

CLAIMS

What is claimed is:

1. A T-cell modulatory multimeric polypeptide comprising at least one heterodimer, wherein the at least one heterodimer comprises:
 - a) a first polypeptide comprising
 - i) a Wilms tumor-1 (WT-1) peptide epitope, wherein the WT-1 peptide epitope has a length from 8 amino acids to 16 amino acids,
 - ii) a beta-2 microglobulin (β 2M) polypeptide, and
 - iii) a linker between the WT-1 peptide and the β 2M polypeptide, wherein the linker comprises a Cys residue; and
 - b) a second polypeptide comprising
 - i) a major histocompatibility complex (MHC) class I heavy chain polypeptide,
 - ii) an immunoglobulin (Ig) Fc polypeptide, and
 - iii) at least one activating immunomodulatory polypeptide,wherein the heterodimer comprises a first disulfide bond formed between (i) the Cys residue in the 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,
wherein the heterodimer comprises a second disulfide bond formed between a Cys residue in the β 2M polypeptide and a Cys residue in the MHC class I heavy chain polypeptide, and
wherein the Ig Fc polypeptide does not comprise a C-terminal lysine.
2. A T-cell modulatory multimeric polypeptide 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 at least one immunomodulatory polypeptide;
 - 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 immunomodulatory polypeptide, the TMMP may comprise one or more linkers between the immunomodulatory polypeptides.

3. A T-cell modulatory multimeric polypeptide 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 at least one immunomodulatory polypeptide,

wherein, when the TMMP comprises more than one immunomodulatory polypeptide, the TMMP may comprise one or more linkers between the immunomodulatory polypeptides.

4. A T-cell modulatory multimeric polypeptide of any one of claims 1-3, wherein the at least one immunomodulatory polypeptide is selected from the group consisting of a cytokine, a 4-1BBL polypeptide, an ICOS-L polypeptide, an OX-40L polypeptide, a CD80 polypeptide, a CD86 polypeptide, a CD40 polypeptide, a CD70 polypeptide, and combinations thereof.

5. A T-cell modulatory multimeric polypeptide of any one of claims 1-4, wherein the at least one immunomodulatory polypeptide comprises an IL-2 polypeptide.

6. A T-cell modulatory multimeric polypeptide of any one of claims 1-5, wherein the multimeric polypeptide comprises at least two immunomodulatory polypeptides, and wherein at least two of the immunomodulatory polypeptides are the same, optionally wherein the 2 or more immunomodulatory polypeptides are in tandem.

7. A T-cell modulatory multimeric polypeptide of any one of claims 1-6, wherein one or more of the at least one immunomodulatory polypeptide is a variant IL-2 polypeptide that exhibits reduced affinity to an IL-2 receptor compared to the affinity of a wild-type IL-2 polypeptide for the IL-2 receptor, optionally wherein the variant IL-2 polypeptide comprises: i) an H16 substitution and an F42 substitution.

8. A T-cell modulatory multimeric polypeptide of claim 7, wherein the one or more variant IL-2 polypeptides comprises: i) an H16A substitution and an F42A substitution; or ii) an H16T substitution and an F42A substitution.
9. A T-cell modulatory multimeric polypeptide of any one of claims 1-8, wherein:
- a) the first disulfide bond is between: i) the Cys present in the 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
 - b) the second disulfide bond is between: i) a Cys residue at position 12 of the β 2M polypeptide; and ii) a Cys residue at position 236 of the MHC class I heavy chain polypeptide.
10. A T-cell modulatory multimeric polypeptide of any one of claims 1-9, wherein the linker between the WT-1 peptide epitope and the β 2M polypeptide is GCGGS(GGGGS) n (SEQ ID NO:319), where n is 1, 2, 3, 4, 5, 6, 7, 8, or 9.
11. A T-cell modulatory multimeric polypeptide of any one of claims 1-10, wherein the WT-1 peptide epitope has a length of 9 or 10 amino acids.
12. A T-cell modulatory multimeric polypeptide of any one of claims 1-11, wherein the Ig Fc polypeptide comprises an amino acid sequence having at least 95% sequence identity to one of the amino acid sequences depicted in FIG. 5D, FIG. 5E, FIG. 5F, FIG. 5G, and FIG. 5H.
13. A T-cell modulatory multimeric polypeptide of any one of claims 1-12, wherein the first or the second MHC polypeptide comprises an amino acid sequence having at least 95% amino acid sequence identity to SEQ ID NO:455, or at least 95% amino acid sequence identity to SEQ ID NO:342, or at least 95% amino acid sequence identity to SEQ ID NO:344.
14. A T-cell modulatory multimeric polypeptide of claim 1, wherein the first polypeptide of the at least one heterodimer has the amino acid sequence set forth in SEQ ID NO:423; and the second polypeptide of the at least one heterodimer has the amino acid sequence set forth in SEQ ID NO:486.
15. A T-cell modulatory multimeric polypeptide of any one of claims 1-14, wherein the multimeric polypeptide comprises a first and a second heterodimer, and wherein the first and second heterodimers

are covalently bound by one or more disulfide bonds between the Ig Fc polypeptides of the first and second heterodimers.

16. A T-cell modulatory multimeric polypeptide of claim 15,
wherein the first polypeptide of both the first and second heterodimers has the amino acid sequence set forth in SEQ ID NO:423,
wherein the second polypeptide of both the first and second heterodimers has the amino acid sequence set forth in SEQ ID NO:486.
17. A composition comprising one or more nucleic acids encoding the first and second polypeptides of a T cell modulatory multimeric polypeptide according to any one of claims 1-14.
18. A composition comprising one or more expression vectors comprising the one or more nucleic acids of claim 17.
19. Use of a T-cell modulatory multimeric polypeptide according to any one of claims 1-16 in the manufacture of a medicament for selectively modulating the activity of a T cell specific for a Wilms tumor-1 (WT-1) epitope.
20. Use of a pharmaceutical composition comprising T-cell modulatory multimeric polypeptide according to any one of claims 1-16 in the manufacture of a medicament for treating cancer in a patient.
21. The use of claim 20, wherein the cancer is acute myeloid leukemia, myeloma, ovarian cancer, pancreatic cancer, non-small cell lung cancer, colorectal cancer, breast cancer, Wilms tumor, mesothelioma, soft tissue sarcoma, neuroblastoma, or nephroblastoma.
22. The use of claim of 20 or 21, wherein the patient also receives one or more checkpoint inhibitors.
23. The use of claim 22, wherein the checkpoint inhibitor is an antibody that binds to a polypeptide selected from the group consisting of CD27, CD28, CD40, CD122, CD96, CD73, CD47, OX40, GITR, CSF1R, JAK, PI3K delta, PI3K gamma, TAM, arginase, CD137, ICOS, A2AR, B7-H3, B7-H4, BTLA, CTLA-4, LAG3, TIM3, VISTA, CD96, TIGIT, CD122, PD-1, PD-L1, and PD-L2.

24. The use of claim 23, wherein the checkpoint inhibitor is an antibody specific for PD-1, PD-L1, or CTLA4.
25. The use of claim 23 or 24, wherein the one or more checkpoint inhibitors is selected from the group consisting of nivolumab, pembrolizumab, pidilizumab, AMP-224, MPDL3280A, MDX-1105, MEDI-4736, arelumab, ipilimumab, tremelimumab, pidilizumab, IMP321, MGA271, BMS-986016, lirilumab, urelumab, PF-05082566, IPH2101, MEDI-6469, CP-870,893, Mogamulizumab, Varlilumab, Avelumab, Galiximab, AMP-514, AUNP 12, Indoximod, NLG-919, INCB024360, KN035, and combinations thereof.
26. Use of a T-cell modulatory multimeric polypeptide of any one of claims 1-16 in the manufacture of a medicament for delivering an immunomodulatory polypeptide selectively to a target T cell in a mixed population comprising non-target T cells and the target T cell, wherein the target T cell is specific for the WT-1 epitope present within the T-cell modulatory multimeric polypeptide, and wherein said immunomodulatory polypeptide present within the T-cell modulatory multimeric polypeptide is delivered to the target T cell.
27. A method of detecting, in a mixed population of T cells obtained from an individual, the presence of a target T cell that binds a WT-1 epitope, the method comprising:
- a) contacting *in vitro* the mixed population of T cells with the T-cell modulatory multimeric polypeptide of any one of claims 1-16, wherein the T-cell modulatory multimeric polypeptide comprises the WT-1 epitope; and
 - b) detecting activation and/or proliferation of T cells in response to said contacting, wherein activated and/or proliferated T cells indicates the presence of the target T cell.