nucleotide sequences encoding the T-cell modulatory multimeric polypeptides, and host cells comprising the nucleic acids. The present disclosure provides methods of modulating the activity of a T cell; the methods comprise contacting the T cell with a T-cell modulatory multimeric polypeptide of the present disclosure.

[0004a] In a first aspect, there is provided a multimeric polypeptide comprising a heterodimeric polypeptide, wherein the heterodimeric polypeptide comprises:

a) a first polypeptide comprising:

i) a peptide epitope comprising a human papilloma virus E7 epitope; and

ii) a first major histocompatibility complex (MHC) polypeptide, wherein the first MHC polypeptide is a β2-microglobulin polypeptide; and

b) a second polypeptide comprising:

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

ii) an immunoglobulin (Ig) Fc polypeptide,

wherein the multimeric polypeptide comprises one or more immunomodulatory domains, wherein the one or more immunomodulatory domain is:

A) at the C-terminus of the first polypeptide;

B) at the N-terminus of the second polypeptide;

C) at the C-terminus of the second polypeptide; or

D) at the C-terminus of the first polypeptide and at the N-terminus of the second polypeptide, and

wherein at least one of the one or more immunomodulatory domains is a variant IL2 polypeptide that exhibits reduced binding affinity to an IL-2 receptor (IL2R) comprising alpha, beta, and gamma polypeptides having amino acid sequences depicted in FIG. 3A-3C, compared to the binding affinity of the IL2 amino acid sequence set forth in SEQ ID NO:1 for the IL2R.

[0004b] In a second aspect, there is provided a protein comprising two of the multimeric polypeptides of the first aspect, wherein each of the two multimeric polypeptides comprises an immunoglobulin (Ig) Fc polypeptide.

[0004c] In a third aspect, there is provided a nucleic acid comprising a nucleotide sequence encoding a first and/or second polypeptide of the first aspect.

[0004d] In a fourth aspect, there is provided an expression vector comprising the nucleic acid of the third aspect.

[0004e] In a fifth aspect, there is provided a host cell genetically modified with the expression vector of the fourth aspect.

[0004f] In a sixth aspect, there is provided a method of producing the multimeric polypeptide of the first aspect or a protein of the second aspect, the method comprising culturing a host cell of the fifth aspect *in vitro* in a culture medium under conditions such that the host cell synthesizes the multimeric polypeptide.

[0004g] In a seventh aspect, there is provided a method of selectively activating an epitope-specific T cell, the method comprising contacting the T cell with the multimeric polypeptide of the first aspect or a protein of the second aspect, wherein said contacting selectively activates the epitope-specific T cell.

[0004h] In an eighth aspect, there is provided a composition comprising:

a) the multimeric polypeptide of the first aspect or a protein of the second aspect; and

b) a pharmaceutically acceptable excipient.

[0004i] In a ninth aspect, there is provided use of the multimeric polypeptide of the first aspect or a protein of the second aspect, or a composition of the eighth aspect in the manufacture of a medicament for treating cancer in an individual.

[0004j] In a tenth aspect, there is provided a multimeric polypeptide comprising a heterodimeric polypeptide, wherein the heterodimeric polypeptide comprises:

a) a first polypeptide comprising

i) a peptide comprising an epitope present in a cancer-associated antigen; and

ii) a first major histocompatibility complex (MHC) polypeptide, wherein the first MHC polypeptide is a β2-microglobulin polypeptide;

b) a second polypeptide comprising:

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

ii) an immunoglobulin (Ig) Fc polypeptide,

wherein the multimeric polypeptide comprises one or more immunomodulatory domains,

wherein at least one of the one or more immunomodulatory domains is a variant IL2 polypeptide comprising an amino acid sequence having at least 85% amino acid sequence identity to set forth in SEQ ID NO:1,

wherein the variant IL-2 polypeptide has one or more amino acid substitutions relative to the sequence set forth in SEQ ID NO:1, and

wherein the variant IL-2 polypeptide exhibits reduced binding affinity to an IL-2 receptor (IL2R) comprising alpha, beta, and gamma polypeptides having amino acid sequences depicted in FIG. 3A-3C, compared to the binding affinity of the IL-2 amino acid sequence set forth in SEQ ID NO: 1 for the IL2R.

[0004k] In an eleventh aspect, there is provided a protein comprising two of the multimeric polypeptides of the tenth aspect, wherein each of the two multimeric polypeptides comprises an immunoglobulin (Ig) Fc polypeptide, and

optionally wherein the two heterodimers are disulfide linked to one another via the Fc polypeptides present in the heterodimers.

[0004l] In a twelfth aspect, there is provided a nucleic acid comprising a nucleotide sequence encoding a first and/or second polypeptide according to the tenth aspect.

[0004m] In a thirteenth aspect, there is provided an expression vector comprising the nucleic acid of the twelfth aspect.

[0004n] In a fourteenth aspect, there is provided a host cell genetically modified with the expression vector of the thirteenth aspect.

[0004o] In a fifteenth aspect, there is provided a method of producing the multimeric polypeptide of the tenth aspect, or a protein of the eleventh aspect, the method comprising culturing a host cell of the fourteenth aspect *in vitro* in a culture medium under conditions such that the host cell synthesizes the multimeric polypeptide.

[0004p] In a sixteenth, there is provided a method of selectively activating an epitope-specific T cell, the method comprising contacting the T cell with the multimeric polypeptide of the tenth aspect or a protein of the eleventh aspect, wherein said contacting selectively activates the epitope-specific T cell, and wherein said contacting is *in vitro* or *in vivo*.

[0004q] In a seventeenth aspect, there is provided a composition comprising:

a) the multimeric polypeptide of the tenth aspect or a protein of the eleventh aspect; and

b) a pharmaceutically acceptable excipient.

[0004r] In an eighteenth aspect, there is provided use of the composition of the seventeenth aspect in the manufacture of a medicament for treating cancer in an individual.

BRIEF DESCRIPTION OF THE DRAWINGS

[0005] **FIG. 1A-1D** schematically depict various embodiments of a T-cell modulatory multimeric polypeptide of the present disclosure. In these embodiments, disulfide bonds are formed between MHC (e.g., HLA) polypeptides present in separate polypeptides.

[0006] **FIG. 2A-2Q** provide an amino acid sequence of wild-type human IL-2 (FIG. 2A); and amino acid sequences of variant IL-2 polypeptides (FIG. 2B-2Q).

[0007] **FIG. 3A-3C** provide amino acid sequences of IL-2 receptor alpha chain (FIG. 3A), beta chain (FIG. 3B), and gamma chain (FIG. 3C).

[0008] **FIG. 4A-4C** provide amino acid sequences of immunoglobulin Fc polypeptides.

[0009] **FIG. 5A-5C** provide amino acid sequences of human leukocyte antigen (HLA) Class I heavy chain polypeptides. Signal sequences are underlined.

[0010] **FIG. 6** provides a multiple amino acid sequence alignment of beta-2 microglobulin (β2M) precursors (i.e., including the leader sequence) from *Homo sapiens* (NP_004039.1; SEQ ID NO:95), *Pan troglodytes* (NP_001009066.1; SEQ ID NO:96), *Macaca mulatta* (NP_001040602.1; SEQ ID NO:97), Bos Taurus (NP_776318.1; SEQ ID NO:98) and Mus musculus (NP_033865.2; SEQ ID NO:99). Amino acids 1-20 are a signal peptide.

[0011] **FIG. 7A-7B** depict production of IL-2/synTacs ("Cue-IL-2-a" and Cue-IL-2-b") of the present disclosure following transient transfection. FIG. 7A depicts unpurified yields; FIG. 7B depicts purified product.

[0012] **FIG. 8A-8B** depict production of IL-2/synTacs of the present disclosure, in which the IL-2 polypeptide is present on the light chain (the polypeptide chain with the light chain (e.g., β2M) of an MHC Class I molecule) or on the heavy chain (the polypeptide chain with the heavy chain of an MHC Class I molecule).

[0013] **FIG. 9** depicts the expression level of IL-2/syn-Tacs, in which the IL-2 is wild-type (wt), or comprises various combinations of F42A, D20K, Q126A, E15A, Y45A, and H16A.

[0014] **FIG. 10** depicts expression of IL-2/synTacs of the present disclosure, in which the IL-2 is present in one copy (1X), two copies (2X) or three copies (3X) in the synTac.

CLAIMS

What is claimed is:

1. A multimeric polypeptide comprising a heterodimeric polypeptide, wherein the heterodimeric polypeptide comprises:

a) a first polypeptide comprising:

i) a peptide epitope comprising a human papilloma virus E7 epitope; and

ii) a first major histocompatibility complex (MHC) polypeptide, wherein the first MHC polypeptide is a β2-microglobulin polypeptide; and

b) a second polypeptide comprising:

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

ii) an immunoglobulin (Ig) Fc polypeptide,

wherein the multimeric polypeptide comprises one or more immunomodulatory domains, wherein the one or more immunomodulatory domain is:

A) at the C-terminus of the first polypeptide;

B) at the N-terminus of the second polypeptide;

C) at the C-terminus of the second polypeptide; or

D) at the C-terminus of the first polypeptide and at the N-terminus of the second polypeptide, and

wherein at least one of the one or more immunomodulatory domains is a variant IL2 polypeptide that exhibits reduced binding affinity to an IL-2 receptor (IL2R) comprising alpha, beta, and gamma polypeptides having amino acid sequences depicted in FIG. 3A-3C, compared to the binding affinity of the IL2 amino acid sequence set forth in SEQ ID NO:1 for the IL2R.

2. A multimeric polypeptide of claim 1, wherein the second polypeptide comprises one or two immunomodulatory polypeptides, and wherein each immunomodulatory polypeptide is selected from the group consisting of

a variant IL-2 polypeptide comprising an H16A substitution relative to set forth in SEQ ID NO:1; and

a variant IL-2 polypeptide comprising an H16A substitution and an F42A substitution relative to set forth in SEQ ID NO:1.

3. A multimeric polypeptide of claim 1 or 2, wherein the peptide epitope comprises the amino acid sequence selected from the group consisting of HPV16E7/82-90 (LLMGTLGIV; SEQ ID NO:11), HPV16E7/86-93 (TLGIVCPI; SEQ ID NO:12), HPV16E7/ll-20 (YMLDLQPETT; SEQ ID NO:13), and HPV16E7/ll-19 (YMLDLQPET; SEQ ID NO:87).

4. A multimeric polypeptide of any of claims 1-3, wherein the Ig Fc polypeptide comprises L234A and L235A substitutions.

5. A multimeric polypeptide of any one of claims 1-4, wherein the β2-microglobulin polypeptide comprises amino acids 21-119 of any one of the amino acid sequences set forth in FIG.6, optionally wherein the amino acid sequence comprises an R12C substitution.

6. A multimeric polypeptide of any one of claims 1-5, wherein the MHC class I heavy chain polypeptide is an HLA-A heavy chain.

7. A multimeric polypeptide of any one of claims 1-6, wherein the MHC class I heavy chain polypeptide comprises amino acids 25 to 365 of the amino acid sequence set forth in FIG. 5A (SEQ ID NO:65), optionally wherein the amino acid sequence comprises a Y84A substitution, an A236C substitution, or both a Y84A substitution and an A236C substitution.

8. A multimeric polypeptide of any one of claims 1-7, wherein the first polypeptide and the second polypeptide are non-covalently associated.

9. A multimeric polypeptide of any one of claims 1-8, wherein the first polypeptide and the second polypeptide are covalently linked to one another.

10. A multimeric polypeptide of claim 9, wherein the covalent linkage is via a disulfide bond.

11. A multimeric polypeptide of claim 10, wherein a disulfide bond joins a Cys residue in the β2M polypeptide and a Cys residue in the MHC heavy chain polypeptide.

12. A multimeric polypeptide of claim 11, wherein a Cys at amino acid residue 12 of the β2M polypeptide is disulfide bonded to a Cys at amino acid residue 236 of the MHC heavy chain polypeptide.

13. A multimeric polypeptide of claim 10, wherein the first polypeptide chain comprises a linker between the peptide epitope and the β2M polypeptide, and wherein the disulfide bond links a Cys present in the linker with a Cys of the MHC heavy chain polypeptide.

14. A multimeric polypeptide of claim 1, comprising:

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

i) a peptide epitope, wherein the peptide epitope comprises the amino acid sequence YMLDLQPETT (SEQ ID NO:13) and has a length of 10 amino acids; and

ii) a first major histocompatibility complex (MHC) polypeptide, wherein the first MHC polypeptide is a β2-microglobulin polypeptide;

b) a second polypeptide comprising in order from N-terminus to C-terminus:

i) a first immunomodulatory polypeptide, wherein the first immunomodulatory polypeptide is a variant IL-2 polypeptide comprising an H16A substitution and an F42A substitution relative to set forth in SEQ ID NO:1;

ii) a second immunomodulatory polypeptide, wherein the second immunomodulatory polypeptide is a variant IL-2 polypeptide comprising an H16A substitution and an F42A substitution relative to set forth in SEQ ID NO:1;

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

iv) an immunoglobulin (Ig) Fc polypeptide,

and wherein:

the β2-microglobulin polypeptide comprises amino acids 21-119 of any one of the amino acid sequences set forth in FIG.6, optionally wherein the amino acid sequence comprises an R12C substitution,

the MHC class I heavy chain polypeptide comprises amino acids 25 to 365 of the amino acid sequence set forth in FIG. 5A (SEQ ID NO:65), optionally wherein the amino acid sequence comprises a Y84A substitution, an A236C substitution, or both a Y84A substitution and an A236C substitution, and

the first polypeptide and the second polypeptide are covalently linked to one another via a disulfide bond, and wherein the disulfide bond joins Cys at amino acid residue 12 of the β2M polypeptide to a Cys at amino acid residue 236 of the MHC heavy chain polypeptide.

15. A multimeric polypeptide of claim 14, wherein

i.) the β2-microglobulin polypeptide comprises the amino acid sequence depicted in FIG. 34A;

ii.) the first and second variant IL-2 polypeptide comprise the amino acid sequence depicted in FIG. 34B;

iii) the MHC Class I heavy chain polypeptide comprises the amino acid sequence depicted in FIG. 34C; and

iv) the Ig Fc polypeptide comprises the amino acid sequence depicted in FIG. 33A, 33B, 33C, or 33D.

16. A multimeric polypeptide of claim 14, wherein the first polypeptide comprises a linker between the epitope and the β2-microglobulin polypeptide, and the second polypeptide comprises a peptide linker between one or more of:

a) a first copy of the variant IL-2 polypeptide and a second copy of the variant IL-2 polypeptide;

b) the variant IL-2 polypeptide and the MHC heavy chain polypeptide; and

c) the MHC heavy chain polypeptide and the IgGl Fe polypeptide.

17. A multimeric polypeptide of claim 16, wherein the peptide linker is selected from $(GGGGS)_3$, $(GGGGS)_4$, and AAAGG.

18. A multimeric polypeptide of any of claims 14-17, comprising:

a) a first polypeptide comprising the amino acid sequence depicted in FIG. 31, and

b) a second polypeptide comprising the amino acid sequence depicted in FIG. 22,

or

a) a first polypeptide comprising the amino acid sequence depicted in FIG. 31, and

b) a second polypeptide comprising the amino acid sequence depicted in FIG. 25,

or

a) a first polypeptide comprising the amino acid sequence depicted in FIG. 31, and

b) a second polypeptide comprising the amino acid sequence depicted in FIG. 28.

19. A protein comprising two of the multimeric polypeptides of any one of claims 1 to 18, wherein each of the two multimeric polypeptides comprises an immunoglobulin (Ig) Fc polypeptide.

20. A protein of claim 19, wherein the two heterodimers are disulfide linked to one another via the Fc polypeptides present in the heterodimers.

21. A nucleic acid comprising a nucleotide sequence encoding a first and/or second polypeptide according to any one of claims 1-18.

22. An expression vector comprising the nucleic acid of claim 21.

23. A host cell genetically modified with the expression vector of claim 22.

24. A method of producing the multimeric polypeptide of any one of claims 1-18 or a protein of claim 19 or 20, the method comprising culturing a host cell of claim 23 *in vitro* in a culture medium under conditions such that the host cell synthesizes the multimeric polypeptide.

25. A method of selectively activating an epitope-specific T cell, the method comprising contacting the T cell with the multimeric polypeptide of any one of claims 1-18 or a protein of claim 19 or 20, wherein said contacting selectively activates the epitope-specific T cell.

26. A method of claim 25, wherein said contacting is *in vitro.*

27. A method of claim 25, wherein said contacting is *in vivo.*

28. A composition comprising:

a) the multimeric polypeptide of any one of claims 1-18 or a protein of claim 19 or 20; and

b) a pharmaceutically acceptable excipient.

29. Use of the multimeric polypeptide of any one of claims 1-18 or a protein of claim 19 or 20, or a composition of claim 28 in the manufacture of a medicament for treating cancer in an individual.

30. The use of claim 29, wherein the medicament is to be administered subcutaneously, intravenously, or peritumorally.

31. The use of claim 29 or 30, wherein the medicament is to be administered systemically, distally to a treatment site, locally, or at or near a treatment site.

32. The use of claim 30, wherein the medicament is to be administered intravenously.

33. A multimeric polypeptide comprising a heterodimeric polypeptide, wherein the heterodimeric polypeptide comprises:

a) a first polypeptide comprising

i) a peptide comprising an epitope present in a cancer-associated antigen; and

ii) a first major histocompatibility complex (MHC) polypeptide, wherein the first MHC polypeptide is a β2-microglobulin polypeptide;

b) a second polypeptide comprising:

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

ii) an immunoglobulin (Ig) Fc polypeptide,

wherein the multimeric polypeptide comprises one or more immunomodulatory domains,

wherein at least one of the one or more immunomodulatory domains is a variant IL2 polypeptide comprising an amino acid sequence having at least 85% amino acid sequence identity to set forth in SEQ ID NO:1,

wherein the variant IL-2 polypeptide has one or more amino acid substitutions relative to the sequence set forth in SEQ ID NO:1, and

wherein the variant IL-2 polypeptide exhibits reduced binding affinity to an IL-2 receptor (IL2R) comprising alpha, beta, and gamma polypeptides having amino acid sequences depicted in FIG. 3A-3C, compared to the binding affinity of the IL-2 amino acid sequence set forth in SEQ ID NO: 1 for the IL2R.

34. A multimeric polypeptide of claim 33, wherein the second polypeptide comprises

i) a first immunomodulatory polypeptide, wherein the first immunomodulatory polypeptide is a variant IL-2 polypeptide comprising an H16A substitution and an F42A substitution relative to set forth in SEQ ID NO:1; and

ii) a second immunomodulatory polypeptide, wherein the second immunomodulatory polypeptide is a variant IL-2 polypeptide comprising an H16A substitution and an F42A substitution relative to set forth in SEQ ID NO:1.

35. A multimeric polypeptide of any one of claims 33-34, wherein the first polypeptide and the second polypeptide are covalently linked to one another via a disulfide bond that joins a Cys residue in the β2M polypeptide and a Cys residue in the MHC heavy chain polypeptide,

and optionally wherein a Cys at amino acid residue 12 of the β2M polypeptide is disulfide bonded to a Cys at amino acid residue 236 of the MHC heavy chain polypeptide.

36. A multimeric polypeptide of any one of claims 33-35, wherein the first polypeptide comprises a linker between the epitope and the β2-microglobulin polypeptide, and the second polypeptide comprises a peptide linker between one or more of:

a) a first copy of the variant IL-2 polypeptide and a second copy of the variant IL-2 polypeptide;

b) the variant IL-2 polypeptide and the MHC heavy chain polypeptide; and

c) the MHC heavy chain polypeptide and the IgGl Fc polypeptide,

and optionally wherein the peptide linker is optionally selected from $(GGGGS)_3$, $(GGGGS)_4$, and AAAGG.

37. A protein comprising two of the multimeric polypeptides of any one of claims 33 to 36, wherein each of the two multimeric polypeptides comprises an immunoglobulin (Ig) Fc polypeptide, and

optionally wherein the two heterodimers are disulfide linked to one another via the Fc polypeptides present in the heterodimers.

38. A nucleic acid comprising a nucleotide sequence encoding a first and/or second polypeptide according to any one of claims 33-36.

39. An expression vector comprising the nucleic acid of claim 38.

40. A host cell genetically modified with the expression vector of claim 39.

41. A method of producing the multimeric polypeptide of any one of claims 33-36, or a protein of claim 37, the method comprising culturing a host cell of claim 40 *in vitro* in a culture medium under conditions such that the host cell synthesizes the multimeric polypeptide.

42. A method of selectively activating an epitope-specific T cell, the method comprising contacting the T cell with the multimeric polypeptide of any one of claims 33-36 or

a protein of claim 37, wherein said contacting selectively activates the epitope-specific T cell, and wherein said contacting is *in vitro* or *in vivo*.

43. A composition comprising:

a) the multimeric polypeptide of any one of claims 33-36 or a protein of claim 37; and

b) a pharmaceutically acceptable excipient.

44. Use of the composition of claim 43 in the manufacture of a medicament for treating cancer in an individual.