguous aas) of that sequence (e.g., it may comprise 1-30, 1-5, 5-10, 10-15, 15-20, 20-25, or 25-30 aa insertions, deletions, and/or substitutions). In an embodiment, where the MOUSE H2K heavy chain polypeptide of a T-Cell-MP 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 and/or139 selected from: a tyrosine to alanine at position 84 (Y84A); a tyrosine to cysteine at position 84 (Y84C); and an alanine to cysteine at position 139 (A139C). The MOUSE H2K heavy chain polypeptide sequence of a T-Cell-MP may comprise the Y84C and A139C substitutions.

(vi) The Effect of Amino Acid Substitutions in MHC Polypeptides on T-Cell-MPs

(a) Substitutions at Positions 84 and 139

[00188] 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 a peptide epitope) 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-MP. Alternatively, the MHC-H (e.g., HLA-heavy chain) of a T-Cell-MP may be substituted with cysteines to form an intrachain disulfide bond between a cysteine substituted into the carboxyl end portion of the α1 helix and a cysteine in the amino end portion of the α2-1 helix (e.g., amino acids 84 and 139). Such disulfide bonds stabilize the MHC-H polypeptide sequence of a T-Cell-MP, and permit its translation, cellular processing, and excretion from eukaryotic cells in the absence of a bound peptide epitope (or null peptide). Any combination of substitutions provided in the table provide below at residues 84 and 130 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

[00189] Any MHC Class I heavy chain sequences (including those disclosed above for: the HLA-A*0101; HLA-A*0201; HLA-A*1101; HLA-A*2402; HLA-A*3303; HLA-B; HLA-C; Mouse H2K, or any of the other HLA-A, B, C, E, F, and/or G sequence disclosed herein) may further comprise a cysteine substitution at position 116 (e.g., Y116C) or at position 167.

[00190] As with aa position 84 substitutions that open one end of the MHC-H binding pocket (e.g., Y84A or its equivalent), substitution of an alanine or glycine at position 167 (e.g., a W167A substitution or its equivalent) opens the other end of the MHC binding pocket, creating a groove that permits greater variation (e.g., longer length) of the peptide epitopes that may be presented by the T-Cell-MP-KRAS-epitope conjugates. Substitutions at positions 84 and/or 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. A cysteine substitution at positions 116 (e.g., Y116C) and/or 167 (e.g., W167C) may be used separately or in combination to anchor epitopes (e.g., peptide epitopes) in one or two locations (e.g., the ends of the epitope containing peptide). Substitutions at positions 116 and/or 167 may be combined with substitutions including those at positions 84 and/or 139 described above.

[00191] The Table below lists some MHC heavy chain sequence modifications that may be incorporated into a T-Cell-MPs.

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

Entry	HLA Heavy Chain Sequence From FIGs. 3D-H	Sequence Identity Range□	Substitutions at aa positions 84 and/or 139	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; or (Y84C & A139C)	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; or (Y84C & A139C)	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 insertions, deletions, and/or substitutions (not counting variable residues)	None; Y84C; Y84A; A139C; or (Y84C & A139C)	None; Y116C; W167A; W167C; or (Y116C & W167C)
4	B*0702, B*0801, B*1502, B*3501, B*3802, B*4001, B*4402, B*4403, B*4601, B*5301, or B*5801	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; or (Y84C & A139C)	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; or (Y84C & A139C)	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; or (Y84C & A139C)	None; Y116C; W167A; W167C; or (Y116C & W167C)

Entry	HLA Heavy Chain Sequence From FIGs. 3D-H	Sequence Identity Range□	Substitutions at aa positions 84 and/or 139	Substitutions at positions 116 and/or 167
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; or (Y84C & A139C)	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; or (Y84C & A139C)	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-MP relative to the corresponding portion of the sequences listed in FIG. 3D-3H not counting the variable residues when the consensus sequences are used for the comparison.

b. MHC Class I β2-Microglobins and Combinations with MHC-H Polypeptides

[00192] A β2M polypeptide of a T-Cell-MP can be a human β2M polypeptide, a non-human primate β2M polypeptide, a murine β2M polypeptide, and the like. In some instances, a β2M polypeptide comprises an aa 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% aa sequence identity to a β2M aa sequence (e.g., a mature β2M sequence) depicted in FIG. 4. The β2M polypeptide of a T-Cell-MP may comprise an aa 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% aa sequence identity to aas 21 to 119 of a β2M aa sequence depicted in FIG. 4, which may include a cysteine or other aa substitution or insertion as a chemical conjugation site for epitope attachment (e.g., and E44C substitution) when the identity is less than 100%. Chemical conjugation sites may be located at, for example, solvent accessible locations in the β2M polypeptide sequence.

[00193] The β2M polypeptide sequence of a T-Cell-MP may have at least 90% (e.g., at least 95% or 98%) or 100% sequence identity to at least 70 (e.g., at least 80, 90, 96, 97, 98 or all) contiguous aas of a mature human β2M polypeptide (e.g., aas 21-119 of NCBI accession number NP_004039.1 provided in FIG. 4). By way of example, a β2M polypeptide sequence of a T-Cell-MP may have up to six (e.g., 1, 2, 3, 4, 5, or 6) aa substitutions within an aa segment of at least 70 (e.g., at least 80, 90, 96, 97, or 98 or all) contiguous aas of a mature human β2M polypeptide (e.g., aas 21-119 of NCBI accession number NP_004039.1 provided in FIG. 4), and may comprise the chemical conjugation site for attachment of an epitope (e.g., an E44C substitution in the mature peptide). As noted above, in such β2M polypeptide sequences the chemical conjugation sites of epitopes may be located at a variety of locations including solvent accessible aa positions. For example, a cysteine or other amino acid substitution or insertion at a solvent accessible amino acid position can provide a chemical conjugation site for direct or indirect (e.g., through a peptide linker) attachment of an epitope.

[00194] Some solvent accessible positions of mature β2M polypeptides lacking their leader sequence include aa positions 2, 14, 16, 34, 36, 44, 45, 47, 48, 50, 58, 74, 77, 85, 88, 89, 91, 94, and 98 (Gln 2, Pro 14, Glu 16, Asp 34, Glu 36, Glu 44, Arg 45, Glu 47, Arg 48, Glu 50, Lys 58, Glu 74, Glu 77, Val 85, Ser 88, Gln 89, Lys 91, Lys 94, and Asp 98) of the mature peptide from NP_004039.1, or their corresponding amino acids in other β2M sequences (see the sequence alignment in FIG. 4). The solvent accessible locations for chemical conjugation sites (e.g., a cysteine or another reactive aa substitution) may be selected from positions 2, 44, 50, 77, 85, 88, 91, or 98 of a mature β2M polypeptide sequence such as NP_004039.1, or the corresponding aa positions in other β2M sequences such as those in FIG. 4. The solvent accessible locations for chemical conjugation sites (e.g., a cysteine or another reactive aa substitution) may also be selected from positions 2, 44, 50, or 98 of a mature β2M polypeptide sequence such as NP_004039.1, or the corresponding aa positions in other β2M sequences such as those in FIG. 4. The solvent accessible locations for chemical conjugation sites (e.g., a cysteine or another reactive aa substitution) may be selected from positions 2 or 44 (Glu 2 or Glu 44) of a mature β2M polypeptide sequence such as NP_004039.1, or the corresponding aa positions in other β2M sequences such as those in FIG. 4.

[00195] A β2M polypeptide sequence may comprise a single cysteine substituted into a wt. β2M polypeptide (e.g., a β2M sequence in FIG. 4). Such cysteine residues, when present in a T-Cell-MP polypeptide, can act as a chemical conjugation site for the covalent coupling of an epitope (either directly or indirectly through a linker). The covalent attachment may be in the form of a bond made to a reactive group in or attached to the epitope, such as a maleimide group incorporated into the epitope or a linker attached to the peptide epitope, or in the form of a disulfide bond. For example, in some cases, one of amino acids 43, 44, or 45 of the mature β2M lacking its signal sequence (residues 63, 64, and 65 of the unprocessed proteins as shown with their signal sequences in FIG. 4) may be substituted with a cysteine residue. The aa position substituted with a cysteine may be position 44 (e.g., an E44C substitution of the mature human protein NP_004039.1 or a corresponding aa substitution in a β2M sequence such as those in FIG. 4). Alternatively, the aa position substituted with a cysteine may be position 2 (e.g., a Q44C substitution of the mature human protein NP_004039.1 or a corresponding aa substitution in a β2M sequence such as those in FIG. 4).

c. Some Combinations of Substitutions in the MHC-H and the β2M polypeptide sequences

[00196] Separately, or in addition to, any cysteine residues inserted into the MHC-H or β2M polypeptide sequence of a T-Cell-MP that may function as a chemical conjugation site for an epitope or a payload (e.g., an E44C substitution in a β2M polypeptide sequence that provides a chemical conjugation site for an epitope), a T-Cell-MP may comprise an intrachain disulfide bond between a cysteine substituted into the carboxyl end portion of the α1 helix and a cysteine in the amino end portion of the α2-1 helix (e.g., amino acids at aa positions 84 and 139, such as Y84C and A139C). The carboxyl end portion of the α1 helix is from about aa position 79 to about aa position 89 and the amino end portion of the α2-1 helix is from about aa position 134 to about aa position 144 of the MHC-H chain (the aa

positions are determined based on the sequence of the heavy chains without their leader sequence (*see, e.g.*, FIGs. 3D-3H). Accordingly, a disulfide bond may be 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-H polypeptide sequence. For example, in a T-Cell-MP 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 of the MHC-H polypeptide sequence. In one aspect, the MHC-H intrachain disulfide bond is between cysteines substituted at positions 84 and 139 of any of the heavy chain sequences set forth in FIGs. 3D-3H.

[00197] A T-Cell-MP may comprise a combination of: (i) a mature β2M polypeptide sequence having at least 90% (e.g., at least 95% or 98%) sequence identity to at least 70 (e.g., at least 80, 90, 96, 97, 98 or all) of aas 21-119 of NP_004039.1 with an E44C (or another cysteine substitution) as a chemical conjugation site for an epitope; and (ii) a HLA Class I heavy chain polypeptide sequence having at least 90% sequence identity (e.g., at least 95%, 98%, or 100% sequence identity) excluding variable aa clusters 1-4 to: GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEY WDGETRKVKAHSQTHRVDL(aa cluster 1){C}(aa cluster 2)AGSHTVQRMYGCDVGSDWRFLRGY HQYAYDGKDYIALKEDLRSW(aa cluster 3){C}(aa cluster 4))HKWEAAHVAEQLRAYLEGTCVEW LRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTEL(aa cluster 5)(C)(aa cluster 6)QKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWEP (SEQ ID NO:84); where the cysteine residues indicated as {C} form a disulfide bond between the α1 and α2-1 helices.

[00198] 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 aa residues, wherein the aa residues are each selected independently from i) any naturally occurring (proteogenic) aa or ii) any naturally occurring aa except proline or glycine. The MHC- H polypeptide sequence may be an HLA-A chain, wherein:

aa cluster 1 may be the amino acid sequence GTLRG (SEQ ID NO:85) or that sequence with one or two aas deleted or substituted with other naturally occurring aas (e.g., L replaced by I, V, A or F);

aa cluster 2 may be the amino acid sequence YNQSE (SEQ ID NO:86) or that sequence with one or two aas deleted or substituted with other naturally occurring aas (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 (SEQ ID NO:87) or that sequence with one or two aas deleted or substituted with other naturally occurring aas (e.g., T replaced by S, A replaced by G, D replaced by E, and/or M replaced by L, V, or I); and/or aa cluster 4 may be the amino acid sequence AQTTK (SEQ ID NO:88) or that sequence with one or two aas deleted or substituted with other naturally occurring aas (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 (SEQ ID NO:235) or that sequence with one or two aas deleted or substituted with other naturally occurring aas (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 (SEQ ID NO:236) or that sequence with one or two aas deleted or substituted with other naturally occurring aas (e.g., D replaced by E, T replaced by S, or F replaced by L, W, or Y).

In an embodiment cluster 5 is VETRP (SEQ ID NO:235), position 236 is an A, and cluster 6 is GDGTF (SEQ ID NO:236).

[00199] As noted above, any of the MHC-H intrachain disulfide bonds, including a disulfide bond between cysteines at 84 and 139 (a Y84C and A139C disulfide), may be combined with substitutions that permit incorporation of a peptide epitope into a T-Cell-MP. Accordingly, the present disclosure includes and provides for T-Cell-MPs and their higher order complexes (e.g., duplexes) comprising one or more T-Cell-MP polypeptides having an MHC-H polypeptide sequence with an intrachain Y84C A139C disulfide bond and an E44C substitution in the β2M polypeptide sequence. T-Cell-MPs and their higher order complexes (e.g., duplexes) may comprise: (i) a mature β2M polypeptide sequence with an E44C substitution having at least 90% (e.g., at least 95% or 98%) sequence identity to at least 70 (e.g., at least 80, 90, 96, 97, 98 or all) of 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:61 to 65 , see FIG. 4); and (ii) an MHC-H sequence with Y84C and A139C substitutions (that form a disulfide bond) may have at least 85% (e.g., at least 90%, at least 95% or 98%) or 100% sequence identity to at least 200 (e.g., at least 225, at least 250, at least 260, or at least 275) contiguous aas of the α1, α2, and α3 domains an HLA-A, -B, -C, -E, -F, or -G sequences in FIGs. 3D-3H. The MHC-H polypeptide sequence may be a HLA- A*0101, HLA-A*0201, HLA-A*1101, HLA-A*2402, HLA-A*3303, or HLA-A*3401polypeptide sequence having Y84C and A139C substitutions (see FIG. 3E). The MHC-H polypeptide sequence may be a HLA-B*0702, HLA-B*0801, HLA-B*1502, B27 (subtypes HLA-B*2701-2759), HLA-B*3802, HLA-B*4001, HLA-B*4601, or HLA-B*5301 polypeptide sequence having Y84C and A139C substitutions (see, e.g., FIG. 3F). The MHC-H polypeptide sequence may be a HLA-C*0102, HLA-C*0303, HLA-C*0304, HLA-C*0401, HLA-C*0602, HLA-C*0701, HLA-C*0702, HLA-C*0801, or HLA-C*1502 polypeptide sequence having Y84C and A139C substitutions (see, e.g., FIG. 3G).

4 Scaffold polypeptides

[00200] T-Cell-MPs and T-Cell-MP-KRAS-epitope conjugates may comprise an immunoglobulin heavy chain constant region ("Ig Fc" or "Fc") polypeptide, or may comprise another suitable scaffold polypeptide. Where scaffold polypeptide sequences are identical and pair or multimerize (e.g., some Ig Fc sequences or leucine zipper sequences), they can form symmetrical pairs or multimers (e.g., homodimers, see e.g., FIG. 9 with an Fc scaffold). In contrast, where an asymmetric pairing between two T-Cell-MP molecules is desired (e.g., to produce a duplex T-Cell-MP with each bearing one or more different MODs), the scaffold polypeptides present in the T-Cell-MP may comprise interspecific binding sequences. Interspecific binding sequences are non-identical polypeptide sequences that selectively interact with their specific complementary counterpart sequence to form asymmetric pairs (heterodimers, see e.g., FIG. 10 with an interspecific Fc scaffold). Interspecific binding sequences may in some instances form some amount of homodimers, but preferentially dimerize by binding more strongly) with

their counterpart interspecific binding sequence. Accordingly, specific heterodimers tend to be formed when an interspecific dimerization sequence and its counterpart interspecific binding sequence are incorporated into a pair of polypeptides. By way of example, where an interspecific dimerization sequence and its counterpart are incorporated into a pair of polypeptides they may selectively form greater than 70%, 80%, 90%, 95%, 98% or 99% heterodimers when an equimolar mixture of the polypeptides are combined. The remainder of the polypeptides may be present as monomers or homodimers, which may be separated from the heterodimer. Moreover, because interspecific sequences are selective for their counterpart sequence, they can limit the interaction with other proteins expressed by cells (e.g., in culture or in a subject) particularly where the interspecific sequences are not naturally occurring or are variants of naturally occurring protein sequences.

[00201] Scaffold polypeptide sequences generally may be less than 300 aa (e.g., about 100 to about 300 aa). Scaffold polypeptide sequences may be less than 250 aa (e.g., about 75 to about 250 aa). Scaffold polypeptide sequences may be less than 200 aa (e.g., about 60 to about 200 aa). Scaffold polypeptide sequences may be less than 150 aa (e.g., about 50 to about 150 aa).

[00202] Scaffold polypeptide sequences include, but are not limited to, interspecific and non-interspecific Ig Fc polypeptide sequences, however, polypeptide sequences other than Ig Fc polypeptide sequences (non-Immunoglobulin sequences) may be used as scaffolds.

a. Non-Immunoglobulin Fc Scaffold Polypeptides

[00203] Non-immunoglobulin Fc scaffold polypeptides include, but are not limited to: albumin, XTEN (extended recombinant); transferrin; Fc receptor, elastin-like; albumin-binding; silk-like (*see*, e.g., Valluzzi et al. (2002) *Philos Trans R Soc Lond B Biol Sci.* 357:165); a silk-elastin-like (SELP; *see*, e.g., Megeed et al. (2002) *Adv Drug Deliv Rev.* 54:1075) polypeptides; 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. Suitable elastin-like polypeptides are described, for example, in Hassouneh et al. (2012) *Methods Enzymol.* 502:215.

[00204] Other non-immunoglobulin Fc scaffold polypeptide sequences include but are not limited to: polypeptides of the collectin family (e.g., ACRP30 or ACRP30-like proteins) that contain collagen domains consisting of collagen repeats Gly-Xaa-Yaa and/or Gly-Xaa-Pro (which may be repeated from 10-40 times); coiled-coil domains; leucine-zipper domains; Fos/Jun binding pairs; Ig CH1 and light chain constant region C_L sequences (Ig CH1/C_L pairs such as a Ig CH1 sequence paired with a Ig C_L κ or C_L λ light chain constant region sequence).

[00205] Non-immunoglobulin Fc scaffold polypeptides can be interspecific or non-interspecific in nature. For example, both Fos/Jun binding pairs and Ig CH1 polypeptide sequences and light chain constant region C_L sequences form interspecific binding pairs. Coiled-coil sequences, including leucine zipper sequences, can be either interspecific leucine zipper or non-interspecific leucine zipper sequences. See e.g., Zeng et al., (1997) PNAS (USA) 94:3673-3678; and Li et al., (2012), Nature Comms. 3:662.

[00206] The scaffold polypeptides of a duplex T-Cell-MP may each comprise a leucine zipper polypeptide sequence. The leucine zipper polypeptides bind to one another to form a dimer. Non-limiting examples of leucine-zipper polypeptides include a peptide comprising any one of the following aa sequences: RMKQIEDKIEEILSKIYHIENEIARIKKLIGER (SEQ ID NO:89); LSSIEKKQEEQTS-WLIWISNELTLIRNELAQS (SEQ ID NO:90); LSSIEKKLEEITSQLIQISNELTLIRNELAQ (SEQ ID NO:91; LSSIEKKLEEITSQLIQIRNELTLIRNELAQ (SEQ ID NO:92); LSSIEKKLEEITSQLQQ-IRNELTLIRNELAQ (SEQ ID NO:93); LSSLEKKLEELTSQLIQLRNELTLLRNELAQ (SEQ ID NO:94); ISSLEKKIEELTSQIQQLRNEITLLRNEIAQ (SEQ ID NO:95). In some cases, a leucine zipper polypeptide comprises the following aa sequence: LEIEAAFLERENTALETRVAELRQRVQRLRNRV-SQYRTRYGPLGGGK (SEQ ID NO:96). Additional leucine-zipper polypeptides are known in the art, a number of which are suitable for use as scaffold polypeptide sequences.

[00207] The scaffold polypeptide of a T-Cell-MP may comprise a coiled-coil polypeptide sequence that forms a dimer. Non-limiting examples of coiled-coil polypeptides include, for example, a peptide of any one of the following aa sequences: LKSVENRLAVVENQLKTVIEELKTVKDLLSN (SEQ ID NO:97); LARIEEKLKTIKAQLSEIASTLNMIREQLAQ (SEQ ID NO:98); VSRLEEKVKTLKSQVTELAS-TVSLLREQVAQ (SEQ ID NO:99); IQSEKKIEDISSLIGQIQSEITLIRNEIAQ (SEQ ID NO:100); and LMSLEKKLEELTQTLMQLQNELSMLKNELAQ (SEQ ID NO:101).

[00208] The T-Cell-MPs of a T cell MP duplex may comprise a pair of scaffold polypeptide sequences that each comprise at least one cysteine residue that can form a disulfide bond permitting homodimerization or heterodimerization of those polypeptides stabilized by an interchain disulfide bond between the cysteine residues. Examples of such aa sequences include: VDLEGSTSNGRQCAGIRL (SEQ ID NO:102); EDDVTTTEELAPALVPPPKGTCAGWMA (SEQ ID NO:103); and GHDQE-TTTQGPGVLLPLPKGACTGQMA (SEQ ID NO:104).

[00209] Some scaffold polypeptide sequences permit formation of T-Cell-MP complexes of higher order than duplexes, such as triplexes, tetraplexes, pentaplexes or hexaplexes. Such aa sequences include, but are not limited to, IgM constant regions (discussed below). Collagen domains, which form trimers, can also be employed. Collagen domains may comprise the three aa sequence Gly-Xaa-Xaa and/or GlyXaaYaa, where Xaa and Yaa are independently any aa, with the sequence appear or are repeated multiple times (e.g., from 10 to 40 times). In Gly-Xaa-Yaa sequences, Xaa and Yaa are frequently proline and hydroxyproline respectively in greater than 25%, 50%, 75%, 80% 90% or 95% of the Gly-Xaa-Yaa occurrences, or in each of the Gly-Xaa-Yaa occurrences. In some cases, a collagen domain comprises the sequence Gly-Xaa-Pro repeated from 10 to 40 times. A collagen oligomerization peptide can comprise the following aa sequence: VTAFSNMDDMLQKAHLVIEGTFIYLRDSTEFFIRVRDGW-KKLQLGELIPIPADSPPPPALSSNP (SEQ ID NO:105).

b. Immunoglobulin Fc Scaffold Polypeptides

(i) Non-Interspecific Immunoglobulin Fc Scaffold Polypeptides

[00210] The scaffold polypeptide sequences of a T-Cell-MP or its corresponding T-Cell-MP-KRAS-epitope conjugate may comprise a Fc polypeptide. The Fc polypeptide of a T-Cell-MP or T-Cell-MP-

KRAS-epitope conjugate can be, for example, from an IgA, IgD, IgE, IgG, or IgM, any of which may be 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. In some cases, the Fc polypeptide comprises an aa sequence having at least about 70% (e.g., at least about 75%, 80%, 85%, 90%, 95%, 98%, or 99%), or 100% aa sequence identity to at least 125 contiguous aas (e.g., at least 150, at least 175, at least 200, or at least 210 contiguous aas), or all aas of an aa sequence of a Fc region depicted in FIGs. 2A-2H. Such immunoglobulin sequences can interact forming a duplex or higher order structure from T-Cell-MP molecules. In some instances, the Fc scaffold polypeptide sequences include naturally occurring cysteine residues (or non-naturally occurring cysteine residues provided by protein engineering) that are capable of forming interchain disulfide bonds covalently linking two T-Cell-MP polypeptides together. Unless stated otherwise, the Fc polypeptides used in the T-Cell-MPs and their epitope conjugates do not comprise a transmembrane anchoring domain or a portion thereof sufficient to anchor the T-Cell-MP to a cell membrane.

[00211] Most immunoglobulin Fc scaffold polypeptides, particularly those comprising only or largely wt. sequences, may spontaneously link together via disulfide bonds to form homodimers resulting in duplex T-Cell-MPs. In the case of IgM heavy chain constant regions, in the presences of a J-chains, higher order complexes may be formed. Scaffold polypeptides may comprise an aa sequence having 100% aa sequence identity to the wt. human IgG1 Fc polypeptide depicted in FIG. 2D. A scaffold polypeptide may comprise an aa sequence having at least about 70% (e.g., at least about 80%, 90%, 95%, 98%, or 99%) or 100% aa sequence identity to at least 125 contiguous aas (e.g., at least 150, at least 175, at least 200, or at least 210 contiguous aas), or all aas, of the wt. human IgG1 Fc polypeptide depicted in FIG. 2D. Such scaffold sequences may include a substitution of N297 (N77 as numbered in FIG. 2D, SEQ ID NO:7) with an aa other than asparagine. In one case, N297 is substituted by alanine, (N297A). Substitutions at N297 lead to the removal of carbohydrate modifications and result antibody sequences with reduced complement component 1q ("C1q") binding compared to the wt. protein, and accordingly a reduction in complement-dependent cytotoxicity (CDC). K322 (e.g., K322A) substitutions shows a substantial reduction in reduction in FcγR binding affinity and ADCC, with the C1q binding and CDC functions substantially or completely eliminated. Hezareh et al., (2001) J. Virol. 75:12161-168.

[00212] Amino acid L234 and other aas in the lower hinge region (e.g., aas 234 to 239, such as L235, G236, G237, P238, S239) which correspond to aas 14-19 of SEQ ID NO:4) of IgG are involved in binding to the Fc gamma receptor (FcγR), and accordingly, mutations at that location reduce binding to the receptor (relative to the wt. protein) and resulting in a reduction in antibody-dependent cellular cytotoxicity (ADCC). Hezareh et al., (2001) have demonstrated that the double mutant (L234A, L235A) does not effectively bind either FcγR or C1q, and both ADCC and CDC functions were substantially or completely abolished. A scaffold polypeptide with a substitution in the lower hinge region may comprise an aa sequence having at least about 70% (e.g., at least about 80%, 90%, 95%, 98%, or 99%) aa sequence

identity to at least 125 contiguous aas (e.g., at least 150, at least 175, at least 200, or at least 210 contiguous aas), or all aas, of the wt. human IgG1 Fc polypeptide depicted in FIG. 2D, that includes a substitution of L234 (L14 of the aa sequence depicted in FIG. 2D) with an aa other than leucine.

[00213] A scaffold polypeptide with a substitution in the lower hinge region may comprise an aa sequence having at least about 70% (e.g., at least about 80%, 90%, 95%, 98%, or 99%) aa sequence identity to at least 125 contiguous aas (e.g., at least 150, at least 175, at least 200, or at least 210 contiguous aas), or all aas, of the wt. human IgG1 Fc polypeptide depicted in FIG. 2D, that includes a substitution of L235 (L15 of the aa sequence depicted in FIG. 22D) with an aa other than leucine. In some cases, the scaffold polypeptide present in a T-Cell-MP with substitutions in the lower hinge region includes L234A and L235A ("LALA") substitutions (the positions corresponding to positions 14 and 15 of the wt. aa sequence depicted in FIG. 2D; see, e.g., SEQ ID NO:8).

[00214] A scaffold polypeptide with a substitution in the lower hinge region may comprise an aa sequence having at least about 70% (e.g., at least about 80%, 90%, 95%, 98%, or 99%) aa sequence identity to at least 125 contiguous aas (e.g., at least 150, at least 175, at least 200, or at least 210 contiguous aas), or all aas of the wt. human IgG1 Fc polypeptide depicted in FIG. 2D, that includes a substitution of P331 (P111 of the aa sequence depicted in FIG. 2D) with an aa other than proline. Substitutions at P331, like those at N297, lead to reduced binding to C1q relative to the wt. protein, and thus a reduction in complement dependent cytotoxicity. In one embodiment, the substitution is a P331S substitution. In another embodiment, the substitution is a P331A substitution.

[00215] A scaffold polypeptide may comprise an aa sequence having at least about 70% (e.g., at least about 80%, 90%, 95%, 98%, or 99%) aa sequence identity to at least 125 contiguous aas (e.g., at least 150, at least 175, at least 200, or at least 210 contiguous aas), or all aas, of the wt. human IgG1 Fc polypeptide depicted in FIG. 2D, and include substitutions of D270, K322, and/or P329 (corresponding to D50, K102, and P109 of SEQ ID NO:4 in FIG. 2D) that reduce binding to C1q protein relative to the wt. proteins.

[00216] A scaffold polypeptide may comprise an aa sequence having at least about 70% (e.g., at least about 80%, 90%, 95%, 98%, or 99%) aa sequence identity to at least 125 contiguous aas (e.g., at least 150, at least 175, at least 200, or at least 210 contiguous aas), or all aas, of the wt. human IgG1 Fc polypeptide depicted in FIG. 2D, including substitutions at L234 and/or L235 (L14 and/or L15 of the aa sequence depicted in FIG. 2D) with aas other than leucine (such as L234A and L235A substitutions), and a substitution of P331 (P111 of the aa sequence depicted in FIG. 2D) with an aa other than proline such as P331S. In one instance, a scaffold polypeptide present in a T-Cell-MP comprises the "Triple Mutant" aa sequence (SEQ ID NO:6) depicted in FIG. 2D (human IgG1 Fc) having L234F, L235E, and P331S substitutions (corresponding to aa positions 14, 15, and 111 of the aa sequence depicted in FIG. 2D).

[00217] The scaffold Fc polypeptide of a T-Cell-MP may comprise an aa sequence having at least about 70% (e.g., at least about 75%, 80%, 85%, 90%, 95%, 98%, or 99%), or 100% aa, sequence identity to at least 125 contiguous aas (e.g., at least 150, at least 175, at least 200, or at least 210 contiguous aas), or all aas, of a human IgG2 Fc polypeptide depicted in FIG. 2E. The scaffold Fc polypeptide of a T-Cell-MP

may comprise an aa sequence having at least about 70% (e.g., at least about 75%, 80%, 85%, 90%, 95%, 98%, or 99%), or 100% aa, sequence identity to at least 125 contiguous aas (e.g., at least 150, at least 175, at least 200, or at least 210 contiguous aas), or all aas, of a human IgG3 Fc polypeptide depicted in FIG. 2F. The scaffold Fc polypeptide of a T-Cell-MP may comprise an aa sequence having at least about 70% (e.g., at least about 75%, 80%, 85%, 90%, 95%, 98%, or 99%), or 100% aa, sequence identity to at least 125 contiguous aas (e.g., at least 150, at least 175, at least 200, or at least 210 contiguous aas), or all aas, of a human IgG4 Fc polypeptide depicted in FIG. 2G. The scaffold Fc polypeptide of a T-Cell-MP may comprise an aa sequence having at least about 70% (e.g., at least about 75%, 80%, 85%, 90%, 95%, 98%, or 99%), or 100% aa sequence identity to at least 125 contiguous aas (e.g., at least 150, at least 175, at least 200, or at least 210 contiguous aas e.g., aas 99 to 327 or 111 to 327), or all of the GenBank P01861 human IgG4 Fc polypeptide depicted in FIG. 2G.

[00218] The scaffold Fc polypeptide of a T-Cell-MP may comprise IgM heavy chain constant regions (see e.g., FIG 2H), which forms hexamer, or pentamers (particularly when combined with a mature j-chain peptide lacking a signal sequence such as that provided in FIG. 2I.

(ii) Interspecific Immunoglobulin Fc Scaffold Polypeptides

[00219] Where an asymmetric pairing between two T-Cell-MP molecules is desired (e.g., to produce a duplex T-Cell-MP with different MODs), a scaffold polypeptide present in a T-Cell-MP may comprise, consist essentially of, or consist of an interspecific Ig Fc polypeptides) sequence variants. Such interspecific polypeptide sequences include, but are not limited to, knob-in-hole without (KiH) or with (KiHs-s) a stabilizing disulfide bond, HA-TF, ZW-1, 7.8.60, DD-KK, EW-RVT, EW-RVTs-s, and A107 sequences. One interspecific binding pair comprises a T366Y and Y407T mutant pair in the CH3 domain interface of IgG1, or the corresponding residues of other immunoglobulins. See Ridgway et al., Protein Engineering 9:7, 617-621 (1996). A second interspecific binding pair involves the formation of a knob by a T366W substitution, and a hole by the triple substitutions T366S, L368A and Y407V on the complementary Ig Fc sequence. See Xu et al. mAbs 7:1, 231-242 (2015). Another interspecific binding pair has a first Ig Fc polypeptide with Y349C, T366S, L368A, and Y407V substitutions and a second Ig Fc polypeptide with S354C, and T366W substitutions (disulfide bonds can form between the Y349C and the S354C). See e.g., Brinkmann and Konthermann, *mAbs* 9:2, 182–212 (2015). Ig Fc polypeptide sequences, either with or without knob-in-hole modifications, can be stabilized by the formation of disulfide bonds between the Ig Fc polypeptides (e.g., the hinge region disulfide bonds). Several interspecific binding sequences based upon immunoglobulin sequences are summarized in the table that follows, with cross reference to the numbering of the aa positions as they appear in the wt. IgG1 sequence (SEQ ID NO:4) set forth in FIG. 2D shown in brackets "{}".

Table 1. Interspecific immunoglobulin sequences and their cognate counterpart interspecific sequences

Interspecific Pair Name	Substitutions in the first interspecific polypeptide sequence	Substitutions in the second (counterpart) interspecific polypeptide sequence	Comments
KiH	T366W {T146W}	T366S/L368A/Y407V {T146S/L148A/Y187V}	Hydrophobic/steric complementarity
KiHs-s	T366W/S354C* {T146W/S134C*}	T366S/L368A/Y407V/Y349C {T146S/L148A/Y187V/Y129C}	KiH + inter-CH3 domain S-S bond
HA-TF	S364H/F405A {S144H/F185A}	Y349T/T394F {Y129T/T174F}	Hydrophobic/steric complementarity
ZW1	T350V/L351Y/F405A/ Y407V {T130V/L131Y/F185A/Y187V}	T350V/T366L/K392L/T394W {T130V/T146L/K172L/T174W}	Hydrophobic/steric complementarity
7.8.60	K360D/D399M/Y407A {K140D/D179M/Y187A}	E345R/Q347R/T366V/K409V {E125R/Q127R/T146V/K189V}	Hydrophobic/steric complementarity + electrostatic complementarity
DD-KK	K409D/K392D {K189D/K172D}	D399K/E356K {D179K/E136K}	Electrostatic complementarity
EW-RVT	K360E/K409W {K140E/K189W}	Q347R/D399V/F405T {Q127R/D179V/F185T}	Hydrophobic/steric complementarity & long-range electro-static interaction
EW-RVTs-s	K360E/K409W/Y349C* {K140E/K189W/Y129C*}	Q347R/D399V/F405T/S354C {Q127R/D179V/F185T/S134C}	EW-RVT + inter-CH3 domain S-S bond
A107	K370E/K409W {K150E/K189W}	E357N/D399V/F405T {E137N/D179V/F185T}	Hydrophobic/steric complementarity + hydrogen bonding complementarity

Table 1 is modified from Ha et al., *Frontiers in Immunol.*7:1-16 (2016).

* aa forms a stabilizing disulfide bond.

[00220] In addition to the interspecific pairs of sequences in Table 1, scaffold polypeptides may include interspecific "SEED" sequences having 45 residues derived from IgA in an IgG1 CH3 domain of the interspecific sequence, and 57 residues derived from IgG1 in the IgA CH3 in its counterpart interspecific sequence. See Ha et al., *Frontiers in Immunol.*7:1-16 (2016).

[00221] Interspecific immunoglobulin sequences my include substitutions described above for non-interspecific immunoglobulin sequences that inhibit binding either or both of the FcγR or C1q, and reduce or abolish ADCC and/or CDC function.

[00222] In an embodiment, a scaffold polypeptide found in a T-Cell-MP may comprise an interspecific binding sequence or its counterpart interspecific binding sequence selected from the group consisting of:

knob-in-hole (KiH); knob-in-hole with a stabilizing disulfide (KiHs-s); HA-TF; ZW-1; 7.8.60; DD-KK; EW-RVT; EW-RVTs-s; A107; or SEED sequences.

[00223] In an embodiment, a T-Cell-MP comprises a scaffold polypeptide comprising an IgG1 sequence with a T146W KiH sequence substitution, and its counterpart interspecific binding partner polypeptide comprises an IgG1 sequence having T146W, L148A, and Y187V KiH sequence substitutions, where the scaffold polypeptides comprises a sequence having at least 80%, at least 90%, at least 95%, or at least 97% sequence identity to at least 100 (e.g., at least 125, 150, 170, 180, 190, 200, 210, 220, or all 227) contiguous aas of the wt. IgG1 of FIG. 2D. Scaffold polypeptides optionally comprise substitutions at one of more of: L234 and L235 (e.g., L234A/L235A "LALA" or L234F/L235E); N297 (e.g., N297A); P331 (e.g. P331S); L351 (e.g., L351K); T366 (e.g., T366S); P395 (e.g., P395V); F405 (e.g., F405R); Y407 (e.g., Y407A); and K409 (e.g., K409Y). Those substitutions appear at: L14 and L15 (e.g., L14A/L15A "LALA" or L14F/L15E); N77 (e.g., N77A); P111 (e.g. P111S) L131 (e.g., L131K); T146 (e.g., T146S); P175 (e.g., P175V); F185 (e.g., F185R); Y187 (e.g., Y187A); and K189 (e.g., K189Y) in the wt. IgG1 sequence of FIG 2D.

[00224] In an embodiment, a T-Cell-MP or duplex T-Cell-MP comprises a scaffold polypeptide comprising an IgG1 sequence with a T146W KiH sequence substitution, and its counterpart interspecific binding partner polypeptide comprises an IgG1 sequence having T146S, L148A, and Y187V KiH sequence substitutions, where the scaffold polypeptides comprise a sequence having at least 80%, at least 90%, at least 95%, or at least 97% sequence identity to at least 100 (e.g., at least 125, 150, 170, 180, 190, 200, 210, 220, or all 227) contiguous aas of the wt. IgG1 of FIG. 2D; where one or both (in the case of duplex T-Cell-MP) scaffold polypeptide sequence(s) may comprise additional substitutions such as L14 and/or L15 substitutions (e.g., "LALA" substitutions L234A and L235A), and/or N77 (N297 e.g., N297A or N297G).

[00225] In an embodiment, a T-Cell-MP or duplex T-Cell-MP comprises a scaffold polypeptide comprising an IgG1 sequence with a T146W and S134C KiHs-s substitutions, and its counterpart interspecific binding partner polypeptide comprises an IgG1 sequence having T146S, L148A, Y187V and Y129C KiHs-s substitutions, where the scaffold polypeptides comprise a sequence having at least 80%, at least 90%, at least 95%, or at least 97% sequence identity to at least 100 (e.g., at least 125, 150, 170, 180, 190, 200, 210, 220, or all 227) contiguous aas of the wt. IgG1 of FIG. 2D; where one or both (in the case of duplex T-Cell-MP) scaffold polypeptide sequence(s) sequences may comprise additional substitutions such as L14 and/or L15 substitutions (e.g., "LALA" substitutions L234A and L235A), and/or N77 (N297 e.g., N297A or N297G).

[00226] In an embodiment, a T-Cell-MP comprises a scaffold polypeptide comprising an IgG1 sequence with a S144H and F185A HA-TF substitutions, and its counterpart interspecific binding partner polypeptide comprises an IgG1 sequence having Y129T and T174F HA-TF substitutions, where the scaffold polypeptides comprise a sequence having at least 80%, at least 90%, at least 95%, or at least 97% sequence identity to at least 100 (e.g., at least 125, 150, 170, 180, 190, 200, 210, 220, or all 227) contiguous aas of the wt. IgG1 of FIG. 2D; where one or both (in the case of duplex T-Cell-MP) scaffold

polypeptide sequence(s) may comprise additional substitutions such as L14 and/or L15 substitutions (e.g., "LALA" substitutions L234A and L235A), and/or N77 (N297 e.g., N297A or N297G).

[00227] In an embodiment, a T-Cell-MP or duplex T-Cell-MP comprises a scaffold polypeptide comprising an IgG1 sequence with a T130V, L131Y, F185A, and Y187V ZW1 substitutions, and its counterpart interspecific binding partner polypeptide comprises an IgG1 sequence havingT130V, T146L, K172L, and T174W ZW1 substitutions, where the scaffold polypeptides comprise a sequence having at least 80%, at least 90%, at least 95%, or at least 97% sequence identity to at least 100 (e.g., at least 125, 150, 170, 180, 190, 200, 210, 220, or all 227) contiguous aas of the wt. IgG1 of FIG. 2D; where one or both (in the case of duplex T-Cell-MP) scaffold polypeptide sequence(s) may comprise additional substitutions such as L14 and/or L15 substitutions (e.g., "LALA" substitutions L234A and L235A), and/or N77 (N297 e.g., N297A or N297G).

[00228] In an embodiment, a T-Cell-MP or duplex T-Cell-MP comprises a scaffold polypeptide comprising an IgG1 sequence with a K140D, D179M, and Y187A 7.8.60 substitutions, and its counterpart interspecific binding partner polypeptide comprises an IgG1 sequence havingT130V E125R, Q127R, T146V, and K189V 7.8.60 substitutions, where the scaffold polypeptides comprise a sequence having at least 80%, at least 90%, at least 95%, or at least 97% sequence identity to at least 100 (e.g., at least 125, 150, 170, 180, 190, 200, 210, 220, or all 227) contiguous aas of the wt. IgG1 of FIG. 2D; where one or both (in the case of duplex T-Cell-MP) scaffold polypeptide sequence(s) may comprise additional substitutions such as L14 and/or L15 substitutions (e.g., "LALA" substitutions L234A and L235A), and/or N77 (N297 e.g., N297A or N297G).

[00229] In an embodiment, a T-Cell-MP or duplex T-Cell-MP comprises a scaffold polypeptide comprising an IgG1 sequence with a K189D, and K172D DD-KK substitutions, and its counterpart interspecific binding partner polypeptide comprises an IgG1 sequence havingT130V D179K and E136K DD-KK substitutions, where the scaffold polypeptides comprise a sequence having at least 80%, at least 90%, at least 95%, or at least 97% sequence identity to at least 100 (e.g., at least 125, 150, 170, 180, 190, 200, 210, 220, or all 227) contiguous aas of the wt. IgG1 of FIG. 2D; where one or both (in the case of duplex T-Cell-MP) scaffold polypeptide sequence(s) may comprise additional substitutions such as L14 and/or L15 substitutions (e.g., "LALA" substitutions L234A and L235A), and/or N77 (N297 e.g., N297A or N297G).

[00230] In an embodiment, a T-Cell-MP or duplex T-Cell-MP comprises a scaffold polypeptide comprising an IgG1 sequence with a K140E and K189W EW-RVT substitutions, its counterpart interspecific binding partner polypeptide comprises an IgG1 sequence havingT130V Q127R, D179V, and F185T EW-RVT substitutions, where the scaffold polypeptides comprise a sequence having at least 80%, at least 90%, at least 95%, or at least 97% sequence identity to at least 100 (e.g., at least 125, 150, 170, 180, 190, 200, 210, 220, or all 227) contiguous aas of the wt. IgG1 of FIG. 2D; where one or both (in the case of duplex T-Cell-MP) scaffold polypeptide sequence(s) may comprise additional substitutions such as L14 and/or L15 substitutions (e.g., "LALA" substitutions L234A and L235A), and/or N77 (N297 e.g., N297A or N297G).

[00231] In an embodiment, a T-Cell-MP or duplex T-Cell-MP comprises a scaffold polypeptide comprising an IgG1 sequence with a K140E, K189W, and Y129C EW-RVTs-s substitutions, its counterpart interspecific binding partner polypeptide comprises an IgG1 sequence havingT130V Q127R, D179V, F185T, and S134C EW-RVTs-s substitutions, where the scaffold polypeptides comprise a sequence having at least 80%, at least 90%, at least 95%, or at least 97% sequence identity to at least 100 (e.g., at least 125, 150, 170, 180, 190, 200, 210, 220, or all 227) contiguous aas of the wt. IgG1 of FIG. 2D; where one or both (in the case of duplex T-Cell-MP) scaffold polypeptide sequence(s) may comprise additional substitutions such as L14 and/or L15 substitutions (e.g., "LALA" substitutions L234A and L235A), and/or N77 (N297 e.g., N297A or N297G).

[00232] In an embodiment, a T-Cell-MP or duplex T-Cell-MP comprises a scaffold polypeptide comprising an IgG1 sequence with a K150E and K189W A107 substitutions, its counterpart interspecific binding partner polypeptide comprises an IgG1 sequence havingT130V E137N, D179V, and F185T A107 substitutions, where the scaffold polypeptides comprise a sequence having at least 80%, at least 90%, at least 95%, or at least 97% sequence identity to at least 100 (e.g., at least 125, 150, 170, 180, 190, 200, 210, 220, or all 227) contiguous aas of the wt. IgG1 of FIG. 2D; where one or both (in the case of duplex T-Cell-MP) scaffold polypeptide sequence(s) may comprise additional substitutions such as L14 and/or L15 substitutions (e.g., "LALA" substitutions L234A and L235A), and/or N77 (N297 e.g., N297A or N297G).

[00233] As an alternative to the use of immunoglobulin CH2 and CH3 heavy chain constant regions as scaffold sequences, immunoglobulin light chain constant regions (See FIG.2K) can be paired with Ig CH1 sequences (See, e.g., FIG. 2J) as interspecific scaffold sequences.

[00234] In an embodiment, a T-Cell-MP scaffold polypeptide comprises an Ig CH1 domain (e.g., the polypeptide of FIG. 2J), and the sequence with which it will form a complex (its counterpart binding partner) comprises is an Ig κ chain constant region sequence, where the scaffold polypeptide comprise a sequence having at least 80%, 85%, 90%, 95%, 98%, 99%, or 100% sequence identity to at least 70, at least 80, at least 90, at least 100, or at least 110 contiguous aas of SEQ ID NOs: 16 and/or 17 resp. See FIG. 2K. The Ig CH1 and Ig κ sequences may be modified to increase their affinity for each other, and accordingly the stability of any heterodimer formed utilizing them. Among the substitutions that increase the stability of CH1- Ig κ heterodimers are those identified as the MD13 combination in Chen et al., *MAbs*, 8(4):761-774 (2016). In the MD13 combination two substitutions are introduced into to each of the IgCH1 and Ig κ sequences. The Ig CH1 sequence is modified to contain S64E and S66V substitutions (S70E and S72V of the sequence shown in FIG 2J). The Ig κ sequence is modified to contain S69L and T71S substitutions (S68L and T70S of the sequence shown in FIG. 2K).

[00235] In another embodiment, a scaffold polypeptide of a T-Cell-MP comprises an Ig CH1 domain (e.g., the polypeptide of FIG. 2J SEQ ID NO:15), and its counterpart sequence comprises an Ig λ chain constant region sequence such as is shown in FIG. 2K (SEQ ID NO:17), where the scaffold polypeptide comprises a sequence having at least 80%, 85%, 90%, 95%, 98%, 99%, or 100% sequence identity to at least 70 (e.g., at least 80, at least 90, or at least 100) contiguous aas of the sequences shown in FIG. 2K.

c. Effects on Stability and Half-Life

[00236] Suitable scaffold polypeptides (e.g., those with an Ig Fc scaffold sequence) will in some cases extend the be half-life of T-Cell-MP polypeptides and their higher order complexes. In some cases, a suitable scaffold polypeptide increases the in vivo half-life (e.g., the serum half-life) of the T-Cell-MP or duplex T-Cell-MP, compared to a control T-Cell-MP or duplex T-Cell-MP lacking the scaffold polypeptide or comprising a control scaffold polypeptide. For example, in some cases, a scaffold polypeptide increases the in vivo half-life (e.g. serum half-life) of a conjugated or unconjugated T-Cell-MP or duplex T-Cell-MP, compared to an otherwise identical control lacking the scaffold polypeptide, or having a control scaffold polypeptide, by at least about 10%, at least about 20%, at least about 30%, at least about 50%, at least about 2-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.

5 Immunomodulatory polypeptides ("MODs")

[00237] MODs that are suitable for inclusion in a T-Cell-MP of the present disclosure include, but are not limited to, wt. and variants of the following immunomodulatory polypeptides IL-1, IL-2, IL-4, IL-6, IL-7, IL-10, IL-12, IL-15, IL-17, IL-21, IL-23, CD7, CD30L, CD40, CD70, CD80, (B7-1), CD83, CD86 (B7-2), HVEM (CD270), ILT3 (immunoglobulin-like transcript 3), ILT4(immunoglobulin-like transcript 4), Fas ligand (FasL), ICAM (intercellular adhesion molecule), ICOS-L (inducible costimulatory ligand), JAG1 (CD339), lymphotoxin beta receptor, 3/TR6, OX40L (CD252), PD-L1, PD-L2, TGF-β1, TGF-β2, TGF-β3, 4-1BBL, and fragments of any thereof, such as ectodomain fragments, capable of engaging and signaling through their cognate receptor). Unless stated otherwise, it is understood that the MODs employed in the T-Cell-MPs of this disclosure may be either wt. and/or variants of wt. immunomodulatory polypeptides, e.g., a variant that selectively binds to a particular Co-MODs and/or has reduced affinity to a particular Co-MOD. Some MOD polypeptides suitable for inclusion in a T-Cell-MP of the present disclosure and their Co-MOD or Co-MODs ("co-immunomodulatory polypeptides" or cognate costimulatory receptors) include polypeptide sequences with T cell modulatory activity from the protein pairs recited in the following table:

Exemplary Pairs of MODs and Co-MODs

a) 4-1BBL (MOD) and 4-1BB (Co-MOD);	m) HVEM (CD270) (MOD) and CD160 (Co-MOD);
b) PD-L1 (MOD) and PD1 (Co-MOD);	n) JAG1 (CD339) (MOD) and Notch (Co-MOD);
c) IL-2 (MOD) and IL-2 receptor (Co-MOD);	o) JAG1 (CD339) (MOD) and CD46 (Co-MOD);
d) CD80 (MOD) and CD28 (Co-MOD);	p) CD70 (MOD) and CD27 (Co-MOD);
e) CD86 (MOD) and CD28 (Co-MOD);	q) CD80 (MOD) and CTLA4 (Co-MOD);
f) OX40L (CD252) (MOD) and OX40 (CD134) (Co-MOD);	r) CD86 (MOD) and CTLA4 (Co-MOD);
g) Fas ligand (MOD) and Fas (Co-MOD);	s) PD-L1(MOD) and CD-80 (Co-MOD); and
h) ICOS-L (MOD) and ICOS (Co-MOD);	t) TGF-β1, TGF-β2, and/or TGF-β3 (MODs) and TGF-β Receptor (e.g., TGFBR1 and/or TGFBR2) (Co-MOD)
i) ICAM (MOD) and LFA-1 (Co-MOD);	
j) CD30L (MOD) and CD30 (Co-MOD);	
k) CD40 (MOD) and CD40L (Co-MOD);	
l) CD83 (MOD) and CD83L (Co-MOD);	

[00238] Typically, the chosen MOD(s) for the T-Cell-MP-KRAS-epitope conjugates of this disclosure will be MODs that cause T cell activation that provides one or more of the properties discussed above, i.e., an increase the activity of ZAP70 protein kinase activity, induction in the proliferation of the T-cell(s), granule-dependent effector actions (e.g., the release of granzymes, perforin, and/or granulysin from cytotoxic T-cells), and/or release of T cell cytokines (e.g., interferon γ from CD8+ cells). In some cases, the MOD is selected from a wt. or variant of an IL-2 polypeptide, a 4-1BBL polypeptide, a B7-1 polypeptide; a B7-2 polypeptide, an ICOS-L polypeptide, an OX-40L polypeptide, a CD80 polypeptide, and a CD86 polypeptide. In some cases, the T-Cell-MP or duplex T-Cell-MP comprises two different MODs, such as an IL-2 MOD or IL-2 variant MOD polypeptide and either a wt. or variant of a CD80 or CD86 MOD polypeptide. In another instance, the T-Cell-MP or duplex T-Cell-MP comprises an IL-2 MOD or IL-2 variant MOD polypeptide and a wt.. In some case MODs, which may be the same or different, are present in a T-Cell-MP or duplex T-Cell-MP in tandem. When MODs are presented in tandem, their sequences are immediately adjacent to each other on a single polypeptide, either without any intervening sequence or separated by only a linker polypeptide (e.g., no MHC sequences or epitope sequences intervene). The MOD polypeptide may comprise all or part of the extracellular portion of a full-length MOD. Thus, for example, the MOD can in some cases exclude one or more of a signal peptide, a transmembrane domain, and an intracellular domain normally found in a naturally-occurring MOD. Unless stated otherwise, a MOD present in a T-Cell-MP or duplex T-Cell-MP does not comprise the signal peptide, intracellular domain, or a sufficient portion of the transmembrane domain to anchor a substantial amount (e.g., more than 5% or 10%) of a T-Cell-MP or duplex T-Cell-MP into a mammalian cell membrane.

[00239] In some cases, a MOD suitable for inclusion in a T-Cell-MP comprises all or a portion of (e.g., an extracellular portion of) the aa sequence of a naturally occurring MOD. In other instances, a MOD

suitable for inclusion in a T-Cell-MP is a variant MOD that comprises at least one aa substitution compared to the aa sequence of a naturally occurring MOD. In some instances, a variant MOD exhibits a binding affinity for a Co-MOD that is lower than the affinity of a corresponding naturally-occurring MOD (e.g., a MOD not comprising the aa substitution(s) present in the variant) for the Co-MODCo-MOD. Suitable variations in MOD polypeptide sequence that alter affinity may be identified by scanning (making aa substitution e.g., alanine substitutions or "alanine scanning" or charged residue changes) along the length of a peptide and testing its affinity. Once key aa positions altering affinity are identified those positions can be subject to a vertical scan in which the effect of one or more aa substitutions other than alanine are tested. The affinity may be determined by BLI as described below

a.MODS and Variant MODs with Reduced Affinity

[00240] Suitable immunomodulatory domains that exhibit reduced affinity for a co-immunomodulatory domain can have from 1 aa to 20 aa differences from a wt. immunomodulatory domain. For example, in some cases, a variant MOD present in a T-Cell-MP differs in aa sequence by 1 aa to 10 aa, or by 11 aa to 20 aa from a corresponding wt. MOD. A variant MOD present in a T-Cell-MP may include a single aa substitution compared to a corresponding reference (e.g., wt.) MOD. A variant MOD present in a T-Cell-MP may include 2 aa substitutions compared to a corresponding reference (e.g., wt.) MOD. A variant MOD present in a T-Cell-MP may include 3 aa substitutions compared to a corresponding reference (e.g., wt.) MOD. A variant MOD present in a T-Cell-MP may include 4 aa substitutions compared to a corresponding reference (e.g., wt.) MOD. A variant MOD present in a T-Cell-MP may include 5 aa substitutions compared to a corresponding reference (e.g., wt.) MOD. A variant MOD present in a T-Cell-MP may include 6 aa or 7 aa substitutions compared to a corresponding reference (e.g., wt.) MOD. A variant MOD present in a T-Cell-MP may include 8 aa, 9 aa, or 10 aa substitutions compared to a corresponding reference (e.g., wt.) MOD. A variant MOD present in a T-Cell-MP may include 11, 12, 13, 14, or 15 aa substitutions compared to a corresponding reference (e.g., wt.) MOD. A variant MOD present in a T-Cell-MP may include 16, 17, 18, 19, or 20 aa substitutions compared to a corresponding reference (e.g., wt.) MOD.

[00241] As discussed above, a variant MOD suitable for inclusion in a T-Cell-MP of the present disclosure may exhibit reduced affinity for a cognate Co-MOD, compared to the affinity of a corresponding wt. MOD for the cognate Co-MOD. In some cases, a variant MOD present in a T-Cell-MP has a binding affinity for a cognate Co-MOD that is from 100 nM to 100 μM. For example, in some cases, a variant MOD present in a T-Cell-MP has a binding affinity for a cognate Co-MOD 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 μM, from about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 20 μM, from about 20 μM to about 30 μM, from about 30 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00242] Alternatively, or additionally to reduced affinity binding, the MOD may be a variant that exhibits selective binding to a Co-MOD. In one aspect, where a MOD can bind to more than one Co-MOD, a variant may be chosen that selectively binds to at least one Co-MOD. For example, wt. PD-L1 binds to both PD-1 and CD80 (also known as B7-1). In such case, a variant PD-L1 MOD may be chosen that selectively (preferentially) binds either to PD-1 or CD80. Likewise, where a wt. MOD may bind to multiple polypeptides within a Co-MOD, a variant may be chosen to selectively bind to only the desired polypeptides with the Co-MOD. For example, IL-2 binds to the alpha, beta and gamma chains of IL-2R. A variant of IL-2 can be chosen that either binds with reduced affinity, or substantially does not bind, to one of the polypeptides, e.g., the alpha chain of IL-2R, or even to two of the chains, , e.g., an IL-2 variant that substantially does not bind to the alpha chain of IL-2R, and has reduced affinity for the ß chain of IL-2R such as the H16A, F42A variant discussed herein..

(i) Determining binding affinity

[00243] Binding affinity between a MOD and its cognate Co-MOD can be determined by bio-layer interferometry (BLI) using purified MOD and purified cognate Co-MOD, following the procedure set forth in published PCT Application WO 2020/132138 A1.

[00244] Unless otherwise stated herein, the affinity of a T-Cell-MP-epitope conjugate of the present disclosure for a Co-MOD, or the affinity of a control T-Cell-MP-epitope conjugate (where a control T-Cell-MP-epitope conjugate comprises a wt. MOD) for a Co-MOD, or the affinity of a MOD and its Co-MOD polypeptide can be determined using BLI following the procedure set forth in published PCT Application WO 2020/132138 A1, mentioned above.

[00245] A variant MOD present in a T-Cell-MP of the present disclosure may bind 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 wt. MOD for the Co-MOD.

~~[00246]~~ In some cases, a variant MOD present in a T-Cell-MP 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-MP 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 μM, from about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 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. In some cases, a variant MOD present in a T-Cell-MP 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.

[00247] Binding affinity of a T-Cell-MP-epitope conjugate of the present disclosure to a target T cell can be measured in the following manner: A) contacting a T-Cell-MP-epitope conjugate of the present disclosure with a target T cell expressing on its surface: i) a Co-MOD that binds to the parental wt. MOD; and ii) a TCR that binds to the epitope, where the T-Cell-MP-epitope conjugate comprises an epitope tag or fluorescent label (e.g., a fluorescent payload or fluorescent protein label, such as green fluorescent protein, as part of the T-Cell-MP), such that the T-Cell-MP-epitope conjugate binds to the target T cell; B) if the T-Cell-MP-epitope conjugate is unlabeled, contacting the target T cell-bound T-Cell-MP-epitope conjugate with a fluorescently labeled binding agent (e.g., a fluorescently labeled antibody) that binds to the epitope tag, generating a T-Cell-MP-epitope conjugate/target T cell/binding agent complex; and C) measuring the mean fluorescence intensity (MFI) of the T-Cell-MP-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-MP-epitope conjugate (library member) provides a measure of the affinity. The MFI measured over a range of concentrations of the T-Cell-MP-epitope conjugate (library member) provides a half maximal effective concentration (EC_{50}) of the T-Cell-MP-epitope conjugate. In some cases, the EC_{50} of a T-Cell-MP-epitope conjugate of the present disclosure for a target T cell is in the nM range; and the EC_{50} of the T-Cell-MP-epitope conjugate for a control T cell (where a control T cell expresses on its surface: i) a Co-MOD that binds the parental wt. MOD; and ii) a T cell receptor that does not bind to the epitope present in the T-Cell-MP-epitope conjugate) is in the μM range. The ratio of the EC_{50} of a T-Cell-MP-epitope conjugate of the present disclosure for a control T cell to the EC_{50} of the T-Cell-MP-epitope conjugate for a target T cell may be 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 x 10^2:1, at least 10^3:1, at least 5 x 10^3:1, at least 10^4:1, at lease 10^5:1, or at least 10^6:1. The ratio of the EC_{50} of a T-Cell-MP-epitope conjugate of the present disclosure for a control T cell to the EC_{50} of the T-Cell-MP-epitope conjugate for a target T cell is an expression of the selectivity of the T-Cell-MP-epitope conjugate.

[00248] In some cases, when measured as described in the preceding paragraph, a T-Cell-MP-epitope conjugate of the present disclosure exhibits selective binding to a target T cell, compared to binding of the T-Cell-MP-epitope conjugate (library member) to a control T cell that comprises: i) the Co-MOD that binds the parental wt. MOD; and ii) a TCR that binds to an epitope other than the epitope present in the T-Cell-MP-epitope conjugate (library member).

[00249] The ratio of: i) the binding affinity of a control T-Cell-MP (where the control T-Cell-MP comprises a wt. MOD) to a cognate Co-MOD to ii) the binding affinity of a T-Cell-MP comprising a variant of the wt. MOD to the cognate Co-MOD, when measured by BLI (as described above), may be 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 x 10^2:1, at least 10^3:1, at least 5 x 10^3:1, at least 10^4:1,

at least 10^5:1, or at least 10^6:1. The ratio of: i) the binding affinity of a control T-Cell-MP (where the control T-Cell-MP comprises a wt. MOD) to a cognate Co-MOD to ii) the binding affinity of a T-Cell-MP comprising a variant of the wt. MOD to the cognate Co-MOD, when measured by BLI, may be 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, from10^3:1 to 10^4:1, from 10^4:1 to 10^5:1, or from 10^5:1 to 10^6:1.

[00250] As an example, where a control T-Cell-MP-epitope conjugate comprises a wt. IL-2 polypeptide, and where a T-Cell-MP-epitope conjugate of the present disclosure comprises a variant IL-2 polypeptide (comprising from 1 to 10 aa substitutions relative to the aa sequence of the wt. IL-2 polypeptide) as the MOD, the ratio of: i) the binding affinity of the control T-Cell-MP-epitope conjugate to an IL-2 receptor (the Co-MOD) to ii) the binding affinity of the T-Cell-MP-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 x 10^2:1, at least 10^3:1, at least 5 x 10^3:1, at least 10^4:1, at least 10^5:1, or at least 10^6:1. Where a control T-Cell-MP-epitope conjugate comprises a wt. IL-2 polypeptide, and where a T-Cell-MP-epitope conjugate of the present disclosure comprises a variant IL-2 polypeptide (comprising from 1 to 10 aa substitutions relative to the aa sequence of the wt. IL-2 polypeptide) as the MOD, the ratio of: i) the binding affinity of the control T-Cell-MP-epitope conjugate to the IL-2 receptor (the Co-MOD) to ii) the binding affinity of the T-Cell-MP-epitope conjugate of the present disclosure to the IL-2 receptor, when measured by BLI, may be 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. Other examples that may have the same ratios of binding affinities include T-Cell-MPs bearing a wt. MOD and T-Cell-MPs bearing a variant MOD where the wt. and variant MODs are selected from: wt. CD80 and variant CD80; wt. CD86 and a variant CD86; or wt. 4-1BBL and variant 4-1BBL.

[00251] A variant MOD present in a T-Cell-MP of the present disclosure may have a binding affinity for a cognate Co-MOD that is from 1 nM to 100 nM, or from 100 nM to 250 μM. For example, a variant MOD present in a T-Cell-MP may have a binding affinity for a cognate Co-MOD that is from about 1 nM to about 10 nM, from about 10 nM to about 100 nM, from about 100 nM to about 500 nM, from about 500 nM to about 750 nM, from about 750 nM 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 100 μM, or from about 100 μM to about 250 μM. A variant MOD present in a T-Cell-MP may have a binding affinity for a cognate 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.

[00252] 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-MP-epitope conjugate of the present disclosure, while still allowing for activity of the MOD. Thus, a T-Cell-MP-epitope conjugate of the present disclosure may bind selectively to a first T cell that displays both: i) a TCR specific for the epitope present in the T-Cell-MP-epitope conjugate; and ii) a Co-MOD that binds to the MOD present in the T-Cell-MP-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-MP-epitope conjugate; and ii) a Co-MOD that binds to the MOD present in the T-Cell-MP-epitope conjugate. For example, a T-Cell-MP-epitope conjugate of the present disclosure may bind 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. See e.g., FIG.1.

[00253] Immunomodulatory polypeptides and variants, including reduced affinity variants, such as CD80, CD86, 4-1BBL and IL-2 are described in the published literature, e.g., published PCT application WO2020132138A1, the disclosure of which as it pertains to immunomodulatory polypeptides and specific variant immunomodulatory polypeptides of CD80, CD86, 4-1BBL, IL-2 are expressly incorporated herein by reference, including specifically paragraphs [00260]-[00455] of WO2020132138A1.

[00254] Of specific interest are MODs that are variants of the cytokine IL-2, discussed below in further detail. Wild-type IL-2 binds to IL-2 receptor (IL-2R) on the surface of a T cell. Wild-type IL-2 has a strong affinity for IL-2R and will bind to activate most or substantially all CD8+ T cells. For this reason, synthetic forms of wt. Il-2 such as the drug Aldesleukin (trade name Proleukin®) are known to have severe side-effects when administered to humans for the treatment of cancer because the IL-2 indiscriminately activates both target and non-target T cells.

[00255] An IL-2 receptor is in some cases a heterotrimeric polypeptide comprising an alpha chain (IL-2Rα; also referred to as CD25), a beta chain (IL-2Rβ; also referred to as CD122: and a gamma chain (IL-2Rγ; also referred to as CD132). Amino acid sequences of human IL-2, human IL-2Rα, IL2Rβ, and IL-2Rγ are known. See, e.g., published PCT application WO2020132138A1, discussed above.

[00256] In some cases, an IL-2 variant MOD of this disclosure exhibits substantially reduced or no binding to IL-2Rα, thereby minimizing or substantially reducing the activation of Tregs by the IL-2 variant. In some cases, an IL-2 variant MOD of this disclosure has reduced affinity to IL-2Rß and/or IL-2Rγ such that the IL-2 variant MOD exhibits an overall reduced affinity for IL-2R. In some cases, an IL-2 variant MOD of this disclosure exhibits both properties, i.e., it exhibits substantially reduced or no binding to IL-2Rα, and also has reduced affinity to IL-2Rß and/or IL-2Rγ such that the IL-2 variant polypeptide exhibits an overall reduced affinity for IL-2R. T-Cell-MP-KRAS-epitope conjugates comprising such variants, including variants that substantially do not bind IL-2Rα and have reduced affinity to IL-2Rß, have shown the ability to preferentially bind to and activate IL-2 receptors on T cells that contain the target TCR that is specific for the peptide epitope on the T-Cell-MP-KRAS-epitope conjugate, and are thus less likely to deliver IL-2 to non-target T cells, i.e., T cells that do not contain a TCR that specifically binds the peptide epitope on the T-Cell-MP-KRAS-epitope conjugate. That is, the binding of the IL-2 variant MOD to its co-MOD on the T cell is substantially driven by the binding of the MHC-epitope moiety rather than by the binding of the Il-2. Suitable IL-2 variant MODs thus include a

polypeptide that comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, at least 99% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:106 for IL-2. One such MOD is a variant IL-2 polypeptide comprises the amino acid sequence of FIG 18 (see, e.g., construct 1694), which includes the sequence of wild-type IL-2 with H16A and F42A substitutions, discussed below.

b. IL-2 and its variants

[00257] As one non-limiting example, a wt. MOD or variant MOD present in a T-Cell-MP is an IL-2 or variant IL-2 polypeptide. In some cases, a variant MOD present in a T-Cell-MP is a variant IL-2 polypeptide. Wild-type IL-2 binds to an IL-2 receptor (IL-2R). A wt. IL-2 aa sequence can be as follows: APTSSSTKKT QLQL**EH**LLL**D** LQMILNGINN YKNPKLTRML T**F**KF**Y**MPKKA TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLIS**N**IN VIVLELKGSE TTFMCEYADE TATIVEFLNR WITFC**Q**SIIS TLT (aa 21-153 of UniProt P60568, SEQ ID NO:106).

[00258] 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-2Rα; also referred to as CD25), a beta chain (IL-2Rβ; also referred to as CD122) and a gamma chain (IL-2Rγ; also referred to as CD132). Amino acid sequences of human IL-2Rα, IL2Rβ, and IL-2Rγ can be as follows.

Human IL-2Rα: ELCDDDPPE IPHATFKAMA YKEGTMLNCE CKRGFRRIKS GSLYMLCTGN SSHSSWDNQC QCTSSATRNT TKQVTPQPEE QKERKTTEMQ SPMQPVDQAS LPGHCREPPP WENEATERIY HFVVGQMVYY QCVQGYRALH RGPAESVCKM THGKTRWTQP QLICTGEMET SQFPGEEKPQ ASPEGRPESE TSCLVTTTDF QIQTEMAATM ETSIFTTEYQ VAVAGCVFLL ISVLLLSGLT WQRRQRKSRR TI (SEQ ID NO:107).

Human IL-2Rβ: VNG TSQFTCFYNS RANISCVWSQ DGALQDTSCQ VHAWPDRRRW NQTCELLPVS QASWACNLIL GAPDSQKLTT VDIVTLRVLC REGVRWRVMA IQDFKPFENL RLMAPISLQV VHVETHRCNI SWEISQASHY FERHLEFEAR TLSPGHTWEE APLLTLKQKQ EWICLETLTP DTQYEFQVRV KPLQGEFTTW SPWSQPLAFR TKPAALGKDT IPWLGHLLVG LSGAFGFIIL VYLLINCRNT GPWLKKVLKC NTPDPSKFFS QLSSEHGGDV QKWLSSPFPS SSFSPGGLAP EISPLEVLER DKVTQLLLQQ DKVPEPASLS SNHSLTSCFT NQGYFFFHLP DALEIEACQV YFTYDPYSEE DPDEGVAGAP TGSSPQPLQP LSGEDDAYCT FPSRDDLLLF SPSLLGGPSP PSTAPGGSGA GEERMPPSLQ ERVPRDWDPQ PLGPPTPGVP DLVDFQPPPE LVLREAGEEV PDAGPREGVS FPWSRPPGQG EFRALNARLP LNTDAYLSLQ ELQGQDPTHL V (SEQ ID NO:108).

Human IL-2Rγ: LNTTILTP NGNEDTTADF FLTTMPTDSL SVSTLPLPEV QCFVFNVEYM NCTWNSSSEP QPTNLTLHYW YKNSDNDKVQ KCSHYLFSEE ITSGCQLQKK EIHLYQTFVV QLQDPREPRR QATQMLKLQN LVIPWAPENL TLHKLSESQL ELNWNNRFLN HCLEHLVQYR TDWDHSWTEQ SVDYRHKFSL PSVDGQKRYT FRVRSRFNPL CGSAQHWSEW SHPIHWGSNT SKENPFLFAL EAVVISVGSM GLIISLLCVY FWLERTMPRI PTLKNLEDLV TEYHGNFSAW SGVSKGLAES LQPDYSERLC LVSEIPPKGG ALGEGPGASP CNQHSPYWAP PCYTLKPET (SEQ ID NO:109).

[00259] In some cases, where a T-Cell-MP comprises a variant IL-2 polypeptide, a cognate Co-MOD is an IL-2R comprising polypeptides comprising the aa sequences of any one of SEQ ID NO:107, SEQ ID :108, and SEQ ID NO:109.

[00260] 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 aa sequence set forth in SEQ ID NO:106. 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 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 an IL-2 polypeptide comprising the aa sequence set forth in SEQ ID NO:106 for an IL-2R (e.g., an IL-2R comprising polypeptides comprising the aa sequence set forth in SEQ ID NOs:107-109), when assayed under the same conditions.

[00261] In some cases, a variant IL-2 polypeptide (e.g., a variant of SEQ ID NO:106) has a binding affinity to IL-2R (e.g., of SEQ ID NOs:107-109) that is from 100 nM to 100 μM. As another example, in some cases, a variant IL-2 polypeptide (e.g., a variant of SEQ ID NO:106) has a binding affinity for IL-2R (e.g., an IL-2R comprising polypeptides comprising the aa sequence set forth in SEQ ID NOs:107-109) that is from about 100 nM to about 200 nM, from about 200 nM to about 400 nM, from about 400 nM to about 600 nM, from about 600 nM to about 800 nM, from about 800 nM 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 20 μM, from about 20 μM to about 40 μM, from about 40 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00262] In some cases, a variant IL-2 polypeptide has a single aa substitution compared to the IL-2 aa sequence set forth in SEQ ID NO:106. In some cases, a variant IL-2 polypeptide has from 2 to 10 aa substitutions compared to the IL-2 aa sequence set forth in SEQ ID NO:106. In some cases, a variant IL-2 polypeptide has 2 aa substitutions compared to the IL-2 aa sequence set forth in SEQ ID NO:106. In some cases, a variant IL-2 polypeptide has 3 aa substitutions compared to the IL-2 aa sequence set forth in SEQ ID NO:106. In some cases, a variant IL-2 polypeptide has 4 aa substitutions compared to the IL-2 aa sequence set forth in SEQ ID NO:106. In some cases, a variant IL-2 polypeptide has 5 aa substitutions compared to the IL-2 aa sequence set forth in SEQ ID NO:106. In some cases, a variant IL-2 polypeptide has 6 or 7 aa substitutions compared to the IL-2 aa sequence set forth in SEQ ID NO:106. In some cases, a variant IL-2 polypeptide has 8, 9, or 10 aa substitutions compared to the IL-2 aa sequence set forth in SEQ ID NO:106.

[00263] Suitable variant IL-2 polypeptide sequences include polypeptide sequences comprising an aa sequence having at least 80% (e.g., at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%) aa sequence identity to at least 80 (e.g., 90, 100, 110, 120, 130 or 133) contiguous aas of SEQ ID NO:106. IL-2 variants include polypeptides having at least 90% (e.g., at least 95%, 98%, or 99%) aa sequence identity to at least 80 (e.g., at least 90, 100, 110, 120, or 130) contiguous aas of SEQ ID NO:106, wherein the aa at position 15 is an aa other than E. In one case, the position of H16 is substituted by Ala. In one case, the position of E15 is substituted by Ala.

[00264] IL-2 variants include polypeptides having at least 90% (e.g., at least 95%, 98%, or 99%) aa sequence identity to at least 80 (e.g., at least 90, 100, 110, 120, or 130) contiguous aas of SEQ ID NO:106, wherein the aa at position 16 is an aa other than H. In one case, the position of H16 is substituted by Asn, Cys, Gln, Met, Val, or Trp. In one case, the position of H16 is substituted by Ala. In another case, the position of H16 is substituted by Thr. IL-2 variants include polypeptides having at least 90% (e.g., at least 95%, 98%, or 99%) aa sequence identity to at least 80 (e.g., at least 90, 100, 110, 120, or 130) contiguous aas of SEQ ID NO:106, wherein the aa at position 20 is an aa other than D. In one case, the position of D20 is substituted by Ala.

[00265] IL-2 variants include polypeptides having at least 90% (e.g., at least 95%, 98%, or 99%) aa sequence identity to at least 80 (e.g., at least 90, 100, 110, 120, or 130) contiguous aas of SEQ ID NO:106, wherein the aa at position 42 is an aa other than F. In one case, the position of F42 is substituted by Met, Pro, Ser, Thr, Trp, Tyr, Val, or His. In one case, the position of F42 is substituted by Ala. IL-2 variants include polypeptides having at least 90% (e.g., at least 95%, 98%, or 99%) aa sequence identity to at least 80 (e.g., at least 90, 100, 110, 120, or 130) contiguous aas of SEQ ID NO:106, wherein the aa at position 45 is an aa other than Y. In one case, the position of Y45 is substituted by Ala.

[00266] IL-2 variants include polypeptides having at least 90% (e.g., at least 95%, 98%, or 99%) aa sequence identity to at least 80 (e.g., at least 90, 100, 110, 120, or 130) contiguous aas of SEQ ID NO:106, wherein the aa at position 88 is an aa other than N. In one case, the position of N88 is substituted by Ala. In another case, the position of N88 is substituted by Arg. IL-2 variants include polypeptides having at least 90% (e.g., at least 95%, 98%, or 99%) aa sequence identity to at least 80 (e.g., at least 90, 100, 110, 120, or 130) contiguous aas of SEQ ID NO:106, wherein the aa at position 126 is an aa other than Q. In one case, the position of Q126 is substituted by Ala.

[00267] IL-2 variants include polypeptides having at least 90% (e.g., at least 95%, 98%, or 99%) aa sequence identity to at least 80 (e.g., at least 100, 110, 120, or 130) contiguous aas of SEQ ID NO:106, wherein the aa at position 16 is an aa other than H and the aa at position 42 is other than F. In one case, the position of H16 is substituted by Ala or Thr and the position of F42 is substituted by Ala or Thr. In one case, the position of H16 is substituted by Ala and the position of F42 is substituted by Ala (an H16A and F42A variant). In one case, the position of H16 is substituted by Thr and the position of F42 is substituted by Ala (an H16T and F42A variant).

[00268] An IL-2 variant may comprise an aa sequence having at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% aa sequence identity to the sequence: APTSSSTKKT QLQLE**X**$_1$LLLD LQMILNGINN YKNPKLTRML T**X**$_2$KFYMPKKA TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNR WITFCQSIIS TLT (SEQ ID NO:110), wherein position 16 and 42 are substituted as follows: X_1 is any aa other than His; and X_2 is any aa other than Phe. A second IL-2 variant comprises the substitutions X_1 is Ala and X_2 is Ala (an H16A and F42A variant). A third IL-2 variant comprise the substitutions X_1 is Thr and X_2 is Ala (an H16T and F42A variant) APTSSSTKKT QLQLE**A**LLLD LQMILNGINN YKNPKLTRML T**A**KFYMPKKA TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE

TATIVEFLNR WITFCQSIIS TLT (SEQ ID NO:233).

[00269] IL-2 variants include polypeptides having at least 90% (e.g., at least 95%, 98%, or 99%) aa sequence identity to at least 80 (e.g., at least 100, 110, 120, or 130) contiguous aas of SEQ ID NO:106, wherein the aa at position 20 is an aa other than D and the aa at position 42 is other than F. In one case, the position of D20 is substituted by Ala and the position of F42 is substituted by Ala (D20A, F42A substitutions).

[00270] IL-2 variants include polypeptides having at least 90% (e.g., at least 95%, 98%, or 99%) aa sequence identity to at least 80 (e.g., at least 100, 110, 120, or 130) contiguous aas of SEQ ID NO:106, wherein the aa at position 15 is other than E, the aa at position 20 is an aa other than D, and the aa at position 42 is other than F. In one case, the position of E15 is substituted by Ala, the position of D20 is substituted by Ala and the position of F42 is substituted by Ala (E15A, D20A, F42A substitutions).

[00271] IL-2 variants include polypeptides having at least 90% (e.g., at least 95%, 98%, or 99%) aa sequence identity to at least 80 (e.g., at least 100, 110, 120, or 130) contiguous aas of SEQ ID NO:106, wherein the aa at position 16 is other than H, the aa at position 20 is an aa other than D, and the aa at position 42 is other than F. In one case, the position of H16 is substituted by Ala, the position of D20 is substituted by Ala and the position of F42 is substituted by Ala (an H16A, D20A, F42A substitution). In another case, the position H16 is substituted by Thr, the position of D20 is substituted by Ala and the position of F42 is substituted by Ala (H16T, D20A, F42A substitutions).

[00272] IL-2 variants include polypeptides having at least 90% (e.g., at least 95%, 98%, or 99%) aa sequence identity to at least 80 (e.g., at least 100, 110, 120, or 130) contiguous aas of SEQ ID NO:106, wherein the aa at position 16 is other than H, the aa at position 42 is other than F, and the aa at position 88 is other than R. In one case, the position of H16 is substituted by Ala or Thr, the position of F42 is substituted by Ala, and the position of N88 is substituted by Arg (H16A, F42A, N88R substitution or H16T, F42A, N88R substitutions).

[00273] IL-2 variants include polypeptides having at least 90% (e.g., at least 95%, 98%, or 99%) aa sequence identity to at least 80 (e.g., at least 100, 110, 120, or 130) contiguous aas of SEQ ID NO:106, wherein the aa at position 16 is other than H, the aa at position 42 is other than F, and the aa at position 126 is other than Q. Such IL-2 variants include those wherein, the position of H16 is substituted by Ala or Thr, the position of F42 is substituted by Ala, and the position of Q126 is substituted by Ala (an H16A, F42A, Q126A substitution or an H16T, F42A, Q126A substitutions).

[00274] IL-2 variants include polypeptides having at least 90% (e.g., at least 95%, 98%, or 99%) aa sequence identity to at least 80 (e.g., at least 100, 110, 120, or 130) contiguous aas of SEQ ID NO:106, wherein the aa at position 20 is other than D, the aa at position 42 is other than F, and the aa at position 126 is other than Q. In one case, the position D20 is substituted by Ala, the position of F42 is substituted by Ala, and the position of Q126 is substituted by Ala (D20A, F42A, Q126A substitutions).

[00275] IL-2 variants include polypeptides having at least 90% (e.g., at least 95%, 98%, or 99%) aa sequence identity to at least 80 (e.g., at least 100, 110, 120, or 130) contiguous aas of SEQ ID NO:106, wherein the aa at position 20 is other than D, the aa at position 42 is other than F, and the aa at position 45

is other than Y. In one case, the position D20 is substituted by Ala, the position of F42 is substituted by Ala, and the position of Y45 is substituted by Ala (D20A, F42A, and Y45A substitutions).

[00276] IL-2 variants include polypeptides having at least 90% (e.g., at least 95%, 98%, or 99%) aa sequence identity to at least 80 (e.g., at least 100, 110, 120, or 130) contiguous aas of SEQ ID NO:106, wherein the aa at position 16 is other than H, the aa at position 20 is other than D, the aa at position 42 is other than F, and the aa at position 45 is other than Y. Such IL-2 variants include those in which the position of H16 is substituted by Ala or Thr, the position D20 is substituted by Ala, the position of F42 is substituted by Ala, and the position of Y45 is substituted by Ala (H16A, D20A, F42A, and Y45A substitution, or H16T, D20A, F42A, and Y45A substitution,).

[00277] IL-2 variants include polypeptides having at least 90% (e.g., at least 95%, 98%, or 99%) aa sequence identity to at least 80 (e.g., at least 100, 110, 120, or 130) contiguous aas of SEQ ID NO:106, wherein the aa at position 20 is other than D, the aa at position 42 is other than F, the aa at position 45 is other than Y, and the aa at position 126 is other than Q. In one case, the position D20 is substituted by Ala, the position of F42 is substituted by Ala, the position of Y45 is substituted by Ala, and the position of Q126 is substituted by Ala (D20A, F42A, Y45A, Q126A substitutions).

[00278] IL-2 variants include polypeptides having at least 90% (e.g., at least 95%, 98%, or 99%) aa sequence identity to at least 80 (e.g., at least 100, 110, 120, or 130) contiguous aas of SEQ ID NO:106, wherein the aa at position 16 is other than H, the aa at position 20 is other than D, the aa at position 42 is other than F, the aa at position 45 is other than Y, and the aa at position 126 is other than Q. In one case, the position of H16 is substituted by Ala or Thr, the position D20 is substituted by Ala, the position of F42 is substituted by Ala, the position of Y45 is substituted by Ala, and the position of Q126 is substituted by Ala (H16A, D20A, F42A, Y45A, Q126A substitutions or H16T, D20A, F42A, Y45A, Q126A substitutions).

6 Linkers

[00279] T-Cell-MPs (and their T-Cell-MP-KRAS-epitope conjugates) can include one or more independently selected linker polypeptide sequences interposed between, for example, any one or more of:

i) two MOD polypeptides located on the N-terminal side of the β2M polypeptide sequence (referred to as an L1linker or position);

(ii) between a MOD and a β2M polypeptide sequence (referred to as an L2 linker or position);

(iii) between a β2M polypeptide sequence and an MHC-H polypeptide sequence (referred to as an L3 linker or position);

(iv) between an MHC-H polypeptide sequence and a scaffold polypeptide sequence (referred to as an L4 linker or position);

(iv) at the carboxyl end of the scaffold or between a scaffold polypeptide sequence and a MOD polypeptide sequence placed carboxy terminal to it (referred to as an L5 linker or position); or

(vi) between two MOD polypeptide sequences placed on the carboxy side of the scaffold (referred to as an L6 linker or position).

See, e.g., FIG. 5.

[00280] Chemical conjugation sites for coupling epitope peptides may be incorporated into linkers (e.g., L1-L6 linkers) including the L3 between the MHC-H and β2M polypeptide sequences. Accordingly, chemical conjugation sites including, but not limited to: sulfatase, sortase, transglutaminase, selenocysteine, non-natural amino acids, and naturally occurring proteinogenic amino acids (e.g., cysteine residues) etc. may be incorporated into linkers, including the L3 linker. Polypeptide linkers placed at either the N- or C- termini provide locations to couple additional polypeptides (e.g., histidine tags), payloads and the like, and to protect the polypeptide from exo-proteases.

[00281] Linkers may also be utilized between the peptide epitope and any reactive chemical moiety (group) used to couple the peptide epitope to the chemical conjugation site of an unconjugated T-Cell-MP (see e.g., FIG. 10). Linkers utilized between epitope (e.g., peptide epitope) and a reactive chemical moiety may be peptide/polypeptide linkers, and/or other chemical linkers (e.g., non-peptide linkers in the form of homo or hetero bifunctional linkers that comprise an alkyl group as a spacer, see e.g., FIG. 10 at entries d and e).

[00282] Suitable polypeptide 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 50 aa, from 1aa to 5 aa, from 1 aa to 15 aa, from 2 aa to 15 aa, from 2 aa to 25 aa, from 3 aa to 12 aa, from 4 aa to 10 aa, from 4 aa to 35 aa, from 5 aa to 35 aa, from 5 aa to 10 aa, from 5 aa to 20 aa, from 6 aa to 25 aa, from 7 aa to 35 aa, from 8aa to 40 aa, from 9 aa to 45 aa, from 10 to 15 aa, from 10 aa to 50 aa, from 15 to 20 aa,, from 20 to 40 aa, or from 40 to 50 aa. Suitable polypeptide linkers in the range from 10 to 50 aas in length may be from 10 to 20, from 10 to 25, from 15 to 25, from 20 to 30, from 25 to 35, from 25 to 50 30 to 35, from 35 to 45, or from 40 to 50) 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, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 aa in length. A polypeptide linker may have a length of from 15 aa to 50 aa, e.g., from 20-35, from 25 to 30, from 25 to 45, from 30 to 35, from 35 to 40, from 40 to 45, or from 45 to 50 aa in length.

[00283] Polypeptide linkers in the T-Cell-MP may include, for example, polypeptides that comprise, consist essentially of, or consists of: i) Gly and/or Ser; ii) Ala and Ser; iii) Gly, Ala, and Ser; iv) Gly, Ser, and Cys (e.g., a single Cys residue); v) Ala, Ser, and Cys (e.g., a single Cys residue); and vi) Gly, Ala, Ser, and Cys (e.g., a single Cys residue). Exemplary linkers may comprise glycine polymers, glycine-serine polymers, glycine-alanine polymers; alanine-serine polymers (including, for example polymers comprising the sequences GA, AG, AS, SA, GS, GSGGS (SEQ ID NO:111) or GGGS (SEQ ID NO:112), any of which may be repeated from 1 to 10 times (e.g., repeated 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 times); and other flexible linkers known in the art. Glycine and glycine-serine polymers can both be used as 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 polymers, and are much less restricted than residues with longer side chains (*see* Scheraga, *Rev. Computational Chem.* 11173-142 (1992)). Exemplary linkers may also comprise an aa sequence comprising, but not limited to, GGSG

(SEQ ID NO:113), GGSGG (SEQ ID NO:114), GSGSG (SEQ ID NO:115), GSGGG (SEQ ID NO:116), GGGSG (SEQ ID NO:117), GSSSG (SEQ ID NO:118), any of which may be repeated from 1 to 15 times (e.g., repeated 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 times), or combinations thereof, and the like. Linkers can also comprise the sequence $Gly(Ser)_4$ (SEQ ID NO:119) or $(Gly)_4Ser$ (SEQ ID NO:120), either of which may be repeated from 1 to 10 times (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 times). In an embodiment, the linker comprises X1-X2-X3-X4-X5 where X1-X5 are selected from glycine and serine, and one of which may be a leucine, cysteine, methionine or alanine (SEQ ID NO:121). In one embodiment the linker comprises the aa sequence AAAGG (SEQ ID NO:122), which may be repeated from 1 to 10 times.

[00284] In some cases, a linker polypeptide, present in a T-Cell-MP includes a cysteine residue that can form a disulfide bond with a cysteine residue present in another T-Cell-MP or act as a chemical conjugation site for the coupling of an epitope (e.g., via reaction with a maleimide). In some cases, for example, the linker comprises Gly, Ser and a single Cys, such as in the aa sequence G***C***GGS(G_4S) (SEQ ID NO:123) where the G_4S unit may be repeated from 1 to 10 times (e.g., repeated 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 times), G***C***GASGGGGSGGGGS (SEQ ID NO:124), the sequence G***C***GGSGGGGSGGGGSGGGGS (SEQ ID NO:125) or the sequence G***C***GGSGGGGSGGGGS (SEQ ID NO:126).

[00285] A linker may comprise the aa sequence (GGGGS) (SEQ ID NO:120, also be represented as Gly_4Ser or G_4S), 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 embodiments a linker comprising G_4S repeats has one glycine or serine residue replaced by a leucine or methionine. A first T-Cell-MP comprising a Gly_4Ser containing linker polypeptide that includes a cysteine residue may, when duplexed with a second T-Cell-MP, form a disulfide bond with a cysteine residue present in the second T-Cell-MP of the duplex T-Cell-MP. Such cysteine residues present in linkers (particularly the L3 linker) may also be utilized as a chemical conjugation site for the attachment of an epitope (e.g., a peptide epitope), such as by reaction with a maleimide functionality that is part of, or indirectly connected by a linker to, the epitope. In some cases, for example, the linker comprises the aa sequence GCGGS(G_4S) (SEQ ID NO:123) where the G_4S unit may be repeated from 1 to 10 times (e.g., repeated 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 times), G***C***GASGGGGSGGGGS (SEQ ID NO:124), the sequence G***C***GGSGGGGSGGGGSGGGGS (SEQ ID NO:125) or the sequence G***C***GGSGGGGSGGGGS (SEQ ID NO:126).

[00286] Rigid polypeptide linkers comprise a sequence of amino acids that effectively separates protein domains by maintaining a substantially fixed distance/spatial separation between the domains, thereby reducing or substantially eliminating unfavorable interactions between such domains. Rigid polypeptide linkers thus may be employed where it is desired to minimize the interaction between the domains of the T-Cell-MP, in particular the interactions between MOD aa sequences (e.g., IL-2) and other aas sequences of the T-Cell-MP. Rigid peptide linkers include peptide linkers rich in proline, and peptide linkers having an inflexible helical structure, such as an α-helical structure. Examples of rigid peptide linkers include, e.g., (EAAAK) (SEQ ID NO:205), A(EAAAK)A (SEQ ID NO:206), A(EAAAK)ALEA(EAAAK)A

(SEQ ID NO:207), (Lys-Pro), (Glu-Pro), (Thr-Pro-Arg), and (Ala-Pro) where the bracketed sequences may be repeated or appear from 1 to 20 (e.g., n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20). Non-limiting examples of suitable rigid linkers comprising EAAAK (SEQ ID NO:208) include EAAAK (SEQ ID NO:208), (EAAAK)2 (SEQ ID NO:209), (EAAAK)3 (SEQ ID NO:210), A(EAAAK)ALEA(EAAAK)A where the EAAAK sequence may be repeated or appear 1-4 times (SEQ ID NO:211), and AEAAAKEAAAKA (SEQ ID NO:212). Non-limiting examples of suitable rigid linkers comprising (AP)n include APAP (SEQ ID NO:213; also referred to herein as "(AP)2"); APAPAPAP (SEQ ID NO:214; also referred to herein as "(AP)4"); APAPAPAPAPAP (SEQ ID NO:215; also referred to herein as "(AP)6"); APAPAPAPAPAPAPAP (SEQ ID NO:216; also referred to herein as "(AP)8"); and APAPAPAPAPAPAPAPAPAP (SEQ ID NO:217; also referred to herein as "(AP)10"). Non-limiting examples of suitable rigid linkers comprising (KP)n include KPKP (SEQ ID NO:218; also referred to herein as "(KP)2"); KPKPKPKP (SEQ ID NO:219; also referred to herein as "(KP)4"); KPKPKPKPKPKP (SEQ ID NO:220; also referred to herein as "(KP)6"); KPKPKPKPKPKPKPKP (SEQ ID NO:221; also referred to herein as "(KP)8"); and KPKPKPKPKPKPKPKPKPKP (SEQ ID NO:222; also referred to herein as "(KP)10"). Non-limiting examples of suitable rigid linkers comprising (EP)n include EPEP (SEQ ID NO:223; also referred to herein as "(EP)2"); EPEPEPEP (SEQ ID NO:224; also referred to herein as "(EP)4"); EPEPEPEPEPEP (SEQ ID NO:225; also referred to herein as "(EP)6"); EPEPEPEPEPEPEPEP (SEQ ID NO:226; also referred to herein as "(EP)8"); and EPEPEPEPEPEPEPEPEPEP (SEQ ID NO:227; also referred to herein as "(EP)10").

[00287] Non-peptide linkers that may be used to covalently attach epitopes, targeting sequences and/or payloads (e.g., a drug or labeling agent) to a T-Cell-MP (including its peptide linkers) may take a variety of forms, including, but not limited to, alkyl, poly(ethylene glycol), disulfide groups, thioether groups, acid labile groups, photolabile groups, peptidase labile groups, and esterase labile groups. The non-peptide linkers (or "crosslinkers") may also be, for example, homobifunctional or heterobifunctional linkers that comprise reactive end groups such as N-hydroxysuccinimide esters, maleimide, iodoacetate esters, and the like. Examples of suitable cross-linkers include: N-succinimidyl-[(N-maleimidopropion-amido)-tetraethyleneglycol]ester (NHS-PEG4-maleimide); N-succinimidyl 4-(2-pyridyldithio)butanoate (SPDB); N-succinimidyl 4-(2-pyridyldithio)2-sulfobutanoate (sulfo-SPDB); N-succinimidyl 4-(2-pyridyldithio) pentanoate (SPP); N-succinimidyl-4-(N-maleimidomethyl)-cyclohexane-1-carboxy-(6-amidocaproate) (LC-SMCC); 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-maleimidoacetoxy)-succinimide ester (AMAS); succinimidyl-6-(β-maleimidopropionamide)hexanoate (SMPH); N-succinimidyl 4-(p-maleimidophenyl)butyrate (SMPB); N-(p-maleimidophenyl)isocyanate (PMPI); 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); 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).

7 Additional Polypeptide Sequences

[00288] A polypeptide chain of a T-Cell-MP can include one or more polypeptides in addition to those described above. Suitable additional polypeptides include epitope tags, affinity domains, and fluorescent protein sequences (e.g., green fluorescent protein). 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.

a. Epitope Tags and Affinity Domains

[00289] Suitable epitope tags include, but are not limited to, hemagglutinin (HA; e.g., YPYDVPDYA (SEQ ID NO:127)); c-myc (e.g., EQKLISEEDL; SEQ ID NO:128)), and the like.

[00290] 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:129), HisX6 (HHHHHH) (SEQ ID NO:130), C-myc (EQKLISEEDL) (SEQ ID NO:128), Flag (DYKDDDDK) (SEQ ID NO:131, StrepTag (WSHPQFEK) (SEQ ID NO:132), hemagglutinin (e.g., HA Tag (YPYDVPDYA) (SEQ ID NO:127)), glutathione-S-transferase (GST), thioredoxin, cellulose binding domain, RYIRS (SEQ ID NO:133), Phe-His-His-Thr (SEQ ID NO:134), chitin binding domain, S-peptide, T7 peptide, SH2 domain, C-end RNA tag, WEAAAREACCRECCARA (SEQ ID NO:135), 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.

b. Targeting Sequences

[00291] T-Cell-MPs of the present disclosure may include one or more targeting polypeptide sequence(s) or "targeting sequence(s)." Targeting sequences may be located anywhere within the T-Cell-MP polypeptide, for example within, at, or near the carboxyl terminal end of a scaffold peptide (e.g., translated with the scaffold in place of a C-terminal MOD in FIGs. 5 or 6 or attached to an L5 linker). Alternatively, a targeting sequence, such as an antibody antigen-binding fragment (Fab), may be covalently or non-covalently attached to a T-Cell-MP. Covalent attachment of a targeting sequence may be made at a chemical conjugation site (e.g., a chemical conjugation site in a scaffold polypeptide), where the targeting sequence effectively becomes a payload-like molecule attached to the T-Cell-MP. Targeting sequences may also be non-covalently bound to a T-Cell-MP (e.g., a T-Cell-MP having a biotin labeled

scaffold may be non-covalently attached to an avidin labeled targeting antibody or Fab directed to a cancer antigen. A bispecific antibody (e.g., a bispecific IgG or humanized antibody) having a first antigen binding site directed to a part of the T-cell-MP (e.g., the scaffold) may also be employed to non-covalently attach a T-Cell-MP to a targeting sequence (the second bispecific antibody binding site) directed to a target (e.g., a cancer antigen). Targeting sequences serve to bind or "localize" T-Cell-MPs to cells and/or tissues displaying the protein (or other molecule) to which the targeting sequence binds. A targeting sequence may be an antibody or antigen binding fragment thereof. A targeting sequence may also be a single-chain T cell receptor (scTCR).

8 Epitopes and their assessment

[00292] An unconjugated T-Cell-MP of the present disclosure may be conjutated at a chemical conjugation site to a variety of KRAS-related molecules that present an antigenic determinate to form a T-Cell-MP-KRAS-epitope conjugate. The molecules that may be conjugated to an unconjugated T-Cell-MP include those presenting non-peptide epitopes (e.g., carbohydrate epitopes), and peptide epitopes. Other molecules that may be conjugated to a T-Cell-MP to form an epitope conjugate include phosphopeptides epitope, glycosylated peptides (glycopeptides)epitope, carbohydrate, and lipopeptideepitopes, which include peptides modified with fatty acids (e.g., palmitoylation), isoprenoids (e.g., farnesylation and/or geranylgeranylation), sterols, phospholipids, or glycosylphosphatidyl inositol). Collectively, the epitope presenting molecules that may be bound to an unconjugated T-Cell-MP are referred to as an "epitope" or "epitopes." The epitope presenting sequence of the peptide, phospho-peptide, lipopeptide, or glycopeptide) present in a T-Cell-MP-KRAS-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 aa to 25 aa, from 7 aa to 12 aa, from 7 aa to 25 aa, from 10 aa to 15 aa, from 15 aa to 20 aa, or from 20 aa to 25 aa).

[00293] Epitopes of a T-Cell-MP-KRAS-epitope conjugate are not part of the T-Cell-MP as translated from mRNA, but, as indicated above, are added to a T-Cell-MP at a chemical conjugation site. Selection of candidate MHC allele and peptide (e.g., phosphopeptide, lipopeptides or glycopeptide) epitope combinations for effective presentation to a TCR by a T-Cell-MP-KRAS-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-MP in which it will be presented as part of the epitope conjugate.

[00294] It is possible to determine if the peptide in combination with the specific heavy chain allele and β2M 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 including BLI assays utilized for assessing binding affinity of T-Cell-MPs with wt. and variant MODs discussed above. The epitope (e.g., peptide epitope) that will be used to prepare a T-Cell-MP-KRAS-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 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 epitope binds to a TCR on a T cell with an affinity of from about 10^{-4} M to about 10^{-5} M, from about 10^{-5} M to

about 10^{-6} M, from about 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-MP binds to a TCR on a T cell with an affinity of from about 1 nM to about to about 10 nM, from about 10 nM to about 100 nM, from about 0.1 μM to about 1 μM, from about 1 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.

a. Cell-based binding assays

[00295] 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-MP-KRAS-epitope conjugate. The binding assay can be used in the selection of peptides for incorporation into a T-Cell-MP-KRAS-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 or similar TAP deficient cells to express the HLA H allele of interest.

[00296] 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 μM, 100 μM, 20 μM and 2 μM. The cells are mixed 1:1 with each peptide dilution to give a final volume of 200 μL and final peptide concentrations of 100 μM, 50 μM, 10 μM and 1 μM. A HLA A*0201 binding peptide, GILGFVFTL (SEQ ID NO:233), and a non-HLA A*0201-restricted peptide, HPVGEADYF (HLA-B*3501; SEQ ID NO:234), are included as positive and negative controls, respectively. The cell/peptide mixtures are kept at 37°C in 5% CO_2 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.

[00297] Labeled (e.g., a radio or fluorescently labeled payload) T-Cell-MP-KRAS-epitope conjugates including MOD-less T-Cell-MP-KRAS-epitope conjugates, particularly in the form higher order complexes (e.g., duplexes, tetramers or pentamers) may be used in vitro to establish epitope specific

binding between a T-Cell-MP-KRAS-epitope conjugate and a T cell. T cell binding by T-MP-epitope conjugates and/or MOD-less T-Cell-epitope conjugates is not, however, limited to in vitro appli+cations. Binding, particularly by higher order complexes of T-Cell-MP-KRAS-epitope conjugates may be conducted in vivo or ex vivo to, for example, track epitope specific T cell movement and localization. The use of MOD-less molecules is advantageous as it limits the potential interference due to interactions between a MOD on a T-Cell-MP-KRAS-epitope conjugate and Co-MOD on cells that are not of interest. In such in vivo or ex vivo binding assessments a labeled (e.g., fluorescent or radio labeled) T-Cell-MP-KRAS-epitope conjugate, which may be MOD-less, is administered to a subject in vivo, or contacted with a tissue ex vivo. Once the T-Cell-MP-KRAS-epitope conjugate binds a T-cell in the subject or tissue it will effectively label the T cell which may circulate or be localized as evidenced by the localization of the label. Accordingly, such labeled T-Cell-MP-KRAS-epitope conjugates, including their MOD-less variants, find use both in research and as companion diagnostics. The label permits evaluation of epitope specific binding between the T-Cell-MP-KRAS-epitope conjugate and target T cells and tracking of epitope specific T cells to determine of their fate. The label also permits a determination of the localization of the T-Cell-MP-KRAS-epitope conjugate in vivo and/or ex vivo, which may be used to determine if a T-Cell-MP-KRAS-epitope conjugate is localized to a tissue, including tissues to which a medical treatment is desired (e.g., tumor tissue).

b. Biochemical binding assays

[00298] MHC Class I complexes comprising a β2M polypeptide complexed with an HLA heavy chain polypeptide of a specific allele intended for use in construction of a T-Cell-MP 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.

[00299] As another example, a peptide of interest can be incubated with a MHC Class I complex (containing an HLA heavy chain peptide and β2M) 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

[00300] Whether a given peptide binds a MHC Class I complex (comprising an HLA heavy chain and a β2M 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 (IFNγ) production, cytotoxic activity, and the like.

(i) ELISPOT assays

[00301] Suitable T cell activation assays include, e.g., an enzyme linked immunospot (ELISPOT) assay where production of a product by target cells (e.g., IFNγ 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 the target cell produced factor (e.g., IFNγ) 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 released factor (e.g., IFNγ) bound to the immobilized antibody is detected using a detectably labeled antibody. A colorimetric assay can be used. For example, where IFNγ release is measured, a detectably labeled anti-IFNγ antibody can be a biotin-labeled anti-IFNγ antibody, which can be detected using, e.g., streptavidin conjugated to alkaline phosphatase, with a BCIP/NBT (5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium) solution added, to develop the assay. The presence of IFNγ-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

[00302] Whether a given epitope (e.g., peptide) binds to a particular MHC class I heavy chain allele complexed with β2M, 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 β2M, and the epitope and MHC heavy chain allele combination to be tested. The target cells can be radioactively labeled, e.g., with ^{51}Cr. If the target cell effectively presents the epitope to a TCR on the cytotoxic $CD8^+$ T cell, it induces cytotoxic activity by the $CD8^+$ T cell toward the target cell, which 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

[00303] As another example, multimers (e.g., dimers, tetramers, or pentamers) of peptide-MHC complexes are generated with a label or tag (e.g., 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. KRAS Epitopes

[00304] An epitope present in a T-Cell-MP-KRAS-epitope conjugate may be bound in an epitope-specific manner by a T cell (i.e., the epitope is specifically bound by an epitope-specific T cell whose TCR recognizes the peptide). An epitope-specific T cell binds an epitope having a reference aa sequence in the context of a specific MHC-H allele polypeptide/ β2M complex, but does not substantially bind an epitope that differs from the reference aa sequence presented in the same context. For example, an epitope-specific T cell may bind an epitope in the context of a specific MHC-H allele polypeptide/ β2M complex having a reference aa sequence, and may bind an epitope that differs from the reference aa sequence presented in the same context, 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 may bind an epitope (e.g., a peptide presenting an epitope of interest) 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.

[00305] In some cases, the peptide epitope present in a T-Cell-MP-KRAS-epitope conjugate presents an epitope-specific to an HLA-A, -B, -C, -E, -F or -G allele. In an embodiment, the peptide epitope present in a T-Cell-MP presents an epitope restricted to HLA-A*0101, A*0201, A*0203, A*0301, A*1101, A*2301, A*2402, A*2407, A*3303, A*3401, and/or A*5801. In an embodiment, the peptide epitope present in a T-Cell-MP presents an epitope restricted to HLA- B*0702, B*0801, B*1502, B*2705, B*3802, B*3901, B*3902, B*4001, B*4601, and/or B*5301. In an embodiment, the peptide epitope present in a T-Cell-MP 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.

[00306] The present disclosure provides a T-Cell-MP-KRAS-epitope conjugate comprising a KRAS peptide that, when bound to major histocompatibility complex (MHC) polypeptides, presents an KRAS epitope to a T-cell receptor (TCR). As used herein, the term "KRAS peptide" means a peptide having a length of at least 4 amino acids, e.g., from 4 amino acids to about 25 amino acids (e.g., 4 amino acids (aa), 5 aa, 6 aa, 7 aa, 8 aa, 9 aa, 10 aa, 11 aa, 12 aa, 13 aa, 14 aa, 15 aa, 16 aa, 17 aa, 18 aa, 19 aa, 20 aa, 21 aa, 22 aa, 23 aa, 24 aa, or 25 aa, including within a range of from 4 to 20 amino acids, from 6 to 18 amino acids, from 8 to 15 amino acids, from 8 to 12 amino acids, from 9-10 amino acids, from 5 to 10 amino acids, from 10 to 20 amino acids, and from 15 to 25 amino acids in length) that presents a KRAS epitope to a TCR when the KRAS peptide is bound to an MHC complex. As used herein, the term "KRAS epitope" means an epitope found on a KRAS protein. As used herein, the terms "KRAS" and "KRAS protein" are synonymous and mean a protein having an amino acid sequence present in one of the following: (i) a KRAS4A polypeptide; (ii) a KRAS4B; and (iii) variants of (i) and (ii) that occur in human cancers, including, e.g., mutated forms. As used herein, the term "KRAS polypeptide" means a polypeptide having a sequence of amino acids found in all or a part of a KRAS protein, or where specified, a polypeptide having at least 80% (e.g., at least 90%, 95%, 98% or more) amino acid sequence identity to a sequence of amino acids found in all or a part of a KRAS protein. KRAS epitopes of interest include peptides that have sequence variations (e.g., substitutions, deletions, insertions etc.) not found in wild type RAS proteins and that have been associated neoplastic behavior when RAS/KRAS proteins

bearing those sequence variations are introduced into mammalian cells. The peptides may include posttranslational modifications including phosphorylation, glycosylation, and/or lipidation (e.g., palmitoylation, glycosylation, and/or farnesylation).

[00307] KRAS (also known as "KRAS proto-oncogene, GTPase," Kirsten rat sarcoma viral oncogene homolog," and "K-Ras P21 protein") is a GTPase that controls cell proliferation. When mutated, KRAS can fail to control cell proliferation, leading to cancer.

[00308] A wild-type (normal; non-cancer-associated) KRAS polypeptide can have the following amino acid sequence: MTEYKLVVVG A**GG**VGKSALT IQLIQNHFVD EYDP**T**IEDSY RKQVVIDG**E**T CLWDILDTAG **Q**EEYSAMRDQ YMRTGEGFLC VFAINNTKSF EDIHHYREQI KRVKDSEDVP MVLVGNKCDL PSRTVDT**K**QA QDLARSYGIP FIETSAKTRQ GVDD**A**FYTLV REIRKHKEKM SKDGKKKKKK SKTKCVIM (SEQ ID NO:136).

[00309] A wild-type (normal; non-cancer-associated) KRAS polypeptide can have the following amino acid sequence: MTEYKLVVVG A**GG**VGKSALT IQLIQNHFVD EYDP**TI**EDSY RKQVVIDG**E**T CLLDILDTAG **Q**EEYSAMRDQ YMRTGEGFLC VFAINNTKSF EDIHHYREQI KRVKDSEDVP MVLVGNKCDL PSRTVDT**K**QA QDLARSYGIP FIETSAKTRQ RVED**A**FYTLV REIRQYRLKK ISKEEKTPGC VKIKKCIIM (SEQ ID NO:137).

[00310] Mutated forms of KRAS are associated with certain cancers; and at least a portion of the mutated form of KRAS is present on the surface of certain cancer cells. See, e.g., Prior et al. (2012) *Cancer Res.* 72:2457; and Warren and Holt (2010) *Human Immunology* 71:245. In SEQ ID NO:136 and SEQ ID NO:137, amino acids G12, G13, T35, I36, E49, Q61, K127, and A156 are in bold and underlined; substitutions of one or more of these residues can be present in a cancer-associated form of a KRAS polypeptide. A cancer-associated KRAS polypeptide can include one or more of: i) a substitution of G12 (e.g. G12C, G12V, G125, G12A, G12R, G12F, or G12D); ii) a substitution of G13 (e.g. G13C, G13D, G13R, G13V, G13S, or G13A); iii) a substitution of T35 (e.g., T35I); iv) a substitution of I36 (e.g., I36L or I36M); v) a substitution of E49 (e.g., E49K); vi) a substitution of Q61 (e.g. Q61H, Q61R, Q61P, Q61E, Q61K, Q61L, or Q61K); vii) a substitution of K117 (e.g., K117N); and viii) a substitution of A146 (e.g. A146T or A146V); where the amino acid numbering is as set out in SEQ ID NO:136 and SEQ ID NO:137. See, e.g., U.S. 2019/0194192. Peptides bearing such substitutions may be incorporated into an unconjugated T-Cell-MP as a peptide epitope to from the corresponding T-Cell-MP-KRAS-epitope conjugate.

[00311] For example, a cancer-associated, mutated form of a KRAS polypeptide, or peptides that acts as the presented epitope in a T-Cell-MP-KRAS-epitope conjugate, can have one or more amino acid substitutions compared to the amino acid sequence set forth in SEQ ID NO:136 or SEQ ID NO:137. In some cases, a cancer-associated, mutated form of a KRAS polypeptide or peptide epitope has only a single amino acid substitution compared to the amino acid sequence set forth in SEQ ID NO:136 or SEQ ID NO:137. In some cases, a cancer-associated, mutated form of a KRAS polypeptide or peptide epitope has only two amino acid substitutions compared to the amino acid sequence set forth in SEQ ID NO:136 or SEQ ID NO:137. In some cases, a cancer-associated, mutated form of a KRAS polypeptide or peptide

epitope has only three amino acid substitutions compared to the amino acid sequence set forth in SEQ ID NO:136 or SEQ ID NO:137. In some cases, a cancer-associated, mutated form of a KRAS polypeptide or peptide epitope has only four amino acid substitutions compared to the amino acid sequence set forth in SEQ ID NO:136 or SEQ ID NO:137. In some cases, a cancer-associated, mutated form of a KRAS polypeptide or peptide epitope has only five amino acid substitutions compared to the amino acid sequence set forth in SEQ ID NO:136 or SEQ ID NO:137.

[00312] For example, KRAS(G12D) (a KRAS polypeptide having a G-to-D substitution at amino acid position 12, based on the amino acid numbering set forth in SEQ ID NO:136) is associated with pancreatic ductal adenocarcinoma (PDAC). KRAS(G12V) (a KRAS polypeptide having a G-to-V substitution at amino acid position 12, based on the amino acid numbering set forth in SEQ ID NO:136 or SEQ ID NO:137) is also associated with pancreatic cancer. KRAS(G12R) (a KRAS polypeptide having a G-to-R substitution at amino acid position 12, based on the amino acid numbering set forth in SEQ ID NO:136 or SEQ ID NO:137) is also associated with pancreatic cancer. See, e.g., Waters and Der (2018) *Cold Spring Harb. Perspect. Med.* 8:(9). pii: a031435. doi: 10.1101/cshperspect.a031435. As another example, KRAS(G12C) (a KRAS polypeptide having a G-to-C substitution at amino acid position 12, based on the amino acid numbering set forth in SEQ ID NO:136 or SEQ ID NO:137) is associated with lung cancer, e.g., non-small cell lung cancer. See, e.g., Román et al. (2018) *Mol. Cancer* 17:33. Other mutated forms of KRAS (e.g., G12A; G12C; G12D; G12R; G12S; G12V; G13A; G13C; G13D; G13R; G13S; G13V) are associated with various cancers; where such cancers include, e.g., bile duct carcinoma, gall bladder carcinoma, adenocarcinoma, rectal adenocarcinoma, endometrial carcinoma, hematopoietic neoplasms, and lung cancer. See, e.g., Prior et al. (20120 *Cancer Res.* 72:2457.

[00313] As another example, a cancer-associated, mutated form of a KRAS polypeptide can have an amino acid substitution at amino acid 61 of a KRAS polypeptide (e.g., a KRAS polypeptide having the amino acid sequence set forth in SEQ ID NO:136 or SEQ ID NO:137). For example, a cancer-associated, mutated form of a KRAS polypeptide can have an amino acid substitution such as Q61H, Q61L, Q61E, Q61R, or Q61K.

[00314] As discussed above, a T-Cell-MP-KRAS-epitope conjugate of the present disclosure comprises a KRAS peptide that is typically at least about 4 amino acids in length, and presents a KRAS epitope to a T cell when in an MHC/peptide complex (e.g., an HLA/peptide complex). The KRAS epitope may include one or more aa substitutions associated with a benign neoplasm or cancer (malignant neoplasm).

[00315] A KRAS epitope present in a T-Cell-MP-KRAS-epitope conjugate of the present disclosure is a peptide specifically bound by a T-cell, i.e., the epitope is specifically bound by an 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.

[00316] In some cases, a suitable KRAS peptide is a peptide of at least 4 amino acids in length, (e.g., from 4 amino acids to about 25 amino acids (e.g., 4 amino acids (aa), 5 aa, 6 aa, 7 aa, 8 aa, 9 aa, 10 aa, 11 aa, 12 aa, 13 aa, 14 aa, 15 aa, 16 aa, 17 aa, 18 aa, 19 aa, 20 aa, 21 aa, 22 aa, 23 aa, 24 aa, or 25 aa, including within a range of from 4 to 20 amino acids, from 6 to 18 amino acids, from 8 to 15 amino acids, from 8 to 12 amino acids, from 9-10 amino acids, from 5 to 10 amino acids, from 10 to 20 amino acids, and from 15 to 25 amino acids in length) of a KRAS polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to a portion of the KRAS sequence provided in SEQ ID NO:136, where the KRAS polypeptide comprises one or more (e.g., 1, 2, 3, 4, or 5) amino acid substitutions compared to the amino acid sequence forth in SEQ ID NO:136. The one or more amino acid substitutions can include substitutions associated with cancer; e.g., substitutions that are found in a KRAS polypeptide in a cancer cell.

[00317] In some cases, a suitable KRAS peptide is a peptide of at least 4 amino acids in length, e.g., from 4 amino acids to about 25 amino acids (e.g., 4 amino acids (aa), 5 aa, 6 aa, 7 aa, 8 aa, 9 aa, 10 aa, 11 aa, 12 aa, 13 aa, 14 aa, 15 aa, 16 aa, 17 aa, 18 aa, 19 aa, 20 aa, 21 aa, 22 aa, 23 aa, 24 aa, or 25 aa, including within a range of from 4 to 20 amino acids, from 6 to 18 amino acids, from 8 to 15 amino acids, from 8 to 12 amino acids, from 9-10 amino acids, from 5 to 10 amino acids, from 10 to 20 amino acids, and from 15 to 25 amino acids in length) of a KRAS polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to a portion of the KRAS sequence provided in SEQ ID NO:137, where the KRAS polypeptide comprises one or more (e.g., 1, 2, 3, 4, or 5) amino acid substitutions compared to the amino acid sequence forth in SEQ ID NO:136. The one or more amino acid substitutions can include substitutions associated with cancer; e.g., substitutions that are found in a KRAS polypeptide in a cancer cell.

[00318] In some cases, a suitable KRAS peptide is a peptide of at least 4 amino acids in length, e.g., from 4 amino acids to about 25 amino acids (e.g., 4 amino acids (aa), 5 aa, 6 aa, 7 aa, 8 aa, 9 aa, 10 aa, 11 aa, 12 aa, 13 aa, 14 aa, 15 aa, 16 aa, 17 aa, 18 aa, 19 aa, 20 aa, 21 aa, 22 aa, 23 aa, 24 aa, or 25 aa, including within a range of from 4 to 20 amino acids, from 6 to 18 amino acids, from 8 to 15 amino acids, from 8 to 12 amino acids, from 9-10 amino acids, from 5 to 10 amino acids, from 10 to 20 amino acids, and from 15 to 25 amino acids in length) of a KRAS polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to a portion of the following KRAS amino acid sequence: MTEY(X1)L(X2)(X3)(X4)GA(X5)(X6)VGKSALT IQLIQNHFVD EYDPTIEDSY RKQVVIDGET CLWDILDTAG QEEYSAMRDQ YMRTGEGFLC VFAINNTKSF EDIHHYREQI KRVKDSEDVP MVLVGNKCDL PSRTVDTKQA QDLARSYGIP FIETSAKTRQ GVDDAFYTLV REIRKHKEKM SKDGKKKKKK SKTKCVIM (SEQ ID NO:138), where X1 is Lys, Phe, or Leu; X2 is Val or Leu; X3 is Val or Thr; X4 is Val or Thr; X5 is Gly, Asp, Cys, Val, or Ser; and X6 is Gly, Cys, or Asp; where one or both of X5 and X6 is not a Cys.

[00319] Non-limiting examples of suitable KRAS peptides for incorporation into a T-Cell-MP include: VVGA**D**GVGK (SEQ ID NO:139), VVGA**C**GVGK (SEQ ID NO:140), VVGA**V**GVGK (SEQ ID

NO:141), VVVGA**D**GVGK (SEQ ID NO:142), VVVGA**V**GVGK (SEQ ID NO:143), VVVGA**C**GVGK (SEQ ID NO:144), V**T**GA**D**GVGK (SEQ ID NO:145), V**T**GA**V**GVGK (SEQ ID NO:146), V**T**GA**C**GVGK (SEQ ID NO:147), V**T**VGA**D**GVGK (SEQ ID NO:148), V**T**VGA**V**GVGK (SEQ ID NO:149), and V**T**VGA**C**GVGK (SEQ ID NO:150); where the KRAS peptide has a length of 9 amino acids or 10 amino acids.

[00320] Additional non-limiting examples of suitable KRAS peptides include: VVVGAG**D**VGK (SEQ ID NO:151); VVGAGDVGK (SEQ ID NO:152); VVVGARGVGK (SEQ ID NO:153); and VVGARGVGK (SEQ ID NO:154); where the KRAS peptide has a length of 9 amino acids or 10 amino acids.

[00321] Non-limiting examples of suitable KRAS peptides include: LVVVGA**D**GV (SEQ ID NO:155), LVVVGA**V**GV (SEQ ID NO:156), LVVVGA**C**GV (SEQ ID NO:157), KLVVVGA**D**GV (SEQ ID NO:158), KLVVVGA**V**GV (SEQ ID NO:159), KLVVVGA**C**GV (SEQ ID NO:160), L**L**VVGA**D**GV (SEQ ID NO:161), L**L**VVGA**V**GV (SEQ ID NO:162), L**L**VVGA**C**GV (SEQ ID NO:163), **F**LVVVGA**D**GV (SEQ ID NO:164), **F**LVVVGA**V**GV (SEQ ID NO:165), and **F**LVVVGA**C**GV (SEQ ID NO:188); where the KRAS peptide has a length of 9 amino acids or 10 amino acids.

[00322] Additional non-limiting examples of suitable KRAS peptides include: KLVVVGAGDV (SEQ ID NO:166); and KLVVVGARGV (SEQ ID NO:167); where the KRAS peptide has a length of 9 amino acids or 10 amino acids.

[00323] Additional non-limiting examples of suitable KRAS peptides include: GAGDVGKSAL (SEQ ID NO:168); AGDVGKSAL (SEQ ID NO:169); DVGKSALTI (SEQ ID NO:170); GAVGVGKSAL (SEQ ID NO:171); AVGVGKSAL (SEQ ID NO:172); YKLVVVGAV (SEQ ID NO:173); ARGVGKSAL (SEQ ID NO:174); GARGVGKSAL (SEQ ID NO:175); EYKLVVVGAR (SEQ ID NO:176); RGVGKSALTI (SEQ ID NO:177); LVVVGARGV (SEQ ID NO:178); GADGVGKSAL (SEQ ID NO:179); ACGVGKSAL (SEQ ID NO:180); and GACGVGKSAL (SEQ ID NO:181).

[00324] In some cases, a T-Cell-MP-KRAS-epitope conjugate of the present disclosure modulates the activity of a T cell that comprises a TCR that is specific for a G12V form of a KRAS polypeptide, as described above. In such cases, the KRAS peptide present in a T-Cell-MP-KRAS-epitope conjugate of the present disclosure can comprise, for example, one of the following amino acid sequences: VVGA**V**GVGK (SEQ ID NO:141), VVVGA**V**GVGK (SEQ ID NO:143), VGA**V**GVGKS (SEQ ID NO:182), VGA**V**GVGKSA (SEQ ID NO:183), A**V**GVGKSAL (SEQ ID NO:172), A**V**GVGKSALT (SEQ ID NO:184), GA**V**GVGKSAL (SEQ ID NO:171), GA**V**GVGKSA (SEQ ID NO:185), LVVVGA**V**GVG (SEQ ID NO:186), LVVVGA**V**GV (SEQ ID NO:156), KLVVVGA**V**GV (SEQ ID NO:159), and KLVVVGA**V**G (SEQ ID NO:187); where the KRAS peptide has a length of 9 amino acids or 10 amino acids.

[00325] In some cases, the KRAS peptide present in a T-Cell-MP-KRAS-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 KRAS peptide present in a T-Cell-MP-KRAS-epitope conjugate presents an epitope restricted to HLA-A*0101, A*0201, A*0203, A*0301, A*1101, A*2301, A*2402, A*2407, A*3101, A*3303, A*3401, and/or A*6801. In an embodiment, the KRAS epitope peptide present in a T-Cell-MP-KRAS-epitope

conjugate presents an epitope restricted to HLA- B*0702, B*0801, B*1502, B*2705, B*3802, B*3802, B*3901, B*3902, B*4001, B*4601, B*5101, and/or B*5301. In an embodiment, the KRAS epitope peptide present in a T-Cell-MP-KRAS-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.

[00326] As non-limiting examples, the KRAS peptides VVGA**D**GVGK (SEQ ID NO:139), VVGA**C**GVGK (SEQ ID NO:140), VVGA**V**GVGK (SEQ ID NO:141), VVVGA**D**GVGK (SEQ ID NO:142), VVVGA**V**GVGK (SEQ ID NO:143), VVVGA**C**GVGK (SEQ ID NO:144), V**T**GA**D**GVGK (SEQ ID NO:145), V**T**GA**V**GVGK (SEQ ID NO:146), V**T**GA**C**GVGK (SEQ ID NO:147), V**T**VGA**D**GVGK (SEQ ID NO:148), V**T**VGA**V**GVGK (SEQ ID NO:149), V**T**VGA**C**GVGK (SEQ ID NO:150), VVVGAG**D**VGK (SEQ ID NO:151), VVGAGDVGK (SEQ ID NO:152), VVVGARGVGK (SEQ ID NO:153), and VVGARGVGK (SEQ ID NO:154) present an epitope when bound to an HLA complex comprising a β2M polypeptide and an A*1101 HLA-A heavy chain. Such peptides may also be presented in complex with an HLA complex comprising a β2M polypeptide and an A*6801 HLA-A heavy chain.

[00327] As non-limiting examples, the KRAS peptides LVVVGA**D**GV (SEQ ID NO:155), LVVVGA**V**GV (SEQ ID NO:156), LVVVGA**C**GV (SEQ ID NO:157), KLVVVGA**D**GV (SEQ ID NO:158), KLVVVGA**V**GV (SEQ ID NO:159), KLVVVGA**C**GV (SEQ ID NO:160), L**L**VVGA**D**GV (SEQ ID NO:161), L**L**VVGA**V**GV (SEQ ID NO:162), L**L**VVGA**C**GV (SEQ ID NO:163), **F**LVVVGA**D**GV (SEQ ID NO:164), **F**LVVVGA**V**GV (SEQ ID NO:165), and FLVVVGACGV (SEQ ID NO:188) present an epitope when bound to an HLA complex comprising a β2M polypeptide and an A*0201 HLA-A heavy chain.

[00328] Another group of KRAS peptides suitable as epitopes includes KLVVGADGV (SEQ ID NO:189) , KLVVGAVGV (SEQ ID NO:190) , KLVVVAVGV (SEQ ID NO:191) , and KLVVVADGV (SEQ ID NO:192).

[00329] As additional examples, the following KRAS peptides can present an epitope when bound to an HLA complex comprising a β2M polypeptide and an HLA-A heavy chain as follows: GAGDVGKSAL (SEQ ID NO:168), which can present an epitope when bound to an HLA complex comprising a β2M polypeptide and a B*3801 HLA-A heavy chain; AGDVGKSAL (SEQ ID NO:169), which can present an epitope when bound to an HLA complex comprising a β2M polypeptide and a B0702, a B*3801, or a B*3901 HLA-A heavy chain; DVGKSALTI (SEQ ID NO:170), which can present an epitope when bound to an HLA complex comprising a β2M polypeptide and a B*5101 HLA-A heavy chain; GAVGVGKSAL (SEQ ID NO:171), which can present an epitope when bound to an HLA complex comprising a β2M polypeptide and a B*0702 or a B*3801 HLA-A heavy chain; AVGVGKSAL (SEQ ID NO:172), which can present an epitope when bound to an HLA complex comprising a β2M polypeptide and a B*0702 HLA-A heavy chain; YKLVVVGAV (SEQ ID NO:173), which can present an epitope when bound to an HLA complex comprising a β2M polypeptide and an A*0203 or a B*3902 HLA-A heavy chain; ARGVGKSAL (SEQ ID NO:174), which can present an epitope when bound to an HLA complex comprising a β2M polypeptide and a B*0702, a B*2705, or a B*3901 HLA-A heavy chain;

GARGVGKSAL (SEQ ID NO:175), which can present an epitope when bound to an HLA complex comprising a β2M polypeptide and a B*0702 HLA-A heavy chain; EYKLVVVGAR (SEQ ID NO:176), which can present an epitope when bound to an HLA complex comprising a β2M polypeptide and an A*3101 HLA-A heavy chain; RGVGKSALTI (SEQ ID NO:177), which can present an epitope when bound to an HLA complex comprising a β2M polypeptide and a B*0702 HLA-A heavy chain; LVVVGARGV (SEQ ID NO:178), which can present an epitope when bound to an HLA complex comprising a β2M polypeptide and an A*0203 HLA-A heavy chain; GADGVGKSAL (SEQ ID NO:179), which can present an epitope when bound to an HLA complex comprising a β2M polypeptide and a B*3801 HLA-A heavy chain; ACGVGKSAL (SEQ ID NO:180), which can present an epitope when bound to an HLA complex comprising a β2M polypeptide and a B*0702 HLA-A heavy chain; and GACGVGKSAL (SEQ ID NO:181), which can present an epitope when bound to an HLA complex comprising a β2M polypeptide and a B*3801 HLA-A heavy chain.

9 Payloads--Drug And Other Conjugates

[00330] A polypeptide chain of a T-Cell-MP can comprise an attached payload such as a therapeutic (e.g., a small molecule drug or therapeutic) a label (e.g., a fluorescent label or radio label), or other biologically active agent that is linked (e.g., covalently attached) to the polypeptide chain at a chemical conjugation site. For example, where a T-Cell-MP comprises an Fc polypeptide, the Fc polypeptide may comprise a covalently linked payload molecule that treats a cancer or an infectious disease, or is an agent that relieves a symptom of such diseases.

[00331] A payload can be linked directly or indirectly to a chemical conjuation site that is part of the polypeptide chain of a T-Cell-MP of the present disclosure (e.g., to scaffold such as an Ig Fc polypeptide). Direct linkage can involve linkage directly to an aa side chain. Indirect linkage can be linkage via a cross-linker, such as a bifunctional cross cross-linker. A payload can be linked to a T-Cell-MP by any acceptable chemical linkage including, but not limited to a thioether bond, an amide bond, a carbamate bond, a disulfide bond, or an ether bond formed by reaction with a crosslinking agent.

[00332] Crosslinkers (crosslinking agents) include cleavable cross-linkers and non-cleavable cross-linkers may be used to link payloads and/or targeting sequences to a T-Cell-MP polypeptide. The crosslinkers may comprise reactive NHS, maleimide, iodoacetate, bromoacetate and/or carboxlyate groups. In some cases, the cross-linker is a protease-cleavable cross-linker. Suitable cross-linkers may include, for example, peptides (e.g., from 2 to 10 aas in length; e.g., 2, 3, 4, 5, 6, 7, 8, 9, or 10 aas 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 example of suitable cross-linkers are: N-succinimidyl-[(N-maleimidopropionamido)-tetraethyleneglycol]ester (NHS-PEG4-maleimide); N-succinimidyl 4-(2-pyridyldithio)butanoate (SPDB); N-succinimidyl 4-(2-pyridyldithio)2-sulfobutanoate (sulfo-SPDB); N-succinimidyl 4-(2-pyridyldithio) pentanoate (SPP); N-succinimidyl-4-(N-maleimidomethyl)-cyclohexane-1-carboxy-(6-amidocaproate) (LC-SMCC); 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-maleimidoacetoxy)-succinimide ester (AMAS); succinimidyl-6-(β-maleimidopropionamide)hexanoate (SMPH); N-succinimidyl 4-(p-maleimidophenyl)butyrate (SMPB); N-(p-maleimidophenyl)isocyanate (PMPI); 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); 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).

[00333] T-Cell-MP-payload conjugates may be formed by reaction of a T-Cell-MP polypeptide (e.g., an Ig Fc polypeptide of a T-Cell-MP) with a crosslinking reagent to introduce 1-10 reactive groups. The polypeptide is then reacted with the molecule to be conjugated (e.g., a thiol-containing payload drug, label or agent) to produce a T-Cell-MP-payload conjugate. For example, where a T-Cell-MP of the present disclosure comprises an Ig Fc polypeptide, the conjugate can be of the form (A)-(L)-(C), where (A) is the polypeptide chain comprising the Ig Fc polypeptide; where (L), if present, is a cross-linker; and where (C) is a payload. (L), if present, links (A) to (C). In some cases, the T-Cell-MP includes an Ig Fc polypeptide sequence that comprises one or more (e.g., 2, 3, 4, 5, or more than 5) molecules of a payload. Introducing payloads into a T-Cell-MP using an excess of crosslinking agents can result in multiple molecules of payload being incorporated into the T-Cell-MP.

[00334] Suitable payloads (e.g., drugs) include virtually any small molecule (e.g., less than 2,000 Daltons in molecular weight) approved by the U.S. Food and Drug Administration, and/or listed in the 2020 U.S. Pharmacopeia or National Formulary. In an embodiment, those drugs are less than 1,000 molecular weight. Suitable drugs include chemotherapeutic (antineoplastic). Suitable chemotherapeutics may be alkylating agents, cytoskeletal disruptors (taxanes), epothilone, histone deacetylase inhibitors, topoisomerase I inhibitors, topoisomerase II inhibitors, kinase inhibitors, nucleotide analog or precursor analogs, peptide antineoplastic antibiotics (e.g. bleomycin or actinomycin), platinum-based agents, retinoids, or vinca alkaloids. Suitable chemotherapeutics also include alkylating agents, cytoskeletal disruptors (taxanes), epothilone, histone deacetylase inhibitors, topoisomerase I inhibitors, topoisomerase II inhibitors, kinase inhibitors, nucleotide analog or precursor analogs, peptide antineoplastic antibiotics (e.g. bleomycin or actinomycin), platinum-based agents, retinoids, or vinca alkaloids.

[00335] 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, particles bearing nucleic acids or other molecules), and combinations thereof.

[00336] 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, antibioticcell 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.

[00337] In an embodiment the payload is a label, selected independently from the group consisting of photo detectable 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. In some embodiments, the payload is a label that is or includes a radioisotope. Examples of radioisotopes or other labels include, but are not limited to, ^{3}H, ^{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 ^{153}Pb.

II. Nucleic Acids

[00338] The present disclosure provides a nucleic acid comprising a nucleotide sequence encoding a T-Cell-MP or more than one T-Cell-MP (e.g., a pair of T-Cell-MPs that form an interspecific heterodimer). The individual T-Cell-MPs of heteromer (e.g., an interspecific pair forming a heteroduplex) may be encoded in separate nucleic acids. Alternatively, the T-Cell-MPs of a heteromeric T-Cell-MP (e.g., an interspecific pair) may also be encoded in a single nucleic acid. Such nucleic acids include those comprising a nucleotide sequence encoding a T-Cell-MP having chemical conjugation sites (e.g., cysteine residues) that are provided in the MHC-H, β2M or scaffold polypeptide sequences of the T-Cell-MP, or into any linker (e.g., a L3 linker) joining those polypeptide sequences.

A. Nucleic acids encoding unconjugated T-Cell-MPs

[00339] The present disclosure provides nucleic acids comprising nucleotide sequences encoding an unconjugated T-Cell-MP that may form higher order complexes (e.g., duplexes). The nucleotide sequences encoding an unconjugated T-Cell-MP may be 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. As noted above, in some cases, the individual unconjugated T-Cell-MPs form heteromeric complexes (e.g., a heteroduplex T-Cell-MP comprising an interspecific scaffold pair). Heteromeric unconjugated T-Cell-MPs may be encoded in a single polycistronic nucleic acid sequence. Alternatively, heteromeric T cell-MPs may be encoded in separate monocistronic nucleic acid sequences with expression driven by separate transcriptional control elements. Where separate monocistronic sequences are utilized, they may be present in a single vector or in separate vectors.

[00340] The present disclosure includes and provides for a nucleic acid sequence encoding an unconjugated T-Cell-MP polypeptide that comprises (e.g., from N-terminus to C-terminus): (i) optionally one or more MOD polypeptide sequences (e.g., two or more MOD polypeptide sequences, such as in tandem, wherein when there are two or more MOD polypeptide sequences they are optionally joined to each other by independently selected L1 linkers); (ii) an optional linker L2 polypeptide sequence joining

the one or more MOD polypeptide sequences to a β2M polypeptide sequence; (iii) the β2M polypeptide sequence; (iv) an optional L3 linker polypeptide sequence (e.g., from 10-50 aa in length); (v) a class I MHC-H polypeptide sequence; (vi) an optional L4 linker polypeptide sequence; (vii) a scaffold polypeptide sequence (e.g., an immunoglobulin Fc sequence); (viii) an optional L5 linker polypeptide sequence; and (ix) optionally one or more MOD polypeptide sequence (e.g., two or more MOD polypeptide sequences, such as in tandem, wherein when there are two or more MOD polypeptide sequences they are optionally joined to each other by independently selected L6 linkers); wherein the unconjugated T cell modulatory polypeptide comprises at least one MOD polypeptide sequence (e.g., the MOD(s) of element (i) and/or (ix)); and wherein at least one of the β2M polypeptide sequence, L3 linker polypeptide sequence, and/or the MHC-H polypeptide sequence comprises a chemical conjugation site for epitope conjugation.

[00341] The present disclosure includes and provides for a nucleic acid sequence encoding an unconjugated T-Cell-MP polypeptide that comprises from N- to C-terminus: (i) optionally one or more MOD polypeptide sequences (e.g., two or more MOD polypeptide sequences, such as in tandem, wherein when there are two or more MOD polypeptide sequences they are optionally joined to each other by independently selected L1 linkers); (ii) an optional linker L2 polypeptide sequence; (iii) a β2M polypeptide sequence; (iv) an optional L3 linker polypeptide sequence (e.g., from 10-50 aa in length); (v) a class I MHC-H polypeptide sequence; (vi) an optional L4 linker polypeptide sequence; (vii) a scaffold polypeptide sequence (e.g., an immunoglobulin Fc sequence); (viii) an optional L5 linker polypeptide sequence; and (ix) optionally one or more MOD polypeptide sequence (e.g., two or more MOD polypeptide sequences, such as in tandem, wherein when there are two or more MOD polypeptide sequences they are optionally joined to each other by independently selected L6 linkers); wherein the unconjugated T cell modulatory polypeptide comprises at least one MOD polypeptide sequence (e.g., the MOD(s) of element (i) and/or (ix)); and wherein at least one of the β2M polypeptide sequence, L3 linker polypeptide sequence, and/or the MHC-H polypeptide sequence comprises a chemical conjugation site for epitope conjugation.

[00342] The present disclosure includes and provides for a nucleic acid sequence encoding an unconjugated T-Cell-MP polypeptide that comprises from N- to C-terminus: (i) one or more MOD polypeptide sequences (e.g., two or more MOD polypeptide sequences, such as in tandem, wherein when there are two or more MOD polypeptide sequences they are optionally joined to each other by independently selected L1 linkers); (ii) an optional linker L2 polypeptide sequence; (iii) a β2M polypeptide sequence; (iv) an optional L3 linker polypeptide sequence (e.g., from 10-50 aa in length); (v) a class I MHC-H polypeptide sequence; (vi) an optional L4 linker polypeptide sequence; (vii) a scaffold polypeptide sequence (e.g., an immunoglobulin Fc sequence); (viii) an optional L5 linker polypeptide sequence; and (ix) optionally one or more MOD polypeptide sequence (e.g., two or more MOD polypeptide sequences, such as in tandem, wherein when there are two or more MOD polypeptide sequences they are optionally joined to each other by independently selected L6 linkers); wherein the unconjugated T cell modulatory polypeptide comprises at least one MOD polypeptide sequence (e.g., the

MOD(s) of element (i) and/or (ix)); and wherein at least one of the β2M polypeptide sequence, L3 linker polypeptide sequence, and/or the MHC-H polypeptide sequence comprises a chemical conjugation site for epitope conjugation.

[00343] Suitable MHC-H, β2-microglobulin (β2M) polypeptide, and scaffold polypeptides are described above. The MHC-H polypeptide may be a HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, or HLA-G heavy chain. In some cases, the MHC-H polypeptide comprises an amino acid sequence having at least 85% aa sequence identity to the amino acid sequence depicted in any one of FIGs. 3A-3H. In such an embodiment the MHC Class I heavy chain polypeptide may not include a transmembrane anchoring domain and intracellular domain (see, *e.g.*, the MHC-H polypeptides in FIG. 3D). In some cases, the first MHC polypeptide comprises a β2-microglobulin (β2M) polypeptide; and the second MHC polypeptide comprises a MHC Class I heavy chain polypeptide. In some cases, the β2M polypeptide comprises an amino acid sequence having at least about 85% (e.g., at lease about 90%, 95%, 98%, 99%, or even 100%) aa sequence identity to a β2M amino acid sequence depicted in FIG. 4

B. Recombinant expression vectors

[00344] The present disclosure provides recombinant expression vectors comprising nucleic acid sequence encoding T-Cell-MPs 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.*

[00345] 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 Opthalmol Vis Sci* 35:2543 2549, 1994; Borras et al., *Gene Ther* 6:515 524, 1999; Li and Davidson, *PNAS* 92:7700 7704, 1995; Sakamoto et al., *H 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 Opthalmol 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.

[00346] 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.

[00347] 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).

[00348] 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.

III. Methods of Generating and Selecting T-Cell-MPs

[00349] The present disclosure provides a method of obtaining T-Cell-MPs (both unconjugated T-Cell-MPs and/or T-Cell-MP-KRAS-epitope conjugates) including in duplex and other higher order aggregates, which may include one or more wt. MOD polypeptide sequences and/or one or more variant MOD polypeptide sequences that exhibit lower affinity for a Co-MOD compared to the affinity of the corresponding wt. MOD polypeptide sequence for the Co-MOD, the method comprising:

A) generating a T-Cell-MP (or a higher order complex such as a duplex) by introducing into cells or cell-free systems one or more nucleic acids encoding an unconjugated T-Cell-MP or each of the unconjugated T-Cell-MPs that make up a heteromer (e.g., a heterodimeric duplex of unconjugated T-Cell-MPs);

wherein when the T-Cell-MP comprises one or more nascent chemical conjugation sites, the nascent chemical conjugation site may be activated to produce an unconjugated T-Cell-MP with chemical conjugation site (e.g., reacting sulfatase motifs with an FGE to convert a Cys residue to a fGly residue if the cells translating the T-Cell-MP nucleic acids do not express a formylglycine generating enzyme).

The above-mentioned method of generating T-Cell-MPs may further comprise providing one or more nucleic acids encoding the unconjugated T-Cell-MP, including those specifically described in the present disclosure, which may be present in a recombinant expression vector and/or operably linked to a transcriptional control elements such as those functional in a eukaryotic cell. The method may be stopped at this point and the unconjugated T-Cell-MP (e.g., unconjugated duplex T-Cell-MP) that is unpurified (including cell lysates and unpurified media) may be obtained. Alternatively, the unconjugated T-Cell-MP may be purified using, for example, one or more of salt precipitation (e.g., ammonium sulfate), affinity chromatography, and/or size exclusion chromatography, to produce crude (less than 60% by weight), initially refined (at least 60% by weight), partly refined (at least 80% by weight), substantially refined (at least 95% by weight), partially pure or partially purified (at least 98% by weight), substantially pure or substantially purified (at least 99% by weight), essentially pure or essentially purified (at least 99.5% by weight) or purified (at least 99.8%) or highly purified (at least 99.9% by weight) of the unconjugated T-Cell-MP based on the total weight of protein present in the sample may be

obtained by purification. Where a T-Cell-MP-KRAS-epitope conjugate is desired, the method may be continued by reacting anywhere from a crude preparation to a highly purified preparation T-Cell-MP with an epitope presenting molecule as in step B:

B) providing a KRAS epitope (e.g., an KRAS epitope-presenting peptide) suitable for conjugation with the chemical conjugation site present in the unconjugated T-Cell-MP of step A (e.g., a hydrazinyl or hydrazinyl indole modified peptide for reaction with a formyl glycine of a sulfatase motif or a maleimide containing peptide for reaction with a cysteine residue), and contacting the epitope with the T-Cell-MP (e.g., under suitable reaction conditions) to produce a T-Cell-MP-KRAS-epitope conjugate.

The choice of how purified the unconjugated material entered into the reaction needs to be depends on a number of factors including the conjugation reaction and conditions, the potential for side reactions, and the degree to which the final epitope conjugate will need to be purified.

[00350] The T-Cell-MP-KRAS-epitope conjugate (e.g., as a duplex or a higher order complex) may be purified by, for example, salt precipitation, size separation, and/or affinity chromatography, so that it is at least partly refined (at least 80% by weight of protein present in the sample), substantially refined (at least 95% by weight), partially pure or partially purified (at least 98% by weight), substantially pure or substantially purified (at least 99% by weight), essentially pure or essentially purified (at least 99.5% by weight), purified (at least 99.8%), or highly purified (at least 99.9% by weight) of the T-Cell-MP-KRAS-epitope conjugate based on the total weight of protein present in the sample.

[00351] Where it is desirable for a T-Cell-MP or higher order complexes to contain a payload, the payload may be reacted with the unconjugated T-Cell-MP or the T-Cell-MP-KRAS-epitope conjugate. The selectivity of the epitope and the payload for different 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. The payload may be a cytotoxic agent that is selected from, for example, 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. The payload may be a retinoid. When possible, a single purification scheme that removes reagents and other materials present from the conjugation of the epitope and attachment of the payload is employed to minimize loss of the protein.

[00352] A variety of cells and cell-free systems may be used for the preparation of unconjugated T-Cell-MPs. As discussed in the section titled "Genetically Modified Host cells," the cells may be eukaryotic origin, and more specifically of mammalian, primate or even human origin.

[00353] The present disclosure provides a method of obtaining an unconjugated T-Cell-MP or T-Cell-MP-KRAS-epitope conjugate (or their higher order complexes, such as duplexes) comprising one or more wt. MODs and/or variant MODs that exhibit reduced affinity for a Co-MOD compared to the affinity of the corresponding parental wt. MOD for the Co-MOD. Where a variant MOD having reduced affinity is desired, the method can comprise preparing a library of variant MOD polypeptides (e.g., that have at least

one insertion, deletion or substitution) and selecting from the library of MOD polypeptides a plurality of members that exhibit reduced affinity for their Co-MOD (such as by BLI as described above). Once a variant MOD is selected a nucleic acid encoding the unconjugated T-Cell-MP including the variant MOD is prepared and expressed. After the unconjugated T-Cell-MP has been expressed it can be purified, and if desired conjugated to an epitope to produce the selected T-Cell-MP-KRAS-epitope conjugate. The process may be repeated to prepare a library of unconjugated T-Cell-MPs or their epitope conjugates.

[00354] The present disclosure provides a method of obtaining a T-Cell-MP-KRAS-epitope conjugate or its higher order complexes, such as a duplex) that exhibits selective binding to a T cell, the method comprising:

A) generating a library of T-Cell-MP-KRAS-epitope conjugates (or their higher order complexes) comprising a plurality of members, wherein each member comprises a different variant MOD on the T-Cell-MP-KRAS-epitope conjugate, wherein the variant MOD differs in amino acid sequence (e.g., by from 1 aa to 10 aas) from its parental wt. MOD , and wherein the T-Cell-MP-KRAS-epitope conjugate library members further comprise an epitope tag or a fluorescent label), and

B) contacting a T-Cell-MP-KRAS-epitope conjugate library member with a target T cell expressing on its surface: i) a Co-MOD that binds the parental wt. MOD; and ii) a TCR that binds to the epitope;

C) selecting a T-Cell-MP-KRAS-epitope conjugate library member that selectively binds the target T cell relative to its binding under the same conditions to a control T cell that comprises: i) the Co-MOD that binds the parental wt. MOD; and ii) a TCR that binds to an epitope other than the epitope present in the T-Cell-MP library member (e.g., choosing the T-Cell-MP-KRAS-epitope conjugate that has higher avidity or affinity for the target T cell than the control T cell such as by BLI as described above).

A T-Cell-MP-KRAS-epitope conjugate library member that is identified as selectively binding to a target T cell may be isolated from the library.

[00355] When the T-Cell-MP-KRAS-epitope conjugate comprises an epitope tag or label, identifying a T-Cell-MP-KRAS-epitope conjugate selective for a target T cell may comprise detecting the epitope tag or label associated with target and control T cells by using, for example, flow cytometry. While labeled T-Cell-MPs (e.g., fluorescently labeled) do not require modification to be detected, epitope tagged molecules may require contacting with an agent that renders the epitope tag visible (e.g., a fluorescent agent that binds the epitope tag). The affinity/avidity of the T-Cell-MP-KRAS-epitope conjugate can be determined by measuring the agent or label associated with target and control T cells (e.g., by measuring the mean fluorescence intensity using flow cytometry) over a range of concentrations. The T-Cell-MP-KRAS-epitope conjugate that binds with the highest affinity or avidity to the target T cell relative to the control T cell is understood to selectively bind to the target T cell.

[00356] MOD and Co-MOD pairs, including wt. and variant MOD and Co-MOD pairs, utilized in the methods of obtaining T-Cell-MPs and methods of obtaining a T-Cell-MP-KRAS-epitope conjugate that exhibits selective binding to a T cell may be selected from, e.g.,: IL-2 and IL-2 receptor; 4-1BBL and 4-1BB; 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; and CD70 and CD27. Alternatively, they may be selected from IL-2 and IL-2 receptor; 4-1BBL and 4-1BB; CD80 and CD28; and CD86 and CD28. In some cases, the variant MODs present in a T-Cell-MP, which are independently selected, comprise from 1 to 20 aa independently selected sequence variations (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 aa substitutions, deletions, or insertions) compared to the corresponding parental wt. MOD.

[00357] A T-Cell-MP (unconjugated T cell-MP or T-Cell-MP-KRAS-epitope conjugate) may comprise two or more wt. and/or variant MODs. The two or more MODs may comprise the same or different amino acid sequence. The two or more MODs may be on the same T-Cell-MP (e.g., in tandem) of a T cell-MP- duplex. The first of two or more MODs may be on the first T-Cell-MP of a T-Cell-MP duplex and the second of two variant MODs may be on the second T-Cell-MP of the duplex.

IV. Genetically Modified Host cells

[00358] The present disclosure provides a genetically modified host cell, where the host cell is genetically modified with a nucleic acid of the present disclosure (e.g., a nucleic acid encoding an unconjugated T-Cell-MP that may be operably linked to a promoter). Where such cell express T-Cell-MPs they may be utilized in methods of generating and selecting T-Cell-MPs as discussed in the preceding section.

[00359] 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. CCLI.3), human embryonic kidney (HEK) cells (ATCC No. CRL1573), HLHepG2 cells, and the like.

[00360] In some cases, the host cell is a mammalian cell that has been genetically modified such that it does not synthesize endogenous β2M 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-MPs 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 β2M and MHC-H proteins.

V. Compositions and Formulations

[00361] The present disclosure provides compositions and formulations, including pharmaceutical compositions and formulations. Compositions may comprise: a) a T-Cell-MP and b) an excipient. Where the excipient(s) present in a composition or formulation are pharmaceutically acceptable excipients, the composition may be a pharmaceutically composition or formulation. Pharmaceutical compositions or

formulations may also be sterile and/or pyrogen free. Some pharmaceutically acceptable excipients are provided below. The present disclosure also provides compositions and formulations, including pharmaceutical compositions, comprising a nucleic acid or a recombinant expression vector, where the nucleic acid or expression nucleic acid encodes all or part of a T-Cell-MP or its higher order complexes (e.g., one T-Cell-MP of a heterodimeric T-Cell-MP duplex).

A. Compositions comprising T-Cell-MP-KRAS-epitope conjugates

[00362] Compositions of the present disclosure may comprise, in addition to a T-Cell-MP-KRAS-epitope conjugate, one or more of: a salt, e.g., NaCl, $MgCl_2$, $CaCl_2$, KCl, $MgSO_4$, sodium acetate, sodium lactate, *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 (surfactants), e.g., a non-ionic detergent such as Tween-20, *etc.*; a protease inhibitor; glycerol; and the like; any or all of which may be in the form of solvates (e.g., mixed ionic salts with water and/or organic solvents), hydrates, or the like.

[00363] A pharmaceutically acceptable compositions comprising a T-Cell-MP-KRAS-epitope conjugate may comprise, in addition to the T-Cell-MP, a pharmaceutically acceptable excipient, a variety of which are known in the art and need not be discussed in detail herein. Pharmaceutically acceptable compositions (e.g., injectable formulations) may be sterile and/or free of pyrogens and other materials detrimental to administration to patients or subjects (e.g., lipopolysaccharides). 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.

[00364] A subject pharmaceutical composition may be suitable for administration to a subject, e.g., will generally 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. A pharmaceutical composition may be suitable for use ex vivo or in vitro (ex vivo treatment of cells) where, for example, it may be contacted with cells and then subsequently removed prior to administration of the cells to a subject.

[00365] The T-Cell-MP compositions, including pharmaceutical compositions, may also comprise components, such as mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, glycerol, magnesium, carbonate, and the like, any or all of which may be pharmaceutical grade.

[00366] Compositions may 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.

[00367] Where a T-Cell-MP-KRAS-epitope conjugate 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. T-Cell-MP formulations may also be provided so as to enhance serum half-life of the subject protein following administration. For example, the T-Cell-MP 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.

[00368] Other examples of formulations suitable for parenteral administration include those comprising sterile injection solutions, salts, anti-oxidants, bacteriostats, and/or solutes that render the formulation isotonic with the blood of the intended recipient. Such parenteral formulations may also include one or more independently selected suspending agents, solubilizers, thickening agents, stabilizers, and preservatives.

[00369] Formulations or pharmaceutical composition comprising a T-Cell-MP can be present in a container, e.g., a sterile container, such as a syringe. The formulations can also be presented in unit-dose or multi-dose sealed containers, such as ampules and vials, any of which may be sterile. The formulation or pharmaceutical compositions may be stored in a sterile 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 solutions, powders, granules, and/or tablets that comprise the T-Cell-MP.

[00370] The concentration of a T-Cell-MP 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.

[00371] In some cases, a T-Cell-MP is present in a liquid composition. Thus, the present disclosure provides compositions (e.g., liquid compositions, including pharmaceutical compositions) comprising a T-Cell-MP of the present disclosure. The present disclosure also provides a composition comprising: a) a T-Cell-MP of the present disclosure; and b) saline (e.g., 0.9% or about 0.9% NaCl). In some cases, the composition is sterile. The composition may 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. Thus, the present disclosure provides a composition comprising: a) a T-Cell-MP-KRAS-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

[00372] The present disclosure provides compositions (e.g., pharmaceutical compositions) comprising a nucleic acid or a recombinant expression vector of the present disclosure (see, e.g., supra) that comprise

one or more nucleic acid sequences encoding any one or more T-Cell-MP polypeptide (or each of the polypeptides of a duplex T-Cell-MP multimer such as a heterodimer). Pharmaceutically acceptable excipients are known in the art and 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.

[00373] 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-MP polypeptide (or all polypeptides of a T-Cell-MP) of the present disclosure; and b) one or more of: a salt, 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, , and a preservative. Suitable buffers include, but are not limited to, (such as N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES), bis(2-hydroxyethyl)amino-tris(hydroxymethyl)methane (BIS-Tris), N-(2-hydroxyethyl)piperazine-N'3-propanesulfonic acid (EPPS or HEPPS), glycylglycine, N-2-hydroxyehtylpiperazine-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, $MgCl_2$, KCl, $MgSO_4$, etc.

[00374] 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, formulation may comprise a subject nucleic acid or subject recombinant expression vector of the present disclosure.

[00375] 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.

[00376] 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 a solution or suspensions in aqueous, non-aqueous or mixed media.

[00377] A formulation comprising a subject nucleic acid or recombinant expression vector can be a liposomal formulation. As used herein, the term "liposome" includes unilamellar or multilamellar

vesicles having an aqueous interior that may contain the composition (e.g., a subject nucleic acid) to be delivered. Cationic liposomes comprise positively charged lipids 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 lipids, which may form liposomes, can be used to deliver a subject nucleic acid or recombinant expression vector in vitro, ex vivo, or in vivo.

[00378] 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 include those comprising one or more glycolipids and those comprising lipids derivatized with one or more hydrophilic polymers (e.g., a polyethylene glycol (PEG) moiety). Liposomes and their uses are further described, for example, in U.S. Pat. No. 6,287,860.

[00379] Penetration enhancers may be included in compositions comprising a subject nucleic acid or expression vector to effect their efficient delivery of the nucleic acids. In addition to aiding the diffusion of non-lipophilic drugs such as nucleic acids across cell membranes, penetration enhancers also enhance the permeability of lipophilic drugs, such as those that may co-administered with a subject nucleic acid. 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, for example, in U.S. Pat. No. 6,287,860.

[00380] 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 minitablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable. Suitable oral formulations include those in which a subject 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, dimethyl sulfoxide, triethanolamine, N,N-dimethylacetamide, N,N-dimethylformamide, 2-pyrrolidone and derivatives thereof, tetrahydrofurfuryl alcohol, and AZONE™.

VI. Methods of Modulating Immune Responses and Treating Diseases and Disorders

[00381] T-Cell-MPs and higher order T-Cell-MP complexes (e.g., duplex T-Cell-MP) of the present disclosure are useful for modulating an activity of a T cell, and directly or indirectly modulating the activity of other cells of the immune system. The present disclosure provides methods of modulating an activity of a T cell selective for a epitope (e.g., an “epitope-specific T cell” or an “epitope selective T cell”), the methods generally involving contacting a target T cell with a T-Cell-MP-KRAS-epitope

conjugate or a higher order complex of T-Cell-MP-KRAS-epitope conjugates (e.g., duplex T-Cell-MP-KRAS-epitope conjugates) of the present disclosure. A T-Cell-MP-KRAS-epitope conjugate or its higher order complexes may comprise one or more independently selected MODs that activate an epitope-specific T cell that recognizes a cancer, or benign (non-malignant) neoplasm. In some cases, the activated T cells are cytotoxic T cells (e.g., $CD8^+$ cells). Accordingly, the disclosure includes and provides for a method of treating a cancer,or benign neoplasm (e.g., a non-malignant but inoperable tumor) the method comprising administering to an individual in need thereof an effective amount of a T-Cell-MP-KRAS-epitope conjugate or a higher order complex thereof that comprises one or more independently selected MODs that activate an epitope-specific T cell that recognizes an epitope specific to the cancer or neoplasm. An effective amount of such a T-Cell-MP-KRAS-epitope conjugate or its higher order complex may be an amount that activates a $CD8^+$ T cell specific to the conjugated epitope (e.g., increasing proliferation of the $CD8^+$ T cells, increasing release of their cytotoxic agents such as granzyme, and/or inducing or enhancing release of their cytokines such as interferon γ).

[00382] A T-Cell-MP-KRAS-epitope conjugate or its higher order complexes may also comprise one or more independently selected MODs that inhibit an epitope-specific T cell. Such T-Cell-MP-KRAS-epitope conjugates are useful for the treatment of disease and disorders where the subject fails to make a sufficient immune response due to, for example, CD8+ T reg cell suppression as may occur in various tumors.

[00383] In addition to the foregoing, this disclosure contemplates and provides for the use of T-Cell-MPs for the delivery of MOD polypeptides. The delivery of MODs may be accomplished in epitope selective manner using a T-Cell-MP-KRAS-epitope conjugate, and may also be accomplished in a non-specific manner using an unconjugated T-Cell-MP. The methods of delivering MODs may be utilized in the treatment of diseases or disorders affecting mammalian subjects (e.g., human patients in need of treatment).

A. Methods of modulating T cell activity

[00384] The present disclosure provides a method of selectively modulating the activity of a T cell, the method comprising contacting or administering to a subject a T-Cell-MP-KRAS-epitope conjugate or a higher order complex thereof, in some instances with a payload. The contacting or administration may occur in vivo where the molecule is administered to an animal (e.g., a mammal such as a human, rat, mouse, dog, cat, pig, horse, or primate), in vitro, or ex vivo; where it may constitute all or part of a method of treating a disease or disorder as discussed further below. The T cells subject to modulation may be, for example, CD8+ T cells, a NK-T cells, and/or T reg cells. In some cases, the T cell is a CD8+ effector T cell. T-Cell-MP-KRAS-epitope conjugates of this disclosure also can be used to cause proliferation of CAR-T cells *in vivo*, thereby reducing the number of CAR-T cells that are required to be administered to a patient having a cancer associated with a KRAS mutation.

[00385] The present disclosure provides a method of selectively modulating the activity of an epitope-specific T cell. The method comprises contacting the T cell with a T-Cell-MP-KRAS-epitope conjugate (e.g., in duplex form) bearing a KRAS epitope recognized by the epitope-specific T-Cell. The contacting

results in selectively modulating the activity of the epitope-specific T cell with the selectivity driven by the epitope and the resultant activation driven, at least in part, by the MOD polypeptide sequence of the T-Cell-MP-KRAS-epitope conjugate. Contacting T cells with T-Cell-MP-KRAS-epitope conjugates, or higher order T-Cell-MP complexes (e.g., duplex T-Cell-MP-KRAS-epitope conjugates) can result in activation or suppression of T cells expressing a TCR specific for the conjugated epitope (an epitope-specific T cell) including induction or suppression of granule dependent and independent responses. Granule-independent responses include, but are not limited to, changes in the number or percentage of epitope-specific CD 8+ T cell (e.g., in a population of cells such as in blood, lymphatics, and/or in a target tissue), changes in the expression of Fas ligand (Fas-L, which can result in activation of caspases and target cell death through apoptosis), and cytokine/chemokine production (e.g., production and release of interferon gamma (IFN-γ). Granule-dependent effector actions include the release of granzymes, perforin, and/or granulysin. Activation of epitope-specific $CD8^+$ cytotoxic T cells (e.g., $CD8^+$ cytotoxic effector T cells) can result in the targeted killing of, for example, cancer cells by epitope-specific T cells that recognize the epitope presented by the T-Cell-MP-KRAS-epitope conjugate (or higher order complex thereof (e.g., a duplex) through granule-dependent and/or independent responses.

[00386] Contacting a T-Cell-MP-KRAS-epitope conjugate or higher order complex thereof (e.g., a duplex) bearing an activating MOD, where the T-Cell-MP is conjugated to a KRAS epitope recognize by the TCR of a target T cell (an epitope specific T cell), may result in one or more of: i) proliferation of the epitope-specific T cell (e.g., CD8+ cytotoxic T cells); ii) epitope-specific induction cytotoxic activity; iii) release of one or more cytotoxic molecules (e.g., a perforin; a granzyme; a granulysin) by the epitope specific cytotoxic (e.g., CD8+) T cell. In contrast, contacting a T-Cell-MP-KRAS-epitope conjugate or higher order complex thereof (e.g., a duplex) bearing an inhibitory MOD, where the T-Cell-MP is conjugated to an epitope recognize by TCR of a target T cell (an epitope specific T cell), may result in one or more of: i) suppression of proliferation and/or reduction the number of the epitope-specific T cells (e.g., CD8+ cytotoxic T cells); ii) epitope-specific suppression of a cytotoxic activity; iii) suppression the production and/or release of one or more cytotoxic molecules (e.g., a perforin; a granzyme; a granulysin) by the epitope specific cytotoxic (e.g., CD8+) T cell.

[00387] In some cases, a T-Cell-MP-KRAS-epitope conjugate (or higher order complex thereof (e.g., a duplex) comprises a cancer epitope and it induces a $CD8^+$ T cell response (e.g., a cytotoxic $CD8^+$ T cell response to a cancer cell).

[00388] The present disclosure provides a method of increasing the proliferation (e.g., proliferation rate) and/or the total number of CD 8+ effector T cells in an animal or tissue that are specific to the KRAS epitope presented by a T-Cell-MP-KRAS-epitope conjugate or higher order complex thereof (e.g., a duplex) bearing an activating MOD such as IL-2. A method of increasing T cell proliferation or numbers comprises contacting (e.g., in vitro, in vivo, or ex vivo) T cells with a T-Cell-MP-KRAS-epitope conjugate or higher order a complex thereof. Contacting may occur, for example, by administering to a subject in one or more doses a T-Cell-MP-KRAS-epitope conjugate). The contacting or administering may increase the number of CD8+ effector T cells having a TCR capable of binding the epitope present in

the T-Cell-MP -epitope conjugate relative to the number (e.g., total number or percentage) of T cells present in a tissue (e.g., in a population of cells such as in blood, lymphatics, and/or in a target tissue such as a tumor). For example, the absolute or relative number of CD 8+ effector T cells specific to the epitope presented by the T-Cell-MP-KRAS-epitope conjugate or its higher order complex (e.g., duplex) can be increased by at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, least 75%, at least 100%, at least 2-fold, at least 2.5-fold, at least 5-fold, at least 10-fold, or more than 10-fold following one or more contacts with doses or administrations of the T-Cell-MP-KRAS-epitope conjugate or a higher order complex thereof. The increase may be calculated relative the CD8+ T cell numbers present prior to the contacting or administrations, or relative to the population of T cells present in a sample (e.g., a sample of blood or tissue) that has not been contacted with the T-Cell-MP-KRAS-epitope conjugate or is higher order complex.

[00389] The present disclosure provides a method of increasing granule-dependent and/or granule-independent responses of epitope-specific CD 8+ T cell comprising contacting or administering (e.g., in vitro, in vivo, or ex vivo) T cells with a T-Cell-MP conjugated to a KRAS epitope (e.g., a KRAS peptide bearing a mutation associated with a benign neoplasm or cancer) or a higher order complex thereof, (e.g., with a CD80, and/or CD86 MOD). The contacting or administering may result in, for example, an increased expression of Fas ligand expression, cytokines/chemokines (e.g., IL-2, IL-4, and/or IL-5), release of interferons (e.g., IFN-γ), release of granzymes, release of perforin, and/or release of granulysin. For example, contacting a CD 8+ effector cell with a T-Cell-MP-KRAS-epitope conjugate or complex thereof (e.g., a duplex) presenting epitope-specific to the effector cell can increase one or more of Fas ligand expression, interferon gamma (IFN-γ) release, granzyme release, perforin release, and/or granulysin release by at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, least 75%, at least 100%, at least 2-fold, at least 2.5-fold, at least 5-fold, at least 10-fold, or more than 10-fold. The increase may be calculated relative the level of expression or release prior to the contacting or administrations, or relative to the population of T cells present in a sample (e.g., a sample of blood or tissue) that has not been contacted with the T-Cell-MP-KRAS-epitope conjugate or a complex thereof.

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

[00390] The present disclosure provides a method of delivering a MOD (a costimulatory polypeptide) such as IL-2, 4-1BBL, CD-80, or CD-86, or a reduced-affinity variant of any thereof (e.g., an IL-2 variant disclosed herein) to a selected T cell or a selected T cell population having a TCR specific for a given KRAS epitope (e.g., KRAS epitope peptides including one, two or more mutations associated with the formation of neoplasms). The method comprises contacting (such as by administration to a subject) a population of T cells with a T-Cell-MP-KRAS-epitope conjugate or a higher order complex thereof (e.g., a duplex). The population of T cells can be a mixed population that comprises: i) the target T cell with a TCR specific to a target KRAS epitope; and ii) non-target T cells that are not specific for the target epitope (e.g., T cells that are specific for epitope(s) other than the KRAS epitope to which the epitope-specific T cell binds). The epitope-specific T cell is specific for the KRAS epitope present in and presented by the T-Cell-MP-KRAS-epitope conjugate, or a higher order complex thereof, and binds to the

peptide MHC complex provided by the T-Cell-MP-KRAS-epitope conjugate, thereby selectively delivering the MODs present in the T-Cell-MP-KRAS-epitope conjugate to the target T cell(s). The contacting or administration may be conducted in vitro, ex vivo, or in vivo, and may constitute all or part of a method of treatment. Thus, for example, the present disclosure provides a method of delivering a costimulatory polypeptide such as IL-2, or a reduced-affinity variant of a naturally occurring costimulatory polypeptide such as a IL-2 variant disclosed herein, or a combination of both, selectively to a target T cell, which form part of a treatment of a disease or disorder.

[00391] By way of example, a T-Cell-MP-KRAS-epitope conjugate or a higher order complex thereof (e.g., a duplex) is contacted with a population of T cells comprising: i) a target T cell(s) that is/are specific for the KRAS epitope present in the epitope conjugate; and ii) a non-target T cell(s), e.g., a T cell(s) that is specific for a second epitope(s) that is not the KRAS epitope present in the epitope conjugate. Contacting the population results in selective delivery of the MOD(s) or reduced-affinity variant MOD(s) to the target T cell. Less than 50%, less than 40%, less than 30%, less than 25%, less than 20%, less than 15%, less than 10%, less than 5%, or less than 4%, 3%, 2% or 1%, of the T-Cell-MP-KRAS-epitope conjugate or higher order complex there of (e.g., duplex T-Cell-MP) may bind to non-target T cells and, as a result, the MOD(s) is/are selectively delivered to target T cell (and accordingly, substantially not delivered to the non-target T cells).

[00392] In some cases, the population of T cells to which the MOD(s) and/or variant MOD(s) is/are delivered is present in vitro or ex vivo, and a biological response (e.g., T cell activation, expansion, and/or phenotypic differentiation) of the target T cell population to the T-Cell-MP-KRAS-epitope conjugate or a higher order complex thereof (e.g., a duplex) is elicited in the context of an in vitro or ex vivo setting. For example, a mixed population of T cells can be obtained from an individual and can be contacted with the T-Cell-MP-KRAS-epitope conjugate or a higher order complex thereof (e.g., a duplex) *in vitro* or *ex vivo*. Such contacting can comprise single or multiple exposures of the population of T cells to a defined dose(s) and/or exposure schedule(s). In some cases, said contacting results in selectively binding/activating and/or expanding target T cells within the population of T cells, and results in generation of a population of activated and/or expanded target T cells. As an example, a mixed population of T cells can be peripheral blood mononuclear cells (PBMC). For example, PBMCs from a patient can be obtained by standard blood drawing and PBMC enrichment techniques before being exposed to 0.1-1000 nM of a T-Cell-MP-KRAS-epitope conjugate or a higher order complex thereof (e.g., a duplex) under standard lymphocyte culture conditions. At time points before, during, and after exposure of the mixed T cell population at a defined dose and schedule, the abundance of target T cells in the in vitro culture can be monitored by specific peptide-MHC multimers, phenotypic markers, and/or functional activity (e.g. cytokine ELISpot assays). In some cases, upon achieving an optimal abundance and/or phenotype of antigen specific cells in vitro, all or a portion of the population of activated and/or expanded target T cells is administered to an individual (e.g., the individual from whom the mixed population of T cells was obtained as a treatment for a disease of disorder).

[00393] For example, a mixed population of T cells is obtained from an individual and is contacted with a T-Cell-MP-KRAS-epitope conjugate or a higher order complex thereof (e.g., a duplex) in vitro. Such contacting, which can comprise single or multiple exposures of the T cells to a defined dose(s) and/or exposure schedule(s) in the context of in vitro cell culture, can be used to determine whether the mixed population of T cells includes T cells that are specific for the KRAS epitope presented by the T-Cell-MP-KRAS-epitope conjugate or higher order complex. The presence of T cells that are specific for the KRAS epitope of the T-Cell-MP or higher order complex can be determined by assaying a sample comprising a mixed population of T cells, which population of T cells comprises T cells that are not specific for the KRAS epitope (non-target T cells) and may comprise T cells that are specific for the KRAS epitope (target T cells). Known assays can be used to detect activation and/or proliferation of the target T cells, thereby providing an ex vivo assay that can determine whether a particular T-Cell-MP-KRAS-epitope conjugate or a higher order complex thereof possesses an epitope that binds to T cells present in the individual, and thus whether the epitope conjugate has potential use as a therapeutic composition for that individual. Suitable known assays for detection of activation and/or proliferation of target T cells include, e.g., flow cytometric characterization of T cell phenotype and/or antigen specificity and/or proliferation. Such an assay to detect the presence of epitope-specific T cells, e.g., a companion diagnostic, may further include additional assays (e.g. effector cytokine ELISpot assays) and/or appropriate controls (e.g. antigen-specific and antigen-nonspecific multimeric peptide-HLA staining reagents) to determine whether the T-Cell-MP-KRAS-epitope conjugate or a higher order complex thereof (e.g., a duplex) is selectively binding, modulating (activating or inhibiting), and/or expanding the target T cells. Thus, for example, the present disclosure provides a method of detecting, in a mixed population of T cells obtained from an individual, the presence of a target T cell that binds an KRAS epitope of interest, the method comprising: a) contacting in vitro the mixed population of T cells with a T-Cell-MP-KRAS-epitope conjugate bearing the KRAS epitope, or a higher order complex thereof (e.g., a duplex); and b) detecting modulation (activation or inhibition) and/or proliferation of the T cells in response to said contacting, wherein modulation of and/or proliferation of T cells indicates the presence of the target T cell. Alternatively, or in addition, if activation and/or expansion (proliferation) of the desired T cell population is obtained using a T-Cell-MP-KRAS-epitope conjugate or a higher order complex thereof (e.g., a duplex), then all or a portion of the population of T cells comprising the activated/expanded T cells can be administered back to the individual as a therapy.

[00394] In some instances, the population of T cells is in vivo in an individual. In such instances, a method of the present disclosure for selectively delivering one or more costimulatory polypeptides (e.g., an IL-2 or reduced-affinity IL-2) to an epitope-specific T cell comprises administering the T-Cell-MP-KRAS-epitope conjugate or a higher order complex thereof (e.g., duplex) to the individual. In some instances, the epitope-specific T cell to which one or more MOD polypeptide sequences (e.g., a wild-type or reduced-affinity variant of IL-2) is/are being selectively delivered is a target T cell.

C. Methods of Treatment

[00395] The present disclosure provides methods of treatment for a variety of diseases and disorders. The diseases and/or disorders that can be treated include benign neoplasms (e.g., non-malignant neoplasms) and malignant neoplasm (e.g., cancers). The methods of treatment may comprise administering to an individual an effective amount of a T-Cell-MP-KRAS-epitope conjugate, or a higher order complex thereof (e.g., a duplex). Where it is desirable to selectively modulate the activity of an KRAS epitope-specific T cell in an individual and thereby effect a method of treating a disease or condition, a T-Cell-MP-KRAS-epitope conjugate or a higher order complex thereof (e.g., a duplex) may be administered to the individual. T-Cell-MP-KRAS-epitope conjugates utilized in methods of treatment may comprise one or more (e.g., two or more) independently selected MOD and/or variant MOD polypeptide sequences.

[00396] The present disclosure provides a method of selectively modulating the activity of an KRAS epitope-specific T cell in an individual, thereby effecting a treatment, the method comprising administering to the individual an effective amount of a T-Cell-MP-KRAS-epitope conjugate or a higher order complex thereof (e.g., a duplex), where the administered molecule selectively modulates the activity of the KRAS epitope-specific T cell in the individual, thereby treating the 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-MP-KRAS-epitope conjugate or a higher order complex thereof sufficient to effect treatment. Administering the T-Cell-MP-KRAS-epitope conjugate induces a KRAS epitope-specific T cell response and may also induce an epitope-non-specific T cell response, where the ratio of the KRAS 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 KRAS 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 KRAS 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 KRAS 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 KRAS 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 KRAS 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 , e.g., a cell expressing a cancer antigen that displays the same KRAS epitope displayed by the peptide epitope present in the T-Cell-MP-KRAS-epitope conjugate. As discussed below, in some cases, the administering is intravenous, subcutaneous, intramuscular, systemic, intralymphatic, distal to a treatment site, local, or at or near a treatment site the doses needed to administer an effective amount of the administered molecule are discussed herein below.

[00397] A T-Cell-MP-KRAS-epitope conjugate or a higher order complex thereof (e.g., a homoduplex or heteroduplex) may be administered alone or with one or more additional therapeutic agents or drugs. The therapeutic agents (e.g., antibodies against check point inhibitors such as: anti-PD-1, for example Nivolumab, Cemiplimab, and Pembrolizumab; anti-PDL-1 such as Atezolizumab, Avelumab, or Durvalumab; or anti-CTLA-4, for example Ipilimumab, which, along with others, are further described

below) may be administered before, during, or subsequent to T-Cell-MP administration. When an additional therapeutic agent or drug is administered with a composition or formulation comprising a T-Cell-MP-KRAS-epitope conjugate, or a higher order complex thereof (e.g., a duplex), the therapeutic agent or drug may be administered concurrently with any of those molecules. Alternatively, the therapeutic agents may be co-administered with the T-Cell-MP-KRAS-epitope conjugate as part of a single formulation or composition (e.g., a pharmaceutical composition).

[00398] Because the KRAS epitopes are associated with neoplasms including cancerous cells or tissues the T-Cell-MP-KRAS-epitope conjugates described herein may be utilized in methods of treating various neoplasms or cancers.

1 Neoplasms and Cancers

[00399] Cancers (e.g., malignant neoplasms) and neoplasms (e.g., benign neoplasms or benign tumors) that can be treated with a methods of the present disclosure include those expressing KRAS epitopes associated with with their abnormal growth. Cancers and benign neoplasms that can be treated with a method of the present disclosure include solid tumors. Cancers that may be treated include carcinomas, sarcomas, melanoma, leukemias, and lymphomas. Cancers that can be treated with the methods of the present disclosure include non-solid cancers (e.g., leukemia, lymphoma and myeloma). In some cases, a T-Cell-MP-KRAS-epitope conjugate or a higher order complex thereof (e.g., a duplex) comprises (i) a KRAS epitope associated with a cancer, and (ii) one or more independently selected activating MOD polypeptide sequences that activates an epitope-specific T cell (e.g., activating effector functions and/or proliferation). Where the T cells are cytotoxic T cells (e.g., CD8+ cells), such a T-Cell-MP-KRAS-epitope conjugate or its higher order complexes may increase the number and/or activity of a CD8+ effector T cell specific for a cancer cell cell expressing the KRAS epitope. Activation of CD8+ T cells can result in increased proliferation of the CD8+ T cells and/or inducing or enhancing release of chemokines and/or cytokines by CD8+ T cells. Accordingly, the disclosure provides a method of treating a cancer that includes administering to an individual in need thereof an effective amount of a T-Cell-MP-KRAS epitope KRAS conjugate or a higher order complex thereof (e.g., a duplex) comprising: (i) a KRAS epitope associated with the cancerous growth; and (ii) one or more independently selected activating MOD polypeptide sequences that activates a T cell specific for the conjugated KRAS epitope. In some instances, an effective amount of a T-Cell-MP-KRAS-epitope conjugate or a higher order complex thereof (e.g., a duplex) is an amount that increases the number or activity of CD8+ effector cells.

[00400] The doses and routes of administration required to provide an effective amount of a T-Cell-MP to effect a treatment are discussed below.

[00401] Carcinomas that express a KRAS epitope associated with malignant growth can be treated by a method disclosed herein. Such carcinomas may include, but are not limited to, esophageal carcinoma, hepatocellular carcinoma, basal cell carcinoma (a form of skin cancer), squamous cell carcinoma (various tissues), bladder carcinoma, including transitional cell carcinoma (a malignant neoplasm of the bladder), bronchogenic carcinoma, colon carcinoma, colorectal carcinoma, gastric carcinoma, lung carcinoma, including small cell carcinoma and non-small cell carcinoma of the lung, adrenocortical carcinoma,

thyroid carcinoma, pancreatic carcinoma, breast carcinoma, ovarian carcinoma, prostate carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinoma, cystadenocarcinoma, medullary carcinoma, renal cell carcinoma, ductal carcinoma in situ or bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical carcinoma, uterine carcinoma, testicular carcinoma, osteogenic carcinoma, epithelial carcinoma, and nasopharyngeal carcinoma.

[00402] Sarcomas that express a KRAS epitope associated with malignant growth can be treated by a method disclosed herein. Such sarcomas may include, but are not limited to, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, chordoma, osteogenic sarcoma, osteosarcoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's sarcoma, leiomyosarcoma, rhabdomyosarcoma, and other soft tissue sarcomas that express a KRAS epitope associated with malignant growth.

[00403] Other solid tumors that express a KRAS epitope associated with malignant growth can be treated by a method disclosed herein. Such solid tumors may include, but are not limited to, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma, and retinoblastoma.

[00404] Leukemias that express a KRAS epitope associated with malignant growth can be amenable to therapy by a method disclosed herein. Such leukemias may include, but are not limited to, a) chronic myeloproliferative syndromes (neoplastic disorders of multipotential hematopoietic stem cells); b) acute myelogenous leukemias (neoplastic transformation of a multipotential hematopoietic stem cell or a hematopoietic cell of restricted lineage potential; c) chronic lymphocytic leukemias (CLL; clonal proliferation of immunologically immature and functionally incompetent small lymphocytes), including B-cell CLL, T cell CLL prolymphocytic leukemia, and hairy cell leukemia; and d) acute lymphoblastic leukemias (characterized by accumulation of lymphoblasts). Lymphomas that can be treated using a subject method include, but are not limited to, B-cell lymphomas (e.g., Burkitt's lymphoma); Hodgkin's lymphoma; non-Hodgkin's lymphoma, and the like.

[00405] In an embodiment, the cancers that can be treated with with the methods of the present disclosure include colorectal cancer, pancreatic cancer, lung cancer, bile duct carcinoma, gall bladder carcinoma, adenocarcinoma, rectal adenocarcinoma, endometrial carcinoma, hematopoietic neoplasms. In an embodiment, the cancers that can be treated with the methods of the present disclosure include non-small cell lung cancer, lung adenocarcinoma, mucinous adenoma, ductal carcinoma of the pancreas, colorectal cancer, or leukemia.

[00406] Other cancers that express a KRAS epitope associated with malignant growth that can be treated according to the methods disclosed herein. Such cancers can include atypical meningioma, islet cell carcinoma, medullary carcinoma of the thyroid, mesenchymoma, hepatocellular carcinoma, hepatoblastoma, clear cell carcinoma of the kidney, and neurofibroma mediastinum.

[00407] As noted above, in some cases, in carrying out a subject treatment method, a T-Cell-MP-KRAS-epitope conjugate or a higher order complex thereof (e.g., a duplex) of the present disclosure is administered to an individual in need thereof, as the polypeptide per se.

[00408] In addition to the administration of a T-Cell-MP-KRAS-epitope conjugate, methods of treating a cancer or benign neoplasm may further comprising administering one or more therapeutic agents that, for example, enhance CD 8+ T cell functions (e.g., effector function) and/or otherwise treat the cancer or benign neoplasm or alleviate their symptoms. Accordingly, an anti-TGF-β antibody such as Metelimumab (CAT192) directed against TGF-β1 and Fresolimub directed against TGF-β1 and TGF-β2, or a TGF-β trap may be administered in conjunction with a T cell-MP-KRAS-epitope conjugate for treatment of a cancer or benign neoplasm. Treatment with an anti- TGF-β antibody may be subject to the proviso that the T-Cell-MP does not comprise an aa sequence to which the antibodies or TGF-β trap bind).

[00409] Other therapeutic agents that enhances CD 8+ function that may be administered in conjunction with a T cell-MP or a higher order complex thereof (e.g., a duplex)for the treatment of a cancer or neoplasm neoplasm include, but are not limited to checkpoint inhibitors (discussed below), antibodies directed against: B lymphocyte antigens (e.g., ibritumomab, tiuxetan, obinutuzumab, ofatumumab, rituximab to CD20, brentuximab vedotin directed against CD30, and alemtuzumab to CD52); EGFR (e.g., cetuximab, panitumumab, and necitumumab); VEGF (e.g., bevacizumab); VEGFR2 (e.g., ramucirumab); HER2 (e.g., pertuzumab, trastuzumab, and ado-trastuzumab); PD-1 (e.g., nivolumab and pembrolizumab targeting a check point inhibition); RANKL (e.g., denosumab); CTLA-4 (e.g., ipilimumab targeting check point inhibition); IL-6 (e.g., siltuximab); disialoganglioside (GD2), (e.g., dinutuximab) disialoganglioside (GD2); CD38 (e.g., daratumumab); SLAMF7 (Elotuzumab); both EpCAM and CD3 (e.g., catumaxomab); or both CD19 and CD3 (blinatumomab) (optionally subject to the proviso that the T-Cell-MP or duplexed T-Cell-MP does not comprise a aa sequence to which the antibodies bind).

[00410] Chemotherapeutic agents that may be administered in conjunction with a T-Cell-MP-KRAS-epitope conjugate for the treatment of cancers and neoplasms include, but are not limited to, alkylating agents, cytoskeletal disruptors (taxane), epothilones, histone deacetylase inhibitors, topoisomerase I inhibitors, topoisomerase II inhibitors, kinase inhibitors, nucleotide analog or precursor analogs, peptide antineoplastic antibiotics (e.g. bleomycin or actinomycin), platinum-based agents, retinoids, or vinca alkaloids and their derivatives. The chemotherapeutic agents may be selected from the group consisting of actinomycin all-trans retinoic acid, azacytidine, azathioprine, bleomycin, bortezomib, carboplatin, capecitabine, cisplatin, chlorambucil, cyclophosphamide, cytarabine, daunorubicin, docetaxel, doxifluridine, doxorubicin, epirubicin, epothilone, etoposide, fluorouracil, gemcitabine, hydroxyurea, idarubicin, imatinib, irinotecan, mechlorethamine, mercaptopurine, methotrexate, mitoxantrone, oxaliplatin, paclitaxel, pemetrexed, teniposide, tioguanine, topotecan, valrubicin, vemurafenib, vinblastine, vincristine, and vindesine.

2. Immune checkpoint inhibitors

[00411] As noted above, one type of therapeutic agent that may be administered in conjunction with a T cell-MP or a higher order complex thereof (e.g., a duplex) for the treatment of a cancer or benign neoplasm is an immune checkpoint inhibitor. Exemplary immune checkpoint inhibitors include inhibitors that target immune checkpoint polypeptide such as CD27, CD28, CD40, CD122, CD96, CD73, CD47, OX40, GITR, CSF1R, JAK, PI3K delta, PI3K gamma, TAM, arginase, CD137 (also known as 4-1BB), ICOS, A2AR, B7-H3, B7-H4, BTLA, CTLA-4, LAG3, TIM3, VISTA, CD96, TIGIT, CD122, PD-1, PD-L1 and PD-L2. In some cases, the immune checkpoint polypeptide is a stimulatory checkpoint molecule selected from CD27, CD28, CD40, ICOS, OX40, GITR, CD122 and CD137. In some cases, the immune checkpoint polypeptide is an inhibitory checkpoint molecule selected from A2AR, B7-H3, B7-H4, BTLA, CTLA-4, IDO, KIR, LAG3, PD-1, TIM3, CD96, TIGIT and VISTA.

[00412] In some cases, the immune checkpoint inhibitor is an antibody specific for an immune checkpoint, e.g., a monoclonal antibody. The anti-immune checkpoint antibody may be a fully human, humanized, or de-immunized such that the antibody does not substantially elicit an immune response in a human. In some cases, the anti-immune checkpoint antibody inhibits binding of the immune checkpoint polypeptide to a ligand for the immune checkpoint polypeptide. In some cases, the anti-immune checkpoint antibody inhibits binding of the immune checkpoint polypeptide to a receptor for the immune checkpoint polypeptide.

[00413] Antibodies, e.g., monoclonal antibodies, that are specific for immune checkpoints and that function as immune checkpoint inhibitors, are known in the art. See, e.g., Wurz et al. (2016) *Ther. Adv. Med. Oncol.* 8:4; and Naidoo et al. (2015) *Ann. Oncol.* 26:2375. Suitable anti-immune checkpoint antibodies include, but are not limited to, nivolumab (Bristol-Myers Squibb), pembrolizumab (Merck), pidilizumab (Curetech), AMP-224 (GlaxoSmithKline/Amplimmune), MPDL3280A (Roche), MDX-1105 (Medarex, Inc./Bristol Myer Squibb), MEDI-4736 (Medimmune/AstraZeneca), arelumab (Merck Serono), ipilimumab (YERVOY, (Bristol-Myers Squibb), tremelimumab (Pfizer), pidilizumab (CureTech, Ltd.), IMP321 (Immutep S.A.), MGA271 (Macrogenics), BMS-986016 (Bristol-Meyers Squibb), lirilumab (Bristol-Myers Squibb), urelumab (Bristol-Meyers Squibb), PF-05082566 (Pfizer), IPH2101 (Innate Pharma/Bristol-Myers Squibb), MEDI-6469 (MedImmune/AZ), CP-870,893 (Genentech), Mogamulizumab (Kyowa Hakko Kirin), Varlilumab (CelIDex Therapeutics), Avelumab (EMD Serono), Galiximab (Biogen Idec), AMP-514 (Amplimmune/AZ), AUNP 12 (Aurigene and Pierre Fabre), Indoximod (NewLink Genetics), NLG-919 (NewLink Genetics), INCB024360 (Incyte) and combinations thereof. Suitable anti-LAG3 antibodies include, e.g., BMS-986016 and LAG525. Suitable anti-GITR antibodies include, e.g., TRX518, MK-4166, INCAGN01876, and MK-1248. Suitable anti-OX40 antibodies include, e.g., MEDI0562, INCAGN01949, GSK2831781, GSK-3174998, MOXR-0916, PF-04518600, and LAG525. Suitable anti-VISTA antibodies are provided in, e.g., WO 2015/097536.

[00414] A suitable dosage of an anti-immune checkpoint antibody is from about 1 mg/kg to about 2400 mg/kg per day, such as from about 1 mg/kg to about 1200 mg/kg per day, including from about 50 mg/kg to about 1200 mg/kg per day. Other representative dosages of such agents include about 5 mg/kg, 10

mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg, 35 mg/kg, 40 mg/kg, 45 mg/kg, 50 mg/kg, 60 mg/kg, 70 mg/kg, 80 mg/kg, 90 mg/kg, 100 mg/kg, 125 mg/kg, 150 mg/kg, 175 mg/kg, 200 mg/kg, 250 mg/kg, 300 mg/kg, 400 mg/kg, 500 mg/kg, 600 mg/kg, 700 mg/kg, 800 mg/kg, 900 mg/kg, 1000 mg/kg, 1100 mg/kg, 1200 mg/kg, 1300 mg/kg, 1400 mg/kg, 1500 mg/kg, 1600 mg/kg, 1700 mg/kg, 1800 mg/kg, 1900 mg/kg, 2000 mg/kg, 2100 mg/kg, 2200 mg/kg, and 2300 mg/kg per day. The effective dose of the antibody may be administered as two, three, four, five, six or more sub-doses, administered separately at appropriate intervals throughout the day.

[00415] In some cases, an immune checkpoint inhibitor is an anti-PD-1 antibody. Suitable anti-PD-1 antibodies include, e.g., nivolumab, pembrolizumab (also known as MK-3475), pidilizumab, SHR-1210, PDR001, and AMP-224. In some cases, the anti-PD-1 monoclonal antibody is nivolumab, pembrolizumab or PDR001. Suitable anti-PD1 antibodies are described in U.S. Patent Publicatio No. 2017/0044259. For pidilizumab, see, e.g., Rosenblatt et al. (2011) *J. Immunother.* 34:409-18.

[00416] In some cases, the anti-PD1 antibody is pembrolizumab. In some cases, the anti-PD-1 antibody is nivolumab (also known as MDX-1106 or BMS-936558; see, e.g., Topalian et al. (2012) *N. Eng. J. Med.* 366:2443-2454; and U.S. Patent No. 8,008,449). In some cases, the anti-CTLA-4 antibody is ipilimumab or tremelimumab. For tremelimumab, see, e.g., Ribas et al. (2013) *J. Clin. Oncol.* 31:616-22.

[00417] In some cases, the immune checkpoint inhibitor is an anti-PD-L1 monoclonal antibody. In some cases, the anti-PD-L1 monoclonal antibody is BMS-935559, MEDI4736, MPDL3280A (also known as RG7446), or MSB0010718C. In some embodiments, the anti-PD-L1 monoclonal antibody is MPDL3280A (atezolizumab) or MEDI4736 (durvalumab). For durvalumab, see, e.g., WO 2011/066389. For atezolizumab, see, e.g., U.S. Patent No. 8,217,149.

[00418] In some cases, the anti-PD-L1 antibody is atezolizumab.

3. Additional therapeutic agents for use in method of treatment

Suitable therapeutic agents or drugs that may be administered with a T-Cell-MP-KRAS-epitope conjugate, or higher order complex thereof, include virtually any therapeutic agent. Suitable therapeutic agents or drugs include but are not limited to, small molecule therapeutics (e.g., less than 2,000 Daltons in molecular weight) approved by the U.S. Food and Drug Administration, and/or listed in the 2020 U.S. Pharmacopeia or National Formulary. In an embodiment, those therapeutic agents or drugs are less than 1,000 molecular weight. Suitable drugs include, but are not limited to chemotherapeutic (antineoplastic) agents and the like . Suitable chemotherapeutics may be alkylating agents, cytoskeletal disruptors (taxanes), epothilones, histone deacetylase inhibitors, topoisomerase I inhibitors, topoisomerase II inhibitors, kinase inhibitors, nucleotide analog or precursor analogs, peptide antineoplastic antibiotics (e.g. bleomycin or actinomycin), platinum-based agents, retinoids, or vinca alkaloidsIn an embodiment, the chemotherapeutic agents are selected from actinomycin all-trans retinoic acid, azacytidine, azathioprine, bleomycin, bortezomib, carboplatin, capecitabine, cisplatin, chlorambucil, cyclophosphamide, cytarabine, daunorubicin, docetaxel, doxifluridine, doxorubicin, epirubicin, epothilone, etoposide, fluorouracil, gemcitabine, hydroxyurea, idarubicin, imatinib, irinotecan,

mechlorethamine, mercaptopurine, methotrexate, mitoxantrone, oxaliplatin, paclitaxel, pemetrexed, teniposide, tioguanine, topotecan, valrubicin, vemurafenib, vinblastine, vincristine, and vindesine.

[00419] In an embodiment, a suitable therapeutic agent that may be administered with a T-Cell-MP-KRAS-epitope conjugate, or its higher order complexes, comprises an anti-TGF-β antibody, such as Metelimumab (CAT192) directed against TGF-β1 and/or Fresolimub directed against TGF-β1 and TGF-β2, or a TGF-β trap (e.g., Cablivi® caplacizumab-yhdp). Such antibodies would, as a generality, not be administered in conjunction with a T-Cell-MP or higher order T-Cell-MP complex that comprise a sequence to which the antibodies bind such as a TGF-β1 or TGF-β2 MOD.

[00420] In an embodiment, a suitable therapeutic agent that may be administered with a T-Cell-MP-KRAS-epitope conjugate, or higher order complex thereof, comprises one or more antibodies directed against: B lymphocyte antigens (e.g., ibritumomab tiuxetan, obinutuzumab, ofatumumab, rituximab to CD20, brentuximab vedotin directed against CD30, and alemtuzumab to CD52); EGFR (e.g., cetuximab, panitumumab, and necitumumab); VEGF (e.g., bevacizumab); VEGFR2 (e.g., ramucirumab); HER2 (e.g., pertuzumab, trastuzumab, and ado-trastuzumab); PD-1 (e.g., nivolumab and pembrolizumab targeting a check point inhibition); RANKL (e.g., denosumab); CTLA-4 (e.g., ipilimumab targeting check point inhibition); IL-6 (e.g., siltuximab); disialoganglioside (GD2), (e.g., dinutuximab) disialoganglioside (GD2); CD38 (e.g., daratumumab); SLAMF7 (Elotuzumab); both EpCAM and CD3 (e.g., catumaxomab); or both CD19 and CD3 (blinatumomab). Such antibodies would, as a generality, not be administered in conjunction with a T-Cell-MP or higher order T-Cell-MP complex (e.g., a duplexed T-Cell-MP) that comprise a sequence to which any of the administered antibodies bind.

[00421] In an embodiment a suitable therapeutic agent that may be administered with a T-Cell-MP-KRAS-epitope conjugate, or higher order complex thereof (e.g., a duplex), comprises a KRAS(G12C) inhibitor such as Sotorasib or MRTX849.

VII. Subjects suitable for treatment

[00422] Subjects suitable for treatment, e.g., by selectively delivering a MOD to a T cell or by modulating their T cell activity, include those with a neoplasm, such as benign neoplasm or a malignant neoplasm (e.g., a cancer in the form of a solid malignant tumor).

[00423] Subjects suitable for treatment who have a cancer include, but are not limited to, individuals who have been provided other treatments for the cancer but who failed to respond to the treatment, or who have responded to the treatment for some period of time but have become refractory to the treatment, are unable to tolerate the treatment, or experience disease progression while on the treatment. Cancers and neoplasms that can be treated with a method of the present disclosure include, but are not limited to, those displaying any of the KRAS cancer epitopes recited herein (see, e.g., the epitopes recited in Section I.A.8) and those cancers and neoplasms recited in the methods of treatment described herein (see, e.g., Section VI).

VIII. Dosages and Routes of Administration

A. Dosages

[00424] A suitable dosage of a T-Cell-MP (e.g., a T-Cell-MP-KRAS-epitope conjugate) 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 T-Cell-MP-KRAS-epitope conjugate to be administered, sex of the patient, time, route of administration, general health, and other drugs being administered concurrently. Those of skill will also appreciate that dose levels can vary as a function of the specific T-Cell-MP being administered, 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.

[00425] A T-Cell-MP-KRAS-epitope conjugate may be administered in amounts between 1 ng/kg body weight and 100 mg/kg body weight per dose, e.g., from 0.01 μg to 100 mg per kg of body weight, from 0.1 μg to 10 mg per kg of body weight, from 1 μg to 50 mg per kg of body weight, from 10 μg to 20 mg per kg of body weight, from 100 μg to 15 mg per kg of body weight, from 500 μg to 10 mg per kg of body weight (e.g., from 0.1-0.5 mg per kg of body weight, 0.5-1.0 mg per kg of body weight, 1.0 to 5.0 mg per kg of body weight, 5.0 to 10.0 mg per kg of body weight, 5.0 to 15.0 mg per kg of body weight, 10.0 to 15.0 mg per kg of body weight, 15.0 to 20.0 mg per kg of body weight, 20-25 mg per kg of body weight, 1.0-3.0 mg per kg of body weight, 2.0-4.0 mg per kg of body weight, 3.0-5.0 mg per kg of body weight, 4.0-6.0 mg per kg of body weight, 5.0- 7.0 mg per kg of body weight, 6.0- 8.0 mg per kg, 7.0- 9.0 mg per kg of body weight, and 8.0- 10.0 mg per kg of body weight), or from 0.5 mg/kg body weight to 5 mg/kg body weight; however, doses below or above these exemplary ranges are envisioned, especially considering the aforementioned factors. If the regimen is a continuous infusion the above-mentioned doses can be utilized, or doses can be, for example, in the range of 1 μg to 10 mg per kilogram of body weight per minute. A T-Cell-MP-KRAS-epitope conjugate can also be administered in an amount of from about 0.1 mg/kg body weight to 50 mg/kg body weight, e.g., from about 0.1 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 5 mg/kg body weight to about 15 mg/kg body weight, from about 10.0 to about 15.0 mg per kg of body weight, from about 15.0 to about 20.0 mg per kg of body weight, from about 20-25 mg per kg of body weight,from about 10 mg/kg body weight to about 20 mg/kg body weight, from about 20 mg/kg body weight to about 30 mg/kg body weight, from about 30 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. 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.

[00426] Following successful treatment, it may be desirable to have the patient undergo maintenance therapy, i.e., periodic administrations intended to prevent the recurrence of the disease state, wherein a T-Cell-MP-KRAS-epitope conjugate is administered in maintenance doses, for example, ranging from 0.01 μg to 100 mg per kg of body weight, from 0.1 μg to 100 mg per kg of body weight, from 1 μg to 50 mg

per kg of body weight, from 10 μg to 20 mg per kg of body weight, from 100 μg to 15 mg per kg of body weight, or from 500 μg to 10 mg per kg of body weight (e.g., from 0.1-0.5 mg per kg, 0.5-1.0 mg per kg, 1.0-3.0 mg per kg, 2.0-4.0 mg per kg, 3.0-5.0 mg per kg, 4.0-6.0 mg per kg, 5.0- 7.0 mg per kg, 6.0- 8.0 mg per kg, 7.0- 9.0 mg per kg, and 8.0- 10.0 mg per kg), 5.0 to 10.0 mg per kg of body weight, 5.0 to 15.0 mg per kg of body weight, 10.0 to 15.0 mg per kg of body weight, 15.0 to 20.0 mg per kg of body weight, 20-25 mg per kg of body weight.

[00427] The frequency of administration of a T-Cell-MP-KRAS-epitope conjugate can vary depending on any of a variety of factors, e.g., severity of the symptoms, *etc.* For example, in some embodiments, a T-Cell-MP is administered once every two months, once per month, twice per month, once every two weeks, three times per month, once every three weeks, every other week (qow), once every week, 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).

[00428] The duration of administration of a T-Cell-MP-KRAS-epitope conjugate of the present disclosure (e.g., the period of time over which a T-Cell-MP is administered in one or more doses) can vary depending on any of a variety of factors including patient response, *etc.* For example, a T-Cell-MP-KRAS-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

[00429] A T-Cell-MP-KRAS-epitope conjugate of the present disclosure may be administered to an individual using any available method and route suitable for delivery, including *in vivo* and *ex vivo* methods, as well as systemic and localized routes of administration.

[00430] A T-Cell-MP-KRAS-epitope conjugate of the present disclosure may be administered to a host using any available 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. Some acceptable routes of administration include intratumoral, peritumoral, intramuscular, intralymphatic, intratracheal, intracranial, subcutaneous, intradermal, topical, intravenous, intra-arterial, 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-MP-KRAS-epitope conjugate administered and/or the desired effect. A T-Cell-MP-KRAS-epitope conjugate can be administered in a single dose or in multiple doses.

[00431] A T-Cell-MP-KRAS-epitope conjugate may be administered intravenously. A T-Cell-MP-KRAS-epitope conjugate may be administered intramuscularly. A T-Cell-MP-KRAS-epitope conjugate may be administered intralymphatically. A T-Cell-MP-KRAS-epitope conjugate may be administered locally (e.g., pulmonary administration such as in a nebulized or other aerosolized form). A T-Cell-MP-

KRAS-epitope conjugate may be administered intracranially. A T-Cell-MP-KRAS-epitope conjugate may be administered subcutaneously.

[00432] 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-MP-KRAS-epitope conjugate. Where systemic delivery is desired, administration typically involves invasive or systemically absorbed topical or mucosal administration of pharmaceutical preparations.

IX. Certain Aspects

[00433] 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 modulatory polypeptide (T-Cell-MP) KRAS-epitope conjugate (T-Cell-MP-KRAS-epitope conjugate), the T-Cell-MP polypeptide comprising:

(e.g., from N-terminus to C-terminus):

(i) optionally one or more MOD polypeptide sequences (e.g., two or more MOD polypeptide sequences, such as in tandem, wherein when there are two or more MOD polypeptide sequences, they are optionally joined to each other by independently selected L1 linkers);

(ii) an optional L2 linker polypeptide sequence joining the one or more MOD polypeptide sequences to a β2M polypeptide sequence;

(iii) the β2M polypeptide sequence;

(iv) an optional L3 linker polypeptide sequence (e.g., from 10-50 aa in length);

(v) a class I MHC-H polypeptide sequence;

(vi) an optional L4 linker polypeptide sequence;

(vii) a scaffold polypeptide sequence (e.g., an immunoglobulin Fc sequence);

(viii) an optional L5 linker polypeptide sequence; and

(ix) optionally one or more MOD polypeptide sequences (e.g., two or more MOD polypeptide sequences, such as in tandem, wherein when there are two or more MOD polypeptide sequences they are optionally joined to each other by independently selected L6 linkers);

wherein the T-Cell-MP-KRAS-epitope conjugate comprises at least one MOD polypeptide sequence (e.g., the MOD(s) of element (i) or (ix)); and

wherein at least one of the β2M polypeptide sequence, L3 linker polypeptide sequence, and/or MHC-H polypeptide sequence comprises one or more chemical conjugation sites at which a KRAS

epitope presenting molecule (KRAS epitope) is covalently attached either directly, or indirectly through a linker, to form a T-Cell-MP-KRAS-epitope conjugate.

The one or more MOD polypeptide sequences may be polypeptides such as wt. IL-2 or a variant of wt. IL-2 (e.g., that comprises an H16A or T substitution and an F42A substitution) that result in T cell activation, wherein T cell activation may result in one or more of the following: an increase in the activity of ZAP70 protein kinase activity, induction in the proliferation of the T-cell(s), granule-dependent effector actions (e.g., the release of granzymes, perforin, and/or granulysin from cytotoxic T-cells), and/or release of T cell cytokines (e.g., interferon γ from CD8+ cells).

2. The T-Cell-MP-KRAS-epitope conjugate of aspect 1, the T-Cell-MP polypeptide comprising from N-terminus to C-terminus:

(i) optionally one or more MOD polypeptide sequences (e.g., two or more MOD polypeptide sequences, such as in tandem, wherein when there are two or more MOD polypeptide sequences they are optionally joined to each other by independently selected L1 linkers);

(ii) an optional L2 linker polypeptide sequence;

(iii) a β2M polypeptide sequence;

(iv) an optional L3 linker polypeptide sequence (e.g., from 10-50 aa in length);

(v) a class I MHC-H polypeptide sequence;

(vi) an optional L4 linker polypeptide sequence;

(vii) a scaffold polypeptide sequence (e.g., an immunoglobulin Fc sequence);

(viii) an optional L5 linker polypeptide sequence; and

(ix) optionally one or more MOD polypeptide sequences (e.g., two or more MOD polypeptide sequences, such as in tandem, wherein when there are two or more MOD polypeptide sequences they are optionally joined to each other by independently selected L6 linkers);

wherein the T-Cell-MP-KRAS-epitope conjugate comprises at least one MOD polypeptide sequence (e.g., the MOD(s) of element (i) or (ix)); and

wherein the chemical conjugation site at which the KRAS epitope is covalently attached is within the β2M polypeptide sequence, L3 linker polypeptide sequence, and/or MHC-H polypeptide sequence.

The chemical conjugation site of the KRAS-epitope conjugates of aspects 1 and 2 permit the covalent attachment of a KRAS epitope presenting molecule (e.g., a KRAS peptide epitope) to the T-Cell-MP such that it can be bound by the MHC-H polypeptide and presented to a TCR. It is understood that the T-Cell-MP-KRAS-epitope conjugates of aspects 1 and 2 comprise a KRAS epitope covalently attached to a T-Cell-MP polypeptide that can be located in the binding cleft of the MHC-H/β2M polypeptide sequences and presented to a TCR.

3. The T-Cell-MP-KRAS-epitope conjugate of aspect 1 or aspect 2, wherein:

the MHC-H polypeptide sequence comprises a human class I MHC-H chain polypeptide sequence selected from HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, and HLA-G MHC-H polypeptide sequences having at least 85% (e.g., at least 90%, at least 95% or 98%) or 100% sequence identity to

at least 200 (e.g., at least 225, at least 250, at least 260, or at least 275) contiguous aas of an MHC-H polypeptide provided in any of FIGs. 3A-3H.

4. The T-Cell-MP-KRAS-epitope conjugate of any preceding aspect, wherein the MHC-H sequence does not include the MHC-H transmembrane domain, or a portion thereof, that will anchor the T-Cell-MP in a cell membrane.
5. The T-Cell-MP-KRAS-epitope conjugate of any preceding aspect, wherein the MHC-H polypeptide sequence has at least 85% (e.g., at least 90%, at least 95% or 98%) or 100% sequence identity to at least 200 (e.g., at least 225, at least 250, at least 260, or at least 275) contiguous aas of the α1, α2, and α3 domains of an HLA-A allele.
6. The T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-5, wherein the MHC-H polypeptide sequence has at least 85% (e.g., at least 90%, at least 95% or 98%) or 100% sequence identity to at least 200 (e.g., at least 225, at least 250, at least 260, or at least 275) contiguous aas of an HLA-A*0101, HLA-A*0201, HLA-A*0301, HLA-A*1101, HLA-A*2301, HLA-A*2402, HLA-A*2407, HLA-A*3303, or HLA-A*3401 polypeptide sequence provided in FIG. 3E.
7. The T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-6, wherein the MHC-H polypeptide sequence has at least 85% (e.g., at least 90%, at least 95% or 98%) or 100% sequence identity to at least 200 (e.g., at least 225, at least 250, at least 260, or at least 275) contiguous aas of an HLA-A*0101, HLA-A*0201, HLA-A*1101, HLA-A*2402, HLA-A*3303, or HLA-A*3401 polypeptide sequence (e.g., as provided in FIG. 3E).
8. The T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-4, wherein the MHC-H polypeptide sequence has at least 85% (e.g., at least 90%, at least 95% or 98%) or 100% sequence identity to at least 200 (e.g., at least 225, at least 250, at least 260, or at least 275) contiguous aas of the α1, α2, and α3 domains of an HLA-B allele.
9. The T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-4 or 8, wherein the MHC-H polypeptide sequence has at least 85% (e.g., at least 90%, at least 95% or 98%) or 100% sequence identity to at least 200 (e.g., at least 225, at least 250, at least 260, or at least 275) contiguous aas of an HLA-B*0702, HLA-B*0801, HLA-B*1502, B27 (subtypes HLA-B*2701-2759), HLA-B*3802, HLA-B*4001, HLA-B*4601, or HLA-B*5301 polypeptide sequence (e.g., as provided in FIG. 3F).
10. The T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-4 or 8, wherein the MHC-H sequence has at least 85% (e.g., at least 90%, at least 95% or 98%) or 100% sequence identity to at least 200 (e.g., at least 225, at least 250, at least 260, or at least 275) contiguous aas of HLA-B*0702.
11. The T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-4, wherein the MHC-H polypeptide sequence has at least 85% (e.g., at least 90%, at least 95% or 98%) or 100% sequence identity to at least 200 (e.g., at least 225, at least 250, at least 260, or at least 275) contiguous aas of the α1, α2, and α3 domains of an HLA-C allele.
12. The T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-4 or 11, wherein the MHC-H sequence has at least 85% (e.g., at least 90%, at least 95% or 98%) or 100% sequence identity to at least 200 (e.g., at least 225, at least 250, at least 260, or at least 275) contiguous aas of an HLA-C*0102, HLA-

C*0303, HLA-C*0304, HLA-C*0401, HLA-C*0602, HLA-C*0701, HLA-C*0702, HLA-C*0801, or HLA-C*1502 polypeptide sequence (e.g., as provided in FIG. 3G).

13. The T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-4 or 11, wherein the MHC-H polypeptide sequence has at least 85% (e.g., at least 90%, at least 95% or 98%) or 100% sequence identity to at least 200 (e.g., at least 225, at least 250, at least 260, or at least 275) contiguous aas of HLA-C*0701.
14. The T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-4, wherein the MHC-H polypeptide sequence has at least 85% (e.g., at least 90%, at least 95% or 98%) or 100% sequence identity to at least 200 (e.g., at least 225, at least 250, at least 260, or at least 275) contiguous aas of the α1, α2, and α3 domains of an HLA-E allele.
15. The T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-4 or 14, wherein the MHC-H polypeptide sequence has at least 85% (e.g., at least 90%, at least 95% or 98%) or 100% sequence identity to at least 200 (e.g., at least 225, at least 250, at least 260, or at least 275) contiguous aas of an HLA-E*0101, HLA-E*01:03, HLA-E*01:04, HLA-E*01:05, HLA-E*01:06, HLA-E*01:07, HLA-E*01:09, or HLA-E*01:10 polypeptide sequence (e.g., as provided in FIG. 3H), optionally wherein the MHC-H polypeptide sequence has at least 85% (e.g., at least 90%, at least 95% or 98%) or 100% sequence identity to at least 200 (e.g., at least 225, at least 250, at least 260, or at least 275) contiguous aas of anHLA-E*0101 or HLA-E*01:03.
16. The T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-4 or 14, wherein the MHC-H polypeptide sequence has at least 85% (e.g., at least 90%, at least 95% or 98%) or 100% sequence identity to at least 200 (e.g., at least 225, at least 250, at least 260, or at least 275) contiguous aas of the HLA-E allele consensus sequence:

GSHSLKYFHT SVSRPGRGEP RFISVGYVDD TQFVRFDNDA ASPRMVPRAP
WMEQEGSEYW DRETRSARDT AQIFRVNLRT LRG**Y**YNQS**X1**A GSHTLQWMHG
CELGPD**X2**RFL RGYEQFAYDG KDYLTLNEDL RSWTAVDT**A**A QISEQKSNDA
SEAEHQ**X3X4**YL EDTCVEWLHK YLEKGKETLL HLEPPKTHVT HHPISDHEAT
LRCWALGFYP AEITLTWQQD GEGHTQDTEL VETRP**A**GDGT FQKWAAVVVP
SGEE**X5**RYTCH VQHEGL**X6**EPV TLRWKPASQP TIPI,

wherein 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.

17. The T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-4, wherein the MHC-H polypeptide sequence has at least 85% (e.g., at least 90%, at least 95% or 98%) or 100% sequence identity to at least 200 (e.g., at least 225, at least 250, at least 260, or at least 275) contiguous aas of the α1, α2, and α3 domains of an HLA-F allele.
18. The T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-4 or 17, wherein the MHC-H polypeptide sequence has at least 85% (e.g., at least 90%, at least 95% or 98%) or 100% sequence identity to at least 200 (e.g., at least 225, at least 250, at least 260, or at least 275) contiguous aas of an 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, or HLA-F*01:06 polypeptide sequence (e.g., as provided in FIG. 3H).

19. The T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-4 or 17, wherein the MHC-H polypeptide sequence has at least 85% (e.g., at least 90%, at least 95% or 98%) or 100% sequence identity to at least 200 (e.g., at least 225, at least 250, at least 260, or at least 275) contiguous aas of the HLA-F allele consensus sequence:

GSHSLR**X1**FST AVSRPGRGEP RYIAVEYVDD TQFLRFDSDA AIPRMEPRE**X2**
WVEQEGPQYW EWTTGYAKAN AQTDRVALRN LLRRYNQSEA GSHTLQGMNG
CDMGPDGRLL RGYHQHAYDG KDYISLNEDL RSWTAADTVA QITQRFYEAE
EYAEEFRTYL EGECLELLRR YLENGKETLQ RADPPKAHVA HHPISDHEAT
LRCWALGFYP AEITLTWQRD GEEQTQDTEL VETRPAGDGT FQKWAAVVVP
X3GEEQRYTCH VQHEGLPQPL ILRWEQS**X4**QP TIPI,

wherein X1= Y or F; X2= P or Q; X3= S or P; and X4= P or L.

20. The T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-4, wherein the MHC-H polypeptide sequence has at least 85% (e.g., at least 90%, at least 95% or 98%) or 100% sequence identity to at least 200 (e.g., at least 225, at least 250, at least 260, or at least 275) contiguous aas of the α1, α2, and α3 domains of an HLA-G allele.

21. The T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-4 or 20, wherein the MHC-H polypeptide sequence has at least 85% (e.g., at least 90%, at least 95% or 98%) or 100% sequence identity to at least 200 (e.g., at least 225, at least 250, at least 260, or at least 275) contiguous aas of an 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*01:11, 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, or HLA-G*01:22 polypeptide sequence (e.g., as provided in FIG. 3H).

22. The T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-4 or 20, wherein the MHC-H polypeptide sequence has at least 85% (e.g., at least 90%, at least 95% or 98%) or 100% sequence identity to at least 200 (e.g., at least 225, at least 250, at least 260, or at least 275) contiguous aas of the HLA-G allele consensus sequence:

GSHSMRYFSA AV**X1**RPGRGEP RFIAMG**X2**VDD **X3**QF**X4**RFDSDS ACPRMEPRAP
WVE**X5**EGPEYW EEETRNTKAH AQTDRMNLQT **X6**RG**Y**YNQSEA SSHTLQWMI**X7**
CDL**X8X9**DGRL**X10** RGYEQYAYDG KDYLALNEDL RSWTAADT**A**A QISKRKCEAA
NVAEQRRA**X11**L EGTCVEWL**X12**R **X13**LENGKE**X14**LQ RADP**X15**KTHVT HHPVFDYEAT
LRCWALGFYP AEIILTWQ**X16**D GEDQTQDVEL VETRP**A**GDGT FQKWAAVVVP
SGEEQRY**X17**CH VQHEGLPEPL MLRW**X18**QSSLP TIPI,

wherein 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, and X18= K or E.

23. The T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-22, wherein the MHC-H polypeptide sequence comprises at least one mutation (e.g., two or three mutations) selected from the group consisting of: an alanine at position 84 (e.g., Y84A or R84A in the case of HLA-F), a cysteine at

position 84 (e.g., Y84C or R84C in the case of HLA-F), a cysteine at position 139 (e.g., A139C or V139C in the case of HLA-F), and a cysteine at position 236 (e.g., A236C). See FIG 3I for the location of those aa positions.

24. The T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-23, wherein the MHC-H polypeptide sequence comprises a combination of mutations selected from the group consisting of: Y84A and A139C; Y84A and A236C; Y84C and A139C; Y84C and A236C; and Y84C, A139C and A236C.
25. The T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-23, wherein the MHC-H polypeptide sequence comprises: a cysteine at position 84 (e.g., Y84C or R84C in the case of HLA-F), a cysteine at position 139 (e.g., A139C or V139C in the case of HLA-F), and optionally a cysteine at position 236 (e.g., A236C). See FIG 3I for the location of those aa positions.
26. The T-Cell-MP-KRAS-epitope conjugate of any preceding aspect, wherein the β2M sequence has at least 90% (e.g., at least 95% or 98%) or 100% sequence identity to at least 50 (e.g., 60, 70, 80, 90, 96, 97, 98, or all) contiguous aas of a mature human β2M polypeptide (e.g., aas 21-119 of NCBI accession number NP_004039.1 provided in FIG. 4).
27. The T-Cell-MP-KRAS-epitope conjugate of any preceding aspect, wherein the β2M sequence has up to 6 (e.g., 1, 2, 3, 4, or 5) aa substitutions within an aa segment of at least 70 (e.g., at least 80, 90, 96, 97, 98, or all) contiguous aas of a mature human β2M polypeptide (e.g., aas 21-119 of NCBI accession number NP_004039.1 provided in FIG. 4).
28. The T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-27, wherein the T-Cell-MP-KRAS-epitope conjugate comprises at least one linker sequence comprising, consisting essentially of, or consisting of: i) Gly and/or Ser; ii) Ala and Ser; iii) Gly, Ala, and Ser; iv) Gly, Ser, and Cys (e.g, a single Cys residue); v) Ala, Ser, and Cys (e.g., a single Cys residue); and vi) Gly, Ala, Ser, and Cys (e.g., a single Cys residue).
29. The T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-27, wherein the T-Cell-MP-KRAS-epitope conjugate comprises at least one linker (e.g., any of linkers L1-L6) that comprises one or more sequences selected from: polyG (e.g., polyglycine comprising 1-10 Gly residues), GA, AG, AS, SA, GS, GSGGS (SEQ ID NO:111), GGGS (SEQ ID NO:112), GGSG (SEQ ID NO:113), GGSGG (SEQ ID NO:114), GSGSG (SEQ ID NO:115), GSGGG (SEQ ID NO:116), GGGSG (SEQ ID NO:117), GSSSG (SEQ ID NO:118), GGGGS (SEQ ID NO:120), or AAAGG (SEQ ID NO:122), any of which may be repeated 2, 3, 4, 5, 6, 7, 8, 9, or 10 times.
30. The T-Cell-MP-KRAS-epitope conjugate of any preceding aspect, wherein the T-Cell-MP-KRAS-epitope conjugate comprises at least one linker comprising a G_4S (SEQ ID NO:120) or an AAAGG (SEQ ID NO:122) sequence that may be repeated from 1-10 times (e.g., repeated 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 times).
31. The T-Cell-MP-KRAS-epitope conjugate of any preceding aspect, wherein the scaffold polypeptide sequences are independently selected from non-interspecific sequences or interspecific sequences.
32. The T-Cell-MP-KRAS-epitope conjugate of aspect 31, wherein the interspecific and non-interspecific sequences are selected from the group consisting of: immunoglobulin heavy chain

constant regions (Ig Fc, e.g., CH2-CH3) (see, e.g., SEQ ID NOs:1-11), collectin polypeptides, coiled-coil domains (see, e.g., SEQ ID NOs:97-101), leucine-zipper domains(see, e.g., SEQ ID NOs:89-90 and 92-96), Fos polypeptides, Jun polypeptides, Ig CH1, Ig C_L κ, Ig C_L λ, knob-in-hole without disulfide (KiH), knob-in hole with a stabilizing disulfide bond (KiHs-s), HA-TF, ZW-1, 7.8.60, DD-KK, EW-RVT, EW-RVTs-s, and A107 sequences.

33. The T-Cell-MP-KRAS-epitope conjugate of any preceding aspect, complexed to form a duplex T-Cell-MP-KRAS-epitope conjugate or higher order T-Cell-MP-KRAS-epitope conjugate comprising at least a first T-Cell-MP-KRAS-epitope conjugate and a second T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-32, wherein:

    (i) the first T-Cell-MP-KRAS-epitope conjugate comprises a first β2M polypeptide sequence, a first class I MHC-H polypeptide sequence, and a first scaffold polypeptide; and

    (ii) the second T-Cell-MP-KRAS-epitope conjugate comprises a second β2M polypeptide sequence, a second class I MHC-H polypeptide sequence, and a second scaffold polypeptide; and

    wherein the first and second T-Cell-MP-KRAS-epitope conjugates associate by binding interactions between the first and second scaffold polypeptides that optionally include one or more interchain covalent bonds (e.g., one or two disulfide bonds). See e.g., the duplexes in FIGs. 8 and 9.

34. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any preceding aspect, wherein the scaffold comprises a non-immunoglobulin polypeptide sequence.

35. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of aspect 34, wherein the non-immunoglobulin polypeptide sequence is a non-interspecific polypeptide sequence (e.g., a non-interspecific coiled-coil or leucine zipper sequence).

36. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of aspect 34, wherein the non-immunoglobulin polypeptide sequence is an interspecific polypeptide sequence (e.g., an interspecific coiled-coil or leucine zipper sequence, Fos polypeptides that pair with Jun protein sequences, Jun polypeptides that pair with Fos protein sequences).

37. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-33, wherein the scaffold comprises an immunoglobulin polypeptide sequence.

38. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of aspect 37, wherein the immunoglobulin polypeptide sequence comprises one or more substitutions that reduce the binding with Ig Fc receptors and/or complement C1q protein relative to a T-Cell-MP where the immunoglobulin polypeptide sequence is unsubstituted.

39. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of aspect 37 or 38, wherein the scaffold comprises a non-interspecific immunoglobulin polypeptide sequence.

40. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of aspect 39, wherein the non-interspecific immunoglobulin polypeptide sequence comprises a human IgA Fc, IgD Fc, or IgE Fc (e.g., comprising an aa sequence having at least about 70% (e.g., at least about 75%, 80%, 85%, 90%, 95%, 98%, or 99%), or 100% aa sequence identity to at least 125 contiguous

aas (e.g., at least 150, at least 175, at least 200, or at least 210 contiguous aas), or all aas of an aa sequence of an Ig Fc region depicted in FIGs. 2A-2C).

41. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of aspect 39, wherein the non-interspecific immunoglobulin polypeptide sequence comprises a human IgG1 Fc, IgG2 Fc, IgG3 Fc, or IgG4 Fc (e.g., comprising an aa sequence having at least about 70% (e.g., at least about 75%, 80%, 85%, 90%, 95%, 98%, or 99%), or 100% aa sequence identity to at least 125 contiguous aas (e.g., at least 150, at least 175, at least 200, or at least 210 contiguous aas), or all aas of an aa sequence of an Ig Fc region depicted in FIGs. 2D-2G).
42. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 39-41, wherein the non-interspecific immunoglobulin polypeptide sequence comprises a human IgG1 Fc (e.g., comprising an aa sequence having at least about 70% (e.g., at least about 75%, 80%, 85%, 90%, 95%, 98%, or 99%), or 100% aa sequence identity to at least 125 contiguous aas (e.g., at least 150, at least 175, at least 200, or at least 210 contiguous aas), or all aas of an aa sequence of the wt. Ig Fc sequence depicted in FIG. 2D).
43. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of aspect 42, wherein the non-interspecific immunoglobulin polypeptide comprises at least one substitution at L234, L235, G236, G237, P238, S239, D270, N297, K322, P329, and/or P331 (respectively, aas L14, L15, G16, G17, P18, S19, D50, N77, K102, P109, and P111 of the wt. IgG1 aa sequence in FIG. 2D) or another substitution (e.g., a corresponding substitution) that reduces binding to the Fc λ receptor and/or the C1q protein relative to the same sequence without the substitution(s).
44. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of aspect 42, comprising: (i) a substitution of N297 (e.g., N297A, see, e.g., **SEQ ID NO:7**); (ii) a substitution of any of aas 234 to 239; (iii) a substitution at L234; (iv) a substitution at L235; (v) a substitution at L234 and L235 (e.g., L234A and L235A or "LALA" substitutions, see, e.g., SEQ ID NO:8); (vi) a substitution of P331; (vii) substitutions of D270, K322, and/or P329; or (viii) substitutions at L234 and/or L235 and a substitution at P331 (e.g., L234F, L235E, and P331S substitutions, see, e.g., SEQ ID NO:6).
45. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of aspect 39, wherein the scaffold sequence comprises an IgM heavy chain constant region.
46. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-33, wherein the scaffold comprises an interspecific immunoglobulin polypeptide sequence.
47. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of aspect 46, wherein the interspecific immunoglobulin sequence is selected from the group consisting of immunoglobulin heavy chain constant regions (Ig Fc CH2-CH3), Ig CH1, Ig C_L κ, Ig C_L λ, a knob-in-hole without disulfide (KiH), a knob-in hole with a stabilizing disulfide bond (KiHs-s), HA-TF, ZW-1, 7.8.60, DD-KK, EW-RVT, EW-RVTs-s, and A107 sequences.

48. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of aspect 46, wherein the interspecific immunoglobulin sequence comprises a KIH or a KIHs-s polypeptide sequence.
49. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of aspect 46, wherein the interspecific immunoglobulin sequence comprises an EW-RVT or an EW-RVTs-s polypeptide sequence.
50. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of aspect 46, wherein the interspecific immunoglobulin sequence comprises an HA-TF, ZW-1, 7.8.60, DD-KK, or A107 polypeptide sequence.
51. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 46-50, further comprising one or more substitutions that reduce binding to the Fc λ receptor and/or the C1q protein (e.g., substations at IgG1 aa L234 and/or L235, or K322) relative to the same sequence without the substitution(s).
52. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 46-50, further comprising one or more substitutions that limit complement activation (e.g., reduce binding to the complement C1q protein such as by substations at IgG D270, N297, K322, P329, and/or P331) relative to the same sequence without the substitution(s).
53. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 46-52, wherein the interspecific immunoglobulin polypeptide sequence comprises a human IgG1 Fc (e.g., comprising an aa sequence having at least about 70% (e.g., at least about 75%, 80%, 85%, 90%, or 95%) aa sequence identity to at least 125 contiguous aas (e.g., at least 150, at least 175, at least 200, or at least 210 contiguous aas) of the wt. Ig Gg1 Fc sequence in FIG. 2D.
54. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of aspect 53, wherein the interspecific immunoglobulin polypeptide sequence comprises one or more Ig Fc regions, comprising at least one substitution at L234, L235, G236, G237, P238, S239, D270, N297, K322, P329, and/or P331 (respectively, aas L14, L15, G16, G17, P18, S19, D50, N77, K102, P109, and P111 of the wt. IgG1 aa sequence in FIG. 2D) or another substitution (e.g., a corresponding substitution) that reduces binding to the Fc λ receptor and/or the C1q protein relative to the same sequence without the substitution(s).
55. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of aspect 53, comprising: (i) a substitution of N297 (e.g., N297A); (ii) a substitution of any of aas 234 to 239; (iii) a substitution at L234; (iv) a substitution at L235; (v) a substitution at L234 and L235 (e.g., an L234A and L235A or "LALA" substitution); (vi) a substitution at P331; (vii) substitutions of D270, K322, and/or P329; or (viii) substitutions at L234 and/or L235, and a substitution at P331 (e.g., L234F, L235E, and P331S substitutions).
56. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-55, comprising at least one (e.g., at least two or at least three) wt. MOD or variant MOD polypeptide sequence selected independently from the group consisting of: IL-1, IL-2, IL-4, IL-6, IL-

7, IL-10, IL-12, IL-15, IL-17, IL-21, IL-23, CD7, CD30L, CD40, CD70, CD80 (B7-1), CD83, CD86 (B7-2), HVEM (CD270), ILT3 (immunoglobulin-like transcript 3), ILT4 (immunoglobulin-like transcript 4), Fas ligand (FasL), ICAM (intercellular adhesion molecule), ICOS-L (inducible costimulatory ligand), JAG1 (CD339), lymphotoxin beta receptor, 3/TR6, OX40L (CD252), PD-L1, PD-L2, TGF-β1, TGF-β2, TGF-β3, and 4-1BBL polypeptide sequences.

57. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any preceding aspect, comprising at least one (e.g., at least two or at least three) wt. MOD or variant MOD polypeptide sequence selected independently from the group consisting of: 4-1BBL, PD-L1, IL-2, CD80, CD86, OX40L (CD252), Fas ligand (FasL), ICOS-L, ICAM, CD30L, CD40, CD83, HVEM (CD270), JAG1 (CD339), CD70, TGF-β1, TGF-β2, and TGF-β3 polypeptide sequences.
58. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any preceding aspect, comprising at least one (e.g., at least two or at least three) wt. MOD or variant MOD polypeptide sequence selected independently from the group consisting of 4-1BBL, PD-L1, IL-2, CD80, CD86 and FasL wt. MOD or variant MOD polypeptide sequences. For example, the T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate may comprise at least one wt. MOD and/or variant IL-2 MOD polypeptide sequence, and at least one wt. CD80, wt. CD86, variant CD80 or variant CD86 polypeptide sequence.
59. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any preceding aspect, comprising at least one wt. IL-2 or variant IL-2 MOD (e.g., comprising an H16A or T substitution and an F42A substitution) polypeptide sequence, or at least one pair of wt. IL-2 MOD or variant IL-2 MOD polypeptide sequences in tandem.
60. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of aspect 59, further comprising at least one wt. or variant: (i) CD80 or CD86 MOD polypeptide sequence; (ii) PD-L1 MOD polypeptide sequence; and/or (iii) FasL MOD polypeptide sequence.
61. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any preceding aspect, further comprising an intrachain disulfide bond between a cysteine substituted into the carboxyl end portion of the α1 helix and a cysteine present or substituted into the amino end portion of the α2-1 helix of the MHC-H polypeptide sequence.
62. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any preceding aspect, comprising an intrachain disulfide bond between a cysteine substituted into the carboxyl end portion of the α1 helix at position 84 and a cysteine substituted into the amino end portion of the α2-1 helix at position 139 of the MHC-H polypeptide sequence;

wherein the five residue clusters amino and carboxyl to position 84 (denoted aac 1 and aac 2, respectively), the five residue clusters amino and carboxyl to position 139 (denoted aac 3 and aac 4, respectively), and the five residue clusters amino and carboxyl to position 236 (denoted aac 5 and aac 6, respectively) may each be substituted with 1 to 5 independently selected naturally occurring aas.

63. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of aspect 62, wherein aac 1 to aac 6 may each be substituted with 1 to 5 independently selected naturally occurring aas other than proline.
64. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of aspect 62, wherein the carboxyl end portion of the α1 helix comprises a first sequence CYNQSE (SEQ ID NO:237) and the amino end portion of the α2-1 helix of the MHC-H polypeptide sequence comprises a second sequence D(M/T)CAQ (SEQ ID NO:238), and wherein the intrachain disulfide bond is formed between the cysteines in the first and second sequence. See aac 1 to aac 4 of FIG. 3I.
65. The duplex T-Cell-MP-KRAS-epitope conjugates of any of aspects 33-64, wherein the first T-Cell-MP and the second T-Cell-MP are not linked by disulfide bonds.
66. The duplex T-Cell-MP-KRAS-epitope conjugates of any of aspects 33-64, wherein the first T-Cell-MP and the second T-Cell-MP are covalently linked by at least one (e.g., two) disulfide bond(s).
67. The duplex T-Cell-MP-KRAS-epitope conjugates of aspect 66, wherein the first T-Cell-MP and the second T-Cell-MP are covalently linked by at least one (e.g., two) disulfide bond(s) between the scaffold polypeptide sequence of the first T-Cell-MP and second T-Cell-MP.
68. The duplex T-Cell-MP-KRAS-epitope conjugates of any of aspects 1-66, wherein at least one (e.g., both) of the first T-Cell-MP-KRAS-epitope conjugate and the second T-Cell-MP-KRAS-epitope conjugate sequences do not comprise Ig CH1 domain polypeptide sequences.
69. The unconjugated duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 33-35 and 37-45, wherein the first T-Cell-MP and the second T-Cell-MP are identical, and the unconjugated duplex T-Cell-MP-KRAS-epitope conjugate is a homodimer. See, e.g., FIG. 6, structures A and B for an exemplary unconjugated monomeric T-Cell-MPs that can form such an unconjugated duplex.
70. The T-Cell-MP-KRAS-epitope conjugate of aspect 69, comprising at least one (e.g., at least two, or at least three) wt. MOD or variant MOD polypeptide sequence selected independently from the group consisting of: IL-1, IL-2, IL-4, IL-6, IL-7, IL-10, IL-12, IL-15, IL-17, IL-21, IL-23, CD7, CD30L, CD40, CD70, CD80 (B7-1), CD83, CD86 (B7-2), HVEM (CD270), ILT3 (immunoglobulin-like transcript 3), ILT4 (immunoglobulin-like transcript 4), Fas ligand (FasL), ICAM (intercellular adhesion molecule), ICOS-L (inducible costimulatory ligand), JAG1 (CD339), lymphotoxin beta receptor, 3/TR6, OX40L (CD252), PD-L1, PD-L2, TGF-β1, TGF-β2, TGF-β3, and 4-1BBL polypeptide sequences.
71. The unconjugated duplex T-Cell-MP-KRAS-epitope conjugate of aspect 69, comprising at least one (e.g., at least two or at least three) wt. MOD or variant MOD polypeptide sequence selected independently from the group consisting of: 4-1BBL, IL-2, CD80, and CD86 wt. MOD or variant MOD polypeptide sequences. For example, the T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate may comprise at least one IL-2 wt. MOD or variant MOD polypeptide sequence, and at least one CD80, CD86, variant CD80 or variant CD86 polypeptide sequence.

72. The unconjugated duplex T-Cell-MP-KRAS-epitope conjugate of aspect 69, comprising at least one IL-2 wt. MOD or variant MOD (e.g., comprising an H16A or T substitution and an F42A substitution) polypeptide sequence, or at least one pair of IL-2 wt. MOD or variant MOD polypeptide sequences in tandem.
73. The unconjugated duplex T-Cell-MP-KRAS-epitope conjugate of aspect 69, further comprising at least one CD80 and/or CD86 wt. MOD or variant MOD polypeptide sequence.
74. The unconjugated duplex T-Cell-MP-KRAS-epitope conjugate of any one of aspects 33-34, 36 and 46-68, wherein the scaffold polypeptides of the first T-Cell-MP and the second T-Cell-MP are a pair of interspecific polypeptide sequences and the unconjugated duplex T-Cell-MP-KRAS-epitope conjugate is a heterodimer.
75. The unconjugated duplex T-Cell-MP-KRAS-epitope conjugate of aspect 74, wherein at least one (e.g., at least two) of the first T-Cell-MP and the second T-Cell-MP comprises at least one wt. MOD or variant MOD polypeptide sequence selected independently from the group consisting of: IL-1, IL-2, IL-4, IL-6, IL-7, IL-10, IL-12, IL-15, IL-17, IL-21, IL-23, CD7, CD30L, CD40, CD70, CD80 (B7-1), CD83, CD86 (B7-2), HVEM (CD270), ILT3 (immunoglobulin-like transcript 3), ILT4 (immunoglobulin-like transcript 4), ICAM (intercellular adhesion molecule), ICOS-L (inducible costimulatory ligand), JAG1 (CD339), lymphotoxin beta receptor, 3/TR6, OX40L (CD252), TGF-β1, TGF-β2, TGF-β3, and 4-1BBL polypeptide sequences.
76. The unconjugated duplex T-Cell-MP-KRAS-epitope conjugate of aspect 74, wherein at least one (e.g., at least two) of the first T-Cell-MP and the second T-Cell-MP comprises at least one wt. MOD or variant MOD polypeptide sequence selected independently from the group consisting of: 4-1BBL, IL-2, CD80, and CD86 wt. MOD or variant MOD polypeptide sequences. For example, the unconjugated duplex T-Cell-MP-KRAS-epitope conjugate may comprise at least one IL-2 wt. MOD or variant MOD polypeptide sequence, and at least one CD80, CD86, variant CD80 or variant CD86 polypeptide sequence.
77. The unconjugated duplex T-Cell-MP-KRAS-epitope conjugate of aspect 74, wherein at least one (e.g., at least two) of the first T-Cell-MP and the second T-Cell-MP comprises at least one IL-2 wt. MOD or variant MOD polypeptide sequence, or at least one pair of IL-2 wt. MOD or variant MOD polypeptide sequences in tandem.
78. The unconjugated duplex T-Cell-MP-KRAS-epitope conjugate of aspect 74, wherein at least one (e.g., at least two) of the first T-Cell-MP and the second T-Cell-MP comprises at least one CD80 and/or CD86 wt. MOD or variant MOD polypeptide sequence.
79. The unconjugated duplex T-Cell-MP-KRAS-epitope conjugate of aspect 74, wherein at least one (e.g., at least two) of the first T-Cell-MP and the second T-Cell-MP comprises at least one CD80 and/or CD86 wt. MOD or variant MOD polypeptide sequence.
80. The unconjugated duplex T-Cell-MP-KRAS-epitope conjugate of aspect 74, wherein at least one (e.g., at least two) of the first T-Cell-MP and the second T-Cell-MP comprises at least one (e.g., IL-2) wt. MOD or variant MOD polypeptide sequence.

81. The unconjugated duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 74-80, wherein: (i) the first T-Cell-MP and the second T-Cell-MP do not comprise the same MODs; (ii) the first T-Cell-MP and the second T-Cell-MP do not comprise the same number of MODs; or (iii) the MODs are placed in different locations of the first T-Cell-MP and the second T-Cell-MP.
82. The T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-64, complexed to form a triplex T-Cell-MP of three heterodimers, a quadraplex T-Cell-MP of four heterodimers, a pentaplex T-Cell-MP of five heterodimers, or a hexaplex T-Cell-MP of six dimers.
83. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any one of aspects 1-82, wherein each chemical conjugation site is jointly or independently selected from: a) amino acid chemical conjugation sites; b) non-natural amino acids and/or selenocysteines; c) peptide sequences that act as an enzymatic modification sequence (e.g., a sulfatase motif); d) carbohydrate or oligosaccharide moieties; and/or e) IgG nucleotide binding sites.
84. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any one of aspects 1-83, wherein at least one (e.g., two or more) chemical conjugation site comprises an enzymatic modification sequence.
85. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-84, wherein at least one (e.g., two) chemical conjugation site comprises a sulfatase motif.
86. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of aspect 85, wherein the sulfatase motif comprises the sequence X1Z1X2Z2X3Z3 (SEQ ID NO:66) 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.
87. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of aspect 86, wherein at least one Z1 residue has been converted into an fGly amino acid residue.
88. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-84, wherein:

    at least one (e.g., two or more) of the chemical conjugation sites comprises a Sortase A enzyme site (e.g., comprising the amino acid sequence LP(X5)TG, LP(X5)TA, or LPETGG) positioned at the C-terminus of at least one (e.g., two or more) T-Cell-MP; and

at least one of the chemical conjugation sites is a Sortase A enzyme site comprising an oligoglycine (e.g., $(G)_{2, 3, 4, \text{ or } 5}$) or an oligo alanine (e.g., $(A)_{2, 3, 4, \text{ or } 5}$) at the amino terminus of the T-Cell-MP polypeptide(s).

89. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-84, wherein at least one (e.g., two or more) chemical conjugation site comprises a transglutaminase site (e.g., selected from the group consisting of: LQG, LLQGG, LLQG, LSLSQG, and LLQLQG).
90. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-84, wherein at least one (e.g., two or more) chemical conjugation site comprises a selenocysteine, or an amino acid sequence containing one or more independently selected non-natural amino acids.
91. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of aspect 90, wherein at least one of the one or more non-natural amino acids (e.g., two or more) is selected from the group consisting of para-acetylphenylalanine, para-azido phenylalanine and propynyl-tyrosine.
92. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-84, wherein at least one (e.g., two or more) chemical conjugation site comprises a carbohydrate, monosaccharide, disaccharide and/or oligosaccharide.
93. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-84, wherein at least one (e.g., two or more) chemical conjugation site comprises one or more IgG nucleotide binding sites.
94. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-84, wherein at least one (e.g., two or more) chemical conjugation site comprises an amino acid conjugation site (e.g., a cysteine provided in a T-Cell-MP by protein engineering of its sequence).
95. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of aspect 94, wherein the at least one (e.g., two or more) chemical conjugation site comprises the epsilon amino group of a lysine provided in a T-Cell MP polypeptide sequence (e.g., provided in a T-Cell-MP by protein engineering of its polypeptide sequence).
96. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of aspect 94, wherein the at least one (e.g., two or more) chemical conjugation site comprises a selenol group of selenocysteine and/or a sulfhydryl group of a cysteine provided in a T-Cell MP polypeptide sequence (e.g., provided in a T-Cell-MP by protein engineering of its polypeptide sequence).
97. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of aspect 94, wherein the at least one chemical conjugation site comprises a sulfhydryl group of a cysteine provided in a T-Cell MP polypeptide sequence, or in the polypeptide sequence of each of the first T-Cell-MP and the second T-Cell-MP of a duplex T-Cell-MP-KRAS-epitope conjugate (e.g., provided in a T-Cell-MP by protein engineering of the polypeptide sequence(s)).

98. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-97, wherein each chemical conjugation site (e.g., for the conjugation of an epitope) present in the T-Cell-MP-KRAS-epitope conjugate or duplexed T-Cell-MP-KRAS-epitope conjugate is selected to be the same (e.g., both conjugations sites of a duplex T-Cell-MP are the sulfhydryl of a cysteine provided in a T-Cell-MP polypeptide sequence by protein engineering of the polypeptide sequence(s)).

99. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-98, wherein a chemical conjugation site (e.g., for the conjugation of an epitope) is located at the N- or C-terminus of a T-Cell-MP, or, if present, attached to or within a linker located at the N- or C-terminus of the T-Cell-MP.

100. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-98, wherein a chemical conjugation site is located in a linker of the T-Cell-MP (e.g., an L1-L6 linker).

101. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-98, wherein the chemical conjugation site (e.g., for the conjugation of an epitope) is located in the MHC-H polypeptide sequence, the β2M polypeptide sequence, or a linker sequence joining the MHC-H and β2M polypeptide sequences (the L3 linker).

102. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-98, wherein the chemical conjugation site (e.g., for the conjugation of an epitope) is located in a linker sequence joining the MHC-H and β2M polypeptide sequences (the L3 linker).

103. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of aspect 102, wherein the chemical conjugation site is a sulfhydryl of a cysteine present in the linker sequence joining the MHC-H and β2M polypeptide sequences.

104. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of aspect 103, wherein the linker sequence joining the MHC-H and β2M polypeptide sequences further comprises a glycine, glycine and serine, alanine, alanine and serine, or alanine glycine and serine containing polypeptide sequence.

105. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of aspect 103, wherein the linker sequence joining the MHC-H and β2M polypeptide sequences comprises the polypeptide sequence GGGS (SEQ ID NO:112) or GGGGS (SEQ ID NO:120).

106. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of aspect 103, wherein the linker sequence joining the MHC-H and β2M polypeptide sequences comprises a polypeptide sequence selected from the group consisting of: G***C***GGS(G_4S) (SEQ ID NO:123) where the G_4S unit may be repeated from 1 to 10 times (e.g., repeated 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 times), G***C***GASGGGGSGGGGS (SEQ ID NO:124), G***C***GGSGGGGSGGGG SGGGGS (SEQ ID NO:125) or G***C***GGSGGGGSGGGGS (SEQ ID NO:126).

107. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 101-106, wherein the linker sequence joining the MHC-H and β2M polypeptide sequences comprises from 15 to 50 aas.

108. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 101-106, wherein the linker sequence joining the MHC-H and β2M polypeptide sequences (the L3 linker) comprises from 10 to 50 aas.

109. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-98, wherein the chemical conjugation site (e.g., for the conjugation of an epitope) is located in the MHC-H polypeptide sequence, which has at least 85% (e.g., at least 90%, 95%, 98% or 99%, or even 100%) aa sequence identity to at least 150, 175, 200, or 225 contiguous aas of an MHC-H sequence shown in FIGs. 3A-3I.

110. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of aspect 109, wherein the chemical conjugation site comprises a cysteine or selenocysteine.

111. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of aspect 110, wherein at least one cysteine or selenocysteine chemical conjugation site is located at position 2, 5, 7, 59, 84, 116, 139, 167, 168, 170, or 171 of an MHC-H polypeptide with the numbering as in FIGs. 3D-3I.

112. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-98, wherein a chemical conjugation site (e.g., for the conjugation of an epitope) is located in the β2M polypeptide sequence, which has at least 85% (e.g., at least 90%, 95%, 98% or 99%, or even 100%) aa sequence identity to at least 50 (e.g., at least 60, 70, 80, 90, 96, 97, 98 or all) contiguous aas of a mature β2M polypeptide sequence shown in FIG. 4 (e.g., the sequences shown in FIG. 4 starting at aa 21 and ending at their C-terminus).

113. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of aspect 112, wherein the chemical conjugation site is located between aas 35-55 (e.g., 40 to 50) of the human mature β2M polypeptide sequence of Fig 4, having 0 to 15 aa substitutions.

114. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of aspect 112, wherein at least one cysteine or selenocysteine chemical conjugation site is located at position 2, 44, 50, 77, 85, 88, 91, or 98 of the mature β2M polypeptides (aas 22, 64, 70, 97, 105, 108, 111, or 118 of the β2M sequences as shown in FIG. 4).

115. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-98, wherein a chemical conjugation site (e.g., for the conjugation of an epitope) is located in the β2M polypeptide sequence, which has 1 to 15 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15) aa deletions, insertions and/or changes compared with a mature β2M polypeptide set forth in FIG. 4 (starting at aa 21 and ending at its C-terminus).

116. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 112-115, wherein the chemical conjugation site is a cysteine.

117. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 112-115, wherein the β2M polypeptide sequence is the mature human β2M sequence of FIG 4.

118. The duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 33-117, wherein at least the first T-Cell-MP polypeptide sequence comprises, and optionally the first and second T-Cell-MP polypeptide sequences comprise, from N-terminus to C-terminus:

(i) one or more MOD polypeptide sequences optionally joined by L1 linkers;

(ii) an L2 polypeptide;

(iii) a β2M polypeptide sequence;

(iv) an L3 linker polypeptide sequence comprising from 10 to 50 (e.g., from 10 to 20, from 10 to 25, from 15 to 25, from 20 to 30, from 25 to 35, from 25 to 50, from 30 to 35, from 35 to 45, or from 40 to 50) amino acids;

(v) a class I MHC-H polypeptide sequence comprising cysteines substituted at positions 84 and 139 (see FIGs. 3E-3H, e.g., Y84C and A139C substitutions) and forming a disulfide bond;

(vi) an L4 linker polypeptide sequence;

(vii) an interspecific or non-interspecific immunoglobulin Fc scaffold sequence;

(viii) an optional L5 linker polypeptide sequence;

(ix) optionally one or more MOD polypeptide sequences (e.g., two or more MOD polypeptide sequences, such as in tandem optionally joined by L6 linkers);

wherein at least one of the β2M polypeptide sequence, L3 linker polypeptide sequence, or MHC-H polypeptide sequence comprises a chemical conjugation site (e.g., added by protein engineering) at which a KRAS epitope presenting molecule (e.g., a KRAS peptide, phosphopeptide, or lipopeptide presenting a KRAS epitope) is covalently attached either directly, or indirectly through a linker, to form a T-Cell-MP-KRAS-epitope;

wherein the first and second T-Cell-MPs are covalently linked through at least one disulfide bond between their Ig Fc scaffold sequences, and

optionally wherein one or more MOD polypeptide sequences comprises a polypeptide such as wt. IL-2 or a variant of wt. IL-2 (e.g., that comprises an H16A or T substitution and an F42A substitution) that results in T cell activation, wherein T cell activation may result in one or more of the following: an increase in the activity of ZAP70 protein kinase activity, induction in the proliferation of the T-cell(s), granule-dependent effector actions (e.g., the release of granzymes, perforin, and/or granulysin from cytotoxic T-cells), and/or release of T cell cytokines (e.g., interferon γ from CD8+ cells).

119. The duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 33-117, wherein at least the first T-Cell-MP polypeptide sequence comprises, and optionally the first and second T-Cell-MP polypeptide sequences comprise:

(i) optionally one or more MOD polypeptide sequences optionally joined by L1 linkers;

(ii) an optional L2 polypeptide;

(iii) a β2M polypeptide sequence;

(iv) an L3 linker polypeptide sequence comprising from 10 to 50 amino acids;

(v) a class I MHC-H polypeptide sequence comprising cysteines substituted at positions 84 and 139 (see FIGs. 3E-3H, e.g., Y84C and A139C substitutions) and forming a disulfide bond;

(vi) an L4 linker polypeptide sequence;

(vii) an interspecific or non-interspecific immunoglobulin Fc scaffold sequence;

(viii) an L5 linker polypeptide sequence; and

(ix) one or more MOD polypeptide sequences joined by L6 linker polypeptides;

wherein at least one of the β2M polypeptide sequence, L3 linker polypeptide sequence, or MHC-H polypeptide sequence comprises a chemical conjugation site (e.g., added by protein engineering) at which a KRAS epitope presenting molecule (e.g., a KRAS peptide, phosphopeptide, or lipopeptide presenting a KRAS epitope) is covalently attached either directly, or indirectly through a linker, to form a T-Cell-MP-KRAS-epitope;

wherein the first and second T-Cell-MPs are covalently linked through at least one disulfide bond between their Ig Fc scaffold sequences, and

optionally wherein one or more MOD polypeptide sequences comprises a polypeptide such as wt. IL-2 or a variant of wt. IL-2 (e.g., that comprises an H16A or T substitution and an F42A substitution) that results in T cell activation, wherein T cell activation may result in one or more of the following: an increase in the activity of ZAP70 protein kinase activity, induction in the proliferation of the T-cell(s), granule-dependent effector actions (e.g., the release of granzymes, perforin, and/or granulysin from cytotoxic T-cells), and/or release of T cell cytokines (e.g., interferon γ from CD8+ cells).

120. The duplex T-Cell-MP-KRAS-epitope conjugate of aspects 118 or 119, wherein the chemical conjugation sites of the first and second T-Cell-MP polypeptides are within the L3 linkers.

121. The duplex T-Cell-MP-KRAS-epitope conjugate of aspect 120, wherein the chemical conjugation sites of the first and second T-Cell-MP polypeptides are the sulfhydryl of a cysteine present in L3 linkers comprising, consisting essentially (predominantly) of, or otherwise consisting of a glycine, serine and/or alanine.

122. The duplex T-Cell-MP-KRAS-epitope conjugate of aspects 118 or 119, wherein the chemical conjugation sites of the first and second T-Cell-MP polypeptides arewithin the β2M polypeptide sequence (e.g., an E44C substitution in a mature β2M polypeptide sequence provided in FIG. 4).

123. The duplex T-Cell-MP-KRAS-epitope conjugate of aspect 122, wherein the chemical conjugation sites of the first and second T-Cell-MP polypeptides are the sulfhydryl of a cysteine provided in the β2M polypeptide sequence.

124. The duplex T-Cell-MP-KRAS-epitope conjugate of aspect 123, wherein the chemical conjugation sites of the first and second T-Cell-MP polypeptides are the sulfhydryl of a cysteine provided in a β2M polypeptide at position 44 of a mature β2M polypeptide sequence provided in FIG. 4.

125. The duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 118-124, wherein the one or more MOD polypeptide sequences comprises at least one (e.g., two or more) wt. IL-2 or variant IL-2 sequence (e.g., comprising an H16A or T substitution and an F42A substitution).

126. The duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 118-125, wherein the one or more MOD polypeptide sequences comprises at least one wt. or variant CD80 or CD86 sequence.

127. The duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 118-126, wherein the one or more MOD polypeptide sequences comprises at least one wt. or variant PD-L1 sequence.

128. The duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 118-127, wherein the one or more MOD polypeptide sequences comprises at least one wt. or variant 4-1BBL or PD-L1 sequence.

129. The duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 118-128, wherein:

(i) the immunoglobulin Fc scaffold is a non-interspecific scaffold polypeptide and the duplex is a homodimer comprising identical first and second T-Cell-MP polypeptides; or

(ii) the first and second scaffold polypeptides are an interspecific pair of immunoglobulin Fc scaffold polypeptides (e.g., a KIH or KIH-ss pair), and the duplex is a heterodimer.

130. The duplex T-Cell-MP-KRAS-epitope conjugate of aspect 129, wherein the first and second scaffold polypeptides are an interspecific pair of immunoglobulin Fc scaffold polypeptides, and the first T-Cell-MP polypeptide sequence comprises at least one MOD polypeptide sequence not present in the second T-Cell-MP polypeptide sequence.

131. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any preceding aspect further comprising an additional peptide and/or a payload covalently linked to a T-Cell-MP.

132. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of aspect 131, wherein the additional peptide is an epitope tag or an affinity domain.

133. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of aspect 131, wherein the additional peptide is a targeting sequence. wherein the payload is a therapeutic agent, chemotherapeutic agent, diagnostic agent, or label.

134. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of aspect 131, wherein the payload is a therapeutic agent, chemotherapeutic agent, diagnostic agent, or label.

135. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-134, wherein at least one MHC-H polypeptide (e.g., each MHC-H polypeptide) comprises:

a) an amino acid sequence having at least 95% aa sequence identity to the HLA-A*0101, HLA-A*0201, HLA-A*0301, HLA-A*1101, HLA-A*2301, HLA-A*2402, HLA-A*2407, HLA-A*3303, or HLA-A*3401 amino acid sequence depicted in FIG. 3E; or

b) an amino acid sequence having at least 95% aa sequence identity to the HLA-B*0702, HLA-B*0801, HLA-B*1502, HLA-B*3802, HLA-B*4001, HLA-B*4601, or HLA-B*5301 amino acid sequence depicted in FIG. 3F; or

c) an amino acid sequence having at least 95% aa sequence identity to the HLA-C*0102, HLA-C*0303, HLA-C*0304, HLA-C*0401, HLA-C*0602, HLA-C*0701, HLA-C*0702, HLA-C*0801, or HLA-C*1502 depicted in FIG. 3G.

136. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-135, wherein the KRAS epitope comprises a KRAS epitope aa sequence set forth in Section I.A.8.d. of this disclosure.

137. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-135, wherein the KRAS epitope comprises the aa sequence: VVGA**D**GVGK (SEQ ID NO:139), VVGA**C**GVGK (SEQ ID NO:140),VVGA**V**GVGK (SEQ ID NO:141), VVVGA**D**GVGK (SEQ ID NO:142), VVVGA**C**GVGK (SEQ ID NO:144), or VVVGA**V**GVGK (SEQ ID NO:143), and has a length of 9 or 10 amino acids.

138. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-135, wherein the KRAS epitope comprises the aa sequence: V**T**GA**D**GVGK (SEQ ID NO:145), V**T**GA**C**GVGK (SEQ ID NO:147), or V**T**GA**V**GVGK (SEQ ID NO:146), and has a length of 9 amino acids.

139. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-135, wherein the KRAS epitope comprises the aa sequence: VTVGADGVGK (SEQ ID NO:148), VTVGACGVGK (SEQ ID NO:150), or VTVGAVGVGK (SEQ ID NO:149), and has a length of 10 amino acids.

140. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 136-139, wherein each MHC-H polypeptide comprises a sequence having at least 95% (e.g., at least 96%, 97%, 98%, 99%, or 100%) sequence identity to HLA-A*1101 polypeptide sequence SEQ ID NO:32).

141. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-135, wherein the KRAS epitope comprises the aa sequence: LVVVGADGV (SEQ ID NO:155), LVVVGAVGV (SEQ ID NO:156), LVVVGACGV (SEQ ID NO:157), KLVVGADGV (SEQ ID NO:189), KLVVGAVGV (SEQ ID NO:190), KLVVVAVGV (SEQ ID NO:191), or KLVVVADGV (SEQ ID NO:192), and has a length of 9 amino acids.

142. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-135, wherein the KRAS epitope comprises the aa sequence: KLVVVGADGV (SEQ ID NO:158), KLVVVGAVGV (SEQ ID NO:159), or KLVVVGACGV (SEQ ID NO:160), and has a length of 10 amino acids.

143. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-135, wherein the KRAS epitope comprises the aa sequence: LLVVGADGV (SEQ ID NO:161), LLVVGAVGV (SEQ ID NO:162), or LLVVGACGV (SEQ ID NO:163), and has a length of 9 amino acids.

144. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-135, wherein the KRAS epitope comprises the aa sequence: FLVVVGADGV (SEQ ID

NO:164), FLVVVGAVGV (SEQ ID NO:165), or FLVVVGACGV (SEQ ID NO:188), and has a length of 10 amino acids.

145. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of any of aspects 1-144, wherein the KRAS epitope is a peptide, phosphopeptide, or lipopeptide that comprises from about 4 aas to about 25 aas (e.g., 4 aas, 5 aas, 6 aas, 7 aas, 8 aas, 9 aa, 10 aas, 11 aas, 12 aas, 13 aas, 14 aas, 15 aas, 16 aas, 17 aas, 18 aas, 19 aas, 20 aas, 21 aas, 22 aas, 23 aas, 24 aas, or 25 aas or the KRAS epitope can have a length of from about 4 aa to about 10 aa, from about 6 aa to about 12 aa, from about 10 aas to about 15 aas, from about 15 aas to about 20 aas, or from about 20 aa sto about 25 aas).

146. A method of treatment or prophylaxis of a disease or condition comprising:

(i) administering to a patient/subject (e.g., a patient in need thereof) an effective amount of one or more T-Cell-MP-KRAS-epitope conjugates and/or duplex T-Cell-MP-KRAS-epitope conjugates of any of aspects 1-144; or

(ii) contacting a cell or tissue in vitro, in vivo, or ex vivo with one or more T-Cell-MP-KRAS-epitope conjugates and/or duplex T-Cell-MP-KRAS-epitope conjugates according to any of aspects 1-144 and administering the cell, tissue, or progeny thereof to the patient/subject.

147. The method of aspect 146, wherein the administration is to a mammalian patient or subject.

148. The method of aspect 147, wherein the patient or subject is human.

149. The method of aspect 147, wherein the patient or subject is non-human (e.g., rodent, lagomorph, bovine, canine, feline, rodent, murine, caprine, simian, ovine, equine, lappine, porcine, etc.).

150. The method of any one of aspects 146-149, wherein the disease or condition is a neoplasm (e.g., benign neoplasm or malignant neoplasm/cancer).

151. The method of aspect 150, wherein the disease or condition is a cancer.

152. The method of aspect 151, wherein the cancer is non-small cell lung cancer, lung adenocarcinoma, mucinous adenoma, ductal carcinoma of the pancreas, colorectal cancer, or leukemia.

153. The method of any of aspects 146-152, further comprising administering one or more therapeutic agents that enhances CD 8+ T cell functions (e.g., effector function) and/or treats the disease or condition.

154. The method of aspect 153, wherein the therapeutic agent that enhances CD 8+ function and/or treats the disease or condition comprises an anti-TGF-β antibody such as Metelimumab (CAT192) directed against TGF-β1 and Fresolimub directed against TGF-β1 and TGF-β2, or a TGF-β trap (optionally subject to the proviso that the T-Cell-MP or duplexed T-Cell-MP does not comprise an aa sequence to which the antibodies or TGF-β trap bind, such as a TGF-β1 or TGF-β2 wt. MOD or variant MOD aa sequence).

155. The method of aspect 153 or 154, wherein the therapeutic agent that enhances CD 8+ function and/or treats the disease or condition comprises one or more antibodies directed against: B lymphocyte antigens (e.g., ibritumomab tiuxetan, obinutuzumab, ofatumumab, rituximab to CD20, brentuximab vedotin directed against CD30, and alemtuzumab to CD52); EGFR (e.g., cetuximab, panitumumab,

and necitumumab); VEGF (e.g., bevacizumab); VEGFR2 (e.g., ramucirumab); HER2 (e.g., pertuzumab, trastuzumab, and ado-trastuzumab); PD-1 (e.g., nivolumab and pembrolizumab targeting a check point inhibition); RANKL (e.g., denosumab); CTLA-4 (e.g., ipilimumab targeting check point inhibition); IL-6 (e.g., siltuximab); disialoganglioside (GD2) (e.g., dinutuximab);CD38 (e.g., daratumumab); SLAMF7 (Elotuzumab); both EpCAM and CD3 (e.g., catumaxomab); or both CD19 and CD3 (blinatumomab) (optionally subject to the proviso that the T-Cell-MP or duplexed T-Cell-MP does not comprise an aa sequence to which the antibodies bind).

156. The method of any of aspects 146-155, further comprising administering one or more chemotherapeutic agents.

157. The method of aspect 156, wherein the one or more chemotherapeutic agents are selected from: alkylating agents, cytoskeletal disruptors (taxane), epothilones, histone deacetylase inhibitors, topoisomerase I inhibitors, topoisomerase II inhibitors, kinase inhibitors, nucleotide analogs or precursor analogs, peptide antineoplastic antibiotics (e.g. bleomycin or actinomycin), platinum-based agents, retinoids, or vinca alkaloids and their derivatives.

158. The method of aspect 156, wherein the one or more chemotherapeutic agents are selected from the group consisting of actinomycin all-trans retinoic acid, azacytidine, azathioprine, bleomycin, bortezomib, carboplatin, capecitabine, cisplatin, chlorambucil, cyclophosphamide, cytarabine, daunorubicin, docetaxel, doxifluridine, doxorubicin, epirubicin, epothilone, etoposide, fluorouracil, gemcitabine, hydroxyurea, idarubicin, imatinib, irinotecan, mechlorethamine, mercaptopurine, methotrexate, mitoxantrone, oxaliplatin, paclitaxel, pemetrexed, teniposide, tioguanine, topotecan, valrubicin, vemurafenib, vinblastine, vincristine, and vindesine.

159. A method of producing a T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate, the method comprising:

introducing one or more nucleic acids encoding an unconjugated T-Cell-MP into cells in vitro or ex vivo;

optionally selecting for cells that produce the unconjugated T-Cell-MP and/or optionally selecting for cells comprising all or part of the one or more nucleic acids either unintegrated or integrated into at least one cellular chromosome; and

conjugating a KRAS epitope (e.g., a peptide expressing a KRAS epitope) to the unconjugated T-Cell-MP.

160. The method of aspect 159, wherein the cells are cells of a mammalian cell line selected from HeLa cells, CHO cells, 293 cells, Vero cells, NIH 3T3 cells, Huh-7 cells, BHK cells, PC12 cells, COS cells, COS-7 cells, RAT1 cells, mouse L cells, human embryonic kidney (HEK) cells, and HLHepG2 cells.

161. The cells of aspect 160, wherein cells express from about 20 to about 200 (e.g., 20-40, 40-80, 80-100, 100-120, 120-140, 140-160. 160-180 or 180-200) mg/liter or more of the T-Cell-MP.

162. The cells of aspect 161, wherein the cells express from about 20 to about 200 mg/liter or more of the T-Cell-MP-without a substantial reduction (less than a 5%, 10%, or 15% reduction) in cell viability relative to otherwise identical cells not expressing the T-Cell-MP or duplex T-Cell-MP.

163. A method of selectively delivering (i) a KRAS epitope and (ii) one or more (e.g., two or more) wt. MOD polypeptides and/or variant MOD polypeptides to one or more cells or tissues of a patient or subject, the method comprising:

(i) administering to a patient/subject (e.g., a patient in need thereof) an effective amount of one or more T-Cell-MP-KRAS-epitope conjugates or duplex T-Cell-MP-KRAS-epitope conjugates of any of aspects 1-145;

wherein at least one T-cell present in a tissue of the patient/subject is selective (e.g., specific) for the KRAS epitope present in the T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate; and

wherein the T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate comprises one or more wt. MODs or variant MODs.

164. The method of aspect 163, wherein the one or more wt. MOD polypeptides and/or variant MOD polypeptides are selected independently from the group consisting of: 4-1BBL, IL-2, CD80, CD86, OX40L (CD252), ICOS-L, ICAM, CD30L, CD40, CD83, HVEM (CD270), JAG1 (CD339), CD70, TGF-β1, TGF-β2, and TGF-β3 wt. MOD or variant MOD polypeptide sequences.

165. The method of aspect 163 or 164, wherein the one or more wt. MOD polypeptides and/or variant MOD polypeptides are selected independently from the group consisting of: 4-1BBL, IL-2, CD80, and CD86 wt. MOD and variant MOD polypeptide sequences of any thereof.

166. The method of any of aspects 163 to 165, wherein the T-Cell-MP or duplex T-Cell-MP comprises at least one wt. IL-2 MOD or variant IL-2 MOD (e.g., comprising an H16A or T substitution and an F42A substitution) polypeptide sequence, and/or at least one CD80, CD86, variant CD80 or variant CD86 polypeptide sequence.

167. The method of any of aspects 163 to 166, wherein the T-Cell-MP or duplex T-Cell-MP comprises at least one wt. IL-2 MOD or variant IL-2 MOD (e.g., comprising an H16A or T substitution and an F42A substitution) polypeptide sequence, or at least one pair of wt. IL-2 MOD or variant IL-2 MOD (e.g., comprising an H16A or T substitution and an F42A substitution) polypeptide sequences in tandem.

168. The method of any of aspects 163 to 167, wherein the T-Cell-MP or duplex T-Cell-MP comprises at least one CD80 and/or CD86 wt. MOD or variant MOD polypeptide sequence.

X. Examples

Example 1

[00434] Nucleic acids were prepared encoding a series of constructs comprising a HLA-A*02:01(HLA-A02) class I heavy chain polypeptide sequence, a human β2M polypeptide sequence, and an IgG scaffold sequence, as core elements of split chain or single chain constructs shown as duplexes in Fig. 12 at A, B and C.

[00435] Each of the split chain constructs (structures A or B) has a first polypeptide sequence that comprises from the N-terminus to the C-terminus tandem human IL-2 polypeptide sequences (2xhIL2) with F42A,H16A substitutions, HLA-A*02:01 (A02) α1, α2, and α3 domains, and a human IgG1

scaffold with L234A and L235A substitutions. The 1694 first polypeptide appearing in most of the split chain constructs comprises an A236C,Y84C and A139C substitutions 2xhIL2 (F42A, H16A)-$(G_4S)_4$-HLA-A02 (A236C, Y84C, A139C)-AAAGG-IgG1 (L234A, L235A): APTSSSTKKTQLQLEALLLDLQ MILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLIS NINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGGS*** APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELK PLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL T***GGGGSGGGGSGGGGSGGGGS***GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQ RMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRG***C***YNQSEAGSHTVQRMYGCDVGS DWRFLRGYHQYAYDGKDYIALKEDLRSWTAADM***C***AQTTKHKWEAAHVAEQLRAYLEGTCVE WLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTE LVETRP***C***GDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWE***AAAGG***DKTHTCPPCPAP EAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK (linker sequences are indicated in bold and italics) (SEQ ID NO:193).

[00436] The 4008 polypeptide appearing in two split chain constructs parallels the 1694 construct, but comprises A236C, Y85C, and D137C substitutions in the HLA-A02 sequence - 2xhIL2 (F42A, H16A)-$(G_4S)_4$-HLA-A02 (A236C, Y85C, D137C)-AAAGG-IgG1 (L234A, L235A): APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELK PLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL T***GGGGSGGGGSGGGGSGGGGS***APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAK FYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYA DETATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGGS***GSHSMRYFFTSVSRPGRGEPR FIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRGY CNQSEAGSHTVQRMYGCDVGSDWRFLRGYHQYAYDGKDYIALKEDLRSWTAA***C***MAAQTTKH KWEAAHVAEQLRAYLEGTCVEWLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALS FYPAEITLTWQRDGEDQTQDTELVETRPCGDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPL TLRWE***AAAGG***DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:194).

[00437] Each of the split chain constructs (structures A and B) in FIG. 12 comprises a second polypeptide comprising a β2M polypeptide sequence having R12C and E44C substitutions: IQRTPKIQVYSCHPAENGKSNFLNCYVSGFHPSDIEVDLLKNGCRIEKVEHSDLSFSKDWSFYLLY YTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDM (SEQ ID NO:195); to which either the indicated

linker, or CMV epitope peptide NLVPMVATV (SEQ ID NO:196) and linker, is added at their N-termini as indicated in the table provided below.

[00438] The unconjugated T-Cell-MP conjugates listed in FIG 12 each comprise as a single polypeptide chain from N-terminus to C-terminus IL-2, β2M, HLA-A*02:01 (A02) α1, α2, and α3 domains, and human IgG1 polypeptide sequences. The aa sequence of the 3861 construct is provided below, and the remainder of the single chain T-Cell-MP constructs may be considered variations of the 3861 construct, which has tandem 2xIL-2 sequences with F42A and H16A substitutions- a $(G_4S)_4$ linker-β2M (E44C)-a $(G_4S)_3$ linker-HLA-A02 with Y84C, A139C- an AAAGG (SEQ ID NO:122) linker-and an IgG1 with L234A and L235A substitutions: APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAK FYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYA DETATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGGS***APTSSSTKKTQLQLEALLLDL QMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDL ISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGG S***IQRTPKIQVYSRHPAENGKSNFLNCYVSGFHPSDIEVDLLKNGCRIEKVEHSDLSFSKDWSFYLL YYTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDM***GGGGSGGGGSGGGGS***GSHSMRYFFTSVSR PGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVD LGTLRGCYNQSEAGSHTVQRMYGCDVGSDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADM CAQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRYLENGKETLQRTDAPKTHMTHHAVSDHEAT LRCWALSFYPAEITLTWQRDGEDQTQDTELVETRPAGDGTFQKWAAVVVPSGQEQRYTCHVQH EGLPKPLTLRWE***AAAGG***DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA PIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:197).

[00439] Where tandem IL-2 sequences are present in the constructs of this example, they are separated by a $(G_4S)_4$ linker. Each of the sequences other than 3861 has variations in the linkers present between the IL-2 and β2M, and/or β2M and HLA-A02 sequences (the L3 linker) as indicated. Additionally, construct 3984 has only a single IL-2 sequence, and each of 3999-4002 have an additional aa substitution in the HLA-A02 polypeptide sequence as indicated in the table that follows.

Construct		Form in FIG. 12	Construct Content and Organization
Split Chain Construct).			
1694	2686	B	β2M (R12C, E44C)
	839	A	CMV-GGGASGGGGSGGGGS-β2M (R12C)
	3993	B	GM-$(G_4S)_3$-β2M (R12C, E44C)
	3994	B	GMGGS-$(G_4S)_2$-β2M (R12C, E44C)
	3995	B	GMS-(G_4S)2-β2M (R12C, E44C)
	3996	B	GMGGGS-(G_4S)-β2M (R12C, E44C)
	3997	B	GMGS-(G_4S)-β2M (R12C, E44C)
	3998	B	GM-(G_4S)-β2M (R12C, E44C)

Construct		Form in FIG. 12	Construct Content and Organization
4008	4002	A	CMV-(G_3AS)-(G4S)$_2$-β2M (R12C, E44C)
	4003	A	CMV-(G_3AS)-(G_4S)-FC1-(G_4S)-β2M (R12C, E44C)
	839	A	CMV-GGGASGGGGSGGGGS-β2M (R12C)
	2686	B	β2M (R12C, E44C)
Unconjugated T-Cell-MP (Single Chain Construct)			
3984		C	1xhIL2(F42A, H16A)-(G_4S)$_4$-β2M (E44C)-(G_4S)$_3$-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)
3985		C	hIL2 (F42A, H16A)-(G_4S)$_4$-hIL2 (F42A, H16A)-(G_4S)-GLGGS-(G_4S)$_2$-β2M (E44C)-(G_4S)$_3$-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)
3986		C	hIL2 (F42A, H16A)-(G_4S)$_4$-hIL2 (F42A, H16A)-(G_4S)$_2$-GLGGS-(G_4S)-β2M (E44C)-(G_4S)$_3$-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)
3987		C	hIL2 (F42A, H16A)-(G_4S)$_4$-hIL2 (F42A, H16A)-(G_4S)$_3$-GLGGS-β2M (E44C)-(G_4S)$_3$-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)
3988		C	hIL2 (F42A, H16A)-(G_4S)$_4$-hIL2 (F42A, H16A)-(G_4S)-GMGGSGGGGS-(G_4S)-β2M (E44C)-(G_4S)$_3$-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)
3989		C	hIL2 (F42A, H16A)-(G_4S)$_4$-hIL2 (F42A, H16A)-(G_4S)-GGGMSGGGGS-(G_4S)-β2M (E44C)-(G_4S)$_3$-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)
3990		C	hIL2 (F42A, H16A)-(G_4S)$_4$-hIL2 (F42A, H16A)-(G4S)-GGGGSMGGGS-(G4S)-β2M (E44C)-(G4S)3-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)
3991		C	hIL2 (F42A, H16A)-(G_4S)$_4$-hIL2 (F42A, H16A)-(G_4S)-GGGGSGMGGS-(G_4S)-β2M (E44C)-(G_4S)$_3$-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)
3992		C	hIL2 (F42A, H16A)-(G_4S)$_4$-hIL2 (F42A, H16A)-(G_4S)-GGGGSGGGGM-(G_4S)-β2M (E44C)-(G_4S)$_3$-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)
3861		C	hIL2 (F42A, H16A)-(G_4S)$_4$-hIL2 (F42A, H16A)-(G_4S)$_4$-β2M (E44C)-(G_4S)$_3$-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)
4004		C	hIL2 (F42A, H16A)-(G_4S)$_4$-hIL2 (F42A, H16A)-(G_4S)$_5$-β2M (E44C)-(G_4S)$_3$-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)
4005		C	hIL2 (F42A, H16A)-(G_4S)$_4$-hIL2 (F42A, H16A)-(G_4S)$_6$-β2M (E44C)-(G_4S)$_3$-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)
4006		C	hIL2 (F42A, H16A)-(G_4S)$_4$-hIL2 (F42A, H16A)-(G_4S)$_4$-β2M (E44C)-(G_4S)$_2$-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)
4007		C	hIL2 (F42A, H16A)-(G_4S)$_4$-hIL2 (F42A, H16A)-(G_4S)$_4$-β2M (E44C)-(G_4S)-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)
3999		C	hIL2 (F42A, H16A)-(G_4S)$_4$-hIL2 (F42A, H16A)-(G_4S)$_4$-β2M (E44C)-(G_4S)$_3$-HLA-A02(Y84C, A139C, T143L)-AAAGG-IgG1(L234A, L235A)

Construct	Form in FIG. 12	Construct Content and Organization
4000	C	hIL2 (F42A, H16A)-$(G_4S)_4$-hIL2 (F42A, H16A)-$(G_4S)_4$-β2M (E44C)-$(G_4S)_3$-HLA-A02(Y84C, A139C, T143M)-AAAGG-IgG1(L234A, L235A)
4001	C	hIL2 (F42A, H16A)-$(G_4S)_4$-hIL2 (F42A, H16A)-$(G_4S)_4$-β2M (E44C)-$(G_4S)_3$-HLA-A02(L81M, Y84C, A139C)-AAAGG-IgG1(L234A, L235A)

[00440] The nucleic acids encoding the protein constructs were transfected into and expressed by CHO cells as soluble protein in the culture media. The level of protein expressed in the culture media after 7days was determined by BLI assay using protein A to capture the expressed protein (FIG. 12 at D). The fraction of protein appearing in unaggregated duplex form is assessed by isolating the protein from the culture media using magnetic protein A beads. After washing, the bound protein is eluted from the beads by reducing the pH, and then subject to analytical size exclusion chromatography using UV detection on an AGILENT® chromatography system. The fraction of unaggregated protein reported in FIG. 12 at E is based on the area of the peak corresponding to the molecular weight of the duplex relative to the total area of the chromatographed protein.

[00441] The results indicate that unconjugated single chain T-Cell-MP constructs appear to be expressed more uniformly at higher levels than their unconjugated split chain construct counterparts.

Example 2

[00442] The effect of time in culture, cell culture density, and culture temperature on unconjugated T-Cell-MPs was examined by transiently expressing the construct 3861 (see Example 1) in CHO cells at 28 and 32 °C. Transfection was accomplished with expiCHO® transfection kits (Gibco™/ ThermoFisher Scientific, Skokie, IL) using a recombinant pTT5 vector into which the cassette encoding the polypeptide was cloned. The transfected cells were diluted to 2, 4 or 6 million cells per milliliter and T-Cell-MP 3861expression levels and the fraction of unaggregated protein in duplex form determined at days 2, 4, 7, and/or 9 as indicated by removing a portion of the culture. Analyses were conducted as in Example 1 and are shown in FIG. 13 (at A and B) with the number of cells and culture temperature shown below each histogram set (e.g., six million cells at 32 °C denoted as 6M/32C). Also shown in Fig. 13 are size exclusion chromatograms (C and D) of the unconjugated 3861 T-Cell-MP harvested from a culture using protein A and after further purification by size exclusion chromatography (upper and lower chromatograms respectively). Coomassie blue stained SDS PAGE analysis (at E) confirms the purity and homogeneity of the purified material, samples of which were applied to the gel in reduced (R) and non-reduced form (NR).

Example 3

[00443] The specific interaction of T-Cell-MP-epitope conjugates and control constructs with epitope specific T cells was assessed by incubating the molecules with T cells responsive to either the CMV peptide NLVPMVATV SEQ ID NO:196) (black bars) or the Melan-A and Mucin Related Peptide (MART-1) ELAGIGILTV (SEQ ID NO:198) (white bars) in the histogram of Elispot data provided FIG.

14A. Control samples of the unconjugated 3861 T-Cell-MP duplex (see FIG. 12 at C for the general structure) group 1, and an unconjugated split chain construct comprising polypeptides 1694 and 2686 (duplexed as in FIG. 12 at B) group 2 were run in parallel with test samples. T-Cell-MP and split chain constructs conjugated to the E44C position of β2M through a $(G_4S)_3$ linker by a maleimide group are shown in groups 3 and 4. The effect of control construct split chain fusion proteins (FIG 12 structure A) having a CMV or MART-1 polypeptide as part of the fusion protein are shown in groups 5 and 6 respectively. Control stimulation by CMV and MART-1 peptides is shown in groups 7 and 8 respectively. The histogram indicates the number of spots due to captured interferon gamma indicating activation of the T cells by the treatments.

[00444] The SDS-PAGE gel shown in FIG. 14 B provides an analysis of reducing and non-reducing samples of the epitope conjugates and fusion proteins, indicating their purity and homogeneity.

Example 4

[00445] Ficoll-Paque® purified samples of leukocytes from CMV responsive donors (Donors 8, 10, 38, and 39) and MART-1 responsive donors (Donors 17 and 18) were prepared and used to demonstrate the ability of T-Cell-MP-epitope conjugates to expand T cells specific to CMV or MART-1 specific epitopes. MART-1 responsive Donor 18 also displays some responsiveness to the CMV peptide. Positive and negative control treatments included: treatment with split chain constructs conjugated to CMV and MART-1 peptides; treatment with the CMV or MART-1 peptides in culture media; and media only control treatment. For the experiments, leukocytes were suspended at 2.5 x 10^6 cells per ml in ImmunoCult™ media (Stemcell Technologies, Vancouver, British Columbia) containing the indicated amounts of the control or T-Cell-MP-epitope conjugate or control treatments. After 10 days in culture the number of cells responsive to CMV or MART-1 were assessed by Flow cytometry using CMV or MART-1 tetramers purchased from MBL International Corp. The results indicate that both the T-Cell-MP and split chain constructs conjugated to the CMV peptide, and to a lesser degree CMV peptide, stimulate expansion of CMV specific T cells from CMV responsive donors in a concentration dependent manner. T-Cell-MP and split chain constructs conjugated to the MART-1 peptide, and to a lesser degree the MART-1 peptide stimulate expansion of MART-1 specific T cells from MART-1 responsive donors in a concentration dependent manner. In each instance, CMV peptide conjugates selectively stimulated T cells from CMV responsive donors but not MART-1 responsive donors and vice versa. Free peptide in the absence of IL-2 failed to produce an effect the was equal to the effect observed with the T-Cell-MP-epitope conjugates. Results are provided in Fig. 15.

[00446] The T-Cell-MP-epitope conjugate employed for the assays was a duplex of the 3186 polypeptide (see Example 1 and FIG. 12 structure C for the general form of the unconjugated duplex) conjugated at a cysteine (E44C) in the β2M polypeptide sequence to a either a CMV (NLVPMVATV, SEQ ID NO:196) or MART-1 (ELAGIGILTV, SEQ ID NO:198) peptide via a $(G_4S)_3$ linker bearing a maleimide group (e.g., for the CMV peptide NLVPMVATV-$(G_4S)_3$-lysine-epsilon amino-maleimide). The split chain epitope conjugate was a duplex of two split chain constructs each comprising a1694 and 2686 polypeptide (see Example 1 and FIG. 12 structure B for the general form of the unconjugated duplex), which was

conjugated at a cysteine (E44C) in the β2M polypeptide sequence to a either a CMV (NLVPMVATV, SEQ ID NO:196) or MART-1 (ELAGIGILTV, SEQ ID NO:198) peptide via a $(G_4S)_3$ linker bearing a maleimide group. After reduction to remove any capping from the cysteine conjugation sites, the conjugation was conducted as described in for maleimide coupling reactions using at least two additions of the peptide bearing a maleimide group.

[00447] In an additional test, the effect of a construct bearing a $(G_4S)_7$ L3 linker (the linker between the β2M and HLA-A02 sequences), but otherwise identical to 3861, was compared with the 3861 polypeptide duplex (i.e., construct 4125 2xIL2(F42A, H16A)-$(G_4S)_7$-β2M (E44C)-$(G_4S)_3$-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)). Duplexes of both the 3861 and 4125 constructs were conjugated to a CMV or MART-1 peptide by a maleimide terminated $(G_4S)_3$ linker and tested side-by-side for the ability to expand T cells in an epitope specific manner. The assays were conducted as described above for the 3861 epitope conjugates, except only a media alone control was conducted. The results, shown in FIG. 16, indicate that extending the linker length did not substantively alter the expansion of T cells seen with the 3861 epitope conjugates.

Example 5

[00448] In order to examine the effect of L3 linker length on the level of cell expression and the quality (fraction unaggregated) of T-Cell-MP proteins a series of nucleic acids encoding constructs 4125 through 4128 that are related to construct 3861 but with L3 linkers of increasing length were prepared and inserted into an expression vector (pTT5). A second set of constructs (4129-4133) bearing an additional R12C substitution in the β2M polypeptide (R12C, E44C) and an A236C substitution in the HLA-A02 peptide that can form an interchain disulfide bond was also prepared. The vectors were transfected into CHO cells with expiCHO® transfection kits and both the amount of protein expressed in the culture media and the fraction of unaggregated protein after purification using magnetic beads was assessed at days 4, 6, 8, and/or 11 as indicated. The specific constructs included those recited in the following table.

Con-struct	Form in FIG.12		L3 linker $(G_4S)n$	Construct Content and Organization
3861	C		n=3	hIL2 (F42A, H16A)-$(G_4S)_4$-hIL2 (F42A, H16A)-$(G_4S)_4$-β2M (E44C)-$(G_4S)_3$-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)
4128	C		n=4	hIL2 (F42A, H16A)-$(G_4S)_4$-hIL2 (F42A, H16A)-$(G_4S)_4$-β2M (E44C)-$(G_4S)_4$-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)
4127	C		n=5	hIL2 (F42A, H16A)-$(G_4S)_4$-hIL2 (F42A, H16A)-$(G_4S)_4$-β2M (E44C)-$(G_4S)_5$-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)
4126	C		n=6	hIL2 (F42A, H16A)-$(G_4S)_4$-hIL2 (F42A, H16A)-$(G_4S)_4$-β2M (E44C)-$(G_4S)_6$-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)

Con-struct	Form in FIG.12		L3 linker $(G_4S)n$	Construct Content and Organization
4125	C		n=7	hIL2 (F42A, H16A)-$(G_4S)_4$-hIL2 (F42A, H16A)-$(G_4S)_4$-β2M (E44C)-$(G_4S)_7$-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)
4129	C		n=7	hIL2 (F42A, H16A)-$(G_4S)_4$-hIL2 (F42A, H16A)-$(G_4S)_4$-β2M (R12C, E44C)-$(G_4S)_7$-HLA-A02(Y84C, A139C, A236C)-AAAGG-IgG1(L234A, L235A)
4130	C		n=6	hIL2 (F42A, H16A)-$(G_4S)_4$-hIL2 (F42A, H16A)-$(G_4S)_4$-β2M (R12C, E44C)-$(G_4S)_6$-HLA-A02(Y84C, A139C, A236C)-AAAGG-IgG1(L234A, L235A)
4131	C		n=5	hIL2 (F42A, H16A)-$(G_4S)_4$-hIL2 (F42A, H16A)-$(G_4S)_4$-β2M (R12C, E44C)-$(G_4S)_5$-HLA-A02(Y84C, A139C, A236C)-AAAGG-IgG1(L234A, L235A)
4132	C		n=4	hIL2 (F42A, H16A)-$(G_4S)_4$-hIL2 (F42A, H16A)-$(G_4S)_4$-β2M (R12C, E44C)-$(G_4S)_4$-HLA-A02(Y84C, A139C, A236C)-AAAGG-IgG1(L234A, L235A)
4133	C		n=3	hIL2 (F42A, H16A)-$(G_4S)_4$-hIL2 (F42A, H16A)-$(G_4S)_4$-β2M (R12C, E44C)-$(G_4S)_3$-HLA-A02(Y84C, A139C, A236C)-AAAGG-IgG1(L234A, L235A)

[00449] The amount of the expressed unconjugated T-Cell-MP constructs were determined by BLI assay using protein A for capture on a BioForte instrument using the methods described in Example 1. Results are provided in FIG. 17 histogram A.

[00450] The fraction of unconjugated T-Cell-MP that is unaggregated (present in duplex form) after purification on magnetic protein A beads was determined by size exclusion chromatography. The fraction was determined using the area of the chromatographic peak corresponding to the molecular weight of the duplex relative to the area under the chromatogram as described in Example 1. Results are shown in FIG. 17 histogram B.

[00451] Additional optimization indicates that higher yields are possible. Construct 4125 has been observed to reach 200mg/ml and construct 4127 has been observed to reach 170 mg/ml in CHO culture cell media prior to isolation.

What is Claimed Is:

1. A T cell modulatory polypeptide (T-Cell-MP) KRAS-epitope conjugate (T-Cell-MP-KRAS-epitope conjugate), the T-Cell-MP polypeptide comprising:

(i) optionally one or more MOD polypeptide sequences, or two or more MOD polypeptide sequences joined to each other by an independently selected L1 linker;

(ii) optionally an L2 linker polypeptide sequence joining the one or more MOD polypeptide sequences to a β2M polypeptide sequence;

(iii) the β2M polypeptide sequence;

(iv) an L3 linker polypeptide sequence from 10-50 aa in length;

(v) a class I MHC-H polypeptide sequence;

(vi) an L4 linker polypeptide sequence;

(vii) a scaffold polypeptide sequence;

(viii) an optional L5 linker polypeptide sequence; and

(ix) optionally one or more MOD polypeptide sequences;

wherein the T-Cell-MP-KRAS-epitope conjugate comprises at least one MOD polypeptide sequence; and

wherein at least one of the β2M polypeptide sequence, L3 linker polypeptide sequence, and/or MHC-H polypeptide sequence comprises one or more chemical conjugation sites at which a KRAS epitope presenting molecule (KRAS epitope) is covalently attached either directly, or indirectly through a linker, to form a T-Cell-MP-KRAS-epitope conjugate, and

optionally wherein one or more MOD polypeptide sequences comprises a wt. IL-2 or a variant of wt. IL-2.

2. The T-Cell-MP-KRAS-epitope conjugate of claim 1, the T-Cell-MP polypeptide comprising from N-terminus to C-terminus:

(i) optionally one or more MOD polypeptide sequences, or two or more MOD polypeptide sequences joined to each other by an independently selected L1 linker;

(ii) optionally an L2 linker polypeptide sequence joining the one or more MOD polypeptide sequences to a β2M polypeptide sequence;

(iii) the β2M polypeptide sequence;

(iv) an L3 linker polypeptide sequence from 10-50 aa in length;

(v) a class I MHC-H polypeptide sequence;

(vi) an L4 linker polypeptide sequence;

(vii) a scaffold polypeptide sequence;

(viii) an optional L5 linker polypeptide sequence; and

(ix) optionally one or more MOD polypeptide sequences;

wherein the T-Cell-MP-KRAS-epitope conjugate comprises at least one MOD polypeptide sequence; and

wherein the chemical conjugation site at which the KRAS epitope is covalently attached is within the β2M polypeptide sequence, L3 linker polypeptide sequence, and/or MHC-H polypeptide sequence.

3. The T-Cell-MP-KRAS-epitope conjugate of claim 1, the polypeptide comprising from N-terminus to C-terminus:

(i) one or more MOD polypeptide sequences, or two or more MOD polypeptide sequences, in tandem, wherein when there are two or more MOD polypeptide sequences they are optionally joined to each other by independently selected L1 linkers;

(ii) an optional L2 linker polypeptide sequence;

(iii) a β2M polypeptide sequence;

(iv) an L3 linker polypeptide sequence from 10-50 aa in length;

(v) a class I MHC-H polypeptide sequence, wherein the MHC-H polypeptide comprises cysteine substitutions at positions 84 and 139 that form an intrachain disulfide;

(vi) an optional L4 linker polypeptide sequence;

(vii) a scaffold polypeptide sequence;

(viii) an optional L5 linker polypeptide sequence; and

(ix) optionally one or more MOD polypeptide sequences, or two or more MOD polypeptide sequences in tandem, wherein when there are two or more MOD polypeptide sequences they are optionally joined to each other by independently selected L6 linkers;

wherein the unconjugated T cell modulatory polypeptide comprises at least one MOD polypeptide sequence as part of element (i) or (ix).

4. The T-Cell-MP-KRAS-epitope conjugate of claim 1, the polypeptide comprising from N-terminus to C-terminus:

(i) optionally one or more MOD polypeptide sequences, or two or more MOD polypeptide sequences in tandem, wherein, when there are two or more MOD polypeptide sequences, they are optionally joined to each other by independently selected L1 linkers;

(ii) an optional L2 linker polypeptide sequence;

(iii) a β2M polypeptide sequence;

(iv) an L3 linker polypeptide sequence from 10-50 aa in length;

(v) a class I MHC-H polypeptide sequence, wherein the MHC-H polypeptide comprises cysteine substitutions at positions 84 and 139 that form an intrachain disulfide;

(vi) an optional L4 linker polypeptide sequence;

(vii) a scaffold polypeptide sequence;

(viii) an optional L5 linker polypeptide sequence; and

(ix) one or more MOD polypeptide sequences, or two or more MOD polypeptide sequences in tandem, wherein, when there are two or more MOD polypeptide sequences, they are optionally joined to each other by independently selected L6 linkers;

wherein the unconjugated T cell modulatory polypeptide comprises at least one MOD polypeptide sequence as part of element (i) or (ix).

5. The T-Cell-MP-KRAS-epitope conjugate of any preceding claim, wherein at least one of the β2M polypeptide sequence, L3 linker polypeptide sequence, and/or MHC-H polypeptide sequence comprises a chemical conjugation site where the KRAS epitope is conjugated.

6. The T-Cell-MP-KRAS-epitope conjugate of claim 5, wherein the β2M polypeptide sequence or L3 linker polypeptide sequence comprises a chemical conjugation site at which the KRAS epitope is conjugated.

7. The T-Cell-MP-KRAS-epitope conjugate of claim 6, wherein the β2M polypeptide sequence has at least 90% sequence identity to at least 70 contiguous aas of the mature human β2M polypeptide NP_004039.1.

8. The T-Cell-MP-KRAS-epitope conjugate of claim 7, wherein:
the MHC-H polypeptide sequence comprises a human class I MHC-H chain polypeptide sequence selected from HLA-A, HLA-B, HLA-C, HLA-E, and HLA-G MHC-H polypeptide sequences having at least 85% sequence identity to at least 200 contiguous aas of a MHC-H polypeptide provided in any of SEQ ID NOs:18-58 and 60.

9. The T-Cell-MP-KRAS-epitope conjugate of claim 8, wherein the MHC-H polypeptide sequence comprises a disulfide bond between position 84 (e.g., Y84C) and position 139 (e.g., A139C).

10. The T-Cell-MP-KRAS-epitope conjugate of claim 9, wherein the MHC-H polypeptide sequence has at least 85% sequence identity to at least 200 contiguous aas of HLA-A*0101 (SEQ ID NO:24), HLA-A*0201 (SEQ ID NO:27), HLA-A*0301 (SEQ ID NO:35), HLA-A*1101 (SEQ ID NO:32), HLA-A*2301 (SEQ ID NO:36), HLA-A*2402 (SEQ ID NO:33), HLA-A*2407 (SEQ ID NO:37), HLA-A*3303 (SEQ ID NO:34), or HLA-A*3401 (SEQ ID NO:38).

11. The T-Cell-MP-KRAS-epitope conjugate of claim 6, wherein:
the MHC-H polypeptide sequence comprises an HLA-F polypeptide sequence having at least 85% sequence identity to at least 200 contiguous aas of an MHC-H polypeptide provided in SEQ ID NO:59.

12. T-Cell-MP-KRAS-epitope conjugate of claim 11, wherein the MHC-H polypeptide sequence comprises a disulfide bond between position 84 (R84C) and position 139 (V139C).

13. The T-Cell-MP-KRAS-epitope conjugate of claim 10, comprising at least one, at least two, or at least three, wt. MOD or variant MOD polypeptide sequences, and wherein each MOD is selected independently from the group consisting of 4-1BBL, IL-2, CD80, and CD86 wt. MOD or variant MOD polypeptide sequences.

14. The T-Cell-MP-KRAS-epitope conjugate of claim 13, comprising at least one wt. or variant IL-2 MOD polypeptide sequence, or at least one pair of wt. or variant IL-2 MOD polypeptide sequences in tandem.

15. The T-Cell-MP-KRAS-epitope conjugate of claim 14, wherein the T-Cell-MP-KRAS-epitope conjugate comprises a single variant IL-2 MOD or a pair of variant IL-2 MOD polypeptide sequences in tandem, and where the variant IL-2 MOD polypeptide sequences comprise F42A and H16A or T substitutions.

16. The T-Cell-MP-KRAS-epitope conjugate ofclaim 13, wherein the scaffold polypeptide sequence is an interspecific or non-interspecific polypeptide sequence selected from the group consisting of: an immunoglobulin heavy chain constant region; a collectin polypeptide, a coiled-coil domain, a leucine-zipper domain, a Fos polypeptide, a Jun polypeptide, an Ig CH1 polypeptide, an Ig C_L κ polypeptide, an Ig C_L λ polypeptide, a knob-in-hole without disulfide (KiH) polypeptide, a knob-in hole with a stabilizing disulfide bond (KiHs-s) polypeptide, an HA-TF polypeptide, a ZW-1 polypeptide, a 7.8.60 polypeptide, a DD-KK polypeptide, an EW-RVT polypeptide, an EW-RVTs-s polypeptide, and an A107 polypeptide sequence.

17. The T-Cell-MP-KRAS-epitope conjugate of claim 16, complexed to form a duplex T-Cell-MP-KRAS-epitope conjugate, the duplex comprising:

at least a first unconjugated T-Cell-MP and a second unconjugated T-Cell-MP of any preceding claim, wherein

(i) the first unconjugated T-Cell-MP comprises a first β2M polypeptide sequence; a first class I MHC-H polypeptide sequence; and a first scaffold polypeptide sequence; and

(ii) the second unconjugated T-Cell-MP comprises a second β2M polypeptide sequence; a second class I MHC-H polypeptide sequence; and a second scaffold polypeptide sequence; and

wherein the first and second unconjugated T-Cell-MPs associate by binding interactions between the first and second scaffold polypeptide sequences that optionally include one or more interchain covalent bonds therebetween.

18. The duplex T-Cell-MP-KRAS-epitope conjugate of claim 17, wherein the scaffold sequences are immunoglobulin heavy chain constant region polypeptide sequences.

19. The duplex T-Cell-MP-KRAS-epitope conjugate of claim 17, wherein the first and second scaffold sequences are an interspecific pair of sequences selected from the group consisting of: KIH polypeptide sequences, KIHs-s polypeptide sequences, EW-RVT polypeptide sequences, EW-RVTs-s polypeptide sequences, HA-TF polypeptide sequences, ZW-1 polypeptide sequences, 7.8.60 polypeptide sequences, DD-KK polypeptide sequences, and A107 polypeptide sequence.

20. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of claim 17, further comprising one or more substitutions that reduce binding to the Fc λ receptor and/or the C1q protein.

21. TheT-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of claim 17, wherein each chemical conjugation site is jointly or independently selected from: a) amino acid

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

22. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of claim 17, wherein each chemical conjugation site for an epitope conjugation is a cysteine introduced by protein engineering.

23. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of claim 22, wherein the cysteine introduced by protein engineering is within the β2M polypeptide sequence.

24. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of claim 22, wherein the cysteine introduced by protein engineering is within the β2M polypeptide sequence at position 44 of a mature β2M polypeptide sequence of one of SEQ ID NOs:61-65.

25. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of claim 17, further comprising one or more targeting sequences.

26. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of claim 22, wherein the KRAS epitope is directly or indirectly conjugated to a cysteine in the β2M polypeptide sequence via a bond formed between the cysteine and a maleimide group attached to the epitope.

27. The T-Cell-MP-KRAS-epitope conjugate or duplex T-Cell-MP-KRAS-epitope conjugate of claim 26, wherein the KRAS epitope is selected from the group consisting of: VVGA**D**GVGK (SEQ ID NO:139), VVGA**C**GVGK (SEQ ID NO:140), VVGA**V**GVGK (SEQ ID NO:141), VVVGA**D**GVGK (SEQ ID NO:142), VVVGA**C**GVGK (SEQ ID NO:144), VVVGA**V**GVGK (SEQ ID NO:143), V**T**GA**D**GVGK (SEQ ID NO:145), V**T**GA**C**GVGK (SEQ ID NO:147), V**T**GA**V**GVGK (SEQ ID NO:146), V**T**VGA**D**GVGK (SEQ ID NO:148), V**T**VGA**C**GVGK (SEQ ID NO:150), V**T**VGA**V**GVGK (SEQ ID NO:149), LVVVGA**D**GV (SEQ ID NO:155), LVVVGA**V**GV (SEQ ID NO:156), LVVVGA**C**GV (SEQ ID NO:157), KLVVVGA**D**GV (SEQ ID NO:158), KLVVVGA**V**GV (SEQ ID NO:159), KLVVVGA**C**GV (SEQ ID NO:160), L**L**VVGA**D**GV (SEQ ID NO:161), L**L**VVGA**V**GV (SEQ ID NO:162), L**L**VVGA**C**GV (SEQ ID NO:163), **F**LVVVGA**D**GV (SEQ ID NO:164), **F**LVVVGA**V**GV (SEQ ID NO:165), **F**LVVVGA**C**GV (SEQ ID NO:188), KLVVGADGV (SEQ ID NO:189) , KLVVGAVGV (SEQ ID NO:190) , KLVVVAVGV (SEQ ID NO:191) , and KLVVVADGV (SEQ ID NO:192).

28. A method of treating disease comprising administering to a subject in need thereof an effective amount of a T-Cell-MP-KRAS-epitope conjugate of claim 22.

29. The method of claim 28, wherein the subject is suffering from a cancer.

30. The method of claim 29, wherein the cancer is selected from the group consisting of: non-small cell lung cancer, lung adenocarcinoma, mucinous adenoma, ductal carcinoma of the pancreas, colorectal cancer, and leukemia.

31. The method of claim 29, further comprising administering an immune checkpoint inhibitor.

32. The method of claim 31, wherein the immune checkpoint inhibitor is an antibody specific for PD-L1, PD-1, or CTLA4.

33. The method of claim 29, further comprising administering a KRAS(G12C) inhibitor (e.g., Sotorasib or MRTX849).

FIG. 1

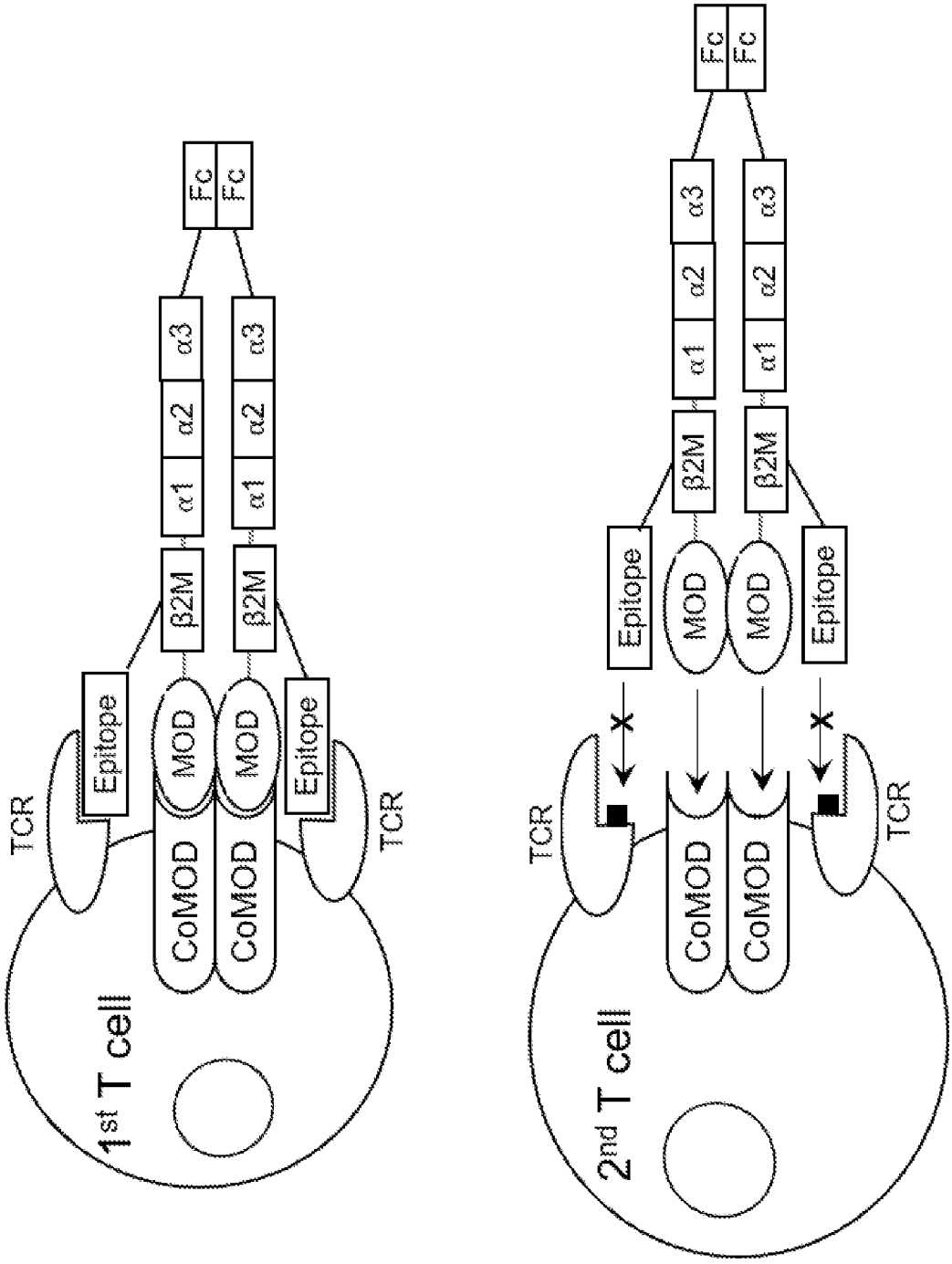

TCR
Epitope
MOD
MOD
Epitope
TCR
β2M
β2M
α1
α2
α3
α1
α2
α3
Fc
Fc
CoMOD
CoMOD
1st T cell
TCR
Epitope
MOD
MOD
Epitope
TCR
β2M
β2M
α1
α2
α3
α1
α2
α3
Fc
Fc
CoMOD
CoMOD
2nd T cell

FIG. 2A

***Homo sapiens* IgA (partial sequence) GenBank P01876 (SEQ ID NO:1)**

```
1   ASPTSPVFP LSLCSTQPDG NVVIACLVQG FFPQEPLSVT WSESGQGVTA RNFPPSQDAS
61  GDLYTTSSQL TLPATQCLAG KSVTCHVKHY TNPSQDVTVP CPVPSTPPTP SPSTPPTPSP
121 SCCHPRLSLH RPALEDLLLG SEANLTCTLT GLRDASGVTF TWTPSSGKSA VQGPPERDLC
181 GCYSVSSVLP GCAEPWNHGK TFTCTAAYPE SKTPLTATLS KSGNTFRPEV HLLPPPSEEL
241 ALNELVTLTC LARGFSPKDV LVRWLQGSQE LPREKYLTWA SRQEPSQGTT TFAVTSILRV
301 AAEDWKKGDT FSCMVGHEAL PLAFTQKTID RLAGKPTHVN VSVVMAEVDG TCY
```

CH1 aas 6-98, Hinge aas 99-121, CH2 aas 122-219, CH3 aas 220-253, (Fc aas 124-353, 210 aa)

FIG. 2B

***Homo sapiens* IgD (partial sequence) GenBank AAA52770 (SEQ ID NO:2)**

```
1   PTKAPDVFPI ISGCRHPKDN SPVVLACLIT GYHPTSVTVT WYMGTQSQPQ RTFPEIQRRD
61  SYYMTSSQLS TPLQQWRQGE YKCVVQHTAS KSKKEIFRWP ESPKAQASSV PTAQPQAEGS
121 LAKATTAPAT TRNTGRGGEE KKKEKEKEEQ EERETKTPEC PSHTQPLGVY LLTPAVQDLW
181 LRDKATFTCF VVGSDLKDAH LTWEVAGKVP TGGVEEGLLE RHSNGSQSQH SRLTLPRSLW
241 NAGTSVTCTL NHPSLPPQRL MALREPAAQA PVKLSLNLLA SSDPPEAASW LLCEVSGFSP
301 PNILLMWLED QREVNTSGFA PARPPPQPRS TTFWAWSVLR VPAPPSPQPA TYTCVVSHED
361 SRTLLNASRS LEVSYVTDHG PMK
```

CH1 aas _1-97, Hinge aas 98-161, CH2 aas 162-267, CH3 aas 268-383 (Fc aas 162-383, 222 aas)

FIG. 2C

***Homo sapiens* IgE (partial sequence) GenBank 1F6A_B) (SEQ ID NO:3)**

```
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
```

CH2 aas 5-114, CH3 aas 113-222 (Fc aas 6-222, 217aas)

FIG. 2D

Wild Type (WT.) Homo sapiens IgG1 (partial sequence) (SEQ ID NO:4)

```
1   DKTHTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
61  GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK
121 GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS
181 DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK
```

Hinge aas 1-10, CH2 aas 11-120, CH3 aas 121-227 (Fc aas 11-227, 217 aas)

***Homo sapiens* IgG1 (partial sequence) Fc GenBank 3S7G_A (SEQ ID NO:5)**

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

Hinge aas 1-10, CH2 aas 11-120, CH3 aas 121-227 (Fc aas 11-227, 217 aas)

***Homo sapiens* IgG1 (partial sequence) Fc Mutant: L234F/L235E/P331S (Triple Mutant "TM") (SEQ ID NO:6)**

```
DKTHTCPPCP APEFEGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA SIEKTISKAK
GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS
DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK
```

Hinge aas 1-10, CH2 aas 11-120, CH3 aas 121-227 (Fc aas 11-227, 217 aas)

FIG. 2D (continued)

***Homo sapiens* IgG1 (partial sequence) Fc Mutant: N297A (SEQ ID NO:7)**

DKTHTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
GVEVHNAKTK PREEQY**A**STY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK
GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS
DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK

Hinge aas 1-10, CH2 aas 11-120, CH3 aas 121-227 (Fc aas 11-227, 217 aas)

***Homo sapiens* IgG1 (partial sequence) Fc Mutant: L234A/L235A ("LALA") (SEQ ID NO:8)**

DKTHTCPPCP APE**AA**GGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK
GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS
DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK

Hinge aas 1-10, CH2 aas 11-120, CH3 aas 121-227 (Fc aas 11-227, 217 aas)

FIG. 2E

***Homo sapiens* IgG2 (partial sequence) GenBank AAN76044 (SEQ ID NO:9)**

1 STKGPSVFPL APCSRSTSES TAALGCLVKD YFPEPVTVSW NSGALTSGVH TFPAVLQSSG
61 LYSLSSVVTV PSSNFGTQTY TCNVDHKPSN TKVDKTVERK CCVECPPCPA PPVAGPSVFL
121 FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VQFNWYVDGV EVHNAKTKPR EEQFNSTFRV
181 VSVLTVVHQD WLNGKEYKCK VSNKGLPAPI EKTISKTKGQ PREPQVYTLP PSREEMTKNQ
241 VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPMLDSDG SFFLYSKLTV DKSRWQQGNV
301 FSCSVMHEAL HNHYTQKSLS LSPGK

CH1 aas 1-97, Hinge aas 98-109, CH2 aas 110-218, CH3 aas 219-325 (Fc aas 110-325, 216 aas)

FIG. 2F

***Homo sapiens* IgG3 (partial sequence) GenBank AAW65947 (SEQ ID NO:10)**

1 HKPSNTKVDK RVELKTPLGD TTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC
61 VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC
121 KVSNKALPAP IEKTISKAKG QPREPQVYTL PPSRDELTKN QVSLTCLVKG FYPSDIAVEW
181 ESNGQPENNY KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL
241 SLSPGK

CH1 aas 1-12, Hinge aas 13-29, CH2 aas 30-139, CH3 aas 140-246 (Fc aas 30-246, 217 aas)

FIG. 2G

***Homo sapiens* IgG4 (partial sequence) GenBank P01861 (SEQ ID NO:11)**

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 YKTTPPVLDS DGSFFLYSRL TVDKSRWQEG
301 NVFSCSVMHE ALHNHYTQKS LSLSLGK

CH1 aas 1-98, Hinge aas 99-110, CH2 aas 111-220, CH3 aas 221-327 (Fc aas 111-327, 217 aas)

***Homo sapiens* IgG4 (partial sequence) Segment (SEQ ID NO:12) 223 aa**

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

Hinge aas 1-7, CH2 aas 8-117, CH3 aas 118-223 (Fc aas 8-223, 216 aas)

FIG. 2H

***Homo sapiens* IgM (partial sequence) UniProtKB P01871.4 (SEQ ID NO:13)**

```
1   GSASAPTLFP LVSCENSPSD TSSVAVGCLA QDFLPDSITF SWKYKNNSDI SSTRGFPSVL
61  RGGKYAATSQ VLLPSKDVMQ GTDEHVVCKV QHPNGNKEKN VPLPVIAELP PKVSVFVPPR
121 DGFFGNPRKS KLICQATGFS PRQIQVSWLR EGKQVGSGVT TDQVQAEAKE SGPTTYKVTS
181 TLTIKESDWL GQSMFTCRVD HRGLTFQQNA SSMCVPDQDT AIRVFAIPPS FASIFLTKST
241 KLTCLVTDLT TYDSVTISWT RQNGEAVKTH TNISESHPNA TFSAVGEASI CEDDWNSGER
301 FTCTVTHTDL PSPLKQTISR PKGVALHRPD VYLLPPAREQ LNLRESATIT CLVTGFSPAD
321 VFVQWMQRGQ PLSPEKYVTS APMPEPQAPG RYFAHSILTV SEEEWNTGET YTCVVAHEAL
381 PNRVTERTVD KSTGKPTLYNVSLVMSDTAGT CY
```

CH1 aas 1-105, CH2 aas 106-217, CH3 aas 218-323, CH4 aas 324-452

FIG. 2I

***Homo sapiens Ig J-chain* (amino acids 23-259) (SEQ ID NO:14)**

```
1   MKNHLLFWGV LAVFIKAVHV KAQEDERIVL VDNKCKCARI TSRIIRSSED PNEDIVERNI
61  RIIVPLNNRE NISDPTSPLR TRFVYHLSDL CKKCDPTEVE LDNQIVTATQ SNICDEDSAT
121 ETCYTYDRNK CYTAVVPLVY GGETKMVETA LTPDACYPD
SIGNAL SEQUENCE AMINO ACIDS 1-22
```

FIG. 2J

Ig CH1 domain Homo sapiens (SEQ ID NO:15)

```
 1 FTVRETASTK GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP
61 AVLQSSGLYS LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KT
```

FIG. 2K

Ig κ chain constant region *Homo sapiens* (SEQ ID NO:16)

```
1  TVAAPSVFIF PPSDEQLKSG TASVVCLLNN FYPREAKVQW KVDNALQSGN SQESVTEQDS
61 KDSTYSLSST LTLSKADYEK HKVYACEVTH QGLSSPVTKS FNRGEC
```

Ig λ chain constant region *Homo sapiens* (SEQ ID NO:17)

```
1  GQPKANPTVT LFPPSSEELQ ANKATLVCLI SDFYPGAVTV AWKADGSPVK AGVETTKPSK
61 QSNNKYAASS YLSLTPEQWK SHRSYSCQVT HEGSTVEKTV APTECS
```

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:18)

```
1   MAVMAPRTLL LLLSGALALT QTWAGSHSMR YFFTSVSRPG RGEPRFIAVG YVDDTQFVRF
61  DSDAASQKME PRAPWIEQEG PEYWDQETRN MKAHSQTDRA NLGTLRGYYN QSEDGSHTIQ
121 IMYGCDVGPD GRFLRGYRQD AYDGKDYIAL NEDLRSWTAA DMAAQITKRK WEAVHAAEQR
181 RVYLEGRCVD GLRRYLENGK ETLQRTDPPK THMTHHPISD HEATLRCWAL GFYPAEITLT
241 WQRDGEDQTQ DTELVETRPA GDGTFQKWAA VVVPSGEEQR YTCHVQHEGL PKPLTLRWEL
301 SSQPTIPIVG IIAGLVLLGA VITGAVVAAV MWRRKSSDRK GGSYTQAASS DSAQGSDVSL
361 TACKV
```

FIG. 3A (continued)

3A.2 *Homo sapiens* HLA-A*1101 NCBI Accession P13746.1 (SEQ ID NO:19)

```
1   MAVMAPRTLL LLLSGALALT QTWAGSHSMR YFYTSVSRPG RGEPRFIAVG YVDDTQFVRF
61  DSDAASQRME PRAPWIEQEG PEYWDQETRN VKAQSQTDRV DLGTLRGYYN QSEDGSHTIQ
121 IMYGCDVGPD GRFLRGYRQD AYDGKDYIAL NEDLRSWTAA DMAAQITKRK WEAAHAAEQQ
181 RAYLEGRCVE WLRRYLENGK ETLQRTDPPK THMTHHPISD HEATLRCWAL GFYPAEITLT
241 WQRDGEDQTQ DTELVETRPA GDGTFQKWAA VVVPSGEEQR YTCHVQHEGL PKPLTLRWEL
301 SSQPTIPIVG IIAGLVLLGA VITGAVVAAV MWRRKSSDRK GGSYTQAASS DSAQGSDVSL
361 TACKV
```

3A.3 *Homo sapiens* HLA-A*2402 NCBI Accession P05534.2 (SEQ ID NO:20)

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

3A.4 *Homo sapiens* HLA-A*3303 NCBI Accession AAA79865.1 (SEQ ID NO:21)

```
1   MAVMAPRTLL LLLLGALALT QTWAGSHSMR YFTTSVSRPG RGEPRFIAVG YVDDTQFVRF
61  DSDAASQRME PRAPWIEQEG PEYWDRNTRN VKAHSQIDRV DLGTLRGYYN QSEAGSHTIQ
121 MMYGCDVGSD GRFLRGYQQD AYDGKDYIAL NEDLRSWTAA DMAAQITQRK WEAARVAEQL
181 RAYLEGTCVE WLRRYLENGK ETLQRTDPPK THMTHHAVSD HEATLRCWAL SFYPAEITLT
241 WQRDGEDQTQ DTELVETRPA GDGTFQKWAS VVVPSGQEQR YTCHVQHEGL PKPLTLRWEP
301 SSQPTIPIVG IIAGLVLFGA VFAGAVVAAV RWRRKSSDRK GGSYSQAASS DSAQGSDMSL
361 TACKV
```

FIG. 3B

***Homo sapiens* HLA-B*07:02:01 GenBank Accession NP_005505.2**
Amino Acids 25-362 (SEQ ID NO:22)

```
1   MLVMAPRTVL LLLSAALALT ETWAGSHSMR YFYTSVSRPG RGEPRFISVG YVDDTQFVRF
61  DSDAASPREE PRAPWIEQEG PEYWDRNTQI YKAQAQTDRE SLRNLRGYYN QSEAGSHTLQ
121 SMYGCDVGPD GRLLRGHDQY AYDGKDYIAL NEDLRSWTAA DTAAQITQRK WEAAREAEQR
181 RAYLEGECVE WLRRYLENGK DKLERADPPK THVTHHPISD HEATLRCWAL GFYPAEITLT
241 WQRDGEDQTQ DTELVETRPA GDRTFQKWAA VVVPSGEEQR YTCHVQHEGL PKPLTLRWEP
301 SSQSTVPIVG IVAGLAVLAV VVIGAVVAAV MCRRKSSGGK GGSYSQAACS DSAQGSDVSL
361 TA
```

FIG. 3C *Homo sapiens* HLA-C*701 (HLA-C*07:01) GenBank Accession NP_001229971.1, Amino acids 25-366 (SEQ ID NO:23)

```
1   MRVMAPRALL LLLSGGLALT ETWACSHSMR YFDTAVSRPG RGEPRFISVG YVDDTQFVRF
61  DSDAASPRGE PRAPWVEQEG PEYWDRETQN YKRQAQADRV SLRNLRGYYN QSEDGSHTLQ
121 RMYGCDLGPD GRLLRGYDQS AYDGKDYIAL NEDLRSWTAA DTAAQITQRK LEAARAAEQL
181 RAYLEGTCVE WLRRYLENGK ETLQRAEPPK THVTHHPLSD HEATLRCWAL GFYPAEITLT
241 WQRDGEDQTQ DTELVETRPA GDGTFQKWAA VVVPSGQEQR YTCHMQHEGL QEPLTLSWEP
301 SSQPTIPIMG IVAGLAVLVV LAVLGAVVTA MMCRRKSSGG KGGSCSQAAC SNSAQGSDES
361 LITCK
```

FIG. 3D (SEQ ID NOs:24-34)

```
HLA-A            GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQKMEPRAPWIEQEGPEYW
HLA-B            GSHSMRYFYTSVSRPGRGEPRFISVGYVDDTQFVRFDSDAASPREEPRAPWIEQEGPEYW
HLA-C            CSHSMRYFDTAVSRPGRGEPRFISVGYVDDTQFVRFDSDAASPRGEPRAPWVEQEGPEYW
HLA_A*0201       GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYW
Mouse H2K        GPHSLRYFVTAVSRPGLGEPRFIAVGYVDDTQFVRFDSDADNPRFEPRAPWMEQEGPEYW
HLA_A(var. 2)    GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYW
HLA_A(var. 2C)   GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYW
HLA-A(var.2CP)   GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYW
HLA-A*1101       GSHSMRYFYTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYW
HLA-A*2402       GSHSMRYFSTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYW
HLA-A*3303       GSHSMRYFTTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYW
                   **:*** *:***** ******:**************** . : ******:********

                                                  84
                                                  ↓
HLA-A            DQETRNMKAHSQTDRANLGTLRGYYNQSEDGSHTIQIMYGCDVGPDGRFLRGYRQDAYDG
HLA-B            DRNTQIYKAQAQTDRESLRNLRGYYNQSEAGSHTLQSMYGCDVGPDGRLLRGHDQYAYDG
HLA-C            DRETQNYKRQAQADRVSLRNLRGYYNQSEDGSHTLQRMYGCDLGPDGRLLRGYDQSAYDG
HLA_A*0201       DGETRKVKAHSQTHRVDLGTLRGYYNQSEAGSHTVQRMYGCDVGSDWRFLRGYHQYAYDG
MOUSE H2K        EEQTQRAKSDEQWFRVSLRTAQRYYNQSKGGSHTFQRMFGCDVGSDWRLLRGYQQFAYDG
HLA_A(var. 2)    DGETRKVKAHSQTHRVDLGTLRGAYNQSEAGSHTVQRMYGCDVGSDWRFLRGYHQYAYDG
HLA_A(var. 2C)   DGETRKVKAHSQTHRVDLGTLRGCYNQSEAGSHTVQRMYGCDVGSDWRFLRGYHQYAYDG
HLA-A(var.2CP)   DGETRKVKAHSQTHRVDLGTLRGAYNQSEAGSHTVQRMYGCDVGSDWRFLRGYHQYAYDG
HLA-A*1101       DQETRNVKAQSQTDRVDLGTLRGYYNQSEDGSHTIQIMYGCDVGPDGRFLRGYRQDAYDG
HLA-A*2402       DEETGKVKAHSQTDRENLRIALRYYNQSEAGSHTLQMMFGCDVGSDGRFLRGYHQYAYDG
HLA-A*3303       DRNTRNVKAHSQIDRVDLGTLRGYYNQSEAGSHTIQMMYGCDVGSDGRFLRGYQQDAYDG
                 : :*   * . *  * .*      ****: ****.* *:***:* * *:***: * ****
                                  aac1  aac2

                                  139
                                  ↓
HLA-A            KDYIALNEDLRSWTAADMAAQITKRKWEAVHAAEQRRVYLEGRCVDGLRRYLENGKETLQ
HLA-B            KDYIALNEDLRSWTAADTAAQITQRKWEAAREAEQRRAYLEGECVEWLRRYLENGKDKLE
HLA-C            KDYIALNEDLRSWTAADTAAQITQRKLEAARAAEQLRAYLEGTCVEWLRRYLENGKETLQ
HLA_A*0201       KDYIALKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRYLENGKETLQ
MOUSE H2K        RDYIALNEDLKTWTAADTAALITRRKWEQAGDAEYYRAYLEGECVEWLRRYLELGNETLL
HLA_A(var. 2)    KDYIALKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRYLENGKETLQ
HLA_A(var. 2C)   KDYIALKEDLRSWTAADMCAQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRYLENGKETLQ
HLA-A(var.2CP)   KDYIALKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRYLENGKETLQ
HLA-A*1101       KDYIALNEDLRSWTAADMAAQITKRKWEAAHAAEQQRAYLEGRCVEWLRRYLENGKETLQ
HLA-A*2402       KDYIALKEDLRSWTAADMAAQITKRKWEAAHVAEQQRAYLEGTCVDGLRRYLENGKETLQ
HLA-A*3303       KDYIALNEDLRSWTAADMAAQITQRKWEAARVAEQLRAYLEGTCVEWLRRYLENGKETLQ
                 :*****:***::***** .*  *::* * .  **  *.**** **: ****** *::.*
                                  aac3  aac4
```

Fig. 3D (continued)

```
                                                                                 236
                                                                                  ↓
HLA-A             RTDPPKTHMTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDGTF
HLA-B             RADPPKTHVTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDRTF
HLA-C             RAEPPKTHVTHHPLSDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDGTF
HLA_A*0201        RTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTELVETRPAGDGTF
MOUSE H2K         RTDSPKAHVTYHPRSQVDVTLRCWALGFYPADITLTWQLNGEDLTQDMELVETRPAGDGTF
HLA_A(var. 2)     RTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTELVETRPCGDGTF
HLA_A(var. 2C)    RTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTELVETRPCGDGTF
HLA-A(var.2CP)    RTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTELVETRPCGDGTF
HLA-A*1101        RTDPPKTHMTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDGTF
HLA-A*2402        RTDPPKTHMTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDGTF
HLA-A*3303        RTDPPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTELVETRPAGDGTF
                  *:: **:*:*:*  *: :.*******.****:******  :***  *** *******.** **
                                                                  aac5   aac6
```

```
HLA-A             QKWAAVVVPSGEEQRYTCHVQHEGLPKPLTLRWE
HLA-B             QKWAAVVVPSGEEQRYTCHVQHEGLPKPLTLRWE
HLA-C             QKWAAVVVPSGQEQRYTCHMQHEGLQEPLTLSWE
HLA_A*0201        QKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWEP
MOUSE H2K         QKWAAVVVPLGKEQNYTCHVHHKGLPEPLTLRW
HLA_A(var. 2)     QKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWE
HLA_A(var. 2C)    QKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWE
HLA- (var.2CP)    QKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWEP
HLA-A*1101        QKWAAVVVPSGEEQRYTCHVQHEGLPKPLTLRWEL
HLA-A*2402        QKWAAVVVPSGEEQRYTCHVQHEGLPKPLTLRWEP
HLA-A*3303        QKWASVVVPSGQEQRYTCHVQHEGLPKPLTLRWEP
                  ****:**** *:**.****::*:** :**** *
```

HLA-A*0101, SEQ ID NO:24; HLA-B*0702, SEQ ID NO:25; HLA-C, SEQ ID NO:26; HLA-A*0201, SEQ ID NO:27; a mouse H2K protein sequence, SEQ ID NO:28; three variants of HLA-A (var.2, var. 2C, and var.2CP, SEQ ID NOs:29, 30, and 31); (HLA-A*1101 (HLA-A11), SEQ ID NO:32; HLA-A*2402 (HLA-A24), SEQ ID NO:33; HLA-A*3303 (HLA-A33), SEQ ID NO:34.

FIG. 3E HLA-A ALLELES

```
A*0101    GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQKMEPRAPWIEQEGPEYW  60
A*0201    GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYW  60
A*0301    GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYW  60
A*1101    GSHSMRYFYTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYW  60
A*2301    GSHSMRYFSTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYW  60
A*2402    GSHSMRYFSTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYW  60
A*2407    GSHSMRYFSTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYW  60
A*3303    GSHSMRYFTTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYW  60
A*3401    GSHSMRYFYTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYW  60
          ******** **********************************:****************
```

FIG. 3E (continued)

HLA-A*0101, SEQ ID NO:24; HLA-A*0201, SEQ ID NO:27; HLA-A*0301, SEQ ID NO:35; HLA-A*1101, SEQ ID NO:32; HLA-A*2301, SEQ ID NO:36; HLA-A*2402, SEQ ID NO:33; HLA-A*2407, SEQ ID NO:37; HLA-A*3303, SEQ ID NO:34; and HLA-A*3401, SEQ ID NO:38;

FIG. 3E (continued) HLA-A ALLELE CONSENSUS SEQUENCE

GSHSMRYF**X1**TSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQ**X2**MEPRAPWIEQEGPEYWD**X3X4**T**X5X6X7**KA**X8**SQ**X9X10**R**X11X12**L**X13X14X15X16X17Y**YNQSE**X18**GSHT**X19**Q**X20**M**X21**GCDVG**X22**D**X23**RFLRGY**X24**Q**X25**AYDGKDYIAL**X26**EDLRSWTAADM**A**AQ**X27**T**X287****X29**KWE**X30X31X32**EAEQ**X33**R**X34**YL**X35**G**X36**CV**X37X38**LRRYLENGKETLQRTD**X39**PK THMTHH**X40X41**SDHEATLRCWAL**X42**FYPAEITLTWQRDGEDQTQDTELVETRP**A**GDGTFQKW A**X43**VVVPSG**X44**EQRYTCHVQHEGLPKPLTLRWE**X45**

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:39.

FIG. 3F HLA-B ALLELES

```
B*0702  GSHSMRYFYTSVSRPGRGEPRFISVGYVDDTQFVRFDSDAASPREEPRAPWIEQEGPEYW   60
B*0801  GSHSMRYFDTAMSRPGRGEPRFISVGYVDDTQFVRFDSDAASPREEPRAPWIEQEGPEYW   60
B*1502  GSHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQEGPEYW   60
B*3802  GSHSMRYFYTSVSRPGRGEPRFISVGYVDDTQFVRFDSDAASPREEPRAPWIEQEGPEYW   60
B*4001  GSHSMRYFHTAMSRPGRGEPRFITVGYVDDTLFVRFDSDATSPRKEPRAPWIEQEGPEYW   60
B*4601  GSHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQEGPEYW   60
B*5301  GSHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRTEPRAPWIEQEGPEYW   60
        ******** *::***********:******* ********:***  **************

                                    84
B*0702  DRNTQIYKAQAQTDRESLRNLRGYYNQSEAGSHTLQSMYGCDVGPDGRLLRGHDQYAYDG   120
B*0801  DRNTQIFKTNTQTDRESLRNLRGYYNQSEAGSHTLQSMYGCDVGPDGRLLRGHNQYAYDG   120
B*1502  DRNTQISKTNTQTYRESLRNLRGYYNQSEAGSHIIQRMYGCDVGPDGRLLRGYDQSAYDG   120
B*3802  DRNTQICKTNTQTYRENLRTALRYYNQSEAGSHTLQRMYGCDVGPDGRLLRGHNQFAYDG   120
B*4001  DRETQISKTNTQTYRESLRNLRGYYNQSEAGSHTLQRMYGCDVGPDGRLLRGHNQYAYDG   120
B*4601  DRETQKYKRQAQTDRVSLRNLRGYYNQSEAGSHTLQRMYGCDVGPDGRLLRGHDQSAYDG   120
B*5301  DRNTQIFKTNTQTYRENLRIALRYYNQSEAGSHIIQRMYGCDLGPDGRLLRGHDQSAYDG   120
        **:**  * ::** * .** *********** :* *****:*********::* ****
                           aac1  aac2

                                139
B*0702  KDYIALNEDLRSWTAADTAAQITQRKWEAAREAEQRRAYLEGECVEWLRRYLENGKDKLE   180
B*0801  KDYIALNEDLRSWTAADTAAQITQRKWEAARVAEQDRAYLEGTCVEWLRRYLENGKDTLE   180
B*1502  KDYIALNEDLSSWTAADTAAQITQRKWEAAREAEQLRAYLEGLCVEWLRRYLENGKETLQ   180
B*3802  KDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRTYLEGTCVEWLRRYLENGKETLQ   180
B*4001  KDYIALNEDLRSWTAADTAAQISQRKLEAARVAEQLRAYLEGECVEWLRRYLENGKDKLE   180
B*4601  KDYIALNEDLSSWTAADTAAQITQRKWEAAREAEQWRAYLEGLCVEWLRRYLENGKETLQ   180
B*5301  KDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGLCVEWLRRYLENGKETLQ   180
        ********** ********** :*** **** *** *:**** *************:.*:
                    aac3  aac4
```

FIG. 3F (continued)

```
                                                                  236
B*0702  RADPPKTHVTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDRTF  241
B*0801  RADPPKTHVTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDRTF  241
B*1502  RADPPKTHVTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDRTF  241
B*3802  RADPPKTHVTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDRTF  241
B*4001  RADPPKTHVTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDRTF  241
B*4601  RADPPKTHVTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDRTF  241
B*5301  RADPPKTHVTHHPVSDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDRTF  241
        *************:************************************************
                                                    aac5  aac6

B*0702  QKWAAVVVPSGEEQRYTCHVQHEGLPKPLTLRWEP  276
B*0801  QKWAAVVVPSGEEQRYTCHVQHEGLPKPLTLRWEP  276
B*1502  QKWAAVVVPSGEEQRYTCHVQHEGLPKPLTLRWEP  276
B*3802  QKWAAVVVPSGEEQRYTCHVQHEGLPKPLTLRWEP  276
B*4001  QKWAAVVVPSGEEQRYTCHVQHEGLPKPLTLRWEP  276
B*4601  QKWAAVVVPSGEEQRYTCHVQHEGLPKPLTLRWEP  276
B*5301  QKWAAVVVPSGEEQRYTCHVQHEGLPKPLTLRWEP  276
        **********************************
```

HLA-B*0702, SEQ ID NO:40; HLA-B*0801, SEQ ID NO:41; HLA-B*1502, SEQ ID NO:42; HLA-B*3802, SEQ ID NO:43; HLA-B*4001, SEQ ID NO:44; HLA-B*4601, SEQ ID NO:45; and HLA-B*5301, SEQ ID NO:46;

HLA-B ALLELE CONSENSUS

GSHSMRYF**X1**T**X2X3**SRPGRGEPRFI**X4**VGYVDDT**X5**FVRFDSDA**X6**SPR**X7X8**PRAPWIEQEG
PEYWDR**X9**TQ**X10X11**KT**X12X13**TQ**X14**Y**X15X16**NL**X17X18X19X20Y**YNQSEAGSH**X21X22QX23**MYGCDLGPDGRLLRGHDQSAYDGKDYIALNEDL**X24**SWTAADT**A**AQI**X25**QRK**X26**EA
AR**X27**AEQ**X28**R**X29**YLEG**X30**CVEWLRRYLENGK**X31X32**L**X33**RADPPKTHVTHHP**X34**SDH
EATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRP**A**GDRTFQKWAAVVVPSGEEQRYTCHV
QHEGLPKPLTLRWEP

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:47.

FIG. 3G HLA-C ALLELES

```
C*0701  CSHSMRYFDTAVSRPGRGEPRFISVGYVDDTQFVRFDSDAASPRGEPRAPWVEQEGPEYW   60
C*0702  CSHSMRYFDTAVSRPGRGEPRFISVGYVDDTQFVRFDSDAASPRGEPRAPWVEQEGPEYW   60
C*0401  GSHSMRYFSTSVSWPGRGEPRFIAVGYVDDTQFVRFDSDAASPRGEPREPWVEQEGPEYW   60
C*0102  CSHSMKYFFTSVSRPGRGEPRFISVGYVDDTQFVRFDSDAASPRGEPRAPWVEQEGPEYW   60
C*0602  CSHSMRYFDTAVSRPGRGEPRFISVGYVDDTQFVRFDSDAASPRGEPRAPWVEQEGPEYW   60
C*0801  CSHSMRYFYTAVSRPGRGEPRFIAVGYVDDTQFVQFDSDAASPRGEPRAPWVEQEGPEYW   60
C*1502  CSHSMRYFYTAVSRPGRGEPHFIAVGYVDDTQFVRFDSDAASPRGEPRAPWVEQEGPEYW   60
C*0303  GSHSMRYFYTAVSRPGRGEPHFIAVGYVDDTQFVRFDSDAASPRGEPRAPWVEQEGPEYW   60
C*0304  GSHSMRYFYTAVSRPGRGEPHFIAVGYVDDTQFVRFDSDAASPRGEPRAPWVEQEGPEYW   60
         ****:** *:** ******:**:**********:************* ***********

                                    84
C*0701  DRETQNYKRQAQADRVSLRNLRGYYNQSEDGSHTLQRMYGCDLGPDGRLLRGYDQSAYDG   120
C*0702  DRETQKYKRQAQADRVSLRNLRGYYNQSEDGSHTLQRMSGCDLGPDGRLLRGYDQSAYDG   120
C*0401  DRETQKYKRQAQADRVNLRKLRGYYNQSEDGSHTLQRMFGCDLGPDGRLLRGYNQFAYDG   120
C*0102  DRETQKYKRQAQTDRVSLRNLRGYYNQSEAGSHTLQWMCGCDLGPDGRLLRGYDQYAYDG   120
C*0602  DRETQKYKRQAQADRVNLRKLRGYYNQSEDGSHTLQWMYGCDLGPDGRLLRGYDQSAYDG   120
C*0801  DRETQKYKRQAQTDRVSLRNLRGYYNQSEAGSHTLQRMYGCDLGPDGRLLRGYNQFAYDG   120
C*1502  DRETQNYKRQAQTDRVNLRKLRGYYNQSEAGSHIIQRMYGCDLGPDGRLLRGHDQLAYDG   120
C*0303  DRETQKYKRQAQTDRVSLRNLRGYYNQSEARSHIIQRMYGCDVGPDGRLLRGYDQYAYDG   120
C*0304  DRETQKYKRQAQTDRVSLRNLRGYYNQSEAGSHIIQRMYGCDVGPDGRLLRGYDQYAYDG   120
        *****:******:***.**:*********  ** :* * ***:*********::* ****
                          aac1  aac2

                                139
C*0701  KDYIALNEDLRSWTAADTAAQITQRKLEAARAAEQLRAYLEGTCVEWLRRYLENGKETLQ   180
C*0702  KDYIALNEDLRSWTAADTAAQITQRKLEAARAAEQLRAYLEGTCVEWLRRYLENGKETLQ   180
C*0401  KDYIALNEDLRSWTAADTAAQITQRKWEAAREAEQRRAYLEGTCVEWLRRYLENGKETLQ   180
C*0102  KDYIALNEDLRSWTAADTAAQITQRKWEAAREAEQRRAYLEGTCVEWLRRYLENGKETLQ   180
C*0602  KDYIALNEDLRSWTAADTAAQITQRKWEAAREAEQWRAYLEGTCVEWLRRYLENGKETLQ   180
C*0801  KDYIALNEDLRSWTAADTAAQITQRKWEAARTAEQLRAYLEGTCVEWLRRYLENGKKTLQ   180
C*1502  KDYIALNEDLRSWTAADTAAQITQRKWEAAREAEQLRAYLEGTCVEWLRRYLENGKETLQ   180
C*0303  KDYIALNEDLRSWTAADTAAQITQRKWEAAREAEQLRAYLEGLCVEWLRRYLKNGKETLQ   180
C*0304  KDYIALNEDLRSWTAADTAAQITQRKWEAAREAEQLRAYLEGLCVEWLRRYLKNGKETLQ   180
        ********************************** **** *** ****** *********:***:***
                          aac3  aac4

                                                                  236
C*0701  RAEPPKTHVTHHPLSDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDGTF   241
C*0702  RAEPPKTHVTHHPLSDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDGTF   241
C*0401  RAEHPKTHVTHHPVSDHEATLRCWALGFYPAEITLTWQWDGEDQTQDTELVETRPAGDGTF   241
C*0102  RAEHPKTHVTHHPVSDHEATLRCWALGFYPAEITLTWQWDGEDQTQDTELVETRPAGDGTF   241
C*0602  RAEHPKTHVTHHPVSDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDGTF   241
C*0801  RAEHPKTHVTHHPVSDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDGTF   241
C*1502  RAEHPKTHVTHHPVSDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDGTF   241
C*0303  RAEHPKTHVTHHPVSDHEATLRCWALGFYPAEITLTWQWDGEDQTQDTELVETRPAGDGTF   241
C*0304  RAEHPKTHVTHHPVSDHEATLRCWALGFYPAEITLTWQWDGEDQTQDTELVETRPAGDGTF   241
        *** *********:************************ ********************
                                                          aac5  aac6
```

HLA-C*0102 , SEQ ID NO:48; HLA-C*0303, SEQ ID NO:49; HLA-C*0304, SEQ ID NO:50; HLA-C*0401, SEQ ID NO:51; HLA-C*0602, SEQ ID NO:52; HLA-C*0701, SEQ ID NO:53; HLA-C*0702, SEQ ID NO:54; HLA-C*0801, SEQ ID NO:55; and HLA-C*1502, SEQ ID NO:56;

FIG. 3G (continued)

HLA-C ALLELE CONSENSUS

X1SHSM**X2**YF**X3**TAVS**X4**PGRGEP**X5**FI**X6**VGYVDDTQFV**X7**FDSDAASPRGEPR**X8**PWVEQEG
PEYWDRETQ**X9**YKRQAQ**X10**DRV**X11**LR**X12**LRG**Y**YNQSE**X13X14**SH**X15X16**Q**X17**M**X18**GC
D**X19**GPDGRLLRG**X20X21**Q**X22**AYDGKDYIALNEDLRSWTAADT**A**AQITQRK**X23**EAAR**X24**A
EQ**X25**RAYLEG**X26**CVEWLRRYL**X27**NGK**X28**TLQRAE**X29**PKTHVTHHP**X30**SDHEATLRCWA
LGFYPAEITLTWQ**X31**DGEDQTQDTELVETRP**A**GDGTFQKWAAV**X32**VPS**GX33**EQRYTCH**X34**
QHEGL**X35**EPLTL**X36**W**X37**P

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 K 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:57.

FIG. 3H - HLA-E, HLA-F. and HLA-G ALLELE CONSENUS SEQUENCES

SEQUENCE

HLA-E

GSHSLKYFHT SVSRPGRGEP RFISVGYVDD TQFVRFDNDA ASPRMVPRAP
WMEQEGSEYW DRETRSARDT AQIFRVNLRT LRG**Y**YNQS**X1**A GSHTLQWMHG
CELGPD**X2**RFL RGYEQFAYDG KDYLTLNEDL RSWTAVDT**A**A QISEQKSNDA
SEAEHQ**X3X4**YL EDTCVEWLHK YLEKGKETLL HLEPPKTHVT HHPISDHEAT
LRCWALGFYP AEITLTWQQD GEGHTQDTEL VETRP**A**GDGT FQKWAAVVVP
SGEE**X5**RYTCH VQHEGL**X6**EPV TLRWKPASQP TIPI

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:58

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**X1**FST AVSRPGRGEP RYIAVEYVDD TQFLRFDSDA AIPRMEPRE**X2**
WVEQEGPQYW EWTTGYAKAN AQTDRVALRN LLR**R**YNQSEA GSHTLQGMNG
CDMGPDGRLL RGYHQHAYDG KDYISLNEDL RSWTAADT**V**A QITQRFYEAE
EYAEEFRTYL EGECLELLRR YLENGKETLQ RADPPKAHVA HHPISDHEAT
LRCWALGFYP AEITLTWQRD GEEQTQDTEL VETRP**A**GDGT FQKWAAVVVP
X3GEEQRYTCH VQHEGLPQPL ILRWEQS**X4**QP TIPI

X1= Y or F; X2= P or Q; X3= S or P; and X4= P or L SEQ ID NO:59

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**X1**RPGRGEP RFIAMG**X2**VDD **X3**QF**X4**RFDSDS ACPRMEPRAP
WVE**X5**EGPEYW EEETRNTKAH AQTDRMNLQT **X6**RG**Y**YNQSEA
SSHTLQWMI**X7** CDL**X8X9**DGRL**X10** RGYEQYAYDG KDYLALNEDL
RSWTAADT**A**A QISKRKCEAA NVAEQRRA**X11**L EGTCVEWL**X12**R
X13LENGKE**X14**LQ RADP**X15**KTHVT HHPVFDYEAT LRCWALGFYP
AEIILTWQ**X16**D GEDQTQDVEL VETRP**A**GDGT FQKWAAVVVP SGEEQRY**X17**CH
VQHEGLPEPL MLRW**X18**QSSLP TIPI

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:60

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

FIG. 3I CONSENSUS SEQUENCE ALIGNMENT

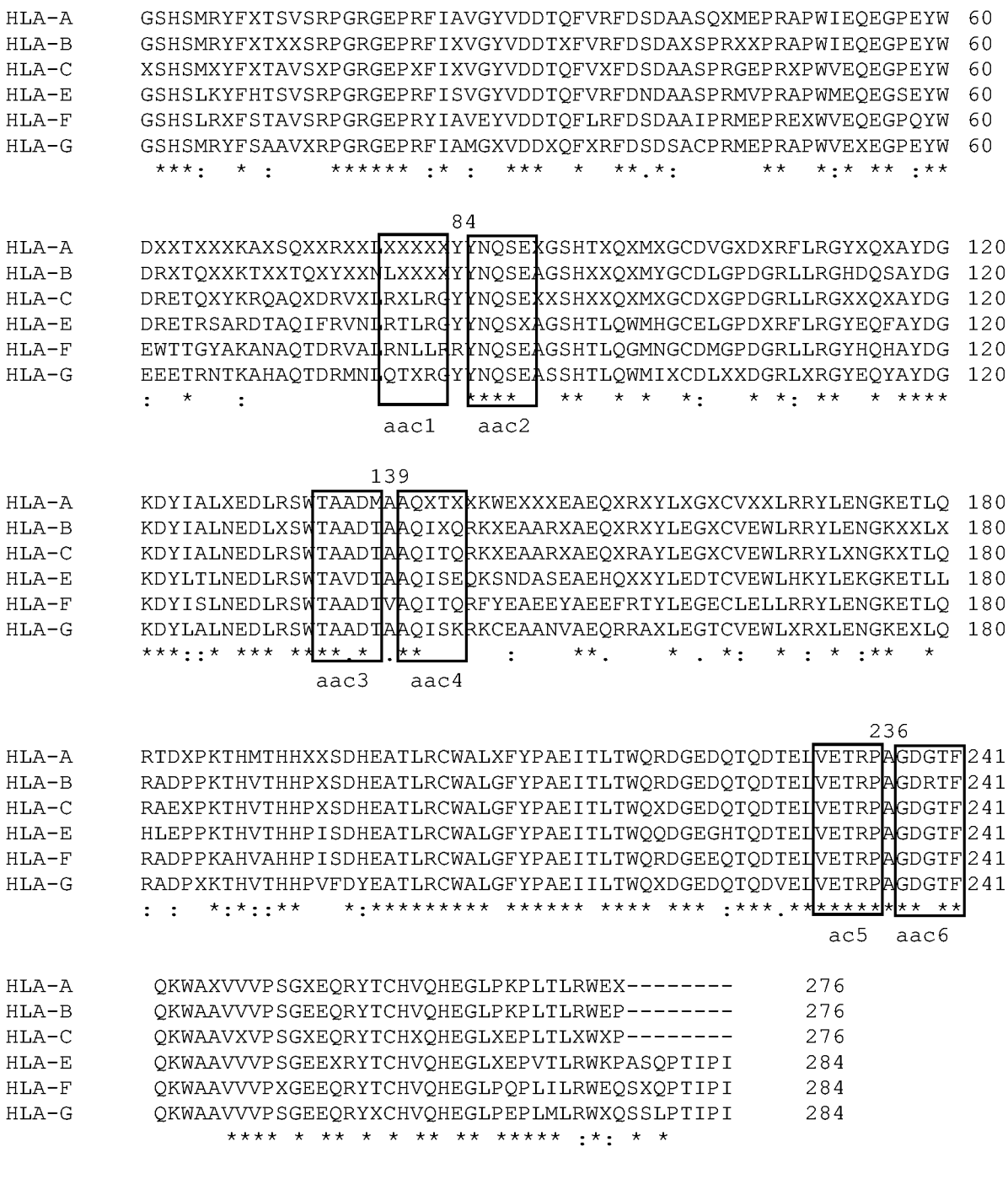
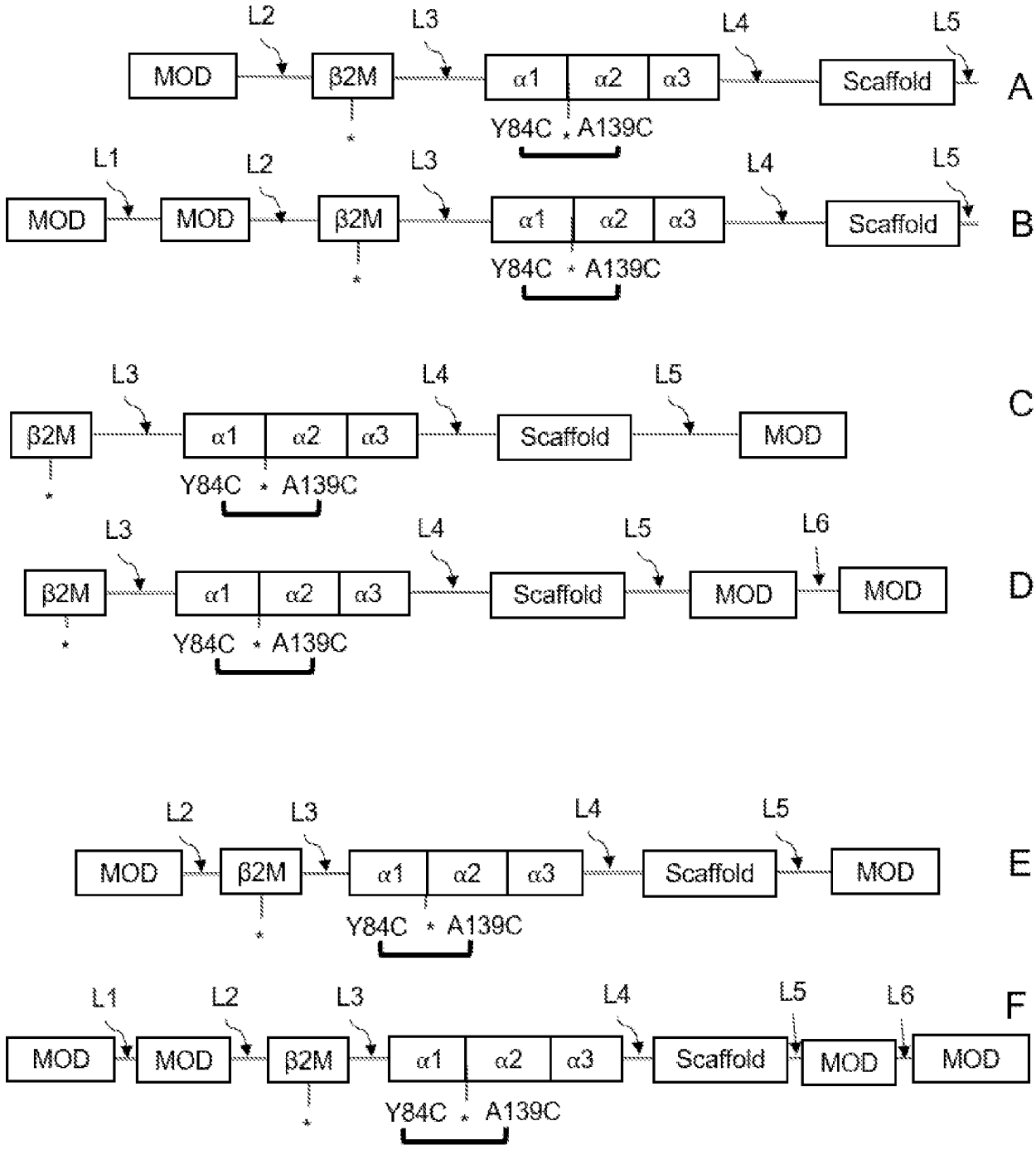


FIG. 4

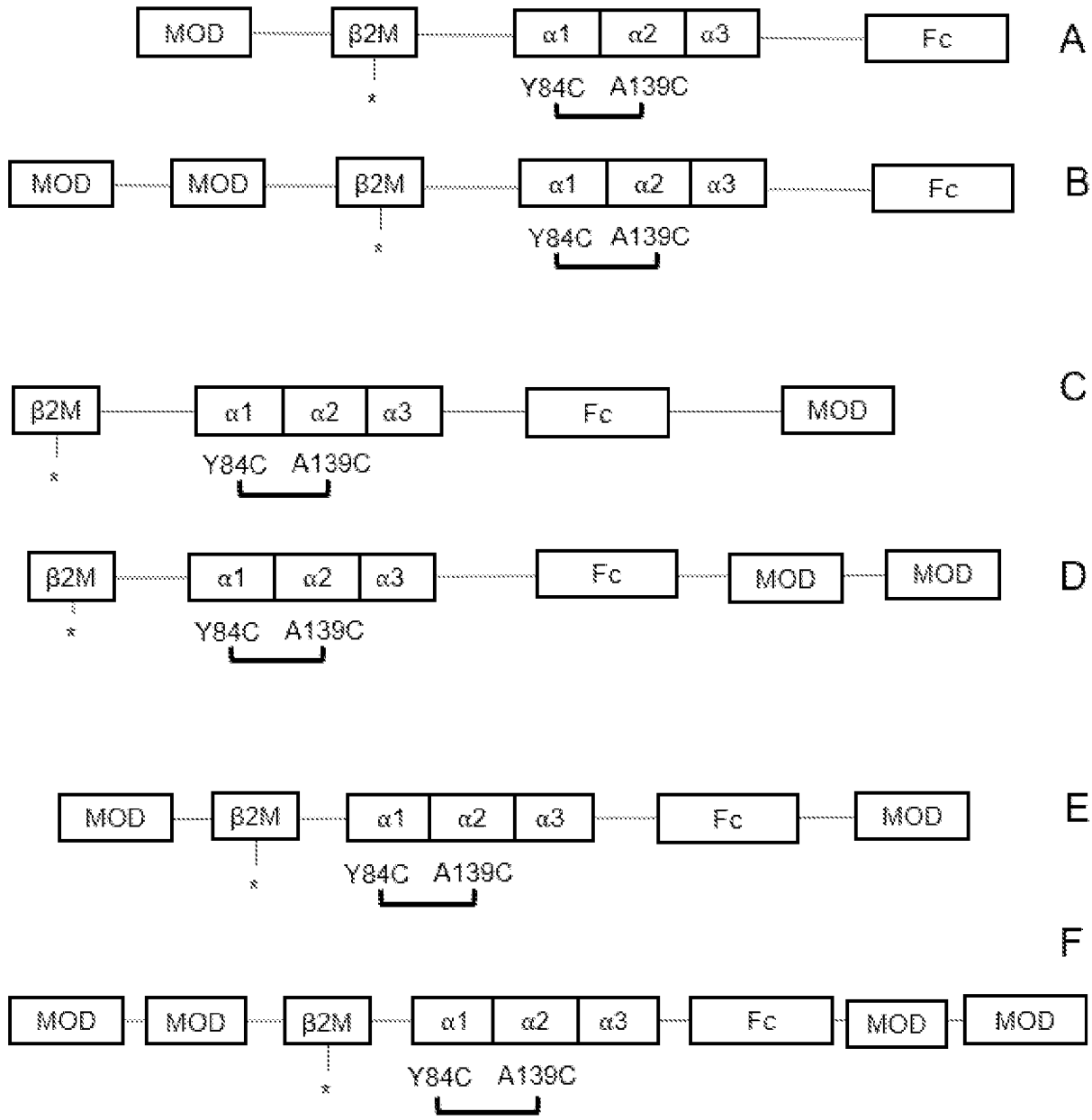
```
NP_004039.1          MSRSVALAVLALLSLSGLEAIQRTPKIQVYSRHPAENGKSNFLNCYVSGFHPSDIEVDLL      60
NP_001009066.1       MSRSVALAVLALLSLSGLEAIQRTPKIQVYSRHPAENGKSNFLNCYVSGFHPSDIEVDLL      60
NP_001040602.1       MSRSVALAVLALLSLSGLEAIQRTPKIQVYSRHPPENGKPNFLNCYVSGFHPSDIEVDLL      60
NP_776318.1          MARFVALVLLGLLSLSGLDAIQRPPKIQVYSRHPPEDGKPNYLNCYVYGFHPPQIEIDLL      60
NP_033865.2          MARSVTLVFLVLVSLTGLYAIQKTPQIQVYSRHPPENGKPNILNCYVTQFHPPHIEIQML      60
                      *:* *:*..* *:**:** ***:.*:********.*:**.* *****  ***..**:::*

NP_004039.1          KNGERIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDM      119
NP_001009066.1       KNGERIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDM      119
NP_001040602.1       KNGEKMGKVEHSDLSFSKDWSFYLLYYTEFTPNEKDEYACRVNHVTLSGPRTVKWDRDM      119
NP_776318.1          KNGEKI-KSEQSDLSFSKDWSFYLLSHAEFTPNSKDQYSCRVKHVTLEQPRIVKWDRDM      118
NP_033865.2          KNGKKIPKVEMSDMSFSKDWSFYILAHTEFTPTETDTYACRVKHASMAEPKTVYWDRDM      119
                     ***::: * * **:**********:* ::****...* *:***:*.::  *: * ****:
```

FIG. 5



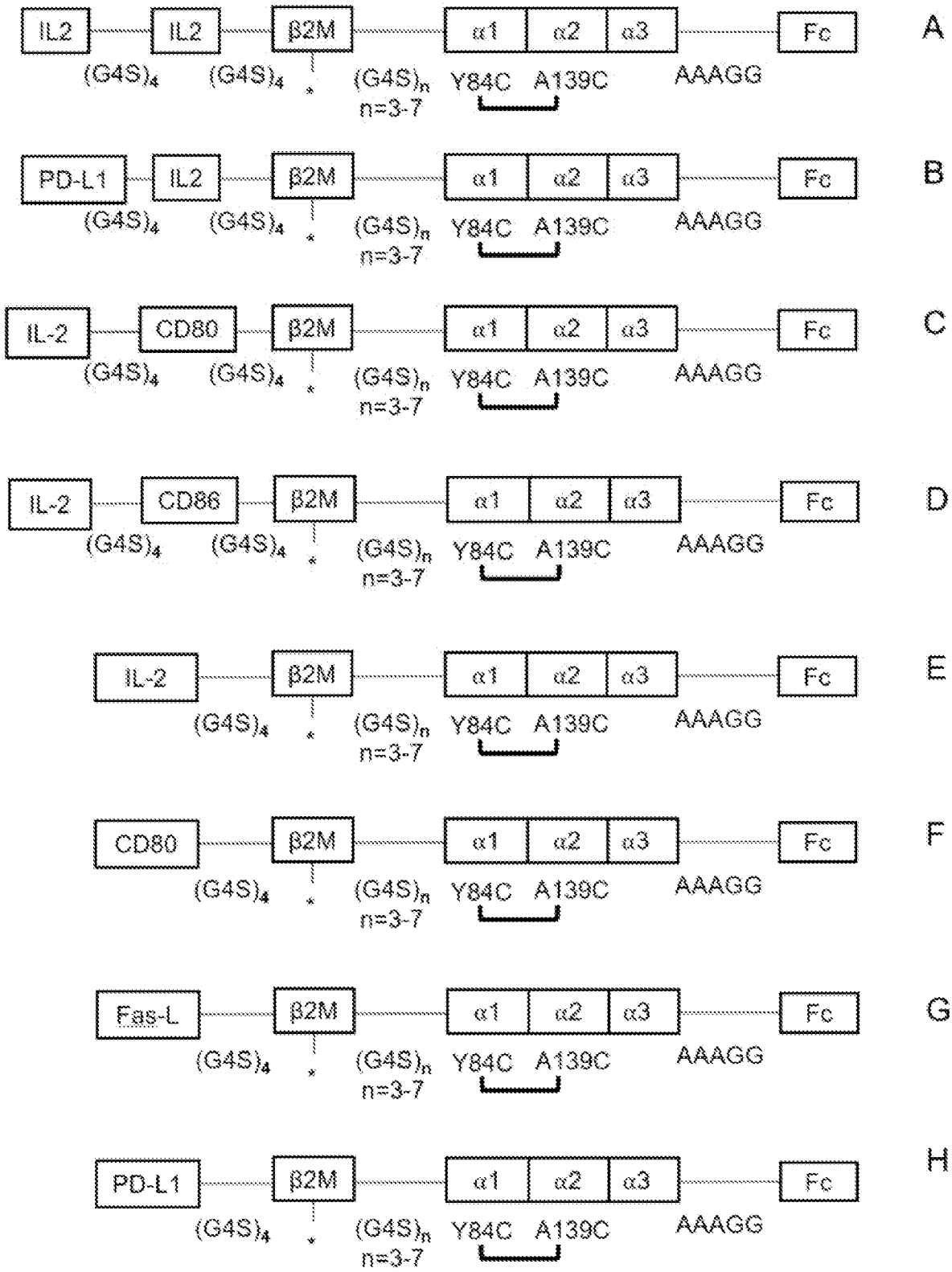
* represents a conjugation site e.g., E44C of β2M and/or a site in the MHC H binding pocket

FIG. 6



* represents a conjugation site in β2M
e.g., E44C of β2M

FIG. 7



* represents a conjugation site e.g., E44C of β2M and/or a site in the MHC H binding pocket

FIG. 8

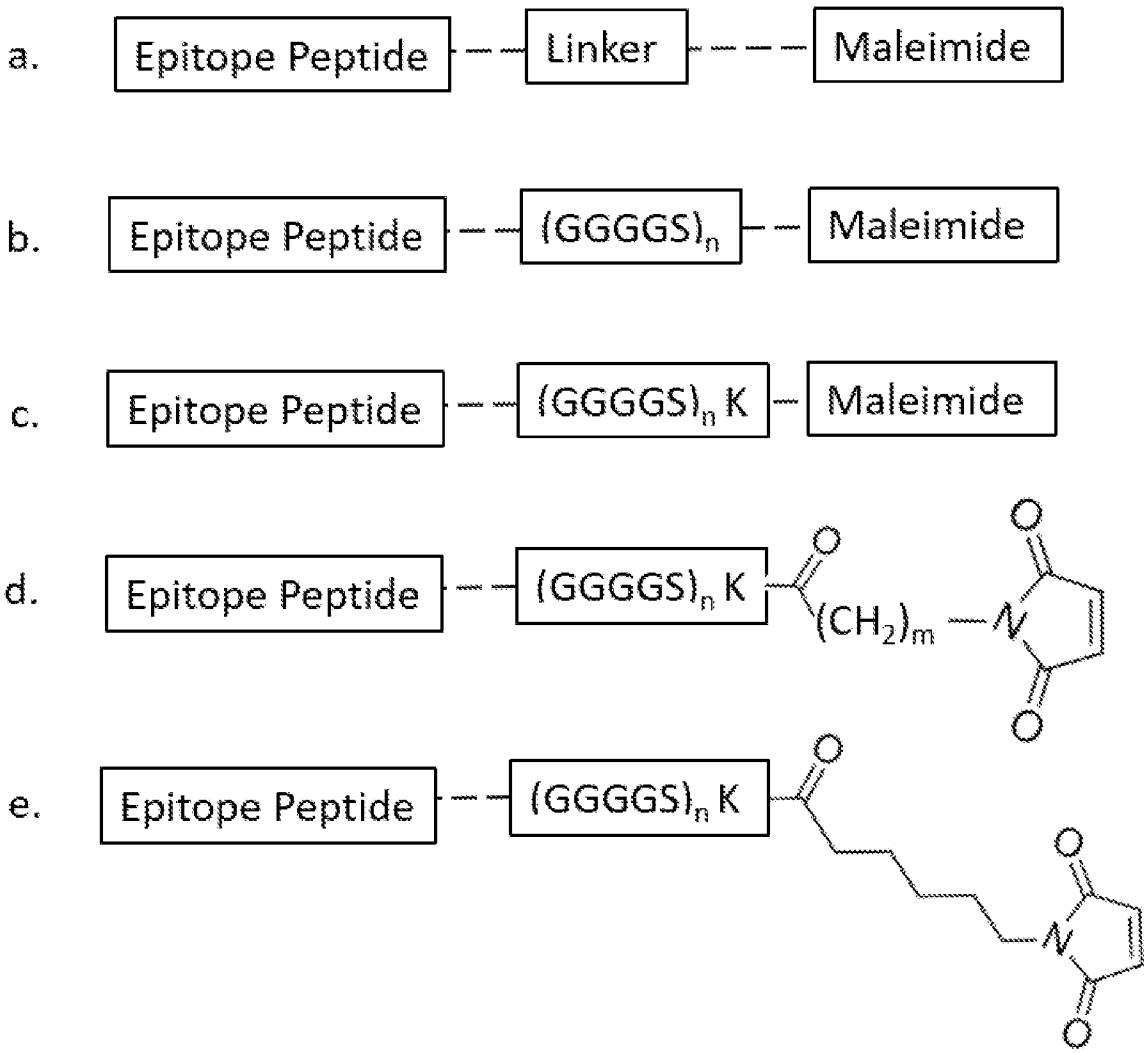

a.
Epitope Peptide
Linker
Maleimide
b.
Epitope Peptide
(GGGGS)n
Maleimide
c.
Epitope Peptide
(GGGGS)n K
Maleimide
d.
Epitope Peptide
(GGGGS)n K
(CH2)m
e.
Epitope Peptide
(GGGGS)n K

FIG. 9

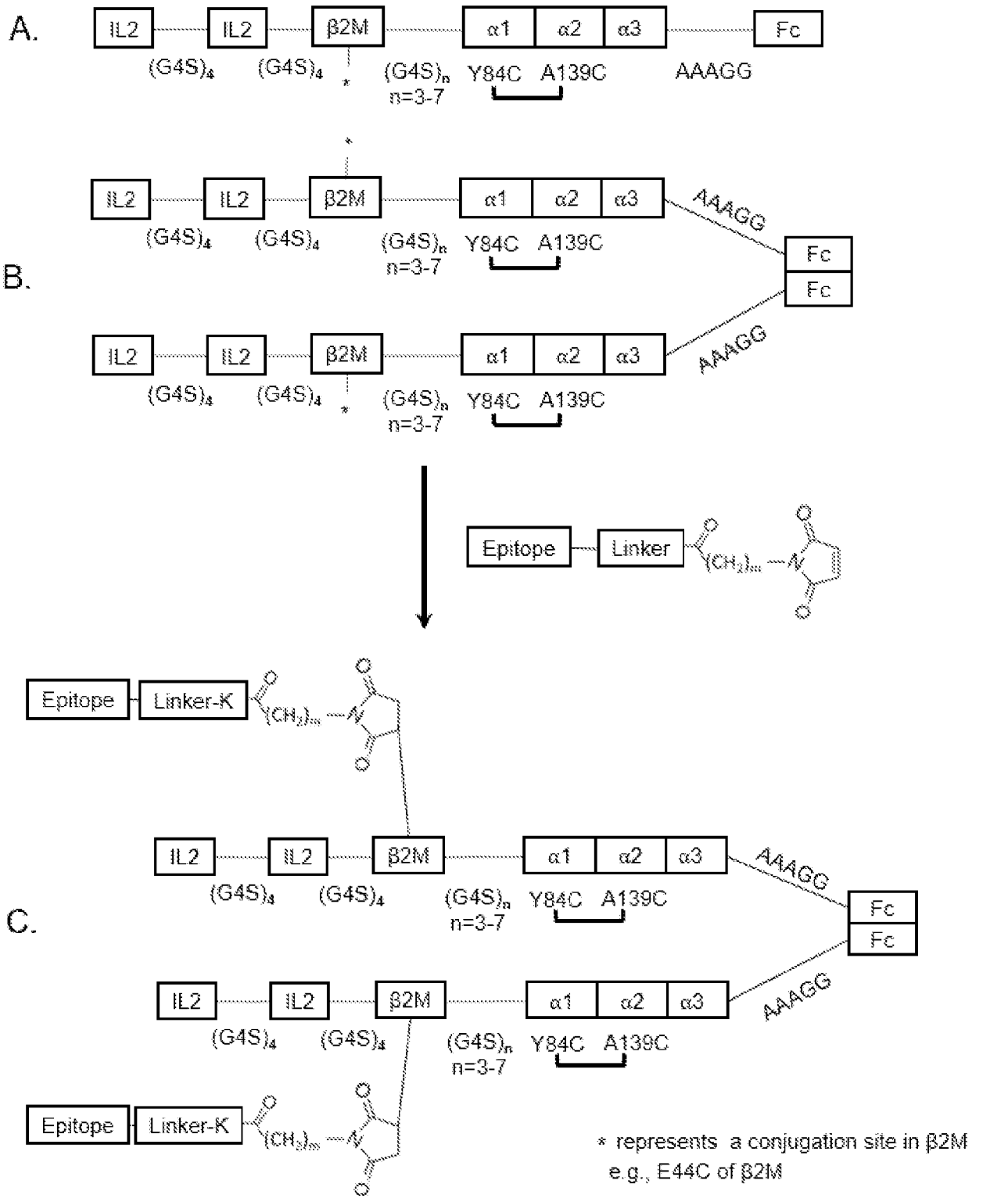

A.
IL2
IL2
β2M
α1
α2
α3
Fc
(G4S)4
(G4S)4
*
(G4S)n
n=3-7
Y84C
A139C
AAAGG
B.
C.
Epitope
Linker
Linker-K
(CH2)m
* represents a conjugation site in β2M
e.g., E44C of β2M
m is any suitable integer e.g., from 1 to 12

FIG. 10

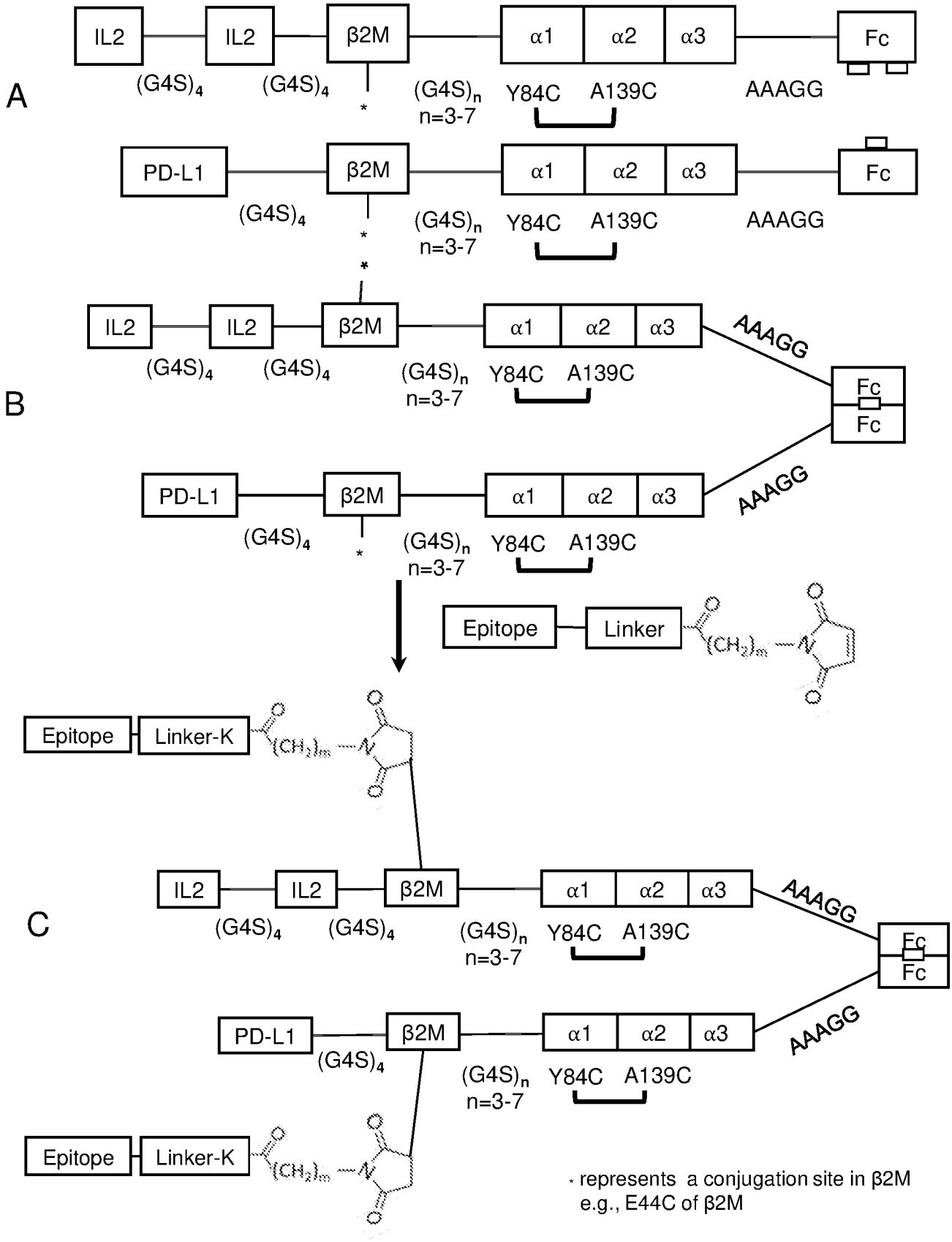

A
IL2
IL2
β2M
α1
α2
α3
Fc
(G4S)4
(G4S)4
(G4S)n
n=3-7
Y84C
A139C
AAAGG
PD-L1
B
C
Epitope
Linker
Linker-K
* represents a conjugation site in β2M
e.g., E44C of β2M
m is any suitable integer e.g., from 1 to 12

FIG. 11

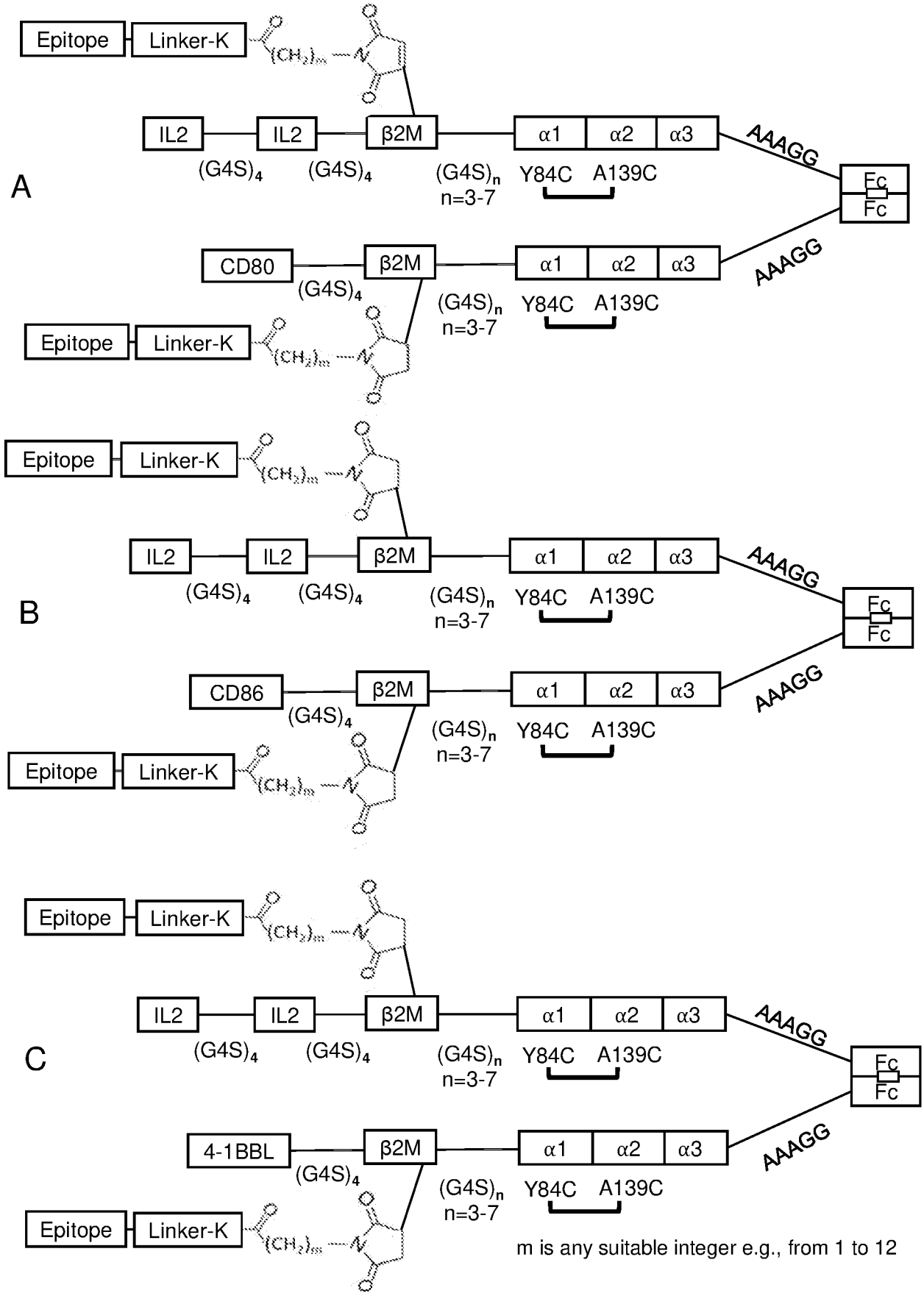

A
Epitope
Linker-K
IL2
IL2
β2M
α1
α2
α3
AAAGG
Fc
Fc
(G4S)4
(G4S)4
(G4S)n
n=3-7
Y84C
A139C
CD80
(G4S)4
B
CD86
C
4-1BBL
m is any suitable integer e.g., from 1 to 12

FIG. 12

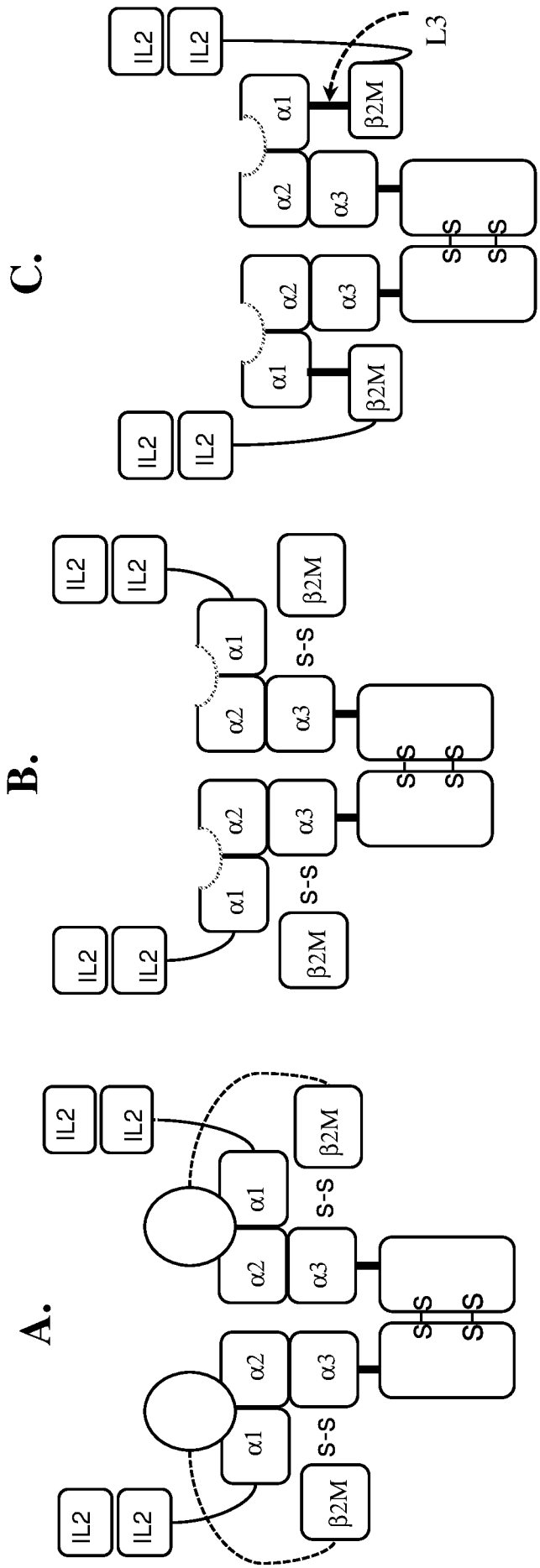
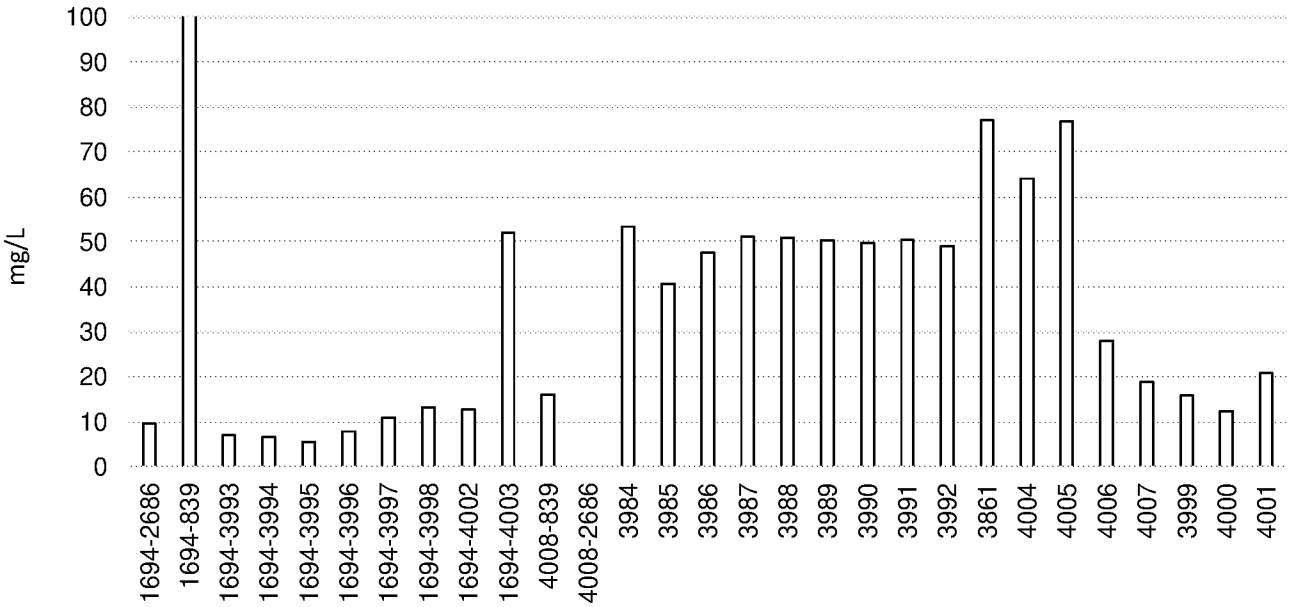

A.
IL2
IL2
α1
α2
α3
β2M
S-S
S-S
S-S
B.
IL2
IL2
α1
α2
α3
β2M
S-S
S-S
S-S
C.
IL2
IL2
α1
α2
α3
β2M
L3
S-S
S-S

FIG. 12 (continued)

D.



E.

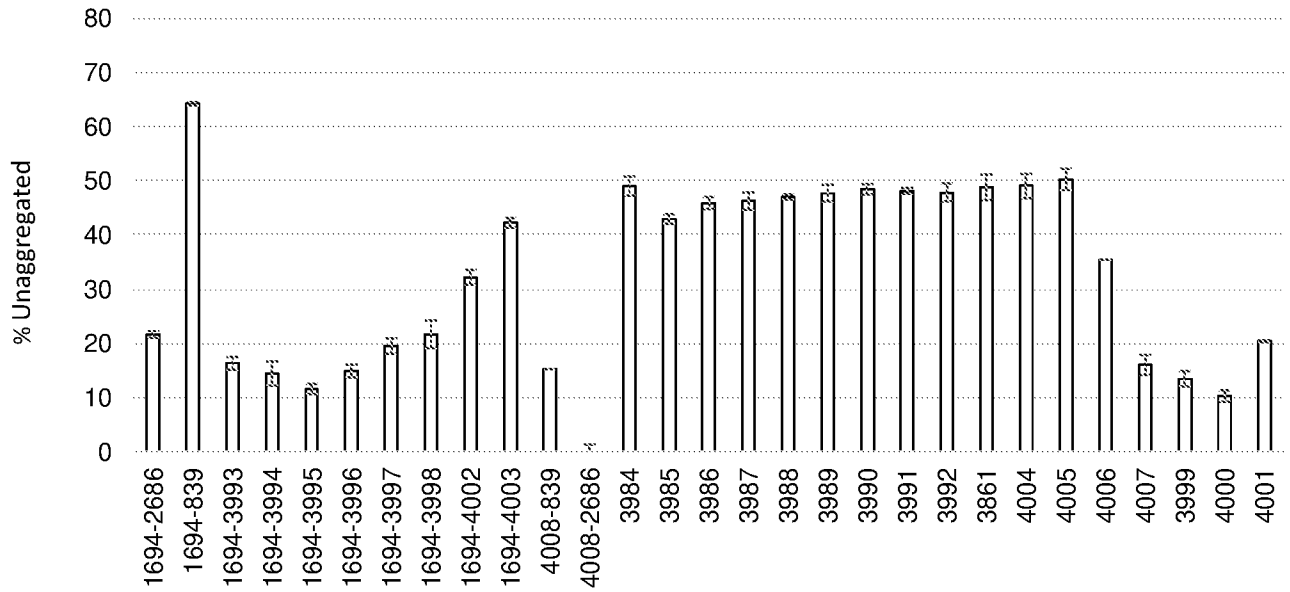


FIG. 13

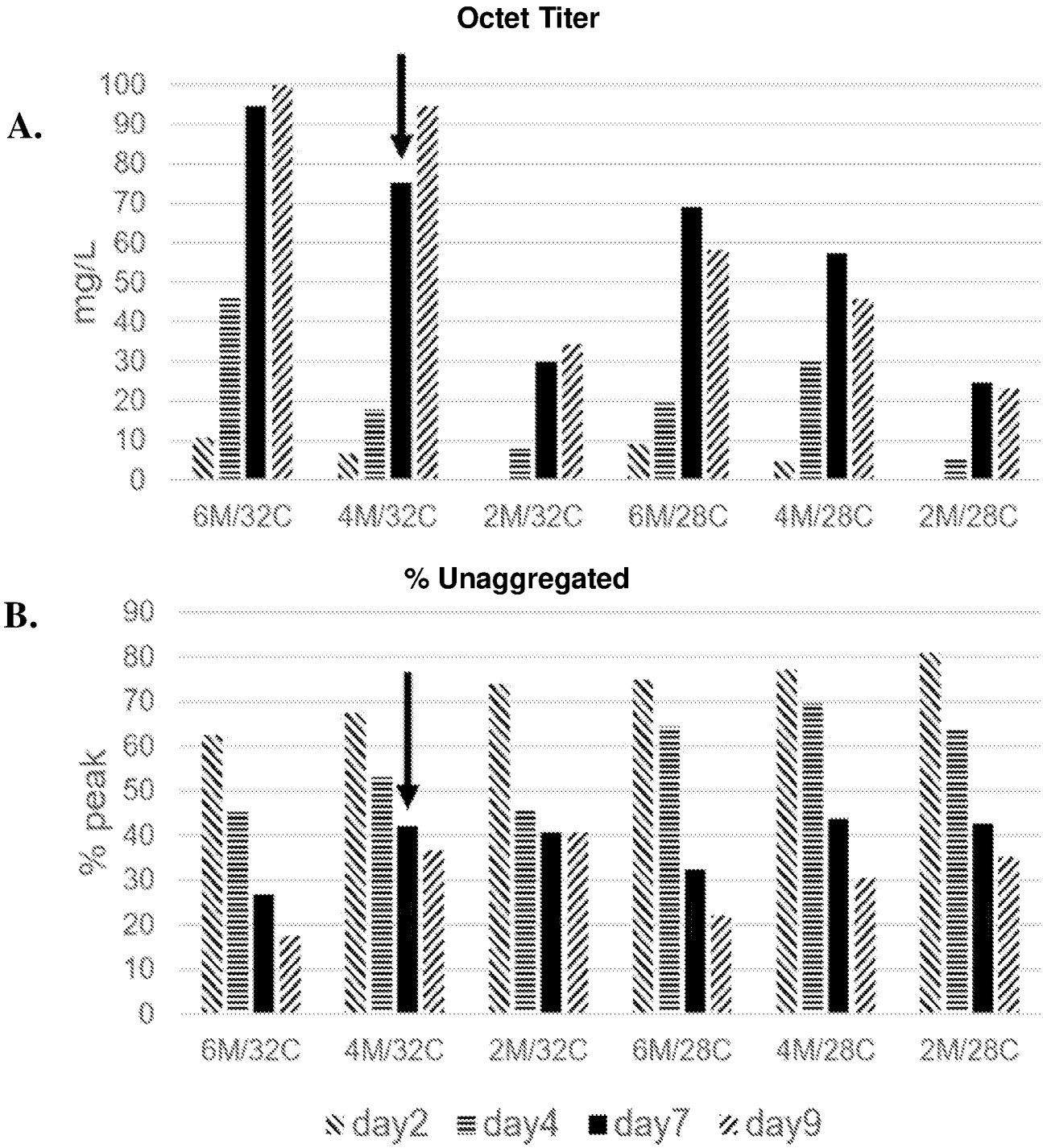
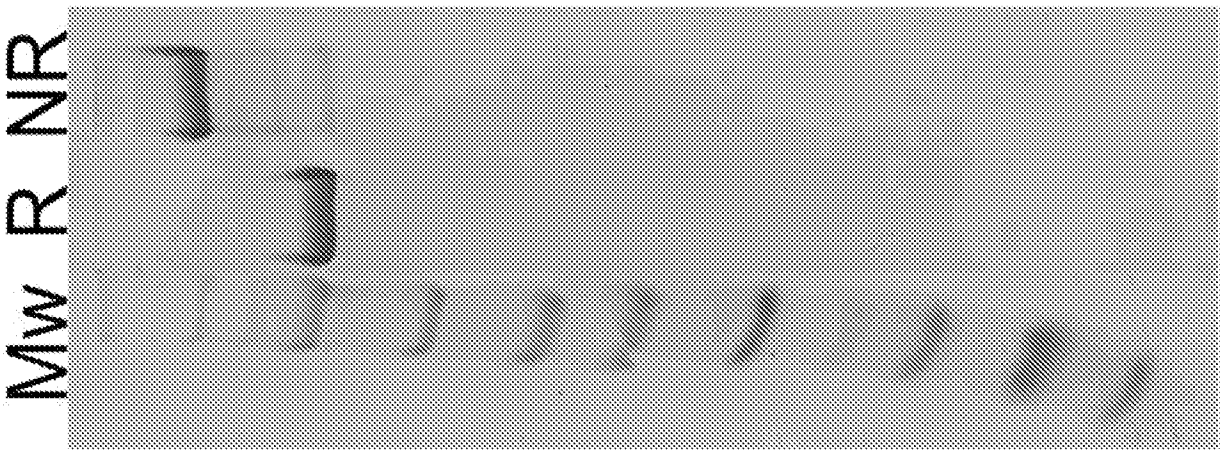
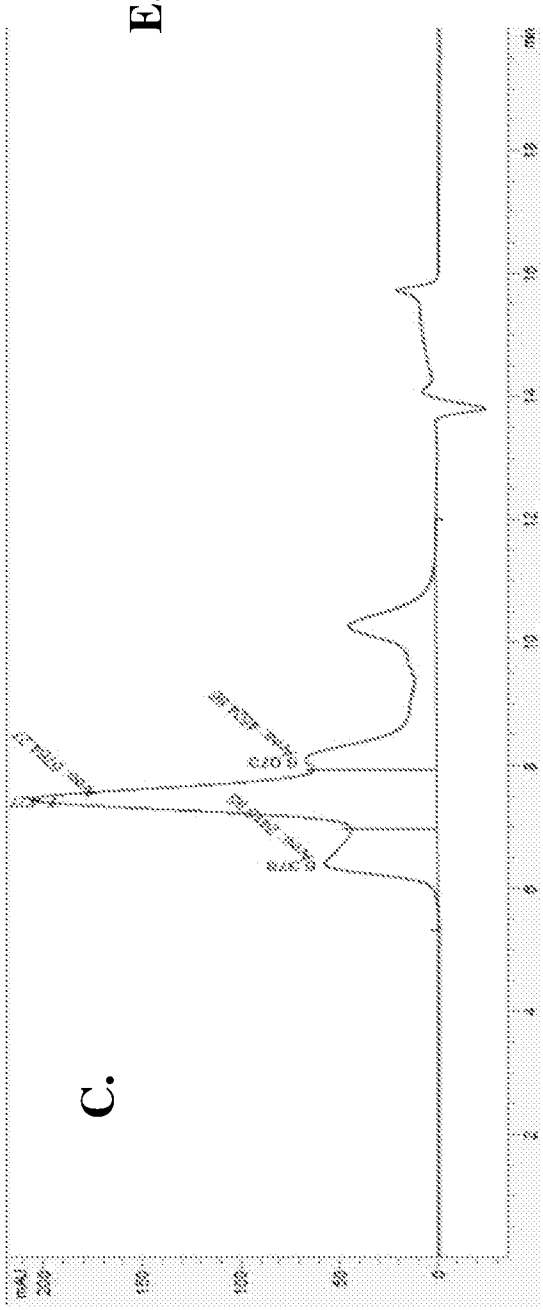

A.
Octet Titer
mg/L
100
90
80
70
60
50
40
30
20
10
0
6M/32C
4M/32C
2M/28C
6M/28C
4M/28C
2M/28C
B.
% Unaggregated
% peak
90
80
70
60
50
40
30
20
10
0
6M/32C
4M/32C
2M/32C
6M/28C
4M/28C
2M/28C
day2
day4
day7
day9

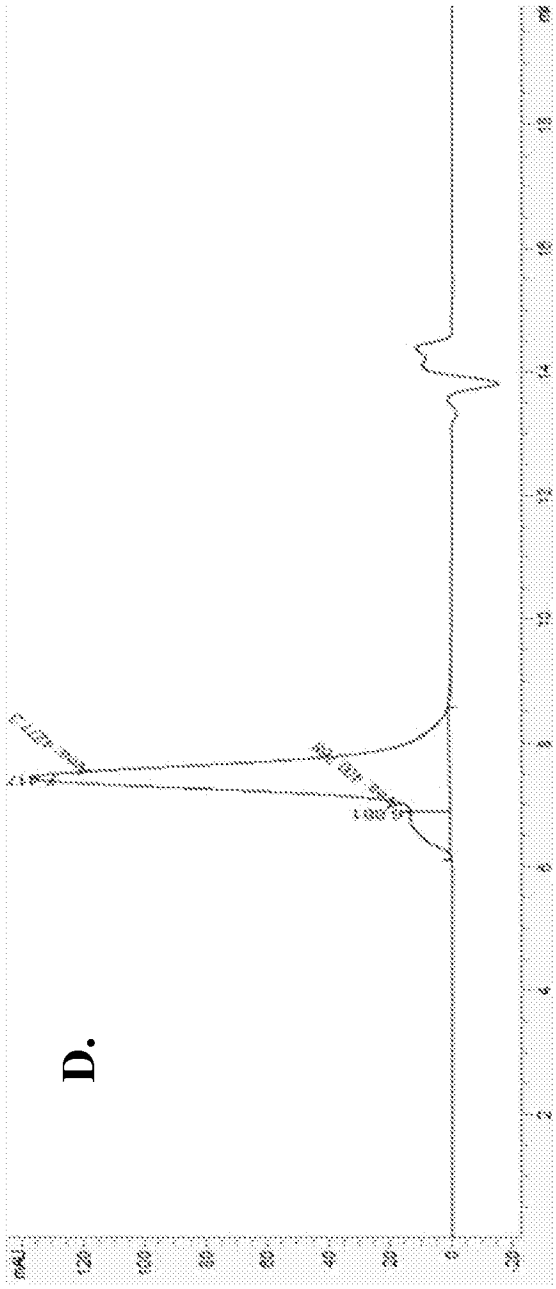
FIG. 13 (continued)



E.



C.

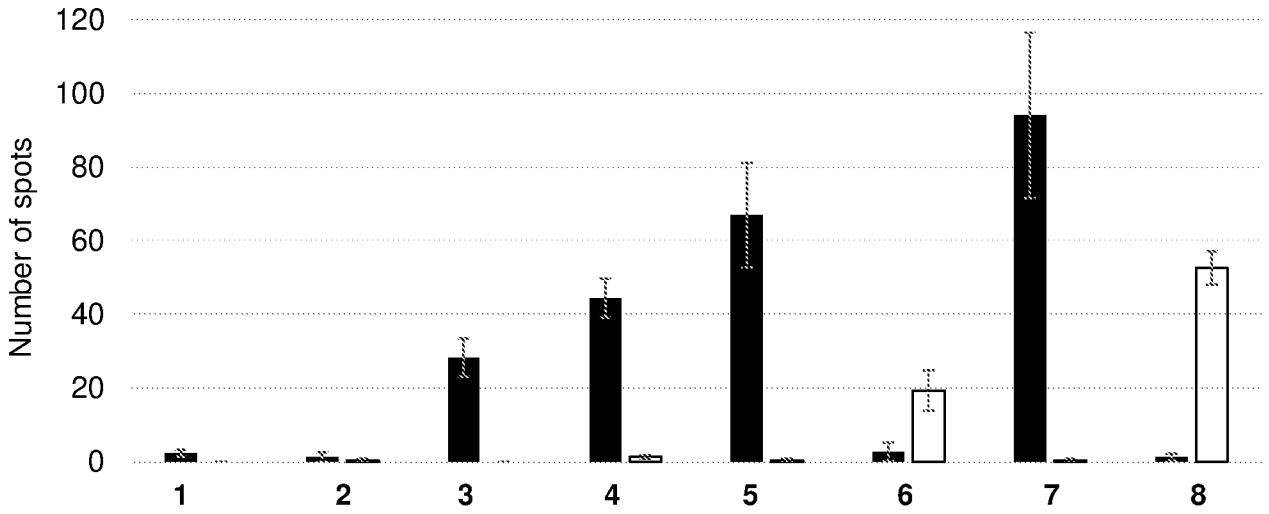


D.

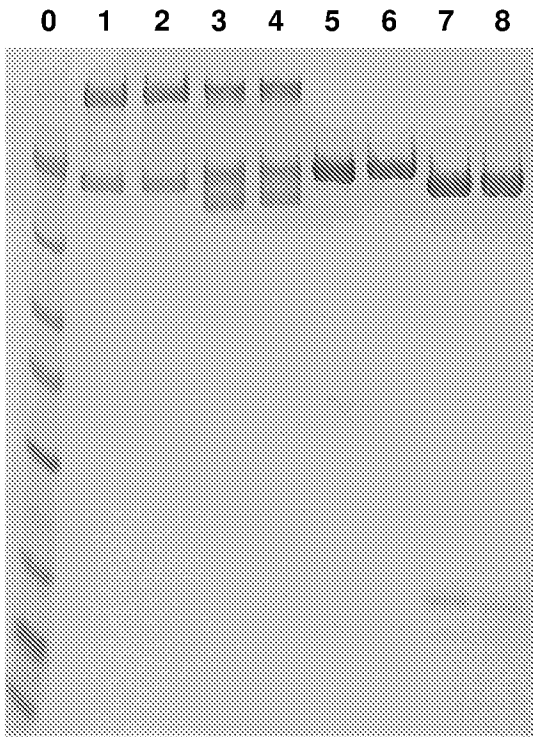
FIG. 14

A.

	1	2	3	4	5	6	7	8
Framework	T-Cell-MP	Split chain	T-Cell-MP	Split Chain	Split Chain	Split chain	—	—
Construct Type	Unconju-gated	Unconju-gated	Epitope conjugate	Epitope conjugate	Fusion protein	Fusion protein	–	–
Epitope Peptide	-	-	(G4S)3 CMV	(G4S)3 CMV	(G4S)3 CMV	(G4S)3 MART-1	CMV	MART-1



B.



Non-Reduced
0- MW Standards
1- T-Cell-MP-CMV epitope conjugate
2- T-Cell-MP-MART-1 epitope conjugate
3- Split-chain fusion protein with CMV
4- Split-chain fusion protein with MART1

Reduced
5- T-Cell-MP-CMV epitope conjugate
6- T-Cell-MP-MART-1 epitope conjugate
7- Split-chain fusion protein with CMV
8- Split-chain fusion protein with MART1

FIG. 15

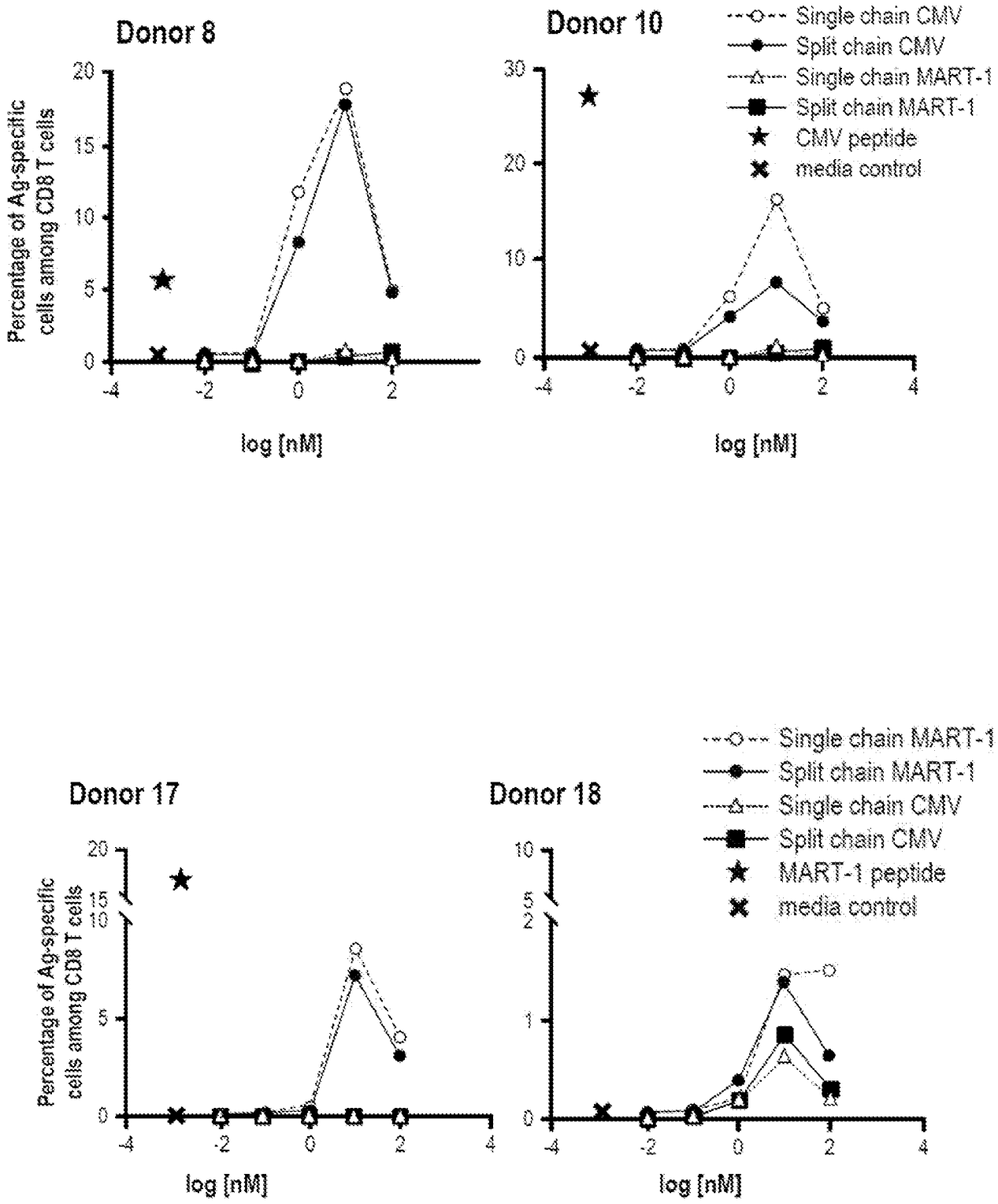

Donor 8
Donor 10
Donor 17
Donor 18
Single chain CMV
Split chain CMV
Single chain MART-1
Split chain MART-1
CMV peptide
MART-1 peptide
media control
Percentage of Ag-specific cells among CD8 T cells
log [nM]

FIG. 16

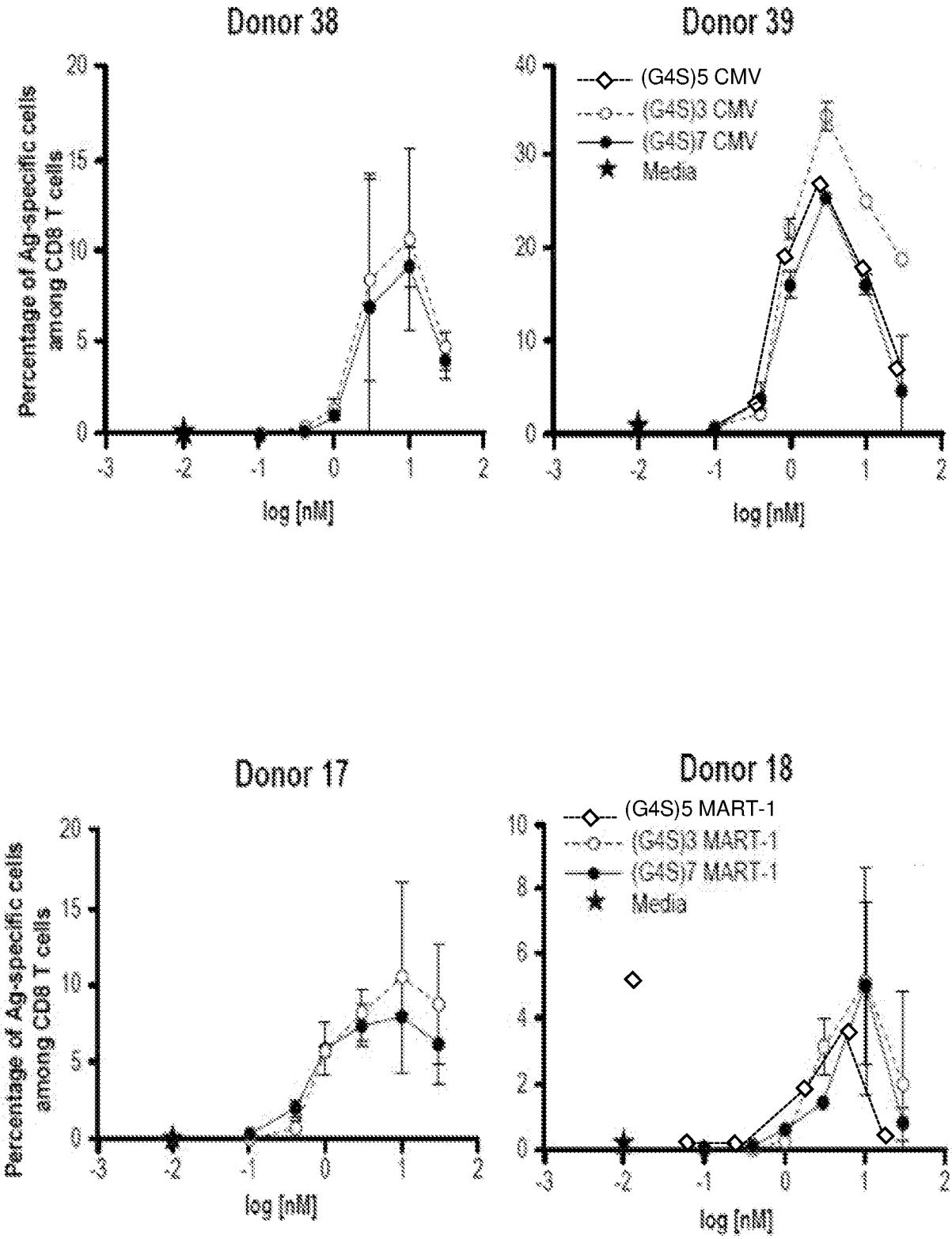
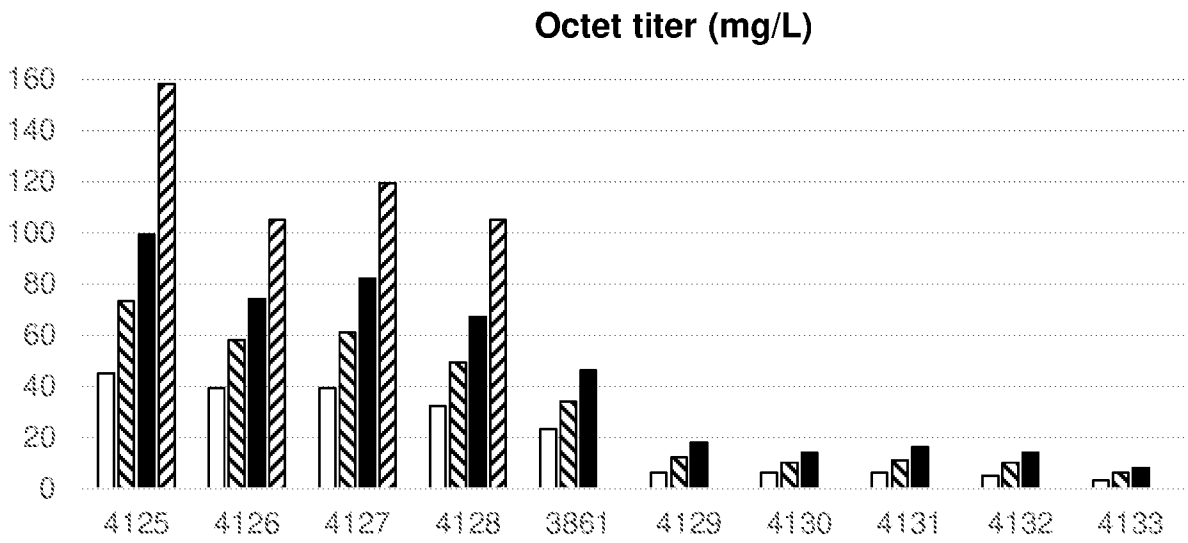

Donor 38
Donor 39
Donor 17
Donor 18
Percentage of Ag-specific cells among CD8 T cells
log [nM]
(G4S)5 CMV
(G4S)3 CMV
(G4S)7 CMV
Media
(G4S)5 MART-1
(G4S)3 MART-1
(G4S)7 MART-1
Media

FIG. 17

A.


Octet titer (mg/L)
160
140
120
100
80
60
40
20
0
4125
4126
4127
4128
3861
4129
4130
4131
4132
4133

B.

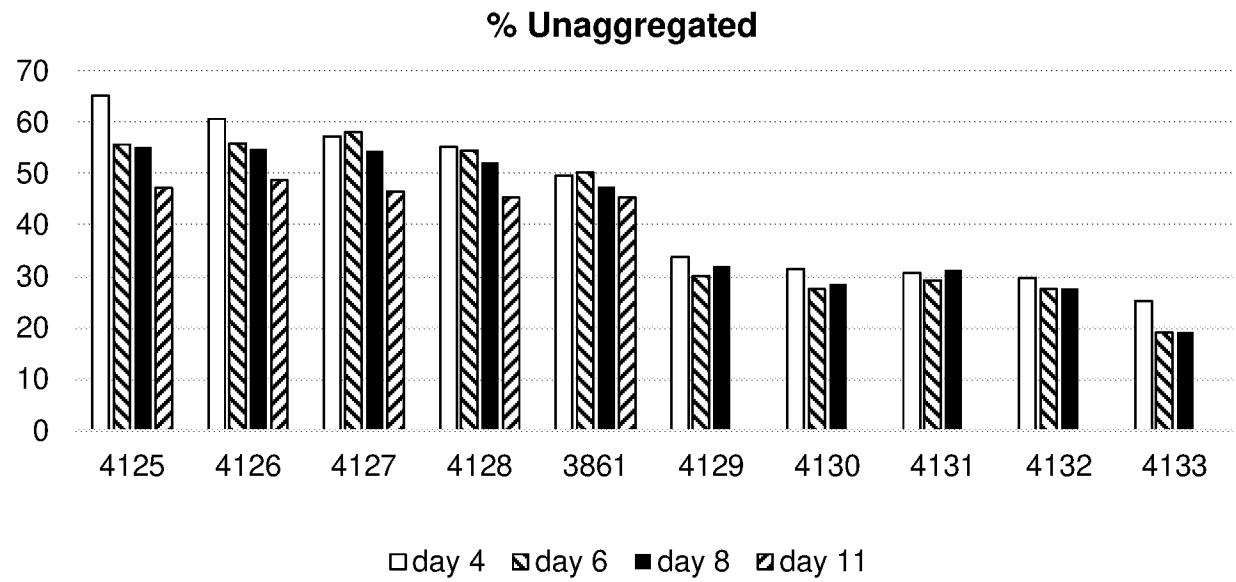

% Unaggregated
70
60
50
40
30
20
10
0
4125
4126
4127
4128
3861
4129
4130
4131
4132
4133
day 4
day 6
day 8
day 11

FIG. 18

Construct 839 CMV-GGGASGGGGSGGGGS-β2M (R12C)

NLVPMVATV***GGGASGGGGSGGGG***SIQRTPKIQVYSCHPAENGKSNFLNCYVSGFHPSDIEVDLLK NGERIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDM (SEQ ID NO:198).

Construct 1694 hIL2 (F42A, H16A)-$(G_4S)_4$-hIL2 (F42A, H16A)-$(G_4S)_4$-HLA-A02 (A236C, Y84C, A139C)-AAAGG-IgG1 (L234A, L235A)

APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKP LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGGS***APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFY MPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGGS***GSHSMRYFFTSVSRPGRGEPRFIAV GYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRGCYNQS EAGSHTVQRMYGCDVGSDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADMCAQTTKHKWEA AHVAEQLRAYLEGTCVEWLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEI TLTWQRDGEDQTQDTELVETRPCGDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWE***AAAGG***DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:193).

Construct 2686 β2M (R12C, E44C)

IQRTPKIQVYSCHPAENGKSNFLNCYVSGFHPSDIEVDLLKNGCRIEKVEHSDLSFSKDWSFYLLYY TEFTPTEKDEYACRVNHVTLSQPKIVKWDRDM (SEQ ID NO:195).

Construct 3861 hIL2 (F42A, H16A)-$(G_4S)_4$-hIL2 (F42A, H16A)-$(G_4S)_4$-β2M (E44C)-$(G_4S)_3$-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)

APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKP LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGGS***APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFY MPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGGS***IQRTPKIQVYSRHPAENGKSNFLNC YVSGFHPSDIEVDLLKNGCRIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPK IVKWDRDM***GGGGSGGGGSGGGGS***GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAA SQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRGCYNQSEAGSHTVQRMYGCDVG SDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADMCAQTTKHKWEAAHVAEQLRAYLEGTCVE WLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTEL VETRPAGDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWE***AAAGG***DKTHTCPPCPAPEA AGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH EALHNHYTQKSLSLSPG (SEQ ID NO:197).

FIG. 18 (continued)

Construct 3984 1xhIL2(F42A, H16A)-(G_4S)$_4$-β2M (E44C)-(G_4S)$_3$-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)

APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKP
LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGGS***IQRTPKIQVYSRHPAENGKSNFLNCYVSGFHPSDIEVDLLKNGCRIE
KVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDM***GGGGSGGGGSGG GGS***GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDG
ETRKVKAHSQTHRVDLGTLRG**C**YNQSEAGSHTVQRMYGCDVGSDWRFLRGYHQYAYDGKDYI
ALKEDLRSWTAADM**C**AQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRYLENGKETLQRTDAPK
THMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTELVETRPAGDGTFQKWAAVVV
PSGQEQRYTCHVQHEGLPKPLTLRWE***AAAGG***DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISR
TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
(SEQ ID NO:199).

Construct 3985 hIL2 (F42A, H16A)-(G_4S)$_4$-hIL2 (F42A, H16A)-(G_4S)-GLGGS-(G_4S)$_2$-β2M (E44C)-(G_4S)$_3$-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)

APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKP
LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGGS***APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFY
MPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADET
ATIVEFLNRWITFCQSIISTLT***GGGGSGLGGSGGGGSGGGGS***IQRTPKIQVYSRHPAENGKSNFLNC
YVSGFHPSDIEVDLLKNGCRIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPK
IVKWDRDM***GGGGSGGGGSGGGGS***GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAA
SQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRG**C**YNQSEAGSHTVQRMYGCDVG
SDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADM**C**AQTTKHKWEAAHVAEQLRAYLEGTCVE
WLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTEL
VETRPAGDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWE***AAAGG***DKTHTCPPCPAPEA
AGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
EALHNHYTQKSLSLSPG (SEQ ID NO:200).

Construct 3986 hIL2 (F42A, H16A)-(G_4S)$_4$-hIL2 (F42A, H16A)-(G_4S)$_2$-GLGGS-(G_4S)-β2M (E44C)-(G_4S)$_3$-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)

APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKP
LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGGS***APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFY
MPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADET
ATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGLGGSGGGGS***IQRTPKIQVYSRHPAENGKSNFLNC
YVSGFHPSDIEVDLLKNGCRIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPK
IVKWDRDM***GGGGSGGGGSGGGGS***GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAA
SQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRG**C**YNQSEAGSHTVQRMYGCDVG
SDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADM**C**AQTTKHKWEAAHVAEQLRAYLEGTCVE
WLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTEL
VETRPAGDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWE***AAAGG***DKTHTCPPCPAPEA
AGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
EALHNHYTQKSLSLSPG (SEQ ID NO:201).

FIG. 18 (continued)

Construct 3987 hIL2 (F42A, H16A)-(G_4S)$_4$-hIL2 (F42A, H16A)-(G_4S)$_3$-GLGGS-β2M (E44C)-(G_4S)$_3$-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)

APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKP
LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT***G***
GGGSGGGGSGGGGSGGGGSAPTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFY
MPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADET
ATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGLGGS***IQRTPKIQVYSRHPAENGKSNFLNC
YVSGFHPSDIEVDLLKNGCRIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPK
IVKWDRDM***GGGGSGGGGSGGGGS***GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAA
SQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRG**C**YNQSEAGSHTVQRMYGCDVG
SDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADM**C**AQTTKHKWEAAHVAEQLRAYLEGTCVE
WLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTEL
VETRPAGDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWE***AAAGG***DKTHTCPPCPAPEA
AGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
EALHNHYTQKSLSLSPG (SEQ ID NO:202).

Construct 3988 hIL2 (F42A, H16A)-(G_4S)$_4$-hIL2 (F42A, H16A)-(G_4S)-GMGGSGGGGS-(G_4S)-β2M (E44C)-(G_4S)$_3$-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)

APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKP
LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT***G***
GGGSGGGGSGGGGSGGGGSAPTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFY
MPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADET
ATIVEFLNRWITFCQSIISTLT***GGGGSGMGGSGGGGSGGGGS***IQRTPKIQVYSRHPAENGKSNFLNC
YVSGFHPSDIEVDLLKNGCRIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPK
IVKWDRDM***GGGGSGGGGSGGGGS***GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAA
SQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRG**C**YNQSEAGSHTVQRMYGCDVG
SDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADM**C**AQTTKHKWEAAHVAEQLRAYLEGTCVE
WLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTEL
VETRPAGDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWE***AAAGG***DKTHTCPPCPAPEA
AGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
EALHNHYTQKSLSLSPG (SEQ ID NO:203).

Construct 3989 hIL2 (F42A, H16A)-(G_4S)$_4$-hIL2 (F42A, H16A)-(G_4S)-GGGMSGGGGS-(G_4S)-β2M (E44C)-(G_4S)$_3$-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)

APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKP
LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT***G***
GGGSGGGGSGGGGSGGGGSAPTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFY
MPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADET
ATIVEFLNRWITFCQSIISTLT***GGGGSGGGMSGGGGSGGGGS***IQRTPKIQVYSRHPAENGKSNFLNC
YVSGFHPSDIEVDLLKNGCRIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPK
IVKWDRDM***GGGGSGGGGSGGGGS***GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAA
SQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRG**C**YNQSEAGSHTVQRMYGCDVG
SDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADM**C**AQTTKHKWEAAHVAEQLRAYLEGTCVE
WLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTEL
VETRPAGDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWE***AAAGG***DKTHTCPPCPAPEA
AGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
EALHNHYTQKSLSLSPG (SEQ ID NO:204).

FIG. 18 (continued)

Construct 3990 hIL2 (F42A, H16A)-(G_4S)$_4$-hIL2 (F42A, H16A)-(G_4S)-GGGGSMGGGS-(G_4S)-β2M (E44C)-(G_4S)$_3$-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)

APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKP
LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT***G***
GGGSGGGGSGGGGSGGGGSAPTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFY
MPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADET
ATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSMGGGSGGGGS***IQRTPKIQVYSRHPAENGKSNFLNC
YVSGFHPSDIEVDLLKNGCRIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPK
IVKWDRDM***GGGGSGGGGSGGGGS***GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAA
SQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRG**C**YNQSEAGSHTVQRMYGCDVG
SDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADM**C**AQTTKHKWEAAHVAEQLRAYLEGTCVE
WLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTEL
VETRPAGDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWE***AAAGG***DKTHTCPPCPAPEA
AGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
EALHNHYTQKSLSLSPG (SEQ ID NO:205).

Construct 3991 hIL2 (F42A, H16A)-(G_4S)$_4$-hIL2 (F42A, H16A)-(G_4S)-GGGGSGMGGS-(G_4S)-β2M (E44C)-(G_4S)$_3$-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)

APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKP
LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT***G***
GGGSGGGGSGGGGSGGGGSAPTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFY
MPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADET
ATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGMGGSGGGGS***IQRTPKIQVYSRHPAENGKSNFLNC
YVSGFHPSDIEVDLLKNGCRIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPK
IVKWDRDM***GGGGSGGGGSGGGGS***GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAA
SQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRG**C**YNQSEAGSHTVQRMYGCDVG
SDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADM**C**AQTTKHKWEAAHVAEQLRAYLEGTCVE
WLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTEL
VETRPAGDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWE***AAAGG***DKTHTCPPCPAPEA
AGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
EALHNHYTQKSLSLSPG (SEQ ID NO:206).

Construct 3992 hIL2 (F42A, H16A)-(G_4S)$_4$-hIL2 (F42A, H16A)-(G_4S)-GGGGSGGGGM-(G_4S)-β2M (E44C)-(G_4S)$_3$-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)

APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKP
LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT***G***
GGGSGGGGSGGGGSGGGGSAPTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFY
MPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADET
ATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGMGGGGS***IQRTPKIQVYSRHPAENGKSNFLNC
YVSGFHPSDIEVDLLKNGCRIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPK
IVKWDRDM***GGGGSGGGGSGGGGS***GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAA
SQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRG**C**YNQSEAGSHTVQRMYGCDVG
SDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADM**C**AQTTKHKWEAAHVAEQLRAYLEGTCVE
WLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTEL
VETRPAGDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWE***AAAGG***DKTHTCPPCPAPEA
AGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
EALHNHYTQKSLSLSPG (SEQ ID NO:207).

FIG. 18 (continued)

Construct 3993 GM-$(G_4S)_3$-β2M (R12C, E44C)
GM***GGGGSGGGGSGGGGS***IQRTPKIQVYSCHPAENGKSNFLNCYVSGFHPSDIEVDLLKNGCRIEK VEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDM (SEQ ID NO:208).

Construct 3994 GMGGS-$(G_4S)_2$-β2M (R12C, E44C)
GMGGS***GGGGSGGGGS***IQRTPKIQVYSCHPAENGKSNFLNCYVSGFHPSDIEVDLLKNGCRIEKVE HSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDM (SEQ ID NO:209).

Construct 3995 GMS-$(G_4S)_2$-β2M (R12C, E44C)
GMS***GGGGSGGGGS***IQRTPKIQVYSCHPAENGKSNFLNCYVSGFHPSDIEVDLLKNGCRIEKVEHSD LSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDM (SEQ ID NO:210).

Construct 3996 GMGGGS-(G_4S)-β2M (R12C, E44C)
GMGGGS***GGGGS***IQRTPKIQVYSCHPAENGKSNFLNCYVSGFHPSDIEVDLLKNGCRIEKVEHSDLS FSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDM (SEQ ID NO:211).

Construct 3997 GMGS-(G_4S)-β2M (R12C, E44C)
GMGS***GGGGS***IQRTPKIQVYSCHPAENGKSNFLNCYVSGFHPSDIEVDLLKNGCRIEKVEHSDLSFS KDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDM (SEQ ID NO:212).

Construct 3998 GM-(G_4S)-β2M (R12C, E44C)
GM***GGGGS***IQRTPKIQVYSCHPAENGKSNFLNCYVSGFHPSDIEVDLLKNGCRIEKVEHSDLSFSKD WSFYLLYYTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDM (SEQ ID NO:213).

Construct 3999 hIL2 (F42A, H16A)-$(G_4S)_4$-hIL2 (F42A, H16A)-$(G_4S)_4$-β2M (E44C)-$(G_4S)_3$-HLA-A02(Y84C, A139C, T143L)-AAAGG-IgG1(L234A, L235A)
APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKP LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT***G GGGSGGGGSGGGGSGGGGS***APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFY MPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGGS***IQRTPKIQVYSRHPAENGKSNFLNC YVSGFHPSDIEVDLLKNGCRIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPK IVKWDRDM***GGGGSGGGGSGGGGS***GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAA SQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRG**C**YNQSEAGSHTVQRMYGCDVG SDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADM**C**AQT**L**KHKWEAAHVAEQLRAYLEGTCVE WLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTEL VETRPAGDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWE***AAAGG***DKTHTCPPCPAPEA AGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH EALHNHYTQKSLSLSPG (SEQ ID NO:214).

FIG. 18 (continued)

Construct 4000 hIL2 (F42A, H16A)-$(G_4S)_4$-hIL2 (F42A, H16A)-$(G_4S)_4$-β2M (E44C)-$(G_4S)_3$-HLA-A02(Y84C, A139C, T143M)-AAAGG-IgG1(L234A, L235A)

APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKP
LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGGS***APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFY
MPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADET
ATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGGS***IQRTPKIQVYSRHPAENGKSNFLNC
YVSGFHPSDIEVDLLKNGCRIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPK
IVKWDRDM***GGGGSGGGGSGGGGS***GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAA
SQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRG**C**YNQSEAGSHTVQRMYGCDVG
SDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADM**C**AQT**M**KHKWEAAHVAEQLRAYLEGTCV
EWLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTE
LVETRPAGDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWE***AAAGG***DKTHTCPPCPAPE
AAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ
VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV
MHEALHNHYTQKSLSLSPG (SEQ ID NO:215).

Construct 4001 hIL2 (F42A, H16A)-$(G_4S)_4$-hIL2 (F42A, H16A)-$(G_4S)_4$-β2M (E44C)-$(G_4S)_3$-HLA-A02(L81M, Y84C, A139C)-AAAGG-IgG1(L234A, L235A)

APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKP
LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGGS***APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFY
MPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADET
ATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGGS***IQRTPKIQVYSRHPAENGKSNFLNC
YVSGFHPSDIEVDLLKNGCRIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPK
IVKWDRDM***GGGGSGGGGSGGGGS***GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAA
SQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGT**M**RG**C**YNQSEAGSHTVQRMYGCDV
GSDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADM**C**AQTTKHKWEAAHVAEQLRAYLEGTCV
EWLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTE
LVETRPAGDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWE***AAAGG***DKTHTCPPCPAPE
AAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ
VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV
MHEALHNHYTQKSLSLSPG (SEQ ID NO:216).

Construct 4002 CMV-(G_3AS)-$(G_4S)_2$-β2M (R12C, E44C)

NLVPMVATVGGGAS***GGGGSGGGGS***IQRTPKIQVYSCHPAENGKSNFLNCYVSGFHPSDIEVDLLK
NGCRIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDM (SEQ ID
NO:217).

Construct 4003 CMV-(G_3AS)-(G_4S)-FC1-(G_4S)-β2M (R12C, E44C)

NLVPMVATVGGGAS***GGGGS***HVVPYGLGSPRSKRALENLL***GGGGS***IQRTPKIQVYSCHPAENGKS
NFLNCYVSGFHPSDIEVDLLKNGCRIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVT
LSQPKIVKWDRDM (SEQ ID NO:218).

FIG. 18 (continued)

Construct 4004 hIL2 (F42A, H16A)-$(G_4S)_4$-hIL2 (F42A, H16A)-$(G_4S)_5$-β2M (E44C)-$(G_4S)_3$-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)

APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKP LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGGS***APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFY MPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGGSGGGGS***IQRTPKIQVYSRHPAENGKS NFLNCYVSGFHPSDIEVDLLKNGCRIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVT LSQPKIVKWDRDM***GGGGSGGGGSGGGGS***GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRF DSDAASQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRG**C**YNQSEAGSHTVQRMY GCDVGSDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADM**C**AQTTKHKWEAAHVAEQLRAYL EGTCVEWLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGED QTQDTELVETRPAGDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWE***AAAGG***DKTHTC PPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:219).

Construct 4005 hIL2 (F42A, H16A)-$(G_4S)_4$-hIL2 (F42A, H16A)-$(G_4S)_6$-β2M (E44C)-$(G_4S)_3$-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)

APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKP LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGGS***APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFY MPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGGSGGGGSGGGGS***IQRTPKIQVYSRHPA ENGKSNFLNCYVSGFHPSDIEVDLLKNGCRIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACR VNHVTLSQPKIVKWDRDM***GGGGSGGGGSGGGGS***GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDT QFVRFDSDAASQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRG**C**YNQSEAGSHT VQRMYGCDVGSDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADM**C**AQTTKHKWEAAHVAEQ LRAYLEGTCVEWLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQR DGEDQTQDTELVETRPAGDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWE***AAAGG***DK THTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:220).

Construct 4006 hIL2 (F42A, H16A)-$(G_4S)_4$-hIL2 (F42A, H16A)-$(G_4S)_4$-β2M (E44C)-$(G_4S)_2$-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)

APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKP LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGGS***APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFY MPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGGS***IQRTPKIQVYSRHPAENGKSNFLNC YVSGFHPSDIEVDLLKNGCRIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPK IVKWDRDM***GGGGSGGGGS***GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRME PRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRG**C**YNQSEAGSHTVQRMYGCDVGSDWRF LRGYHQYAYDGKDYIALKEDLRSWTAADM**C**AQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRY LENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTELVETRP AGDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWE***AAAGG***DKTHTCPPCPAPEAAGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPG (SEQ ID NO:221).

FIG. 18 (continued)

Construct 4007 hIL2 (F42A, H16A)-$(G_4S)_4$-hIL2 (F42A, H16A)-$(G_4S)_4$-β2M (E44C)-(G_4S)-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)

APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKP
LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT***G***
GGGSGGGGSGGGGSGGGGSAPTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFY
MPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADET
ATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGGS***IQRTPKIQVYSRHPAENGKSNFLNC
YVSGFHPSDIEVDLLKNGCRIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPK
IVKWDRDM***GGGGS***GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPW
IEQEGPEYWDGETRKVKAHSQTHRVDLGTLRG**C**YNQSEAGSHTVQRMYGCDVGSDWRFLRGYH
QYAYDGKDYIALKEDLRSWTAADM**C**AQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRYLENGK
ETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTELVETRPAGDGT
FQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWE***AAAGG***DKTHTCPPCPAPEAAGGPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV
LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPG (SEQ ID NO:222).

Construct 4008 2xhIL2 (F42A, H16A)-$(G_4S)_4$-HLA-A02 (A236C, Y85C, D137C)-AAAGG-IgG1 (L234A, L235A)

APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKP
LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT***G***
GGGSGGGGSGGGGSGGGGSAPTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFY
MPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADET
ATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGGS***GSHSMRYFFTSVSRPGRGEPRFIAV
GYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRGY**C**NQS
EAGSHTVQRMYGCDVGSDWRFLRGYHQYAYDGKDYIALKEDLRSWTAA**C**MAAQTTKHKWEA
AHVAEQLRAYLEGTCVEWLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEI
TLTWQRDGEDQTQDTELVETRP**C**GDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWE***A***
AAGGDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG
VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE
PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:194).

Construct 4125 hIL2 (F42A, H16A)-$(G_4S)_4$-hIL2 (F42A, H16A)-$(G_4S)_4$-β2M (E44C)-$(G_4S)_7$-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)

APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKP
LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT***G***
GGGSGGGGSGGGGSGGGGSAPTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFY
MPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADET
ATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGGS***IQRTPKIQVYSRHPAENGKSNFLNC
YVSGFHPSDIEVDLLKNGCRIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPK
IVKWDRDM***GGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGS***GSHSMRYFFTSVSRPGRGEP
RFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRG**C**
YNQSEAGSHTVQRMYGCDVGSDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADM**C**AQTTKHK
WEAAHVAEQLRAYLEGTCVEWLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFY
PAEITLTWQRDGEDQTQDTELVETRPAGDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLR
WE***AAAGG***DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS
KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:224).

FIG. 18 (continued)

Construct 4126 hIL2 (F42A, H16A)-$(G_4S)_4$-hIL2 (F42A, H16A)-$(G_4S)_4$-β2M (E44C)-$(G_4S)_6$-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)

APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKP
LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT***G***
GGGSGGGGSGGGGSGGGGSAPTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFY
MPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADET
ATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGGS***IQRTPKIQVYSRHPAENGKSNFLNC
YVSGFHPSDIEVDLLKNGCRIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPK
IVKWDRDM***GGGGSGGGGSGGGGSGGGGSGGGGSGGGGS***GSHSMRYFFTSVSRPGRGEPRFIAVG
YVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRG**C**YNQSE
AGSHTVQRMYGCDVGSDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADM**C**AQTTKHKWEAA
HVAEQLRAYLEGTCVEWLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEIT
LTWQRDGEDQTQDTELVETRPAGDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWE***AA***
AGGDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV
DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:225).

Construct 4127 hIL2 (F42A, H16A)-$(G_4S)_4$-hIL2 (F42A, H16A)-$(G_4S)_4$-β2M (E44C)-$(G_4S)_5$-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)

APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKP
LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT***G***
GGGSGGGGSGGGGSGGGGSAPTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFY
MPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADET
ATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGGS***IQRTPKIQVYSRHPAENGKSNFLNC
YVSGFHPSDIEVDLLKNGCRIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPK
IVKWDRDM***GGGGSGGGGSGGGGSGGGGSGGGGS***GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDT
QFVRFDSDAASQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRG**C**YNQSEAGSHT
VQRMYGCDVGSDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADM**C**AQTTKHKWEAAHVAEQ
LRAYLEGTCVEWLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQR
DGEDQTQDTELVETRPAGDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWE***AAAGG***DK
THTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP
PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:226).

Construct 4128 hIL2 (F42A, H16A)-$(G_4S)_4$-hIL2 (F42A, H16A)-$(G_4S)_4$-β2M (E44C)-$(G_4S)_4$-HLA-A02(Y84C, A139C)-AAAGG-IgG1(L234A, L235A)

APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKP
LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT***G***
GGGSGGGGSGGGGSGGGGSAPTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFY
MPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADET
ATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGGS***IQRTPKIQVYSRHPAENGKSNFLNC
YVSGFHPSDIEVDLLKNGCRIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPK
IVKWDRDM***GGGGSGGGGSGGGGSGGGGS***GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRF
DSDAASQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRG**C**YNQSEAGSHTVQRMY
GCDVGSDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADM**C**AQTTKHKWEAAHVAEQLRAYL
EGTCVEWLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGED
QTQDTELVETRPAGDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWE***AAAGG***DKTHTC
PPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:227).

FIG. 18 (continued)

Construct 4129 hIL2 (F42A, H16A)-$(G_4S)_4$-hIL2 (F42A, H16A)-$(G_4S)_4$-β2M (R12C, E44C)-$(G_4S)_7$-HLA-A02(Y84C, A139C, A236C)-AAAGG-IgG1(L234A, L235A)

APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKP
LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGGS***APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFY
MPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADET
ATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGGS***IQRTPKIQVYSCHPAENGKSNFLNC
YVSGFHPSDIEVDLLKNGCRIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPK
IVKWDRDM***GGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGS***GSHSMRYFFTSVSRPGRGEP
RFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRG**C**
YNQSEAGSHTVQRMYGCDVGSDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADM**C**AQTTKHK
WEAAHVAEQLRAYLEGTCVEWLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFY
PAEITLTWQRDGEDQTQDTELVETRP**C**GDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLR
WE***AAAGG***DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS
KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:228).

Construct 4130 hIL2 (F42A, H16A)-$(G_4S)_4$-hIL2 (F42A, H16A)-$(G_4S)_4$-β2M (R12C, E44C)-$(G_4S)_6$-HLA-A02(Y84C, A139C, A236C)-AAAGG-IgG1(L234A, L235A

APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKP
LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGGS***APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFY
MPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADET
ATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGGS***IQRTPKIQVYSCHPAENGKSNFLNC
YVSGFHPSDIEVDLLKNGCRIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPK
IVKWDRDM***GGGGSGGGGSGGGGSGGGGSGGGGSGGGGS***GSHSMRYFFTSVSRPGRGEPRFIAVG
YVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRG**C**YNQSE
AGSHTVQRMYGCDVGSDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADM**C**AQTTKHKWEAA
HVAEQLRAYLEGTCVEWLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEIT
LTWQRDGEDQTQDTELVETRP**C**GDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWE***AA***
AGGDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV
DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:229).

Construct 4131 hIL2 (F42A, H16A)-$(G_4S)_4$-hIL2 (F42A, H16A)-$(G_4S)_4$-β2M (R12C, E44C)-$(G_4S)_5$-HLA-A02(Y84C, A139C, A236C)-AAAGG-IgG1(L234A, L235A)

APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKP
LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGGS***APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFY
MPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADET
ATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGGS***IQRTPKIQVYSCHPAENGKSNFLNC
YVSGFHPSDIEVDLLKNGCRIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPK
IVKWDRDM***GGGGSGGGGSGGGGSGGGGSGGGGS***GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDT
QFVRFDSDAASQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRG**C**YNQSEAGSHT
VQRMYGCDVGSDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADM**C**AQTTKHKWEAAHVAEQ
LRAYLEGTCVEWLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQR
DGEDQTQDTELVETRP**C**GDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWE***AAAGG***DK
THTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP
PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:230).

FIG. 18 (continued)

Construct 4132 hIL2 (F42A, H16A)-$(G_4S)_4$-hIL2 (F42A, H16A)-$(G_4S)_4$-β2M (R12C, E44C)-$(G_4S)_4$-HLA-A02(Y84C, A139C, A236C)-AAAGG-IgG1(L234A, L235A)

APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKP
LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT***G***
GGGSGGGGSGGGGSGGGGSAPTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFY
MPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADET
ATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGGS***IQRTPKIQVYSCHPAENGKSNFLNC
YVSGFHPSDIEVDLLKNGCRIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPK
IVKWDRDM***GGGGSGGGGSGGGGSGGGGS***GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRF
DSDAASQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRG**C**YNQSEAGSHTVQRMY
GCDVGSDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADM**C**AQTTKHKWEAAHVAEQLRAYL
EGTCVEWLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGED
QTQDTELVETRP**C**GDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWE***AAAGG***DKTHTC
PPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:231).

Construct 4133 hIL2 (F42A, H16A)-$(G_4S)_4$-hIL2 (F42A, H16A)-$(G_4S)_4$-β2M (R12C, E44C)-$(G_4S)_3$-HLA-A02(Y84C, A139C, A236C)-AAAGG-IgG1(L234A, L235A)

APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKP
LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT***G***
GGGSGGGGSGGGGSGGGGSAPTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFY
MPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADET
ATIVEFLNRWITFCQSIISTLT***GGGGSGGGGSGGGGSGGGGS***IQRTPKIQVYSCHPAENGKSNFLNC
YVSGFHPSDIEVDLLKNGCRIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPK
IVKWDRDM***GGGGSGGGGSGGGGS***GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAA
SQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRG**C**YNQSEAGSHTVQRMYGCDVG
SDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADM**C**AQTTKHKWEAAHVAEQLRAYLEGTCVE
WLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTEL
VETRP**C**GDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWE***AAAGG***DKTHTCPPCPAPEA
AGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
EALHNHYTQKSLSLSPG (SEQ ID NO:232).