

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 selected from K9, H16, L19, D20, N26, N88, E100, and N119, wherein the residue positions correspond to positions 9, 16, 19, 20, 26, 88, 100, and 119 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 K9 and H16, wherein the residue positions correspond to positions 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)sulfonyl)propanoic acid, or 2-amino-3-(phenylsulfonyl)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-2R $\beta\gamma$, or reduces recruitment of the IL-2R γ subunit to the IL-2/IL-2R β 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-2R $\alpha\beta\gamma$ 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-2R $\alpha\beta\gamma$ 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-2R $\beta\gamma$ and a second receptor signaling potency to IL-2R $\alpha\beta\gamma$, 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-2R $\beta\gamma$.
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-2R $\alpha\beta\gamma$.
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-2R α subunit to the IL-2 polypeptide leading to activation of interleukin 2 $\alpha\beta\gamma$ receptor (IL-2R $\alpha\beta\gamma$), wherein the increase in recruitment is compared to a recruitment of an IL-2R α 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-2R γ subunit to the IL-2/IL-2R β complex, wherein the reduced recruitment is compared to a recruitment of an IL-2R β subunit and/or IL-2R γ 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 thrombocytopenic 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-2R $\alpha\beta\gamma$, 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-2R $\alpha\beta\gamma$, 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.