

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. 63/023,834, filed May 12, 2020, and U.S. Provisional Patent Application No. 63/041,451, filed June 19, 2020, which applications are incorporated herein by reference in their entirety.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING PROVIDED AS A TEXT FILE

[0002] A Sequence Listing is provided herewith as a text file, "CUEB-133WO_SEQ_LIST2_ST25.txt" created on May 7, 2021 and having a size of 800 KB. The contents of the text file are incorporated by reference herein in their entirety.

INTRODUCTION

[0003] 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 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 not epitope specific and instead is generally expressed on all T cells or on large T cell subsets.

SUMMARY

[0004] 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.

[0004a] In one example, the present disclosure refers to 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.

In one aspect, the present disclosure refers to 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,

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.

[0004b] In one aspect, the present disclosure refers to a composition comprising one or more nucleic acids encoding the first and second polypeptides of a T cell modulatory multimeric polypeptide as disclosed herein.

[0004c] In one aspect, the present disclosure refers to a composition comprising one or more expression vectors comprising the one or more nucleic acids as disclosed herein.

[0004d] In one aspect, the present disclosure refers to use of a T-cell modulatory multimeric polypeptide as disclosed herein in the manufacture of a medicament for selectively modulating the activity of a T cell specific for a Wilms tumor-1 (WT-1) epitope.

[0004e] In one example, the present disclosure refers to use of a pharmaceutical composition comprising T-cell modulatory multimeric polypeptide as disclosed herein in the manufacture of a medicament for treating cancer in a patient. In one aspect, the present disclosure refers to use of a pharmaceutical composition comprising T-cell modulatory multimeric polypeptide as disclosed herein in the manufacture of a medicament for treating a Wilms tumor-1 cancer in a patient.

[0004f] In one aspect, the present disclosure refers to use of a T-cell modulatory multimeric polypeptide as disclosed herein 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.

[0004g] 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 the T-cell modulatory multimeric polypeptide as disclosed herein, 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.

BRIEF DESCRIPTION OF THE DRAWINGS

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

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,
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.
2. A T-cell modulatory multimeric polypeptide of claim 1, 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.
3. A composition comprising one or more nucleic acids encoding the first and second polypeptides of a T cell modulatory multimeric polypeptide according to claim 1.

4. A composition comprising one or more expression vectors comprising the one or more nucleic acids of claim 3.
5. Use of a T-cell modulatory multimeric polypeptide according to claim 1 or claim 2 in the manufacture of a medicament for selectively modulating the activity of a T cell specific for a Wilms tumor-1 (WT-1) epitope.
6. Use of a pharmaceutical composition comprising T-cell modulatory multimeric polypeptide according to claim 1 or claim 2 in the manufacture of a medicament for treating a Wilms tumor-1 cancer in a patient.
7. The use of claim 6, wherein the Wilms tumor-1 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.
8. The use of claim 6 or claim 7, wherein the patient also receives one or more checkpoint inhibitors.
9. The use of claim 8, 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.
10. The use of claim 9, wherein the checkpoint inhibitor is an antibody specific for PD-1, PD-L1, or CTLA4.
11. The use of claim 9 or 10, 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.
12. Use of a T-cell modulatory multimeric polypeptide of claim 1 or claim 2 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.

13. 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 claim 1 or claim 2, 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.