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, 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 (β2M) 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 polypeptides, wherein the one or more immunomodulatory polypeptides 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 polypeptides is a variant IL2 polypeptide that comprises an H16A substitution and an F42A substitution relative to set forth in SEQ ID NO:1 and 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 IL-2 amino acid sequence set forth in SEQ ID NO: 1 for the IL2R, and

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

[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 a 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 an *in vitro* 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: (i) the peptide epitope comprises the amino acid sequence YMLDLQPETT (SEQ ID NO:13) and has a length of 10 amino acids, and (ii) the multimeric polypeptide comprises two copies of a variant IL-2 polypeptide comprising an H16A substitution and an F42A substitution, and~~ 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~~, wherein: (i) the peptide epitope comprises the amino acid sequence YMLDLQPETT (SEQ ID NO:13) and has a length of 10 amino acids, and (ii) the multimeric polypeptide comprises two copies of a variant IL-2 polypeptide comprising an H16A substitution and an F42A substitution~~.

~~**[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 polypeptides,~~

~~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 one example, 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*.

[0004q] In a sixteenth 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 another example, there is provided use of the composition of the sixteenth 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.

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, 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 (β2M) 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 polypeptides, wherein the one or more immunomodulatory polypeptides 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 polypeptides is a variant IL2 polypeptide that comprises an H16A substitution and an F42A substitution relative to set forth in SEQ ID NO:1 and 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 IL-2 amino acid sequence set forth in SEQ ID NO: 1 for the IL2R, and

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

~~2. A multimeric polypeptide of claim 1, wherein the second polypeptide comprises the one or more 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), HPV16E7/11-20 (YMLDLQPETT; SEQ ID NO:13), and HPV16E7/11-19 (YMLDLQPET; SEQ ID NO:87).~~

~~4~~2. A multimeric polypeptide of ~~any of claims 1-3~~claim 1, wherein the Ig Fc polypeptide comprises L234A and L235A substitutions.

~~5~~3. A multimeric polypeptide of ~~any one of claims 1-4~~claim 1 or 2, wherein the β2M ~~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 based on the amino acid numbering of the amino acid sequence set forth in SEQ ID NO:16.

~~6~~4. A multimeric polypeptide of any one of claims 1-~~5~~3, wherein the MHC class I heavy chain polypeptide is an HLA-A heavy chain.

~~7~~5. A multimeric polypeptide of any one of claims 1-~~6~~4, 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 SEQ ID NO:14, optionally wherein the MHC class I heavy chain polypeptide comprises the amino acid sequence set forth in SEQ ID NO:19.

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

~~9~~6. A multimeric polypeptide of any one of claims 1-~~7~~5, wherein the first polypeptide and the second polypeptide are covalently linked to one another.

~~10~~7. A multimeric polypeptide of claim ~~9~~6, wherein the covalent linkage is via a disulfide bond.

~~11~~8. A multimeric polypeptide of claim ~~10~~7, wherein the disulfide bond joins a Cys residue in the β2M polypeptide and a Cys residue in the MHC heavy chain polypeptide.

~~12~~9. A multimeric polypeptide of claim ~~11~~8, wherein a Cys at amino acid residue 12, based on the amino acid numbering of SEQ ID NO:17, of the β2M polypeptide is disulfide bonded to a Cys at amino acid residue 236, based on the amino acid numbering of SEQ ID NO:19, of the MHC heavy chain polypeptide.

~~13~~10. A multimeric polypeptide of claim ~~10~~7, 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~~11. A multimeric polypeptide of claim 1, comprising:

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

i) the peptide epitope~~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) the β2M polypeptide~~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~~ the MHC class I heavy chain polypeptide; and

iv) ~~an immunoglobulin (~~the Ig~~)~~ Fc polypeptide,

and wherein:

the β2M ~~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 based on the amino acid numbering of SEQ ID NO:16,

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:14, optionally wherein

the MHC class I heavy chain polypeptide comprises the amino acid sequence set forth in SEQ ID NO:19, 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~~12. A multimeric polypeptide of claim ~~14~~11, wherein

i.) the β2M ~~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 an amino acid sequence having at least 95% amino acid sequence identity to the amino acid sequence depicted in FIG. 33A, 33B, 33C, or 33D.

~~16~~13. A multimeric polypeptide of claim ~~14~~ 11 or ~~15~~12, wherein the first polypeptide comprises a linker between the epitope and the β2M ~~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 class I heavy chain polypeptide; and

c) the MHC heavy chain polypeptide and the Ig~~G1~~ Fc polypeptide.

~~17~~14. A multimeric polypeptide of claim ~~16~~13, wherein the peptide linker is selected from $(GGGGS)_3$, $(GGGGS)_4$, and AAAGG.

~~18~~15. A multimeric polypeptide of any of claims ~~14~~11-~~17~~14, comprising:

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

i) an HPV16 E7 epitope comprising amino acid sequence YMLDLQPETT (SEQ ID NO:13);

ii) a linker comprising the amino acid sequence GGGGSGGGGSGGGGS (SEQ ID NO:89); and

iii) a β2M polypeptide comprising the amino acid sequence set forth in SEQ ID NO:17; and

b) a second polypeptide comprising an amino acid sequence selected from the amino acid sequence depicted in FIG. 22, the amino acid sequence depicted in FIG. 25, and the amino acid sequence depicted in FIG. 28.

~~19~~16. A protein comprising two of the multimeric polypeptides of any one of claims 1 to ~~18~~15~~,~~ ~~wherein each of the two multimeric polypeptides comprises an immunoglobulin (Ig) Fc polypeptide~~.

~~20~~17. A protein of claim ~~19~~16, wherein the two heterodimers are disulfide linked to one another via the Fc polypeptides present in the heterodimers.

~~21~~18. A nucleic acid comprising a nucleotide sequence encoding a first and a second polypeptide according to any one of claims 1-~~18~~15.

~~22~~19. An expression vector comprising the nucleic acid of claim ~~21~~18.

~~23~~20. A host cell genetically modified with the expression vector of claim ~~22~~19.

~~24~~21. A method of producing the multimeric polypeptide of any one of claims 1-~~18~~ 15 or a protein of claim ~~19~~ 16 or ~~20~~17, the method comprising culturing a host cell of claim ~~23~~ 20 *in vitro* in a culture medium under conditions such that the host cell synthesizes the multimeric polypeptide.

~~25~~22. An *in vitro* 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~~ 15 or a protein of claim ~~19~~ 16 or ~~20~~17~~, wherein:~~

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

~~(ii) the multimeric polypeptide comprises two copies of a variant IL-2 polypeptide comprising an H16A substitution and an F42A substitution, and~~

wherein said contacting selectively activates the epitope-specific T cell.

~~26~~23. A composition comprising:

a) the multimeric polypeptide of any one of claims 1-~~18~~ 15 or a protein of claim ~~19~~ 16 or ~~20~~17; and

b) a pharmaceutically acceptable excipient.

~~27~~24. Use of the multimeric polypeptide of any one of claims 1-~~18~~ 15 or a protein of claim ~~19~~ 16 or ~~20~~17, or a composition of claim ~~26~~ 23 in the manufacture of a medicament for treating cancer in an individual~~, wherein:~~

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

~~(ii) the multimeric polypeptide comprises two copies of a variant IL-2 polypeptide comprising an H16A substitution and an F42A substitution~~.

~~28~~25. The use of claim ~~27~~24, wherein the medicament is to be administered subcutaneously, intravenously, or peritumorally.

~~29~~26. The use of claim ~~27~~ 24 or ~~28~~25, wherein the medicament is to be administered systemically, distally to a treatment site, locally, or at or near a treatment site.

~~30~~27. The use of claim ~~28~~25, wherein the medicament is to be administered intravenously.

~~31. 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 polypeptides,~~

~~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.

32. A multimeric polypeptide of claim 31, wherein the second polypeptide comprises two immunomodulatory polypeptides comprising a first and a second immunomodulatory polypeptide, wherein the second polypeptide, wherein:

i) 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) 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.

33. A multimeric polypeptide of any one of claims 31-32, 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, based on the amino acid numbering of SEQ ID NO:17, of the β2M polypeptide is disulfide bonded to a Cys at amino acid residue 236, based on the amino acid numbering of SEQ ID NO:19, of the MHC heavy chain polypeptide.

34. A multimeric polypeptide of any one of claims 31-33, 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 IgG1 Fc polypeptide,

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

35. A protein comprising two of the multimeric polypeptides of any one of claims 31 to 34, 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.

36. A nucleic acid comprising a nucleotide sequence encoding a first and second polypeptide according to any one of claims 31-34.

~~37. An expression vector comprising the nucleic acid of claim 36.~~

~~38. A host cell genetically modified with the expression vector of claim 37.~~

~~39. A method of producing the multimeric polypeptide of any one of claims 31-34, or a protein of claim 35, the method comprising culturing a host cell of claim 38 *in vitro* in a culture medium under conditions such that the host cell synthesizes the multimeric polypeptide.~~

~~40. A composition comprising:~~

~~a) the multimeric polypeptide of any one of claims 31-34 or a protein of claim 35; and~~

~~b) a pharmaceutically acceptable excipient.~~