

[0006] There exists in the art a need for improved modified interleukin 2 (IL-2) polypeptides with or without conjugates. The present invention addresses this and other related needs in the art.

Summary

[0007] The present ~~disclosure~~invention is directed to modified interleukin 2 (IL-2) polypeptides, polynucleotides, *e.g.*, DNA, RNA or viral vector, that encode the modified IL-2 polypeptide and are configured to express said modified IL-2 polypeptide *in vitro* and/or *in vivo*, conjugates comprising the modified IL-2 polypeptides, and uses thereof.

[0008] In one aspect, the present ~~disclosure~~invention is directed to a modified interleukin 2 (IL-2) polypeptide, which comprises an amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 and a substitution with a natural amino acid or an unnatural amino acid at a position selected from the group consisting of Q13, L19, N29, N30, Y31, K32, N33, P34, K35, T37, R38, T41, F42, K43, Y45, K48, K49, E62, K64, P65, N71, Q74, K76, R81, L85, S87, V91, I92, V93 and a combination thereof, wherein said modified IL-2 polypeptide: a) is configured to be unconjugated or conjugated to a water-soluble polymer, a lipid, or a polypeptide, *e.g.*, a protein or a peptide; b) has reduced binding to an interleukin 2 receptor α (IL-2R α) compared to a comparable IL-2 polypeptide comprising an amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 without the substitution; c) has reduced receptor signaling potency to IL-2R $\alpha\beta\gamma$ compared to a comparable IL-2 polypeptide comprising an amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 without the substitution, d) has increased ratio of signaling potency to IL-2R $\beta\gamma$ over signaling potency to IL-2R $\alpha\beta\gamma$ (*i.e.*, increased ratio of signaling potency to IL-2R $\beta\gamma$ / signaling potency to IL-2R $\alpha\beta\gamma$) compared to a comparable IL-2 polypeptide comprising an amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 without the substitution, and/or e) has enhanced receptor signaling potency to IL-2R $\beta\gamma$ compared to a comparable IL-2 polypeptide comprising an amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 without the substitution, and provided that when said modified IL-2 polypeptide comprises a substitution with an unnatural amino acid, said modified IL-2 polypeptide comprises a substitution at a position selected from the group consisting of N29, N30, Y31, K32, N33, P34, K35, R38, T41, F42, K43, Y45, K48, K49, E62, K64, P65, N71, Q74, K76 and a combination thereof, and a substitution with a natural amino acid or an unnatural amino acid at a position within IL-2R α interaction region, IL-2R β interaction region and/or IL-2R γ interaction region, and provided that said modified IL-2

polypeptide has at least about 80% sequence identity in the region of amino acid residues 10-25, 80-100 and/or 100-134 to the corresponding region of a comparable IL-2 polypeptide comprising an amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 without the substitution, and said modified IL-2 polypeptide has at least about 50% sequence identity to a comparable IL-2 polypeptide comprising an amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 without the substitution.

[0009] In another aspectexample, the present disclosureinvention is directed to a polynucleotide, *e.g.*, DNA, RNA or viral vector, that encodes the modified IL-2 polypeptide and is configured to express said modified IL-2 polypeptide *in vitro* and/or *in vivo*. In some embodimentexamples, the modified IL-2 polypeptide, as described above, with or without conjugate, can be applied in the format of a protein, a fusion protein, a protein conjugate, or as part of nanoparticles. In some embodimentexamples, the above polynucleotide, *e.g.*, DNA, RNA or viral vector, that encodes the modified IL-2 polypeptide and is configured to express said modified IL-2 polypeptide *in vitro* and/or *in vivo*, can be applied to cell(s), tissue(s), organ(s), or subject(s), *e.g.*, human subject(s).

[0010] In still another exampleaspect, the present invention-disclosure is directed to a modified IL-2 polypeptide conjugate, which comprises a modified IL-2 polypeptide, as described above, that is conjugated to a water-soluble polymer, a lipid, a polypeptide, *e.g.*, a protein, or a peptide.

[0011] In yet another aspectexample, the present invention-disclosure is directed to a pharmaceutical composition comprising an effective amount of a modified IL-2 polypeptide, a polynucleotide, *e.g.*, DNA, RNA or viral vector, or a modified IL-2 polypeptide conjugate, as described above, and a pharmaceutically acceptable carrier or excipient.

[0012] In yet another exampleaspect, the present disclosureinvention is directed to a method for treating or preventing a disease or a disorder, *e.g.*, a proliferation disease or disorder, an autoimmune or inflammatory disease or disorder, or an infectious disease or disorder, in a subject in need comprising administering to said subject an effective amount of a modified IL-2, a polynucleotide, *e.g.*, DNA, RNA or viral vector, a modified IL-2 polypeptide conjugate or a pharmaceutical composition, as described above.

[0013] In yet another exampleaspect, the present disclosureinvention is directed to an use of an effective amount of a modified IL-2 polypeptide, a polynucleotide, *e.g.*, DNA, RNA or viral vector, or a modified IL-2 polypeptide conjugate, as described above, for the manufacture of a

medicament for treating or preventing a disease or a disorder, *e.g.*, a proliferation disease or disorder, an autoimmune or inflammatory disease or disorder, or an infectious disease or disorder, in a subject.

[0014] In yet another exampleaspect, the present disclosureinvention is directed to a method of expanding a CD4⁺ helper cell, CD8⁺ effector naive and memory cell, Natural Killer (NK) cell, or Natural killer T (NKT) cell population, which comprises contacting a cell population with an effective amount of a modified IL-2 polypeptide, a polynucleotide, *e.g.*, DNA, RNA or viral vector, or a modified IL-2 polypeptide conjugate, as described above, or a pharmaceutical composition comprising the modified IL-2 polypeptide, a polynucleotide, *e.g.*, DNA, RNA or viral vector, or modified IL-2 polypeptide conjugate for a time sufficient to induce formation of a complex with an IL-2R $\beta\gamma$, thereby stimulating the expansion of the T cell, NK cell, and/or NKT cell population.

[0015] In yet another exampleaspect, the present disclosureinvention is directed to a method of expanding a CD4⁺ helper cell, CD8⁺ effector naive and memory cell, Treg cells, Natural Killer (NK) cell, or Natural killer T (NKT) cell population, which comprises contacting a cell population with an effective amount of a modified IL-2 polypeptide, a polynucleotide, *e.g.*, DNA, RNA or viral vector, or a modified IL-2 polypeptide conjugate, as described above, or a pharmaceutical composition comprising the modified IL-2 polypeptide, a polynucleotide, *e.g.*, DNA, RNA or viral vector, or modified IL-2 polypeptide conjugate for a time sufficient to induce formation of a complex with an IL-2R $\beta\gamma$, thereby stimulating the expansion of the T cell, Treg cell, NK cell, and/or NKT cell population with reduced cell death by 10% to 100%.

[0016] In yet another exampleaspect, the present disclosureinvention is directed to an use of an effective amount of a modified IL-2 polypeptide, a polynucleotide, *e.g.*, DNA, RNA or viral vector, or a modified IL-2 polypeptide conjugate, as described above, for the manufacture of a medicament for expanding a CD4⁺ helper cell, CD8⁺ effector naive and memory cell, Treg cell, Natural Killer (NK) cell, or Natural killer T (NKT) cell in a cell population.

[0016a] In another aspect, the present disclosure refers to a polynucleotide that encodes a modified IL-2 polypeptide as described herein and is configured to express said modified IL-2 polypeptide *in vitro* and/or *in vivo*, wherein the polynucleotide comprises:

a) DNA; and/or

b) RNA; and/or

c) a viral vector; and/or

combinations of a)-c).

[0016b] In another aspect, the present disclosure refers to a modified IL-2 polypeptide conjugate comprising a modified IL-2 polypeptide as described herein, conjugated to a water-soluble polymer, a lipid, a polypeptide, a protein, or a peptide.

[0016c] In another aspect, the present disclosure refers to a pharmaceutical composition comprising an effective amount of a modified IL-2 polypeptide as described herein, a polynucleotide as described herein, or a modified IL-2 polypeptide conjugate as described herein, and a pharmaceutically acceptable carrier or excipient.

[0016d] In another aspect, the present disclosure refers to an effective amount of a modified IL-2 polypeptide as described herein, a polynucleotide as described herein, a modified IL-2 polypeptide conjugate as described herein, or a pharmaceutical composition as described herein for use in treating or preventing a disease or a disorder.

[0016e] In yet another aspect, the present disclosure refers to a use of an effective amount of a modified IL-2 polypeptide as described herein, a polynucleotide as described herein, a modified IL-2 polypeptide conjugate as described herein, or a pharmaceutical composition as described herein in the manufacture of a medicament for treating or preventing a disease or a disorder in a subject, wherein the disease or disorder comprises: a proliferation disease or disorder; an autoimmune or inflammatory disease or disorder; or an infectious disease or disorder, wherein the subject has, is suspected of having, or at risk of having the disease or disorder, and wherein the medicament is to be administered to the subject.

[0016f] In yet another aspect, the present disclosure refers to a method of expanding *in vitro*, or *ex vivo*, a CD4⁺ helper cell, CD8⁺ effector naive and memory cell, Natural Killer (NK) cell, or Natural killer T (NKT) cell population, which comprises contacting a cell population with an effective amount of a modified IL-2 polypeptide as described herein, a polynucleotide as described herein, a modified IL-2 polypeptide conjugate as described herein, or a pharmaceutical composition comprising: the modified IL-2 polypeptide; the polynucleotide; or the modified IL-2 polypeptide conjugate, for a time sufficient to induce formation of a complex with an IL-2R $\beta\gamma$, thereby stimulating the expansion of the T cell, NK cell, and/or NKT cell population.

[0016g] In yet another aspect, the present disclosure refers to a use of an effective amount a modified IL-2 polypeptide as described herein, a polynucleotide as described herein, a modified IL-2 polypeptide conjugate as described herein, or a pharmaceutical composition as described herein, for the manufacture of a medicament for expanding, *in vivo*, a CD4⁺ helper cell, CD8⁺ effector naive and memory cell, Natural Killer (NK) cell, or Natural killer T (NKT) cell population, which comprises contacting the cell population for a time sufficient to induce formation of a complex with an IL-2R $\beta\gamma$, thereby stimulating the expansion of the T cell, NK cell, and/or NKT cell population.

[0017] Other ~~examples~~aspects and advantages of the present ~~disclosure~~invention will be apparent from the ~~embodiments and~~ examples provided herein.

[0018] For the sake of brevity, the disclosures of the publications cited in this specification, including patents, are herein incorporated by reference.