

CYTOKINE CONJUGATES FOR THE TREATMENT OF PROLIFERATIVE AND INFECTIOUS DISEASES

CROSS-REFERENCE

[0001] This application claims the benefit of US Provisional Application No. 62/540,781, filed on August 3, 2017, which is incorporated herein by reference in its entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on July 27, 2018, is named 46085-710_601_SL.txt and is 3,703 bytes in size.

BACKGROUND OF THE DISCLOSURE

[0003] Distinct populations of T cells modulate the immune system to maintain immune homeostasis and tolerance. For example, regulatory T (Treg) cells prevent inappropriate responses by the immune system by preventing pathological self-reactivity while cytotoxic T cells target and destroy infected cells and/or cancerous cells. In some instances, modulation of the different populations of T cells provides an option for treatment of a disease or indication.

SUMMARY OF THE DISCLOSURE

[0003a] In one aspect, there is provided a modified interleukin 2 (IL-2) polypeptide comprising at least one unnatural amino acid covalently attached to a conjugating moiety, wherein the position of the at least one unnatural amino acid is K35, T37, R38, T41, F42, K43, F44, Y45, E60, E61, E62, K64, P65, E68, V69, N71, L72, M104, C105, and Y107, wherein the residue positions correspond to positions 35, 37, 38, 41, 42, 43, 44, 45, 61, 62, 64, 65, 68, 69, 71, 72, 104, 105, and 107 as set forth in SEQ ID NO: 1; wherein the conjugating moiety is a water-soluble polymer; and wherein the modified IL-2 polypeptide comprises at least 80% sequence identity to SEQ ID NO: 1.

[0003b] In one aspect, there is provided a pharmaceutical composition comprising the modified IL-2 polypeptide as described herein and a pharmaceutically acceptable excipient.

[0003c] In one aspect, there is provided a use of the modified IL-2 polypeptide as described herein or the pharmaceutical composition as described herein in the manufacture of a

medicament for treating a proliferative disease or condition in a subject in need thereof, wherein the medicament is to be administered to the subject.

[0003d] In one aspect, there is provided an *in vitro* or *ex vivo* method of expanding a CD4+ helper cell, CD8+ effector naïve cell, CD8+ effector memory cell, Natural Killer (NK) cell, or Natural killer T (NKT) cell population, comprising: contacting a cell population with the modified IL-2 polypeptide as described herein or the pharmaceutical composition as described herein for a time sufficient to induce formation of a complex with an IL-2R $\beta\gamma$, thereby stimulating the expansion of the Teff and/or NK cell population.

[0003e] In one aspect, there is provided a use of the modified IL-2 polypeptide as described herein or the pharmaceutical composition as described herein in the manufacture of a medicament for expanding a CD4+ helper cell, CD8+ effector naïve cell, CD8+ effector memory cell, Natural Killer (NK) cell, or Natural killer T (NKT) cell population, wherein the medicament is to be contacted with a cell population for a time sufficient to induce formation of a complex with an IL-2R $\beta\gamma$, thereby stimulating the expansion of the Teff and/or NK cell population.

[0003f] In one aspect, there is provided the modified IL-2 polypeptide as described herein or the pharmaceutical composition as described herein for use in therapy.

[0004] Disclosed herein, in certain embodiments, are cytokine conjugates and use in the treatment of one or more indication. In some embodiments, also described herein include interleukin 2 (IL-2) conjugates and use in the treatment of one or more indications. In some instances, the one or more indications comprise cancer or a pathogenic infection. In some cases, described herein are methods of modulating the interaction between IL-2 and IL-2 receptor to stimulate or expand specific T cell, Natural Killer (NK) cell, and/or Natural killer T (NKT) cell populations. In additional cases, further described herein are pharmaceutical compositions and kits that comprise one or more interleukin conjugates (e.g., IL-2 conjugates) described herein.

[0005] Disclosed herein, in certain embodiments, is an interleukin 2 (IL-2) conjugate comprising: an isolated and purified IL-2 polypeptide; and a conjugating moiety that binds to the isolated and purified IL-2 polypeptide at an amino acid position selected from K35, T37, R38, T41, F42, K43, F44, Y45, E61, E62, E68, K64, P65, V69, L72, and Y107, wherein the numbering of the amino acid residues corresponds to SEQ ID NO: 1. In some embodiments, the amino acid position is selected from T37, R38, T41, F42, F44, Y45, E61, E62, E68, K64, P65, V69, L72, and Y107. In some embodiments, the amino acid position is selected from T37, R38, T41, F42, F44, Y45, E61, E62, E68, P65, V69, L72, and Y107. In some embodiments, the

CLAIMS

WHAT IS CLAIMED IS:

1. A modified interleukin 2 (IL-2) polypeptide comprising
at least one unnatural amino acid covalently attached to a conjugating moiety,

wherein the position of the at least one unnatural amino acid is K35, T37, R38, T41, F42, K43, F44, Y45, E60, E61, E62, K64, P65, E68, V69, N71, L72, M104, C105, and Y107, wherein the residue positions correspond to positions 35, 37, 38, 41, 42, 43, 44, 45, 61, 62, 64, 65, 68, 69, 71, 72, 104, 105, and 107 as set forth in SEQ ID NO: 1;

wherein the conjugating moiety is a water-soluble polymer; and

wherein the modified IL-2 polypeptide comprises at least 80% sequence identity to SEQ ID NO: 1.
2. The modified IL-2 polypeptide of claim 1, wherein the position of the at least one unnatural amino acid is selected from T37, R38, T41, F42, K43, F44, Y45, E61, E62, P65, E68, and L72, wherein the residue positions correspond to the positions 37, 38, 41, 42, 43, 44, 45, 61, 62, 65, 68, and 72 as set forth in SEQ ID NO: 1.
3. The modified IL-2 polypeptide of claim 1, wherein the position of the at least one unnatural amino acid is selected from K35, K64, V69, N71, M104, C105, and Y107, wherein the residue positions correspond to the positions 35, 64, 69, 71, 104, 105, and 107 as set forth in SEQ ID NO: 1.
4. The modified IL-2 polypeptide of claim 1, wherein the position of the at least one unnatural amino acid is selected from T37, R38, T41, Y45, E61, E68, and L72, wherein the residue positions correspond to the positions 37, 38, 41, 45, 61, 68, and 72 as set forth in SEQ ID NO: 1.
5. The modified IL-2 polypeptide of claim 1, wherein the position of the at least one unnatural amino acid is selected from F42, K43, F44, E62, and P65, wherein the residue positions correspond to the positions 42, 43, 44, 62, and 65 as set forth in SEQ ID NO: 1.
6. The modified IL-2 polypeptide of claim 5, wherein the position of the at least one unnatural amino acid is P65, wherein the residue position corresponds to position 65 as set forth in SEQ ID NO: 1.
7. The modified IL-2 polypeptide of any one of claims 1-6, wherein the at least one unnatural amino acid
is a lysine analogue or
comprises an aromatic side chain.

8. The modified IL-2 polypeptide of claim 7, wherein the at least one unnatural amino acid comprises N6-azidoethoxy-L-lysine (AzK), N6-propargylethoxy-L-lysine (PraK), BCN-L-lysine, norbornene lysine, TCO-lysine, methyltetrazine lysine, allyloxycarbonyllysine, p-acetyl-L-phenylalanine, p-azidomethyl-L-phenylalanine (pAMF), p-iodo-L-phenylalanine, m-acetylphenylalanine, p-propargyloxyphenylalanine, p-propargyl-phenylalanine, 3-methyl-phenylalanine, fluorinated phenylalanine, isopropyl-L-phenylalanine, p-azido-L-phenylalanine, p-acyl-L-phenylalanine, p-benzoyl-L-phenylalanine, p-bromophenylalanine, p-amino-L-phenylalanine, isopropyl-L-phenylalanine, O-allyltyrosine, O-methyl-L-tyrosine, O-4-allyl-L-tyrosine, 4-propyl-L-tyrosine, phosphotyrosine, L-3-(2-naphthyl)alanine, 2-amino-3-((2-((3-(benzyloxy)-3-oxopropyl)amino)ethyl)sulfonyl)propanoic acid, or 2-amino-3-(phenylsulfonyl)propanoic acid.
9. The modified IL-2 polypeptide of any one of claims 1-8, wherein the water-soluble polymer comprises polyethylene glycol (PEG), poly(propylene glycol) (PPG), copolymers of ethylene glycol and propylene glycol, poly(oxyethylated polyol), poly(olefinic alcohol), poly(vinylpyrrolidone), poly(hydroxyalkylmethacrylamide), poly(hydroxyalkylmethacrylate), poly(saccharides), poly(α -hydroxy acid), poly(vinyl alcohol), polyphosphazene, polyoxazolines (POZ), poly(N-acryloylmorpholine), or a combination thereof.
10. The modified IL-2 polypeptide of claim 9, wherein the water-soluble polymer comprises PEG.
11. The modified IL-2 polypeptide of claim 10, wherein the PEG has a weight-average molecular weight of from about 20,000 Daltons to about 85,000 Daltons..
12. The modified IL-2 polypeptide of claim 11, wherein the PEG has a weight-average molecular weight of about 20,000 Daltons, about 25,000 Daltons, about 30,000 Daltons, about 35,000 Daltons, about 40,000 Daltons, about 45,000 Daltons, or about 50,000 Daltons.
13. The modified IL-2 polypeptide of any one of claims 1-12, wherein the conjugating moiety is bound to the at least one unnatural amino acid of the modified IL-2 polypeptide through a linker.
14. The modified IL-2 polypeptide of claim 13, wherein the linker comprises a homobifunctional linker, a heterobifunctional linker, a cleavable or a non-cleavable dipeptide linker, a maleimide group, a spacer, or a combination thereof.
15. The modified IL-2 polypeptide of any one of claims 1-14, wherein the modified IL-2 polypeptide comprises an N-terminal deletion.

16. The modified IL-2 polypeptide of claim 15, wherein the N-terminal deletion comprises a deletion of the first 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, or 30 residues from the N-terminus, wherein the residue positions are in reference to the positions in SEQ ID NO: 1.
17. The modified IL-2 polypeptide of any one of claims 1-16, wherein the modified IL-2 polypeptide comprises about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 1.
18. The modified IL-2 polypeptide of any one of claims 1-17, wherein the modified IL-2 polypeptide is capable of expanding CD4+ helper cell, CD8+ effector naïve cell, CD8+ effector memory cell, Natural Killer (NK) cell, or Natural killer T (NKT) cell populations, or a combination thereof.
19. The modified IL-2 polypeptide of any one of claims 1-17, wherein the conjugating moiety or the unnatural amino acid impairs or blocks the binding of IL-2 with IL-2R α .
20. The modified IL-2 polypeptide of any one of claims 1-17, wherein activation of CD4+ helper cell, CD8+ effector naïve cell, CD8+ effector memory cell, Natural Killer (NK) cell, or Natural killer T (NKT) cell populations via the IL-2R $\beta\gamma$ complex by the modified IL-2 polypeptide retains significant potency of activation of said cell population relative to a wild-type IL-2 polypeptide.
21. The modified IL-2 polypeptide of claim 20, wherein the receptor signaling potency of the modified IL-2 polypeptide to the IL-2R $\beta\gamma$ complex is higher than a receptor signaling potency of the wild-type IL-2 polypeptide to the IL-2R $\beta\gamma$ complex.
22. The modified IL-2 polypeptide of claim 20, wherein the receptor signaling potency of the modified IL-2 polypeptide to the IL-2R $\beta\gamma$ complex is lower than a receptor signaling potency of the wild-type IL-2 polypeptide to the IL-2R $\beta\gamma$ complex.
23. The modified IL-2 polypeptide of any one of claims 1-17, wherein the modified IL-2 polypeptide exhibits a first receptor signaling potency to IL-2R $\beta\gamma$ and a second receptor signaling potency to IL-2R $\alpha\beta\gamma$, and wherein the first receptor signaling potency is at least 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 30-fold, 50-fold, 100-fold, 500-fold, or higher than the second receptor signaling potency.
24. The modified IL-2 polypeptide of claim 23, wherein the first receptor signaling potency of the modified IL-2 polypeptide is higher than a receptor signaling potency of the wild-type IL-2 polypeptide to the IL-2R $\beta\gamma$, and the second receptor signaling potency of the modified IL-2 polypeptide is lower than a receptor signaling potency of the wild-type IL-2 polypeptide to the IL-2R $\alpha\beta\gamma$.

25. The modified IL-2 polypeptide of claim 23, wherein the first receptor signaling potency of the modified IL-2 polypeptide is at least 1-fold lower than a receptor signaling potency of the wild-type IL-2 polypeptide.
26. A pharmaceutical composition comprising the modified IL-2 polypeptide of any one of claims 1-25
and
a pharmaceutically acceptable excipient.
27. The pharmaceutical composition of claim 26, wherein the pharmaceutical composition is formulated for systemic delivery.
28. The pharmaceutical composition of claim 26, wherein the pharmaceutical composition is formulated for parenteral administration.
29. Use of the modified IL-2 polypeptide of any one of claims 1-25 or the pharmaceutical composition of any one of claims 26-28 in the manufacture of a medicament for treating a proliferative disease or condition in a subject in need thereof, wherein the medicament is to be administered to the subject.
30. The use of claim 29, wherein the proliferative disease or condition is a cancer.
31. The use of claim 30, wherein the cancer is a solid tumor cancer.
32. The use of claim 31, wherein the solid tumor cancer is bladder cancer, bone cancer, brain cancer, breast cancer, colorectal cancer, esophageal cancer, eye cancer, head and neck cancer, kidney cancer, lung cancer, melanoma, ovarian cancer, pancreatic cancer, or prostate cancer.
33. The use of claim 30, wherein the cancer is a hematologic malignancy.
34. The use of claim 33, wherein the hematologic malignancy is chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), follicular lymphoma (FL), diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma (MCL), Waldenstrom's macroglobulinemia, multiple myeloma, extranodal marginal zone B cell lymphoma, nodal marginal zone B cell lymphoma, Burkitt's lymphoma, non-Burkitt high grade B cell lymphoma, primary mediastinal B-cell lymphoma (PMBL), immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, B cell prolymphocytic leukemia, lymphoplasmacytic lymphoma, splenic marginal zone lymphoma, plasma cell myeloma, plasmacytoma, mediastinal (thymic) large B cell lymphoma, intravascular large B cell lymphoma, primary effusion lymphoma, or lymphomatoid granulomatosis.
35. The use of claim 29, wherein an additional therapeutic agent is to be administered.
36. The use of claim 35, wherein the medicament and the additional therapeutic agent are to be administered simultaneously.

37. The use of claim 35, wherein the medicament and the additional therapeutic agent are to be administered sequentially.
38. The use of claim 35, wherein the medicament is to be administered prior to the additional therapeutic agent.
39. The use of claim 35, wherein the medicament is to be administered after the administration of the additional therapeutic agent.
40. An *in vitro* or *ex vivo* method of expanding a CD4+ helper cell, CD8+ effector naïve cell, CD8+ effector memory cell, Natural Killer (NK) cell, or Natural killer T (NKT) cell population, comprising:
contacting a cell population with the modified IL-2 polypeptide of any one of claims 1-25 or the pharmaceutical composition of any one of claims 26-28 for a time sufficient to induce formation of a complex with an IL-2R $\beta\gamma$, thereby stimulating the expansion of the Teff and/or NK cell population.
41. The method of claim 40, wherein the modified IL-2 polypeptide of any one of claims 1-25 or the pharmaceutical composition of any one of claims 26-28 expands CD4+ T regulatory (Treg) cells by less than 20%, 15%, 10%, 5%, or 1% in the CD3+ cell population compared to an expansion of CD4+ Treg cells in the CD3+ cell population contacted with a wild-type IL-2 polypeptide.
42. The method of claim 40, wherein the modified IL-2 polypeptide of any one of claims 1-25 or the pharmaceutical composition of any one of claims 26-28 does not expand CD4+ Treg cells in the cell population.
43. The method of claim 40, wherein the ratio of the Teff cells to Treg cells in the cell population after incubation with the modified IL-2 polypeptide of any one of claims 1-25 or the pharmaceutical composition of any one of claims 26-28 is about or at least 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 20:1, 50:1, or 100:1.
44. The method of any one of claims 40-43, wherein the subject is a human.
45. Use of the modified IL-2 polypeptide of any one of claims 1-25 or the pharmaceutical composition of any one of claims 26-28 in the manufacture of a medicament for expanding a CD4+ helper cell, CD8+ effector naïve cell, CD8+ effector memory cell, Natural Killer (NK) cell, or Natural killer T (NKT) cell population, wherein the medicament is to be contacted with a cell population for a time sufficient to induce formation of a complex with an IL-2R $\beta\gamma$, thereby stimulating the expansion of the Teff and/or NK cell population.
46. The use of claim 45, wherein the medicament expands CD4+ T regulatory (Treg) cells by less than 20%, 15%, 10%, 5%, or 1% in the CD3+ cell population compared to an

expansion of CD4+ Treg cells in the CD3+ cell population contacted with a wild-type IL-2 polypeptide.

47. The use of claim 45, wherein the medicament does not expand CD4+ Treg cells in the cell population.
48. The use of claim 45, wherein the ratio of the Teff cells to Treg cells in the cell population after incubation with the medicament is about or at least 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 20:1, 50:1, or 100:1.
49. The use of any one of claims 29 to 39 or 45 to 48, wherein the subject is a human.
50. The modified IL-2 polypeptide of any one of claims 1-25 or the pharmaceutical composition of any one of claims 26-28 for use in therapy.