

CLAIMS

WHAT IS CLAIMED IS:

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

wherein the position of the at least one unnatural amino acid is selected from K35, F42, K43, E62, and P65, wherein the residue positions correspond to positions 35, 42, 43, 62, and 65 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 F42, wherein the residue position corresponds to position 42 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 K35, wherein the residue position corresponds to position 35 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 K43, wherein the residue position corresponds to position 43 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 E62, wherein the residue position corresponds to position 62 as set forth in SEQ ID NO: 1.
6. The modified IL-2 polypeptide of claim 1, 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, 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.
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 20 kDa to 85 kDa.
 12. The modified IL-2 polypeptide of claim 11, wherein the PEG has a weight-average molecular weight of about 20 kDa, about 25 kDa, about 30 kDa, about 35 kDa, about 40 kDa, about 45 kDa, or about 50 kDa.
 13. The modified IL-2 polypeptide of claim 12, wherein the PEG has a weight-average molecular weight of about 30 kDa.
 14. The modified IL-2 polypeptide of any one of claims 1-13, wherein the conjugating moiety is bound to the at least one unnatural amino acid of the modified IL-2 polypeptide through a linker.
 15. The modified IL-2 polypeptide of claim 14, wherein the linker comprises a homobifunctional linker, a heterobifunctional linker, a cleavable or a non-cleavable dipeptide linker, a spacer, or a combination thereof.
 16. The modified IL-2 polypeptide of any one of claims 1-15, wherein the modified IL-2 polypeptide comprises an N-terminal deletion.
 17. The modified IL-2 polypeptide of claim 16, 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, or 25 residues from

the N-terminus, wherein the residue positions are in reference to the positions in SEQ ID NO: 1.

18. The modified IL-2 polypeptide of any one of claims 1-17, wherein the modified IL-2 polypeptide comprises about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 1.
19. The modified IL-2 polypeptide of any one of claims 1-18, 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, Natural killer T (NKT) cell populations, or a combination thereof.
20. The modified IL-2 polypeptide of any one of claims 1-18, wherein the conjugating moiety or the unnatural amino acid impairs or blocks the binding of IL-2 with IL-2R α .
21. The modified IL-2 polypeptide of any one of claims 1-18, 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.
22. The modified IL-2 polypeptide of claim 21, 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.
23. The modified IL-2 polypeptide of claim 21, 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.
24. The modified IL-2 polypeptide of any one of claims 1-18, 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, or 500-fold higher than the second receptor signaling potency.
25. The modified IL-2 polypeptide of claim 24, 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 $\alpha\beta\gamma$.

26. The modified IL-2 polypeptide of claim 24, 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.
27. A pharmaceutical composition comprising the modified IL-2 polypeptide of any one of claims 1-26 and a pharmaceutically acceptable excipient.
28. The pharmaceutical composition of claim 27, wherein the pharmaceutical composition is formulated for systemic delivery.
29. The pharmaceutical composition of claim 27, wherein the pharmaceutical composition is formulated for parenteral administration.
30. Use of the modified IL-2 polypeptide of any one of claims 1-26 or the pharmaceutical composition of any one of claims 27-29 in the manufacture of a medicament for treating a cancer in a subject in need thereof, wherein the medicament is to be administered to the subject.
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 30, 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. The use of any one of claims 30-39, wherein in the modified IL-2 polypeptide 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.
41. 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 1-26 or the pharmaceutical composition of any one of claims 27-29 for a time sufficient to induce formation of a complex with an IL-2R $\beta\gamma$, thereby stimulating the expansion of Teff and/or the NK cell population.
42. The *in vitro* or *ex vivo* method of claim 41, wherein the modified IL-2 polypeptide of any one of claims 1-26 or the pharmaceutical composition of any one of claims 27-29 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.
43. The *in vitro* or *ex vivo* method of claim 41, wherein the modified IL-2 polypeptide of any one of claims 1-26 or the pharmaceutical composition of any one of claims 27-29 does not expand CD4+ Treg cells in the cell population.
44. The *in vitro* or *ex vivo* method of claim 41, 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 1-26 or the pharmaceutical composition of any one of claims 27-29 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.
45. The use of any one of claims 30-40, wherein the subject is a human.
46. The modified IL-2 polypeptide of any one of claims 1-26 or the pharmaceutical composition of any one of claims 27-29 for use in therapy.