CYTOKINE CONJUGATES FOR THE TREATMENT OF AUTOIMMUNE 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 August 3, 2018, is named 46085-710_602_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 K8, K9, L12, E15, H16, L19, D20, Q22, M23, N26, D84, N88, E95, E100, D109, N119, T123, Q126, S127, or T131, wherein the residue positions correspond to positions 8, 9, 12, 15, 16, 19, 20, 22, 23, 26, 84, 88, 95, 100, 109, 119, 123, 126, 127, and 131 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 disclosed 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 of as described herein in the manufacture of a

medicament for treating an autoimmune disease or disorder 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 CD4+ regulatory T (Treg) cell population, comprising: contacting a cell 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-2Rαβγ, thereby stimulating the expansion of the Treg 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 CD4+ regulatory T (Treg) cell population, wherein the medicament is to be contacted with a cell for a time sufficient to induce formation of a complex with an IL-2Rαβγ, thereby stimulating the expansion of the Treg cell population.

[0003f] In one aspect, there is provided a 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 an autoimmune disease. In some cases, described herein are IL-2 conjugates for modulating the interaction between IL-2 and IL-2 receptor to stimulate or expand specifically regulatory T cell (Treg cell) populations. In some cases, described herein are IL-2 conjugates with extended in vivo half-life, reduced toxicity, and/or expanded therapeutic windows. In additional cases, described herein are pharmaceutical compositions and kits which comprise one or more interleukin conjugates (e.g., IL-2 conjugates) described herein.

Disclosed herein, in certain embodiments, is an isolated and modified interleukin 2 (IL-2) polypeptide comprising at least one unnatural amino acid at a position that reduces receptor signaling potency to interleukin 2 receptor βγ (IL-2Rβγ) or reduces a recruitment of an IL-2Rγ subunit to the IL-2/IL-2Rβ complex, but retains significant activation of interleukin 2 αβγ receptor (IL-2Rαβγ), wherein the reduced receptor signaling potency is compared to the receptor signaling potency between a wild-type IL-2 polypeptide and IL-2Rβγ, and wherein the

Claims

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 K8, K9, L12, E15, H16, L19, D20, Q22, M23, N26, D84, N88, E95, E100, D109, N119, T123, Q126, S127, or T131, wherein the residue positions correspond to positions 8, 9, 12, 15, 16, 19, 20, 22, 23, 26, 84, 88, 95, 100, 109, 119, 123, 126, 127, and 131 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 K8, K9, and H16, wherein the residue positions correspond to positions 8, 9, and 16 as set forth in SEQ ID NO: 1.
3. The modified IL-2 polypeptide of claim 1 or 2, wherein the position of the at least one unnatural amino acid is K9, wherein the residue position corresponds to position 9 as set forth in SEQ ID NO: 1.
4. The modified IL-2 polypeptide of claim 1 or 2, wherein the position of the at least one unnatural amino acid is H16, wherein the residue position corresponds to position 16 as set forth in SEQ ID NO: 1.
5. The modified IL-2 polypeptide of any one of claim 1-4, wherein the at least one unnatural amino acid
    is a lysine analogue or
    comprises an aromatic side chain.
6. The modified IL-2 polypeptide of any one of claims 1-5, 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, phosphonotyrosine, L-3-(2-naphthyl)alanine, 2-amino-3-((2-((3-(benzyloxy)-3-oxopropyl)amino)ethyl)selanyl)propanoic acid, or 2-amino-3-(phenylselanyl)propanoic.

7. The modified IL-2 polypeptide of any one of claims 1-6 , 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.

8. The modified IL-2 polypeptide of claim 7, wherein the water-soluble polymer comprises PEG.

9. The modified IL-2 polypeptide of claim 8, wherein the PEG has a weight-average molecular weight of from about 10,000 Daltons to about 85,000 Daltons.

10. The modified IL-2 polypeptide of claim 9, 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.

11. The modified IL-2 polypeptide of any one of claims 1-10, wherein the conjugating moiety is indirectly bound to the at least one unnatural amino acid of the modified IL-2 through a linker.

12. The modified IL-2 polypeptide of claim 11, 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.

13. The modified IL-2 polypeptide of any one of claims 1-12, wherein the modified IL-2 polypeptide comprises an N-terminal deletion.

14. The modified IL-2 polypeptide of claim 13, 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.

15. The modified IL-2 polypeptide of any one of claims 1-14, wherein the modified IL-2 polypeptide comprises about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 1.
16. The modified IL-2 polypeptide of any one of claims 1-15, wherein the modified IL-2 is capable of expanding CD4+ T regulatory (Treg) cells.
17. The modified IL-2 polypeptide of any one of claims 1-15, wherein the conjugating moiety impairs or blocks the receptor signaling potency of IL-2 with IL-2Rβγ, or reduces recruitment of the IL-2Rγ subunit to the IL-2/IL-2Rβ complex.
18. The modified IL-2 polypeptide of any one of claims 1-15, wherein CD4+ Treg cell proliferation by the modified IL-2/IL-2Rαβγ complex is equivalent or greater to that of a wild-type IL-2 polypeptide.
19. The modified IL-2 polypeptide of any one of claims 1-15, wherein the modified IL-2/IL-2Rαβγ complex induces proliferation of the CD4+ Treg cells to a population that is sufficient to modulate a disease course in an animal model.
20. The modified IL-2 polypeptide of any one of claims 1-15, wherein the modified IL-2 polypeptide exhibits a first receptor signaling potency to IL-2Rβγ and a second receptor signaling potency to IL-2Rαβγ, 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, 1000-fold, or lower than the second receptor signaling potency.
21. The modified IL-2 polypeptide of claim 20, wherein the first receptor signaling potency of the modified IL-2 polypeptide is lower than a receptor signaling potency of the wild-type IL-2 polypeptide to IL-2Rβγ.
22. The modified IL-2 polypeptide of claim 20, wherein 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 IL-2Rαβγ.
23. The modified IL-2 polypeptide of any one of claims 1-15, wherein the modified IL-2 polypeptide further provides an increase in a recruitment of an IL-2Rα subunit to the IL-2 polypeptide leading to activation of interleukin 2 αβγ receptor (IL-2Rαβγ), wherein the increase in recruitment is compared to a recruitment of an IL-2Rα subunit by a wild-type IL-2 polypeptide.
24. The modified IL-2 polypeptide of any one of claims 1-15, wherein the modified IL-2 polypeptide further provides a decrease in a recruitment of an IL-2Rγ subunit to the

IL-2/IL-2Rβ complex, wherein the reduced recruitment is compared to a recruitment of an IL-2Rβ subunit and/or IL-2Rγ subunit by a wild-type IL-2 polypeptide.

25. A pharmaceutical composition comprising the modified IL-2 polypeptide of any one of claims 1-24

and

a pharmaceutically acceptable excipient.

26. The pharmaceutical composition of claim 25, wherein the pharmaceutical composition is formulated for systemic delivery.

27. The pharmaceutical composition of claim 25, wherein the pharmaceutical composition is formulated for parenteral administration.

28. Use of the modified IL-2 polypeptide of any one of claims 1 to 24, or the pharmaceutical composition of any one of claims 25-27 in the manufacture of a medicament for treating an autoimmune disease or disorder in a subject in need thereof, wherein the medicament is to be administered to the subject.

29. The use of claim 28, wherein the autoimmune disease or disorder comprises alopecia areata, autoimmune hemolytic anemia, autoimmune hepatitis, dermatomyositis, type 1 diabetes, juvenile idiopathic arthritis, glomerulonephritis, Graves' disease, Guillain-Barré syndrome, idiopathic thrombocytepenic purpura, myasthenia gravis, multiple sclerosis, pemphigus/pemphigoid, pernicious anemia, polyarteritis nodosa, polymyositis, primary biliary cirrhosis, psoriasis, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic lupus erythematosus, thyroiditis, uveitis, vitiligo, or Wegener's granulomatosis.

30. The use of claim 28 or 29, wherein an additional therapeutic agent is to be administered.

31. The use of claim 30, wherein the medicament and the additional therapeutic agent are to be administered simultaneously.

32. The use of claim 30, wherein the medicament and the additional therapeutic agent are to be administered sequentially.

33. The use of claim 32, wherein the medicament is to be administered prior to the additional therapeutic agent.

34. The use of claim 32, wherein the medicament is to be administered after the administration of the additional therapeutic agent.

35. The use of any one of the claims 28-34, wherein the subject is a human.

36. An *in vitro* or *ex vivo* method of expanding CD4+ regulatory T (Treg) cell population, comprising:

contacting a cell with the modified IL-2 polypeptide of any one of claims 1 to 24, or the pharmaceutical composition of any one of claims 25-27 for a time sufficient to induce formation of a complex with an IL-2Rαβγ, thereby stimulating the expansion of the Treg cell population.

37. Use of the modified IL-2 polypeptide of any one of claims 1 to 24, or the pharmaceutical composition of any one of claims 25-27 in the manufacture of a medicament for expanding CD4+ regulatory T (Treg) cell population, wherein the medicament is to be contacted with a cell for a time sufficient to induce formation of a complex with an IL-2Rαβγ, thereby stimulating the expansion of the Treg cell population.

38. The modified IL-2 polypeptide of any one of claims 1 to 24, or the pharmaceutical composition of any one of claims 25-27 for use in therapy.