

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) polypeptide, wherein the first class I MHC polypeptide is a β 2-microglobulin (β 2M) polypeptide;

b) a second polypeptide comprising

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

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 the second polypeptide are covalently linked to one another via at least a first and second disulfide bond, wherein the

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

the second disulfide bond is formed between a Cys residue in the β 2M polypeptide and a Cys residue in the MHC class I heavy chain polypeptide.

2. A TMMP of claim 1, wherein:

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

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

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

i) the one or more variant IL-2 polypeptides, wherein each variant IL-2 polypeptide comprises 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;

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 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:

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

i) the WT-1 peptide epitope;

ii) the Cys-containing linker; and

iii) the β 2M polypeptide; and

b) 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 variant IL-2 polypeptides, wherein each variant IL-2

polypeptide comprises 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,

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.

4. A TMMP of any one of claims 1-3, wherein each 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.

6. A TMMP of claim 2, 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 X_1 is Ala and X_2 is Ala, or wherein X_1 is Thr and X_2 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 GSHSMRYFFTSVSRPGRGEPFRFIAVGYYDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDGET RKVKAHSQTHRVDLGLTRGTCYNQSEAGSHTVQRMYGCDVGSDWRFLRGYHQYAYDGKDYIA LKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRLYLENGKETLQRTDAPK THMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTEL VETRPCGDGTFQKWAADV 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 X_1 is Ala and X_2 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 X_1 is Ala and X_2 is Ala, or wherein X_1 is Thr and X_2 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 GSHSMRYFFTSVSRPGRGEPFRFIAVG YVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDGET RKVKAHSQTHRVDLGTLRG CYNQSEAGSHTVQRMYGCDVGSDWRFLRGYHQYAYDGKD YIA LKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLR RYLENGKETLQRTDAPK THMTHHAVSDHEATLRCWALS FYPAEITLTWQRDGEDQTQDTELVETRPCGDGTFQKWA 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.

12. A TMMP of any one of claims 1-11, 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 GSHSMRYFFTSVSRPGRGEPFRFIAVG YVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDGET RKVKAHSQTHRVDLGTLRG CYNQSEAGSHTVQRMYGCDVGSDWRFLRGYHQYAYDGKD YIA LKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLR RYLENGKETLQRTDAPK

THMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTEL VETRPCGDGTFQKWAAVV
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
GSHSMRYFFTSVSRPGRGEPRIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEY
WDGETRKVKAHSQTHRVDLGTLRGCYNQSEAGSHTVQRMYGCDVGSDWRFLRGYH
QYAYDGKDYIALKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLR
RYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQ
DTEL VETRPCGDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWE.

15. A TMMP comprising a first and a second heterodimer according to any one of claims 1-14, 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.

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.

18. A pharmaceutical composition comprising a TMMP of claim 16.

19. A pharmaceutical composition comprising a TMMP of claim 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.
24. The pharmaceutical composition of claim 18 or 19, for use in treating a cancer in an individual.
25. Use of the pharmaceutical composition of claim 18 or 19 in the manufacture of a medicament for treating a cancer in an individual.
26. 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.
27. 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 to be treated with a therapeutically effective amount of an immune checkpoint inhibitor.
28. 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, PD-L1, CTLA4, or TIGIT.
29. 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 to be treated with a therapeutically effective amount of an immune checkpoint inhibitor, and wherein the checkpoint inhibitor is an antibody specific for PD-1, PD-L1, CTLA4, or TIGIT.

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 1-17;
- and
- b) detecting activation and/or proliferation of T cells specific for the 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 to be 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 to be 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 to be treated with a therapeutically effective amount of an immune checkpoint inhibitor, and wherein the checkpoint inhibitor is nivolumab.