

MULTIMERIC T-CELL MODULATORY POLYPEPTIDES AND METHODS OF USE THEREOF

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/782,167, filed December 19, 2018, and U.S. Provisional Patent Application No. 62/814,684, filed March 6, 2019, which applications are incorporated herein by reference in their entirety.

INTRODUCTION

[0002] An adaptive immune response involves the engagement of the T cell receptor (TCR), present on the surface of a T cell, with a small peptide antigen non-covalently presented on the surface of an antigen presenting cell (APC) by a major histocompatibility complex (MHC; also referred to in humans as a human leukocyte antigen (HLA) complex). This engagement represents the immune system's targeting mechanism and is a requisite molecular interaction for T cell modulation (activation or inhibition) and effector function. Following epitope-specific cell targeting, the targeted T cells are activated through engagement of costimulatory proteins found on the APC with counterpart costimulatory proteins on the T cells. Both signals – epitope/TCR binding and engagement of APC costimulatory proteins with T cell costimulatory proteins – are required to drive T cell specificity and activation or inhibition. The TCR is specific for a given epitope; however, the costimulatory protein is not epitope specific and instead is generally expressed on all T cells or on large T cell subsets.

SUMMARY

[0003] The present disclosure provides T-cell modulatory multimeric polypeptides (TMMPs) that comprise an immunomodulatory polypeptide and that comprise an epitope-presenting Wilms tumor peptide. A T-cell modulatory multimeric polypeptide is useful for modulating the activity of a T cell, and for modulating an immune response in an individual.

[0003a] In one aspect, the present disclosure refers to a T-cell modulatory multimeric polypeptide (TMMP) comprising: at least one heterodimer comprising: a) a first polypeptide comprising: i) a Wilms tumor-1 (WT-1) peptide epitope, wherein the WT-1 peptide has a length of from 4 to 25 amino acids; and ii) first class I major histocompatibility complex (MHC) class I polypeptide, wherein the first MHC-class I MHC polypeptide is a β 2-microglobulin (β 2M) polypeptide; b) a second polypeptide comprising: i) a second MHC-class I MHC polypeptide, wherein the second MHC-class I MHC polypeptide is an MHC class I heavy chain polypeptide; and ii) one or more immunomodulatory polypeptides, wherein the one or more immunomodulatory polypeptides is one or more variant IL-2 polypeptides that exhibit reduced affinity to an IL-2 receptor and that comprise a substitution of H16, or a substitution of H16 and a substitution of F42, based on the amino acid numbering of SEQ ID NO:15; and iii) an immunoglobulin (Ig) Fc polypeptide, wherein the first and/or the second polypeptide further comprises are covalently linked to one or more activating immunomodulatory polypeptides, and optionally another via at least a first and second disulfide bond, wherein the TMMP comprises: a first disulfide bond formed between (i) a Cys residue in a Cys-containing linker between the WT-1 peptide epitope and the β 2M polypeptide, and (ii) a Cys residue in the MHC

class I heavy chain polypeptide; and ~~a~~the second disulfide bond is formed between ~~(i)~~ a Cys residue in ~~a linker between the WT 1 peptide epitope and~~ the β 2M polypeptide; and ~~(ii)~~ a Cys residue in the MHC class I heavy chain polypeptide.

[0003b] In one aspect, the present disclosure refers to a TMMP comprising a ~~homodimer of two first and second~~ heterodimers disclosed herein, wherein the ~~first and second~~ heterodimers are ~~the same, and wherein the heterodimers identical in amino acid sequence and~~ are covalently bound to each other by one or more disulfide bonds between the Ig Fc polypeptides of the ~~first and second~~ heterodimers.

~~**[0003c]** In one aspect, the present disclosure refers to a nucleic acid comprising a nucleotide sequence encoding a first or second polypeptide disclosed herein.~~

~~**[0003d]** In one aspect, the present disclosure refers to an expression vector comprising a nucleic acid disclosed herein.~~

~~**[0003e]** In one aspect, the present disclosure refers to a genetically modified host cell, wherein the host cell is genetically modified with a nucleic acid disclosed herein, or an expression vector disclosed herein.~~

~~**[0003f]** In one aspect, the present disclosure refers to a method of making a TMMP disclosed herein comprising culturing a genetically modified host cell *in vitro* in a culture medium under conditions such that the host cell synthesizes the TMMP.~~

[0003g0003c] In one aspect, the present disclosure refers to a pharmaceutical composition comprising a TMMP disclosed herein.

[0003hd] In one aspect, the present disclosure refers to a composition comprising one or more nucleic acids comprising nucleotide sequences encoding the first and second polypeptides of the TMMP disclosed herein~~the TMMP disclosed herein, or a pharmaceutical composition disclosed herein for use in selective modulation of the activity of a T cell that binds to a cancer associated epitope in an individual, wherein said TMMP or pharmaceutical composition is to be administered to selectively modulate the activity of the T cell in the individual.~~

[0003ie] In one aspect, the present disclosure refers to a composition comprising one or more expression vectors, wherein the one or more expression vectors comprise nucleotide sequences encoding the first and second polypeptides of the TMMP disclosed herein~~the use of the TMMP disclosed herein, or a pharmaceutical composition disclosed herein in the manufacture of a medicament for selective modulation of the activity of a T cell that binds to a cancer associated epitope in an individual, wherein said TMMP or pharmaceutical composition is to be administered to selectively modulate the activity of the T cell in the individual.~~

[0003f] In one aspect, the present disclosure refers to a method of producing a TMMP disclosed herein, the method comprising culturing a host cell that is genetically modified with one or more expression vectors comprising nucleotide sequences encoding the first and the second polypeptides of the multimeric polypeptide, under conditions such that the genetically modified host cell produces the TMMP.

[0003g] In one aspect, the present disclosure refers to an in vitro composition of genetically modified host cells, wherein the host cells comprise one or more nucleic acids comprising nucleotide sequences encoding the first and second polypeptides of the TMMP disclosed herein.

[0003jh] In one aspect, the present disclosure refers to the TMMP disclosed herein~~, or a pharmaceutical composition disclosed herein, for use in treating a cancer in an individual.~~

[0003ki] In one aspect, the present disclosure refers to the use of the TMMP disclosed herein~~, or a pharmaceutical composition disclosed herein in the manufacture of a medicament for treating a cancer in an individual.~~

[0003j] In one aspect, the present disclosure refers to the pharmaceutical composition disclosed herein, for use in treating a cancer in an individual, wherein the individual is also being treated with a therapeutically effective amount of an immune checkpoint inhibitor.

[0003km] In one aspect, the present disclosure refers to the use of the pharmaceutical composition disclosed herein in the manufacture of a medicament for treating a cancer in an individual, wherein the individual is also being treated with a therapeutically effective amount of an immune checkpoint inhibitor.

[0003ln] In one aspect, the present disclosure refers to the ~~TMMP disclosed herein, or a~~ pharmaceutical composition disclosed herein, for use in treating a cancer in an individual, wherein the individual is also ~~to be being~~ treated with a therapeutically effective amount of an immune checkpoint inhibitor, ~~optionally and~~ wherein the checkpoint inhibitor is an antibody specific for PD-1, PD-L1, ~~or~~ CTLA4, ~~or~~ TIGIT.

[0003mo] In one aspect, the present disclosure refers to the use of the ~~TMMP disclosed herein, or a~~ pharmaceutical composition disclosed herein in the manufacture of a medicament for treating a cancer in an individual, wherein the individual is also ~~to be being~~ treated with a therapeutically effective amount of an immune checkpoint inhibitor, ~~optionally and~~ wherein the checkpoint inhibitor is an antibody specific for PD-1, PD-L1, ~~or~~ CTLA4, ~~or~~ TIGIT.

[0003np] In one aspect, the present disclosure refers to a method of detecting, in a mixed population of T cells obtained from an individual, the presence of a target T cell that binds a WT-1 epitope, the method comprising: a) contacting *in vitro* the mixed population of T cells with a TMMP disclosed herein; and b) detecting activation and/or proliferation of T cells specific for the ~~TMMP~~WT-1 epitope.

[0003o] In one aspect, the present disclosure refers to the pharmaceutical composition disclosed herein, for use in treating a cancer in an individual, wherein the individual is also being treated with a therapeutically effective amount of an immune checkpoint inhibitor, and wherein the checkpoint inhibitor is an antibody specific for PD-1.

[0003p] In one aspect, the present disclosure refers to the pharmaceutical composition disclosed herein, for use in treating a cancer in an individual, wherein the individual is also being treated with a therapeutically effective amount of an immune checkpoint inhibitor, and wherein the checkpoint inhibitor is pembrolizumab.

[0003q] In one aspect, the present disclosure refers to the pharmaceutical composition disclosed herein, for use in treating a cancer in an individual, wherein the individual is also being treated with a therapeutically effective amount of an immune checkpoint inhibitor, and wherein the checkpoint inhibitor is nivolumab.

[0003r] In one aspect, the present disclosure refers to the use of the pharmaceutical composition disclosed herein, in the manufacture of a medicament for treating a cancer in an individual, wherein the individual is also being treated with a therapeutically effective amount of an immune checkpoint inhibitor, and wherein the checkpoint inhibitor is an antibody specific for PD-1.

[0003s] In one aspect, the present disclosure refers to the use of the pharmaceutical composition disclosed herein, in the manufacture of a medicament for treating a cancer in an individual, wherein the individual is also being treated with a therapeutically effective amount of an immune checkpoint inhibitor, and wherein the checkpoint inhibitor is pembrolizumab.

[0003t] In one aspect, the present disclosure refers to the use of the pharmaceutical composition disclosed herein, in the manufacture of a medicament for treating a cancer in an individual, wherein the individual is also being treated with a therapeutically effective amount of an immune checkpoint inhibitor, and wherein the checkpoint inhibitor is nivolumab.

BRIEF DESCRIPTION OF THE DRAWINGS

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

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

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

CLAIMS

What is claimed is:

1. A T-cell modulatory multimeric polypeptide (TMMP) comprising:
at least one heterodimer comprising:

a) a first polypeptide comprising:

i) a Wilms tumor-1 (WT-1) peptide epitope, wherein the WT-1 peptide has a length of from 4 to 25 amino acids; and

ii) first class I major histocompatibility complex (MHC) ~~class I~~ polypeptide, wherein the first ~~MHC~~ class I MHC polypeptide is a β 2-microglobulin (β 2M) polypeptide;

b) a second polypeptide comprising:

i) a second ~~MHC~~ class I MHC polypeptide, wherein the second ~~MHC~~ class I MHC polypeptide is an MHC class I heavy chain polypeptide; ~~and~~

ii) one or more immunomodulatory polypeptides, wherein the one or more immunomodulatory polypeptides is one or more variant IL-2 polypeptides that exhibit reduced affinity to an IL-2 receptor and that comprise a substitution of H16, or a substitution of H16 and a substitution of F42, based on the amino acid numbering of SEQ ID NO:15; and

iii) an immunoglobulin (Ig) Fc polypeptide,

wherein the first polypeptide and/or the second polypeptide ~~further comprises~~ are covalently linked to one or more activating immunomodulatory polypeptides, and optionally another via at least a first and second disulfide bond, wherein the ~~TMMP comprises~~:

~~a~~ first disulfide bond formed between (i) a Cys residue in a Cys-containing linker between the WT-1 peptide epitope and the β 2M polypeptide, and (ii) a Cys residue in the MHC class I heavy chain polypeptide; and

~~a~~ the second disulfide bond is formed between ~~(i)~~ a Cys residue in ~~a linker between the WT-1 peptide epitope and the β 2M polypeptide; and (ii)~~ a Cys residue in the MHC class I heavy chain polypeptide.

2. ~~A TMMP of claim 1, wherein the WT-1 peptide epitope comprises VLDFAPPGA (SEQ ID NO:259), and~~

~~wherein the second polypeptide comprises:~~

- ~~i) an MHC class I heavy chain polypeptide that comprises an amino acid sequence having at least at least 95% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:449;~~
- ~~ii) the Ig Fc polypeptide; and~~
- ~~iii) the one or more activating immunomodulatory polypeptides.~~

~~32.~~ A TMMP of claim 1 ~~or claim 2~~, wherein:

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

- ~~i) the WT-1 peptide epitope;~~
- ~~ii) a the Cys-containing linker comprising a Cys residue; and~~
- ~~iii) the β 2M polypeptide; and~~

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

- ~~i) the one or more activating immunomodulatory variant IL-2 polypeptides;~~
- ~~ii) an optional linker;~~
- ~~iii) the MHC class I heavy chain polypeptide;~~
- ~~iv) an optional linker; and~~
- ~~v) the Ig Fc polypeptide; or~~

wherein when the TMMP comprises more than one variant IL-2 polypeptide, the TMMP may comprise one or more linkers between the variant IL-2 polypeptides.

3. A TMMP of claim 1, wherein:

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

- ~~i) the WT-1 peptide epitope;~~
- ~~ii) a the Cys-containing linker comprising a Cys residue; and~~
- ~~iii) the β 2M polypeptide; and~~

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

- ~~i) the MHC class I heavy chain polypeptide;~~
- ~~ii) an optional linker;~~
- ~~iii) the one or more activating immunomodulatory Ig Fc polypeptides;~~
- ~~iv) an optional linker; and~~
- ~~v) the Ig Fc polypeptide; or the one or more variant IL-2 polypeptides.~~

~~a3) the first polypeptide comprises, in order from N-terminus to C-terminus:~~

- ~~i) the WT-1 peptide epitope;~~

_____ ii) _____ a linker comprising a Cys residue; and
_____ iii) _____ the β 2M polypeptide; and
b3) the second polypeptide comprises, in order from N-terminus to C-terminus:
_____ i) _____ the MHC class I heavy chain polypeptide;
_____ ii) _____ an optional linker;
_____ iii) _____ the Ig Fc polypeptide;
_____ iv) _____ an optional linker; and
_____ v) _____ the one or more activating immunomodulatory polypeptides; or

a4) the first polypeptide comprises, in order from N-terminus to C-terminus:
_____ i) _____ the one or more activating immunomodulatory polypeptides;
_____ ii) _____ an optional linker;
_____ iii) _____ the WT 1 peptide epitope;
_____ iv) _____ a linker comprising a Cys residue; and
_____ v) _____ the β 2M polypeptide; and

b4) the second polypeptide comprises, in order from N-terminus to C-terminus:
_____ i) _____ the MHC class I heavy chain polypeptide;
_____ ii) _____ an optional linker; and
_____ iii) _____ the Ig Fc polypeptide; or

a5) the first polypeptide comprises, in order from N-terminus to C-terminus:
_____ i) _____ the WT 1 peptide epitope;
_____ ii) _____ a linker comprising a Cys residue;
_____ iii) _____ the β 2M polypeptide;
_____ iv) _____ an optional linker; and
_____ v) _____ the one or more activating immunomodulatory polypeptides; and

b5) a second polypeptide comprises, in order from N-terminus to C-terminus:
_____ i) _____ the MHC class I heavy chain polypeptide;
_____ ii) _____ an optional linker; and
_____ iii) _____ the Ig Fc polypeptide;

-wherein when the TMMP comprises more than one activating immunomodulatory variant IL-2 polypeptide, the TMMP optionally comprises a linker may comprise one or more linkers between the activating immunomodulatory variant IL-2 polypeptides.

4. _____ A TMMP of any one of claims 1-3, wherein the one or more activating immunomodulatory polypeptides are one or more variant IL-2 polypeptides, optionally wherein the one or more variant IL-2 polypeptides exhibit a binding affinity for an IL-2 receptor comprising polypeptides having the amino

acid sequences of SEQ ID NO:16, 17, and 18, and wherein the binding affinity of such one or more variant IL-2 polypeptides is less than the binding affinity of a wild-type human IL-2 polypeptide having the amino acid sequence of SEQ ID NO:15 for the same IL-2 receptor, when assayed under the same conditions in a bio-layer interferometry (BLI) assay.

4. A TMMP of any one of claims 1-3, wherein the variant IL-2 polypeptide comprises: i) an H16A substitution and an F42A substitution; or ii) an H16T substitution and an F42A substitution.

5. A TMMP of any one of claims 1-4, wherein the MHC class I heavy chain polypeptide comprises an amino acid sequence having at least 95% amino acid sequence identity to SEQ ID NO:199, SEQ ID NO:201 or SEQ ID NO:203.

56. A TMMP of ~~any one of claims 1-4~~claim 2, wherein the TMMP comprises two ~~activating immunomodulatory~~variant IL-2 polypeptides ~~that are~~ in tandem ~~and optionally joined by a linker~~, and wherein each of the ~~activating immunomodulatory~~polypeptides is a variant IL-2 polypeptide that comprises an amino acid sequence having at least 95% amino acid sequence identity to ~~the amino acid sequence set forth in~~ SEQ ID NO:188, wherein X₁ is Ala and X₂ is Ala, or wherein X₁ is Thr and X₂ is Ala,

wherein the first disulfide bond is formed between (i) a Cys residue in a linker between the WT-1 peptide epitope and the β 2M polypeptide, and (ii) a Cys residue at position 84 in the MHC Class I heavy chain polypeptide, and wherein the second disulfide bond is formed between a Cys residue at position 12 of the β 2M polypeptide, based on the numbering of SEQ ID NO:311, and a Cys residue at position 236 of the MHC class I heavy chain polypeptide,

wherein the MHC class I heavy chain polypeptide comprises an amino acid sequence having at least 95% amino acid sequence identity to the amino acid sequence GSHSMRYFFTSVSRPGRGEPRIA VGYVDDTQFVRFDSDAASQRM EPRAPWIEQEGPEYWDGET RKVKAHSQTHRVDLGT LRGCYNQSEAGSHTVQRM YGCDVGSDWRFLRGYHQYAYDGKD YIA LKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRYLENGKETLQRTDAPK THMTHHAVSDHEATLRCWALSFP AEITLTWQRDGEDQTQDTEL VETRPCGDGTFQKWA AVV VPSGQEQRYTCHVQHEGLPKPLTLRWE, where amino acid 84 is a Cys and amino acid 236 is a Cys, and

wherein the Ig Fc polypeptide has at least about 95% amino acid sequence identity to the amino acid sequence set forth in any one of SEQ ID NO:418-421.

7. A TMMP of claim 6, wherein the variant IL-2 polypeptides are joined by a linker, and each of the variant IL-2 polypeptides has the amino acid sequence set forth in SEQ ID NO:188, wherein X₁ is Ala and X₂ is Ala.

8. A TMMP of claim 6 or 9, wherein the MHC class I heavy chain polypeptide comprises an amino acid sequence having at least 95% amino acid sequence identity to SEQ ID NO:199, and wherein the epitope is VLDFAPPGA (SEQ ID NO:259).

9. A TMMP of claim 3, wherein the TMMP comprises two variant IL-2 polypeptides that are in tandem and optionally joined by a linker, and wherein each of the variant IL-2 comprises an amino acid sequence having at least 95% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:188, wherein X₁ is Ala and X₂ is Ala, or wherein X₁ is Thr and X₂ is Ala,

wherein the first disulfide bond is formed between (i) a Cys residue in a linker between the WT-1 peptide epitope and the β 2M polypeptide, and (ii) a Cys residue at position 84 in the MHC Class I heavy chain polypeptide, and wherein the second disulfide bond is formed between a Cys residue at position 12 of the β 2M polypeptide, based on the numbering of SEQ ID NO:311, and a Cys residue at position 236 of the MHC class I heavy chain polypeptide,

wherein the MHC class I heavy chain polypeptide comprises an amino acid sequence having at least 95% amino acid sequence identity to the amino acid sequence GSHSMRYFFTSVSRPGRGEPRIA VGYVDDTQFVRFDSDAASQRM EPRAPWIEQEGPEYWDGET RKVKAHSQTHRVDLGT LRGCYNQSEAGSHTVQRM YGCDVGSDWRFLRGYHQYAYDGKD YIA LKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRLRYLENGKETLQRTDAPK THMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTEL VETRPCGDGTFQKWA AVV VPSGQEQRYTCHVQHEGLPKPLTLRWE, where amino acid 84 is a Cys and amino acid 236 is a Cys, and

wherein the Ig Fc polypeptide has at least about 95% amino acid sequence identity to the amino acid sequence set forth in any one of SEQ ID NO:418-421.

10. A TMMP of claim 9, wherein the MHC class I heavy chain polypeptide comprises an amino acid sequence having at least 95% amino acid sequence identity to SEQ ID NO:203.

11. A TMMP of claim 9 or 10, wherein the variant IL-2 polypeptides are joined by a linker, and each of the variant IL-2 polypeptides has the amino acid sequence set forth in SEQ ID NO:188, wherein X₁ is Ala and X₂ is Ala.

~~6. A TMMP of any one of claims 1-5, wherein the TMMP comprises a first disulfide bond and a second disulfide bond, and wherein the first disulfide bond is formed between a Cys residue at position 12 of the β 2M polypeptide and a Cys residue at position 236 of the MHC class I heavy chain polypeptide.~~

~~712. A TMMP of any one of claims 1-611, wherein the TMMP comprises a first disulfide bond and a second disulfide bond, and wherein the second disulfide bond is formed between a Cys residue in the linker between the WT-1 peptide epitope and the β 2M polypeptide and a Cys residue at position 84 in the MHC class I heavy chain polypeptide, and optionally wherein the linker between the WT-1 peptide epitope and the β 2M polypeptide comprises the amino acid sequence set forth in SEQ ID NO:316 or SEQ ID NO:317.~~

13. A TMMP of claim 7, wherein

the first polypeptide comprises the amino acid sequence set forth in SEQ ID NO:423,

the MHC class I heavy chain polypeptide comprises an amino acid sequence having at least 98% amino acid sequence identity to the amino acid sequence
GSHSMRYFFTSVSRPGRGEPRFIAVGYYDDTQFVRFDSDAASQRMETPRAPWIEQEGPEYWDGET
RKVKAHSQTHRVDLGLTRGCYNQSEAGSHTVQRMYGCDVGSDWRFLRGYHQYAYDGKDYL
ALKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRYLENGKETLQRTDAPK
THMTHHAVSDHEATLRCWALSFPYAEITLTWQRDGEDQTQDTELVETRPCGDGTFQKWAADV
VPSGQEQRYTCHVQHEGLPKPLTLRWE, and

the Ig Fc polypeptide comprises an amino acid sequence having at least about 98% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:421.

14. A TMMP of claim 13, wherein the MHC class I heavy chain polypeptide comprises an amino acid sequence having the amino acid sequence

GSHSMRYFFTSVSRPGRGEPRFIAVGYYDDTQFVRFDSDAASQRMETPRAPWIEQEGPEY
WDGETRKVKAHSQTHRVDLGLTRGCYNQSEAGSHTVQRMYGCDVGSDWRFLRGYH
QYAYDGKDYLALKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLR

RYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQ
DTELVETRPCGDGTFQKWA AVVVP SGQEQR YTCHVQHEGLPKPLTLRWE.

8. — A TMMP of any one of claims 1-7, wherein the Ig Fc polypeptide has at least about 95% amino acid sequence identity to an amino acid sequence set forth in any one of SEQ ID NO:410-421, optionally wherein the Ig Fc polypeptide has at least about 98% amino acid sequence identity to an amino acid sequence set forth in any one of SEQ ID NO:418-421.

9. — A TMMP of any one of claims 1-7, wherein the Ig Fc polypeptide has at least about 95% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:421, optionally wherein the Ig Fc polypeptide has at least about 98% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:421.

10. — A TMMP of any one of claims 1 and 3-9, wherein the MHC class I heavy chain polypeptide comprises an amino acid sequence having at least 95% amino acid sequence identity to the amino acid sequence set forth in any one of SEQ ID NO:449, 201, and 203, optionally wherein the MHC class I heavy chain polypeptide comprises an amino acid sequence having at least 98% amino acid sequence identity to the amino acid sequence set forth in any one of SEQ ID NO:449, 201, and 203.

11. — A TMMP of any one of claims 1-10, wherein the WT-1 peptide epitope comprises an amino acid sequence selected from the group consisting of: NLMNLGATL (SEQ ID NO:258), NYMNLGATL (SEQ ID NO:263), CMTWNQMNLGATLKG (SEQ ID NO:223), WNQMNLGATLKGVA (SEQ ID NO:224), CMTWNYMNLGATLKG (SEQ ID NO:225), WNYMNLGATLKGVA (SEQ ID NO:226), MTWNQMNLGATLKG (SEQ ID NO:227), TWNMNLGATLKGVA (SEQ ID NO:228), CMTWNLMNLGATLKG (SEQ ID NO:229), MTWNLMNLGATLKG (SEQ ID NO:230), TWNLNMNLGATLKGVA (SEQ ID NO:231), WNLNMNLGATLKGVA (SEQ ID NO:232), MNLGATL (SEQ ID NO:233), MTWNYMNLGATLKG (SEQ ID NO:234), TWNYMNLGATLKGVA (SEQ ID NO:235), CMTWNQMNLGATLKGVA (SEQ ID NO:236), CMTWNLMNLGATLKGVA (SEQ ID NO:237), CMTWNYMNLGATLKGVA (SEQ ID NO:238), GYLRNPTAC (SEQ ID NO:239), GALRNPTAL (SEQ ID NO:240), YALRNPTAC (SEQ ID NO:241), GLLRNPTAC (SEQ ID NO:242), RYRPHPGAL (SEQ ID NO:243), YQRPHPGAL (SEQ ID NO:244), RLRPHPGAL (SEQ ID NO:245), RIRPHPGAL (SEQ ID NO:246), QFPNHSFKHEDPMGQ (SEQ ID NO:247), HSFKHEDPY (SEQ ID NO:248), QFPNHSFKHEDPM (SEQ ID NO:249), QFPNHSFKHEDPY (SEQ ID NO:250), KRPFMCAYPGCNK (SEQ ID NO:251), KRPFMCAYPGCYK (SEQ ID NO:252), FMCAYPGCY (SEQ ID NO:253), FMCAYPGCK (SEQ ID

~~NO:254), KRPFMCAYPGCNKRY (SEQ ID NO:255), SEKRPFMCAYPGCNK (SEQ ID NO:256), KRPFMCAYPGCYKRY (SEQ ID NO:257), NLMNLGATL (SEQ ID NO:258), VLDFAPPGA (SEQ ID NO:259), RMFPNAPYL (SEQ ID NO:260), CMTWNQMN (SEQ ID NO:261), CYTWNQMNL (SEQ ID NO:269), NYMNLGATL (SEQ ID NO:263), YMFNPAPYL (SEQ ID NO:264), SLGEQQYSV (SEQ ID NO:265), CMTWNQMNL (SEQ ID NO:266), and NQMNLGATL (SEQ ID NO:267).~~

~~12. A TMMP of any of claims 1-11, wherein:~~

~~the first polypeptide comprises, in order from N terminus to C terminus:~~

- ~~i) the WT-1 peptide epitope;~~
- ~~ii) a linker comprising a Cys residue; and~~
- ~~iii) the β 2M polypeptide; and~~

~~the second polypeptide comprises, in order from N terminus to C terminus:~~

- ~~i) the one or more activating immunomodulatory polypeptides;~~
- ~~ii) an optional linker;~~
- ~~iii) the MHC class I heavy chain polypeptide;~~
- ~~iv) an optional linker; and~~
- ~~v) the Ig Fc polypeptide;~~

~~wherein when the TMMP comprises more than one activating immunomodulatory polypeptide, the TMMP optionally comprises a linker between the activating immunomodulatory polypeptides.~~

~~13. A TMMP of any of claims 1-11, wherein:~~

~~the first polypeptide comprises, in order from N terminus to C terminus:~~

- ~~i) the WT-1 peptide epitope;~~
- ~~ii) a linker comprising a Cys residue; and~~
- ~~iii) the β 2M polypeptide; and~~

~~the second polypeptide comprises, in order from N terminus to C terminus:~~

- ~~i) the MHC class I heavy chain polypeptide;~~
- ~~ii) an optional linker;~~
- ~~iii) the Ig Fc polypeptide;~~
- ~~iv) an optional linker; and~~
- ~~v) the one or more activating immunomodulatory polypeptides;~~

~~wherein when the TMMP comprises more than one activating immunomodulatory polypeptide, the TMMP optionally comprises a linker between the activating immunomodulatory polypeptides.~~

14. — A TMMP of claim 12, wherein: the TMMP comprises two activating immunomodulatory polypeptides in tandem, and wherein each of the activating immunomodulatory polypeptides is a variant IL-2 polypeptide that comprises an amino acid sequence having at least 95% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:188, wherein X₁ is Ala and X₂ is Ala, or wherein X₁ is Thr and X₂ is Ala;

—— the TMMP comprises a first disulfide bond and a second disulfide bond, wherein the first disulfide bond is formed between a Cys residue at position 12 of the β 2M polypeptide and a Cys residue at position 236 of the MHC class I heavy chain polypeptide, and wherein the second disulfide bond is formed between (i) a Cys residue in a linker between the WT-1 peptide epitope and the β 2M polypeptide, and (ii) a Cys residue at position 84 in the MHC Class I heavy chain polypeptide;

—— the MHC class I heavy chain polypeptide comprises an amino acid sequence having at least 95% amino acid sequence identity to the amino acid sequence set forth in (SEQ ID NO:451), where amino acid 84 is a Cys and amino acid 236 is a Cys; and

—— the Ig Fc polypeptide has at least about 95% amino acid sequence identity to an amino acid sequence set forth in any one of SEQ ID NO:418-421, optionally wherein the Ig Fc polypeptide has at least about 98% amino acid sequence identity to an amino acid sequence set forth in any one of SEQ ID NO:418-421, and

—— optionally wherein the linker between the WT-1 peptide epitope and the β 2M polypeptide comprises the amino acid sequence set forth in SEQ ID NO:316 or SEQ ID NO:317.

15. — A TMMP of claim 14, wherein the WT-1 peptide epitope comprises VLDFAPPGA (SEQ ID NO:259).

16. — A TMMP of claim 15, wherein:

—— the first polypeptide comprises the amino acid sequence set forth in SEQ ID NO:423;

—— each of the variant IL-2 polypeptide comprises the amino acid sequence set forth in SEQ ID NO:188, wherein X₁ is Ala and X₂ is Ala;

—— the MHC class I heavy chain polypeptide comprises at least about 98% amino acid sequence identity to the amino acid sequence set forth in (SEQ ID NO:451); and

—— the Ig Fc polypeptide has at least about 98% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:421.

17. — A TMMP of claim 13, wherein:

the TMMP comprises two activating immunomodulatory polypeptides in tandem, and wherein each of the activating immunomodulatory polypeptides is a variant IL-2 polypeptide that comprises an

~~amino acid sequence having at least 95% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:188, wherein X₁ is Ala and X₂ is Ala, or wherein X₁ is Thr and X₂ is Ala;~~

~~—— the TMMP comprises a first disulfide bond and a second disulfide bond, wherein the first disulfide bond is formed between a Cys residue at position 12 of the β 2M polypeptide and a Cys residue at position 236 of the MHC class I heavy chain polypeptide, and wherein the second disulfide bond is formed between (i) a Cys residue in a linker between the WT-1 peptide epitope and the β 2M polypeptide and (ii) a Cys residue at position 84 in the MHC Class I heavy chain polypeptide; and~~

~~—— the Ig Fc polypeptide has at least about 95% amino acid sequence identity to an amino acid sequence set forth in any one of SEQ ID NO:418-421, optionally wherein the Ig Fc polypeptide has at least about 98% amino acid sequence identity to an amino acid sequence set forth in any one of SEQ ID NO:418-421.~~

18. ~~A TMMP of claim 17, wherein:~~

~~—— the MHC class I heavy chain polypeptide comprises an amino acid sequence having at least 95% amino acid sequence identity to an amino acid sequence set forth in any one of SEQ ID NO:199, SEQ ID NO:201 or SEQ ID NO:203, optionally wherein the MHC class I heavy chain polypeptide comprises an amino acid sequence having at least 98% amino acid sequence identity to an amino acid sequence set forth in SEQ ID NO:199, SEQ ID NO:201 or SEQ ID NO:203; and~~

~~—— the Ig Fc polypeptide has at least about 95% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:421, optionally wherein the Ig Fc polypeptide has at least about 98% amino acid sequence identity to an amino acid sequence set forth in SEQ ID NO:421.~~

19. ~~A TMMP of claim 18, wherein the MHC class I heavy chain polypeptide comprises an amino acid sequence having at least 95% amino acid sequence identity to an amino acid sequence set forth in SEQ ID NO:344, optionally wherein the MHC class I heavy chain polypeptide comprises an amino acid sequence having at least 98% amino acid sequence identity to an amino acid sequence set forth in SEQ ID NO:344.~~

20. ~~A TMMP of claim 18, wherein the MHC class I heavy chain polypeptide comprises an amino acid sequence having at least 95% amino acid sequence identity to an amino acid sequence set forth in SEQ ID NO:346, optionally wherein the MHC class I heavy chain polypeptide comprises an amino acid sequence having at least 98% amino acid sequence identity to an amino acid sequence set forth in SEQ ID NO:346.~~

~~24~~15. A TMMP comprising a ~~homodimer of two first and a second~~ heterodimers according to any one of claims 1-~~20~~14, wherein the first and second heterodimers are ~~the same, and wherein the heterodimers identical in amino acid sequence and~~ are covalently bound to each other by one or more disulfide bonds between the Ig Fc polypeptides of the first and second heterodimers.

16. A TMMP comprising a first and a second heterodimer according to claim 15, wherein the first and second heterodimers are identical in amino acid sequence and are covalently bound to each other by one or more disulfide bonds between the Ig Fc polypeptides of the first and second heterodimers.

17. A TMMP of claim 16, wherein two disulfide bonds link the Ig Fc polypeptide of one heterodimer to the Ig Fc polypeptide of the other heterodimer.

~~22.~~ A TMMP of claim 21, wherein the TMMP comprises a homodimer of two heterodimers according to claim 15 or 16.

~~23.~~ A nucleic acid comprising a nucleotide sequence encoding a first or second polypeptide according to any one of claims 1-20.

~~24.~~ A nucleic acid according to claim 23, comprising a nucleotide sequence encoding a first or second polypeptide according to claim 16.

~~25.~~ An expression vector comprising a nucleic acid of claim 23 or 24.

~~26.~~ A genetically modified host cell, wherein the host cell is genetically modified with a nucleic acid of claim 23 or 24, or an expression vector of claim 25.

~~27.~~ A method of making a TMMP of any one of claims 1-22, comprising culturing a genetically modified host cell *in vitro* in a culture medium under conditions such that the host cell synthesizes the TMMP.

~~28~~18. A pharmaceutical composition comprising a TMMP of ~~any one of claims 1-21~~claim 16.

~~29~~19. A pharmaceutical composition comprising a TMMP of claim ~~22~~17.

20. A composition comprising one or more nucleic acids comprising nucleotide sequences encoding the first and second polypeptides of the TMMP of any one of any one of claims 1-17.

21. A composition comprising one or more expression vectors, wherein the one or more expression vectors comprise nucleotide sequences encoding the first and second polypeptides of the TMMP of any one of any one of claims 1-17.

22. A method of producing a TMMP of any one of claims 1-17, the method comprising culturing a host cell that is genetically modified with one or more expression vectors comprising nucleotide sequences encoding the first and the second polypeptides of the multimeric polypeptide, under conditions such that the genetically modified host cell produces the TMMP.

23. An in vitro composition of genetically modified host cells, wherein the host cells comprise one or more nucleic acids comprising nucleotide sequences encoding the first and second polypeptides of the TMMP of any one of any one of claims 1-17.

~~30. The TMMP of any one of claims 1-21, or a pharmaceutical composition of claim 28 or 29 for use in selective modulation of the activity of a T cell that binds to a cancer associated epitope in an individual, wherein said TMMP or pharmaceutical composition is to be administered to selectively modulate the activity of the T cell in the individual.~~

~~31. Use of the TMMP of any one of claims 1-21, or a pharmaceutical composition of claim 28 or 29 in the manufacture of a medicament for selective modulation of the activity of a T cell that binds to a cancer associated epitope in an individual, wherein said TMMP or pharmaceutical composition is to be administered to selectively modulate the activity of the T cell in the individual.~~

~~32~~24. The ~~TMMP of any one of claims 1-22, or a~~ pharmaceutical composition of claim ~~28-18 or 19,~~ for use in treating a cancer in an individual.

~~33~~25. Use of the ~~TMMP of any one of claims 1-22, or a~~ pharmaceutical composition of claim ~~28-18 or 19~~ in the manufacture of a medicament for treating a cancer in an individual.

~~34~~26. The pharmaceutical composition of claim ~~29-18 or 19,~~ for use in treating a cancer in an individual, wherein the individual is also being treated with a therapeutically effective amount of an immune checkpoint inhibitor.

~~35~~27. Use of the pharmaceutical composition of claim ~~29-18 or 19~~ in the manufacture of a medicament for treating a cancer in an individual, wherein the individual is also being treated with a therapeutically effective amount of an immune checkpoint inhibitor.

~~36~~28. The ~~TMMP of any one of claims 1-22, or a~~ pharmaceutical composition of claim ~~28-18 or 29-19~~, for use in treating a cancer in an individual, wherein the individual is also ~~to be being~~ treated with a therapeutically effective amount of an immune checkpoint inhibitor, ~~optionally and~~ wherein the checkpoint inhibitor is an antibody specific for PD-1, PD-L1, ~~or~~ CTLA4, or TIGIT.

~~37~~29. Use of the ~~TMMP of any one of claims 1-22, or a~~ pharmaceutical composition of claim ~~28-18 or 19 or 29~~ in the manufacture of a medicament for treating a cancer in an individual, wherein the individual is also ~~to be being~~ treated with a therapeutically effective amount of an immune checkpoint inhibitor, ~~optionally and~~ wherein the checkpoint inhibitor is an antibody specific for PD-1, PD-L1, ~~or~~ CTLA4, or TIGIT.

~~38~~30. A method of detecting, in a mixed population of T cells obtained from an individual, the presence of a target T cell that binds a WT-1 epitope, the method comprising:
a) contacting *in vitro* the mixed population of T cells with a TMMP of any one of claims 1-~~22~~17;
and
b) detecting activation and/or proliferation of T cells specific for the ~~TMMP~~WT-1 epitope.

31. The pharmaceutical composition of claim 18 or 19, for use in treating a cancer in an individual, wherein the individual is also being treated with a therapeutically effective amount of an immune checkpoint inhibitor, and wherein the checkpoint inhibitor is an antibody specific for PD-1.

32. The pharmaceutical composition of claim 18 or 19, for use in treating a cancer in an individual, wherein the individual is also being treated with a therapeutically effective amount of an immune checkpoint inhibitor, and wherein the checkpoint inhibitor is pembrolizumab.

33. The pharmaceutical composition of claim 18 or 19, for use in treating a cancer in an individual, wherein the individual is also being treated with a therapeutically effective amount of an immune checkpoint inhibitor, and wherein the checkpoint inhibitor is nivolumab.

34. Use of the pharmaceutical composition of claim 18 or 19, in the manufacture of a medicament for treating a cancer in an individual, wherein the individual is also being treated with a therapeutically effective amount of an immune checkpoint inhibitor, and wherein the checkpoint inhibitor is an antibody specific for PD-1.

35. Use of the pharmaceutical composition of claim 18 or 19, in the manufacture of a medicament for treating a cancer in an individual, wherein the individual is also being treated with a therapeutically effective amount of an immune checkpoint inhibitor, and wherein the checkpoint inhibitor is pembrolizumab.

36. Use of the pharmaceutical composition of claim 18 or 19, in the manufacture of a medicament for treating a cancer in an individual, wherein the individual is also being treated with a therapeutically effective amount of an immune checkpoint inhibitor, and wherein the checkpoint inhibitor is nivolumab.